In vitro comparative assessment of the mechanical properties of a PMMA cement and a GPC cement for vertebroplasty

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Faculty of Education and Health Sciences,
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Mr Finbarr Condon, MCh, FRCSI, FRCS (Tr & Ortho)

2017
Declaration

I hereby declare that the work contained in this thesis is my own, and was completed with the counsel of my supervisors Professor Colum Dunne and Mr Finbarr Condon. This work has not been submitted to any other University or higher education institution, or for any other academic award in this University.

Where the work of others has been reported, it has been fully acknowledged and referenced.

Omar Abouazza

Mr Omar A. Abouazza
Abstract

Background:
This study examines some of the properties of cements that could be used in vertebroplasty. Polymethylmethacrylate (PMMA) is the gold standard cement, however, some of its biomechanical properties may compromise its deployment. For example, it has a much higher Young’s modulus of elasticity that makes it much stiffer than the bone it is injected into. This can result in further fractures of these surrounding vertebrae.

This study involves the testing of an alternative cement. This testing has uniquely been carried out on human cadaveric bone.

Aims:
The main aim of this study was to further develop an understanding of a new Glass Polyalkenoate Cement (GPC), by carrying out comparative testing with the PMMA cement. Tests were undertaken that were necessary to bring it closer to its end application in a theatre setting.

Methods:
Testing was performed to assess the effect of gamma irradiation used for sterilisation, on the glass transition temperature (Tg), as well as its rheology including working and setting times, thus assessing its handling and finally its mechanical properties, namely the compressive strength (CS) and the biflexural strength (BFS).

In addition, the injectability of the cement was assessed on attempting to load it into syringes with bone trocars attached for vertebroplasty cement injection into the vertebral bodies. Comparison was also made between the GPC cement and the PMMA cement following cement injection into cadaveric human, lower thoracic and lumbar vertebrae looking at the bone mineral density of the injected vertebrae, the zones of cement penetration into the injected vertebrae, the ultimate load failure on compression and the various severity and locations of the vertebral height loss and vertebral compression.

Results:
Gamma irradiation did not affect the glass transition temperature (Tg), which is an indirect measure of the glass’s structure, however, it did cause a colour change in the GPC cement from white to grey. There was no difference in the working times of the GPC cement pre and post-gamma irradiation. More importantly, it was demonstrated that the mechanical properties of the cement were altered with a decrease in both the compression strength and the biflexural strength of the GPC cement post-irradiation compared to pre-irradiation on all tested days. The cement setting medium (blood or saline) was not a statistically significant factor in this analysis (p=0.12).

On testing the cements after injection into cadaveric human vertebrae, there was no statistically significant difference in the mean bone mineral density (BMD) across all the groups for either the superior (p=0.85) or inferior (p=0.24) vertebrae, or the average of the two (p=0.45), thus randomization of vertebral units for testing was effective.

The GPC cement had very short working times which made loading the cement into syringes
quite difficult and resulted in a 100 percent failure of injection via the trocars. Furthermore, it was found to have no penetrance into the anterior third of the vertebrae on bi-pedicular injection directly with syringes. This reflected the poor injectability of the GPC cement. Although the high GPC cement viscosity limited its cadaveric vertebral penetration anteriorly in the sagittal plane, it resulted in a greater penetrance or filling of the segments it did penetrate. There was a trend to a higher failure load required for the GPC cement group compared to the current gold standard PMMA injected group but this was not statistically significant with this small sample size (p=0.92).

Conclusion:
This research has identified several areas where the GPC cement can be further developed in order for it to reach clinical application.
Acknowledgements

A great number of people helped me with this project to whom I am deeply grateful and without whom, this MD would never have been pursued.

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Many thanks to Dr Kieran McDermott and Anatomy Technician Michael Cronin (Department of Anatomy and Biosciences Institute, University College Cork), for their kindness in allowing me the use of their Anatomy Department and cadavers at such short notice and without whom this project could not have been completed.

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And finally, last but not least, my sincerest thanks goes to the people who donated their bodies for the advancement of Medicine and contributed substantially to this research without whom it would not have been possible to carry out this project.

