Development of a mouldable and resorbable bone filler

By
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A thesis submitted for the degree of Doctor of Philosophy

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I hereby declare that this thesis is entirely my own work, with due acknowledgement being made in the text where work has been conducted in collaboration with another. This thesis has not been submitted to any other University or higher education institution, or for any other academic award in this University.

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Imelda Harte
Abstract

The purpose of this study was to develop a resorbable, mouldable bone filler that can be used to repair damaged bone. Work was focused on the use of the resorbable polymer poly DL lactide (PDLLA). The ideal formulation was to be hand mouldable and thus the initial step was to carry out a plasticization study on the polymer. Differential Scanning Calorimetry (DSC), Dynamic Mechanical Thermal Analysis (DMTA) and tensile testing were conducted on plasticized PDLLA to indicate the effect citrate ester plasticizers had on the production of a mouldable PDLLA compound. Blending PDLLA with different citrate esters resulted in sample compositions which were malleable and flexible, with increasing plasticizer content having an increase effect on the hand mouldable characteristics.

This was followed by an investigation into improving the load bearing properties of the plasticized PDLLA, and so an examination into chemically crosslinking the polymer was carried out, initiated by the use of heat, gamma radiation and UV irradiation. The resultant structures were analysed in a series of swelling studies with the most promising crosslinked compositions then analysed by DMTA and tensile testing. Crosslinking was found not to be a viable approach to harden the polylactide filler as not only were high temperatures needed for crosslinking initiation which would cause cellular necrosis, there was also a reduction in the mechanical and physical integrity of the polymer worsening its load bearing properties.

An alternative method of producing a polylactide based bone filler was also explored. By combining polylactide particles with the liquid monomer cyanoacrylate, a hand mouldable and putty-like compound was created. With the onset of polymerization of the monomer, the material then hardened. The composition was modified with the addition of plasticizers and a hydroxyapatite (HA) filler. Initial water absorption and tensile testing conducted found that the addition of the plasticizer and the HA decreased the mechanical properties. A study into optimising the compounds composition was then carried out, and a new source of HA was added to the compositions which helped to increase mechanical properties.

The new material composition was found to be injectable with setting rates which could be altered. Aging the polymer compound in PBS for 8 weeks did not alter the resultant tensile properties of the compound by a noticeable degree. A tensile modulus of 1.3 GPa, a tensile strength of 40MPa, a fracture toughness of 2.5MPa m$^{1/2}$, a flexural modulus of 1.7GPa, and a flexural strength of 37MPa were the highest mechanical results achieved. Some of important mechanical properties are lower then those found in human cortical bone, however the results are higher than many commercially available bone substitutes. With further modification of compositions, the mechanical properties could be further increased producing a material fulfilling requirements for a bone graft substitute which is currently not commercially available.
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Chapter 1
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1.0 Introduction

Bone graft is the second most common transplantation tissue, with blood being the most common. Bone grafting is a surgical procedure that involves filling a bone defect with a bone graft or a substitute (Bashoor-Zadeh et al. 2009) and more than 2.2 million bone grafting procedures are performed annually worldwide in order to repair bone defects in orthopaedics, neurosurgery and dentistry (Giannoudis et al. 2005). The indications for bone grafting include the treatment of post-traumatic skeletal complications such as non-unions, delayed unions and malunions, spinal fusions, joint arthroplasty and to fill defects following removal of bone tumors or defects. The gold standard of bone grafting is harvesting autologous cortical and cancellous bone from the iliac crest, however problems using an autograft exist and alternative bone substitutes are needed (Ilan and Ladd 2003).

There is great research interest in the development of biodegradable synthetic polymers with properties modified to meet the biochemical and biomechanical requirements for various orthopaedic applications such as bone grafting. Biodegradable polymer implants can provide initial structural support for damaged bone and subsequently degrade within the required timeframe for new bone growth and remodelling to take place. Injectable polymer systems have the advantage of employing minimally invasive procedures such as arthroscopic delivery. These systems could help in complex fracture and bone defect repair where substantial amount of bone is lost due to trauma or disease. Furthermore, the injectable polymers may be used to reinforce the fixation of implants such as plates and screws, particularly in patients with osteoporotic bone. Ideally upon injection the polymer should harden without detrimental effects to the surrounding tissue, maintain mechanical and physical integrity and facilitate cell attachment and growth. To date, very few synthetic polymer based injectable systems have been developed that possess suitable properties for orthopaedic applications, such as high strength and controlled degradation rates.
1.1 Aims and objectives

The overall aim of this project is to develop a resorbable and mouldable bone filler that can be used to repair damaged bone. The ideal material should be hand mouldable and easily injectable, have good adhesion to bone and harden in-situ with appropriate load bearing properties. The material should also resorb at a rate equivalent to that of bone re-growth with no harmful degradation products. In this study, work is focused on the use of polymers from the polylactide family as these materials are known to have the necessary mechanical and biological properties needed for such an application.

The ideal formulation should be hand mouldable and easily injectable and so the initial step was to blend the polymer with a plasticizer to create the bone filler base compound. This was followed by an investigation into methods of introducing crosslinks into the polymer material in order to improve mechanical properties in situ. As the crosslinks prevent free chain movement and crosslinked polymers are generally stronger and less flexible than their linear forms, crosslinking the polymer is a possible method of hardening the injected bone filler once it is in place.

An alternative method of producing an injectable polylactide based bone filler was also explored. By combining polylactide particles with a liquid monomer, a base compound could be created which would be hand mouldable and easily injectable. With the onset of polymerization of the monomer, the filler could then harden in situ. Modification of the composition with the addition of plasticizers, fillers and other additives allows for the adjustment of mechanical properties and setting rates and this is also investigated.

1.2 Target material

In order to achieve the aims of this project, some target material properties need to be established for an ideal bone filler product. Ease of handling is of utmost importance for the clinical use of a bone filler. Viscous properties must be balanced between the need for the material to remain at the site of insertion and the need for the surgeon to easily manipulate its placement. Commercial bone cements are usually applied in two ways. Cements can be applied using a syringe or cement gun
to inject the cement. To be injected by hand, the applied force is limited to about 200 N (Bohner and Baroud 2005). Alternatively, the cement is mixed and allowed to reach a doughy state when its viscosity is high enough that it can be manually manipulated and inserted into the bone. A putty-like viscosity within the range of $10^3$ to $10^4$ Pa s is ideal. Working time is also of great importance. Injectable commercial bone cements and void fillers usually have a period of 10 to 15 minutes from the start of mixing of the components, changing the cement from a viscous liquid to a hard, elastic solid. A slightly longer working time may be more appropriate, giving the surgeon a wider period of time to ensure the filler is appropriately inserted.

In general, values for the mechanical properties of bone vary from one bone to another as well as within different regions of the same bone. Properties also differ depending on the direction of loading; if loaded in tension bone is stronger when loaded longitudinally rather than circumferentially. A wide range of properties have been reported in literature depending on the type of bone, with the upper limits quoted for that of cortical bone. In order for a bone void filler to be utilized in load bearing applications in long bones, it is required to meet the mechanical properties quoted for cortical bone. The tensile modulus reported for cortical bone varies from 12 to 20 GPa with the tensile strength varying from 53 to 130 MPa (Currey 2006). The tensile modulus and strength for cancellous bone has been reported to be as low as 0.9 GPa and 2 MPa respectively (Rho et al. 1998). The flexural strength of cortical bone is in the range of 135 to 193 MPa (Wagoner Johnson and Herschler 2011) while flexural moduli in the range of 14 to 21GPa have been reported (Currey 2006). Fracture toughness values have been reported to be in the range of 0.1 MPa m$^{-1/2}$ for cancellous bone and up to 6.0 MPa m$^{-1/2}$ for cortical bone (Moore et al. 2001).

Rate of degradation is also a very important property of the bone filler. The material should provide initial structural support for damaged bone and subsequently degrade within the required timeframe for new bone growth and remodelling to take place. Bone remodelling rate varies in different types of bones, however in a healthy adult an entire remodelling cycle usually requires approximately 4- 6 months. The rate of natural bone fracture healing also varies depending on numerous factors, however the formation of a callus is generally developed at the site of injury in the
first 4 to 8 weeks and has limited strength. Bone remodelling then occurs over months to years with adequate strength usually developing by 6 months (Kalervo Vaananen and Zhao 2008). Therefore, it is important that the bone filler maintains its mechanical integrity throughout the first few months to enable adequate bone healing to occur.

List of properties for ideal mouldable and resorbable bone filler material:
Upon mixing:
- Hand mouldable material with putty-like viscosity within range of $10^2$ to $10^4$ Pa $\cdot$ s
- Injection force not exceeding 200 N
- Working / setting time of approximately 15 - 30 minutes
Once set in situ:
- Tensile modulus in range of 12 to 20 GPa
- Tensile strength in range of 53 to 130 MPa
- Flexural strength in range of 135 to 193 MPa
- Flexural moduli in the range of 14 to 21 GPa
- Fracture toughness of approximately 6.0 MPa $\cdot$ m$^{-1/2}$
- must maintain mechanical integrity for initial months of implantation

1.3 Project Sponsor
The work in this project is carried out on behalf of Stryker Orthopaedics, Limerick. Stryker Corporation is a leader in the worldwide orthopaedic market and is one of the world’s largest medical device companies. It delivers a wide range of capabilities including joint replacements, trauma, spine and micro implant systems, orthobiologics, powered surgical instruments, surgical navigation systems, endoscopic products, as well as patient handling and emergency medical equipment. Through the development of products that combine both natural and synthetic technologies, Stryker has established a position in the marketplace as an innovator in the emerging field of bone substitute technology. Science, technology, and innovation make up the foundation of Stryker’s expanding line of new bone substitutes, which are being embraced by physicians worldwide for their ease of use and potential to improve patient outcomes.
Chapter 1

1.4 Thesis outline

Chapter 1 has given an introduction to this thesis and outlines the aims and objectives.

Chapter 2 gives a review of the current relevant literature starting with an introduction to the mechanisms of human bone remodelling and healing along with an overview of its mechanical and structural properties. An introduction to the surgical procedure of bone grafting is given with particular reference to current methods and materials used and their associated problems. Following this, the review will focus on biodegradable polymers, in particular the polyester polylactide-its synthesis, properties and methods of modification relevant to this research project. Finally the review will concentrate on a polylactide based composite and its constituents with a view to developing a novel synthetic bone graft substitute.

Chapter 3 outlines the different preparation methods, tests and techniques which are employed throughout the thesis in order to evaluate the performance of the materials with potential use as a bone filler substitute.

Chapters 4 to 7 discuss and analyse the results from the research completed

- Chapter 4 focuses on creating a hand mouldable bone filler base compound. The polymer poly (D,L lactide) was blended with different plasticizers and the resultant thermal, dynamic mechanical and tensile properties are analysed.
- Chapter 5 focuses on crosslinking the polymer as a method of hardening the bone filler in situ and improving its load bearing properties. An investigation into introducing crosslinks chemically, through gamma irradiation and UV irradiation was carried out with resultant crosslink densities analysed. The dynamic mechanical and tensile properties of several crosslinked compositions are also examined.
- Chapter 6 focuses on producing an injectable polylactide based bone filler by combining polylactide particles with a liquid monomer. Polylactide particles were blended with various ratios of cyanoacrylate monomer,
plasticizer and hydroxyapatite and the resulting compositions were tensile tested and placed in buffered saline to observe the effects of water absorption and hardness with time.

- Chapter 7 focuses on compositions which showed the most promising results as established in chapter 6. Tensile testing was completed on samples aged in buffered saline while double torsion and flexural testing was also conducted on selected sample compositions. An investigation into the setting times for each composition was completed using a rheometer with the injectability of each sample also analysed. Monomer conversion measurements were also conducted in order to examine the effect sterilization would have on the BCA monomer.

Chapter 8 concludes with the findings and results obtained from this research. Recommendations for future work are also outlined.

1.5 References


Chapter 2
2.0 Literature Review

2.1 Introduction
This review aims to give the reader an introduction to the mechanisms of human bone remodelling and healing along with an overview of bones mechanical and structural properties. An introduction to the surgical procedure of bone grafting is given with particular reference to current methods and materials used and their associated problems. Following this, the review will focus on biodegradable polymers, in particular the polyester polylactide- its synthesis, properties and methods of modification relevant to this research project. Finally the review will concentrate on a polylactide based composite and its constituents with a view to developing a novel synthetic bone graft substitute.

2.2 Bone
Bones provide mechanical support for joints, tendons and ligaments, protect vital organs, and act as a reservoir for calcium and phosphate in the preservation of normal mineral homeostasis (Ralston 2009). It is a viscoelastic composite biomaterial consisting of cells (10%) held within a matrix (90%) which has both organic and inorganic components (Little et al. 2011). Bone is a complex and dynamic tissue that has remarkable ability for self-repair and regeneration after an injury and is able to completely restore its mechanical function on all levels of hierarchical structure (Manjubala et al. 2009).

2.3 Bone remodelling
An important aspect of bone behaviour is its capacity to modify its microstructure and properties according to its environment. It undergoes substantial changes in structure, shape and composition according to mechanical and physiological conditions (Doblare et al. 2004). A decrease in mechanical load causes resorption of bones while an increase leads to bone formation (Chen et al. 2006). There are two different types of remodelling: internal, in which the material properties change with time and external, in which the shape and geometry of the bone changes with time (Garcia et al. 2002). External resorption occurs by resorption or deposition of bone
material on the surface while changes to porosity, mineral content and total weight of
the bone occur during internal remodelling (Chen et al. 2006).

Remodelling enables bones to grow, modify their shape, self repair when fractured
and continuously renew themselves. The cycle is shown below in Figure 2.1. The
process of remodelling is controlled by specialized bone cells, some of which
include osteoblasts, osteocytes, osteoclasts and bone lining cells. These cells operate
together as organised units during remodelling and the process is known to follow a
well defined sequence of activation, resorption and formation (Doblare et al. 2004).

![Figure 2.1 Bone remodelling cycle](Ralston 2009)

2.3.1 Osteoclasts

Osteoclasts are bone destroying cells originating from bone marrow and remove
bone by demineralising it with acid and dissolving collagen with enzymes (Doblare
et al. 2004). They are large multinucleated cells that clamp themselves to the surface
of the bone and dissolve the material underneath. Debris, both organic and mineral is
packed into vesicles which pass through the cell body of the osteoclasts (Currey
2006).
2.3.2 Osteoblasts
Osteoblasts are the cells that form bone with the main secretory product of the cell being type I collagen and other noncollagenous proteins (Donahue et al. 2005). Their initial role is to lay down the collagenous matrix in which mineral is later deposited (Currey 2006). They are differentiated mesenchymal cells created at the periosteum layer or stromal tissue of bone marrow (Doblare et al. 2004).

2.3.3 Osteocytes
Osteocytes are the cells in the body of the bone and are derived from osteoblasts originating from bone marrow (Currey 2006). They are imprisoned in the hard bone tissue located in lacunae and communicate with neighbouring cells via canaliculi. It has been suggested that osteocytes are the cells that control bone remodelling, but this has yet to be proven (Doblare et al. 2004).

2.3.4 Bone lining cells
Bone lining cells cover all surfaces of bones forming a thin continuous layer that controls the movement of ions between the body and the bone (Currey 2006). They are inactive osteoblasts which can be reactivated in response to chemical or mechanical stimuli (Doblare et al. 2004).

2.4 Bone healing
Bone a living tissue, has the capability to regenerate itself and form new tissue where it is damaged or missing. The healing process is complex and involves the coordinated participation of immigration, differentiation and proliferation of inflammatory cells, angioblasts, fibroblasts, chondroblasts and osteoblasts which synthesize and release bioactive substances of extracellular matrix components (Doblare et al. 2004). The stages in bone healing as seen in Figure 2.2 are haematoma formation, inflammation, angiogenesis (formation of new blood vessels), cartilage formation (with subsequent calcification, cartilage removal and bone formation) and bone remodelling (Phillips 2005).

The process of bone fracture healing as described by Doblare starts when blood from ruptured vessels form a haemorrhage at the fracture gap space (Doblare et al. 2004).
Cells called macrophages remove dead tissue and generate a preliminary tissue for the migration of undifferentiated mesenchymal cells where an initial stabilizing callus is formed. The mesenchymal cells may then differentiate into chondrocytes, osteoblasts or fibroblasts depending on the environmental conditions, after which they begin to synthesize an extracellular matrix. Woven bone is produced at each side of the gap site by osteoblasts and advances to the centre of the callus. At the same time cartilage is formed by chondrogenesis at the centre of the callus. Once the callus is filled mainly by cartilage, a process called ossification occurs until all the cartilage has been replaced by bone, achieving good stabilization and sufficient stiffness. The final stage is the remodelling of the fracture site to restore its original internal structure and shape.

![Figure 2.2 The stages of bone healing](Sfeir et al. 2005)

When this natural process does not occur, as in the case of fracture non-unions or large scale traumatic bone injury, surgical intervention and the use of bone graft substitutes is warranted (Khan et al. 2008)
2.5 Bone Grafting

Bone graft is the second most frequent transplantation tissue with blood being the most common. In 2005, 2.2 million bone grafting procedures took place worldwide in order to repair bone defects in orthopaedics, neurosurgery and dentistry (Giannoudis et al. 2005). Bone grafting is a surgical procedure that involves filling a bone defect with a bone graft or a substitute (Bashoor-Zadeh et al. 2009). It is preformed for the treatment of posttraumatic skeletal complications such as non-unions, delayed unions and malunions. Although most fractures heal uncomplicated, 5–10% of patients encounter problems due to bone defects or impaired fracture healing, or a combination of both (Van der Stok et al. 2011). Bone grafting may also be utilized for spinal fusions, joint arthroplasty and to fill defects following removal of bone tumors or defects caused by congenital diseases (Giannoudis et al. 2005).

Marsh (2006) describes the main indications for bone graft substitutes at fracture sites as structural support, for void filling, to help accelerate fracture repair, when healing delays are anticipated and to repair non-unions or delayed unions. A graft can provide structural support in a variety of acute and subacute repair circumstances allowing for patient mobilization. The graft helps to neutralize forces at the fracture site increasing the possibility of uniting the fractured tissue. Void filling is a different type of structural support provided by grafts or graft substitutes in acute trauma. In acute fractures, grafts may accelerate fracture repair in circumstances where high mechanical demands on an implant may otherwise result in implant failure before healing. Bone graft substitutes also may be used in acute or sub-acute trauma if healing delays are anticipated. In the past, grafting was not indicated until a non-union was established after an arbitrary timeframe or a series of radiographs. Currently, surgeons frequently choose to operate earlier in the course of fracture repair when healing delays or failures are anticipated. The classic indication for graft or graft substitutes is the repair of non-unions or delayed unions, usually in combination with internal or external fixation. The greater the mechanical instability and the less the prior osteogenic response, the more certain it is that osteoinductive graft material will be necessary. Grafts are used to fill gaps, span defects or as onlays to exposed surfaces (Marsh 2006). Knowledge of the mechanical and structural properties of bone is needed to identify the optimum requirements of a suitable bone substitute.
2.6 Mechanical Properties and Structure of Bone

The term bone refers to a family of materials each with a somewhat different structural design, but all having a basic building block, the mineralized collagen fibril (Weiner and Wagner 1998). Bone is hierarchical and complex with a varied arrangement of structures at many levels as shown in Figure 2.3. In order to understand the mechanical properties of bone material, it is important to understand the mechanical properties of its component phases and the structural relationship between them at the various levels of structural hierarchical organisation (Rho et al. 1998).

Figure 2.3 Structural organisation of Bone (Rho et al. 1998)

Bone is the internal support system in all higher vertebrates and along with muscles, tendons and cartilage; it forms the human musculoskeletal system (Currey 2006). The main function of bone is to support the body and anchor muscles while enclosing and protecting the brain, lungs and other vital organs. It serves as a site for producing red and other blood cells while also as a mineral reservoir, indirectly helping to maintain body fluids and support metabolic activities. By acting with the skeletal muscles, bone can maintain or change position of body parts and also helps in the transmission of forces from one part of the body to another under controlled strain (Doblare et al. 2004).
The main components of bone include hydroxyapatite, collagen, small amounts of proteoglycans, noncollagenous proteins and water. The mechanical properties of bone are a result of a need for a certain stiffness to reduce strain and a need for ductility to absorb impacts and reduce the risk of fracture. The inorganic components are mainly responsible for the compressive strength and stiffness while the organic components provide the tensile properties (Doblare et al. 2004). At the lowest level bone can be described as being comprised of the fibrous protein collagen stiffened by extremely dense calcium phosphate crystals (Currey 2006).

2.6.1 Collagen matrix

Collagen is the most abundant protein found in animals and it makes up for more than half the protein found in the human body. The chemical structure of type I collagen is shown in Figure 2.4. Only in vertebrates does it undergo a transformation into a mineralized skeletal structure but it can also be found unmineralized when it is required to be flexible but not very extensible (Currey 2006).

![Chemical structure of collagen type I](image)

**Figure 2.4 Chemical structure of collagen type I.** (a) Primary amino acid sequence, (b) tropocollagen molecule, (c) tropocollagen molecules line up in files to form microfibrils (Friess 1998).
Collagen comprises of approximately 90% of the organic matrix in bone (Knott and Bailey 1998) of which the main collagen present is type I (Tzaphlidou 2005). The repetitive nature of the amino acid sequences of collagen, which consists of – (Glycine – X – Y-)n - , where X and Y are frequently proline and hydroxyproline residues, allows the protein to assemble itself into triple helical structures referred to as tropocollagen molecules (Olszta et al. 2007). The tropocollagen molecules line up in files and bond with molecules in neighbouring files to form microfibrils which then in turn aggregate to form fibrils (Currey 2006).

It is very difficult to measure the mechanical properties of an individual fibre of collagen as they almost never exist alone in biological tissues. Also, the packing of fibrils in a fibre may vary from one tissue to another which in turn would influence the resultant mechanical properties (Weiner and Wagner 1998). The remaining 10% organic matrix in bone of which is not collagen consists of noncollagenous proteins (NCP’s). A number of these proteins play a role in the initiation and control of mineralization or reconstruction, while the remainder have a role in binding the collagen and mineral together (Currey 2006).

### 2.6.2 Mineral phase

Impregnating and surrounding the collagen is the bone mineral, which is a variety of calcium phosphate, some in the form of hydroxyapatite (Currey 2006). According to Weiner and Wagner, the apatite crystals are plate shaped with average lengths and widths of 50 X 25 nm (Weiner and Wagner 1998). Isolated crystals from human bone are shown in Figure 2.5

In “Bones” by J.D. Currey the mineralization is explained as a process, by which a matrix of collagen plus a few other organic components called osteoids is initially laid down, mostly on the surface of the bone. Mineral is then deposited in the collagen, at first in the gap zones and then along the length of the collagen fibrils orientated in the direction of the collagen fibril, from this plate like mineral crystals grow and some form large clusters. Mineral is also deposited between the fibrils where the plates are precipitated with one of its long axes aligned with the collagen fibrils (Currey 2006).
Chapter 2

Figure 2.5 Isolated crystals from human bone (Weiner and Wagner 1998)

Reliable measurements of the mechanical properties of biological carbonate apatite are important for the understanding of the properties of bone, but measurements of a single crystal have not been made to date, most likely due to their size. The Young’s modulus of synthetic powdered carbonated apatite has been reported as 109 GPa and at 114 GPa for a large single crystal of hydroxyapatite (Weiner and Wagner 1998).

In “The material bone: Structure-mechanical function relations”, Weiner and Wagner report a study preformed by Currey, in which a large sample of bones with different porosities and structures were mechanically tested. It was found that the Young’s modulus of compact bone in tension increased with increasing mineral content while the ultimate strain and work under the stress-strain curve decreased with mineral content, regardless of the porosity and structure of the bone.

2.6.3 Woven and Lamellar bone

Above the level of the collagen fibril and its associated mineral, mammalian bone exists in two distinct forms; woven bone and lamellar bone (Currey 2006). Woven and lamellar fibril array patterns are shown in Figure 2.6. Woven bone or primary bone is considered immature bone and is characterised by a coarse fibre arrangement with no orientation. The mineral content is variable and cells are randomly arranged (Doll 2005). It is usually laid down quickly, in the foetus and in the callus that is produced during fracture repair (Currey 2006), it is then later replaced by lamellar bone (Doblare et al. 2004). Measurements of the elastic modulus of human foetal woven bone ranged from 4 to 17 GPa (Weiner and Wagner 1998). Lamellar bone is
laid down much slower than woven bone (Currey 2006). It is arranged more precisely, is highly organised and has parallel layers or lamellae that make it stronger than woven bone (Doblare et al. 2004). A Young’s modulus of approximately 22 GPa was reported for a dry specimen of osteon lamellar bone (Rho et al. 1998).

Figure 2.6 Woven and lamellar fibril array patterns of organisation SEM micrographs of fractured surface and schematic illustration (Weiner and Wagner 1998) (a) Woven fibre structure, (b) Lamellar structure

2.6.4 Cancellous bone and Cortical bone

On a macroscopic level, woven and lamellar tissues form two types of bone – cancellous (trabecular) bone and cortical (compact) bone. The distinction between these two types of bone is related to their porosity (Zylberberg 2004). Cancellous bone with 50-95% porosity is usually found in cuboidal bones, flat bones and at the end of long bones, while cortical bone with 5-10% porosity is usually found in the shafts of long bones and surrounding the cancellous bone forming the external shell of flat bones (Doblare et al. 2004). A bone section showing cancellous and cortical bone can be seen in Figure 2.7. The pores in cancellous bone are interconnected and filled with marrow while the bone matrix has a variable arrangement of plates and struts called trabeculae (Doblare et al. 2004). Rho et al. comment on a range of
modulus values reported for single trabeculae in literature, varying from 1 to 20GPa (Rho et al. 1998). They suggest that the discrepancies may be caused by the influence of microstructure, location and density variations of the trabeculae tested.

Figure 2.7 Bone section showing cancellous and cortical bone (Doblare et al. 2004)

Table 2.1 Mechanical properties of single osteon segments (Rho et al. 1998)

<table>
<thead>
<tr>
<th>Osteon segments</th>
<th>Longitudinal lamellar orientation</th>
<th>Lamellar orientation at sharp angles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension</td>
<td>Modulus (GPa)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Strength (MPa)</td>
<td>120</td>
</tr>
<tr>
<td>Compression</td>
<td>Modulus (GPa)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Strength (MPa)</td>
<td>110</td>
</tr>
<tr>
<td>Bending</td>
<td>Modulus (GPa)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Strength (MPa)</td>
<td>390</td>
</tr>
<tr>
<td>Torsion</td>
<td>Modulus (GPa)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Strength (MPa)</td>
<td>200</td>
</tr>
</tbody>
</table>

Cortical bone is comprised of layers of lamellar bone and woven bone with vascular channels located mainly in the woven bone. The most complex type of cortical bone
is Haversian bone, a schematic of which can be seen in Figure 2.8. Vascular channels are surrounded by lamellar bone; this arrangement is called the osteon (Doll 2005). Cortical bone becomes a complex of many adjacent osteons and lamellae. Rho et al. report on a study which preformed mechanical testing on single osteon segments with lamellar orientation in the longitudinal direction and osteons which have adjacent lamella orientation at sharp angles to each other (Rho et al. 1998). Results are shown in the table 2.1.

![Figure 2.8 Microscopic structure of cortical bone](image)

Lamellar orientation within an osteon is fundamental in the resultant mechanical behaviour. From this study it was found that osteons with longitudinal lamellae are better for tension and torsion and perhaps stronger in bending, while osteons with alternating lamella are more suited for compression. It is important to note here that
not all research papers agree that osteon architectures can be classified as neatly as having an orientation in either a longitudinal direction or at sharp angles from one another (Rho et al. 1998).

In cortical bone mechanical properties are influenced greatly by porosity, the mineralization level and the organization of the solid matrix. In general, values for the mechanical properties of bone vary from one bone to another as well within different regions of the same bone (Rho et al. 1998). In “Bones” by J.D. Currey the mechanical properties of human Haversian bone is reported with specimens cut longitudinally and circumferentially with respect to the long axis of the bone. Table 2.2 shows the difference in the properties when bone is loaded in different directions. If bone is loaded in tension it is stronger and has a much higher strain to failure when it is loaded longitudinally rather than circumferentially (Currey 2006).

### Table 2.2 Mechanical properties of human haversian bone (Currey 2006)

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal</th>
<th>Circumferential</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tension</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulus (GPa)</td>
<td>17.7</td>
<td>12.8</td>
</tr>
<tr>
<td>Strength (MPa)</td>
<td>133</td>
<td>53</td>
</tr>
<tr>
<td><strong>Compression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulus (GPa)</td>
<td>18.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Strength (MPa)</td>
<td>205</td>
<td>131</td>
</tr>
</tbody>
</table>

### 2.7 Bone grafting options

A detailed knowledge of the mechanical and structural properties of bone along with a better understanding of the biology of bone healing have led to the development of several bone graft substitute options currently available to orthopaedic surgeons. The gold standard of bone-grafting is harvesting autologous cortical and cancellous bone from the iliac crest (Giannoudis et al. 2005). Problems with using an autograft, however, exist and a great deal of research has been conducted in recent years in order to develop the ideal bone graft substitute. The ideal bone graft material would be osteoconductive, osteoinductive, osteogenic, resorbable in a particular manner, biological acceptable and have a proven safety profile with no adverse local or systemic effects (Boyan et al. 2003).
Osteogenic grafts provide osteoblastic cells with the direct ability to form new bone present within the graft material (Moore et al. 2001), osteoconductive grafts provide a scaffold for new bone to grow in from an adjacent osseous bed while osteoinductive grafts provide factors that induce undifferentiated tissue to differentiate into bone (Heest and Swiontkowski 1999). Finally osteointegration describes the surface bonding between the host bone and the grafting material (Giannoudis et al. 2005). Another requirement of the bone graft substitute is structural support and strength. One of the common reasons for bone grafting is bone loss which indicates a need for mechanical reinforcement (Ilan and Ladd 2003).

Bone graft substitutes consist of several types and encompass various materials, material sources, and origins (natural vs synthetic). Many are formed from composites of one or more types of material; however, the composite is usually built on a base material. Laurencin and Khan (2003) have classified available bone grafting options into five material based categories listed as follows:

1. Harvested bone graft and bone graft substitutes
2. Growth factor based bone graft substitutes
3. Cell based bone graft substitutes
4. Ceramic based bone graft substitutes
5. Polymer based bone graft substitutes (Laurencin and Khan 2003)

2.7.1 Harvested bone graft and bone graft substitutes

Harvested bone graft substitutes include the autograft and the allograft. Autologous bone grafting remains the gold standard in treating bone defects because of its inherent osteoconductivity, osteogenicity and osteoinductivity (Laurencin and Khan 2003). This involves harvesting cortical and cancellous bone, usually from the iliac crest and using this as the graft to repair the defect (Giannoudis et al. 2005). These grafts offer minimum immunological rejection, complete histocompatibility and provide the best osteoconductive, osteogenic and osteoinductive properties (Nandi et al. 2010). However, problems with autologous bone grafting exist with only limited quantities of bone autograft available and also due to the resultant increase in operative time, blood loss, post operational pain, length of hospital stay and cost (Ilan and Ladd 2003).
One alternative to autograft is allograft, or tissue taken from a cadaver. Allograft bone has both osteoinductive and osteoconductive properties, but lacks osteogenic properties because of the absence of viable cells (Nandi et al. 2010). Allogenic bone is available in many forms: demineralized bone matrix, morselized and cancellous chips, corticocancellous and cortical grafts, and osteochondral and whole-bone segments. Although limited supply is less of a problem, there is a risk of disease transmission from donor to recipient and additional complications have been reported after approximately ten years of implantation, with 30 to 60% of allograft implants encountering complications which lead to their failure (Khan et al. 2008).

### 2.7.2 Growth factor based bone graft substitutes

Bone Morphogenetic Proteins (BMP’s) are platelet derived growth and differentiation factors originally isolated from bone matrix based on their ability to induce new bone formation in vivo (Sampath and Reddi 2003). They are components of the transforming growth factor beta ‘superfamily’ which consists of nine members: BMP-1 to BMP-9 (Hollinger and Leong 1996). When implanted with an appropriate carrier matrix at defect sites, BMP’s are capable of inducing new bone formation and restoring lost bone by initiating a cellular process similar to embryonic bone formation (Sampath and Reddi 2003). BMP-7 and BMP-2 are cytokines which have been shown to induce bone formation in tibial non-unions at the same rate as autologous bone graft, and can also induce and accelerate bone healing in fresh tibia fractures (Zimmermann and Moghaddam 2011).

The clinical use of growth factors is mainly limited by the problem of delivery (Nandi et al. 2010). BMPs need to be combined with an appropriate carrier acting as delivery system which also ensures controlled release of the growth factors. The appropriate carrier will retain the growth factors at the site of injury for a prolonged period while also providing initial support for the attachment of cells and formation of regenerated tissue. Controlled delivery systems are necessary in order to avoid uncontrolled bone formation in non-bony tissues (Calori et al. 2011).

Recombinant human BMP-2 and BMP-7 have been approved for limited clinical use. The two commercially available BMP bone substitutes, Infuse, which contains rhBMP-2, and OP-1, which contains rhBMP-7, both use collagen as a scaffold to
permit BMP retention at the wound site. However, both are prepared differently, with rh-BMP-2 soaked onto a collagen sponge immediately before use in the case of the Infuse and rhBMP-7 lyophilized onto collagen granules before use in the case of OP-1 (Barr et al. 2010). Both products application and efficacy has been reduced by delivery problems. As BMP’s have very short biological half-lives and are difficult to retain at sites of local application, large doses are required to induce bone healing. Another disadvantage of using recombinant proteins is their high cost (Dinopoulos and Giannoudis 2007).

### 2.7.3 Cell based bone graft substitutes

Cell-based bone graft substitutes use cells to generate new tissue alone or are seeded onto support matrices. Transplanted cells can either differentiate into bone forming cells or attract other cell types to build up bone tissue and simultaneously resorb bone substitute material. The cells normally used are called mesenchymal stem cells (MSCs) and are able to differentiate into osteoprogenitors or mature osteoblasts and are therefore suitable for such techniques (Cancedda et al. 2003). Bone marrow is a natural reservoir of MSCs and to date MSC-like populations have been successfully isolated from bone marrow aspirates, trabecular bone, adipose tissue and synovial membranes (Janicki and Schmidmaier 2011). In animal trials, MSCs have been combined with suitable three dimensional scaffolds, which promote cell adhesion and differentiation and these studies have shown that MSCs enhance the repair of induced large bony defects (Janicki and Schmidmaier 2011). However, to date, there are only a few clinical trials describing the transplantation of human MSC to treat non-union fractures. Many problems limit the use of MSC for clinical application, such as the sterility technique, long culture times and high cost (Gan et al. 2008).

Osteocel Plus (NuVasive, Inc., San Diego, CA) is an allogenic cellular bone matrix designed to mimic autograft regulated by the FDA as a human cellular and tissue-based product. Indications for use include repair replacement, or reconstruction of musculoskeletal defects. Sourced from cadaveric donors, the material consists of morselized cancellous bone that is treated with proprietary processing to remove potentially immunogenic cells while maintaining viable bone-forming cells, including mesenchymal stem cells and osteoprogenitor cells. The processed tissue is combined with dematerialized cortical bone from the same donor and stored at –
25

80°C as granules in a cryopreservation agent, it is then thawed in the operating room before use (Hollawell 2011).

2.7.4 Ceramic based bone graft substitutes
Ceramics are refractory polycrystalline compounds, usually inorganic, including silicates, metallic oxides, carbides, and various refractory hydrides, sulfides, and selenides (Park 2009). Many ceramics are known to be biocompatible and are used in orthopaedics in various applications. Ceramics such as alumina and yttrium-stabilized zirconia are used in arthroplasty components while ceramics in the form of granules, porous blocks and cements are used as bone graft substitutes (Shors 2003).

Calcium phosphates account for most of the ceramic-based bone graft substitutes currently available, with a main composition of hydroxyapatite (HA), tricalcium phosphate (TPC) or a combination of both (Ilan and Ladd 2003). Tricalcium phosphate has a stoichiometry similar to amorphous bone precursors, whereas hydroxyapatite has a stoichiometry similar to bone mineral (Giannoudis et al. 2005). Hydroxyapatite is a highly crystalline form of calcium phosphate with the nominal composition of Ca₁₀(PO₄)₆(OH)₂. Its chemical similarity with the mineralized phase of bone accounts for its osteoconductive potential and excellent biocompatibility (Nandi et al. 2010). The nominal composition of tri-calcium phosphate is Ca₃(PO₄)₂ which exist in either α or β-crystalline forms. The structure, particularly its porosity, strongly influences its ability to resorb or remodel with the β-crystalline form having a more rapid resorption rate than the α-crystalline form (Ilan and Ladd 2003).

These brittle materials alone posses no osteoinductive properties and demonstrate little tensile strength with minimal immediate structural support. However, when attached to healthy bone, osteoids are produced directly onto the surfaces of the ceramic which then mineralise and the resulting new bone undergoes remodelling (Giannoudis et al. 2005). Vitoss is a synthetic cancellous bone void filler which is a form of beta tricalcium phosphate. It is a porous low density construct prepared by fusing nano particles of approximately 100 nm in diameter. Vitoss contains 39% calcium and 20% phosphorous approximately in a ratio of 1:5 and is available as blocks or morsels. Blocks can be sculpted to fit the shape of the defect while morsels can be gently taped into irregularly shaped defects (Sinha et al. 2009). Calcium
phosphates generally provide limited biomechanical support, because they are brittle and have little tensile strength (Martin et al. 1993). TCPs are less brittle compared with HA; however, their degradation results in subsequent loss of mechanical strength over time (Calori et al. 2011).

Coralline hydroxyapatite is based on natural material derived from sea coral. Certain sea coral species produce a porous structure made by calcium phosphate (coralline) which is similar to human cancellous bone. This is suitable as osteoconductive substitute for bone graft. Coralline substitutes may be natural or manufactured. The natural form is harvested directly from nature, where the manufactured form (coralline-HA), is converted from natural coralline (Giannoudis et al. 2005). Coralline HA marketed as ProOsteon (Interpore International, Irvine, CA, USA) developed in 1971, was the first calcium phosphate-based bone graft substitute to receive FDA approval for fracture treatment in 1992 (Ilan and Ladd 2003). The preparation of ProOsteon involves the processing of the calcium carbonate coral to remove the bulk of its organic matter. It is then subject to both extreme pressure and heat in an aqueous phosphate solution. This converts the calcium carbonate coral skeleton entirely to calcium phosphate (HA) as well as sterilizing it at the same time (Moore et al. 2001). ProOsteon is available in either a particulate or a block form (Ilan and Ladd 2003). Mechanically coralline HA is only slightly greater in compressive strength than cancellous bone. Like the other HA preparations it is weak in tension, brittle and difficult to shape (Moore et al. 2001).

Injectable calcium phosphate is a ceramic composite that combines “cement properties” with those of a void-filler (Giannoudis et al. 2005). Calcium phosphate cements form on mixing one of a range of calcium phosphates with an aqueous solution, resulting in dissolution of the calcium followed by a precipitation reaction in which the calcium phosphate crystals grow and the cement becomes rigid (Boyan et al. 2003). Injectable mineral cements have an advantage over blocks, granules, and pellets, in that a custom fill of the defect is possible (Ilan and Ladd 2003). Mechanical properties vary between the cements and depend to some degree on the composition. They are generally strong in compression but have low tensile strength, making them most suitable for treatment of fractures and defects that are not weight bearing (Boyan et al. 2003).
Three different commercially available cements include BoneSource, HydroSet and Norian Skeletal Repair System. BoneSource powder consists of two powder components; tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) \([\text{CaHPO}_4]\). The liquid used for BoneSource is water based with 0.25 mol/L sodium phosphate solution added (Friedman et al. 1998). HydroSet powder consists of three powder components: dicalcium phosphate dihydrate (DCPD) \([\text{CaHPO}_4\cdot 2\text{H}_2\text{O}]\), tetracalcium phosphate (TTCP) \([\text{Ca}_4(\text{PO}_4)_2\text{O}]\), and tri-sodium citrate \([\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot 2\text{H}_2\text{O}]\). The liquid used for HydroSet is water based with polyvinylpyrrolidone (PVP) \([\text{C}_6\text{H}_9\text{NO}]_x\) and sodium phosphate \([\text{Na}_2\text{HPO}_4]\) added. Norian Skeletal Repair System (SRS) powder consists of three powder components: monocalcium phosphate monohydrate (MCPM) \([\text{Ca}(\text{H}_2\text{PO}_4)_2\cdot \text{H}_2\text{O}]\), \(\alpha\)-Tricalcium phosphate (TCP) \([\text{Ca}_3(\text{PO}_4)_2]\), and calcium carbonate (CC) \([\text{CaCO}_3]\). The liquid used for Norian® SRS® is a sodium phosphate solution (Hannink et al. 2008).

Compressive strength of calcium phosphate cements has been reported to range from 26 to 60 MPa which is comparable to cancellous bone; however their tensile strengths are quite low reported in the range of 1.09 to 1.21 MPa (Welch et al. 2002). A study investigating the mechanical properties of a carbonated apatite cancellous bone cement, Norian SRS reported a compressive strength of 23–55 MPa. The average flexural strength was found to be 0.468 MPa, with a fracture toughness of 0.14 MPa m\(^{1/2}\) (Morgan et al. 1997). BoneSource has a reported compressive strength of 6.3–34 MPa (Van der Stok et al. 2011), while HydroSet has a reported a compressive strength of 14–24 MPa. In the same study the biaxial flexural testing of HydroSet resulted in a flexural modulus of 125–240 MPa and a strength value of 8-10MPa (Clarkin et al. 2009). Calcium phosphate grafts possess compressive strengths comparable to cancellous bone, but their main drawback remains their limited resistance to tensile and shear forces, making them vulnerable to cracking and subsequent material failure (Van der Stok et al. 2011).

Bioactive glass is biocompatible, osteoconductive glass that bonds to bone without an intervening fibrous connective tissue interface (Nandi et al. 2010). It is available in sintered porous bulk or particulate form. Bioactive glass is composed mainly of silica, sodium oxide, calcium oxide and phosphates and by varying the proportions of the composition all range of forms can be produced from soluble to non-
resorbable (Giannoudis et al. 2005). The surface of a bioactive glass takes part in a reaction with the host tissue on implantation, involving dissolution of the surface of the glass and release of mineral ions. A calcium phosphate layer forms which is thought to enhance protein adsorption to the surface of the implant while also enhancing the surface reaction with the host bone (Boyan et al. 2003). After long-term implantation, this biological apatite layer is partially replaced by bone. The behaviour of bioactive glasses is dependent on its composition, the surrounding pH, the temperature, and the surface layers on the glass (Nandi et al. 2010). Although bioactive glasses possess both osteointegrative and osteoconductive properties, some formulations are quite brittle and may form particular debris which contributes to the release of inflammatory cytokines (Boyan et al. 2003). Melt-derived bioactive glass of the original Bioglasss composition (46.1% SiO2, 24.4% NaO, 26.9% CaO and 2.6% P2O5, in mol%) has been used clinically in a powder form since the mid-1980s, as a regenerative bone filler under the product names Perioglas and Novabone (Jones et al. 2007).

Calcium sulphate is also known as plaster of Paris and results from the calcination of gypsum (CaSO4, 2H2O), which partially dehydrates to produce a hemi-hydrate (CaSO4, 1/2H2O). It is a nonphysiologic salt, but has been used for bone implantation for over 100 years and now is primarily used as osteoconductive bone-void filler. Osteoset (Wright Medical Technology, Arlington, TN, USA) is a commercially available calcium sulphate tablet impregnated with tobramycin used for the treatment of bone defects (Moore et al. 2001). It is biocompatible, bioactive, and resorbable with total resorption observed as early as a few weeks to potentially longer times (Ilan and Ladd 2003). Significant loss of its mechanical properties occurs upon its degradation; therefore, it is a questionable choice for load-bearing applications (Nandi et al. 2010). In a study of the healing of large defects treated with calcium sulphate pellets a compressive strength for OsteoSet was attained using in vivo samples which were implanted for 26 weeks in the humerus of dogs. A compressive strength of 0.6–0.9 MPa and a compression modulus of 59 MPa was reported. Calcium sulphates only provide minimal structural support and are not suitable in cases where structural support is required (Urban et al. 2003).
2.7.5 Polymer based bone graft substitutes

Polymers present some options that the other groups do not due to their different physical, mechanical, and chemical properties. The polymers used today can be loosely divided into natural polymers and synthetic polymers. These, in turn, can be divided further into degradable and non degradable types. Non degradable polymers used in orthopaedic applications include ultra high molecular weight polyethylene, as a bearing surface in total joint arthroplasty and polymethyl methacrylate, as acrylic cement for implant fixation and filling defects. These materials are not intended to be replaced with bone, although they may interface with bone tissue (Boyan et al. 2003).

The use of degradable polymers for orthopedic applications has seen a rise in recent decades. For the fixation of fractured bones and joints, biodegradable polymers and copolymers are often currently being used as alternatives to metal implants (Amass et al. 1998). The use of biodegradable orthopaedic fixation devices (pins, rods, screws, tacks, ligaments) will not require a second surgical event for removal. In addition, biodegradation offers other advantages. A fractured bone, fixated with a rigid, non-biodegradable stainless steel implant, has a tendency for re-fracture upon removal of the implant. The bone does not carry sufficient load during the healing process, because the load is carried by the rigid stainless steel. However an implant prepared from biodegradable polymer can be engineered to degrade at a rate that will slowly transfer load to the healing bone (Athanasiou et al. 1998). Currently, only resorbable fixation devices made from homopolymers or copolymers of glycolide, lactide, caprolactone, \( p \)-dioxanone and trimethylene carbonate have been commercialized (Middleton and Tipton 2000).

Due to the limitations in some of the properties of available synthetic substitutes, there has been significant interest in developing resorbable polymers, such as poly-L-lactic acid (PLLA) and poly-L-glycolic acid (PLGA), for structural bone graft substitute applications (Baker et al. 2011). While porous calcium phosphates such as corraline hydroxyapatite have high compressive strengths, the brittle nature of the material can lead to the progressive collapse of the graft, resulting in poor bone healing (Chau and Mobbs 2009). The mechanical characteristics of polymers show converse attributes to ceramics. However, polymers can exhibit a lower elastic
modulus in comparison to native bone and therefore are too flexible for load bearing solutions. The incorporation of high modulus ceramic constituents into the polymer can help to address this problem. The most commonly researched polymer/ceramic composite is polyester with HA constituents (Lichte et al. 2011).

Collagen, a natural polymer is a rational option for use in bone defects healing. It is the major structural protein of bone, and the natural scaffold for osteoblast migration (Rocha et al. 2002). It has received increasing attention over the last years due to its excellent biocompatibility, degradation into physiological end-products, and suitable interaction with cells and other macromolecules. The favourable influence of collagen on cellular infiltration and wound healing is well known (Geiger et al. 2003). Collagen functions poorly as a graft material but once it is coupled with bone morphogenetic proteins, osteoprogenitor precursors, or hydroxyapatite, graft incorporation enhances significantly. Collagen is usually used as composite with other bone substitutes (gel, granules, with biphasic ceramic of HA and tricalcium phosphate, bone marrow, etc.) (Giannoudis et al. 2005).

Polymer-based bone graft substitutes include the following; Healos (DePuy Spine, Raynham, MA, USA), which uses a Type I bovine collagen scaffold with a 5- to 200-mm pore size that has been coated with hydroxyapatite. This minimally immunogenic collagen-hydroxyapatite sponge is intended for use with bone marrow aspirate in non-load-bearing applications such as the posterolateral spine (Carter et al. 2009). Cortoss is a bioactive composite material consisting of an Bisphenol-A-glycidyl dimethacrylated resin reinforced with glass ceramic particles. It is biocompatible and has better radiopacity, a lower polymerization temperature, and greater strength and stiffness compared to PMMA. Clinically, it is as effective as PMMA in alleviating the pain of vertebral fracture (Luo et al. 2007). Cortoss has a reported compressive strength of 91–179 MPa, a biaxial flexural strength of 59-96 MPa and a biaxial flexural modulus of 1.5-2 (GPa) (Boyd et al. 2008). The manufacturer’s information also provides a Young’s modulus of 6400 MPa and a tensile strength of 52 MPa; however, this has not been confirmed in other studies (Van der Stok et al. 2011). Although Cortoss has a compressive strength of 91–197 MPa, its tensile strength does not reach values comparable to cortical bone (Gheduzzi et al. 2006).
BoneTec, Inc (Toronto, Canada) has developed a porous poly (lactic-co-glycolic acid) foam matrix by using a particulate leaching process to induce porosity (Nandi et al. 2010). Immix (Osteobiologics Inc., San Antonio, TX) is a synthetic bone graft scaffold, tissue engineered from amorphous D,L-polylactide-coglycolide, and is designed to resorb within 12 to 20 weeks following implantation. It has been developed as granular particles, resembling allograft bone chips. It provides a porous architecture for the ingrowth of new bone and then fully degrades (Mekhail and Bell 2008).

Synthetic biodegradable polymers and associated composite materials are one of the most promising and ideal biomaterials for bone repair and reconstruction. A number of biodegradable polymers present good biological safety and biocompatibility with degradation products that are non-toxic to human body. The mechanical properties and degradation rate of the polymers can be adjusted and produced according to the different requirements for bone grafting and so the next part of the review will concentrate on biodegradable polymers, in particular poly(lactic acid).

2.8 Biodegradable Polymers

The development of degradable biomaterials is a relatively new area of research but the last few decades has seen a massive shift into the concept of using biodegradable rather then biostable polymers for medical applications (Nair and Laurencin 2007). Developing degradable polymers as medical implants is of great research interest, primarily because secondary surgery is not required for implant removal (Zhang and Goosen 1996). Biodegradation, bioerosion, bioabsorption and bioresorption are four common terms used to indicate that a given material or device will disappear eventually after implantation into a living organism. These terms have been interchanged throughout literature and no clear distinctions are evident in the meaning of these terms. Kohn et al. makes the point that the degradation of a polymer normally refers to a chemical process resulting in the cleavage of covalent bonds, while erosion often refers to the physical changes to the size shape or mass of a device which could be the result of degradation or simply dissolution. The terms bioresorption and bioabsorption are normally used to imply that the polymer or its
degradation products are removed by cellular activity in a biological environment (Kohn et al. 2004).

Within “A review of biodegradable polymers” , Amass et al. define biodegradation as “an event which takes place through the action of enzymes and/ or chemical decomposition associated with living organisms or their secretion products” however they recognise that it is also necessary to consider abiotic reactions (e.g. photodegradation, oxidation and hydrolysis) that may alter the polymer because of environmental factors (Amass et al. 1998). Kohn et al states that as the chemical degradation of the backbone of a polymer such as poly (lactic acid) is controlled by simple hydrolysis and occurs independently of any biological agent, its degradation should not be described as biodegradation (Kohn et al. 2004).

Generally speaking, the term “biodegradable polymer” is normally associated with two fields; one in which the polymer is placed in nature where it interacts with micro-organisms e.g. in landfills, waste tips etc. and one in which polymers interact with mammals. In this study, the term “biodegradable polymer” will be used to identify a polymer which by design degrades in the human body. All polymers are susceptible to degradation but the conditions necessary and associated reaction kinetics can vary from one polymer and environment to the next. Factors that may effect the degradation of implanted biodegradable polymers include chemical structure, the presence of other substances (such as drugs), polymer molecular weight and distribution, the morphological structure and dimensions of the polymer sample and the hydrolysis conditions (Zhang and Goosen 1996).

2.9 Polylactic acid

Of the many polymeric materials which are biodegradable, the α-hydroxy acid - polylactic acid (PLA) is among the most extensively investigated. This is due to its proven biocompatibility and good material properties. Polylactic acid is an aliphatic polyester with hydrolytically labile aliphatic ester linkages in its backbone (Nair and Laurencin 2007). It is considered safe, non-toxic and biocompatible for use as an implantable biomaterial by many regulatory agencies worldwide with currently
approved products available which include sutures, bone pins and implantable drug delivery systems (Kohn et al. 2004).

2.9.1 Lactic Acid
Polylactic acid (PLA) is a biodegradable thermoplastic with its basic building block being lactic acid, a chiral molecule that exists in two stereoisomeric forms; a D(−)-isomer, produced in bacterial systems and an L(+) isomer, produced in humans, other mammals and bacterial systems. The enantiomers of lactic acid can be seen in Figure 2.9.

![Figure 2.9 Lactic acid L- and D- enantiomers](Lim et al. 2008)

Lactic acid (2-hydroxypropionic acid) was first isolated in 1780 from sour milk by the Swedish chemist Scheele and first produced commercially in 1881. Food-related applications are the major use of lactic acid and account for about 85% of the commercially produced product. It is used as a buffering agent, acidic flavouring agent and bacterial inhibitor in many processed foods (Garlotta 2001). Lactic acid can be manufactured either by carbohydrate fermentation or chemical synthesis, although fermentation predominates (Lim et al. 2008).

Chemical synthesis of lactic acid is based on the hydrolysis of lactonitrile, a derivative of petrochemicals, by strong acids, which provides only the racemic mixture of D- and L-lactic acid. Other possible chemical synthesis routes for lactic acid include base-catalyzed degradation of sugars, oxidation of propylene glycol, reaction of acetaldehyde, carbon monoxide, and water at elevated temperatures and pressures, hydrolysis of chloropropionic acid, and nitric acid oxidation of propylene. However, none of these routes have led to technically and economically viable processes (John et al. 2009).
In the fermentation production of lactic acid renewable resources such as starch and cellulose are used as substrates. Cellulose and starch are the most abundant compounds in the world, and when hydrolyzed to mainly glucose they are fermentable by a number of micro-organisms.

Lactic acid production comprises of the following steps: pre-treatment of substrate including hydrolysis to sugars, fermentation of sugars to lactic acid, separation of bacteria and solid particles from the broth, and purification of lactic acid (Hofvendahl and Hahn-Hagerdal 2000). The fermentation processes can be classified according to the type of bacteria used. In the heterolactic fermentative process, equimolar amounts of lactic acid, acetic acid, ethanol, and carbon dioxide are

![Figure 2.10 Metabolic pathway for lactic acid production (A) homolactic fermentation (B) heterolactic fermentation (Lasprilla et al. 2012)]
formed, whereas in the homolactic fermentative process only lactic acid is produced (Lasprilla et al. 2012). Figure 2.10 shows the catabolic pathways for lactic acid production using lactic acid bacteria. Significant advantages of the production of lactic acid by fermentation over chemical synthesis include the use of cheap raw materials, low production temperature and low energy consumption (John et al. 2009). Fermentative production also has the advantage that by choosing a strain of lactic acid bacteria producing only one of the isomers, an optically pure product can be obtained, whereas chemical synthesis always results in a racemic mixture of lactic acid (Hofvendahl and Hahn-Hagerdal 2000).

2.9.2 Production of high molecular weight PLA

In general, there are three methods which can be used to produce high molecular mass PLA (a) direct condensation polymerization; (b) azeotropic dehydrative condensation and (c) polymerization through lactide formation (Lim et al. 2008) as shown in Figure 2.11. Polymerization through lactide formation, patented by Cargill Inc. in 1992, is by and large the current method used for producing PLA (Drumright et al. 2000).

Figure 2.11 Synthesis of PLA from L- and D- lactic acids (Lim et al. 2008)
Direct condensation polymerization is the least expensive route. However, it is very difficult to obtain a solvent-free high molecular weight poly(lactic acid). Therefore, the use of chain coupling agents and adjuvants adds cost and complexity to the process (Zhao 2004). Azeotropic dehydrative condensation of lactic acid can yield high molecular weight poly(lactic acid) without the use of chain extenders or adjuvants. The general procedure consists of reducing the distillation pressure of lactic acid for 2–3 h at 130 °C. The majority of the condensation water is then removed. Catalyst is added along with diphenyl ester. A tube packed with 3Å molecular sieves is attached to the reaction vessel and the solvent returned to the vessel via the molecular sieves for an additional 30–40 hours at 130 °C. Finally, the polymer is isolated as is or dissolved and precipitated for further purification (Auras et al. 2004).

Polymerization through lactide formation involves the prepolymerization of either D-lactic acid, L-lactic acid or a mixture of the two to obtain an intermediate low molecular mass poly(lactic acid), which is then, under lower pressure, catalytically converted into a mixture of lactide stereoisomers (Albertsson and Varma 2003).

Lactide, the cyclic dimer of lactic acid, is formed by the condensation of two lactic acid molecules as follows: L-lactide (two L-lactic acid molecules), D-lactide (two D-lactic acid molecules) and meso-lactide (an L-lactic acid and an D-lactic acid molecule). The chemical structure of L-lactide, Meso-lactide and D-lactide can be seen in Figure 2.12. After vacuum distillation of the lactide, high molecular mass PLA is formed by ring-opening polymerization of the lactides (Auras et al. 2004).
The ring opening polymerization is generally carried out in bulk or in solution (THF, dioxane, toluene, etc.), emulsion or dispersion. The temperature of bulk polymerization is generally in the range of 100-150 °C, whereas in solution polymerization, low temperatures have been used (0-25 °C) to minimize side reactions (Albertsson and Varma 2003). The mechanism of polymerization can be cationic or anionic depending on the type of initiator (Auras et al. 2004). Example of anionic ring opening polymerisation initiators are alkali metal alkoxides. The anionic ring opening polymerisation is initiated when the nucleophilic anion of the initiator attacks the carbonyl group of the lactide, resulting in the cleavage of the carbonyl carbon and the endocyclic oxygen bond. This oxygen becomes a new anion, which continues to propagate (Gupta and Kumar 2007). Figure 2.13 shows the anionic ring opening polymerization of lactide.

Figure 2.13 Anionic ring opening polymerisation (Gupta and Kumar 2007)

Figure 2.14 shows the cationic ring opening polymerization of lactide. Catalyst for the cationic ring opening polymerisation can be carbenium ion donors and a few
strong acids such as triethylxonium tetrafluoroborate, boron trifluoride, and trifluoroacetic acid. The initiation step of cationic polymerisation occurs when the exocyclic oxygen of one of the lactide carbonyls is either alkylated or protonated by the initiator, causing the resulting O–CH bond to become positively charged. Nucleophilic attack by a second monomer breaks this bond to create another electrophilic carbenium ion. The propagation step of this polymerisation repeats as nucleophilic attack by additional monomers continues until the polymerisation is terminated by a monofunctional nucleophile like water (Gupta and Kumar 2007). Ring-opening polymerization can yield high molecular mass polymers under relatively mild conditions. The reactions can be carried out with no or very limited side reactions. This makes it possible to control properties like molecular weight and molecular weight distribution (Amass et al. 1998). Figure 2.15 shows the constitutional unit of polylactide.

2.9.3 PLA structure

The two stereoisomeric forms of lactic acid give rise to four morphologically distinct PLA polymers: D-PLA (PDLA), L-PLA (PLLA), D L-PLA (PDLLA) and meso-PLA (Garlotta 2001). D-PLA and L-PLA are two stereoregular polymers, D L-PLA is a racemic or equimolar mixture of D- lactide and L-lactide (Rissanen et al. 2008) while meso-PLA is obtained from D L-lactide (meso-lactide) but is rarely used for biomedical purposes (Kohn et al. 2004). Because of the stereo regular chain microstructure, D-PLA and L-PLA are semicrystalline materials (Nampoothiri et al. 2010) while D L-PLA is amorphous due to the random distribution of L- and D-lactic acid units (Jiang et al. 2010). It can only become crystalline when the D- and L- unit sequence is completely alternating with each other to form meso-PLA (Urayama et al. 2003). PLLA is more frequently used as a biomaterial rather than
PDLA, since the degradation of PLLA yields L-(+)-lactic acid which is naturally produced in humans.

Due to its amorphous structure, PDLLA is usually considered for applications such as drug delivery as it is important to have a homogeneous dispersion of the drug within the polymer. PLLA is usually used for applications where high mechanical strength and toughness is required (Kohn et al. 2004). Commercial PLA is usually a copolymer of poly(L-lactic acid) (PLLA) and poly(DL-lactic acid) (PDLLA)(Lim et al. 2008). The physical, mechanical and degradation properties of polylactides are strongly influenced by their configurational structure, dependent on the different D/L unit ratio and sequence (Wisniewski et al. 1997). PLA derived from greater than 93% L-lactic acid can be semicrystalline whereas PLA from between 50 and 93% L-lactic acid is strictly amorphous (Auras et al. 2004). Generally, the crystallinity of PLLA decreases with increasing racemic content (Urayama et al. 2003) as both meso and D-lactide induce twists in the otherwise very regular poly(𝐿-lactide) molecular architecture (Auras et al. 2004). PLLA has a crystallinity of around 37%, however by physically blending the polymer PLLA with PDLA, a highly regular stereo complex forms with increased crystallinity (Nampoothiri et al. 2010). Stereocomplexed polylactides melt at about 230°C, a 50°C higher melting temperature than homocrystallized polylactides (Sarasua et al. 2005). Pure poly(D-Lactide) or poly(L-Lactide) have an equilibrium crystalline melting point of 207°C, however typical melting points are in the 170°C – 180°C range due to small and imperfect crystallites, slight racemization and impurities (Garlotta 2001).

PLLA can crystallize into three forms; α, β and γ. The α form is characterised by two antiparallel chains in a left handed helix confirmation packed into an orthorhombic unit cell as shown in Figure 2.16. The β form crystals are generally prepared by stretching their α- counterparts at a high draw ratio and a high drawing temperature (Jiang et al. 2010). The β form is characterised by a left handed 3-fold helix confirmation packed into an orthorhombic unit cell (containing 6 chains). The α structure with a Tm of 185°C is more stable than the β with a Tm of 175°C. The γ form contains two antiparallel helices in the pseudoorthorhombic unit cell (Auras et al. 2004). The melt enthalpy estimated for an enantiopure PLA of 100% crystallinity
(\(\Delta H^\circ_m\)) is 93 J/g; it is the value most often referred to in the literature although higher values (up to 148 J/g) also have been reported (Sodergard and Stolt 2002).

**Figure 2.16 Crystal structure of the \(\alpha\) form of PLLA** (Sasaki and Asakura 2003)

Upper left: ab projection; upper right: ac projection; lower bc projection

The optimum temperature for crystallization of poly(L-lactide) is 105 - 115 \(^\circ\)C and is relatively slow with a half-time of about 2.5 min (Drumright et al. 2000). Quenching the semicrystalline polymer from the melt at a high cooling rate will result in a highly amorphous polymer. PLA has a tendency to crystallise upon reheat, depending on the heating rate as well as the isomer content of the PLA polymer. Recrystallization of PLA articles can be initiated by annealing at temperatures higher than \(T_g\) and below the melting point. Strain induced crystallization also occurs when the polymer is mechanically orientated. The amount of crystallinity attained depends on the mode of stretching with crystallinity also decreasing as the stereoisomeric purity of the polymer decreases (Lim et al. 2008).

### 2.9.4 Thermal Properties of PLA

For amorphous PLA, the glass transition temperature (\(T_g\)) is one of the most important parameters since changes in polymer chain mobility take place at and
above Tg. For semicrystalline PLA, both Tg and melting temperature (Tm) are important physical parameter for predicting PLA behaviour (Lasprilla et al. 2012). For amorphous polylactide, below the $\beta$-relaxation temperature, $T_\beta$, the polymer is completely brittle. Between $T_\beta$ and Tg the amorphous polylactide undergoes physical aging and can show brittle or ductile fracture. The general range in Tg for PDLLA is 55-60°C (Park and Jonnalagadda 2006). In the transition between 110–150 °C, PLA changes from rubbery to viscous and is mainly dependent on the molecular weight and the shear stress. Finally, amorphous PLA decomposes between 215 and 285 °C (Auras et al. 2004). The metastable states of high molecular weight high molecular weight amorphous and semicrystalline PLA can be seen in Figure 2.17

![Figure 2.17 Metastable states of high molecular weight high molecular weight (a) amorphous PLA and (b) semicrystalline PLA (Auras et al. 2004)](image)

For semicrystalline polylactide, the melting temperature is a function of the processing conditions and the stereochemistry of the polymer. Melting temperature increases with a rise in molecular weight (Mw) until the maximum practical value with melting temperature ranging from 130 °C to 180 °C. The Tm depends on the presence of DL-lactide in the structure which produces a depression of the melting temperature. Tg indicates the transition between brittle and ductile fracture and is
also determined by the proportion of different lactides present. The typical PLA glass transition temperature of semi crystalline PLA (Tg) ranges from 50°C to 80°C (Auras et al. 2004).

Table 2.3 Transition temperatures for selected PLA copolymers (Garlotta 2001)

<table>
<thead>
<tr>
<th>Copolymer ratio</th>
<th>Tg (°C)</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/0 (L/DL)-PLA</td>
<td>63</td>
<td>178</td>
</tr>
<tr>
<td>95/5 (L/DL)-PLA</td>
<td>59</td>
<td>164</td>
</tr>
<tr>
<td>90/10 (L/DL)-PLA</td>
<td>56</td>
<td>150</td>
</tr>
<tr>
<td>85/15 (L/DL)-PLA</td>
<td>56</td>
<td>140</td>
</tr>
</tbody>
</table>

2.9.5 Mechanical Properties of PLA

PLA generally has an elastic modulus and tensile strength in the range of 3.2 – 3.7 GPa and 55-60 MPa respectively (Baiardo et al. 2003). However, the mechanical properties of PLA is very dependent on the molecular weight and stereochemical makeup of the backbone. Table 2.4 shows the mechanical properties reported for high molecular weight amorphous and semicrystalline PLA with the wide variance in properties of the semi crystalline PLA due to the degree of orientation and stereochemical composition of various poly(lactic acid) samples.

Table 2.4 Mechanical properties for PLA (Garlotta 2001)

<table>
<thead>
<tr>
<th></th>
<th>Amorphous PLA</th>
<th>Semi-crystalline PLA^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultimate tensile strength (MPa)</td>
<td>47.6 – 53.1</td>
<td>47.6 - 166</td>
</tr>
<tr>
<td>Tensile modulus (MPa)</td>
<td>3447 - 4000</td>
<td>3889 - 4137</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>3.1- 5.8</td>
<td>15- 160</td>
</tr>
</tbody>
</table>

^a Results depend on degree of crystallisation and isomer content

Auras et al. reported that a higher content of L-lactide in the films contributed to a higher tensile strength in samples which were tested containing 98% L-lactide and 94% L-lactide. Although poly(98% L-lactide) had greater elongation at yield than poly(94% L-lactide), the latter has an elongation at break 7 times greater than
poly(98% L-lactide), which indicated that poly(94% L-lactide) was more plastic. Molecular weight also plays a very important role in the resultant mechanical properties. Varying the molecular weight from 50,000, over 150,000 to 200,000 will yield tensile strengths for PLLA of 15.5, 80 and 150 MPa, respectively (Van de Velde and Kiekens 2002). Lim et al. reported polylactide having molecular weights of 35,000 and 55,000 Daltons had an increase of almost 20% in the modulus of elasticity for poly(L-lactide) compared with poly(D,L-lactide) (Lim et al. 2008). Results of mechanical properties for PLA of various molecular weights are shown in table 2.5 (Domb et al. 2002).

| Table 2.5 Mechanical properties for PLA (Domb et al. 2002) |
|-----------------|-------|--------|----------------|
| Polymer          | MW    | Tensile Strength (MPa) | Tensile Modulus (MPa) | Elongation at break (%) |
| PLLA             | 50,000 | 28     | 1200           | 6.0                    |
| PLLA             | 100,000| 50     | 2700           | 3.3                    |
| PLLA             | 300,000| 48     | 3000           | 2.0                    |
| PDLLA            | 107,000| 29     | 1900           | 5.0                    |

Table 2.6 Properties of PLA processed under different conditions (Garlotta 2001)

<table>
<thead>
<tr>
<th></th>
<th>PLLA (Mw 67,000)</th>
<th>Annealed PLLA (Mw 71,000)</th>
<th>PDLLA (Mw 114,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength (MPa)</td>
<td>59</td>
<td>66</td>
<td>44</td>
</tr>
<tr>
<td>Tensile Modulus (MPa)</td>
<td>3750</td>
<td>4150</td>
<td>3900</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>7.0</td>
<td>4.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Flexural strength (MPa)</td>
<td>106</td>
<td>119</td>
<td>88</td>
</tr>
</tbody>
</table>

Annealing PLLA increases the tensile strength due to the increase in the stereoregularity of the chain (Auras et al. 2004). It has been shown that crystallization of injection moulded PLLA parts by annealing at 105 °C for 90 min
increases tensional and flexural elasticity (Lim et al. 2008). Table 2.6 shows the properties for processed PDLLA, PLLA and PLLA which is subsequently annealed.

2.9.6 Degradation of PLA

In tissues and many culture systems PLA degrades slowly into the oligomers of lactic acid and finally into lactic acid itself, which can be metabolized into carbon dioxide and breathed out through the lungs or secreted into urine (Miller et al. 1977). PLA degrades by simple hydrolysis of the ester bond as shown in Figure 2.18 and once implanted occurs independently of any biological agent. Hydrolysis is the scission of susceptible molecular functional groups by reaction with water. It is a single step process in which the rate of chain scission is directly proportional to the rate of initiation of the reaction (Kohn et al. 2004). There are two principle ways in which polymer chains can be hydrolyzed, actively by enzymatic reaction or passively by chemical hydrolysis (Hakkarainen 2002).

![Figure 2.18 PLA hydrolysis and molecular weight loss](Auras et al. 2004)
Chemical hydrolysis of PLA occurs after exposure to moisture as water penetrates the bulk of the polymer matrix and hydrolysis of the ester group takes place. Enzymes such as proteinase K, pronase and bromelain have been used to bring about hydrolysis of polylactide in vivo. However, enzymes are large molecules and are unable to diffuse through the crystalline regions. As expected, little enzymatic degradation occurs at the beginning of the degradation process. Enzymatic involvement can produce pores and fragmentation, making more polymer regions accessible to the enzymes (Auras et al. 2004).

In the case of massive specimens of PLA, the hydrolysis is faster in the centre then at the surface. This is explained by the theory that hydrolysis products are formed at both the surface and the centre. The hydrolysis products at the surface dissolve in the surrounding medium, while the concentration of carboxylic end groups increases at the centre and catalyze ester hydrolysis resulting in surface-centre segregation (Hakkarainen 2002).

The degradation of the semicrystalline PLA occurs in two phases; in the first phase the amorphous regions are hydrolyzed followed by the crystalline regions in the second phase. Most of the mechanical strength is lost after the first phase. Amorphous D, L-PLA only goes through the first phase of degradation (Domb et al. 2002). The composition of PLA polymer chains greatly influences the degradation rate. Results described in “Aliphatic Polyesters: Abiotic and Biotic Degradation and Degradation Products” reported that semicrystalline PLLA containing 100% L-LA units had a degradation time with a half life of 110 weeks in pH7.4 phosphate buffer at 37°C. The incorporation of 50% D-LA units to the polymer greatly increased the degradation rate and the half life decreased to only 10 weeks. The half life corresponds to the time at which half of the initial material is lost (Hakkarainen 2002).

Although the degradation process in PLA is a simple hydrolysis, the degradation rate can be affected by many factors. The polymer degradation rate is mainly determined by polymer reactivity with water and catalysts. Any factor which affects the reactivity and the accessibility, such as particle size and shape, temperature, moisture, crystallinity, % isomer, residual lactic acid concentration, molecular
weight, molecular weight distribution and water diffusion will affect the polymer degradation rate (Auras et al. 2004).

One consequence of hydrolytic degradation of a polymer implant is the release of acidic products and a corresponding fall in the pH of the local tissue which leads to a local inflammatory response. Normally, the buffering capacity of biological fluids can compensate for this but it cannot be accommodated for if the polymeric material undergoes bulk degradation. This acidic loading can be counteracted by the addition of basic salts or calcium compounds such as hydroxyapatite into the polymer (Boyan et al. 2003).

2.10 Modification of PLA

The overall aim of this project is to develop an injectable, resorbable, putty-like bone filler that can be used to repair damaged bone. Polylactide is an ideal candidate as a resorbable substitute however; modification of the polymer is needed to create a mouldable and injectable material which will then harden in-situ.

2.10.1 Copolymerization

In order to enhance the versatility of PLA and to improve the quality and reduce the cost of production, lactic acid can be polymerized with other monomers. PLA has been copolymerized with a range of polyesters and other monomers either through polycondensation of lactic acid with other monomers, producing low molecular weight copolymers, or ring opening copolymerization of lactide with cyclic monomers like glycolide, ε-caprolactone, δ-valerolactone and trimethylene carbonate, as well as linear monomers like ethylene glycol producing high molecular weight copolymers (Rasal et al. 2010). Copolymerization can markedly improve the strength, toughness, hydrophilic and controlled degradable properties of PLA, and at the same time numerous new copolymers in different macromolecular architectures can be obtained (Ren 2011).

Among the co-polyesters investigated, extensive research has been performed in developing a full range of poly(lactide-co-glycolide) polymers (PLGA). Both L- and DL-lactides have been used for co-polymerization. It has been reported that the
50/50 poly(lactide-co-glycolide) is very hydrolytically unstable and the resistance to hydrolytic degradation was found to be more pronounced at either end of the copolymer composition range. Thus, 50/50 poly(DL-lactide-co-glycolide) degrades in approximately 1–2 months, 75/25 in 4–5 months and 85/15 in 5–6 months. Different ratios of poly(lactide-co-glycolides) have been commercially developed and are being investigated for a wide range of biomedical applications (Nair and Laurencin 2007). Since 1970, poly(glycolic acid) has been commercially available as the surgical suture, Dexon, and the copolymer made of 92 mol% GA and 8 mol% LA has had application as the competitive suture, Vicryl, since 1975 (Ren 2011). The major popularity of these biocompatible co-polymers can be attributed in part to their approval by the FDA for use in humans, its good processibility which enables fabrication of a variety of structures and forms and their controllable degradation rates (Nair and Laurencin 2007). The synthesis of the poly(lactide-co-glycolide) copolymer can be seen in Figure 2.19.

Figure 2.19 Synthesis of poly(lactide-co-glycolide) copolymer (Ren 2011)

Polycaprolactone (PCL), as seen in Figure 2.20 is a semicrystalline, highly processible polyester, which has a low melting point (55–60 °C) and glass transition temperature of -60 °C. The polymer undergoes hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages; however, the rate of degradation is rather slow (2–3 years)(Nair and Laurencin 2007).

Figure 2.20 Poly(caprolactone)
PCL and PLA show important differences regarding their physicochemical properties which results in copolymers with degradation rates and a drug permeability that can be adjusted by their compositions (Sabater i Serra et al. 2009). PCL has low tensile strength of approximately 23MPa but an extremely high elongation at breakage (4700%) (Nair and Laurencin 2007) therefore, caprolactone and lactide appear to be suitable comonomers for the preparation of copolymers with mechanical properties ranging from elastomeric to rigid.

Their elasticity and hydrolyzability combine to make copolymers suitable for biomedical applications where flexibility and biodegradability are required in the same product. However, the distribution of the monomer units along the copolymer chain has a profound influence on the properties of these copolymers (Ren 2011). The synthesis of the PLA-co-PLC copolymer can be seen in Figure 2.21.

2.10.2 Plasticization

PLA normally has a glass transition temperature (Tg) greater then 37°C, causing PLA devices to possess brittle characteristics in physiological conditions. Polylactide’s brittleness is a major drawback for many biomedical applications and PLA devices tend to be susceptible to fracture when subjected to tension or load bearing stresses during use (Park and Jonnalagadda 2006). In order to modify its properties, PLA has been blended with other polymers such as poly(vinyl acetate), poly(methyl methacrylate) and poly(ethylene oxide) (Baiardo et al. 2003). Another option is the use of a low molecular weight compound which acts as a plasticizer, example of compounds used with PLA include glycerol, triactine and citrate esters (Ren et al. 2006). Plasticizers can improve the processability, ductility and flexibility of glassy polymers. An effective plasticizer for PLA is expected to reduce the glass transition of the amorphous phase and depress the melting point of the crystalline region (Baiardo et al. 2003).
There are several theories which have been developed to explain the mechanisms of plasticization, with the most widespread concepts being the lubricity theory, the gel theory and the free volume theory. According to the lubricity theory, the function of a plasticizer is to reduce intermolecular friction between polymer molecules (Marcilla and Beltran 2004). The plasticizer molecules act as shields to reduce polymer-polymer interactive forces and prevents the formation of a rigid network, this lowers the glass transition temperature allowing the polymer chains to move rapidly resulting in increased flexibility, softness and elongation (Godwin and Krauskopf 2008).

The gel theory assumes that the rigidity of an unplasticized polymer arises from a three dimensional network of weak secondary bonding forces occurring along the polymer chains. The plasticizer disrupts these polymer-polymer attractions, inserting itself between chains (Carraher and Seymour 2007). Some of the plasticizer molecules will solvate the polymer at a point of attraction, while other plasticizer molecules cause the remaining gel structure to swell (Godwin and Krauskopf 2008). A dynamic equilibrium exists involving the solvation and desolvation of the polymer by the plasticizer and the aggregation and disaggregation of the polymer chains themselves (Marcilla and Beltran 2004). Through the reduction of the gel structure, rigidity is decreased and the polymer becomes more flexible.

Free volume is a measure of the internal space available within a polymer and is the difference between the total volume and the occupied volume, which includes the volume of the polymer molecules calculated from their van der Waals radii plus the volume associated with vibrational motions of the individual bonds (Lutz and Grossman 2001). Plasticizers increase the free volume available to polymer chains and so allow for greater internal chain rotation and unwinding, thus decreasing the rigidity of the chains. According to Marcilla and Beltran (2004), all of the theories discussed above used by themselves do not fully explain the mechanisms of plasticization, but used together they give an approximate picture of the fundamental principles and explain most aspects of the behaviour of plasticized polymers.
The aim of this project is to develop a resorbable, putty like bone filler that can be used to repair damaged bone. By plasticizing PLA, a mouldable polymer could be formed which would allow for the possibility of the bone substitute to be injected into the site of the defect. Upon injection the polymer should harden, possible by the formation of a crosslinked network without detrimental effects to the surrounding tissue, maintain mechanical and physical integrity and facilitate cell attachment and growth. The substitute should then degrade within the required timeframe with no harmful byproducts for new bone growth and remodelling to take place.

There are several important considerations when choosing a plasticizer for PLA for biomedical applications. It should be a non-toxic substance miscible with PLA, thus creating a homogeneous blend. Also, the plasticizer should not be prone to migration as it would cause the material to regain the brittleness of pure PLA (Urayama et al. 2003). Citrate esters are derived from naturally occurring citric acid. They are used as plasticizers with a variety of different polymers such as poly(methyl methacrylate) and cellulose acetates (Labrecque et al. 1997). They are non-toxic and approved for use as additives in food, personal care products and in medical plastics (Gutierrez-Villarreal and Rodríguez-Velazquez 2007).

Table 2.7 Thermal and Mechanical properties of PLA plasticized with different Citrate Esters (Labrecque et al. 1997)

<table>
<thead>
<tr>
<th></th>
<th>Tg (°C)</th>
<th>Tm (°C)</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>59.1</td>
<td>145.2</td>
<td>51.7</td>
<td>7</td>
</tr>
<tr>
<td>Triethyl citrate (TEC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 %</td>
<td>42.1</td>
<td>134.1</td>
<td>28.1</td>
<td>213</td>
</tr>
<tr>
<td>20 %</td>
<td>32.6</td>
<td>130.9</td>
<td>12.6</td>
<td>382</td>
</tr>
<tr>
<td>30 %</td>
<td>22.0</td>
<td>126.8</td>
<td>7.2</td>
<td>610</td>
</tr>
<tr>
<td>Tributyl citrate (TBC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 %</td>
<td>40.4</td>
<td>143.1</td>
<td>22.4</td>
<td>62</td>
</tr>
<tr>
<td>20 %</td>
<td>17.6</td>
<td>139.0</td>
<td>7.1</td>
<td>350</td>
</tr>
<tr>
<td>Acetyl triethyl citrate (ATEC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 %</td>
<td>50.8</td>
<td>141.7</td>
<td>34.5</td>
<td>10</td>
</tr>
<tr>
<td>20 %</td>
<td>30.0</td>
<td>138.1</td>
<td>9.6</td>
<td>320</td>
</tr>
<tr>
<td>30 %</td>
<td>14.2</td>
<td>131.6</td>
<td>7.6</td>
<td>228</td>
</tr>
<tr>
<td>Acetyl tributyl citrate (ATBC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 %</td>
<td>25.4</td>
<td>139.2</td>
<td>17.7</td>
<td>23</td>
</tr>
<tr>
<td>20 %</td>
<td>17.0</td>
<td>138.9</td>
<td>9.2</td>
<td>420</td>
</tr>
</tbody>
</table>
Labrecque et al. (1997) studied the effect that citrate esters had on the thermal and mechanical properties of semi crystalline PLA (Results shown in table 2.7). They found that all plasticizers were miscible with PLA (a composition range of up to 30% plasticizer was analyzed). The reason for good solubility of PLA in citrate plasticizers is due to the polar interactions between the ester groups of PLA and the plasticizer (Ren et al. 2006). As expected, with increasing plasticizer content, the Tg and Tm of the semicrystalline PLA decreased. A significant improvement in elongation at break was also achieved at the expense of tensile strength. Plasticizing polylactide with citrate esters will help improve its thermal properties for use as a base compound in the development of a synthetic bone filler, it would also allow for the possibility of delivering the substitute by injection.

2.10.3 Crosslinking
Crosslinking is the setting up of chemical links between the molecular chains of a polymer having a significant effect on the properties of the material (Rosato et al. 2000). Figure 2.22 shows the changes occurring to a polymer structure as a result of crosslinking. Crosslinking can be introduced into an assembly of polymer molecules either as the polymerisation takes place or as a separate step after the initial macromolecule has been formed (Nicholson 2011). It involves the formation of covalent bonds between the chains to produce a three dimensional network structure (Gowariker et al. 2005).

Figure 2.22 Changes to polymer structure occurring as a result of crosslinking
(Flory 1995)
The formation of the 3D network occurs with the increase of molecular weights and the polydispersity of branched molecules. The largest molecule then progressively becomes larger and larger than any other one in the system until the critical state, the gel point, is reached. At this point, an infinite structure or gel is formed for the first time. Beyond this point, if the degree of conversion and connectivity of the system is further increased, the gel fraction increases at the expense of the molecules which are still soluble (sol). The sol molecules become gradually bound to the gel and, eventually, under certain conditions, all precursor molecules can be parts of the network. All molecules are soluble before the gel point, while beyond the gel point only a part of them are. The sol and gel fractions can be determined by extraction (Dusek and Duskova-Smrckova 2000).

Generally the formation of the 3D network exhibits the following features:
- Increase in molecular weight averages and broadening of the molecular weight distribution.
- Occurrence of the gel point at which an insoluble structure is formed for the first time; the gel point is characterized by a divergence of the weight-average and higher-average molecular weight.
- Transformation of sol molecules into the network structure (gel) by reactions between sol molecules and the gel which results in:
  - decrease in the soluble fraction (sol)
  - decrease in the molecular weight averages of the sol.
  - Build-up of the structure of the gel by gel–gel reactions, thus increasing crosslink density.