Go raibh míle maith agaibh!
To my father, who is a gifted doctor of exceptional integrity, who taught me much of what I know, and to my mother whose amazing grace, enduring patience, support and motivation could always be counted on. To my family and friends for all of their help and patience, without you I would not be here. Thank you.
# Table of Contents

Declaration ................................................................. i  
Abstract ................................................................. ii  
Acknowledgements .................................................... iv  
List of Figures .......................................................... xii  
List of Tables ........................................................... xvi  
List of Appendices .................................................... xvii  
List of Abbreviations .................................................. xviii  

# Chapters

Chapter 1: Introduction ................................................. 1  
1.1 Rationale ............................................................ 2  
1.2 Objectives ........................................................... 3  

Chapter 2: Literature review ............................................ 6  
2.1 – Osteoporosis ....................................................... 7  
2.1.1 Introduction to Osteoporosis: ................................. 7  
2.1.2 Bone Mineral Density ........................................... 11  
2.1.3 The T-Score ........................................................ 13  
2.1.4 The Z-Score ........................................................ 15  
2.1.5 Dual Energy X-ray Absorptiometry ......................... 15  
2.1.6 Osteoporosis Non-Modifiable Risk Factors ............... 18  
2.1.7 Osteoporosis Modifiable Risk Factors ..................... 18  
2.1.8 Treatment of Osteoporosis ..................................... 19  
2.1.9 Bisphosphonates .................................................. 19  
2.1.10 Non-Nitrogen Containing Bisphosphonates ............... 20  
2.1.11 Nitrogen Containing Bisphosphonates ................... 20  
2.1.12 Atypical Subtrochanteric Femoral Shaft Fractures ........ 21  
2.1.13 Strontium Ranelate .............................................. 24  
2.1.14 Hormonal Treatment of Osteoporosis ..................... 27  

2.2 - The Structure of Bone

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.1 The Functions of Bone</td>
<td>28</td>
</tr>
<tr>
<td>2.2.2 The Extracellular Matrix</td>
<td>29</td>
</tr>
<tr>
<td>2.2.3 The Inorganic Matrix</td>
<td>29</td>
</tr>
<tr>
<td>2.2.4 The Organic Matrix</td>
<td>29</td>
</tr>
<tr>
<td>2.2.5 The Cellular Component of Bone</td>
<td>30</td>
</tr>
<tr>
<td>2.2.6 Osteoprogenitor Cells</td>
<td>31</td>
</tr>
<tr>
<td>2.2.7 Osteoblasts</td>
<td>31</td>
</tr>
<tr>
<td>2.2.8 Osteocytes</td>
<td>31</td>
</tr>
<tr>
<td>2.2.9 Osteoclasts</td>
<td>32</td>
</tr>
<tr>
<td>2.2.10 The Bone Remodelling Unit</td>
<td>33</td>
</tr>
<tr>
<td>2.2.11 The Microscopic Structure of Bone</td>
<td>35</td>
</tr>
<tr>
<td>2.2.12 The Macroscopic Structure of Bone</td>
<td>37</td>
</tr>
<tr>
<td>2.2.13 Cortical Bone</td>
<td>37</td>
</tr>
<tr>
<td>2.2.14 Cancellous Bone</td>
<td>37</td>
</tr>
</tbody>
</table>

2.3 - The Structure and Biomechanics of the Spine

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.1 The Structure of the Spine</td>
<td>39</td>
</tr>
<tr>
<td>2.3.2 The Functional Spinal Unit</td>
<td>39</td>
</tr>
<tr>
<td>2.3.3 The Vertebral Body</td>
<td>40</td>
</tr>
<tr>
<td>2.3.4 The Intervertebral Disc</td>
<td>40</td>
</tr>
<tr>
<td>2.3.5 The Vertebral End Plate</td>
<td>42</td>
</tr>
<tr>
<td>2.3.6 The Spinal Ligaments</td>
<td>42</td>
</tr>
<tr>
<td>2.3.7.1 Facet Joint Orientation</td>
<td>45</td>
</tr>
<tr>
<td>2.3.7.2 Curves of the Spine</td>
<td>45</td>
</tr>
<tr>
<td>2.3.7.3 The Line of Gravity of the Body</td>
<td>46</td>
</tr>
<tr>
<td>2.3.8 The Anterior Elements’ disproportionate load</td>
<td>49</td>
</tr>
</tbody>
</table>