Crosslinking can occur through the application of heat, mechanically, through exposure to radiation or active chemical agents or through any combination of these (Carraher and Seymour 2007). Crosslinking agents can be used to initiate crosslinking reactions, bridge polymer molecules together, act as a catalyst for the reaction or to attack the main polymer chain to generate active sites for crosslinking to occur (Brydson 2000). Crosslinking occurs at preferential sites such as double bonds or through the use of other susceptible sites such as tertiary hydrogens (Carraher and Seymour 2007). Mark et al. described the chemical crosslinking of polymers to form a network structure as either random or controlled. Examples of random crosslinking...
include sulphur cures, peroxide cures, high energy radiation and copolymerizations. Whereas examples of controlled crosslinking includes terminal group reactions and side chain reactions (Mark and Erman 2007). The crosslinking process in elastomers is called vulcanization and occurs when sulphur compounds are added to the heated polymer, chains of sulphur atoms bond with adjacent polymer backbone chains and crosslink them. The crosslinking of two chains by a sulphur cure can be seen in Figure 2.23. Crosslink main chain sites are carbon atoms which were double bonded before vulcanization however after vulcanization have become singly bonded (Callister 2003).

![Figure 2.23 The crosslinking of two chains by a sulphur cure](Mark and Erman 2007)

Peroxides can be very useful active site generators, abstracting protons from polymer chains which help to initiate crosslinking (Brydson 2000). Peroxide cures occur when a suitable unstable peroxide is homogeneously cleaved into two free radicals, each of which then abstracts a hydrogen radical from the polymer chain to become the RH stable molecule. The free radical now present on the polymer chain now migrates along the chain until it is in proximity to a similar radical on another chain. Combination of the two radicals results in a covalent bond that crosslinks the two chains as seen in Figure 2.24.

![Figure 2.24 Basic steps in a peroxide cure](Mark and Erman 2007)
The third random technique involves high energy radiation such as electrons (e), gamma photons (γ) and ultraviolet (UV) light. Free radicals are formed and the type of crosslink generated is shown in Figure 2.25. High energy irradiation is the most abusive of the crosslinking techniques and generally causes a great deal of chain scission, resulting in dangling chain irregularities in the network structure.

![Figure 2.25 Crosslinks formed by high energy irradiation](Mark and Erman 2007)

Networks may also be formed by the random copolymerization of monomers at least one type of which has a functionality $\mathcal{O}$ of 3 or greater, where $\mathcal{O}$ is the number of sites from which chains can grow, as shown in Figure 2.26.

![Figure 2.26 Simple condensation polymerization and the addition of a trifunctional comonomer to give a network structure](Mark and Erman 2007)

![Figure 2.27 linking chains through specific side chains](Mark and Erman 2007)
As already stated terminal group reactions and side chain reactions are examples of controlled crosslinking. There are some polymers which cannot be cured easily and so the monomer is copolymerized with a comonomer which introduces sites of unsaturation along the chain (reacting side chains) as shown in Figure 2.27. These sites are then susceptible to the crosslinking techniques mentioned above. Chains can also be end linked with reactive groups, an example of end linking functionality terminated chains is illustrated in Figure 2.28.

![Network of known structure](image.png)

**Figure 2.28 End linking of functionally terminated chains** (Mark and Erman 2007)

As the crosslinks prevent free chain movement, crosslinked polymers are generally stronger and less flexible than their linear forms. The material formed usually cannot be remelted because the bonds are too strong and the greater the degree of crosslinking, the greater the rigidity of the material, the less soluble it is and the less it responds to remelting (Rosato et al. 2000).

Introducing crosslinks into the polymer chain would enable the injected bone filler to harden in-situ with improved load bearing properties as segmental motion would be reduced and the glass transition of the polymer increased. Crosslinked structures of Polylactide (PLA) can be formed by irradiation. Gamma irradiation crosslinks PLA in the presence of crosslinking agent triallyl isocyanurate (TAIC) (Quynh et al. 2007). Chemical crosslinking is another possible method with previous reports of peroxide induced crosslinking of PLA (Nijenhuis et al. 1996; Takamura et al. 2008).

Peroxide induced crosslinking is believed to follow three key steps:
1) The generation of primary radicals derived from thermal decomposition of peroxides
2) Hydrogen abstraction from polymer chains by primary radicals to generate polymer radicals

3) The bimolecular recombination of polymer radicals to form carbon-carbon crosslinks. (Takamura et al. 2008)

Takamura et al. (2008) studied the effect of the type of peroxide on the crosslinking of poly L-lactide (PLLA). Of the peroxides tested, dicumyl peroxide (DCP) had the highest hydrogen abstraction ability and one of the slowest decomposition rates. One of the measurements used to quantify decomposition rate was lifetime, defined as the time when ratio of residual peroxide to initial peroxide was 0.0001. The overall hydrogen abstraction ability of each peroxide was measured using the MSD (methylstyrene dimer) trapping technique, based on addition-fragmentation reactions between free radicals and MSD. They found that the weight average molecular weight of crosslinked PLLA increased with overall abstraction ability of the peroxide, due to the fact that slower decomposition rates caused uniform crosslinking in molten polymer. All crosslinking reactions were carried out using a single-screw extruder with a fixed temperature profile (Zone 1 = 180°C, Zone 2 = 185°C, Zone 3 = 190°C, Die = 190°C). Screw speed was set at 150rpm.

Nijenhuis et al. reported that the addition of DCP at concentrations of 13-25 wt. % to polylactide at high curing temps could produce a polymer with a gel fraction of 100% (Nijenhuis et al. 1996). Although peroxides are used for biomedical applications, the use of high concentrations is undesirable due to possible toxicity of decomposition and undefined degradation products (Nagata and Inaki 2009). Using smaller concentrations of peroxide (up to 1.5 wt. %) with crosslinking agent TAIC (up to 3 wt. %) added to PLA produces a crosslinked system (Yang et al. 2008).

DCP decomposes to form radicals which in turn break the double bonds in TAIC to produce monomer radicals. The peroxide also abstracts hydrogen from PLA to create radicals in the polymer chain. The TAIC monomer radicals then combine with the polymer radicals to form the crosslinked network. By varying the amount of peroxide and crosslinking agent, materials with different gel fractions and crosslink densities can be prepared. See Figure 2.29 for the possible reaction scheme of the crosslinking of TAIC and PLA as purposed by Yang et al. This process occurs at temperatures
above 50°C which may presents a problem for in situ crosslinking by this method. Studies have reported the occurrence of cellular necrosis at temperatures of 50°C. One such study reported that a bone cell necrotizes when it receives 50°C for 360 seconds (Fukushima et al. 2002).

Other means of crosslinking such as photocrosslinking could be a possible method of hardening the polymer is situ. There are very few studies on the photocrosslinking of polylactides to date. Of what has been reported, most involve photocured networks synthesized from polylactide oligomers whose terminal groups were functionalized with an acryloyl chloride or a methyl anhydride. Helminen et al synthesised one such system as seen in Figure 2.30. Polylactide oligomers were polymerised and then functionalized with methacrylic anhydride. The reactivity of the terminal double bonds obtained by functionalization is higher then that of the double bonds along the polymer chains and so a higher crosslinked structure can be obtained. The functionalized oligomer was first mixed with a reactive monomer dimethacrylated butanediol. For photoinitiation, camphorquinone was used as an initiator and ethyl- 4-N, N-dimethylaminobenzoate was used as a promoter. To cure, a high light
instrument emitting blue light (400-500 nm) was used which penetrated the 12 mm thick sample. A 5 minute reaction time was sufficient to crosslink the sample to a high conversion with a gel content of 95% at room temperature (Helminen et al. 2002).

**Functionalization with methacrylic anhydride:**

![Functionalization reaction](image)

**Reaction of double bonds leading to crosslinking:**

![Crosslinking reaction](image)

**Figure 2.30 Functionalization of polylactide oligomers with methacrylic anhydride to obtain reactive terminal double bonds** (Helminen et al. 2002)

Nagata et al. produced a dihydroxy terminated PLA which was then chain extended with diacyl chloride to obtain high molecular weight photocrosslinkable polylactide. This was then irradiated with a 40W high pressure mercury lamp to produce gel films without the use of a photoinitiator (Nagata and Inaki 2009).

Karikari et al. synthesized oligomeric four-arm star shaped PDLLA with the use of an ethoxylated pentaerythritol initiator, which had a low Tg that was suitable for use as a viscous liquid prior to photocrosslinking. The oligomers were then functionalized with either methacrylic anhydride (MAAH) or 2-isocyanatoethyl methacrylate (IEM) as seen in Figure 2.31. The functionalized star polymers were mixed with 2, 2-dimethyl-2-phenylacetophenone (DMPA) and passed through a Fusion UV system at 5 ft/min to achieve an energy dose of 1.63 J/cm² (UVA). Photocrosslinking resulted in
highly crosslinked networks with gel contents ranging from 90 to 99% (Karikari et al. 2005).

Figure 2.31 Functionalized four arm star shaped polylactide and subsequent photocrosslinking (Karikari et al. 2005).

### 2.11 Polymer compound

The overall aim of this project is to develop an injectable, resorbable, putty like bone filler that can be used to repair damaged bone. A review of the biodegradable polymer polylactide has been given with an introduction to the methods which might be used to firstly modify the polymer to create a mouldable and injectable material and secondly the methods which may be used in order to harden this material in-situ. An alternative approach will now be examined in the following section of this review.

Injectable bone substitutes that are self-setting in situ can bring significant benefits in several clinical situations. Injectable systems may be preferred when the fracture, defect or hole must be fixed and posses mechanical resistance immediately, when it is in a position difficult to reach or has a complex shape or simply because of the ease handling and implantation of these systems (Mano et al. 2004). Most injectable bone
substitutes are delivered as one or two dry powders and a fluid which are mixed in the operating room either manually or with a mixing machine. After mixing, the paste-like cement is injectable for a few minutes after which it cures through a slightly exothermic or isothermal reaction (Larsson and Hannink 2011). Calcium phosphate cements are the most widely used injectable bone substitute and have previously been discussed in this review. As already stated, calcium phosphate grafts possess compressive strengths comparable to cancellous bone, but their main drawback remains their limited resistance to tensile and shear forces and they are not suitable for treatment of fractures and defects where structural support is required.

Currently there is great research interest in the compounding of polymers and other materials to obtain composites that attain combinations of mechanical and biological properties similar to those of biological hard tissue. By combining a liquid monomer which could harden upon polymerization and mixing it with particles of polylactide, a bone filler base compound could be created which would be hand mouldable and easily injectable. With the onset of polymerization of the monomer, the filler would then harden in situ. The greatest advantage of composite materials is that they offer the possibility of tailoring its properties by playing with the dimension of the particles and the volume fraction of the discontinuous phase. Modification of the composition with the addition of plasticizers, fillers and other additives allows for the adjustment of mechanical properties and setting rates. The liquid monomer chosen was n-butyl cyanoacrylate. Cyanoacrylates are unique, in that they are single component systems that polymerise at room temperature without the addition of a catalyst, evaporation of a solvent or an external energy source. They also have the advantage of being inexpensive, resorbable and a well established tissue adhesive.

2.11.1 Cyanoacrylates
Alkyl cyanoacrylates represent a class of very reactive monomers widely employed for biomedical purposes as surgical glues for the closure of skin wounds, embolic material for endovascular surgery and for nerve regenerating purposes. Another important application of poly(alkyl cyanoacrylate) is related to the field of drug delivery due to their ability to form submicronic biodegradable nanoparticles (Nicolas et al. 2011). Cyanoacrylates are remarkable compounds, best known as
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instant adhesives or super glues which were discovered by Coover in 1953 (Robello et al. 1999). The uniqueness of cyanoacrylate glue is the extreme reactivity of the cyanoacrylate monomer toward nucleophiles and they have excellent adhesive properties resulting from the bonds of high strength they are able to form with most polar substrates, including living tissues and skin.

The synthesis of alkyl cyanoacrylates is based on a two step procedure as shown in Figure 2.32. The corresponding alkyl cyanoacetate is reacted with formaldehyde in the presence of a basic catalyst, to form poly(alkyl cyanoacrylate) oligomers by Knoevenagel condensation reaction, followed by their thermal depolymerization reaction which leads to the alkyl cyanoacrylate monomer (Nicolas et al. 2011). The resultant cyanoacrylate monomer can subsequently be distilled to achieve purity and remove any toxic byproducts of the synthesis. It can then be formulated with stabilizers, plasticizers, and other additives to improve its biocompatibility, stability, and clinical performance (Singer et al. 2008).

In general, the strength and other physical properties of the cyanoacrylate adhesives are directly related to the length and complexity of their alkyl side chain. Cure speed and adhesive strengths both tend to drop with increasing alkyl chain length (Coover et al. 1990). Short, straight-chain derivatives (ethyl or butyl cyanoacrylate) form tight and stronger bonds compared with complex or long-chain derivatives (Bhatia 2010). Also as the ester chain increases from one carbon to a higher number of carbons, the compound becomes more biocompatible (Lauto et al. 2008). Figure 2.33 shows the structure of different cyanoacrylates.
The reactivity of cyanoacrylates is directly traceable to the presence of two strong electron withdrawing groups; the nitrile group and the alkoylcarbonyl group, which results in the double bond being very polarized and amenable to nucleophilic attack. This permits a weak base, such as water from tissue fluid to initiate and complete the anionic polymerization of the monomer quickly as shown in Figure 2.34. The polymerization process is exothermic with the rate of polymerization inversely proportional to the length of the alkyl side chain and to the amount of monomer (Bhatia 2010).

The anionic polymerization route of cyanoacrylate is by far the most predominant method of polymerization. However, exposure to extended high temperatures or UV light in the presence of peroxides can cause free radical polymerization to be
initiated (Coover et al. 1990). For free-radical polymerization as seen in Figure 2.35, the propagation step is much slower than for anionic polymerization, and the corresponding molecular weights are generally lower (Robello et al. 1999).

![Figure 2.35 Free radical polymerization of alkyl cyanoacrylate](image)

Inhibitors of both anionic and free radical polymerization are added to cyanoacrylate monomers to ensure the shelf life of the adhesive. The most common free radical stabilizer is hydroquinone. Since the anionic cure of cyanoacrylate proceeds as a result of a basic catalysis, acids of either the Lewis or protonic types have been used as anionic polymerization inhibitors (Coover et al. 1990). Acid strength and level are important variables when choosing a stabilizer. High levels of acids can over stabilize and make polymerization speed sluggish. Also, despite the fact that water is known as the most common initiator of polymerization, cyanoacrylates can contain surprisingly high levels of free water. This water in combination with a strong acid can cause hydrolysis of the monomer, forming carboxylic acids which drastically retard cure speed (Coover et al. 1990).

Poly(alkyl cyanoacrylates) undergo hydrolytic degradation which takes place through non-enzymatic reactions with the main degradation products being formaldehyde and the corresponding alkyl cyanoacetate as shown in Figure 2.36. Degradation takes place by breakdown of the polymer backbone and occurs because the methylene hydrogen in the polymer is highly activated inductively by the electron withdrawing neighbouring groups. The degradation rate decreases with an increase in alkyl side chain, as a result of steric hindrance. An alternative degradation mechanism has also been proposed in which the cyanoacrylate polymer degrades by hydrolysis of the ester group to produce cyanoacrylic acid and alcohol as shown in Figure 2.37. This reaction is said to occur in the physiological
environment and may be catalyzed by enzymatic activity (Bhatia 2010). As stated previously, degradation rate is dependent on ester chain length, however it also depends on other factors such as particle size, molecular weight, pH and enzymes (Huang and Lee 2006).

![Diagram of hydrolytic degradation of the cyanoacrylate polymer](image1)

**Figure 2.36** Hydrolytic degradation of the cyanoacrylate polymer releasing formaldehyde and alkyl cyanoacetate as degradation products (Bhatia 2010)

![Diagram of alternative degradation of the cyanoacrylate polymer](image2)

**Figure 2.37** Alternative degradation of the cyanoacrylate polymer. The ester group is hydrolyzed to produce cyanoacrylic acid and alcohol (Bhatia 2010)

Commercially available cyanoacrylate based tissue adhesives are mainly composed of either n-butyl-2-cyanoacrylate or 2-octyl-cyanoacrylate. These cyanoacrylates achieve tissue adhesion through two independent mechanisms. Firstly through molecular interaction to the tissue surface by covalently bonding to functional groups in proteins, in particular amine groups and secondly by mechanical interlocking of the poly(cyanoacrylate) with underlying tissues (Bhatia 2010).
2.11.2 Addition of filler

Polymers generally have insufficient mechanical properties for applications as bone fracture fixation devices in major load-bearing situations. Consequently, various forms of composite technology have been used to enhance the mechanical properties (Nazhat et al. 2001).

There are several advantages in incorporating bioactive ceramics into biodegradable polymers, in order to produce hybrid materials. Calcium phosphate particles, such as hydroxyapatite (HA) or tricalcium phosphate (TCP) improve osteoconductivity and bone bonding properties in polymeric matrix composites (Damien and Parsons 1991). The addition of calcium phosphates into the polymer matrix aids with the formation of a hydroxy apatite layer which provides the bonding interface with bone tissue (Rezwan et al. 2006). Biocompatibility is enhanced as the ceramic particles induce an increased initial flash spread of serum proteins compared to more hydrophobic polymer surfaces (Hutmacher 2000). In a study completed by Rizzi et al., it was shown that the spread of attached human osteoblasts onto PLA and PCL films reinforced with HA is higher than for the polymers alone. They also reported that biochemical assays relating cell activity to DNA content showed that cell activity is also more intense for the composite films (Rizzi et al. 2001). Additionally, foreign body reaction due to the release of acidic degradation products is also minimised by the buffering effect of the basic resorption products of HA or TCP (Agrawal and Athanasiou 1997). The inclusion of HA particles within PLA has also been reported to improve the bulk degradation. Such polymer composites were reported to have hydrolyzed homogeneously due to water penetrating the interfacial regions (Rezwan et al. 2006). HA has also been reported to be efficient at supporting the attachment, differentiation and proliferation of the relevant cells needed for bone regrowth (Bleach et al. 2002).

As well as improved bone bonding properties, the use of a bioactive filler such as hydroxyapatite or Bioglass particles to reinforce a polymer can improve the mechanical properties. The mechanical properties of particulate–polymer composites depend strongly on the particle size, particle–matrix interface adhesion and particle loading. It has been reported that the elastic modulus of polyethylene is increased from 1 GPa to about 8 GPa, which is in the low band of the value for bone, retaining
a fracture toughness comparable to bone with the addition of HA particles to the PE matrix (Liu 2006). Young’s modulus is markedly improved by adding inorganic particles to a polymer matrix since hard particles have much higher stiffness values than the matrix. The elastic modulus of a particulate–polymer composite is generally determined by the elastic properties of its components (particle and matrix), particle loading and aspect ratio (Fu et al. 1999).

For particulate composites, composite strength relies on the effectiveness of stress transfer between matrix and fillers. Factors like particle size, particle/matrix interfacial strength and particle loading significantly affect the composite strength. Smaller calcium carbonate particles provide higher strength of filled polypropylene composites at a given particle loading than larger particle sizes (Lau et al. 2006), while smaller particle sizes yields higher fracture toughness for calcium carbonate filled high density polyethylene (HDPE) (Bartczak et al. 1999). The tensile strength of glass bead filled polystyrene composites depends on the particle–matrix adhesion and increases with it (Dekkers and Heikens 1983). Thus, the use of coupling agents that increase the particle–matrix adhesion leads to higher strength (Spanoudakis and Young 1984). The strength of polyimide/silica composites increases with particle loading to 10 wt% and decreases beyond that (Zhu et al. 1999). Also, the fracture toughness of glass bead filled epoxy composites was shown to increase initially with increasing filler loading till a plateau value is reached at a critical particle volume fraction(Kinloch et al. 1985).

The ultimate strength of a composite depends on the weakest fracture path throughout the material. Hard particles affect the strength in two ways. One is the weakening effect due to the stress concentration they cause, and another is the reinforcing effect since they may serve as barriers to crack growth. In some cases, the weakening effect is predominant and thus the composite strength is lower than the matrix; and in other cases, the reinforcing effect is more significant and then the composites will have strengths higher than the matrix (Fu et al. 2008).

Calcium phosphates as materials for bone grafting substitutes were discussed previously in this review. They were first considered for clinical application as a filler for bone defects in the 1920s and first incorporated in dentistry and
orthopaedics in the 1980s. The calcium phosphate HA is most commonly used in clinical applications and has been used in bone cements for the repair of craniofacial defects, for maxillary sinus floor augmentation and in coatings for hip replacements (Wagoner Johnson and Herschler 2011). In recent years, totally resorbable composite bone substitute materials have been the subjects of intense study in surgical reconstruction and bone tissue engineering. The HA – PLA composite becomes an important representative of these materials since they combine the osteosconductivity and bone bonding ability of HA with the resorbability and the easy processing property of the polymer matrix (Zhang et al. 2005).

2.11.3 Patents
There is a great deal of research being conducted into composites comprising of biodegradable polymers and bioactive ceramic fillers for use as bone substitutes. However, little or no studies into the use of a cyanoacrylate based polymer matrix with a polylactide and HA filler have been conducted. Investigating the literature, a number of patents have been filed which are somewhat similar.

Patent application number 20080220045 was filed for the use of self setting polymeric cyanoacrylate composites as a bone filler and cement. The composite comprises of at least 20% by weight of solid microparticles in a cyanoacrylate matrix. The microparticles should comprise of at least one type of inorganic phosphate and may also comprise of 10% by weight or less of hydroxy-terminated polyglycolide. The matrix may or may not also contain a reinforcing absorbable wrap knitted mesh (Shalaby et al. 2008).

Patent number US7449498 filed by Yesbio Co. Ltd also describes a composite material for bone replacement comprising of cyanoacrylate combined with an inorganic material which has a modified surface to encapsulate hydroxyl groups (inhibits the polymerisation of the cyanoacrylate). The cyanoacrylate and inorganic material can be combined in any ratio but preferably within the range of 1:4 to 1:7 (Park et al. 2008). Patent application number 20090277455 filed by Femasys Inc describes a two component composite comprising of a resorbable polymer (polylactide etc.) and a liquid cyanoacrylate tissue adhesive mixed prior to entry into a catheter for delivery for occluding or of means for opening conduits. The
implantable material may be delivered pre-formed or in situ cured (Lee-Sepsick et al. 2009).

A review of the patent literature indicates that a gap in the market currently exists for a bone substitute composite composed of a cyanoacrylate matrix reinforced with particles of resorbable polylactide along with hydroxyapatite. Modification of the composition would allow for the adjustment of mechanical properties and setting rates and hopefully obtain composites that attain mechanical and biological properties similar to those of biological hard tissue and superior to calcium phosphate cements.
2.12 References


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behavior of CaP and CaP/polymer composites for applications in bone


Chapter 3
3.0 Experimental

3.1 Introduction
In order to evaluate the performance of the materials for use as a bone filler substitute a variety of preparation methods, tests and techniques were employed and are described in this chapter.

3.2 Material preparation
3.2.1 Preparation of polylactide polymer
PDLLA was supplied by Boehringer Ingelheim (Germany) under the trade name of Resomer R 207 S. The first batch of polymer used was in granular form (used in chapters 4-6) with the second batch received (only used in chapter 7) being fibrous in nature. As the polymer is very expensive and only available in small quantities, it was not possible to prepare the polymer by conventional methods. Today the main conversion methods for polylactide are based on melt processing (Lim et al. 2008). A compounded pelletized feed stock is produced that is further processed using techniques such as injection moulding, extrusion, compression moulding and so on (Jiang et al. 2010). Many of these processes require a high volume of material, and so an alternative processing method had to be considered. PDLLA granules were first ground into smaller particles using a coffee grinder. Approximately 10g of polymer was ground for three minutes to give particles sizes of approximately 800µm as indicated in Figure 3.1 below. Particles of this size were used in chapter 4 where the polymer was heated and melt blended in small quantities with plasticizers.

![Figure 3.1 Microscopic view (X5) of PDLLA particles after grinding for 3 minutes](image-url)
In subsequent chapters, the polylactide polymer used was reduced to a powder by continuously regrinding the polymer in a coffee grinder. Approximately 10g of polymer was ground for one minute which was then sieved through a stainless steel sieve with a mesh size of 200µm. Polymer which could not go through the sieve was reground for 1 minute and re-sieved. The above step was repeated numerous times until all the polymer was reduced to a fine powder of particles sizes of approximately 200µm or less as indicated in figure 3.2 below.

![Figure 3.2 Microscopic view (X5) of PDLLA particles after grinding and sieving](image)

### 3.2.2 Gamma Irradiation and photocrosslinking

In chapter 5, an attempt was made to crosslink the polylactide polymer using gamma irradiation or by photocrosslinking. Also in chapter 7, cyanoacrylate samples were sent for gamma irradiation to indicate the effect sterilization would have on the liquid monomer. For gamma irradiation, polymer samples were sent to Isotron in Westport, Co. Mayo and irradiated at 30kGy.

The source of gamma rays used by Isotron are from the radioisotope Cobalt 60. Co60 is an isotope of the stable Co$^{59}$ and is produced in a nuclear reactor by bombarding Co$^{59}$ with neutrons. The decay of each atom of Co$^{60}$ results in 2 photons of gamma radiation, a beta particle and a stable Nickel atom. It is the photons of gamma irradiation that do the work by creating highly active electrons and free radicals in the target material. Products sent to Isotron are loaded onto a pallet with each pallet being programmed to receive a certain exposure (time x source activity). The pallets are placed on a conveyor where they are then moved around the gamma
ray source allowing exposure to all sides of the product. The absorbed dose of radiation is measured in Grays (Gy) with 1 Gray = 1 joule of energy per kg of product. Typical sterilising doses range from 9 - 50 kilo Grays (kGy). The absorbed dose (kGy) is dependant on the source (Co60), energy activity and the exposure period. The exposure period is the main variable which is used to control the absorbed dose. It is the dwell time which is adjusted and monitored to control the dose, i.e. to increase the dose from 10 kGy to 25 kGy the product must be exposed to the source for 2.5 times the dwell period as a 10 kGy dosed product.

The samples which were attempted to be photocrosslinked were placed in a UV (Ultraviolet) box for 20 minutes. The light source had a wavelength of 340nm and an intensity range of 10 to 13.5 mW/cm². Using light instead of heat, UV crosslinking is based on a photochemical reaction. UV light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays, in the range of 10 nm to 400 nm. Using a photoinitiator, crosslinking reactions may happen within the polymer samples as excitation of the photoinitiator occurs with the absorption of electromagnetic radiation from the UV source.

3.3 Test Methods
3.3.1 Differential Scanning Calorimetry (DSC)
Thermal analysis may be defined as the measurement of the physical and chemical properties of materials as a function of temperature or time. DSC is one of the most popular techniques used for the thermal analysis of a polymer and was used in chapter 4 of this report to indicate the effect plasticization had on the Tg of the polymer. Using a constant automatic adjustment of heater power to keep a test sample temperature equal to that of a reference sample, a varying electrical signal that is equal to the changing thermal behaviour of the test sample is recorded. This signal is proportional to the test samples heat capacity and so thermal transitions over a specified temperature range can be accurately measured (Blair 1994). Samples were sealed in an aluminium pan while the reference consisted of an empty aluminium pan. There are two forms of differential scanning calorimetry; power compensating and heat flux. The differential scanning calorimetry (DSC) in this report was preformed using a Perkin-Elmer DSC-1. The Pyris 1 DSC Differential
Scanning Calorimeter is a power compensation DSC. With the power compensated DSC, both the sample side and reference side have their own independently controlled furnaces, which provides a direct calorimetric measure of the true heat flow. In contrast, the heat flux DSC uses a single furnace for both the sample and reference and measures a temperature differential, which is then converted to a heat flow.

![Diagram of heat flux and power compensating DSC](image)

**Figure 3.3 (a) heat flux and (b) power compensating DSC** (Schick 2009)

The resultant graphs are displayed as heat flow versus temperature or time. Exothermic and endothermic transitions in the sample are shown with a positive or negative peak, depending on the software set up. The different thermal properties which can be obtained from DSC analysis include the glass transition temperature and solid–state reactions including nucleation temperatures, crystallisation temperatures and endothermic or melting reactions. The DSC was calibrated using Indium (Tm onset of 156.6°C and ΔH of 28.1 J/g).
3.3.2 Dynamic Mechanical Thermal Analysis (DMTA)

Dynamic mechanical thermal analysis (DMTA) is an important technique capable of providing considerable information on the position of transitions and the mechanical properties of polymers. DMTA measures the storage modulus ($E'$) and the loss modulus ($E''$) as a function of temperature. A sinusoidal mode of deformation is applied to the sample, as the temperature is scanned from well below the glass transition temperature to the point when the sample becomes too soft to test in a given apparatus. DMTA provides information on first and second order transitions (Tm and Tg, respectively), the degree of phase separation, crystallinity and crosslinking of the polymer, and the mechanical properties such as the glassy state and the rubbery plateau modulus.

In DMTA experiments the applied force and the resulting deformation vary sinusoidally with time, at a rate specified by the frequency ($f$) in cycles/sec, or $\omega = 2\pi f$ in radians/sec. For idealised elastic behaviour, the resultant strain will alternate exactly in phase with the applied stress. A completely viscous material will have a strain lagging 90° (out of phase) behind the applied stress. When a sinusoidal stress is applied to a viscoelastic material it will behave neither as a perfectly elastic nor a perfectly viscous body while the resultant stain will lag behind the stress by some angle ($\delta$), where $\delta < 90^\circ$. The magnitude of the loss angle is dependent upon the amount of internal motion occurring (damping).

The stress ($\sigma$) and strain ($\varepsilon$) can be expressed as follows:

$$\sigma = \sigma_0 \sin(\omega t + \delta)$$

$$\varepsilon = \varepsilon_0 \sin(\omega t)$$

Where $\omega$ is the angular frequency and $\delta$ is the phase angle. Then:

$$\sigma = \sigma_0 (\sin \omega t \cos \delta) + \sigma_0 \cos(\omega t) \sin(\delta)$$

The stress may be considered to consist of two components, one in-phase with the strain ($\sigma_0 \cos \delta$) and the other 90° out-of-phase ($\sigma_0 \sin \delta$). When these are divided by the strain, we can separate the modulus into an in-phase (real) and out-of-phase (imaginary) components. The corresponding relationships are:
\[ \sigma = \varepsilon_0 E' \sin(\omega t) + \varepsilon_0 E'' \cos(\omega t) \]

\[ E' = \left( \frac{\sigma_0}{\varepsilon_0} \right) \cos(\delta), \quad E'' = \left( \frac{\sigma_0}{\varepsilon_0} \right) \sin(\delta) \]

Where \( E' \) is the real part of the modulus, and \( E'' \) is the imaginary part.

Alternatively:

\[ E' = \frac{\text{Amplitude of the in phase component}}{\text{Strain amplitude}} \]

\[ E'' = \frac{\text{Amplitude of the out of phase component}}{\text{Strain amplitude}} \]

Complex representation of the modulus can be expressed as:

\[ \sigma = \sigma_0 \exp(it) \]

\[ \varepsilon = \varepsilon \exp(i(\omega t + \delta)) \]

\[ \frac{\sigma}{\varepsilon} = E^* = \text{Dynamic Young's Modulus} = E' + iE'' \]

The phase angle \( \delta \) is defined as:

\[ \tan \delta = \frac{E''}{E'} = \frac{\text{Loss Modulus}}{\text{Storage Modulus}} \]

The real components of the modulus \( E' \) is termed the storage modulus because it is related to the storage of energy in potential form for subsequent release by periodic deformation. The imaginary part of the modulus \( E'' \) is termed the loss modulus and is associated with the dissipation of energy, primarily as heat, upon deformation. The loss tangent, \( \tan \delta \), is described as the internal friction or damping. It corresponds to the ratio of energy dissipated per cycle to the maximum potential energy stored during the cycle. The separation of the two components describing two independent processes within the sample, elasticity (energy storage) and viscosity (energy dissipation), is a fundamental feature that yields sensitivity to both macroscopic and molecular relaxation processes.
Typical events discernible from a DMTA thermogram are displayed in Figure 3.4. From a low temperature, with constant heating rate, resonant induced localised motion of pendant groups can occur and manifests itself in small relative peaks in the damping curve. Correspondingly, there is a slight decrease in slope of the storage modulus (E'). Structural permutations offer additional possibilities that other side groups may vibrate, with local damping maxima and modulus variations. Frequently these are denoted as the γ and β transitions, respectively upon heating. Further heating effects an order of magnitude reduction in the storage modulus.

In the region of the inflexion point where the storage modulus decreases, the damping curve displays a prominent maximum, associated with Tg of the system. Frequently, this is termed the α transition. Additional melting of ordered or partially ordered phases can result in the storage modulus, with corresponding upturns in the damping behaviour. From this event onwards, the extra mobility conferred to the system results in an asymptotic decline in the storage modulus, effectively approaching zero with the advent of Tm. In this state, relative damping increases and reflects the increasing propensity towards viscous behaviour. Accordingly, dynamic mechanical thermal analysis was employed to determine the relative viscoelastic response of the materials over a wide temperature range. The morphology and important second order events were resolved with this technique, enabling correlation with molecular processes.

Figure 3.4 Idealised DMTA behaviour for a viscoelastic material in tension at a fixed frequency
All DMTA was carried out in Athlone Institute of Technology using a Rheometric scientific mark 3 DMTA in tensile mode. The storage and loss moduli and the damping, tan δ, of the material were measured as a function of temperature. In this instrument the upper clamp is driven and the lower clamp us rigidly fixed, all enclosed in a temperature programmable environment chamber.

![Figure 3.5 Rheometric scientific mark III DMTA](image)

Depending upon material stiffness, the frequency of oscillation is selected between 0.01 and 100 Hz, with the ability to multiplex several frequencies. In real time the resultant sample motion is monitored and resolved for moduli and damping calculations. Sub-ambient temperatures were achieved by circulating the liquid nitrogen through a cooling jacket. Due to instrument limitation, the gaseous environment of DMTA chamber was uncontrolled in that a nitrogen purge could not be used. Consequently atmospheric moisture was able to condense on sample substrates upon heating from sub-ambient conditions. However given the relative nature of this technique, all samples were subjected to the same anomaly.

### 3.3.3 Tensile testing

A tensile test can be used to characterise several material properties important to design. A specimen is deformed, usually to fracture with a gradually increasing tensile load that is applied uniaxially along the long axis of the specimen (Callister 2003). The testing apparatus is designed to elongate the specimen at a constant rate and to continuously measure the applied load and resulting elongations. The output of the test results in a graph showing load/force vs. elongation. From these parameters, a graph can be plotted to show tensile stress (σ) against tensile strain (ε).
An Instron 4301 series tensile testing machine was used to test all samples. Specimens were dogbone shaped with a gauge length of 13mm, a width of 3.5mm and thickness of 3mm after ASTM standard D638-08. The samples were tested with a crosshead speed of 10mm/min using a 1kN load cell, which had an accuracy within 1%.

### 3.3.4 Plasticizer migration study

A plasticizer migration study was carried out after standard BS EN ISO 177:2000 “Plastics-Determination of migration of plasticizers”. This standard specifies a method for determination of the tendency of plasticizers to migrate from plastics in which they are contained into other materials when they are brought into close contact. A test specimen cut from a sheet or plate of the material or from the finished product to be tested is placed in close contact with two sheets capable of absorbing plasticizers. It is then subjected to heating under defined conditions. The loss in mass of the test specimen, theoretically equal to the increase in mass of the sheets, is a measure of the migration of the plasticizer. Samples measuring 25.5mm x 3.35mm x 3mm were placed between two cellulose based sheets (tests were ran using both paper and woven fabric). Weights were positioned on each sample and placed in a 37°C oven. The loss in mass of the test specimen was calculated as a measure of the migration of the plasticizer over a period of 30 days.

### 3.3.5 Swelling studies and crosslink density

A crosslinked network structure is defined by several parameters, i.e., the number of cross-links, their functionality and distribution, network defects and entanglements. The most common techniques to determine the crosslink density or the degree of crosslinking in a polymer network is by mechanical measurements and equilibrium swelling studies. After crosslinking the polymer is no longer soluble and when submerged in a suitable solvent, the crosslinked polymer will swell to a certain extent depending on the crosslink density and the interaction between the polymer and solvent.

Flory proposed an analogy between swelling equilibrium and osmotic equilibrium when immersing a polymer network in a liquid. In this analogy the retractive force of the swollen network competes with an osmotic pressure. At equilibrium the osmotic
pressure is sufficient to increase the chemical potential of the solvent in the gel so that the chemical potential is the same as that of the excess solvent surrounding the swollen network. Based on this analogy, the Flory-Rehner equation was formulated relating the equilibrium swelling of a network in any kind of swelling medium with a measure for the crosslink density

\[ n = \frac{-[\ln(1 - \nu_2) + \nu_2 + \chi \nu_2^2]}{V_1 \left( \nu_2^{1/3} - 0.5 \nu_2 \right)} \]

Where \( \nu_2 \) is the polymer network volume fraction at equilibrium swelling, \( \chi \) is the polymer-swelling agent interaction or Flory–Huggins parameter \( V_1 \) is the partial molar volume of the swelling agent and \( n \) is the number of moles of crosslinks per unit volume. This equation expresses crosslink density as a function of swelling ratio and calculations of crosslink density and gel fraction are based on Flory-Rehner theory. Uncrosslinked PDLLA will dissolve in chloroform while crosslinked PDLLA will not. By weighting the sample before and after soaking, gel fractions and degree of swelling were calculated. A high gel fraction and a low degree of swelling indicate a high crosslink density. Gel fraction was measured by the amount of insoluble material in chloroform using the following equation:

**Gel Fraction (%) = \( (W_g / W_o) \times 100 \)**

Where \( W_o \) is the weight (dry) of the crosslinked PDLLA and \( W_g \) is the remaining weight (dry gel component) of the crosslinked PDLLA after dissolving in chloroform for 24 hours. Degree of swelling (volume ratio of absorbed solvent to dry gel) was calculated using the following equation:

**Degree of Swelling = \( [(W_s - W_g) / W_g] (\rho_p / \rho_{CHCl_3}) \)**

Where \( W_g \) is the dry gel component of the crosslinked PDLLA and \( W_s \) is the weight of the swollen gel component. \( \rho_p \) and \( \rho_{CHCl_3} \) are the densities of PDLLA and chloroform respectively. The \( \rho_p \) value for the samples tested changes depending on the polymer-plasticizer composition.
3.3.6 Water Absorption and Hardness
Samples tested for water absorption and hardness over time were placed in phosphate buffered saline (PBS) at a pH of 7.2 and incubated at 37 °C. At selected intervals over an indicated period of time, samples were removed from the PBS, patted dry and weighed. The percentage weight increase is then calculated as a function of time. Shore A hardness values were also measured as a function of time. Hardness may be defined as a material's resistance to permanent indentation. Shore hardness is measured with an apparatus known as a durometer which measures the depth of an indentation in the material created by a given force. This depth is dependent on the hardness of the material, its viscoelastic properties, the shape of the presser foot, and the duration of the test. The hardness measured is a dimensionless quantity but depending on the durometer type used and its spring force and indentor configuration, a specific scale is assigned. The two main scales are Shore A and Shore D with the Shore A scale used for 'softer' polymers while the Shore D scale is used for 'harder' ones. There is no simple relationship between a material's Shore hardness in one scale, and its Shore hardness in any other scale.

3.3.7 Double torsion
The double torsion (DT) test was used to determine the fracture toughness. It has many advantages over other fracture toughness specimen geometries. The crack length is not required in the calculation of the stress intensity factor, since the DT geometry makes it a linear compliance test-piece. It can be used to investigate crack stability in a material, affording an insight into the fracture process. Providing cracks are stable the test-piece can be used to obtain crack velocity-stress intensity factor diagrams, from which static fatigue lifetimes can be predicted. The crack propagates at constant velocity down the DT specimen, the velocity being determined by the crosshead displacement rate, the specimen dimensions and the modulus of the material. The specimens are easy to manufacture and blunt cracks can readily be detected.

The DT test was performed using an Instron Universal testing machine and some specially made fixtures for the support and loading of the samples. These consisted of two parallel rollers of 3mm diameter and spaced 20mm apart and load applied at a constant rate (0.1 mm min\(^{-1}\)) to the slotted end via two 3mm diameter ball bearings.
Chapter 3

spaced 10mm apart. The specimen was therefore subjected to four-point bend loading, during which the crack initiated and propagated, along the centre of the specimen, within the groove. The groove depth and specimen dimensions were chosen to eliminate the need for crack shape corrections. In a DT test the mode I stress intensity factor $K_1$ is independent of crack length and is given by:

$$K_1 = P_c W_m \left( \frac{3(1+\nu)}{W t_n^3} \right)^{\frac{1}{2}}$$

where $W_m$ is the moment arm, $W$ the specimen width, $t$ the specimen thickness, $t_n$ is the thickness in the crack plate and $\nu$ Poisson's ratio (assumed to be 0.33) (Callister 2003). Values for $K_1$, the fracture toughness, were obtained by substituting the appropriate specimen dimensions along with the load at fracture $P_c$ into the equation above.

Figure 3.6 Double torsion fracture toughness test apparatus (Clarkin et al. 2009)

3.3.8 Flexural test

In a three-point bend specimen the relationship between the applied load ($P$) and the deflection at the centre of the specimen ($\delta$) for a specimen of rectangular cross section is given by
\[ P = \frac{4\delta Eb t^3}{s^3} \]

where \( t \) is the beam thickness, \( b \) the beam width and \( s \) the span. A span of 20mm was used, with a crosshead displacement rate of 0.1mm min\(^{-1}\). This gives an almost identical strain rate to that used in the DT tests. The Young’s modulus (E) was calculated from the initial slope of the load deflection plot. The flexural strength is defined by:

\[ \sigma_f = \frac{3Ps}{2bt^2} \]

### 3.3.9 Rheology

To indicate the handling and setting behaviour of the polymer composites in chapter 7, the rheological properties of the compositions while setting were analysed using a TA Instrument AR1500ex Rheometer in parallel plate mode. The diameter of the upper parallel plate measured 20mm and the gap between the two plates was set at 1.5mm. Approximately 2g of each composition was mixed manually and placed between the plates of the rheometer. The top was lowered and any excess material was carefully removed. The rheometer was run at a frequency of 1Hz with a 1% strain at 25°C unless otherwise indicated. The sample components were mixed for 30 seconds with a time of 60 – 100 seconds elapsed from the start of mixing to the beginning of measurements taken by the rheometer. Samples were run for 60 minutes. A sample of the bone cement Simplex from Stryker was also tested for the purpose of comparison. The principles used to measure the handling properties of the polymer compound are the same principles already described in section 3.3.2 for DMTA.

Most polymers are viscoelastic materials and so have viscous and elastic components. These materials change their properties with temperature and time. One way to look at these changes is by measuring the response of the material to deformation by periodic forces, e.g. during forced vibration or small-amplitude oscillatory shear. The response obtained shows that stress and strain are not in phase, the strain lags behind the stress by a phase angle, \( \delta \), known as the loss angle. If the oscillatory shear is sinusoidal, then
where $\tau = \text{shear stress}$; $\tau_0 = \text{stress amplitude}$; $\omega = \text{angular frequency}$; $t = \text{time}$; and
\[
\gamma = \gamma_0 \sin(\omega t - \delta)
\]
where $\gamma = \text{shear strain}$; $\gamma_0 = \text{strain amplitude}$. The equation for stress can be expanded to give
\[
\tau = \tau_0 \sin \omega t \cos \delta + \tau_0 \cos \omega t \sin \delta
\]
This shows that the stress can be resolved into two components: $\tau_0 \cos \delta$ which is in phase with the strain and $\tau_0 \sin \delta$ which is $\pi/2$ out of phase with the strain. Therefore, two dynamic shear moduli ($G$) can be defined: $G'$ which is in phase with the strain and $G''$, which is $\pi/2$ out of phase with the strain. As $G' = (\tau_0 / \gamma_0) \cos \delta$ and $G'' = (\tau_0 / \gamma_0) \sin \delta$, the last equation becomes
\[
\tau = \gamma_0 G' \sin \omega t + \gamma_0 G'' \cos \omega t
\]
This leads to the phase angle $\delta$ being
\[
\tan \delta = G'' / G'
\]
Complex notation is often used and so the stress and strain becomes
\[
\tau = \tau_0 \exp i(\omega t + \delta),
\]
and
\[
\gamma = \gamma_0 \exp i\omega t
\]
Where $i = \sqrt{-1}$
The overall complex modulus $G^* = \tau / \gamma$ is then given by
\[
G^* = \frac{\tau_0}{\gamma_0} \exp i\delta = \frac{\tau_0}{\gamma_0} (\cos \delta + i \sin \delta)
\]
Therefore by using the earlier definitions of $G'$ and $G''$
\[
G^* = G' + iG''
\]
$G'$ and $G''$ are called the real and imaginary components of the modulus, respectively. They are also known as the storage and loss moduli.
Different methods can be used to measure the dynamic viscoelastic behaviour of materials such as oscillatory strain, wave propagation and steady flow. The oscillatory methods involve either free or forced oscillations either in tension or shear. A controlled shear stress rheometer applies a torque (forced oscillation) and measures the resultant displacement. The sample is subjected to a periodic deformation and its periodic response and phase lag (delta) are measured and recorded. From this, the phase angle, complex, elastic and viscous moduli, complex viscosity as well as shear stress and strain, are calculated. The test material can be contained either between two parallel plates, or a cone and plate. There are advantages and disadvantages with each of these methods. The parallel plate system consists of a fixed bottom plate and a rotating upper plate and the sample is placed in between. The gap between the plates can easily be set to a fixed amount. However, the shear rate produced is not constant across the sample, it varies from zero at the centre to a maximum at the edge, and so the software takes an average of this shear rate. One of the main advantages of this system is that it can be used with particulate materials. In the cone and plate system, the shear rate stays nearly constant across the sample, but if particulate materials are being tested the particles may ‘jam’ at the cone apex resulting in noisy data. It can also be difficult to set the correct gap/cone angle. As the polymer composite is a particulate material, the parallel plate system is the most appropriate to use (Nicholas et al. 2007).

3.3.10 Injectability
The injectability of the compositions were analysed in chapter 7 by examining the force needed to extrude the composite through a syringe. The test was performed using an Instron Universal testing machine and a specially made fixture consisting of a long plastic tube which supported the syringe during the injection process. Compositions were mixed by hand for 30 seconds and then transferred into a syringe. The syringe was then placed into the plastic tube and the force needed to inject the sample at a fixed rate of 25 mm/min was measured.

Two separate syringes were used, with both syringes used for Stryker products. The first syringe used is normally used for the product HydroSet and consists of a syringe with a barrel of an inner diameter of 16mm. Connected to this is a cannula with an inner diameter of approximately 2.5mm and 100mm in length. The second syringe
used in this experiment is normally used for the product Spineplex. It consisted of an inner barrel of 16mm with an opening of an inner diameter of 8mm, no cannula was attached to this syringe for this experiment.

3.3.11 Tensile bond strength

To determine the adhesive properties of several compositions of the polymer compound, the tensile bond strength with a biomedical titanium alloy was analysed in chapter 7. The titanium alloy (Ti6Al4V) was formed into two different circular disc sizes. One being a disc with a diameter of 25 mm and a thickness of 1.7 mm, which also had a hole drilled in their centre (10 mm Ø) and the other being a discs with a diameter of 20 mm and a thickness of 6.5 mm. These discs were then ground using 1200 grit silica carbide paper.

Using a layer of the polymer compound applied with a spatula, the discs were bound together and excess material was removed using a scalpel. All bound discs were placed under a mass of 1Kg to produce a sample layer of uniform thickness and the material was allowed to harden for 1 hour. Each set of discs were then placed in 25 ml of distilled water and incubated at 37 °C for 1 day before being removed and their tensile bond strength tested using the plunger apparatus in Figure 3.8. To ensure an even loading, a constant rate $1.0 \text{ mm min}^{-1}$ was applied. Maximum tensile forces

![Figure 3.7 Injectability experimental setup with injection force (F) for a displacement X (Habib et al. 2008)]
were collected using an Instron 4082 Universal testing machine and converted into bond strength using the equation below

\[
\sigma = \frac{F}{A}
\]

where \(\sigma\) is the bond strength (MPa), \(F\) is the maximum force applied (N) and \(A\) is the bonded area (mm\(^2\)).

![Figure 3.8 Tensile bond test apparatus a) schematically b) photographically](Image)

(Clarkin et al. 2009)

### 3.3.12 Cyanoacrylate monomer reactivity measurement

Thermometry was used to evaluate the reactivity of the n-butyl cyanoacrylate (BCA) monomer. Thermometry involves a simple idea in which the change of temperature of a reaction is monitored over time. A thermometer can be used for this process when the reaction itself is not rapid. When monitoring reactions that occur very fast, a more sophisticated method of temperature monitoring is required. The anionic polymerization of cyanoacrylate monomer proceeds at a very fast rate in dilute solution and complete conversion usually occurs after 2-3 seconds, and an electronic thermocouple with a data acquisition system is required to measure the reaction. A PTFE insulated K-type thermocouple was used for the thermometric experiment.
carried out for this study. The thermocouple was then connected to a PC operating with data acquisition software and data was taken at a rate of 1 data point every 0.1 seconds and the temperature rise was plotted as a function of time.

The anionic polymerization of cyanoacrylate monomer proceeds at room temperature and so the Gibbs free energy for polymerization must be considered to have a negative value (-\(\Delta G\)). Chain reaction polymerizations will tend to reduce the translational energy of the monomer on addition to the growing chain and thus reduce the entropy of the system. This process is entropically unfavourable and so a certain amount of monomer unzipping will take place. If a mechanism is present to remove the heat evolved during polymerization (such as a solvent in this case) the reaction will proceed to completion. The second law of thermodynamics states that if the reaction temperature is raised so that \(\Delta H = T\Delta S\) then \(\Delta G = 0\). Thermodynamic equilibrium is therefore established between monomer addition and unzipping and the polymerization rate is equal to the depolymerization rate. The temperature at which this occurs is known as the ceiling temperature, above which depolymerization predominates (Joshi and Zwolinski 1967). The net heat evolved in the system over the period of the reaction, below the ceiling temperature is proportional to the monomer conversion. The fractional conversion at any point in time is read directly from the thermal profile of the reaction thermogram.

The reactivity a number of cyanoacrylate monomer samples were tested by monitoring the change in temperature of the anionic polymerization of each of the samples. Each reaction mixture consisted of \(2.6 \times 10^{-1}\) solution of butyl cyanoacrylate monomer in Tetrahydrofuran (THF) and the polymerization initiator used was tri-phenyl-phosphine (TPP) solution in THF. Each reaction was carried out by addition of 25mls of the reaction mixture to a 50ml insulated glass reaction vessel. A thermocouple was sealed in place and a stirring was set at a constant speed using a magnetic stirrer. The data acquisition was started and the baseline was monitored for 20 minutes to ensure thermal equilibrium had been reached. The reaction was then initiated by a rapid injection of 1ml of the TPP solution into the stirring reaction mixture using a disposable syringe.
3.4 References


Chapter 4
4.0 Plasticization of Poly DL Lactide

4.1 Introduction

The overall aim of this project is to develop a resorbable and mouldable bone filler that can be used to repair bone damaged through injury or disease. This part of the project concerns itself with developing a mouldable compound using the resorbable polymer polylactide. Therefore, the initial step was to blend the polymer with a plasticizer to create this bone filler base compound. As defined by BS 6324-3 “Terms relating to surgical implants”, a plasticizer is a substance incorporated into a plastic material to lower its softening range and to increase its workability, flexibility or extensibility. The addition of a plasticizer is expected to lower the elastic modulus and the Tg of a polymer material while improving processability.

As discussed previously in Chapter 2, there are several theories which have been developed to explain the mechanisms of plasticization, with the most accepted concepts being the lubricity theory, the gel theory and the free volume theory. Each theory used by itself does not fully explain the mechanisms of plasticization, but used together they give an approximate picture of the fundamental principles and explain most aspects of the behaviour of plasticized polymers. Also discussed in chapter 2 are the important considerations when choosing a plasticizer for PLA for biomedical applications. It should be a non-toxic substance miscible with PLA, thus creating a homogeneous blend. Also, the plasticizer should not be prone to migration as it would cause the material to regain the brittleness of pure PLA (Urayama et al. 2003). Previous studies conducted by Labrecque et al. and Ren et al have found that citrate esters are appropriate plasticizers for PLA with good miscibility due to polar interactions between their ester groups. Citrate esters are considered as appropriate plasticizers for polylactide for biomedical applications due to their low order of toxicity, good metabolism and elimination from the body (Guiot and Kennedy 2001). They are approved for use as additives in food, personal care products and in medical plastics by many regulatory agencies worldwide (Gutierrez-Villarreal and Rodríguez-Velazquez 2007).
Poly (D,L lactide) (PDLLA) was blended with three different citrate esters; triethyl citrate (TEC), tributyl citrate (TBC) and acetyl tributyl citrate (ATBC) and the resultant thermal, dynamic mechanical and tensile properties were analysed. The results found experimentally by DSC are also compared with theoretical results calculated for the various plasticized polymer compositions.

### 4.2 Materials and Methods

#### 4.2.1 Blending

The PDLLA investigated in this report supplied by Boehringer Ingelheim (Germany) had a weight average molecular weight of $1.865 \times 10^5$ Da as determined by gel permeation chromatography performed by the University of Manchester. Plasticizers triethyl citrate (TEC), tributyl citrate (TBC) and acetyl tributyl citrate (ATBC) supplied by Sigma Aldrich (Ireland) had molecular weights of 276, 360 and 402 respectively as reported by the supplier. Sample compositions consisted of PDLLA combined with 10 to 30 wt. % plasticizer. All samples were prepared by heating to approximately 150°C, mixed manually with a spatula and transferred to hot moulds (~150°C) to be pressed. With increased plasticizer content, sample compositions became noticeably more malleable and flexible with hand mouldable characteristics increased.

#### 4.2.2 DSC

Differential Scanning Calorimetry (DSC) was performed using a Perkin-Elmer Pyris 1 DSC. Plasticized samples (5mg-6mg) were heated from -60°C to 100°C at a rate of 10°C per minute in a nitrogen atmosphere. PDLLA samples containing all three plasticisers ranging from 10 to 30 wt. % content were analysed. Three samples of each composition were tested. DSC was also performed on each plasticizer within a temperature range of -150°C to 50°C in a helium atmosphere. The glass transition temperature (Tg) was taken as the midpoint of the specific heat increment at the glass transition.

#### 4.2.3 DMTA

DMTA scans were carried out on PDLLA samples containing only the plasticizer TBC using a Rheometric scientific mark 3 DMTA. Analysis was carried out on
specimens measuring 10mm x 4mm x 2.0 mm in tensile mode with a temperature profile ranging from -60 to 120°C at a 2°C per minute heating rate with a frequency of 1Hz. One sample of each composition was tested. Tg was taken as the peak in the tan δ curve accompanied by a step reduction in the storage modulus.

<table>
<thead>
<tr>
<th>Material</th>
<th>Structure</th>
<th>Solubility parameter δ(J cm$^{-3}$)$^{1/2}$</th>
<th>Solubility in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDLLA</td>
<td><img src="image" alt="PDLLA Structure" /></td>
<td>20.1$^a$</td>
<td>-----</td>
</tr>
<tr>
<td>Triethyl citrate (TEC)</td>
<td><img src="image" alt="Triethyl Citrate" /></td>
<td>19.7$^a$</td>
<td>65g/L (25°C)</td>
</tr>
<tr>
<td>Tributyl citrate (TBC)</td>
<td><img src="image" alt="Tributyl Citrate" /></td>
<td>19.6$^a$</td>
<td>insoluble</td>
</tr>
<tr>
<td>Acetyl tributyl citrate (ATBC)</td>
<td><img src="image" alt="Acetyl Tributyl Citrate" /></td>
<td>18.0$^a$</td>
<td>5mg/L (20°C)</td>
</tr>
</tbody>
</table>

$^a$ Ref. (Ljungberg). Calculated with group molar attraction constants from the Hoy series

4.2.4 Tensile testing
Tensile tests were preformed using an Instron 4301 series tensile testing machine. Specimens were dogbone shaped with a gauge length of approximately 13mm, a width of 3.5mm and thickness of 3mm prepared after ASTM standard D638-08. All samples were tested with the same crosshead speed of 10mm/min using a 1kN load cell. Tensile testing was performed on PDLLA samples containing 10, 20 and 30 wt. % of all three plasticizers. Six samples of each composition were tested.
4.2.5 Migration study

Unplasticized PDLLA and PDLLA samples containing all three plasticisers ranging from 10 to 30 wt. % content were prepared for a plasticizer migration study after standard BS EN ISO 177:2000 “Plastics-Determination of migration of plasticizers”. Samples measuring 25.5mm x 3.35mm x 3mm were placed between two sheets capable of absorbing plasticizers. The test was run using cellulose based absorbent sheets of both paper and woven fabric. Weights were positioned on each sample and placed in a 37°C oven. The loss in mass of the test specimen was calculated as a measure of the migration of the plasticizer over a period of 30 days. Three samples of each composition were tested.

4.3 Results and Discussion

4.3.1 DSC

Figures 4.1 to 4.3 illustrate the heat cycle DSC thermoscans for PDLLA and the polymer plasticized with different quantities of each plasticizer. The resultant Tg for all samples analysed is listed in Table 4.2. As expected, by increasing plasticizer content a decrease in Tg occurs which is true for all three plasticizers analysed. The low molecular size of the plasticizer allows them to occupy intermolecular spaces between polymer chains, reducing the energy for molecular motion and the formation of hydrogen bonding between the polymer chains which in turn increases free volume and molecular mobility (Vieira et al. 2011).

Figure 4.1 DSC thermographs of neat PDLLA and PDLLA samples plasticized with 10 wt. % citrate ester
Figure 4.2 DSC thermographs of neat PDLLA and PDLLA samples plasticized with 20 wt. % citrate ester

Figure 4.3 DSC thermographs of neat PDLLA and PDLLA samples plasticized with 30 wt. % citrate ester
Table 4.2 Glass transition temperatures for various blends of polymer and plasticizer found experimentally and theoretically

<table>
<thead>
<tr>
<th>Plasticizer wt. %</th>
<th>TEC</th>
<th>TBC</th>
<th>ATBC</th>
<th>TEC</th>
<th>TBC</th>
<th>ATBC</th>
<th>TBC</th>
</tr>
</thead>
<tbody>
<tr>
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Figure 4.4 Experimental Tg as a function of plasticizer content obtained by DSC
Figure 4.4 illustrates the effect all three plasticizers have on the Tg of the PDLLA as a function of plasticizer content analysed by DSC in this study. The magnitude of the Tg change in the plasticized polymer is a good indicator for the plasticization efficiency of the plasticizer compound (Tarvainen et al. 2001). The most effective plasticizer to decrease Tg was generally ATBC, followed by TBC and lastly TEC. By increasing the molecular weight of the plasticizer, the effectiveness of the citrate plasticizer to reduce the Tg of the PDLLA is generally enhanced.

TEC the least effective plasticizer, only differs structurally from TBC in the number of carbons in its ester chain; TEC containing two carbons while TBC having four (see Table 4.1). Both plasticizers have three ester functionalities, potentially acting as a hydrogen bond acceptor sites and a tertiary hydroxyl group which has both hydrogen bond donating and accepting character (Fadda et al. 2007). Interactions may occur between the hydroxyl groups of TEC/TBC and the hydrogen bond acceptor ester groups of PDLLA. TBC is most likely more effective as a plasticizer due to its larger non polar aliphatic segments. These segments shield dipoles on the PDLLA polymer chains from interacting with adjacent polymer chains, pushing them apart and increasing mobility, thus reducing the Tg more effectively than TEC.

ATBC and TBC structurally differ only by the acetylation of the tertiary hydroxyl group in ATBC. ATBC is exclusively a hydrogen bond acceptor, while TBC having a hydroxyl group, has both hydrogen bond donating and accepting character and has potential to form strong hydrogen bonds with the ester groups of PDLLA. These strong bonds between TBC and the polymer may result in a decrease in polymer chain mobility and flexibility (Tarvainen et al. 2001), causing PDLLA plasticized with TBC to have a higher Tg than PDLLA plasticized with ATBC. ATBC having an extra acetyl group also has an increased associated free volume; the acetyl group may also further shield dipoles on the PDLLA chains, further pushing the polymer chains apart and increasing mobility.

These results are similar to that of Labrecque et al. (1997) who reported that with increasing citrate plasticizer content, the Tg of semicrystalline PLA decreased. They also found that the lower the molecular weight of the plasticizer, the less efficient it was at lowering the Tg of the PLA. The DSC results found experimentally by DSC
were lower than those found by Labrecque et al., this could be due to sample preparation, differences in DSC analysis or differences in the temperature taken as the Tg quoted.

Only one glass transition was noted for each sample of PDLLA blended with TEC, which indicates that this plasticizer was miscible with the polymer for all compositions. While a second slight endothermal shift in the specific heat occurring at the glass transition temperature of PDLLA, was noted to occur in samples containing 20% ATBC, 30% ATBC and 30% TBC. The miscibility of plasticizers and polymers can be estimated by comparing their solubility parameters. The solubility parameters for PDLLA and all three plasticizers is quite similar indicating good compatibility (see Table 4.1). However, the difference between their solubility parameters increases with increasing molecular weight of the plasticizer, resulting in a decrease in solubility and compatibility which may explain the presence of two Tgs in compositions with higher molecular weight plasticizer contents. The lack of miscibility between PDLLA and ATBC at higher concentrations can be seen clearly in Figure 4.4. The Tg of PDLLA plasticized by 20% ATBC is 5.7°C, with the addition of a further 10% ATBC the Tg only decreases slightly to 4.4°C. This differs with work completed by Baiardo et al. who reported to that the solubility limit of ATBC in PLA was 50 wt. %.

4.3.2 DMTA
Figures 4.5 and 4.6 show the tan delta and storage modulus curves obtained from dynamic mechanical analysis of PDLLA plasticised with 10 to 30 wt. % TBC. Table 4.2 indicates the glass transition temperatures for each sample. With increasing plasticizer content a decrease in Tg is obtained and the height of the tan delta peak also decreases and broadens.

As seen in Figure 4.6, at temperatures above the Tg, the storage modulus of each sample drops dramatically due to the softening of the polymer specimens. With increasing plasticizer content there is a decrease in storage modulus values below Tg as the plasticizer continues to increase the mobility of the polymer chains. These results are similar to those obtained by Ren et al. (2006) who plasticized PLA with low molecular weight triacetin and oligomeric poly(1,3-butylene glycol adipate).
Increasing plasticizer content decreased the peak of the tan delta curve while also making them shorter and broader. The storage modulus values were also similar and a decrease could be seen with increasing plasticizer content (Ren et al. 2006).

Figure 4.5 Tan delta curves obtained by DMTA for PDLLA and PDLLA samples plasticized with TBC

Figure 4.6 Storage Modulus curves obtained by DMTA for PDLLA and PDLLA samples plasticized with TBC
4.3.3 Theoretical Tg

There are several equations reported in literature which enable the glass transition temperature of copolymers and polymer blends to be expressed as a function of their composition. Equation (1) was proposed by Fox for the Tg dependence of a binary system:

$$\frac{1}{T_g} = \omega_2 \frac{1}{T_{g_2}} + \omega_1 \frac{1}{T_{g_1}}$$

Where subscripts 1 and 2 refer to plasticizer and polymer respectively and \(\omega\) is the weight fraction (Brostow et al. 2008). This equation was applied to the various compositions of PDLLA with each plasticizer using the Tg obtained for PDLLA and each citrate esters by DSC. Calculated theoretical Tgs are listed in Table 4.2.

![Figure 4.7 Theoretical Tg as a function of plasticizer content](image)

4.3.4 Tg of samples - DMTA vs. DSC vs. Theoretical

Table 4.2 lists the Tg values for PDLLA plasticized with TBC obtained by DMTA and DSC. Since the glass transition is a kinetic process it is affected by the rate at which the sample is heated and different experimental techniques lead to different Tg
values (Scheirs 2000). This is due to different time responses in the motions of side or main chain polar groups and mechanical or thermal stimulation of the motion (Brostow et al. 2008).

The Tg values for PDLLA plasticized with TBC obtained by DMTA are higher than that obtained by DSC. According to Alverous et al., differences in the temperature corresponding to transitions observed by DMTA and DSC are attributed to the frequency of the analysis method (Averous et al. 2000). A clear indication of the glass transition temperature range is more easily observed through DMTA and this result may suggest that the segmental motion of the plasticized PDLLA has only been partially relaxed at the Tg as indicated by DSC.

Figures 4.8 to 4.10 compare the theoretical results calculated for plasticized PDLLA with those found experimentally by DSC. The theoretical results are in good agreement with those obtained experimentally for TEC and TBC at compositions which seemed to be miscible. At a composition of 30% TBC, which showed two separate Tgs, the experimental result was slightly higher than that of the theoretical result calculated most likely due to lack of homogeneity in the sample tested. The results obtained experimentally for ATBC are lower than the theoretical results at compositions of 10 and 20 wt. % ATBC.
He et al. report that the differences between experimental and predicted Tg values is sometimes considered as a measure of the strength of interactions between the blend components (He et al. 2004). Glass transition temperatures found experimentally which are lower than those predicted may be attributed to weak specific interactions between the blends (Lu and Weiss 1992). At a composition of 30% ATBC, the
experimental result was higher than that of the theoretical result calculated which may also be due to lack of homogeneity in the sample.

4.3.5 Tensile testing

Figures 4.11 to 4.13 illustrate the stress strain curves of PDLLA samples un-plasticized and plasticized by the three different citrate esters. Un-plasticized PDLLA is quite brittle as seen in figure 4.11 and fractured without yielding. The 10% TEC plasticized sample was less brittle than pure PDLLA and had some necking, while 10% TBC and ATBC plasticized samples were more ductile than the 10% TEC sample with necking and further extension occurring at lower stress, occurring more so in the TBC plasticized sample than the ATBC plasticized sample.

Figures 4.12 and 4.13 show that with the increase in plasticizer content there is a general increase in ductility and decrease in strength. Percentage elongation at break increases at the expense of Young’s modulus and tensile strength. At compositions above 10 wt. % plasticizer both the stiffness and strength of the samples drop dramatically and at compositions of 30 wt. % plasticizer they are very low. These results are similar to those reported by Labrecque et al. (1997) who found that the addition of 10% citrate esters to PLA decreased tensile strength by approximately 50% with the deterioration larger at higher concentrations of plasticizer.
Figure 4.12 Stress strain curves of PDLLA samples plasticized with 20 wt. % citrate ester

Figure 4.13 Stress strain curves of PDLLA samples plasticized with 30 wt. % citrate ester
Figures 4.14 to 4.16 show the average Young’s modulus, tensile strength and percentage elongation at break for unplasticized and plasticized PDLLA as a function of plasticizer content with the average results for all samples tested listed in Table 4.3.

At 10 wt. % plasticizer content, samples plasticized with TEC had the greatest stiffness and strength followed by ATBC plasticized samples and lastly TBC plasticized samples. TBC plasticized samples also had the greatest elongation at this plasticizer concentration. These results do not fully correlate with the experiment Tg measurements, as it would be expected that the samples containing 10% ATBC which had the lowest Tg would also have the lowest stiffness and strength and the greatest elongation. However the results do correlate with the theoretical Tgs calculated, which predicted samples plasticised with 10 wt. % TBC to have the greatest chain mobility at that plasticizer content (see figure 4.7).

As already stated, the Tg found experimentally for PDLLA plasticized with ATBC was lower than those predicted which may be attributed to weak specific interactions between ATBC and PDLLA. This may have resulted from the migration of the plasticizer ATBC causing the material to regain some of the rigidity of pure PDLLA. TEC plasticized samples displayed the greatest elongation as the weight percentage content of plasticizer increased to 20 and 30% while TBC and ATBC were not as effective. The larger non polar aliphatic segments of ATBC and TBC may have shielded dipoles on the PDLLA polymer chains from interacting with adjacent polymer chains and this disruption to the polymer system may have caused a decrease in elongation compared with samples plasticized by TEC.
Figure 4.14 Young’s modulus of neat PDLLA and samples plasticized with different citrate esters as a function of plasticizer content

Figure 4.15 Tensile strength of neat PDLLA and samples plasticized with different citrate esters as a function of plasticizer content
Figure 4.16 Percentage elongation of neat PDLLA and samples plasticized with different citrate esters as a function of plasticizer content

Table 4.3 Tensile testing results

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<th>Tensile strength (MPa)</th>
<th>Std dev (+/-)</th>
<th>Elongation to break (%)</th>
<th>Std dev (+/-)</th>
<th>Young’s Modulus (MPa)</th>
<th>Std dev (+/-)</th>
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4.3.6 Migration study

Loss of plasticizer could not be accurately measured by the method described in section 4.2.5. This was due to plasticized polymer sticking to the sheets used to absorb the migrated plasticizer giving inaccurate results. The experiment was run using cellulose based sheets of both paper and woven fabric, however in both cases adhesion between the sheets and the polymer was such as to render gravimetric analysis impossible. This adhesion between the polymer and the sheets would suggest that migration of the plasticizer did occur, however quantification of the extent of migration was not possible.

4.4 Conclusion

Blending PDLLA with citrate esters results in sample compositions which are malleable and flexible, with increasing plasticizer content having an increase effect on the hand mouldable characteristics. Plasticizing PDLLA results in a decrease in Tg with increasing citrate ester plasticizer content a further decrease in Tg occurs. By increasing the molecular weight of the plasticizer, their effectiveness to reduce the Tg of the PDLLA is generally enhanced but miscibility is decreased and two Tgs occurred in samples containing higher concentrations of higher molecular weight plasticizer.

DMTA of TBC plasticised PDLLA indicates a decrease in Tg is obtained with increasing plasticizer content and a decrease in storage modulus values below Tg. The Tg values obtained by DMTA are higher than that obtained by DSC, which is attributed to the frequency of the analysis method. Theoretical Tg results for plasticized PDLLA are in good agreement with those obtained experimentally for TEC and TBC at compositions which were miscible. Weak specific interactions between ATBC and PDLLA may account for differences in experimentally and theoretical Tgs calculated. As expected with the addition of a plasticizer there is a general increase in percentage elongation at the expense of Young’s modulus and tensile strength. The addition of 10% citrate esters to PDLLA decreases Young’s modulus and nearly halves the tensile strength, with the deterioration larger at higher concentrations of plasticizer.
4.5 References


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Chapter 5
5.0 Crosslinking of Plasticized Poly DL Lactide

5.1 Introduction
This part of the project concerns itself with the development of a method for the mouldable bone filler to harden in-situ with the appropriate load bearing properties. After initially blending the polymer with a plasticizer to create the mouldable bone filler base compound, a method of improving its load bearing properties in situ needed to be devised. An investigation into crosslinking the polymer was carried out with the view that upon injection, the polymer could harden by this method without detrimental effects to the surrounding tissue, maintain mechanical and physical integrity and facilitate cell attachment and growth.

As previously discussed in chapter 2, crosslinking is the setting up of chemical links between the molecular chains of a polymer having a significant effect on the properties of the material (Rosato et al. 2000). Crosslinking can be introduced into an assembly of polymer molecules either as the polymerisation takes place or as a separate step after the initial macromolecule has been formed (Nicholson 2011). It involves the formation of covalent bonds between the chains to produce a three dimensional network structure (Gowariker et al. 2005). As the crosslinks prevent free chain movement, crosslinked polymers are generally stronger and less flexible than their linear forms. With a greater degree of crosslinking there is a greater rigidity of the material and the less soluble it is (Rosato et al. 2000).

Crosslinking can occur through the application of heat, mechanically, through exposure to radiation or active chemical agents or through any combination of these (Carraher and Seymour 2007). Crosslinking agents can be used to initiate crosslinking reactions and peroxides can be very useful active site generators, abstracting protons from polymer chains which help to initiate crosslinking (Brydson 2000). Also previously discussed in Chapter 2 were studies which have reported the chemical crosslinking of polylactide with peroxides (Nijenhuis et al. 1996; Takamura et al. 2008). Crosslinked structures of polylactide can also be formed by irradiation. Quynh et al. reported the crosslinking of PLA in the presence of crosslinking agent triallyl isocyanurate by gamma irradiation (Quynh et al. 2007). Other means of crosslinking
such as photocrosslinking could be a possible method of hardening the polymer in situ, but there are very few studies on the photocrosslinking of polylactides to date.

In this study poly (D,L lactide) (PDLLA) was blended with peroxides and crosslinking agent triallyl isocyanurate and the effects of heat and gamma irradiation were analysed by conducting swelling studies to examine resultant crosslink densities. An investigation into chemically crosslinking the copolymer polylactide-co-caprolactone was also conducted. This was carried out to see if a higher crosslinked density could be achieved by crosslinking the copolymer, as it has a larger quantity of sites which are susceptible to crosslinking. DMTA was carried out on PDLLA samples crosslinked by the crosslinking agents and heat. Samples containing photoinitiator Irgacure 369 and crosslinking agents were prepared and UV cured in an attempt to photocrosslink the poly (D,L lactide). Crosslink densities of these samples were examined and tensile testing was carried out.

Only tributyl citrate (TBC) was used as a plasticizer for the samples analysed in this chapter. From chapter 4 it was shown that the effectiveness of the citrate esters to plasticizers increased with increasing molecular weight, however their tendency to migrate also increased with increasing molecular weight. TBC was chosen as the appropriate plasticizer as it is the citrate ester with the intermediate molecular weight of the plasticizers tested. By using TBC, the effectiveness to plasticize the polymer was greater than the lower molecular weight triethyl citrate and its tendency to migrate is lower than that of the higher molecular weight plasticizer acetyl tributyl citrate.

5.2 Materials and Methods

5.2.1 Blending procedure for chemical crosslinking

PDLLA was supplied by Boehringer Ingelheim (Germany). Tributyl citrate (TBC), triallyl isocyanurate (TAIC), dicumyl peroxide (DCP), benzyol peroxide (BPO), 2-benzyl -2-(dimethylamino)-4-morpholinobutyrophenone (Irgacure 369) and chloroform (CHCl$_3$) were supplied by Sigma Aldrich (Ireland). As the polymer is very expensive and only available in small quantities, it was not possible to blend the polymer, plasticizer and crosslinking agents by conventional methods. The methods
for preparing the polymer are described in chapter 3, however they are explained here also as polymer preparation had a great effect on the outcome of the crosslinked polymer. PDLLA granules were first ground into smaller particles using a coffee grinder. Approximately 10g of polymer was ground for three minutes to give particles sizes of approximately 800µm as indicated in Figure 5.1 below.

![Figure 5.1 Microscopic view (X5) of PDLLA particles after grinding for 3 minutes](image)

Various concentrations of plasticizer TBC and crosslinking agents TAIC and DCP were added and the samples were mixed and heated. It was identified that the blending procedure had to be altered to ensure a more uniformed crosslinked mixture was obtained and so, two different blending procedures were tested as described below.

**Method 1**

- PDLLA granules were first ground into smaller particles using a coffee grinder. Approximately 10g of polymer was ground for 3 minutes to produce particles of irregular shape with an average size of 800µm as indicated in figure 5.1.
- 100g of ground PDLLA granules were then mixed in a Brevell food mixer with 43mls of TBC plasticizer at a low speed for 30 minutes to produce a 70% polymer, 30% plasticizer composition.
- The mixture was then halved and heated in a beaker to 140°C for 15 minutes while being continuously stirred. This was then repeated with remaining mixture.
- The polymer/plasticizer mixture was then placed in containers and left to cool in a desiccator.
Once cooled, the mixture was then weighted into 3g samples and the appropriate volume of TAIC and TBC was added to each sample. This was then heated to 150°C (or as indicated) for 2 minutes while being continuously stirred.

The appropriate amount of DCP was then added and mixture was heated and stirred for a further 30 seconds.

The mixture was then placed into an air tight container and left in desiccator to cool.

Table 5.1 lists the concentrations of the various samples produced by this method.

Method 2

PDLLA granules were first ground into smaller particles using a coffee grinder. Approximately 10g of polymer was ground for 1 minute.

The ground polymer was then sieved through a stainless steel sieve with a mesh size of 200µm. Polymer which could not go through the sieve was reground for 1 minute and re-sieved.

The above step was repeated numerous times until all the polymer was reduced to a fine powder of particles sizes of approximately 200µm or less as indicated in figure 5.2 below.

Figure 5.2 Microscopic view (X5) of PDLLA particles after grinding and sieving

- DCP crystals were finely ground using a mortar and pestle.
- 4mls of TAIC was placed in a container and left in a freezer for 2 hours which transformed the liquid into a powder.
- Required amounts of each powder; PDLLA, DCP and TAIC were accurately weighed and placed into containers which were then agitated and mixed thoroughly
before appropriate volumes of plasticizer were added. The samples were again mixed thoroughly.

- Each sample was heated for 2 minutes 40 seconds to 3 minutes until it reached 150°C, at which point it was mixed thoroughly and left at that heat for a further 30 seconds.
- The mixture was then placed into an air tight container and left in desiccator to cool.

<table>
<thead>
<tr>
<th>Table 5.1 Composition of Samples prepared by Method 1 and 2</th>
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5.2.2 Swelling studies and crosslink density

Uncrosslinked PDLLA will dissolve in chloroform while crosslinked PDLLA will not. By weighting the sample before and after soaking, gel fractions and degree of swelling were calculated. A high gel fraction and a low degree of swelling indicate a high crosslink density.

Three 0.05g samples of each blend were transferred to separate glass vials (labelled A, B and C) 15ml of chloroform was then added. The vials were sealed and left at room temperature for 24 hours. The mixture was then filtered and the remaining swollen gel was weighed. The gel was then left in a desiccator for 48 hours and reweighed. Gel fraction was measured by the amount of insoluble material in chloroform using the following equation:
Chapter 5

Gel Fraction (%) = \( \frac{W_g}{W_o} \times 100 \) \hspace{1cm} (1)

Where \( W_o \) is the weight (dry) of the crosslinked PDLLA and \( W_g \) is the remaining weight (dry gel component) of the crosslinked PDLLA after dissolving in chloroform for 24 hours.

Degree of swelling (volume ratio of absorbed solvent to dry gel) was calculated using the following equation:

\[
\text{Degree of Swelling} = \frac{\left( W_s - W_g \right)}{W_g} \left( \frac{\rho_p}{\rho_{\text{CHCl}_3}} \right) \hspace{1cm} (2)
\]

Where \( W_g \) is the dry gel component of the crosslinked PDLLA and \( W_s \) is the weight of the swollen gel component. \( \rho_p \) and \( \rho_{\text{CHCl}_3} \) are the densities of PDLLA/plasticizer blend and chloroform respectively.

5.2.3 Swelling studies of chemically crosslinked PDLLA by application of heat

A series of PDLLA samples were blended with different concentrations of plasticizer TBC and crosslinking agents TAIC, DCP and/or BPO prepared by method 2 as described in section 2.1. Samples were blended at three different temperatures and gel fractions and degree of swelling were calculated. Samples prepared are listed in tables 2 to 5 below. Three samples of each composition were analyzed, with three specimens taken from each sample.

| Table 5.2 Composition of samples prepared at lower temperatures |
|-------------------|----------------|-------------|---------|--------|
| PDLLA (wt. %)     | TBC (wt. %)   | DCP (wt. %) | TAIC (wt. %) | Temp (°C) |
| 60                | 30            | 5           | 5        | 50     |
| 60                | 30            | 5           | 5        | 75     |
| 60                | 30            | 5           | 5        | 100    |

| Table 5.3 Composition of samples prepared with BPO |
|-------------------|----------------|-------------|---------|--------|
| PDLLA (wt. %)     | TBC (wt. %)   | BPO (wt. %) | TAIC (wt. %) | Temp (°C) |
| 60                | 30            | 5           | 5        | 50     |
| 60                | 30            | 5           | 5        | 75     |
| 60                | 30            | 5           | 5        | 100    |
### Table 5.4 Composition of Samples prepared at 150°C

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<th>PDLLA (wt. %)</th>
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<th>DCP (wt. %)</th>
<th>TAIC (wt. %)</th>
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### Table 5.5 Composition of Samples prepared at 150°C

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<th>TAIC (wt. %)</th>
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5.2.4 Chemical crosslinking of PDLLA by Gamma Irradiation

PDLLA samples containing TBC, DCP and TAIC were prepared and mixed as described using method 2 blending procedure in section 5.2.1. However, these samples were not heated but placed in moulds to be pressed and left over night. The samples were removed from the moulds and sent to Isotron for gamma irradiation at 30kGy. A set of samples was also prepared using BPO instead of DCP. The gel fraction and degree of swelling was calculated for each sample irradiated. Tables 5.6 to 5.8 list the compositions tested. One sample of each composition was analysed, with three specimens taken from each sample.

### Table 5.6 Composition of samples gamma irradiated

<table>
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<tr>
<th>PDLLA (wt. %)</th>
<th>TBC (wt. %)</th>
<th>DCP (wt. %)</th>
<th>TAIC (wt. %)</th>
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### Table 5.7 Composition of samples gamma irradiated

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Table 5.8 Composition of samples gamma irradiated

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<th>PDLLA (wt. %)</th>
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5.2.5 Swelling studies of chemically crosslinked Polylactide-co-caprolactone by application of heat

Polylactide-co-caprolactone (PLCL) with a ratio of 70% polylactide to 30% caprolactone, supplied by Pharma Polymers (Germany) was blended with various concentrations of plasticizer TBC and crosslinking agents TAIC and DCP. Samples were heated to 150°C with a selection of compositions also prepared at temperatures of 50°C and 100°C. The gel fraction and degree of swelling was calculated for each sample. Tables 5.9 to 5.11 list the compositions tested. Three samples of each composition were analysed, with three specimens taken from each sample.

Table 5.9 Composition of crosslinked copolymer samples prepared at 150°C

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Table 5.10 Composition of crosslinked copolymer samples prepared at lower temperatures

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Table 5.11 Composition of crosslinked copolymer samples prepared at 150°C

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<th>TAIC (wt. %)</th>
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5.2.6 DMTA of chemically crosslinked PDLLA

DMTA scans were carried out on PDLLA samples containing TBC, DCP and TAIC prepared using method 2 blending procedure described in section 5.2.1. Scans were also carried out on pure PDLLA and plasticized PDLLA samples which were not crosslinked. Analysis was carried out using a Rheometric scientific mark 3 DMTA on specimens measuring 10mm x 4mm x 2.0 mm in tensile mode with a temperature profile ranging from -60 to 100°C at a 2°C per minute heating rate and a frequency of 1Hz. Tg was taken as the peak in the tan δ curve accompanied by a step reduction in the storage modulus. Table 5.12 lists the compositions tested. One sample of each composition was analysed.

Table 5.12 Composition of Samples prepared for DMTA

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<tr>
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5.2.7 Photocrosslinking

PDLLA samples containing photoinitiator Irgacure 369, TBC, DCP and TAIC were prepared and mixed as described using method 2 blending procedure in section 5.2.1. These samples were not heated but placed in moulds to be pressed and left overnight. The samples were removed from the moulds and placed in a UV box for 20 minutes. The light source had a wavelength of 340nm and an intensity range of 10 to 13.5 mW/cm². A sample that was plasticized with 30% TBC was prepared by the same method, but was not irradiated. The gel fraction and degree of swelling was calculated for each sample. Table 5.13 lists the compositions tested. One sample of each composition was analysed with three specimens from each sample tested.

Table 5.13 Composition of Samples prepared for UV crosslinking

<table>
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<tr>
<th>PDLLA (wt. %)</th>
<th>TBC (wt. %)</th>
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<th>TAIC (wt. %)</th>
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5.2.8 Tensile testing of UV irradiated samples

Tensile tests were performed using an Instron 4301 series tensile testing machine. Specimens were dogbone shaped with a gauge length of approximately 13mm, a width of 3.5mm and thickness of 3mm prepared after ASTM standard D638-08. All samples were tested with the same crosshead speed of 10mm/min using a 1kN load cell. Tensile tests were performed on UV cured samples as listed in table 5.13 and on the sample which was plasticized with 30% TBC and prepared by the same method, but not irradiated. One sample of each composition was analysed.
5.3 Results and Discussion

5.3.1 Blending procedure for chemical crosslinking and swelling studies

A series of PDLLA samples were blended with different concentrations of plasticizer TBC and crosslinking agents TAIC and/or DCP. According to Takamura et al. peroxide induced crosslinking follows three key steps:

1) The generation of primary radicals derived from thermal decomposition of peroxides
2) Hydrogen abstraction from polymer chains by primary radicals to generate polymer radicals
3) The bimolecular recombination of polymer radicals to form carbon-carbon crosslinks. (Takamura et al. 2008)

Using peroxides and crosslinking agent TAIC, Yang et al. proposed a possible reaction scheme for the crosslinking of PLA as seen in figure 5.3

Figure 5.3 Possible reaction scheme of the crosslinking of TAIC and PLA (Yang et al. 2008)
Each sample was then soaked in chloroform for 24 hours to confirm if it had successfully crosslinked. Uncrosslinked PDLLA will dissolve in chloroform while crosslinked PDLLA will not. By weighting the sample before and after soaking, gel fractions and degree of swelling were calculated.

From initial studies on the different crosslinked systems, it was identified that the blending procedure had to be altered to ensure a more uniformed crosslinked mixture was obtained as the swelling studies produced samples that sometimes didn’t crosslink or samples that did not have reproducible crosslink densities. And so, two different blending procedures were tested as described in sections 5.2.1.

Method 1 procedure produced a large bulk supply of polymer/plasticizer system, from which smaller samples were taken and crosslinking agents were then added. Method 2 describes a process where the polymer, crosslinker and peroxide were transformed into powder form and mixed before the addition of the plasticizer and the application of heat. It was hoped that by increasing the surface area of the polymer particles by having a smaller particle size and by having the crosslinking agents and polymer in powder form, a more uniformed crosslinking structure with higher crosslink densities could be achieved by mixing the constituents thoroughly before heating.

Figures 5.4 and 5.5 show the gel fraction for samples prepared by method 1 and method 2. The data is represented in bar chart form to clearly illustrate the difference in crosslinking for samples prepared by the two different methods. All of the samples prepared by method 2 crosslinked and had higher gel fractions with lower standard deviations then that of samples prepared by method 1. This indicates that method 2 is a more appropriate form of blending as these samples had a higher crosslink density and were more uniformly crosslinked. This method of blending was used for all of the following swelling studies, unless otherwise stated.
Figure 5.4 Gel Fraction of Samples containing 2 wt. % DCP, 30 wt. % TBC and different concentrations of TAIC prepared using Method 1 and Method 2

Figure 5.5 Gel Fraction of Samples containing 4 wt. % DCP, 30 wt. % TBC and different concentrations of TAIC prepared using Method 1 and Method 2
5.3.2 Swelling studies of chemically crosslinked PDLLA by application of heat

A series of PDLLA samples were blended with different concentrations of plasticizer TBC and crosslinking agents TAIC, DCP and/or BPO prepared by method 2 as described in section 5.2.1. Samples were blended at three different temperatures and gel fractions and degree of swelling were calculated.

All samples prepared at 50°C and 100°C using TAIC and/or DCP as crosslinking agents and samples prepared at 150°C using only TAIC as a crosslinking agent dissolved completely in chloroform which indicated that they did not crosslink. This suggests that the crosslinking process is dependent not only on DCP, but also the generation of primary radicals with the decomposition of DCP requires temperatures higher than 100°C. DCP has been shown to be an effective crosslinking agent for a great number of polymers and of the peroxides used to crosslink polylactide as investigated by Takamura et al., it had a high hydrogen abstraction ability with one of the slowest decomposition rates, ensuring uniform crosslinking in molten polymer. Nijenhuis et al. found when using DCP as a crosslinking agent for PLA, there was a dependence of gel formation on curing temperature. They reported that at DCP concentrations of 5 wt. % and curing temperatures of 175°C and 192°C, a gel fraction of 80% and 90% was obtained respectively. They also found at such high temperatures, chain scission occurred at long reaction times decreasing the gel fraction. All samples blended at 150°C which contained DCP crosslinked to some extent (Nijenhuis et al. 1996). This suggests that the crosslinking process is dependent on the formation of cumyloxy and methyl radicals by the degradation of DCP as reported by Takamura et al (2008).

Samples were also prepared using BPO, to establish if crosslinking could occur at temperatures of 100°C or lower. BPO has a much faster decomposition rate compared to DCP (Takamura et al. 2008). However, all of these samples dissolved completely in chloroform indicating that they did not crosslink. This suggests that BPO also requires temperatures greater than 100°C to initiate crosslinking in PDLLA. Figures 5.6 and 5.7 illustrate the effect plasticizer content has on the gel fraction and degree of swelling of crosslinked samples containing 2 wt. % DCP and various concentrations of TAIC.
Figure 5.6 Gel fraction of PDLLA samples containing various concentrations of plasticizer TBC and 2 wt. % DCP as a function of TAIC content

Figure 5.7 Degree of swelling for PDLLA samples containing various concentrations of plasticizer TBC and 2 wt. % DCP as a function of TAIC content
Increasing the plasticizer content generally decreases the gel fraction and increases the degree of swelling. The plasticizer was initially used to decrease the glass transition of the polymer by embedding itself between chains and increasing free volume. Increasing free volume with the addition of extra plasticizer may hinder the formation of crosslinks. With a lower crosslink density and increased free volume there is a higher degree of swelling. It should be noted here that the values as found from the swelling studies are qualitative rather than quantitative results. The plasticizer used (TBC) is soluble in chloroform, therefore some of the plasticizer may have been extracted during the experiments. However, the extent of swelling which occurred and the percentage gel fraction of each sample is a good indicator to the extent of the crosslink density.

Figures 5.8 and 5.9 illustrate the effect DCP content has on the gel fraction and degree of swelling of crosslinked samples containing 30 wt. % TBC and various concentrations of TAIC. The effect peroxide content has on the gel fraction is more clearly presented in figures 5.10 to 5.12, while figures 5.13 to 5.15 clearly present the effect peroxide content had on the degree of swelling.

![Figure 5.8 Gel fractions of PDLLA samples containing various concentrations of DCP and 30 wt. % TBC as a function of TAIC content](image-url)
Figure 5.9 Degree of swelling for PDLLA samples containing various concentrations of DCP and 30 wt. % TBC as a function of TAIC content

Figure 5.10 Gel fractions of PDLLA samples containing 30 wt. % TBC and 2 wt. % and 4 wt. % DCP as a function of TAIC content
Figure 5.11 Gel fractions of PDLLA samples containing 30 wt. % TBC and 4 wt. % and 6 wt. % DCP as a function of TAIC content

Figure 5.12 Gel fractions of PDLLA samples containing 30 wt. % TBC and 6 wt. % and 8 wt. % DCP as a function of TAIC content
As DCP content increases from 2 to 4 wt. %, gel fraction increases with samples having the highest gel fractions having between 4 to 6 wt. % TAIC content. Both curves show a gradual increase in gel fraction followed by a slight decline with increasing TAIC concentration.

Increasing DCP content from 4 to 6 wt. % increases gel fractions at low TAIC concentrations. As the TAIC content increases, the samples containing less DCP have slightly higher gel fractions.

This trend can also be seen in figure 5.12, by increasing DCP content from 6 to 8 wt. %, a higher gel fraction is achieved at low concentrations of 2 and 4 wt. % TAIC, as the TAIC content increases, the samples containing less DCP (6 wt. %) have a slightly higher gel fraction than the samples containing more DCP (8 wt. %).

Figure 5.13 Degree of swelling for PDLLA samples containing 30 wt. % TBC and 2 wt. % and 4 wt. % DCP as a function of TAIC content
Figure 5.14 Degree of swelling for PDLLA samples containing 30 wt. % TBC and 4 wt. % and 6 wt. % DCP as a function of TAIC content

Figure 5.15 Degree of swelling for PDLLA samples containing 30 wt. % TBC and 6 wt. % and 8 wt. % DCP as a function of TAIC content
Figure 5.13 confirms that with increasing the peroxide content from 2 to 4 wt. %, the degree of swelling decreases and crosslink density increases, with a lower degree of swelling occurring in samples containing 4 to 8 wt. % TAIC.

Figure 5.14 illustrates that by further increasing DCP content a decrease in degree of swelling occurs. At higher concentrations of TAIC the samples containing less DCP had slightly higher gel fractions while also having a higher degree of swelling. The samples containing higher concentrations of DCP which only had a slightly lower gel fraction and a much lower degree of swelling had a higher crosslink density.

Figure 5.15 illustrates that by increasing DCP content from 6 to 8 wt. %, a lower degree of swelling is achieved at a concentration of up to 4 wt. % TAIC, as the TAIC content increases, the samples containing less DCP have a lower degree of swelling.

Figures 5.16 and 5.17 illustrate the effect TAIC content has on the gel fraction and degree of swelling of crosslinked samples containing 30 wt. % TBC and various concentrations of DCP. The effect TAIC content has on the gel fraction is more clearly presented in figures 5.18 to 5.21, while figures 5.22 to 5.25 clearly present the effect had on the degree of swelling.

Figure 5.16 Gel fractions of PDLLA samples containing various concentrations of TAIC and 30 wt. % TBC as a function of DCP content
Figure 5.17 Degree of swelling for PDLLA samples containing various concentrations of TAIC and 30 wt. % TBC as a function of DCP content.

Figure 5.18 Gel fractions of PDLLA samples containing 30 wt. % TBC, no TAIC and 2 wt. % TAIC as a function of DCP content.
Figure 5.19 Gel fractions of PDLLA samples containing 30 wt. % TBC, 2 and 4 wt. % TAIC as a function of DCP content.

Figure 5.20 Gel fractions of PDLLA samples containing 30 wt. % TBC, 4 and 6 wt. % TAIC as a function of DCP content.
Figure 5.21 Gel fractions of PDLLA samples containing 30 wt. % TBC, 6 and 8 wt. % TAIC as a function of DCP content

Figure 5.18 illustrates that the addition of 2 wt. % TAIC increases gel fraction by 20% or more, with the greatest gel fraction in samples containing high concentrations of peroxide. Increasing TAIC content by a further 2% to 4 wt. % further increases gel fraction as observed in figure 18.

A similar trend can be seen in figure 5.20, with samples containing 6 wt. % TAIC generally having a higher gel fraction than samples with 4 wt. % TAIC, although at high concentrations of peroxide, the gel fraction in samples containing the most TAIC starts to decline. Increasing the TAIC content further to 8 wt. % results in a decrease in gel fraction as observed in figure 5.21.
Figure 5.22 Degree of swelling for PDLLA samples containing 30 wt. % TBC, no TAIC and 2 wt. % TAIC as a function of DCP content.

Figure 5.23 Degree of swelling for PDLLA samples containing 30 wt. % TBC, 2 and 4 wt. % TAIC as a function of DCP content.
Figure 5.24 Degree of swelling for PDLLA samples containing 30 wt. % TBC, 4 and 6 wt. % TAIC as a function of DCP content.

Figure 5.25 Degree of swelling for PDLLA samples containing 30 wt. % TBC, 6 and 8 wt. % TAIC as a function of DCP content.
Figures 5.22 and 5.23 confirm that by increasing TAIC content from 0 to 4 wt. % an increase in crosslink density occurs as degree of swelling decreases. While figure 5.24 illustrates that at lower concentrations of peroxide, samples containing less TAIC (4 wt. %) had a lower degree of swelling than samples containing more TAIC (6 wt. %), however as peroxide content increases the degree of swelling is lowest in samples with higher TAIC content. Figure 5.25 illustrates that the highest crosslink density is achieved in samples containing 6 wt. % TAIC at high concentrations of peroxide.

Conducting the swelling studies on PDLLA has demonstrated that the addition of plasticizers hinders the crosslinking process with a decreasing crosslink density observed in samples with increasing plasticizer content. As already stated, the plasticizer was initially used to decrease the glass transition of the polymer by embedding itself between chains and increasing the free volume. Increasing the free volume may hinder the formation of crosslinks. With an increased free volume there is a higher degree of swelling and a lower crosslink density. Crosslinked structures can be achieved through the use of peroxides and temperatures of 150°C. Previous studies conducted by Nijenhuis et al. found when using DCP as a crosslinking agent for PLA, there was a dependence of gel formation on curing temperature. They reported that at DCP concentrations of 5 wt% and curing temperatures of 175°C and 192°C, a gel fraction of 80% and 90% was obtained respectively. In this study samples were prepared by making chloroform solutions of PLLA/DCP mixtures which were then dried in a Petri dish for 14h at 40°C and a final pressure of 50 × 10² Pa. Strips of the resulting film were cured under vacuum in an oven at predetermined times and temperatures.

The use of crosslinking agent TAIC alone with the application of heat will not crosslink the polymer, however high crosslink densities are achieved at high concentrations of TAIC (6 – 8 wt. %) and 6 wt. % DCP. Yang et al. (2008) who crosslinked PLA with concentrations of up to 1.5 wt % DCP and 3 wt % TAIC and the application of heat reported that the crosslink density of PLA increased with increasing content of DCP and TAIC. In this study PLA samples containing different concentrations of TAIC and DCP were mixed in a Haake melt mixer at 50 rpm, 180 °C for 10 min. The samples were hot-pressed at 190°C for 3 min followed by cold-
pressing at room temperature for 3 min to form the sheets with thickness of 0.8 mm. Differences in the results reported in the present study to those reported in literature may be due to the different processing techniques used.

5.3.3 Chemical crosslinking of PDLLA by Gamma Irradiation

A series of PDLLA samples were mixed with different concentrations of plasticizer TBC and crosslinking agents TAIC and/or DCP and pressed into moulds before being removed and sent to Isotron for gamma irradiation. Swelling studies were conducted with gel fractions and degree of swelling calculated for each sample.

Samples which contained only peroxides (DCP or BPO) and no other crosslinking agents dissolved completely in chloroform indicating that they did not crosslink. Crosslinking only occurred in samples which contained crosslinking agent TAIC. Samples containing TAIC and no plasticizer also did not crosslink. This suggests that the plasticizer was needed to ensure crosslinking occurred either by allowing for a more effective distribution of the crosslinking agent TAIC throughout the samples or by acting as a sort of binder to the powder constituents of the sample. The samples had not been heated (to prevent heat activation of the crosslinking agents) and so the plasticizer may have helped in creating solidification or cohesion of the sample which enabled crosslinking to occur.

The effect of concentrations of plasticizer on the crosslinking of gamma irradiated samples can be seen in figures 5.26 and 5.27. Although high plasticizer content was needed for crosslinking to occur, increasing TBC from 30 to 50 wt. % decreases gel fraction and increases degree of swelling. Highest crosslink densities were achieved in samples containing 30 wt. % TBC at high concentrations of TAIC.
Figure 5.26 Gel fraction of irradiated PDLLA samples containing 30 and 50 wt. % TBC and 2 wt. % DCP as a function of TAIC content.

Figure 5.27 Degree of swelling for irradiated PDLLA samples containing 30 and 50 wt. % TBC and 2 wt. % DCP as a function of TAIC content.
Figures 5.28 and 5.29 illustrate the effect DCP content has on irradiated samples containing 30 wt. % TBC and various concentrations of TAIC. Increasing DCP content generally seems to decrease gel fraction. The greatest gel fractions are attained in samples containing no DCP while the samples with the highest DCP content have the lowest gel fraction. A deviation from this trend can be seen with samples containing 2 wt. % DCP having a higher gel fraction than that of samples containing 1 wt. % DCP. The degree of swelling recorded for these samples confirms that samples containing no DCP have the highest crosslink density with the lowest degree of swelling while samples containing the most DCP have the lowest crosslink densities. The highest crosslink densities are attained in samples with no DCP and high concentrations of TAIC. High energy irradiation is the most abusive of polymer crosslinking techniques. Irradiating polymers creates very energetic ions and excited states, which decay to reactive free radicals. These intermediate species can follow several reaction paths, resulting in not only the formation of new bonds but also chain scission. Scissioning and crosslinking occur at the same time where one may predominate over the other. The presence of the DCP in the irradiated samples may have caused more chain scission to occur, decreasing the crosslink density.

![Figure 5.28 Gel fractions of irradiated PDLLA samples containing various concentrations of DCP and 30 wt. % TBC as a function of TAIC content](image)
Figure 5.29 Degree of swelling for irradiated PDLLA samples containing various concentrations of DCP and 30 wt. % TBC as a function of TAIC content

Figure 5.30 Gel fractions of irradiated PDLLA samples containing various concentrations of TAIC and 30 wt. % TBC as a function of DCP content
Figures 5.30 and 5.31 illustrate the effect TAIC content has on irradiated samples containing 30 wt. % TBC and various concentrations of DCP. At low concentrations of peroxide, samples containing the most TAIC had the highest gel fraction and the lowest degree of swelling while at higher concentrations of peroxide, samples containing less TAIC had higher gel fractions. These figures clearly illustrate that the highest crosslink densities are achieved at low or no peroxide content, most likely due to the occurrence of chain scission with the presence of DCP in the irradiated samples.

Figures 5.32 and 5.33 illustrate the difference in crosslink densities for samples containing the same quantity of crosslinkers and plasticizer with crosslinking being initiated by irradiation or heat. The gel fractions are similar with the irradiated samples being slightly greater and having a lower standard deviation. Irradiated samples also generally had a low degree of swelling with lower standard deviations. This indicates that gamma irradiation yields samples with higher crosslink densities which were more uniformly crosslinked.
Figure 5.32 Gel fractions of irradiated PDLLA and PDLLA samples crosslinked by heat containing 2 wt. % DCP and 30 wt. % TBC as a function of TAIC content

Figure 5.33 Degree of swelling for irradiated PDLLA and PDLLA samples crosslinked by heat containing 2 wt. % DCP and 30 wt. % TBC as a function of TAIC content
Conducting swelling studies on the gamma irradiated PDLLA has indicated that unlike the heat activated crosslinked structures, the crosslinking agent TAIC alone is required to produce a crosslinked network and the addition of DCP actually decreases crosslink density. The radiation induced crosslinking of PLLA in the presence of TAIC was investigated by Jin et al. who reported that a 100% gel fraction was achieved using 3 wt. % TAIC at an irradiation dose of 10kGy or 5 wt. % TAIC at a dose of 5kGy. While Mitomo et al. reported a gel fraction of 67% in PLLA irradiated at 20kGy containing 5 wt. % TAIC (Mitomo et al. 2005). Differences in gel fractions achieved in each study may be due to different PLA sample preparation, differences in the radiation source or the dispersion of TAIC during blending.

Plasticizers were required to ensure TAIC was distributed throughout the sample for crosslinking to occur, most likely due to sample preparation issues. However as with the heat activated crosslinked PDLLA, increasing plasticizer content to high concentrations retards the crosslinking process. Gamma irradiated samples had higher crosslink densities then those of samples which had chemically crosslinked through heat with the highest crosslink densities achieved in samples with no peroxides and increased TAIC content. Quynh et al. who investigated the properties of PLLA / PLDA stereo complex crosslinked with TAIC by irradiation also found that crosslink density of the samples increased with increased TAIC content (concentrations of up to 5 wt. % were studied).

5.3.4 Chemical crosslinking of Polylactide-co-caprolactone by peroxides and the application of heat

A series of copolymer polylactide-co-caprolactone samples were blended with different concentrations of plasticizer (TBC) and crosslinking agents (TAIC and/or DCP) prepared at three different temperatures, and gel fractions and degree of swelling were calculated. As with the PDLLA samples, PLCL samples prepared at temperatures under 150°C using TAIC and/or DCP as crosslinking agents and samples prepared at 150°C using only TAIC as a crosslinking agent dissolved completely in chloroform which indicated that they did not crosslink. Figures 5.34 and 5.35 below illustrate the effect plasticizer content has on the gel fraction and
degree of swelling of crosslinked samples containing various concentrations of peroxide DCP.

Figure 5.34 Gel fraction of PLCL samples containing various concentrations of plasticizer TBC as a function of DCP content

Figure 5.35 Degree of swelling for PLCL samples containing various concentrations of plasticizer TBC as a function of DCP content
At low concentrations of 1 to 2 wt. % peroxide, the greatest gel fractions are achieved in samples with no plasticizer, while the lowest gel fractions were observed in samples with the largest plasticizer content. At high concentrations of DCP, samples with 30 wt. % TBC content had a higher average gel fraction than samples containing no TBC. Samples containing 30 wt. % TBC had the highest average degree of swelling, while samples with no plasticizer had a much lower average degree of swelling. Samples containing 50 wt. % TBC also have a low degree of swelling but this is due to the fact that they also had a very low gel fraction. Highest crosslink density was obtained in samples with no plasticizer content and decreased as plasticizer content increased.

Figures 5.36 and 5.37 illustrate the effect DCP content has on the gel fraction and degree of swelling of crosslinked samples containing 30 wt. % TBC and various concentrations of TAIC.

![Figure 5.36 Gel fractions of PLCL samples containing various concentrations of DCP and 30 wt. % TBC as a function of TAIC content](image-url)
Samples with the highest DCP content at concentrations with no TAIC or high TAIC had the greatest gel fraction. At concentrations of 1 to 2 wt. % TAIC, DCP content does not have a significant effect on gel fraction with all resultant gel fractions being somewhat low. Highest crosslink densities were obtained at high concentrations of DCP and TAIC, as these samples had the highest gel fraction and the lowest degree of swelling. Samples which contained no TAIC and high concentrations of peroxide also had a relatively high gel fraction but don’t have a high crosslink density as the degree of swelling was also high.

Figures 5.38 and 5.39 illustrate the effect TAIC content has on the gel fraction and degree of swelling of crosslinked samples containing 30 wt. % TBC and various concentrations of DCP. These figures confirm that the greatest gel fractions are achieved at high concentrations of DCP and either no or high concentrations of TAIC. Samples with concentrations of 1 to 2 wt. % TAIC yield samples with low gel fractions. Highest crosslink densities are obtained in samples with 5 wt. % TAIC as their degree of swelling is much lower than samples containing no TAIC.
Figure 5.38 Gel fractions of PLCL samples containing various concentrations of TAIC and 30 wt. % TBC as a function of DCP content

Figure 5.39 Degree of swelling for PLCL samples containing various concentrations of TAIC and 30 wt. % TBC as a function of DCP content
Figures 5.40 to 5.43 illustrate the difference in crosslink densities for PDLLA samples and PLCL samples containing the same quantity of crosslinkers and plasticizer with crosslinking initiated by heat.

**Figure 5.40** Gel fractions of PLCL and PDLLA samples crosslinked by heat containing no plasticizer as a function of DCP content

**Figure 5.41** Degree of swelling for PLCL and PDLLA samples crosslinked by heat containing no plasticizer as a function of DCP content
Figure 5.42 Gel fractions of PLCL and PDLLA samples crosslinked by heat containing 30 wt. % TBC and 2 wt. % DCP as a function of TAIC content

Figure 5.43 Degree of swelling for PLCL and PDLLA samples crosslinked by heat containing 30 wt. % TBC and 2 wt. % DCP as a function of TAIC content
Similar to the crosslinked structures of PDLLA, crosslinked PLCL was temperature dependent and required the presence of DCP for the process to occur. However, crosslinked PDLLA samples had much higher crosslink densities than the copolymer samples crosslinked by the same quantity of crosslinking agents and by the same method. One of the main problems encountered when trying to crosslink polylactide is the lack of hydrogen’s which would be susceptible to crosslinking along the main chain.

![Figure 5.44 Structure unit of PLA](image)

It was thought that crosslinking the polylactide copolymer PLCL would lead to higher gel fractions as its unit structure has a backbone with a longer carbon chain compared to the unit structure of polylactide. The PLCL copolymer has a greater amount of hydrogens available for abstraction which should enable more crosslinks to form.

![Figure 5.45 Structure unit of PLA-co-PCL](image)

However in all cases, crosslinked PDLLA had a much higher gel fraction than PLCL, with both sets of samples having similar degrees of swelling, the polylactide had a much higher degree of crosslinking. As these samples had the highest gel fractions, DMTA was preformed on a selection of chemically crosslinked PDLLA samples.
5.3.5 DMTA of chemically crosslinked PDLLA

Figures 5.46 and 5.47 show the tan delta and storage modulus curves obtained from Dynamic Mechanical analysis of PDLLA plasticised with 30 wt. % TBC and crosslinked with various concentrations of DCP and TAIC. Table 5.14 indicates the glass transition temperatures taken as the peak of the tan delta plot, and the resultant gel fraction for each sample. Figure 4.48 and 4.49 show tan delta and the storage modulus curves obtained for crosslinked PDLLA plasticised with 30 wt. % TBC which had the highest crosslink density, uncrosslinked PDLLA plasticised with 30 wt. % TBC and pure uncrosslinked PDLLA.

Table 5.14 Glass Transition temperatures for crosslinked PDLLA samples determined by DMTA

<table>
<thead>
<tr>
<th>Sample ( wt. % )</th>
<th>Gel Fraction ( % )</th>
<th>Tg ( °C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% TBC / 4% DCP / 4% TAIC / 62% PDLLA</td>
<td>68.26</td>
<td>28.48</td>
</tr>
<tr>
<td>30% TBC / 6% DCP / 4% TAIC / 60% PDLLA</td>
<td>58.51</td>
<td>34.18</td>
</tr>
<tr>
<td>30% TBC / 8% DCP / 4% TAIC / 58% PDLLA</td>
<td>66.49</td>
<td>32.8</td>
</tr>
<tr>
<td>30% TBC / 4% DCP / 6% TAIC / 60% PDLLA</td>
<td>73.75</td>
<td>35.78</td>
</tr>
<tr>
<td>30% TBC / 70% PDLLA</td>
<td>0</td>
<td>36.1</td>
</tr>
<tr>
<td>100% PDLLA</td>
<td>0</td>
<td>74.3</td>
</tr>
</tbody>
</table>
Figure 5.46 Tan delta curves obtained from dynamic mechanical analysis of PDLLA plasticised with 30 wt. % TBC and crosslinked with different concentrations of DCP and TAIC.

Figure 5.47 Storage modulus curves obtained from dynamic mechanical analysis of PDLLA plasticised with 30 wt. % TBC and crosslinked with different concentrations of DCP and TAIC.
Figure 5.48 Tan delta curves obtained from dynamic mechanical analysis of pure PDLLA, PDLLA plasticised with 30 wt. % TBC and PDLLA plasticized with 30 wt. % TBC and crosslinked with DCP and TAIC.

Figure 5.49 Storage modulus curves obtained from dynamic mechanical analysis of pure PDLLA, PDLLA plasticised with 30 wt. % TBC and PDLLA plasticized with 30 wt. % TBC and crosslinked with DCP and TAIC.
The Tg of crosslinked samples should shift to higher temperatures as the crosslink density increases, due to the resultant increase in the hindrance of chain relaxation (Zuza et al. 2008). This was not evident however in the chemically crosslinked samples analysed. The samples showed no clear trend, with the highest gel fraction tested having a Tg slightly lower the that of the uncrosslinked sample. There were some incidents where the crosslinked samples Tg was higher than that of the uncrosslinked sample but no more than by 1°C. Yang et al. reported a decrease in Tg and Tm of semicrystalline PLA with increasing gel fraction and crosslink density. This was accounted for by the fact that increasing chemical crosslinks will decrease the crystallinity of the polymer and therefore decrease the Tg and Tm. The net result may be that the Tg does not change significantly. Nijenhuis et al. also reported similar results for crosslinked semicrystalline PLLA. The polylactide crosslinked in this study was initially amorphous so it is thought that crosslinking should significantly increase the Tg.

Figure 5.49 show the storage modulus curves obtained for crosslinked PDLLA plasticised with 30 wt. % TBC and uncrosslinked PDLLA plasticised with 30 wt. % TBC and uncrosslinked PDLLA. There is a dramatic drop in the storage modulus values for the samples which have been crosslinked. Yang et al. found that increasing peroxide concentrations resulted in a steep drop in tensile strength and a high crosslink density made the material brittle (Yang et al. 2008). Nijenhuis et al. found that the crosslinking of PLA by electron beam irradiation caused polymer degradation with the evolution of gaseous degradation products and high temperatures that caused the formation of voids, which in turn lead to high stress concentrations, strained areas and an over all embrittlement of the material (Nijenhuis et al. 1996).

Crosslinking the PDLLA with DCP and TAIC at high temperatures caused the material to foam and voids were formed in the test specimens as seen in figures 5.50 to 5.52. The formation of these voids had a large decreasing effect on the modulus of the crosslinked polymer and also cancelled out some of the effects crosslinking had on Tg. As a result, there were no significant differences in Tg values for crosslinked and uncrosslinked samples.
5.3.6 Macroscopic and microscopic images of crosslinked PDLLA

Figures 5.50 and 5.51 macroscopic images of peroxide crosslinked DMTA samples

Figure 5.52 Microscopic view (X5) of crosslinked sample containing 4 wt. % DCP / 6 wt. % TAIC
5.3.7 Photocrosslinking

Photoinitiators are compounds that break down into free radicals upon exposure to ultraviolet radiation. The absorption bands of the photoinitiator must overlap with the emission spectrum of the UV source and there must be minimal competing absorption by the components of the formulation at the wavelengths corresponding to photoinitiator excitation in order for photoinitiation to proceed efficiently. As the light source had a wavelength of 340nm, Irgacure 369 was chosen as a crosslinking agent as due to its UV absorbance spectra illustrated in figure 5.53.

![UV absorbance spectra for Irgacure 369](image)

**Figure 5.53 UV absorbance spectra for Irgacure 369** (Aldrich Chemical Co. 2008)

According to their mechanism of actions, the photoinitiators can be divided in two main categories: those (type I) that undergo a photoinduced $\alpha$-cleavage and (type II) that involve hydrogen abstraction by photoexcited molecules. 2-benzyl-2-dimethylamino-40-morpholinobutyrophenone (commercially known as Irgacure 369) is a type I photoinitiator that undergoes an $\alpha$-cleavage with formation of benzoyl and $\alpha$-aminoalkyl radicals (Alberti et al. 2008). It was thought that the
formation of these free radicals with the addition of peroxides and crosslinking agent TAIC should help to trigger a crosslinking reaction.

![Chemical structure of Irgacure 369 and radicals](image)

**Figure 5.54 Breakdown of Irgacure 360 by UV radiation into benzoyl and α-aminoalkyl radicals**

All samples which were UV cured dissolved completely in chloroform indicating that they did not crosslink. Nijenhuis et al. suggest that for aliphatic polyesters, the ratio of the backbone carbon atoms to the backbone ester groups should at least be equal to 3 in order for gel formation by irradiation to be possible, for PDLLA this ratio is equal to 2 (Nijenhuis et al. 1996). The UV irradiation did have some effect on the structure of the polymer as clearly seen in figures 5.55 to 5.57.

**5.3.8 Microscopic images of PDLLA (UV irradiated and non-UV irradiated)**

![Microscopic image](image)

**Figure 5.55 Microscopic image (X5) of 30 wt. % TBC 70 wt. % PDLLA sample (not irradiated)**
Figures 5.56 and 5.57 Microscopic images (X5) of UV irradiated PDLLA containing 1 wt. % Irgacure with 30 wt. % TBC and 2 wt. % Irgacure with 30 wt. % TBC respectively

Figure 5.55 illustrates a microscopic view of the plasticized sample which was not UV cured. Two distinct separate phases can be observed with large black pools of plasticizer dispersed throughout the polymer material. Comparing figure 5.55 to the microscopic views of UV irradiated samples (figures 5.56 and 5.57) a clear difference can be seen as the plasticizer seems to be more evenly dispersed. Increasing photoinitiator content form 1 to 2 wt. % has an even greater effect on the dispersion of the plasticized phase. To indicate the extent of the effect of plasticizer distribution, tensile testing was conducted on UV irradiated samples.

5.3. 9 Tensile testing of UV irradiated samples

The stress strain curves obtained from the tensile testing of the irradiated samples can be seen in figure 5.58. A plasticized sample which was not irradiated was also tested. The un-irradiated sample had the highest tensile strength of all samples tested at 0.94 MPa. Yang et al. tensile tested crosslinked PLA and found that the introduction of crosslinks resulted in an increase of tensile modulus and a decrease in elongation at break. Increasing the crosslink density resulted in a brittle polymer (Yang et al. 2008). Quynh et al. found similar results with PLA material crosslinked by irradiation and TAIC. They also found that an increase in TAIC content increased the tensile modulus (Quynh et al. 2007).
The UV irradiated samples as shown in figure 5.58 have a decrease in tensile strength and modulus but an increase in elongation at break as compared to the non-irradiated sample. The same effects can be seen when increasing the plasticization of a polymer sample (Baiardo et al. 2003). All samples were plasticized by the same percentage of TBC and manufactured by the same process, the only difference being that the UV irradiation step which helped to diffuse the plasticizer throughout the sample.

Figure 5.58 Stress-Strain curves for sample plasticized with 30 wt. % TBC with UV irradiated samples which also contain 30 wt. % TBC
5.4 Conclusions

The swelling studies carried out on PDLLA demonstrate that crosslinked structures are achieved through the use of peroxides and temperatures of 150°C. Sample preparation has a major effect on the crosslink densities of the resultant structures with all of the samples prepared by method 2 having higher gel fractions with lower standard deviations then that of samples prepared by method 1. Method 2 describes a process where the polymer, crosslinker and peroxide were transformed into powder form and mixed before the addition of the plasticizer and the application of heat. The addition of plasticizers hinders the crosslinking process with a decreasing crosslink density observed in samples with increasing plasticizer content. The use of crosslinking agent TAIC alone with the application of heat will not crosslink the polymer PDLLA, however high crosslink densities are achieved at high concentrations of TAIC and DCP.

The swelling studies conducted on the gamma irradiated PDLLA indicates that unlike the heat activated crosslinked structures, the crosslinking agent TAIC alone is required to produce a crosslinked network and the addition of DCP decreases crosslink density. Plasticizers are required to ensure TAIC is distributed throughout the sample for crosslinking to occur due to sample preparation issues. However as with the heat activated crosslinked PDLLA, increasing plasticizer content to high concentrations retards the crosslinking process. Irradiated samples have higher crosslink densities then those of samples which are chemically crosslinked through heat. The highest crosslink densities are achieved in samples with no peroxides and increased TAIC content. Differences in gel fractions achieved in this study and other reported studies may be due to different PLA sample preparation, differences in the radiation source or the dispersion of TAIC during blending.

Similar to the crosslinked structures of PDLLA, crosslinked PLCL is temperature dependent and requires the presence of DCP for the process to occur. Highest crosslink densities are obtained at high concentrations of DCP and TAIC. Samples which contain no TAIC and high concentrations of peroxide also have a relatively high gel fraction but don’t have a high crosslink density as the degree of swelling is also high. Crosslinked PDLLA samples have much higher crosslink densities than
copolymer samples crosslinked by the same quantity of crosslinking agents and by the same method.

DMTA of crosslinked PDLLA indicates that no significant effect occurs to the Tg of the polymer while there is a dramatic drop in the storage modulus. Crosslinking PDLLA with DCP and TAIC at high temperatures causes the material to foam and voids are formed which lead to high stress concentrations.

An attempt to UV crosslink the PDLLA was unsuccessful. The UV irradiation has an apparent plasticizing effect on the polymer samples tested with samples having a decreased tensile strength and modulus but an increased elongation at break as compared to the non-irradiated sample.

One of the main aims of this project was to develop an injectable bone filler which would harden in-situ with appropriate load bearing properties without detrimental effects to the surrounding tissue. Crosslinking is not a viable approach to harden the injected polylactide filler as not only are high temperatures needed for crosslinking initiation which would cause cellular necrosis, there is also a reduction in the mechanical and physical integrity of the polymer worsening its load bearing properties. Therefore an alternative method will need to be considered in order to produce an injectable polylactide based filler which will harden in situ.
5.5 References


Chapter 6
6.0 Preliminary Polylactide Compound Formulation

6.1 Introduction

Initial work was concentrated on blending the polymer Poly (D,L) lactide (PDLLA), with different citrate ester plasticizers to produce polymer-plasticizer mixtures of various concentrations which could be easily mouldable. Following this, an investigation into crosslinking the polymer was carried out with the view that upon injection, the polymer could harden by this method. Problems became apparent with this approach, as high temperatures were needed for crosslinking initiation and a reduction in mechanical properties was observed.

The intention of the project is to produce a bone filler, that once injected would harden with improved mechanical properties and with initiation temperatures of below 50°C, to avoid the occurrence of cellular necrosis. Therefore an alternative method was considered by using an injectable monomer which could harden upon polymerization in-situ. This part of the project concerns itself with developing a bone filler combining polylactide particles with the liquid monomer cyanoacrylate. Cyanoacrylates are unique, in that they are single component systems that polymerise at room temperature without the addition of a catalyst, evaporation of a solvent or an external energy source. They also have the advantage of being relatively inexpensive, resorbable and a well established tissue adhesive. By combining a cyanoacrylate monomer with a polylactide powder, a base compound could be created which would be hand mouldable and easily injectable. With the onset of polymerization of the monomer, the filler would then harden in situ below 50°C. Modification of the composition with the addition of plasticizers, fillers and other additives allows for the adjustment of mechanical properties and setting rates.

In this study, polylactide powder was blended with various ratios of cyanoacrylate monomer, plasticizer and hydroxyapatite. Once set, the resulting compositions were tensile tested and placed in buffered saline to observe the effects of water absorption and hardness with time. From these initial results it is hoped to establish an appropriate sample range upon which further investigation will be completed.
6.2 Materials and Methods

6.2.1 Blending

PDLLA granules, supplied by Boehringer Ingelheim (Germany) were ground into smaller particles using a coffee grinder. The ground polymer was then sieved through a stainless steel sieve with a mesh size of 500µm. Polymer which could not go through the sieve was reground for 1 minute and re-sieved. This was repeated until all the polymer was reduced to a fine powder of particles sizes of approximately 500µm or less. The plasticizer tributyl citrate (TBC) was supplied by Sigma Aldrich (Ireland) while the cyanoacrylate monomer used was n-butyl 2 cyanoacrylate (BCA) supplied by Henkel Ireland Ltd. Hydroxyapatite supplied by Stryker Orthopaedics Limerick was first gyro-milled then sieved using a 45µm mesh. It was then placed in a vacuum oven for 4 hrs at 140°C.

Samples of compositions as indicated in table 6.1 were prepared for tensile testing and water absorption analysis.

Table 6.1 Composition of samples prepared

<table>
<thead>
<tr>
<th>Sample</th>
<th>PDLLA wt. %</th>
<th>BCA wt. %</th>
<th>TBC wt. %</th>
<th>HA wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
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<td>0</td>
</tr>
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<td>3</td>
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<td>0</td>
</tr>
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<td>4</td>
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<td>40</td>
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<td>0</td>
</tr>
<tr>
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</tbody>
</table>

Samples numbered 1 to 3 consisted of different ratios of PDLLA and BCA only. Samples numbered 4 to 15 consisted of a 50 wt. % liquid component (BCA and/or TBC of different ratios) and a 50 wt. % powder component (PDLLA and/or HA of
different ratios). Each composition could be easily mixed by hand to create homogenous blends in which the liquid components completely wetted all the dry powder components. All samples were simply mixed manually using a spatula at ambient temperature and placed in moulds to be pressed and left to set.

6.2.2 Water Absorption and Hardness
Water absorption and hardness tests were completed to identify the swelling characteristics of each of the compositions over ten weeks. Samples with compositions as indicated in table 1 were prepared by mixing the components manually and transferring the pastes to moulds measuring 25 x 65 x 3mm. The samples were then pressed and left to set over night. All samples were placed in phosphate buffered saline (PBS) at a pH of 7.2 and incubated at 37 °C. At selected intervals over a ten week period, samples were removed from the PBS, patted dry and weighed. Shore A hardness values were also measured. One sample of each composition was analysed.

6.2.3 Tensile testing
Tensile tests were completed to identify important mechanical characteristics such as modulus and strength for each of the sample compositions as indicated in table 1. The components for each sample were mixed manually and transferred to a mould, which was then pressed and left to set. Specimens were dogbone shaped with a gauge length of approximately 13mm, a width of 3.5mm and thickness of 3mm. Six specimens of each composition were prepared and all were tested with the same crosshead speed of 10mm/min using a 1kN load cell.

6.3 Results and discussion
6.3.1 Blending
All compositions as indicated in table 6.1 mixed to produce samples which were of a putty-like consistency. All samples were extremely hand malleable once mixed, however with the onset of polymerization of the monomer, the material slowly but gradually hardened with samples containing HA setting faster than samples which did not contain HA. A slight exotherm was evident as the samples set, however each sample could be handled with ease throughout the setting process.
6.3.2 Water Absorption and Hardness

Water absorption and hardness testing was completed to identify the swelling characteristics of each of the compositions over a ten week period. Ideally, a bone filler should have limited swelling and maintain its shape and mechanical integrity once placed in situ and during the initial stages of bone healing. Sample swelling and reductions in sample hardness due to water absorption give a good indication of the degradation rate of the samples, which should be limited within the first ten weeks. Figures 6.1 to 6.7 show the weight change of samples prepared as in section 6.2.1 and placed in PBS at 37°C over 71 days. Figures 6.1 to 6.3 clearly illustrate the effect TBC and BCA content has on the percentage weight change over the ten week period. Figure 6.1 shows that water absorption is greatest and also increases over time with increasing plasticizer content and decreasing cyanoacrylate content. For a sample containing no TBC and 50 wt. % BCA, percentage weight gain increased from 0.67% on day 10 to 0.84% on day 44, compared to a sample containing 30 wt. % TBC and 20 wt. % BCA which had a percentage weight change of + 1.65% on day 10 to + 8.99% on day 44.

![Graph showing weight change as a function of time for samples consisting of 50% PDLLA and different BCA/TBC combinations.](image)

**Figure 6.1** Percentage weight change as a function of time for samples consisting of 50% PDLLA and different BCA/TBC combinations

The trend seen in figure 6.1 is also evident in figures 6.2 and 6.3 with the greatest percentage weight changes being in samples containing more TBC and less BCA.
For samples containing smaller amounts or no plasticizer, water absorption increases steadily through out the 10 week period. For samples containing larger amounts of plasticizer, water absorption is gradual up until about day 38, after which percentage weight change increases more rapidly.

Figure 6.2 Percentage weight change as a function of time for samples consisting of 40% PDLLA, 10% HA and different BCA /TBC combinations

Figure 6.3: Percentage weight change as a function of time for samples consisting of 30% PDLLA, 20% HA and different BCA /TBC combinations
Figures 6.4 to 6.6 illustrate the effect PDLLA and HA content has on the percentage weight change of a sample over ten weeks in PBS at 37°C. Figure 6.4 shows that samples containing no plasticizer have a gradual increase in water absorption over time. For the sample containing 10 wt. % HA, water absorption is similar to that of the sample containing no HA. By increasing the amount of HA to 20 wt. %, there is a higher percentage of water absorption over the 10 week period. Figures 6.5 and 6.6 show that water absorption is similar and gradually increasing for all samples up to day 44, after which there is more of a rapid climb in percentage weight change, increasing with HA content. By increasing HA content there is an increase in water absorption.

![Figure 6.4 Percentage weight change as a function of time for samples consisting of 50% BCA and different PDLLA /HA combinations](image)
Figure 6.5 Percentage weight change as a function of time for samples consisting of 40% BCA, 10% TBC and different PDLLA /HA combinations

Figure 6.6 Percentage weight change as a function of time for samples consisting of 30% BCA, 20% TBC and different PDLLA /HA combinations

Figure 6.7 shows percentage weight change of samples containing only PDLLA and BCA in different % wt. ratios. All samples follow a gradual increasing water absorption trend. The sample with the lowest content of cyanoacrylate (the highest polylactide content) had the greatest percentage weight increase over all.
Figures 6.8 to 6.14 show the Shore A hardness of samples placed in PBS at 37°C over 71 days. Figures 6.8 to 6.10 clearly illustrate the effect BCA and TBC content has on the Shore A hardness values. In figure 6.8, samples containing little or no plasticizer have the highest hardness values and maintain this throughout the 10 week period, with just a slight decrease in the hardness for the sample containing 10% TBC at the end of the 10 weeks. By increasing the plasticizer content to 20 wt. % and decreasing the BCA content, the hardness dramatically decreases and there is a steady decrease in hardness throughout the 10 week period. For the sample containing 30 wt. % plasticizer, all hardness is lost after 44 days (sample was too soft for reading to be taken). For the sample containing 40 wt. % plasticizer hardness is lost 20 days earlier on day 24. Figures 6.9 and 6.10 show a similar trend with samples containing less plasticizer and more cyanoacrylate being harder and maintaining that hardness for longer.
Figure 6.8 Shore A hardness as a function of time for samples consisting of 50% PDLLA and different BCA/TBC combinations.

Figure 6.9 Shore A hardness as a function of time for samples consisting of 40% PDLLA, 10% HA and different BCA/TBC combinations.
Figures 6.11 to 6.13 illustrate the effect PDLLA and HA content has on the shore A hardness values. In figure 6.11, samples containing no or little HA had higher hardness values and somewhat maintain this hardness through out the 10 week period. The sample containing the most HA had lower hardness values with larger standard deviations. Due to the higher HA content, the sample set a lot quicker and was harder to mould. Resultant samples had a rough surface area and were not uniform. Therefore, accurate Shore A hardness values were difficult to measure, giving a large standard deviation.
Figure 6.11 Shore A hardness as a function of time for samples consisting of 50% BCA and different PDLLA /HA combinations

Figure 6.12 Shore A hardness as a function of time for samples consisting of 40% BCA, 10% TBC and different PDLLA /HA combinations
In figure 6.12 all samples have similar hardness values for 44 days, with samples containing no HA and 10 wt. % HA having slightly higher values than the 20 wt. % HA sample. After 10 weeks, the hardness values decrease with the samples containing HA having the biggest decline. Figure 6.13 shows that the sample having the largest content of HA has slightly higher hardness values. The samples containing 10 wt. % HA and no HA follow the same trend with the latter having slightly higher hardness values. There is a gradual decline in the hardness of all the samples throughout the 10 week period.

Figure 6.14 illustrates the hardness values of samples containing only PDLLA and BCA in different % wt. ratios. These samples mostly maintained their hardness values throughout the 10 week period, with samples containing the least amount of BCA having slightly lower values. The sample containing the most BCA had hardness values with large standard deviations. Due to the larger volume of BCA, the PDLLA wasn’t evenly dispersed throughout and the sample wasn’t uniform giving a wider range of hardness values.
Plasticizer content has a great effect on the water absorption and hardness of samples placed in PBS over a ten week period at 37°C. The plasticizer increases water absorption and decreases hardness, increasing the content of the plasticizer also increases the rate of water absorption and hardness loss. The primary role of plasticizers is to improve processability and flexibility of the polymer by lowering the Tg. The low molecular size of the plasticizer allows them to occupy intermolecular spaces between polymer chains, reducing the energy for molecular motion and the formation of hydrogen bonding between the chains which in turn increases free volume and molecular mobility (Vieira et al. 2011). Therefore a reduction in hardness is seen as plasticiser content increases. The increase in free volume by the disruption of hydrogen bonding and the spreading apart of polymer chains also increases water permeability (Martelli et al. 2006). This accounts for the rise in water absorption with the increase of plasticizer content. Vieira et al. (2011) and Andreuccetti et al. (2009) also found that an increase in quantities of plasticizer in polymer samples lead to an increase of water permeability (Andreuccetti et al. 2009). Hoglund et al. (2009) studied the migration and hydrolysis of hydrophobic plasticizer acetyl tributyl citrate (ATBC) in PLA. They found that despite the low water solubility of ATBC (5mg/L), it migrates from the polymer to the surrounding
water (Hoglund et al. 2010). If the plasticizer diffuses out of the polymer there is a faster permeation of buffer into polymer (Labrecque et al. 1997). The plasticizer TBC used in this study is hydrophobic and water insoluble, however may also have migrated out of the samples leading to permeation of the buffer into the polymer causing the weight gain in plasticized samples.

It can also be seen that increasing HA content slightly increases water absorption with a more apparent water uptake after a period of 44 days. Work completed by Deb et al. (1995) found that in general, water uptake is reduced by the presence of HA in polymer composites. Domingo et al. (2003) found that midway filled composite resins loaded with micro HA particles displayed lower percent water uptake values than unfilled resins. Although they also found that composites containing only nanometric particles of HA as filler showed a higher solubility in water (Domingo et al. 2003). In a study completed by Santos et al. (2001), the water uptake in the resin phase of a composite was slightly higher than that of the water uptake of the unfilled resin. They accounted for the increase in water absorption due to the porosity in the HA and inclusions or filler particle aggregates. They observed that the inclusions appeared loosely embedded in the matrix and concluded that the additional amount of water could be accommodated at the interface between the agglomerates and the matrix. These were weak links which could provide paths of diffusion towards the insides of the aggregates, where the presence of microvoids was quite probable due to a lack of impregnation of filler particles with the polymer matrix (Santos et al. 2002). Deb et al. (1995) also detected a higher water uptake in a composite with treated HA. They attributed this to the introduction of water soluble impurities during the treatment process of the HA (Deb et al. 1995). Therefore, the increase in water absorption observed in samples with HA in this study may be due to HA agglomerates, porosity or soluble impurities in the HA.

HA does not have an impact on hardness as substantial as plasticizer content does, although in samples with low plasticizer content or no plasticizer content it does seem to decrease the hardness slightly. This may be due to the slight increase in water absorption caused by the HA. The increased water content will act as a plasticizer decreasing the hardness of the samples.
For samples containing only PDLLA and BCA, differences in water absorption and hardness are slight. The sample with the least amount of BCA had lower hardness values and a higher percentage of water absorption. Water uptake in a polymer network is related to resin polarity and chain topology. Resin polarity influences the number of hydrogen bonding sites and the attraction between the polymer and water molecules, while chain topology determines the special configuration of the molecular segments and the availability of nanopores within the polymer structure (Yiu et al. 2004). In a study completed by Yiu et al. (2004) it was concluded that resin hydrophilicity is the prime factor in determining the extent of water sorption, with the more hydrophilic polymer absorbing more water. A decrease in hardness is observed with increase in water absorption due to the plasticization action of the water by swelling the polymer network and reducing the frictional forces between the polymer chains. The sample with the highest amount of BCA had hardness values with a high standard deviation due to an inconsistent moulded sample.

### 6.3.3 Tensile testing

Figures 6.15 to 6.23 show the results obtained from tensile testing. Figure 6.15 shows the Young’s modulus values of all samples tested, as a function of TBC / BCA and PDLLA / HA content. The greatest Young’s modulus values were obtained for samples which contained no HA and the modulus decreased with increasing HA content. This is also true for plasticizer content. As the TBC content increased, the Young’s modulus decreased. The highest Young’s modulus value was obtained for the sample containing no HA and no TBC.

Figure 6.16 shows the modulus values for samples which contained 50 wt. % PDLLA and no HA as a function of TBC and BCA content, and clearly illustrates the decrease in modulus with increasing plasticizer content and decreasing BCA content. The same trend was seen in samples which contained 40 wt. % PDLLA / 10 wt. % HA and 30 wt. % PDLLA / 20 wt. % HA with decreasing values as HA content increases.
Figure 6.15 Young’s modulus of samples as a function of TBC/BCA and PDLLA/HA wt. % content

Figure 6.16 Young’s modulus as a function of increasing TBC and decreasing BCA content for samples which contain 50 wt. % PDLLA
Figure 6.17 shows the tensile strength values obtained for each sample tested, as a function of TBC / BCA and PDLLA / HA content. The results are similar to that obtained for the Young’s modulus. The greatest tensile strength values were obtained for samples which contained no HA and these results decreased with increasing HA content. This is also true for plasticizer content. As the TBC content increased, the tensile strength decreased. The highest tensile strength value was obtained for the sample containing no HA and no TBC.

Figure 6.18 shows the strength values for samples which contained 50 wt. % PDLLA and no HA as a function of TBC and BCA content and clearly illustrates the decrease in tensile strength with increasing plasticizer content and decreasing BCA content. The same trend was seen in samples which contained 40 wt. % PDLLA / 10 wt. % HA and 30 wt. % PDLLA / 20 wt. % HA with decreasing values as HA content increases.
Figure 6.18 Tensile strength as a function of increasing TBC and decreasing BCA content for samples which contain 50 wt. % PDLLA

Figure 6.19 shows the percentage elongation values obtained for each sample tested, as a function of TBC / BCA and PDLLA / HA content. As expected, elongation increases with increasing plasticizer content. Percentage elongation was the greatest for samples which didn’t contain HA and decreased with HA content, apart from one sample which contained 40% PDLLA, 10% HA, 20% BCA and 30% TBC and had the highest percentage elongation.

Figure 6.20 shows the strength values for samples which contained 50 wt. % PDLLA and no HA as a function of TBC and BCA content and clearly illustrates the increase in elongation with increasing plasticizer content and decreasing BCA content. The same trend was seen in samples which contained 40 wt. % PDLLA / 10 wt. % HA and 30 wt. % PDLLA / 20 wt. % HA with decreasing values as HA content increases.
Figure 6.19 Percentage elongation of samples as a function of TBC/BCA and PDLLA/HA wt. % content

Figure 6.20 Percentage Elongation as a function of increasing TBC and decreasing BCA content for samples which contain 50 wt. % PDLLA
Figures 6.21 to 6.23 show the tensile testing results obtained for samples containing only BCA and PDLLA. The sample containing 50 wt. % PDLLA and 50 wt. % BCA preformed best throughout, having the highest Young’s modulus, tensile strength and percentage elongation. The sample containing 60 wt. % PDLLA and 40 wt. % BCA had only slightly lower tensile strength and Young’s modulus and a percentage elongation approximately 3% lower than the 50-50 sample. The 40 wt. % PDLLA and 60 wt. % BCA sample had nearly half the tensile strength and Young’s modulus of the 50-50 sample but had a similar percentage elongation.

Figure 6.21 Young’s modulus for samples only containing PDLLA and BCA as a function of increasing PDLLA and decreasing BCA content
Figure 6.22 Tensile strength for samples only containing PDLLA and BCA as a function of increasing PDLLA and decreasing BCA content.

Figure 6.23 Percentage Elongation for samples only containing PDLLA and BCA as a function of increasing PDLLA and decreasing BCA content.
Table 6.2 Tensile testing results

<table>
<thead>
<tr>
<th>Composition (wt. %)</th>
<th>Tensile strength (MPa)</th>
<th>Std dev (+/-)</th>
<th>Elongation to break (%)</th>
<th>Std dev (+/-)</th>
<th>Young's Modulus (GPa)</th>
<th>Std dev (+/-)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TBC 60</td>
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<td>0.020</td>
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6.3.4 Consequences of tensile results

The use of materials that are not only stronger than bone but also much stiffer is the cause of failure for many implants. Living bone is responsive to its environment and implants that are stiffer than bone bear a greater proportion of the load, shielding the surrounding tissue from its normal stress levels. The result is that the surrounding tissue is resorbed and the implant becomes loose over time, often requiring revision surgery (Neuendorf et al. 2008). The table below lists some of the materials currently used and their tensile properties.

### Table 6.3 Mechanical properties of materials used in specific clinical applications (Park 2000)

<table>
<thead>
<tr>
<th>Material</th>
<th>Tensile strength (MPa)</th>
<th>Elongation to break (%)</th>
<th>Young’s Modulus (GPa)</th>
<th>Clinical application</th>
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<td>PMMA bone cement</td>
<td>30</td>
<td>3</td>
<td>2</td>
<td>Bone cement</td>
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<tr>
<td>Ti6Al4V</td>
<td>860 - 990</td>
<td>10 - 14</td>
<td>110</td>
<td>Femoral stem, knee</td>
</tr>
<tr>
<td>Stainless Steel 316L</td>
<td>1000</td>
<td>9</td>
<td>200</td>
<td>Femoral stem, bone plate for fracture fixation</td>
</tr>
<tr>
<td>UHMWPE</td>
<td>30</td>
<td>200</td>
<td>1</td>
<td>Cemented acetabular cup</td>
</tr>
<tr>
<td>Polysulphone</td>
<td>70</td>
<td>50</td>
<td>2.5</td>
<td>Bone plates, screws</td>
</tr>
</tbody>
</table>

In general, values for the mechanical properties of bone vary from one bone to another as well within different regions of the same bone. Rho et al. comment on a range of modulus values reported for trabecular bone in literature, varying from 1 to 20GPa (Rho et al. 1998). According to Neuendorf et al. (2008) natural bone elasticity modulus is in the interval of 0.09–18.6 GPa (Neuendorf et al. 2008), while the ultimate tensile strength of natural bone in tension is in the interval of 2 –130 MPa (Park 2000). Currently, there is a great deal of research concerning artificial
bone substitutes capable of performing in load-bearing situations. It is believed that a strong organic/inorganic composite would combine the flexibility, toughness and bioresorbability of a polymer with the stiffness, strength and osteoconductivity of a ceramic, creating a suitable substitution for bone.

In this study, samples which contained no plasticizers or HA provided the most satisfactory tensile results. A composition of 50 wt. % PDLLA and 50 wt. % BCA had a Young’s modulus of 0.26GPa and a tensile strength of 42.72MPa. While a composition of 40 wt. % BCA and 60 wt. % PDLLA had a Young’s modulus of 0.23GPa and a tensile strength of 34.61MPa. The sample containing 60 wt. % BCA and 40 wt. % PDLLA had the lowest performance with a Young’s modulus of 0.14GPa and a tensile strength of 23.93MPa. This may be due to an uneven dispersion of PDLLA throughout the sample. These results are within range of the tensile properties reported for bone.

<table>
<thead>
<tr>
<th>Composition (Wt. %)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation to break (%)</th>
<th>Young’s Modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDLLA 50</td>
<td>BCA 50</td>
<td>42.72</td>
<td>21.2</td>
</tr>
<tr>
<td>PDLLA 60</td>
<td>BCA 40</td>
<td>34.61</td>
<td>18.2</td>
</tr>
<tr>
<td>PDLLA 40</td>
<td>BCA 60</td>
<td>23.93</td>
<td>20.7</td>
</tr>
</tbody>
</table>

The addition of TBC decreased the Young’s modulus and tensile strength of the samples, while increasing percentage elongation. This is as expected due to the increase in free volume and molecular mobility with the addition of a plasticizer. It was anticipated that the addition of HA filler would increase the modulus of the samples, however the incorporation of HA decreased the Young’s modulus and tensile strength. Elongation generally decreased with HA content, as increasing the amount of filler decreased the amount of polymer available for elongation. HA particles behave as load carriers leading to good mechanical properties if they are present in small amounts and distributed homogeneously in the polymer matrix. If
aggregation or non-homogeneous distribution of the HA particles occurs, phase segregation may arise leading to non-homogeneity in the structure and poor adhesion to the matrix.

When HA begins to aggregate, it behaves like voids and weakens the composite, resulting in a decrease in mechanical properties (Serbetci et al. 2004). Debonding of the matrix from the particles also results in void formation, which lowers the tensile strength since cracks can more easily propagate through regions containing voids (Bleach et al. 2002). Therefore, HA agglomeration and poor adhesion between the HA and the polymer matrix would account for the reduction in tensile properties. It has been reported that the chemical treatment of HA used for such applications can cause an increase in the mechanical properties of the composite. PLA can be bonded chemically to HA surfaces by silane coupling agents, improving the interfacial property between the ceramic phase and the polymer matrix (Zhang et al. 2005).

6.4 Conclusions

From placing the samples in PBS for 10 weeks it was shown that the plasticizer increases water absorption and decreases hardness, while increasing the content of the plasticizer also increases the rate of water absorption and hardness loss. A decrease in hardness is observed with increase in water absorption due to the plasticization action of the water. Increasing HA content increases water absorption, this may be due to HA agglomerates, porosity or soluble impurities in the HA. HA decreases the hardness of the samples in PBS slightly, this may be due to the slight increase in the water absorption. The sample with the least amount of BCA had lower hardness values and a higher percentage of water absorption. Resin polarity influences extent of water sorption.

Samples which contained no plasticizers or HA provided the most satisfactory tensile results which were within range of the tensile properties reported for bone. The addition of TBC decreased the Young’s modulus and tensile strength of the samples, while increasing percentage elongation. Incorporation of HA decreased the Young’s modulus and tensile strength and decreased percentage elongation. HA
agglomeration and poor adhesion between the HA and the polymer matrix could account for the reduction in tensile properties. Chemical treatment of HA could cause an increase in the mechanical properties.

The results indicate that these compositions are promising materials which could be developed to produce a bone substitute fulfilling all of the objectives set out for this project. Upon mixing all of the constituents a putty like substance is achieved and with the adhesive properties of the cyanoacrylate it should have good adhesion to bone. Upon polymerization of the monomer the material then hardens. The composition which had the most appropriate properties were samples which contained no HA or plasticizers and comprised of a ratio of 50 wt. % PDLLA to 50 wt. % BCA. Using a purer form of HA which has less porosity and preventing HA agglomeration could improve the water absorption and hardness values as well as increasing the mechanical properties. Further analysis is needed with an alternative source of HA to indicate if a suitable bone filler substitute can be developed, with the appropriate load bearing properties.
6.5 References


Chapter 7
Chapter 7

7.0 Polylactide Compound Formulation Optimisation

7.1 Introduction
As indicated in the previous chapter, polylactide powder was blended with various ratios of cyanoacrylate monomer, plasticizer and hydroxyapatite. The resulting compositions were tensile tested and placed in buffered saline to observe the effects of water absorption and hardness with time. The compositions which had the most appropriate properties were the samples which contained no HA or plasticizers and comprised of a ratio of 0.5 PDLLA to 0.5 BCA. In this chapter further testing on this composition was completed with the addition of a new source of HA. The addition of the original source of HA in chapter 6 caused a deterioration in the sample properties due to HA agglomerates, porosity or soluble impurities. The new source of HA consisted of perfectly spherical particles which were homogenous in size, and it was hoped that the addition of this HA would help to improve the compounds properties.

A further composition of a ratio of 0.4 PDLLA to 0.6 BCA with the addition of the new source of HA was also tested. Although this ratio of PDLLA to BCA had inferior results to the properties of samples which consisted of a ratio of 0.5 PDLLA to 0.5 BCA (as reported in chapter 6), it was hoped that with the increased monomer liquid content, a higher weight percentage of HA could be worked into the matrix. With a higher content of HA, mechanical properties of the compound may be further improved. No plasticizers were used in any of the compositions, as the outcome of the testing as indicated in chapter 6 suggests that their addition is not favourable on the resulting properties.

The new HA used was supplied by Premier Biomaterials and as the original polymer stock had run out, a new batch of the same grade of polymer was purchased. Tensile samples were prepared and tested, with further tensile specimens also prepared and placed in buffered saline and tested at selected intervals. The effect of water absorption and hardness with time was also examined for these tensile samples. Double torsion and flexural testing was conducted on selected sample compositions. An investigation into the setting times for each composition was completed using a rheometer. The injectability and the tensile bond strength on selected sample
compositions were also analysed. Monomer conversion measurements were conducted in order to examine the effect sterilization would have on the reactivity of the BCA monomer.

### 7.2 Materials and Methods

#### 7.2.1 Materials

A new batch of the same grade of PDLLA polymer (Resomer R207S) was supplied by Boehringer Ingelheim (Germany). The polymer was fibrous in nature it could not be ground into a powder and so it was first heated to 120°C and allowed to cool to a dense amorphous solid, before being ground into smaller particles using a coffee grinder as described in chapter 3. The new batch of polymer was compared to the old batch using GPC which indicated that the two batches were similar, the old batch having a weight average molecular weight of $1.865 \times 10^5$ Da and the new batch having a weight average molecular weight of $2.028 \times 10^5$ Da. GPC analysis was preformed by the University of Manchester.

![Figure 7.1 Molecular weight distribution of old and new batch of PDLLA as determined by GPC](image)

The new HA supplied by Premier Biomaterials was manufactured using a controlled plasma sintered process which resulted in completely spherical particles of HA of less
than 10 µm as indicated in figure 7.2. Before use it was placed in a vacuum oven for 4 hrs at 140°C to remove any water present.

Also, a new batch of n-butyl 2 cyanoacrylate (BCA monomer) was used as supplied by Henkel Ireland Ltd. p-Toluenesulfonic acid (PTSA) supplied by Sigma Aldrich was added to the cyanoacrylate monomer in different quantities allowing the setting rate of the composite to be controlled.

The sample set included a total of 6 different samples compositions as indicated in table 7.1 below.

Table 7.1 Composition of samples prepared

<table>
<thead>
<tr>
<th>Sample</th>
<th>PDLLA wt. %</th>
<th>BCA wt. %</th>
<th>HA wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.5: 0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>30</td>
<td>40</td>
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<tr>
<td>2</td>
<td>35</td>
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<td>3</td>
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<td>40</td>
<td>20</td>
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<tr>
<td></td>
<td>(0.6:0.4)</td>
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<tr>
<td>4</td>
<td>20</td>
<td>30</td>
<td>50</td>
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<tr>
<td>5</td>
<td>24</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>42</td>
<td>30</td>
</tr>
</tbody>
</table>

The first three samples have a ratio of 0.5 PDLLA to 0.5 BCA with the addition of 20 to 40 wt. % HA. These compositions were chosen as 40 wt. % was the highest
percentage of HA which could be physically incorporated into the polymer system. Compositions with lower weight percentages of HA were then chosen for comparative reasons. Samples 4 to 6 have a ratio of 0.4 PDLLA to 0.6 BCA with the addition of 30 to 50 wt. % HA. 50 wt. % was the highest percentage of HA which could be physically incorporated into this BCA-PDLLA composition. Each composition could be easily mixed by hand to create homogenous blends in which the liquid components completely wetted all the dry powder components. All samples were prepared simply by mixing manually using a spatula at ambient temperature.

7.2.2 Tensile testing, Water Absorption and Hardness
Specimens of each composition as indicated in table 7.1 were prepared for tensile testing. The components were mixed manually and transferred to a mould, which was then pressed and left to set. Specimens were dogbone shaped with a gauge length of approximately 13mm, a width of 3.5mm and thickness of 3mm after ASTM standard D638-08. The samples were then tensile tested with a crosshead speed of 10mm/min using a 1kN load cell. Three samples of each composition were tested.

Further samples of the same compositions were prepared and placed in phosphate buffered saline (PBS) at a pH of 7.2 and incubated at 37 °C. At selected intervals over an eight week period, samples were removed from the PBS, patted dry and weighed. Shore A hardness values were also measured. The samples were then tensile tested with the same crosshead speed of 10mm/min using a 1kN load cell. Three samples of each composition were tested.

7.2.3 Double torsion
Double torsion specimens of samples 1, 3, 4 and 6 as indicated in table 7.1 were prepared. The components were mixed manually and transferred to a mould, which was then pressed and left to set. Samples measured 3.0 x 65 x 25mm and a sharp groove 0.5mm deep was cut down the centre of each specimen with a slot also cut at one end of the specimen using a diamond wafer blade. The DT test was performed using an Instron Universal testing machine and some specially made fixtures for the support and loading of the samples. These consisted of two parallel rollers of 3mm diameter and spaced 20mm apart and load applied at a constant rate (0.1 mm min⁻¹) to
the slotted end via two 3mm diameter ball bearings spaced 10mm apart. The specimen was subjected to four-point bend loading, during which the crack initiated and propagated, along the centre of the specimen, within the groove. The values of the fracture toughness could then be calculated with three samples of each composition tested.

7.2.4 Flexural test
Immediately after testing the DT specimens, the broken halves were cut up into three-point bend specimens, measuring 3.0 x 10.0 x 65mm. The three-point bend test was performed using an Instron Universal testing machine and some specially made fixtures for the support and loading of the samples. Four specimens were tested for each of the compositions 1, 3, 4 and 6 as indicated in table 7.1.

7.2.5 Rheology
The rheological properties of the compositions as indicated in table 7.1 while setting were analysed using a TA Instrument AR1500ex Rheometer in parallel plate mode. The diameter of the upper parallel plate measured 20mm and the gap between the two plates was set at 1.5mm. Approximately 2g of each composition was mixed manually and placed between the plates of the rheometer. The top was lowered and any excess material was carefully removed. The rheometer was run at a frequency of 1Hz with a 1% strain at 25°C unless otherwise indicated. The sample components were mixed for 30 seconds with a time of 60 – 100 seconds elapsed from the start of mixing to the beginning of measurements taken by the rheometer. Samples were run for 60 minutes. A sample of the bone cement Simplex from Stryker was also tested for comparative reasons. One sample of each composition was analysed.

7.2.6 Injectability
The injectability of the compositions were analysed by examining the force needed to extrude the composite through a syringe. The test was performed using an Instron Universal testing machine and a specially made fixture consisting of a long plastic tube which supported the syringe during the injection process. Compositions were mixed by hand for 30 seconds and then transferred into a syringe. The syringe was then placed into the plastic tube and the force needed to inject the sample at a fixed rate of 25 mm/min was measured. Two separate syringes were used, with both
syringes used for Stryker products. The first syringe used is normally used for the product HydroSet and consists of a syringe with a barrel of an inner diameter of 16mm. Connected to this is a cannula with an inner diameter of approximately 2.5mm and 100mm in length. The second syringe used in this experiment is normally used for the product Spineplex. It consisted of an inner barrel of 16mm with an opening of an inner diameter of 8mm, no cannula was attached to this syringe for this experiment. One sample of each composition was analysed.

7.2.7 Tensile bond strength
To determine the adhesive properties of the polymer compound, the tensile bond strength of samples of samples 1, 3, 4 and 6 as indicated in table 7.1 were analyzed with a biomedical titanium alloy. Two circular disc sizes were formed with one having a hole drilled in its centre. Using a layer of the polymer compound, the discs were bound together and excess material was removed. All bound discs were placed under a mass of 1Kg to produce a sample layer of uniform thickness and the material was allowed to harden for 1 hour. Each set of discs were then placed in 25 ml of distilled water and incubated at 37 °C for 1 day before being removed. Tensile bond strength was tested using an Instron 4082 Universal testing machine and a plunger apparatus to separate the discs. To ensure an even loading, a constant rate 1.0 mm min$^{-1}$ was applied. Maximum tensile forces were collected and converted into bond strength. One sample of each composition was analysed.

7.2.8 Monomer reactivity measurement
In order to examine the effect sterilization would have on the BCA monomer, four BCA samples were sent to Isotron for gamma irradiation at 30kGry. Sterilization of the monomer by gamma irradiation may cause the monomer to polymerize, changing the viscosity of the monomer and in turn effect the polymer compound properties. The first sample was a vial of the BCA monomer as received from the supplier Henkel. The remaining BCA samples each contained one of three different free radical stabilizers; hydroquinone, benzoquinone and tertbutylhydroquinone at a concentration of 5000ppm. All of the samples sent for irradiation were composed of the old batch of BCA. The reactivity of each of the gamma irradiated samples along with two samples which had not been gamma irradiated (the old batch and the new batch) were then tested by monitoring the change in temperature of the anionic
polymerization of each of the samples. The BCA as supplied by Henkel is manufactured by a batch process and the reactivity of the monomer can vary from batch to batch. Each reaction mixture consisted of 2.6 x 10^-1 solution of butyl cyanoacrylate monomer in THF and the initiator used was tri-phenyl-phosphine (TPP) solution in THF. Each reaction was carried out by addition of 25mls of the reaction mixture to a 50ml insulated glass reaction vessel. A thermocouple was sealed in place and a stirring was set at a constant speed using a magnetic stirrer. The data acquisition was started and the baseline was monitored for 20 minutes to ensure thermal equilibrium had been reached. The reaction was initiated by a rapid injection of 1ml of the TPP solution into the stirring reaction mixture using a disposable syringe. One sample of each composition was analysed.

7.3 Results and Discussion

7.3.1 Water absorption

Figures 7.3 and 7.4 show the percentage weight gain for samples which were stored in PBS for 8 weeks at 37°C.

Figure 7.3 Percentage weight change as a function of time for samples containing a ratio of 0.5 PDLLA to 0.5 BCA and different wt. % of HA which have been stored in PBS @ 37°C
Figure 7.3 shows that samples containing the least amount of HA have the greatest percentage weight increase. After the eight week period, samples with the most HA (30 wt. % and 40 wt. %) have a weight gain of approximately 1.1 %, while the sample with the least amount of HA has a slightly higher weight gain of 1.3%.

Samples in figure 7.3 contain an equal weight ratio of BCA to PDLLA with various amounts of HA, while samples in figure 7.4 contain a ratio of 0.6 BCA to 0.4 PDLLA and various wt. % of HA. Figure 7.4 shows that samples containing a higher amount of BCA have a slightly higher water uptake. Similarly, these samples show the same trend to that of samples in figure 7.3 with a lower HA content leading to a greater water uptake and a higher percentage weight gain. After the eight week period, samples containing 30 wt. % HA had a percentage weight increase of approximately 2% while samples containing 50 wt. % HA had a weight gain of just 1.25%.

![Figure 7.4 Percentage weight change as a function of time for samples containing a ratio of 0.4 PDLLA to 0.6 BCA and different wt. % of HA which have been stored in PBS @ 37°C](image)

In the initial studies conducted in chapter 6 with similar compositions it was noted that increasing HA content slightly increased water absorption with a more apparent water uptake after a period of 44 days. The resultant increase in water absorption observed was most likely due to HA agglomerates, porosity or soluble impurities in
the HA. As a different source of HA was used in these samples and the HA used consisted of perfectly spherical particles which were homogenous in size, the opposite effect was evident and water absorption decreased as HA content increased. This is similar to work completed by Deb et al. (1995), who found that in general, water uptake is reduced by the presence of HA in polymer composites.

Figures 7.5 and 7.6 show the percentage weight gain for samples containing the same wt. % of HA but different ratios of PDLLA to BCA. The initial water absorption testing completed in chapter 6, showed that samples containing only PDLLA and BCA in different % wt. ratios had a gradual increasing trend in water absorption. The sample with the lowest content of BCA (40 wt. %) had the greatest percentage weight increase over all. However, the sample containing 60 wt. % BCA and 40 wt. % PDLLA had a slightly higher percentage weight gain compared to the 50 wt. % BCA - 50 wt. % PDLLA sample, similar to the results found with the new compositions as shown in figures 7.5 and 7.6.

![Figure 7.5 Percentage weight change as a function of time for samples containing 30% HA and two different ratios of PDLLA to BCA which have been stored in PBS @ 37°C](image)

Figure 7.5 Percentage weight change as a function of time for samples containing 30% HA and two different ratios of PDLLA to BCA which have been stored in PBS @ 37°C
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7.3.2 Hardness Shore A

Figures 7.7 and 7.8 show the shore A hardness values for samples which were stored in PBS for 8 weeks at 37°C. There is little difference in the values obtained after 7 days of the test compared with the hardness values obtained at the end of the eight week period.

![Diagram](image_url)

Figure 7.6 Percentage weight change as a function of time for samples containing 40 wt. % HA and two different ratios of PDLLA to BCA which have been stored in PBS @ 37°C

![Diagram](image_url)

Figure 7.7 Shore A hardness values as a function of time for samples containing a ratio of 0.5 PDLLA to 0.5 BCA and different wt. % of HA which have been stored in PBS @ 37°C
7.3.3 Tensile testing

Figures 7.9 to 7.11 show the tensile properties for samples tested as a function of HA content. All BCA used to prepare the samples contained 0.1 to 0.2% PTSA which helped to slow down the polymerization process, allowing adequate time for samples to be moulded.

Figure 7.9 shows that with increased HA content there is an increase in Young’s modulus. Statistical analysis preformed on the results show that this is true as the variance ratio $F$ is greater than the critical variance ratio. The statistical analysis can be seen in table 1 and 2 in appendix B. The greatest modulus of 1.3GPa was achieved in the samples consisting of a ratio of 0.6 BCA to 0.4 PDLLA with 50 wt. % HA, the sample composition with the highest HA content. Samples with a ratio of 0.5 BCA to 0.5 PDLLA generally had greater average moduli than those of samples with a ratio of 0.6 BCA to 0.4 PDLLA, which contained the same wt. % of HA. However statically there is no difference between the modulus of samples with a different polymer ratio and the same HA content (see table 25, appendix B). The elastic modulus of a particulate–polymer composite is generally determined by the elastic properties of its components (particle and matrix), particle loading and aspect ratio. Young’s modulus is markedly improved by adding inorganic particles to a
polymer matrix since the hard particles have much higher stiffness values than the matrix (Fu et al. 1999).

Figure 7.9 Young’s modulus as a function of HA content

Figure 7.10 shows that the greatest tensile strengths are achieved in samples which do not contain any HA, with the highest average tensile strength of 40MPa achieved for samples which contained a ratio of 0.5 BCA to 0.5 PDLLA and no HA. Although the addition of HA initially decreases tensile strength by nearly half, as the percentage weight content of HA increases the general trend is for the average tensile strength also to increase slightly. However statistical analysis shows that with the addition of HA there is no statistical difference achieved by further increasing HA content (see tables 3 and 4 in appendix B). As with the modulus values, samples consisting of a ratio of 0.5 BCA to 0.5 PDLLA had higher average tensile strengths than those of samples with a ratio of 0.6 BCA to 0.4 PDLLA, which contained the same wt. % of HA. However statically there is no difference between the strengths of these samples (see table 26, appendix B). For particulate composites, composite strength relies on the effectiveness of stress transfer between matrix and fillers. Factors like particle size, particle/matrix interfacial strength and particle loading significantly affect the composite strength. The ultimate strength of a composite depends on the weakest fracture path throughout the material. Hard particles affect the strength in two ways. One is the weakening effect due to the stress concentration...
they cause, and another is the reinforcing effect since they may serve as barriers to crack growth. In some cases, the weakening effect is predominant and thus the composite strength is lower than the matrix; and in other cases, the reinforcing effect is more significant and then the composites will have strengths higher than the matrix (Fu et al. 2008).

Sample compositions tested in chapter 6 had a Young’s modulus and tensile strength which decreased with the incorporation of HA. HA agglomeration was responsible for this reduction in tensile properties. The HA incorporated into these samples now being discussed, is purer and consists of perfectly spherical particles which are homogenous in size, allowing for homogeneous distribution which helped increase Young’s modulus and tensile strength.

![Figure 7.10 Tensile strength as a function of HA content](image)

Figure 7.11 shows the percentage elongation to break for samples as a function of HA content. As expected the addition of HA decreased average percentage elongation to break, with increasing the HA content having a further decreasing effect. Statistical analysis preformed on the results show that this is true for the samples containing a ratio of 0.5 BCA to 0.5 PDLLA, as the variance ratio F is greater than the critical variance ratio (see table 5, appendix B). Samples containing a ratio of 0.6 BCA to 0.4 PDLLA and no HA had an average elongation to break of
25%. The addition of 30 wt. % HA decreases this to an average of 11% and with the addition of a further 10 wt. % HA, percentage elongation to break is decreased to 4% at 40 wt. % HA content. At a HA content of 50 wt. %, elongation to break is decreased further to an average of 2%. However, statistical analysis preformed on these results shows this not to be true with no statistical difference between the elongation to break as a function of HA content (see table 6 appendix B).

According to Neuendorf et al. (2008) natural bone elasticity modulus is in the interval of 0.09–18.6 GPa (Neuendorf et al. 2008), while the ultimate tensile strength of natural bone in tension is in the interval of 2 –130 MPa (Park 2000). A tensile strength of 40MPa was achieved for samples which contained a ratio of 0.5 BCA to 0.5 PDLLA and no HA while the same samples had a Young’s modulus of 0.25 GPa. Samples with the greatest modulus contained a ratio of 0.6 BCA to 0.4 PDLLA and 50 wt. % HA with a value of 1.3GPa, the same samples had an average tensile strength of 20MPa. While these values are lower then those found in human cortical bone, the results are higher then a lot of commercially available bone substitutes. The market leaders of injectable bone graft substitutes are calcium phosphate pastes which are generally strong in compression but have low tensile strength. Compressive strength of calcium phosphate cements has been reported to range from 26 to 60 MPa which is comparable to cancellous bone; however their tensile strength is significantly lower.
strengths are reported in the range of 1.09 to 1.21 MPa (Welch et al. 2002). Their main drawback remains their limited resistance to tensile and shear forces, making them vulnerable to cracking and subsequent material failure (Van der Stok et al. 2011).

As the tensile testing was conducted, it was noted that deviations of results could occur depending on the time elapsed between sample preparation and testing. After noting this, an attempt was made to ensure all samples had the same time delay between preparation and testing.

Figure 7.12 Stress strain curve for samples containing 40 wt. % HA, 30 wt. % BCA (0.2% PTSA) and 30 wt.% PDLLA with samples A to C prepared one week before testing and sample D prepared 2 days before testing.

Figure 7.12 shows the stress strain curves for samples of the same composition, with A to C prepared one week before testing and sample D prepared two days before testing. Although sample D appeared to be fully set at the time of testing, it is suspected that residual monomer was still present in the sample after two days, allowed for a slight plasticization of the sample decreasing strength and increasing elongation to break.
Figures 7.13 to 7.15 show the tensile properties for samples consisting of 50 wt. % HA, 20 wt. % PDLLA and 30 wt. % BCA tested as a function of the % wt. content of PTSA in the BCA.

Figure 7.13 Young’s modulus of samples containing 50 wt. % HA and a ratio of 0.6 BCA to 0.4 PDLLA as a function of PTSA content in the BCA used.

Figure 7.14 Tensile strength of samples containing 50 wt. % HA and a ratio of 0.6 BCA to 0.4 PDLLA as a function of PTSA content in the BCA used.
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The addition of PTSA inhibits the polymerisation of the BCA monomer allowing more working time for the composite material. Since the anionic cure of cyanoacrylate proceeds as a result of a basic catalysis, acids such as PTSA are used as anionic polymerization inhibitors (Coover et al. 1990). Acid strength and level are important variables when choosing a stabilizer. High levels of acids can over stabilize and make polymerization speed sluggish. Tensile testing of samples with various concentrations of PTSA was completed to identify if the percentage of PTSA used had any effect on the tensile properties once the samples had fully set. Samples were prepared and left in a desiccator for five weeks to ensure enough time had elapsed to allow the monomer to fully polymerise.

Figures 7.13 to 7.15 show that with increasing PTSA content there is a decrease in the tensile properties. An average Young’s modulus of 1.3GPa is obtained for samples which contain BCA with a concentration of 0.1% PTSA. Increasing the PTSA content to 0.2% decreases the Young’s modulus to an average of 0.8GPa, further increasing the PTSA content to 0.5% does not have any further effect on the Young’s modulus with an average of 0.8GPa also obtained for these samples. There is also a decreasing effect on the tensile strength with average results ranging from 20MPa for 0.1% PTSA samples, 16MPa for 0.2% PTSA samples and 11MPa for
0.5% PTSA samples. The percentage elongation to break also decreases as PTSA content increases. An average of 5% is obtained for samples containing 0.1% PTSA, this is reduced to 2% for samples containing 0.5% PTSA.

7.3.4 Tensile results of samples in PBS

Figures 7.16 to 7.24 illustrate the tensile properties for samples which have been stored in PBS at 37°C as a function of time. Day 0 identifies samples tested which have not been stored in PBS. All BCA monomer used contained 0.1 wt. % PTSA. Figures 7.16 and 7.17 show the effect had on the Young’s modulus over an eight week period. As expected, samples with a greater HA content had higher modulus values. Overall, the young’s modulus did not seem to be effected over time with the results gathered for samples on day 0 (samples which had not been placed in PBS) generally in the same range as the results gathered for the same sample compositions which had spent 56 days in PBS at 37°C. Samples consisting of a ratio of 0.5 BCA to 0.5 PDLLA and 20wt. % HA had a Young’s modulus of 0.75GPa while samples stored in PBS at 37°C after 56 days had a Young’s modulus of 0.8GPa. Statistical analysis performed on the results shows that there is no difference in the modulus values obtained as a function of time stored in PBS at 37°C as the variance ratio F is less than the critical variance ratio. The statistical analysis for the Young’s modulus of the various samples can be seen in tables 7 to 12 in appendix B.

![Figure 7.16 Young’s modulus as a function of time for samples containing a ratio of 0.5 BCA to 0.5 PDLLA and various wt. % of HA which have been stored in PBS @ 37°C](image-url)
Figure 7.17 Young’s modulus as a function of time for samples containing a ratio of 0.6 BCA to 0.4 PDLLA and various wt. % of HA which have been stored in PBS @ 37°C

Figure 7.18 Tensile strength as a function of time for samples containing a ratio of 0.5 BCA to 0.5 PDLLA and various wt. % of HA which have been stored in PBS @ 37°C
Figures 7.18 and 7.19 show the effect had on tensile strength for the same samples. Both figures show a slight reduction in the average tensile strength, more evident in samples containing the highest percentage weight of HA after the eight week period, these samples also initially had lower tensile strengths. For samples containing 50 wt. % HA and a ratio of 0.6 BCA to 0.4 PDLLA an average tensile strength achieve on day 0 of 20MPa is reduced to an average of 14MPa after 56 days in PBS at 37°C. Statistical analysis performed on the tensile results of each of the sample compositions as a function of time showed that at low HA contents there was no change in the tensile strength, however at the highest HA contents, there was a decrease in tensile strength as a function of time. See tables 13 to 18 in appendix B for the statistical analysis of each sample as a function of time stored in PBS. Composite strength relies on the effectiveness of stress transfer between matrix and fillers and factors like particle/matrix interfacial strength significantly affects the composite strength. With the absorption of water over the eight week period can act to further weaken the particle/matrix interfacial bond further decreasing the strength.
Figures 7.20 and 7.21 illustrate the effect had on percentage elongation to break for samples stored in PBS at 37°C as a function of time. Figure 7.20 clearly shows an increase in percentage elongation as a function of days stored in PBS solution. Samples containing a ratio of 0.5 BCA to 0.5 PDLLA and 20 wt. % HA had an initial average elongation to break of 4%, which was increased to 16% after 56 days in PBS at 37°C. In figure 7.21 samples which contain the most HA (50 wt. %) had an increase in percentage elongation to break over the eight week period, these samples had an average elongation to break of 3% at day 0 which doubled to 6% after 56 days in PBS at 37°C. For samples which contain less HA (30 and 40 wt. %), the data is quite scattered with high standard deviations, but there is a slight increase at the end of the eight week period. Statistical analysis performed on each composition as a function of time showed that there was a difference in the elongation to break over the 56 day period. See tables 19 to 24 in appendix B for these results.
Figures 7.22 to 7.24 show the tensile properties of samples all containing the same weight percentage of HA (30 wt. %) to clearly illustrate the effect the two different ratios of BCA to PDLLA had on the resultant properties. The average Young’s modulus as illustrated in figure 7.22 is generally greater for the samples with a ratio of 0.5 BCA to 0.5 PDLLA compared to samples containing a ratio of 0.6 BCA to 0.4 PDLLA throughout the eight week period. However statistical analysis preformed on the young’s modulus results as a function of BCA to PDLLA ratio show that there is no statistical difference in the results obtained for a polymer ratio of 0.5 BCA- 0.5 PDLLA to results found for a polymer ratio of 0.6 BCA-0.4 PDLLA having the same HA content. See table 25 in appendix B for the statistical results. As stated earlier, the modulus values obtained at the end of the 56 days are within range of the values obtained for samples which had not been aged in PBS.
Figure 7.22 Young’s modulus as a function of time for samples containing 30 wt.% HA and two different ratios of BCA to PDLLA which have been stored in PBS @ 37°C.

Figure 7.23 shows the tensile strength for samples as a function of time and again, samples with a ratio of 0.5 BCA to 0.5 PDLLA had higher average strength values compared to samples containing a ratio of 0.6 BCA to 0.4 PDLLA throughout the eight week period. However, statistical analysis preformed on the tensile strength results as a function of BCA to PDLLA ratio show that there is no statistical difference in the results obtained for a polymer ratio of 0.5 BCA-0.5 PDLLA to results found for a polymer ratio of 0.6 BCA-0.4 PDLLA having the same HA content. See table 26 in appendix B for the statistical results. As stated earlier, the tensile strength values obtained at the end of the 56 days are slightly lower than the values obtained for samples which had not been aged in PBS for samples with the highest HA contents.

Figure 7.24 shows the percentage elongation at break for the same samples over the eight week period. Samples containing a ratio of 0.5 BCA to 0.5 PDLLA have average values lower than that of samples with a ratio of 0.6 BCA to 0.4 PDLLA after day 7 of being stored in PBS at 37°C. At day 28 samples with a 50-50 ratio have a similar average percentage elongation to break, however, these average values are greatly increased compared with the 60-40 ratio samples after 56 days. Statistical analysis preformed on the percentage elongation to break results as a function of
BCA to PDLLA ratio show that there is no statistical difference in the results obtained for a polymer ratio of 0.5 BCA- 0.5 PDLLA to results found for a polymer ratio of 0.6 BCA-0.4 PDLLA having the same HA content. See table 27 in appendix B for the statistical results.

Figure 7.23 Tensile strength as a function of time for samples containing 30 wt. % HA and two different ratios of BCA to PDLLA which have been stored in PBS @ 37°C

Figure 7.24 Percentage elongation to break as a function of time for samples containing 30 wt. % HA and two different ratios of BCA to PDLLA which have been stored in PBS @ 37°C
7.3.5 Fracture toughness and Flexure

Figures 7.25 to 7.27 illustrate the results obtained from the double torsion and flexural testing of the samples 1, 3, 4 and 6 as indicated in table 7.1. All BCA monomer used contained 0.1% PTSA.

![Graph showing fracture toughness values](image)

**Figure 7.25 Fracture toughness values obtained from double torsion testing**

Figure 7.25 shows the fracture toughness values obtained from double torsion testing. For samples which have the same ratio of BCA to PDLLA, average fracture toughness values increased as HA content increased. In brittle matrices, the reverse holds and the brittleness is reduced. Samples containing 40 wt. % HA and a ratio of 0.5 BCA to 0.5 PDLLA had a higher average $K_{IC}$ at 2.5MPa m$^{-1/2}$ than samples containing more HA with 50 wt. % HA and a ratio of 0.6 BCA to 0.4 PDLLA which had an average $K_{IC}$ value of 2MPa m$^{-1/2}$. This was also true for samples with 20 wt. % HA and a ratio of 0.5 BCA to 0.5 PDLLA having an average higher $K_{IC}$ of 2.3MPa m$^{-1/2}$ than samples containing more HA with 30 wt. % HA and a ratio of 0.6 BCA to 0.4 PDLLA which had an average $K_{IC}$ value of 1.6MPa m$^{-1/2}$. Statistical analysis of the results showed that there was an increase in fracture toughness with increased HA content for samples with a ratio of 0.6 BCA to 0.4 PDLLA, however there was no statistical difference in fracture toughness by increasing HA content in
samples with a polymer ratio of 0.5 BCA to 0.5 PDLLA. See tables 28 and 29 in appendix B for the statistical analysis of the fracture toughness results.

Figure 7.26 and 7.27 illustrate the flexural modulus and the flexural strength values obtained from three point bend testing respectively. Increasing the HA content increases the average modulus with samples containing the most HA having the highest average modulus values. For samples which contain a ratio of 0.6 BCA to 0.4 PDLLA increasing the HA content from 30 wt. % to 50 wt. % increases the average flexural modulus from 1.3 to 1.7GPa. For samples containing a ratio of 0.5 BCA to 0.5 PDLLA increasing the HA content from 20 wt. % to 40 wt. % does not have the same effect with the average modulus value of 1.5GPa obtained for both sets of samples. The modulus is increased by adding HA to a polymer matrix since the particles have much higher stiffness values than the matrix. By having a ratio of 0.6 BCA to 0.4 PDLLA, a greater weight percentage of HA can be worked into the matrix giving the highest average modulus obtained at 1.7GPa. However statistical analysis preformed on the flexural modulus results as a function of HA content show that there is no statistical difference in the results obtained. See table 30 and 31 in appendix B for the statistical results.

![Figure 7.26 Flexural modulus values obtained from three point bend testing](image-url)
The average flexural strength for samples as shown in figure 7.27 were greatest for samples with the least amount of HA and in both sets of samples of different polymer ratios, increasing HA content decreases strength. Hard particles can weaken the material due to the stress concentration they cause. This decrease in strength was shown to be statistically untrue for samples containing a ratio of 0.5 BCA to 0.5 PDLLA. For samples containing a ratio of 0.6 BCA to 0.4 PDLLA the decrease in average strength is greater and increasing the HA content from 30 wt. % to 50 wt. % the average strength decreases from 37MPa to 28MPa, which was shown to be a statistically true result. See tables 32 and 33 in appendix B for the statistical analysis.

Figure 7.27 Flexural strength values obtained from three point bend testing

Moore et al. have reported a fracture toughness in the range of 0.1 MPa m$^{-1/2}$ for cancellous bone to 6.0 MPa m$^{-1/2}$ for cortical bone (Moore et al. 2001). The flexural strength of cortical bone has been reported in the range of 135 to 193 MPa (Wagoner Johnson and Herschler 2011) while the flexural modulus has been reported in the range of 14 to 21GPa (Currey 2006). One of the best performing samples had a composition of 40 wt. % HA with a ratio of 0.5 BCA to 0.5 PDLLA. Results showed it had an average flexural modulus of 1.5GPa, an average flexural strength of 34MPa and an average fracture toughness of 2.5MPa m$^{-1/2}$. While these are lower then those reported for cortical bone, they are much higher than those reported for calcium phosphate pastes. A study investigating the mechanical
properties of injectable bone graft substitute Norian SRS reported an average flexural strength of 0.468 MPa with a fracture toughness of 0.14 MPa m$^{1/2}$ (Morgan et al. 1997). HydroSet has a reported flexural modulus of 125–240 MPa and a flexural strength of 8-10MPa, while the same study, reported that no significant fracture toughness was obtained for HydroSet (Clarkin et al. 2009).

### 7.3.6 Rheology

The rheological properties of the compositions as indicated in table 7.1 while setting were analysed using a TA Instrument AR1500ex Rheometer. In this system the test material is held between two parallel plates, the bottom of which is fixed. A torque is applied to the top plate, forcing it to oscillate and the response of the material is measured by the rotation of the same upper plate. The rheometer was run at a frequency of 1Hz with a 1% strain at 25°C unless otherwise indicated. Figures 7.28 to 7.36 illustrate the storage modulus of samples after the components were initially mixed as a function of time. A time of 60 – 100 seconds has elapsed from the start of mixing to the beginning of measurements taken by the rheometer. The storage modulus corresponding to the elastic behaviour of the material, and each graph initially shows a low storage modulus which gradually increases as the material hardens and changes from having predominantly viscous properties after mixing, to having predominantly elastic properties once set, giving an indication of the working times for each of the samples. All testing was carried out at 25°C unless otherwise stated as in figure 7.27 and all BCA monomer used contained 0.1% PTSA unless otherwise stated as in figures 7.33 and 7.34.

From figure 7.28 it can be clearly seen that HA content has a great effect on setting times as the sample containing the most HA sets the fastest and with decreased HA content working times are increased. This is also clearly shown in figures 7.29 and 7.30. Figure 7.29 illustrates the storage modulus for samples which all contain the same ratio of 0.6 BCA to 0.4 PDLLA but with different HA content. As the HA content increases, setting times decrease. The same trend can be seen in figure 7.30 for samples which contain a ratio of 0.5 BCA to 0.5 PDLLA with various HA content.
Figure 7.28 Storage modulus for all six samples after the mixing of components analysed using a TA Instrument AR1500ex Rheometer.
Figures 7.29 and 7.30 illustrate the effect had on storage modulus for samples containing different ratios of BCA to PDLLA and the same % wt. content of HA. Figure 7.31 shows that samples which have a ratio of 0.5 BCA to 0.5 PDLLA set
faster than samples which have a ratio of 0.6 BCA to 0.4 PDLLA. The same trend can be seen in figure 7.32 for samples which contain a higher wt. % of HA, as samples with a higher ratio of BCA take longer to polymerise.

Figure 7.31 Storage modulus for samples containing 30 wt. % HA with two different ratios of BCA to PDLLA after mixing

Figure 7.32 Storage modulus for samples containing 40 wt. % HA with two different ratios of BCA to PDLLA after mixing

Figures 7.33 and 7.34 illustrate the effect on the storage modulus for samples of the same basic composition (50 wt. % HA, 0.6 BCA-0.4 PDLLA), the only difference
being the content of PTSA in the monomer. Three different concentrations of PTSA were added to the BCA, 0.1, 0.2 and 0.3 w/w %. As expected increasing PTSA content increases setting times as it retards polymerization of the BCA monomer. Using 0.1% PTSA, the samples set within 10 minutes. Doubling that concentration to 0.2% increases setting time to approximately 50 minutes, while further increasing the concentration to 0.5% results in samples which have not set after 1 hour.

Figure 7.33 Storage modulus for samples consisting of a ratio of 0.6 BCA to 0.4 PDLLA with 50wt. % HA using BCA with three different PTSA contents

Figure 7.34 Storage modulus for samples consisting of a ratio of 0.6 BCA to 0.4 PDLLA with 50wt. % HA using BCA with three different PTSA contents
Figures 7.35 and 7.36 illustrate the storage modulus of the fastest setting sample (50 wt. % HA, 0.6 BCA-0.4 PDLLA) compared with the storage modulus of Simplex bone cement. Simplex has a working time of approximately 10 minutes. From figure 7.36, after 10 minutes the storage modulus of simplex is approximately 1MPa, from this the working time of the composite sample can be identified. At the corresponding storage modulus, the fastest setting sample has a working time of approximately 8.5 minutes.

**Figure 7.35** Storage modulus for samples consisting of a ratio of 0.6 BCA to 0.4 PDLLA with 50wt. % HA (+0.1%PTSA) and a sample of Simplex

**Figure 7.36** Storage modulus for samples consisting of a ratio of 0.6 BCA to 0.4 PDLLA with 50wt. % HA (+0.1%PTSA) and a sample of Simplex
Figure 7.37 illustrates the storage modulus of samples as a function of time conducted at two different temperatures. For the initial setting times, a temperature rise does not have an effect on the working times.

![Storage modulus for samples containing 40 wt. % HA with a ratio of 0.5 BCA to 0.5 PDLLA after mixing carried out at 25°C and 37°C](image)

The rheological properties of any injectable bone substitute during its curing phase is very important in determining its mixing/handling characteristics and viscoelastic properties. With these compositions we can see that HA and BCA content have the greatest effect on setting times. The addition of an acid to the BCA monomer further allows for the adjustment of these times to times of over one hour, however to much acid may lead to a decrease in the resultant products mechanical properties as seen in section 7.3.3.

Table 7.2 lists the mechanical properties of all six samples and their setting times (using the working time of simplex as a reference). All results are average values and are for compositions in which the BCA monomer used contained 0.1 wt. % PTSA.
Table 7.2 Mechanical properties and setting times for all samples (with a 0.1% PTSA content in BCA monomer)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Young’s modulus (GPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation at break (%)</th>
<th>Fracture toughness MPa m$^{1/2}$</th>
<th>Flexural Modulus (GPa)</th>
<th>Flexural Strength (MPa)</th>
<th>Setting time (min) using simplex as ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 wt. % HA</td>
<td>0.75</td>
<td>21</td>
<td>4</td>
<td>2.28</td>
<td>1.54</td>
<td>37</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>30 wt. % HA</td>
<td>0.87</td>
<td>25</td>
<td>5</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>23.6</td>
</tr>
<tr>
<td>40 wt. % HA</td>
<td>0.93</td>
<td>22</td>
<td>4</td>
<td>2.50</td>
<td>1.52</td>
<td>34</td>
<td>13.8</td>
</tr>
<tr>
<td>0.6 BCA - 0.4 PDLLA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 wt. % HA</td>
<td>0.72</td>
<td>15</td>
<td>11</td>
<td>1.63</td>
<td>1.29</td>
<td>37</td>
<td>43.3</td>
</tr>
<tr>
<td>40 wt. % HA</td>
<td>0.84</td>
<td>17</td>
<td>4</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>21.2</td>
</tr>
<tr>
<td>50 wt. % HA</td>
<td>1.31</td>
<td>20</td>
<td>3</td>
<td>1.96</td>
<td>1.69</td>
<td>28</td>
<td>8.8</td>
</tr>
</tbody>
</table>
7.3.7 Injectability

The injectability of the compositions were analysed by examining the force needed to extrude the composite through a syringe. Compositions were mixed by hand for 30 seconds and then transferred into a syringe. The force needed to inject the sample at a fixed rate of 25 mm/min was then measured. To be injected by hand, the applied force is limited to about 200 N. However, in surgical practice many bone cements are applied under high pressures by means of injection guns (Bohner and Baroud 2005).

![Figure 7.38 extrusion forces for samples extruded using HydroSet syringes](image)

Figure 7.38 shows the extrusion forces measured for different compositions using the HydroSet syringe as a function of the overall percentage displacement for each sample. The HydroSet syringe consists of a barrel with an inner diameter of 16mm and a cannula with an inner diameter of approximately 2.5mm and 100mm in length. For all compositions tested the force needed to extrude the samples exceeded 200 N. All tests were stopped before all of the sample could be completely extruded as the syringe itself started to buckle as the force increased.

Figure 7.39 shows the extrusion forces measured for different compositions using the Spineplex syringe as a function of the overall percentage displacement for each sample. The Spineplex syringe consists of a barrel with an inner diameter of 16mm
and no cannula. Each composition was easily extruded through the opening and a max force of just 31 N was needed for both samples. This suggests that the inner diameter of the HydroSet cannula is too small to enable effective extrusion by hand. Further investigation is needed to find the optimum syringe/cannula diameters to ensure effective injectability for each composition.

![Graph showing extrusion forces for samples extruded using Spineplex syringes](image)

**Figure 7.39 extrusion forces for samples extruded using Spineplex syringes**

### 7.3.8 Tensile bond strength

Figure 7.40 illustrates the results obtained from the tensile bond strength testing of the samples 1, 3, 4 and 6 as indicated in table 7.1. All BCA monomer used contained 0.1% PTSA. Preliminary tensile bond strength tests were conducted to evaluate the adhesive properties of the samples compositions in comparison to other commercial products. Testing was based on a study conducted by Clarkin et al, who reported the tensile bond strength of Stryker products HydroSet and Simplex P. They reported bond strengths of approximately 0.1 and 0.2MPa respectively for each product, tested after a one day incubation period in distilled water at 37 °C. All of the samples tested exhibited superior tensile bond strength to the reported strengths of the commercial cements, which is as expected given the established adhesive properties of cyanoacrylates. Bond strengths ranged from approximately 1MPa to 3MPa for the samples tested with the sample containing the highest weight percentage of HA and
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the higher ratio of BCA to PDLLA having the greatest tensile bond strength, however no other apparent trend is evident relating to sample composition.

![Graph showing tensile bond strength of four sample compositions](image)

**Figure 7.40 Tensile bond strength of four sample compositions**

These initial results may contain some discrepancies due to the variation of sample thickness between discs which will have an effect on the adhesive properties. However they give a good indication of the superior adhesive properties of the samples compared with commercial products. Further analysis is needed to establish more accurate tensile bond strengths with bone.

**7.3.9 Monomer conversion**

Figures 7.41 to 7.43 show the percentage monomer conversion plots for several BCA samples as a function of time. In order to examine the effect sterilization would have on the BCA monomer, four BCA samples were sent to Isotron for gamma irradiation at 30kGry. This included a sample of the old batch of BCA, and three more old batch samples each containing 5000ppm of a different free radical stabilizer; hydroquinone, benzoquinone or tertbutylhydroquinone. The reactivity of each of the gamma irradiated samples along with two samples which had not been gamma irradiated (the old batch and the new batch) were then tested by monitoring the change in temperature in the induced anionic polymerization of each of the samples. The old batch of BCA polymer which did not contain any stabilizers polymerized during irradiation and so was not tested along with the other samples.
Figure 7.41 shows the resultant plots for all samples. The addition of stabilizers to the irradiated BCA did have an effect on the resultant monomer as it prevented each of the samples from polymerizing. On return from sterilization each of the samples with stabilizers had a viscosity similar to that of BCA samples which had not been gamma irradiated, where as the BCA sample which had not contained stabilizers had completely hardened. Figure 7.41 shows that the irradiation process did have some effect on the same as the monomer converted at a faster rate than the same BCA batch which had not sent for sterilization.

![Figure 7.41 Percentage monomer conversion for all samples tested as a function of time](image)

Figure 7.42 clearly shows the conversion plots for each of the irradiated samples which contained each of the three different stabilizers. The samples which initially showed the fastest conversion rates were those which contained both benzoquinone and hydroquinone, with the sample containing tertbutylhydroquinone having a slightly slower conversion rate. However, samples containing both tertbutylhydroquinone and benzoquinone took approximately 2.5 seconds to reach 100% conversion whereas the sample containing hydroquinone took double that at a time of 5 seconds to reach 100% conversion.
Figure 7.42 Percentage monomer conversion for irradiated BCA samples containing stabilizers as a function of time

Figure 7.43 Percentage monomer conversion for an old and new batch of BCA as a function of time

Figure 7.43 shows the percentage conversion for monomers from two different batches which had not been irradiated. The new batch of BCA takes just over 2 seconds to reach 100% conversion whereas the old batch takes approximately 18 seconds. Despite the fact that water is known as the most common initiator of
polymerization, cyanoacrylates can contain surprisingly high levels of free water. This water can cause hydrolysis of the monomer over time which drastically retard cure speed. This needs to taken into account regarding the shelf life of the BCA monomer.

7.4 Conclusions

Water absorption and hardness testing showed that samples containing the least amount of HA have the greatest percentage weight increase. While the opposite effect was evident to samples in chapter 6 as water absorption decreased as HA content increased. This was due to the use of a impure HA which resulted in HA agglomeration. Samples containing a higher amount of BCA have a slightly higher water uptake after the eight week period. Testing also found that there is little difference in the hardness values obtained after 7 days of the test compared with the hardness values obtained at the end of the eight week period.

Tensile testing revealed that with increased HA content there is an increase in Young’s modulus while the greatest tensile strengths are achieved in samples which do not contain any HA with the addition of HA decreasing tensile strength by nearly half. Tensile results were much improved from sample compositions tested in chapter 6, which had a Young’s modulus and tensile strength which decreased with the incorporation of HA due to HA agglomeration. The addition of HA decreased percentage elongation to break for samples with a polymer ratio of 0.5 BCA to 0.5 PDLLA. Differing polymer ratio from 0.5 BCA to 0.5 PDLLA to a ratio of 0.6 BCA to 0.4 PDLLA had no effect on the moduli and tensile strengths or percentage elongation at break for samples which contained the same wt. % of HA. Deviations in tensile results were seen to occur depending on the time taken to prepare the samples to when the samples were tested. Also, the addition of high quantities of PTSA which prolongs the working time of the composite causes a decrease in the tensile properties. Young’s modulus did not decrease for samples which were placed in PBS at 37°C for eight weeks; however there was a slight reduction in tensile strength for samples with the highest HA contents and an increase in percentage elongation was also evident.
For samples which have the same ratio of BCA to PDLLA, flexural modulus did not change as HA content increased. While flexural strength was greatest and the fracture toughness was the lowest for samples with the least amount of HA content in samples with a ratio of 0.6 BCA to 0.4 PDLLA. Changing HA content in samples with a ratio of 0.5 BCA to 0.5 PDLLA did not have an effect on there fracture toughness or flexural strength.

Rheological testing completed showed that HA and BCA content have the greatest effect on setting times, with increased HA content increasing the setting rate and increased BCA content decreasing the setting rate. The addition of an acid to the BCA monomer further allows for the adjustment of these times from approximately 8 minutes to over one hour, however as already stated, the addition of high concentrations of acid lead to a decrease in the resultant products mechanical properties.

Sterilization of the product was also considered with the polymerization of the BCA monomer of primary concern. However the addition of stabilizers such as hydroquinone was confirmed to prevent the free radical polymerization of the BCA monomer. Further study into the shelf life of the monomer will have to be carried out as the free water present caused hydrolysis of the monomer over time which will have an effect on setting rate and possibly resultant mechanical properties.

The overall results show promising features of a potential bone graft substitute. All compositions mixed to produce samples of a putty-like consistency, which were extremely hand malleable. The material is injectable with setting rates which can be altered and compositions have apparent adhesive properties greater than those of commercial products HydroSet or Simplex. With the onset of polymerization of the monomer, the material slowly but gradually hardens. The mechanical properties were lower than those found in human cortical bone, however the results are higher than a lot of commercially available bone substitutes. The market leaders of injectable bone graft substitutes are calcium phosphate pastes which are generally strong in compression but have much lower tensile, fracture toughness and flexural properties compared with the samples tested. Perhaps with further optimisation of compositions, the mechanical properties can be further increased.
7.5 References


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8.0 Conclusions

8.1 Introduction
The overall aim of this project was to develop a mouldable, resorbable bone filler that could be used to repair damaged bone. The ideal material was to be hand mouldable and easily injectable, have good adhesion to bone and harden in-situ with appropriate load bearing properties. The material was also to resorb at a rate equivalent to that of bone re-growth with no harmful degradation products. In this study, work was focused on the use of polymers from the polylactide family as these materials are known to have the necessary mechanical and biological properties needed for such an application.

8.2 Main findings
The ideal formulation should be hand mouldable and easily injectable and so the initial step was to blend the polymer with a plasticizer to create the bone filler base compound. Citrate esters are appropriate plasticizers for PLA with good miscibility and non toxic degradation products. Plasticizing PDLLA with citrate esters results in a decrease in Tg with increasing plasticizer content a further decrease in Tg occurs. By increasing the molecular weight of the plasticizer, their effectiveness to reduce the Tg of the PDLLA is generally enhanced but miscibility is decreased and two Tgs occurred in samples containing higher concentrations of higher molecular weight plasticizer. With the addition of the plasticizers there was also a general increase in percentage elongation at the expense of Young’s modulus and tensile strength. Plasticizing PDLLA with citrate esters resulted in a mouldable substance which could be used as the bone filler base compound.

Following the plasticization of the polymer, an investigation into methods of introducing crosslinks into the polymer material in order to improve mechanical properties in situ was carried out. As crosslinks prevent free chain movement and crosslinked polymers are generally stronger and less flexible than their linear forms, crosslinking the polymer was seen as a possible method of hardening the injected bone filler once it is in place. Swelling studies carried out on PDLLA demonstrated that crosslinked structures were achieved through the use of peroxides and
temperatures of 150°C. The addition of plasticizers hindered the crosslinking process with a decreasing crosslink density observed in samples with increasing plasticizer content. The use of crosslinking agent TAIC alone with the application of heat did not crosslink the polymer PDLLA, however high crosslink densities were achieved at high concentrations of TAIC and DCP. The swelling studies conducted on the gamma irradiated PDLLA indicated that unlike the heat activated crosslinked structures, the crosslinking agent TAIC alone was required to produce a crosslinked network and the addition of DCP decreased crosslink density. Irradiated samples had higher crosslink densities then those of samples which were chemically crosslinked through heat. An attempt to UV crosslink the PDLLA was unsuccessful. The UV irradiation only had an apparent plasticizing effect on the polymer samples tested. DMTA of crosslinked PDLLA indicated that no significant effect occurred to the Tg of the polymer while there was a dramatic drop in the storage modulus. Crosslinking PDLLA with DCP and TAIC at high temperatures caused the material to foam and voids were formed which lead to high stress concentrations.

The main conclusions drawn from this was that crosslinking was not a viable approach to harden the injected polylactide filler as not only were high temperatures needed for crosslinking initiation which would cause cellular necrosis, there was also a reduction in the mechanical and physical integrity of the polymer worsening its load bearing properties. Therefore an alternative method was considered in order to produce an injectable polylactide based filler which would harden in situ.

An alternative method of producing an injectable polylactide based bone filler was explored by combining polylactide particles with a liquid monomer cyanoacrylate, to create a base compound which was hand mouldable and easily injectable. With the onset of polymerization of the monomer, the filler then harden in situ. Modification of the composition with the addition of plasticizers, fillers such as HA allowed for the adjustment of mechanical properties and setting rates. Initial results indicated that these compositions are promising materials which could be developed to produce a bone substitute fulfilling all of the objectives set out in this project. Upon mixing all of the constituents a putty like substance is achieved and with the adhesive properties of the cyanoacrylate it should have good adhesion to bone, while upon polymerization of the monomer the material hardens. The composition which had the
most appropriate properties were samples which contained no HA or plasticizers and comprised of a ratio of 50 wt. % PDLLA to 50 wt. % BCA. Initial testing on these compositions concluded that a more homogeneous form of HA filler which had less porosity and less agglomeration could improve the water absorption and hardness values as well as increasing the mechanical properties and further investigation needed to be conducted on the compositions with the most appropriate initial results.

From further investigation, it was found that a new source of HA improved the water absorption and hardness results. Tensile testing of sample compositions found that with increased HA content there was an increase in Young’s modulus while the greatest tensile strengths are achieved in samples which did not contain any HA. Tensile results were much improved from earlier sample compositions tested, which had a Young’s modulus and tensile strength which decreased with the incorporation of HA due to HA agglomeration. Tensile results were seen to vary with time between sample preparation to when the samples were tested. Also the addition of high quantities of PTSA which prolongs the working time of the composite caused a decrease in the tensile properties. Young’s modulus did not decrease for samples which were placed in PBS at 37°C for eight weeks; however there was a slight reduction in tensile strength was observed in samples with the highest HA content and an overall increase in percentage elongation was also evident.

For samples which had a ratio of 0.6 BCA to 0.4 PDLLA, fracture toughness increased and flexural strength decreased as HA content increased. While increasing HA content to the polymer ratio of 0.5 BCA to 0.5 PDLLA had no effect on the fracture toughness or flexural strength. Increasing HA content also had no effect on the flexural modulus for samples with a polymer ratio of 0.6 BCA to 0.4 PDLLA or a ratio of 0.5 BCA to 0.5 PDLLA. Rheological testing completed showed that HA and BCA content had the greatest effect on setting times, with increased HA content increasing the setting rate and increased BCA content decreasing the setting rate. The addition of an acid to the BCA monomer further allowed for the adjustment of these times from approximately 8 minutes to over one hour, however as already stated, the addition of high concentrations of acid lead to a decrease in the resultant products mechanical properties.
Sterilization of the new materials was also considered with the polymerization of the BCA monomer of primary concern. However the addition of stabilizers such as hydroquinone was confirmed to prevent the free radical polymerization of the BCA monomer. Further study into the shelf life of the monomer will have to be investigated as the free water present caused hydrolysis of the monomer over time which has an effect on setting rate and possibly resultant mechanical properties.

8.3 Target material

In chapter 1, a set of target material properties were established for a bone filler which could be used to repair defects in damaged cortical bone. The mechanical values listed indicate the upper limit of material property values which are necessary for a bone filler with such an application. Ideally the material should be injectable while also having the initial viscous properties to remain at the site of insertion and be easily manipulated by the surgeon at the site of placement. All compositions mixed to produce samples of a putty-like consistency, which were extremely hand malleable. The material is injectable at a force of less than 200N, depending on the syringe diameter used and has apparent adhesive properties greater than those of commercial products HydroSet or Simplex. With the onset of polymerization of the monomer, the material slowly but gradually hardened. Compared with the working time of Simplex bone cement, the fastest setting sample has a working time of approximately 8.5 minutes while this working time can be altered to times of over 1 hour if necessary.

The target tensile properties in chapter 1 reported a modulus in range of 12 to 20GPa and a strength in the range of 53 to 130MPa. In this study a max tensile strength of 40MPa was achieved with the same samples having a Young’s modulus of 0.25 GPa. The highest modulus of 1.3 GPa was achieved for samples which had an average tensile strength of 20MPa. While these values are lower than those found in human cortical bone, the results are higher than a lot of commercially available bone substitutes. The market leaders of injectable bone graft substitutes are calcium phosphate pastes with tensile strengths reported in the range of 1.09 to 1.21 MPa. Aging the polymer compound in PBS at 37°C for 8 weeks resulted in modulus values which were within range of the values obtained for samples which had not
been aged in PBS, while a slight reduction in tensile strength did occur. This indicates that the mechanical integrity of the polymer compound would be maintained for the first two months of implantation.

Ideally, a fracture toughness of approximately $6.0 \text{ MPa m}^{-1/2}$, a flexural modulus in the range of 14 to 21GPa and a flexural strength in range of 135 to 193 MPa is desirable as indicated in chapter 1. In this study the highest average fracture toughness of $K_{IC}$ at $2.5 \text{MPa m}^{-1/2}$ was achieved. The highest average flexural modulus obtained was 1.7GPa, while the greatest flexural strength of 37MPa was achieved. While these are lower then those reported for cortical bone, they are much higher than those reported for calcium phosphate pastes. A study investigating the mechanical properties of injectable bone graft substitute Norian SRS reported an average flexural strength of 0.468 MPa with a fracture toughness of 0.14 MPa m$^{-1/2}$. HydroSet has a reported flexural modulus of 125–240 MPa and a flexural strength of 8-10MPa, while the same study, reported that no significant fracture toughness was obtained for HydroSet.

The overall results show promising features of a potential bone graft substitute. The material is injectable with setting rates which can be altered. Perhaps with further modification of compositions, the mechanical properties can be further increased. While some of important mechanical properties are lower then those found in human cortical bone, the results are higher then a lot of commercially available bone substitutes. The market leaders of injectable bone graft substitutes are calcium phosphate pastes which are generally strong in compression but have much lower tensile, fracture toughness and flexural properties compared with the samples tested in this study.

### 8.4 Future work

Further work will have to be completed on the degradation rates of the compositions indicated. The modulus of the samples remains constant for eight weeks, however further investigate will need to be conducted to indicate at what point the compositions start to drastically lose their mechanical properties. A variety of different polylactide family polymers may be used instead of PDLLA which would
further change degradation rates. Further investigation will also need to be conducted using a variety of different fillers. In this study HA was used however using a variety of different ceramic particle may yield very different results regarding mechanical properties and degradation properties. A closer examination into particle size, loading and particle – matrix interface adhesion should also be conducted to indicate what the effect may be. Further analysis into the setting properties in situ of the material will also need to be conducted as the BCA monomer is known to polymerize once in contact with substances such as blood, so this will have a major effect on setting rates. An examination into the materials tensile bond strength with bone will also need to be conducted. The adhesive properties of cyanoacrylates with soft tissue is well established, however an investigation into the polymer compounds reactivity with bone will need to be carried out while also investigating the rate of loss of degradation and its effect on the tensile bond strength. Further investigation into the injectability of the compound with the appropriate delivery device will need to be determined. Also further examination into the shelf life of the BCA monomer and its effect on setting times and resultant mechanical properties will need to be conducted.

Finally, a full set of biocompatibility testing will also need to be carried out. Each of the main components are used in medical devices which are currently on the market. PDLLA and HA are utilized in current bone graft substitutes, while cyanoacrylates are approved for use in topical skin closures, as medical sealants used to help stop leaks in blood vessels, and as an artificial material used to block blood flow in the treatment of malformed blood vessels in the brain. However, biocompatibility testing will need to be conducted to ensure the polymer compound is safe for human use as a bone filler. Completion of this work could lead to a potential product fulfilling requirements for a bone graft substitute which is currently not commercially available.
Appendix A
The Effect of Citrate Ester Plasticizers on the Thermal and Mechanical Properties of Poly(DL-lactide)

Imelda Harte, Colin Birkinshaw, Eric Jones, James Kennedy, Eamonn DeBarra

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2Stryker Osteonics, Raheen, Limerick, Ireland
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4MABE Department, University of Limerick, Limerick, Ireland
Correspondence to: E. DeBarra (E-mail: eamonn.debarra@ul.ie)

ABSTRACT: Citrate esters triethyl citrate, tributyl citrate, and acetyl tributyl citrate were used as plasticizers for amorphous poly(DL-lactide) (PDLLA). The resultant compositions were analyzed by means of differential scanning calorimetry (DSC), dynamic mechanical thermal analysis, and tensile testing to investigate the properties of the blends. Glass transition temperatures (Tg) obtained by DSC were also compared to theoretically calculated Tg. Increasing plasticizer content decreased the resultant Tg of the blend with plasticizer efficiency enhanced as the molecular weight of the citrate ester increased. However, blends with high plasticizer content, a lack of miscibility occurred with increased molecular weight. Theoretical results were comparable with those obtained experimentally at compositions, which were miscible. Increasing plasticizer content increased the ductility and decreased the strength of the polymer. The addition of 10 wt% plasticizer to PDLLA decreased tensile strength by over 50% with the deterioration larger at higher concentrations of plasticizer. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 90:600–608, 2004

KEYWORDS: thermal properties; mechanical properties; polyesters; additives; biomaterials

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INTRODUCTION

Of the many polymeric materials that are biodegradable, the α-hydroxy acid poly lactide (PLA) is among the most extensively investigated. This is due to its proven biocompatibility and good material properties. PLA can exist in three stereoisomeric forms: poly(lactide) (PLLA), poly(DL-lactide) (PDLLA), and poly(ε-caprolactone) (PCL). The polymers in the racemic mixture of l- and d-lactides (a 50:50 mixture of L and D isomers) leads to the synthesis of PDLLA, which is amorphous and is the type of material used in the work reported here. PLA possesses good mechanical properties and generally has an elastic modulus and tensile strength in the ranges of 3.2–3.7 GPa and 55–60 MPa, respectively. In the unalloyed form, it has a glass transition temperature (Tg) greater than 37°C, causing PLA devices to possess brittle characteristics in physiological conditions.

The aim of this research work was to attempt to develop a resorbable bone substitute based on PLA. However, brittleness is a major drawback for many biomedical applications, and PLA devices tend to be susceptible to fracture when subjected to tension or load-bearing stresses during use. To modify its properties, PLA has been blended with other polymers such as poly(ethylene oxide), poly(vinyl acetate), and poly(ethylene glycol) and the citrate esters. Another option is the use of a low-molecular weight compound that acts as a plasticizer. Examples of compounds used with PLA include glycerol, triacetate, polyethylene glycol, and citrate esters. An effective plasticizer for PLA is expected to reduce the glass transition of the amorphous phase and depress the melting point of the crystalline region.

There are several important considerations when choosing a plasticizer for PLA for biomedical applications. It should be nontoxic and approved for use as additives in food, personal care products, and in medical plastics.

Labrecque et al. studied the effect that citrate esters had on the thermal and mechanical properties of semicrystalline PLA...
and found that all plasticizers were miscible with PLA (a composition range of up to 30% plasticizer was analyzed). The reason for good solubility of PLA in citrate plasticizers is due to the polar interactions between the ester groups of PLA and the plasticizer. However, quantitative information on the effects of citrate ester plasticization of PDLLA is not readily available, and, so in this study, amorphous PDLLA was blended with three different citrate esters: triethyl citrate (TEC), tributyl citrate (TBC), and acetyl tributyl citrate (ATBC) and the resultant thermal, dynamic mechanical, and tensile properties were analyzed. The results found experimentally by differential scanning calorimetry (DSC) are also compared to theoretical results calculated for the various plasticized polymer compositions following the methods of Fox.

MATERIALS AND METHODS

Blending

The PDLLA investigated in this report supplied by Boehringer Ingelheim (Germany), had an equimolar mixture of D and L isomers and an average molecular weight of 2.028 x 10^6 Da as determined by gel permeation chromatography using THF as a solvent with a polystyrene calibration applied. Plasticizers TEC, TBC, and ATBC supplied by Sigma Aldrich (Ireland) had molecular weights of 276, 360, and 403, respectively, as reported by the supplier. The polymer was first ground to a fine powder before use and sample compositions consisted of PDLLA combined with 10–30 wt % plasticizer. All samples were prepared by heating to ~150°C in a beaker while being continuously stirred before being transferred to hot molds to be pressed.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a Perkin-Elmer Pyris 1 DSC. Plasticized samples (5–6 mg) were heated from −60 to 100°C at a rate of 10°C per minute under nitrogen atmosphere. PDLLA samples containing all three plasticizers ranging from 10 to 30 wt % content were analyzed. DSC was also performed on each plasticizer within a temperature range of −150 to −50°C under helium atmosphere. The glass transition temperature (T_g) was taken as the midpoint of the specific heat increment at the glass transition.

Dynamic mechanical thermal analysis

Dynamic mechanical thermal analysis (DMTA) scans were carried out on PDLLA samples containing only plasticizer TBC using a Rheometric scientific mark 3 DMTA. Analysis was carried out on specimens measuring 10 mm x 4 mm x 2 mm in tensile mode with a temperature profile ranging from −60 to 120°C at a 2°C per minute heating rate with a frequency of 1 Hz. T_g was taken as the peak in the tan δ curve accompanied by a step reduction in the storage modulus.

Tensile testing

Tensile tests were performed using an Instron 4301 series tensile testing machine. Specimens were dogbone shaped with a gauge length of ~13 mm, a width of 3.5 mm and thickness of 3 mm prepared after ASTM standard D638-08. All samples were tested with the same crosshead speed of 10 mm/min using a 1 kN load cell. Tensile testing was performed on PDLLA samples containing 10, 20, and 30 wt % of all three plasticizers.

RESULTS AND DISCUSSION

Theoretical T_g

There are several equations reported in literature that enable the glass transition temperature of copolymers and polymer blends to be expressed as a function of their composition. Equation (1) was proposed by Fox for the T_g dependence of a binary system:

\[ \frac{1}{T_g} = \frac{\omega_1}{T_{g1}} + \frac{\omega_2}{T_{g2}} \]

where subscripts 1 and 2 refer to plasticizer and polymer, respectively, and \( \omega \) is the weight fraction. This equation was applied to the various compositions of PDLLA with each plasticizer using the T_g obtained for PDLLA and each citrate esters by DSC. Figure 1 illustrates the theoretical T_g calculated as a function of plasticizer content. Calculated theoretical T_g are also listed in Table I.

Differential scanning calorimetry

Figure 2 illustrates the heat cycle differential scanning calorimetry (DSC) thermoscan for PDLLA and the polymer plasticized with different quantities of each plasticizer. The resultant T_g for all samples analyzed is listed in Table I. The glass transition is evident in the pure PDLLA material as a distinct peak whilst in the polymer-plasticizer blends, it is apparent as a significant change in slope. The very small event occurring at 20°C in all scans is an instrumental artifact.

As expected, by increasing plasticizer content, a decrease in T_g occurs, which is true for all three plasticizers analyzed. The low-molecular size of the plasticizer allows it to occupy intermolecular spaces between polymer chains, reducing the energy for molecular motion and the formation of hydrogen bonding between the polymer chains, which in turn increases free volume and molecular mobility.

Figure 3 illustrates the effect all three plasticizers have on the T_g of the PDLLA as a function of plasticizer content analyzed by DSC in this study. The magnitude of the T_g change in the plasticized polymer is a good indicator for the plasticization efficiency of the plasticizer compound. The most effective plasticizer to decrease T_g was generally ATBC, followed by TBC and last TEC. By increasing the molecular weight of the plasticizer, the effectiveness of the citrate plasticizer to reduce the T_g of the PDLLA is generally enhanced.
Table 1. $T_g$ Values for Various Blends of Polymer and Plasticizer Found Experimentally and Theoretically

<table>
<thead>
<tr>
<th>Plasticizer (wt %)</th>
<th>PDLLA</th>
<th>TEC</th>
<th>TBC</th>
<th>ATBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>33.8</td>
<td>29.8</td>
<td>30.6</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>16.7</td>
<td>9.6</td>
<td>11.0</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>1.4</td>
<td>-8.1</td>
<td>-6.2</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TEC, the least effective plasticizer, only differs structurally from TBC in the number of carbons in its ester chain; TBC containing two carbons while TBC having four (see Table II). Both plasticizers have three ester functionalities, potentially acting as a hydrogen bond acceptor sites and a tertiary hydroxyl group, which has both hydrogen bond donating and accepting character. Interactions may occur between the hydroxyl groups of TEC/TBC and the hydrogen bond acceptor ester groups of PDLLA. TBC is more likely to be more effective as a plasticizer due to its larger nonpolar aliphatic segments. These segments shield dipoles on the PDLLA polymer chains from interacting with adjacent polymer chains, pushing them apart, and increasing mobility, thus reducing the $T_g$ more effectively than TEC.

ATBC and TBC structurally differ only by the acetylation of the tertiary hydroxyl group in ATBC. ATBC is exclusively a hydrogen-bond acceptor, while TBC having a hydroxyl group has both hydrogen bond donating and accepting character and has potential to form strong hydrogen bonds with the ester groups of PDLLA. These strong bonds between TBC and the polymer may result in a decrease in polymer chain mobility and flexibility, causing PDLLA plasticized with TBC to have a higher $T_g$ than PDLLA plasticized with ATBC. ATBC having an extra acetyl group also has an increased associated free volume; the acetyl group may also further shield dipoles on the PDLLA chains, further pushing the polymer chains apart and increasing mobility.

These results found for amorphous PLA are similar to that of Labrecque et al., who reported that with increasing citrate plasticizer content, the $T_g$ of semicrystalline PLA decreased. They also found that the lower the molecular weight of the plasticizer, the less efficient it was at lowering the $T_g$ of the PLA. Only one glass transition was noted for each sample of PDLLA blended with TEC, which indicates that this plasticizer was miscible with the polymer for all compositions. Whereas a second slight endothermic shift in the specific heat occurring at the glass transition temperature of PDLLA was noted to occur in samples containing 20% ATBC, 30% ATBC, and 30% TBC. The miscibility of plasticizers and polymers can be estimated by comparing their solubility parameters. The solubility parameters for PDLLA and all three plasticizers are quite similar indicating good compatibility (see Table II). However, the difference between their solubility parameters increases with increasing

![Figure 2](Link to Figure 2)

Figure 2. DSC thermographs of neat PDLLA and PDLLA samples plasticized with (a) 10, (b) 20, and (c) 30 wt % citric ester. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

![Figure 3](Link to Figure 3)

Figure 3. Experimental $T_g$ as a function of plasticizer content obtained by DSC.
molecular weight of the plasticizer, resulting in a decrease in solubility and compatibility, which may explain the presence of two $T_g$s in compositions with higher molecular weight plasticizer contents. The lack of miscibility between PDLLA and ATBC at higher concentrations can be seen clearly in Figure 3. The $T_g$ of PDLLA plasticized by 20% ATBC is 5.7°C; with the addition of a further 10% ATBC, the $T_g$ only decreases slightly to 4.4°C. This differs with work completed on semicrystalline PLA by Bialdo et al., who reported that the solubility limit of ATBC in PLLA was 50 wt %.  

Dynamic mechanical thermal analysis

Figure 4 shows the tan $\delta$ and storage modulus curves obtained from dynamic mechanical analysis of PDLLA and PDLLA plasticized with 10–30 wt % TBC. Table I indicates the glass transition temperatures for each sample. With increasing plasticizer content, a decrease in $T_g$ is obtained and the height of the tan $\delta$ peak also decreases and broadens.

As seen in Figure 4(b), at temperatures above the $T_g$, the storage modulus of each sample drops dramatically due to the softening of the polymer specimens. With increasing plasticizer content, there is a decrease in storage modulus values below $T_g$ as the plasticizer continues to increase the mobility of the polymer chains.

These results for amorphous PLA are similar to those obtained by Ren et al., who plasticized semicrystalline PLA with low-molecular weight triacetin and oligomeric poly(1,3-butylene glycol adipate). Increasing plasticizer content decreased the peak of the tan $\delta$ curve while also making them shorter and broader. The storage modulus values were also similar, and a decrease could be seen with increasing plasticizer content.

Table I lists the $T_g$ values for PDLLA plasticized with TBC obtained by dynamic mechanical thermal analysis (DMTA) and

### Table II. Structure of PDLLA and Each Citrate Ester Plasticizer and Their Corresponding Solubility Parameters as Reported by Ljungberg et al.

<table>
<thead>
<tr>
<th>Plasticizer</th>
<th>Structure</th>
<th>Solubility parameter $\delta$ (J cm$^{-3}$)$^{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>POLLA</td>
<td><img src="image" alt="POLLA structure" /></td>
<td>20.1*</td>
</tr>
<tr>
<td>TEC</td>
<td><img src="image" alt="TEC structure" /></td>
<td>19.7*</td>
</tr>
<tr>
<td>TBC</td>
<td><img src="image" alt="TBC structure" /></td>
<td>19.6*</td>
</tr>
<tr>
<td>ATBC</td>
<td><img src="image" alt="ATBC structure" /></td>
<td>18.0*</td>
</tr>
</tbody>
</table>

*Calculated with group molar attraction constants from the Hoy series.
Figure 4. (a) Tan δ and (b) storage modulus curves obtained by DMTA for PDLLA and PDLLA samples plasticized with TBC.

Figure 5. Theoretical versus experimental $T_g$ of samples plasticized with (a) TEC, (b) TBC, and (c) ATBC as a function of plasticizer content.

Figure 6. Stress-strain curves of neat PDLLA and PDLLA samples plasticized with (a) 0, (b) 10, and (c) 30 wt % citrate ester. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

differential scanning calorimetry (DSC). Because the glass transition is a kinetic process, it is affected by the rate at which the sample is heated, and different experimental techniques lead to different $T_g$ values. This is due to different time responses in the motions of side or main-chain polar groups and mechanical or thermal stimulation of the motion. The $T_g$ values for PDLLA plasticized with TBC obtained by DMTA are higher than that obtained by DSC. According to Averous et al., differences in the temperature corresponding to transitions observed by DMTA and DSC are attributed to the frequency of the analysis method. A clear indication of the glass transition temperature range is more easily observed through DMTA, and this result may suggest that the segmental motion of the plasticized PDLLA has only been partially relaxed at the $T_g$ as indicated by DSC.

Figure 5 compares the theoretical results calculated for plasticized PDLLA with those found experimentally by DSC. The theoretical results are in good agreement with those obtained experimentally for TEC [Fig. 5(a)] and TBC [Fig. 5(b)] at compositions that seemed to be miscible. At a composition of 30% TBC, which showed two separate $T_g$'s, the experimental result was slightly higher than that of the theoretical result calculated most likely due to lack of homogeneity in the sample tested. The results obtained experimentally for ATBC are lower than the theoretical results at compositions of 10 and 20 wt % ATBC.
Table III. Tensile Testing Results

<table>
<thead>
<tr>
<th></th>
<th>Tensile strength (MPa)</th>
<th>Std dev (±)</th>
<th>Elongation to break (%)</th>
<th>Std dev (±)</th>
<th>Young’s modulus (MPa)</th>
<th>Std dev (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDLLA (wt %)</td>
<td>41</td>
<td>6</td>
<td>9</td>
<td>21</td>
<td>870</td>
<td>86</td>
</tr>
<tr>
<td>10 TEC</td>
<td>29</td>
<td>3</td>
<td>78</td>
<td>22</td>
<td>727</td>
<td>39</td>
</tr>
<tr>
<td>TBC</td>
<td>15</td>
<td>1</td>
<td>171</td>
<td>10</td>
<td>325</td>
<td>84</td>
</tr>
<tr>
<td>ATBC</td>
<td>18</td>
<td>1</td>
<td>46</td>
<td>17</td>
<td>525</td>
<td>26</td>
</tr>
<tr>
<td>20 TEC</td>
<td>4</td>
<td>0.3</td>
<td>319</td>
<td>224</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>TBC</td>
<td>4</td>
<td>0.4</td>
<td>138</td>
<td>145</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>ATBC</td>
<td>3</td>
<td>0.9</td>
<td>315</td>
<td>23</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>30 TEC</td>
<td>0.6</td>
<td>0.1</td>
<td>595</td>
<td>201</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TBC</td>
<td>0.4</td>
<td>0.07</td>
<td>337</td>
<td>48</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ATBC</td>
<td>1.2</td>
<td>0.2</td>
<td>225</td>
<td>63</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

[Fig. 5(c)]. He et al. report that the differences between experimental and predicted T_g values are sometimes considered as a measure of the strength of interactions between the blend components. Glass transition temperatures found experimentally that are lower than those predicted may be attributed to weak, specific interactions between the blends. At a composition of 30% ATBC, the measured value was higher than that of the theoretical result, which may also be due to lack of homogeneity in the sample.

**Tensile testing**

Figure 6 illustrates the stress-strain curves of PDLLA samples unplasticized and plasticized by the three different citrate esters. Average Young’s modulus, tensile strength, and percentage elongation at break for all samples tested are listed in Table III.

At 10 wt % plasticizer content [Fig. 6(a)], samples plasticized with TEC had the greatest stiffness and strength followed by ATBC-plasticized samples and last TBC-plasticized samples. TBC-plasticized samples also had the greatest elongation at this plasticizer concentration. These results do not fully correlate with the experiment T_g measurements, as it would be expected that the samples containing 10% ATBC, which had the lowest T_g, would also have the lowest stiffness and strength and the greatest elongation. However, the results do correlate with the theoretical T_g calculated, which predicted samples plasticized with 10 wt % TBC to have the greatest chain mobility at that plasticizer content (see Fig. 1).

As already stated, the T_g found experimentally for PDLLA plasticized with ATBC was lower than those predicted, which may be attributed to weak-specific interactions between ATBC and PDLLA. This may have resulted in the migration of the plasticizer ATBC causing the material to regain some of the rigidity of pure PDLLA. TEC-plasticized samples displayed the greatest elongation as the weight percentage content of plasticizer increased to 20 and 30% while TBC and ATBC were not as effective. The larger nonpolar aliphatic segments of ATBC and TBC may have shielded dipoles on the PDLLA polymer chains from interacting with adjacent polymer chains, and this disruption to the polymer system may have caused a decrease in elongation compared to samples plasticized by TEC.

Unplasticized PDLLA is quite brittle as seen in Figure 6(a) and fractured without yielding. The 10% TEC plasticized sample was less brittle than pure PDLLA and had some necking, while 10% TBC and ATBC-plasticized samples were more ductile than the 10% TEC sample with necking and further extension occurring at lower stress, occurring more so in the TBC plasticized sample than the ATBC-plasticized sample.

Figure 6 shows that with the increase in plasticizer content, there is a general increase in ductility and decrease in strength. Percentage elongation at break increases at the expense of Young’s modulus and tensile strength. At compositions above 10 wt % plasticizer, both the stiffness and strength of the samples drop dramatically and at compositions of 30 wt % plasticizer, these properties are very low. These results for amorphous PLA are similar to those reported by Labreque et al. who found that the addition of 10% citrate esters to semicrystalline PLA decreased tensile strength by ~ 50% with the deterioration larger at higher concentrations of plasticizer.

**CONCLUSION**

Plasticizing amorphous PLA with citrate esters results in a decrease in T_g with increasing plasticizer content. By increasing the molecular weight of the plasticizers, their effectiveness in reducing the T_g of the PDLLA is generally enhanced, but miscibility is decreased. The work is in good agreement with work previously completed on semicrystalline PLA, apart from the resultant solubility limits of the plasticizers within the amorphous polymer, with higher limits previously reported for the semicrystalline PLA form.

DMTA of TBC-plasticized PDLLA indicates that a decrease in T_g is obtained with increasing plasticizer content, and the decrease in storage modulus values below T_g shows that there is no low-temperature antiplasticization effect. The T_g values obtained by DMTA are higher than that obtained by DSC, which is a time-temperature effect. Theoretical T_g results for plasticized PDLLA are in good agreement with those obtained experimentally for TEC and TBC at compositions, which were miscible. Weak-specific interactions between ATBC and PDLLA may account for differences in experimentally observed and theoretical calculated T_g.
As expected with the addition of a plasticizer, there is a general increase in percentage elongation at the expense of Young's modulus and tensile strength. The addition of 10% citrate esters to POE LA decreases Young's modulus and nearly halves the tensile strength, with the deterioration larger at higher concentrations of plasticizer. Migration/retention of plasticizer as a function of time should be quantified, as this is likely to be significant at these plasticizer concentrations.

REFERENCES

Appendix B
Table 1: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>Young's Modulus</th>
<th>0.5 BCA-0.5 PDLLA</th>
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<tbody>
<tr>
<td>0 % HA 20% HA 30% HA 40% HA</td>
<td>0.26 0.743686 0.893451 0.911355</td>
</tr>
<tr>
<td>0.24 0.711472 0.867006 0.916857</td>
<td></td>
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<tr>
<td>0.26 0.791767 0.862989 0.945939</td>
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Anova: Single Factor

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<tr>
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<th>Count</th>
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<td>0.253333</td>
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ANOVA

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<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
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<th>F crit</th>
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<td>Between Groups</td>
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<td>0.282851</td>
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<tr>
<td>Within Groups</td>
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<td>8</td>
<td>0.000596</td>
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<tr>
<td>Total</td>
<td>0.853323</td>
<td>11</td>
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</tr>
</tbody>
</table>

Table 2: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>Young's Modulus</th>
<th>0.6 BCA-0.4 PDLLA</th>
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<tbody>
<tr>
<td>0 % HA 30% HA 40% HA 50% HA</td>
<td>0.16 1.010371 0.561105 1.315619</td>
</tr>
<tr>
<td>0.17 0.581847 0.680669 1.153394</td>
<td></td>
</tr>
<tr>
<td>0.14 0.921444 0.986908 1.552737</td>
<td></td>
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Anova: Single Factor

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<td>0.837887</td>
<td>0.051144</td>
</tr>
<tr>
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<td>3</td>
<td>2.228682</td>
<td>0.742894</td>
<td>0.048231</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>4.02175</td>
<td>1.340583</td>
<td>0.040336</td>
</tr>
</tbody>
</table>

ANOVA

<table>
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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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</thead>
<tbody>
<tr>
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<td>2.121256</td>
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<td>0.707085</td>
<td>20.21042</td>
<td>0.000433</td>
<td>4.066181</td>
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<td>0.279889</td>
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<td>0.034986</td>
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<td></td>
<td></td>
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<td>11</td>
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</tr>
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</table>
**Table 3**: Statistical analysis of the tensile strength for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>Tensile strength</th>
<th>0.5 BCA-0.5 PDLLA</th>
</tr>
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<tbody>
<tr>
<td>20% HA</td>
<td>30% HA</td>
</tr>
<tr>
<td>21.6</td>
<td>25.8</td>
</tr>
<tr>
<td>15.4</td>
<td>24.6</td>
</tr>
<tr>
<td>25.7</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>Column 1</td>
</tr>
<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of Variation</td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

**Table 4**: Statistical analysis of the tensile strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>Tensile strength</th>
<th>0.6 BCA-0.4 PDLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% HA</td>
<td>40% HA</td>
</tr>
<tr>
<td>22.8</td>
<td>12.6</td>
</tr>
<tr>
<td>7.1</td>
<td>22.5</td>
</tr>
<tr>
<td>22.7</td>
<td>20.7</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>Column 1</td>
</tr>
<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of Variation</td>
</tr>
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<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
Table 5: Statistical analysis of the percentage elongation for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>% Elongation</th>
<th>0.5 BCA-0.5 PDLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>4.28</td>
</tr>
<tr>
<td>20.5</td>
<td>3.22</td>
</tr>
<tr>
<td>23</td>
<td>5.38</td>
</tr>
</tbody>
</table>

Anova: Single Factor

**SUMMARY**

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<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>64.5</td>
<td>21.5</td>
<td>1.75</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>12.88</td>
<td>4.293</td>
<td>1.167</td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>14.69</td>
<td>4.897</td>
<td>0.684</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>12.05</td>
<td>4.017</td>
<td>0.003</td>
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**ANOVA**

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<tr>
<th>Source of Variation</th>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>658.9665</td>
<td>3</td>
<td>219.6555</td>
<td>243.7772</td>
<td>3.359E-08</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7.2084</td>
<td>8</td>
<td>0.90105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>666.1749</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Statistical analysis of the tensile strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>% Elongation</th>
<th>0.6 BCA-0.4 PDLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2.73</td>
<td>11.8</td>
</tr>
<tr>
<td>2.45</td>
<td>4.38</td>
</tr>
<tr>
<td>2.79</td>
<td>5.27</td>
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Anova: Single Factor

**SUMMARY**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>7.97</td>
<td>2.65667</td>
<td>0.032933</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>21.45</td>
<td>7.15</td>
<td>16.4149</td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>13.048</td>
<td>4.349333</td>
<td>0.277541</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>7.97</td>
<td>2.65667</td>
<td>0.032933</td>
</tr>
</tbody>
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**ANOVA**

<table>
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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>40.4655</td>
<td>3</td>
<td>13.48852</td>
<td>3.219541</td>
<td>0.082642</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>33.51662</td>
<td>8</td>
<td>4.189577</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73.98216</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 7:** Statistical analysis of the Youngs modulus for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 20% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.5 BCA - 0.5 PDLLA 20 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.743686</td>
</tr>
<tr>
<td></td>
<td>0.711472</td>
</tr>
<tr>
<td></td>
<td>0.791767</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>2.246925</td>
<td>0.748975</td>
<td>0.001633</td>
<td></td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>2.319187</td>
<td>0.773062</td>
<td>0.024905</td>
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</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>1.666586</td>
<td>0.555529</td>
<td>0.107107</td>
<td></td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>2.41564</td>
<td>0.805213</td>
<td>0.008317</td>
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</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.113896</td>
<td>3</td>
<td>0.037965</td>
<td>1.069736</td>
<td>0.41468</td>
<td>4.0661806</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.283924</td>
<td>8</td>
<td>0.03549</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total               | 0.39782 | 11 |          |          |         |        |

**Table 8:** Statistical analysis of the Youngs modulus for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.5 BCA - 0.5 PDLLA 30 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.893451</td>
</tr>
<tr>
<td></td>
<td>0.867006</td>
</tr>
<tr>
<td></td>
<td>0.862989</td>
</tr>
</tbody>
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Anova: Single Factor

<table>
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<tr>
<th>SUMMARY</th>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>2.623446</td>
<td>0.874482</td>
<td>0.000274</td>
<td></td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>2.161292</td>
<td>0.720431</td>
<td>0.005067</td>
<td></td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>2.259115</td>
<td>0.753038</td>
<td>0.131021</td>
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<tr>
<td>Column 4</td>
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<td>2.831564</td>
<td>0.943855</td>
<td>0.018723</td>
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**ANOVA**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
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<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<tbody>
<tr>
<td>Between Groups</td>
<td>0.098014</td>
<td>3</td>
<td>0.032671</td>
<td>0.842674</td>
<td>0.50789</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.310168</td>
<td>8</td>
<td>0.038771</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total               | 0.408182 | 11 |          |          |         |        |
Table 9: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.5 BCA - 0.5 PDLLA 40 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.911355</td>
</tr>
<tr>
<td></td>
<td>0.916857</td>
</tr>
<tr>
<td></td>
<td>0.945939</td>
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Anova: Single Factor

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>Column 2</td>
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<tr>
<td>Column 3</td>
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<tr>
<td>Column 4</td>
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ANOVA

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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.210335</td>
<td>3</td>
<td>0.070112</td>
<td>8.584784</td>
<td>0.006988</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.065336</td>
<td>8</td>
<td>0.008167</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.275671</td>
<td>11</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 10: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.6 BCA - 0.4 PDLLA 30 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.010371</td>
</tr>
<tr>
<td></td>
<td>0.581847</td>
</tr>
<tr>
<td></td>
<td>0.921444</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
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<td>Column 1</td>
</tr>
<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
<tr>
<td>Column 4</td>
</tr>
</tbody>
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ANOVA

<table>
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<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.004178</td>
<td>3</td>
<td>0.001393</td>
<td>0.076836</td>
<td>0.970762</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.144984</td>
<td>8</td>
<td>0.018123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.149161</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
### Table 11: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.6 BCA - 0.4 PDLLA  40 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.561105</td>
</tr>
<tr>
<td></td>
<td>0.680669</td>
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<tr>
<td></td>
<td>0.986908</td>
</tr>
</tbody>
</table>

Anova: Single Factor

**SUMMARY**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>2.228682</td>
<td>0.742894</td>
<td>0.048231</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>2.731785</td>
<td>0.910595</td>
<td>0.004919</td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>2.890829</td>
<td>0.96361</td>
<td>0.009274</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>2.608384</td>
<td>0.869461</td>
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</table>

**ANOVA**

<table>
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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.079669</td>
<td>3</td>
<td>0.026556</td>
<td>1.677117</td>
<td>0.248223</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.126675</td>
<td>8</td>
<td>0.015834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.206344</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

### Table 12: Statistical analysis of the Young’s modulus for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 50% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Youngs modulus</th>
<th>0.6 BCA - 0.4 PDLLA  50 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.315619</td>
</tr>
<tr>
<td></td>
<td>1.153394</td>
</tr>
<tr>
<td></td>
<td>1.552737</td>
</tr>
</tbody>
</table>

Anova: Single Factor

**SUMMARY**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>4.02175</td>
<td>1.340583</td>
<td>0.040336</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>3.11145</td>
<td>1.03715</td>
<td>0.042547</td>
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<tr>
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<td>3.743234</td>
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<td>3.706299</td>
<td>1.235433</td>
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**ANOVA**

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<th>F crit</th>
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**Table 13:** Statistical analysis of the Tensile Strength for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 20% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
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<th>Tensile Strength</th>
<th>0.5 BCA - 0.5 PDLLA 20 wt. % HA</th>
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<tbody>
<tr>
<td>day</td>
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<tr>
<td>Tensile Strength</td>
<td>21.61315</td>
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<td>15.36682</td>
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Anova: Single Factor

**SUMMARY**

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<th>Variance</th>
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<td>3</td>
<td>62.67087</td>
<td>20.89029</td>
<td>27.0386</td>
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<tr>
<td>Column 2</td>
<td>3</td>
<td>73.69901</td>
<td>24.56634</td>
<td>10.12708</td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>60.98142</td>
<td>20.32714</td>
<td>5.906917</td>
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<td>Column 4</td>
<td>3</td>
<td>63.00456</td>
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**ANOVA**

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<th>F crit</th>
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<tr>
<td>Between Groups</td>
<td>33.7322</td>
<td>3</td>
<td>11.24407</td>
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**Table 14:** Statistical analysis of the tensile strength for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Tensile Strength</th>
<th>0.5 BCA - 0.5 PDLLA 30 wt. % HA</th>
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</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td>Tensile Strength</td>
<td>25.8365</td>
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<tr>
<td></td>
<td>24.58826</td>
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<td></td>
<td>23.66852</td>
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Anova: Single Factor

**SUMMARY**

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<th>Variance</th>
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</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>74.09329</td>
<td>24.69776</td>
<td>1.184032</td>
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<td>Column 2</td>
<td>3</td>
<td>58.12644</td>
<td>19.37548</td>
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<tr>
<td>Column 3</td>
<td>3</td>
<td>66.06723</td>
<td>22.02241</td>
<td>6.296952</td>
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<tr>
<td>Column 4</td>
<td>3</td>
<td>63.30611</td>
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**ANOVA**

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<th>P-value</th>
<th>F crit</th>
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<tr>
<td>Between Groups</td>
<td>44.43584</td>
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<td>14.81195</td>
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<td>Within Groups</td>
<td>95.86138</td>
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<td>11.98267</td>
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<td></td>
<td></td>
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<td>Total</td>
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Table 15: Statistical analysis of the tensile strength for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Tensile Strength</th>
<th>0.5 BCA - 0.5 PDLLA  30 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>22.90508</td>
</tr>
<tr>
<td></td>
<td>21.7575</td>
</tr>
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<td></td>
<td>22.48971</td>
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Anova: Single Factor

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ANOVA

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<th>F</th>
<th>P-value</th>
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<td>74.54914</td>
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<td>24.84971</td>
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<td>0.018552</td>
<td>4.066181</td>
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<td>Within Groups</td>
<td>32.7459</td>
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<td>4.093237</td>
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<td>107.295</td>
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Table 16: Statistical analysis of the tensile strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Tensile Strength</th>
<th>0.6 BCA - 0.4 PDLLA  30 wt. % HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>22.7525</td>
</tr>
<tr>
<td></td>
<td>7.141457</td>
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<tr>
<td></td>
<td>22.70792</td>
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Anova: Single Factor

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<td>Groups</td>
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<tr>
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ANOVA

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<th>P-value</th>
<th>F crit</th>
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<tbody>
<tr>
<td>Between Groups</td>
<td>47.43929</td>
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<td>15.8131</td>
<td>0.527198</td>
<td>0.675901</td>
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<td>Within Groups</td>
<td>239.9567</td>
<td>8</td>
<td>29.99459</td>
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Table 17: Statistical analysis of the tensile strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>Tensile Strength</th>
<th>0.6 BCA - 0.4 PDLLA 40 wt. % HA</th>
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</thead>
<tbody>
<tr>
<td>day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>22.50289</td>
</tr>
<tr>
<td></td>
<td>20.74638</td>
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</table>

Anova: Single Factor

<table>
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<tr>
<td>Column 2</td>
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<td>Column 3</td>
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ANOVA

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<th>P-value</th>
<th>F crit</th>
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</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>75.55449</td>
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<td>25.18483</td>
<td>1.952796</td>
<td>0.199762</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>103.1745</td>
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<td>12.89681</td>
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<td>Total</td>
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Table 18: Statistical analysis of the tensile strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 50% HA as a function of time stored in PBS @ 37°C

<table>
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<tr>
<th>Tensile Strength</th>
<th>0.6 BCA - 0.4 PDLLA 50 wt. % HA</th>
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<tr>
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<td></td>
<td>21.19711</td>
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Anova: Single Factor

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<td>Column 2</td>
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<td>Column 3</td>
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<tr>
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ANOVA

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<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<td>119.7317</td>
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<td>39.91058</td>
<td>16.17851</td>
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<tr>
<td>Within Groups</td>
<td>19.73511</td>
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<td>2.466889</td>
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<tr>
<td>Total</td>
<td>139.4669</td>
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</table>
**Table 19:** Statistical analysis of the % elongation for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 20% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>% Elongation</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA 20 wt. % HA</td>
<td>0</td>
<td>7</td>
<td>28</td>
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<tr>
<td></td>
<td>4.28</td>
<td>7.84</td>
<td>12.64</td>
<td>18.27</td>
</tr>
<tr>
<td></td>
<td>3.22</td>
<td>7.27</td>
<td>12.02</td>
<td>16.85</td>
</tr>
<tr>
<td></td>
<td>5.38</td>
<td>6.49</td>
<td>5.87</td>
<td>10.26</td>
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</tbody>
</table>

Anova: Single Factor

**SUMMARY**

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<th>Variance</th>
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<tr>
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<td>12.88</td>
<td>4.293333</td>
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<td>21.6</td>
<td>7.2</td>
<td>0.4593</td>
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<tr>
<td>Column 3</td>
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<td>30.53</td>
<td>10.17667</td>
<td>14.00663</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>45.38</td>
<td>15.12667</td>
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**ANOVA**

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<td>Between Groups</td>
<td>192.4639</td>
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<td>64.15463</td>
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<td>0.01008</td>
<td>4.066181</td>
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<td>Within Groups</td>
<td>67.7998</td>
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</table>

**Table 20:** Statistical analysis of the % elongation for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

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<tr>
<th>% Elongation</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA 30 wt. % HA</td>
<td>0</td>
<td>7</td>
<td>28</td>
<td>56</td>
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<td></td>
<td>5.78</td>
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<td>5.11</td>
<td>18.9</td>
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<td>4.14</td>
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Anova: Single Factor

**SUMMARY**

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<td>14.69</td>
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<td>4.38</td>
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<td>3</td>
<td>13.55</td>
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**ANOVA**

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<th>P-value</th>
<th>F crit</th>
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<tbody>
<tr>
<td>Between Groups</td>
<td>243.894</td>
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<td>81.29801</td>
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<tr>
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Table 21: Statistical analysis of the % elongation for samples containing a ratio of 0.5 BCA to 0.5 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

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<thead>
<tr>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA 40 wt. % HA</td>
</tr>
<tr>
<td>day</td>
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<tr>
<td>4</td>
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<td>3.97</td>
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<tr>
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Anova: Single Factor

SUMMARY

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<th>Average</th>
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</thead>
<tbody>
<tr>
<td>Column 1</td>
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<td>12.05</td>
<td>4.016667</td>
<td>0.003233</td>
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<td>12.86</td>
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ANOVA

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<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>125.4007</td>
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<td>41.80024</td>
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<td>0.000186</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>13.03313</td>
<td>8</td>
<td>1.629142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>138.4339</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22: Statistical analysis of the % elongation for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 30% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 BCA - 0.4 PDLLA 30 wt. % HA</td>
</tr>
<tr>
<td>day</td>
</tr>
<tr>
<td>11.8</td>
</tr>
<tr>
<td>4.38</td>
</tr>
<tr>
<td>5.27</td>
</tr>
</tbody>
</table>

Anova: Single Factor

SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>21.45</td>
<td>7.15</td>
<td>16.4149</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>16.41</td>
<td>5.47</td>
<td>0.4249</td>
</tr>
<tr>
<td>Column 3</td>
<td>3</td>
<td>12.36</td>
<td>4.12</td>
<td>1.4959</td>
</tr>
<tr>
<td>Column 4</td>
<td>3</td>
<td>30.79</td>
<td>10.26333</td>
<td>1.280133</td>
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ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>63.17643</td>
<td>3</td>
<td>21.05881</td>
<td>4.294247</td>
<td>0.044098</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>39.23167</td>
<td>8</td>
<td>4.903958</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>102.4081</td>
<td>11</td>
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</tbody>
</table>
Table 23: Statistical analysis of the % elongation for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 40% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 BCA - 0.4 PDLLA 40 wt.% HA</td>
</tr>
<tr>
<td>day</td>
</tr>
<tr>
<td>4.94</td>
</tr>
<tr>
<td>3.928</td>
</tr>
<tr>
<td>4.18</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Groups</td>
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</tr>
<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
<tr>
<td>Column 4</td>
</tr>
</tbody>
</table>

ANOVA

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<thead>
<tr>
<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>80.49519</td>
<td>3</td>
<td>26.83173</td>
<td>6.550832</td>
<td>0.015106</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>32.76742</td>
<td>8</td>
<td>4.095927</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113.2626</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 24: Statistical analysis of the % elongation for samples containing a ratio of 0.6 BCA to 0.4 PDLLA with 50% HA as a function of time stored in PBS @ 37°C

<table>
<thead>
<tr>
<th>% Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 BCA - 0.4 PDLLA 50 wt.% HA</td>
</tr>
<tr>
<td>day</td>
</tr>
<tr>
<td>2.73</td>
</tr>
<tr>
<td>2.45</td>
</tr>
<tr>
<td>2.79</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
<tr>
<td>Column 4</td>
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</tbody>
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ANOVA

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<th>Source of Variation</th>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>20.36283</td>
<td>3</td>
<td>6.787608</td>
<td>7.573765</td>
<td>0.010065</td>
<td>4.066181</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7.1696</td>
<td>8</td>
<td>0.8962</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.53243</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 25: Statistical analysis of the Young’s modulus for samples containing 30 wt. % Ha as a function of BCA to PDLLA a ratio

<table>
<thead>
<tr>
<th>Young’s Modulus</th>
<th>30% HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA</td>
<td>0.6 BCA - 0.4 PDLLA</td>
</tr>
<tr>
<td>0.893451113</td>
<td>1.010370905</td>
</tr>
<tr>
<td>0.867005695</td>
<td>0.581847322</td>
</tr>
<tr>
<td>0.862989201</td>
<td>0.921443929</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>2.623446</td>
<td>0.874482</td>
<td>0.000274</td>
<td></td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>2.513662</td>
<td>0.837887</td>
<td>0.051144</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.002008749</td>
<td>1</td>
<td>0.002009</td>
<td>0.078134</td>
<td>0.793701</td>
<td>7.708647</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.102836581</td>
<td>4</td>
<td>0.025709</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.10484533</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 26: Statistical analysis of the tensile strength for samples containing 30 wt. % Ha as a function of BCA to PDLLA a ratio

<table>
<thead>
<tr>
<th>Tensile Strength</th>
<th>30% HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA</td>
<td>0.6 BCA - 0.4 PDLLA</td>
</tr>
<tr>
<td>25.8365035</td>
<td>22.7524954</td>
</tr>
<tr>
<td>24.58826307</td>
<td>7.14145658</td>
</tr>
<tr>
<td>23.6685191</td>
<td>22.7079165</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>74.09329</td>
<td>24.69776</td>
<td>1.184032</td>
<td></td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>52.60187</td>
<td>17.53396</td>
<td>81.00353</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>76.980169</td>
<td>1</td>
<td>76.98017</td>
<td>1.87328</td>
<td>0.24293</td>
<td>7.708647</td>
</tr>
<tr>
<td>Within Groups</td>
<td>164.375128</td>
<td>4</td>
<td>41.09378</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>241.355297</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 27: Statistical analysis of the percentage elongation for samples containing 30 wt. % Ha as a function of BCA to PDLLA a ratio

<table>
<thead>
<tr>
<th>% Elongation</th>
<th>30% HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 BCA - 0.5 PDLLA</td>
<td>0.6 BCA - 0.4 PDLLA</td>
</tr>
<tr>
<td>5.78</td>
<td>11.8</td>
</tr>
<tr>
<td>4.14</td>
<td>4.38</td>
</tr>
<tr>
<td>4.77</td>
<td>5.27</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>14.69</td>
<td>4.896677</td>
<td>0.684433</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>21.45</td>
<td>7.15</td>
<td>16.4149</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>7.61626667</td>
<td>1</td>
<td>7.616267</td>
<td>0.890826</td>
<td>0.398696</td>
<td>7.708647</td>
</tr>
<tr>
<td>Within Groups</td>
<td>34.1986667</td>
<td>4</td>
<td>8.549667</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41.8149333</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 28: Statistical analysis of the Fracture toughness for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>Fracture Toughness</th>
<th>(50:50) 40 HA</th>
<th>(50:50) 20 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.420273458</td>
<td>1.247364624</td>
<td></td>
</tr>
<tr>
<td>2.526932024</td>
<td>3.167902388</td>
<td></td>
</tr>
<tr>
<td>2.538058449</td>
<td>2.41346029</td>
<td></td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>7.485264</td>
<td>2.495088</td>
<td>0.004229</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>6.828727</td>
<td>2.276242</td>
<td>0.936238</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.071840057</td>
<td>1</td>
<td>0.07184</td>
<td>0.152775</td>
<td>0.715822</td>
<td>7.708647</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1.88093348</td>
<td>4</td>
<td>0.470233</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.952773537</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 29: Statistical analysis of the Fracture toughness for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>(60:40) 50 HA</th>
<th>(60:40) 30 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.082383887</td>
<td>1.803053167</td>
</tr>
<tr>
<td>1.901204875</td>
<td>1.621862713</td>
</tr>
<tr>
<td>1.970215605</td>
<td>1.453634005</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>COUNT</th>
<th>SUM</th>
<th>AVERAGE</th>
<th>VARIANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>3</td>
<td>5.953804</td>
<td>1.984601</td>
<td>0.008362</td>
</tr>
<tr>
<td>Column 2</td>
<td>3</td>
<td>4.87855</td>
<td>1.626183</td>
<td>0.030537</td>
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</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.192695367</td>
<td>1</td>
<td>0.192695</td>
<td>9.907443</td>
<td>0.034593</td>
<td>7.708647</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.077798223</td>
<td>4</td>
<td>0.01945</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.27049359</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 30: Statistical analysis of the Flexural modulus for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>(50:50) 40 HA</th>
<th>(50:50) 20 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.726733443</td>
<td>1.778665683</td>
</tr>
<tr>
<td>1.52381343</td>
<td>1.783206725</td>
</tr>
<tr>
<td>1.532658291</td>
<td>1.203406965</td>
</tr>
<tr>
<td>1.302452904</td>
<td>1.403352208</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>COUNT</th>
<th>SUM</th>
<th>AVERAGE</th>
<th>VARIANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>4</td>
<td>6.085658</td>
<td>1.521415</td>
<td>0.030077</td>
</tr>
<tr>
<td>Column 2</td>
<td>4</td>
<td>6.168632</td>
<td>1.542158</td>
<td>0.082687</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.000860575</td>
<td>1</td>
<td>0.000861</td>
<td>0.015263</td>
<td>0.90571</td>
<td>5.987378</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.33829191</td>
<td>6</td>
<td>0.056382</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.339152485</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 31: Statistical analysis of the Flexural modulus for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>(60:40) 50 HA</th>
<th>(60:40) 30 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.721239373</td>
<td>0.852712559</td>
</tr>
<tr>
<td>1.262774858</td>
<td>1.399090889</td>
</tr>
<tr>
<td>1.78305009</td>
<td>1.350470428</td>
</tr>
<tr>
<td>2.087738483</td>
<td>1.588415411</td>
</tr>
</tbody>
</table>

Anova: Single Factor

SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>4</td>
<td>6.85508</td>
<td>1.713764</td>
<td>0.116047</td>
</tr>
<tr>
<td>Column 2</td>
<td>4</td>
<td>5.19069</td>
<td>1.297672</td>
<td>0.098531</td>
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ANOVA

<table>
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<tr>
<th>Source of Variation</th>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.346265</td>
<td>1</td>
<td>0.346265</td>
<td>3.227407</td>
<td>0.122545</td>
<td>5.987378</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.643740</td>
<td>6</td>
<td>0.107289</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.989999</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 32: Statistical analysis of the Flexural strength for samples containing a ratio of 0.5 BCA to 0.5 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>(50:50) 40 HA</th>
<th>(50:50) 20 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.77846063</td>
<td>37.95583932</td>
</tr>
<tr>
<td>34.3708787</td>
<td>28.22041427</td>
</tr>
<tr>
<td>35.37514361</td>
<td>40.05228674</td>
</tr>
<tr>
<td>32.21066831</td>
<td>40.47077451</td>
</tr>
</tbody>
</table>

Anova: Single Factor

SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>4</td>
<td>136.7352</td>
<td>34.18379</td>
<td>1.900389</td>
</tr>
<tr>
<td>Column 2</td>
<td>4</td>
<td>146.6993</td>
<td>36.67483</td>
<td>32.97818</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>12.4106</td>
<td>1</td>
<td>12.4106</td>
<td>0.711644</td>
<td>0.431239</td>
<td>5.987378</td>
</tr>
<tr>
<td>Within Groups</td>
<td>104.6357</td>
<td>6</td>
<td>17.43928</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>117.0463</td>
<td>7</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Table 33: Statistical analysis of the Flexural strength for samples containing a ratio of 0.6 BCA to 0.4 PDLLA as a function of HA content

<table>
<thead>
<tr>
<th>(60:40) 50 HA</th>
<th>(60:40) 30 HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.46053708</td>
<td>38.33062331</td>
</tr>
<tr>
<td>30.28458189</td>
<td>36.44672926</td>
</tr>
<tr>
<td>27.51536065</td>
<td>36.5441938</td>
</tr>
<tr>
<td>32.43915352</td>
<td>36.21322346</td>
</tr>
</tbody>
</table>

Anova: Single Factor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>4</td>
<td>120.6996</td>
<td>30.17491</td>
<td>4.097871</td>
</tr>
<tr>
<td>Column 2</td>
<td>4</td>
<td>147.5348</td>
<td>36.88369</td>
<td>0.949778</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>90.01557012</td>
<td>1</td>
<td>90.01557</td>
<td>35.66634</td>
<td>0.000988</td>
<td>5.987378</td>
</tr>
<tr>
<td>Within Groups</td>
<td>15.14294592</td>
<td>6</td>
<td>2.523824</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>105.158516</td>
<td>7</td>
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</tr>
</tbody>
</table>