2.4 - Vertebral Compression Fractures, Vertebroplasty and Kyphoplasty

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.1 Incidence of Vertebral Compression Fractures</td>
<td>51</td>
</tr>
<tr>
<td>2.4.2 Vertebral Compression Fractures</td>
<td>51</td>
</tr>
<tr>
<td>2.4.3 Two Column Theory of Holdsworth</td>
<td>52</td>
</tr>
</tbody>
</table>
2.4.4 Three Column Theory of Denis
2.4.5 Vertebral Compression Fracture Morphology
2.4.6 Intervertebral Disc Pathology Influencing Vertebral Fractures
2.4.7 Vertebroplasty
2.4.8 Vertebroplasty Trials
2.4.9 Future Vertebroplasty Trials
2.4.10 Post-Vertebroplasty Fractures
2.4.11 Young’s Modulus of Elasticity and the Stress Riser Effect
2.4.12 Vertebroplasty and Kyphoplasty Methods
2.4.13 Vertebroplasty versus Kyphoplasty
2.4.14 Patient Related Variables
2.4.15 Procedure Related Variables

2.5 – Cements

2.5.1 PMMA Cement
2.5.2 PMMA Cement Uses
2.5.3 PMMA Liquid Components
2.5.4 PMMA Powder Components
2.5.5 PMMA Porosity
2.5.6 PMMA Polymerisation
2.5.7 PMMA Cement Leakage
2.5.8 PMMA Cement Emboli
2.5.9 PMMA Cement Phases
2.5.10 PMMA and Young’s Modulus of Elasticity
2.5.11 GPC cements
2.5.12 Glass phase of GPC cements
2.5.13 Formation of GPC cements
2.5.14 Polyacrylic Acid
2.5.15 GPC Maturation Time
2.5.16 Osteogenic Properties of GPC Cements
2.5.17 GPC Cements and Ion Leaching
2.5.18 Calcium Phosphate Cements
2.5.19.1 Cement Sterilisation 84
2.5.19.2 Heat Dependent Sterilisation 85
2.5.19.3 Heat Independent Sterilisation 86
2.5.19.4 Chemical Sterilisation 86
2.5.19.5 Radiation Based Sterilisation 87
2.5.19.6 Gamma-Irradiation Sterilisation 87

Chapter 3: Materials and Methods 89

3.1 Cement Synthesis and Sterilisation 90
3.1.1 GPC Cement Synthesis 91
3.1.2 GPC Glass Powder Synthesis 91
3.1.3 Polyacrylic Acid Syntheses 94
3.1.4 PMMA Cement Preparation 94
3.1.5 Differential Thermal Analysis 97
3.1.6 Gamma Sterilisation 98

3.2 Experiment 1: Cement Working and Setting Times with
Injectability assessment 99
3.2.1 Working and Setting Time 100
3.2.2 Injectability 103

3.3 Experiment 2: Compressive Strength Testing 106
3.3.1 Compressive Strength Testing 110

3.4 Experiment 3: Biaxial Flexural Strength Testing 111

3.5 Experiment 4: Cadaveric Vertebrae Compression Testing 121
3.5.1 Preparation of Cadaveric Spines 122
3.5.2 Radiological Assessment of Cadaveric Spines 124
3.5.3 DEXA scanning Cadaveric Spine 128
3.5.4 Division of spine into FSU 137
3.5.5 Cement Injection of Vertebrae 137
3.5.6 Compression Testing of Cadaveric Vertebrae 141
3.5.7 Analysis of Vertebral compression 146

3.6 Statistical analysis 149
Chapter 4: Results and Discussion 153

4.1 Transition Temperature Post-Gamma Irradiation of GPC Cement 154

4.2 Working and Setting Times Pre and Post-Gamma Irradiation Results 156

4.3 Working and Setting Times Pre and Post-Gamma Irradiation Discussion 156

4.4 Cement Injectability Results 161

4.5 Cement Injectability Discussion 165

4.6 Pre and Post-Gamma Irradiation GPC Cement Compressive Strength Results 167

4.7 Compressive Strength Testing in Water and Blood Results 170

4.8 Pre and Post-Gamma Irradiation GPC Cement Biflexural Strength Testing Results 175

4.9 Biflexural Strength Testing in Water and Blood Results 178

4.10 PMMA Mechanical Testing Results 184

4.11 Compressive and Biflexural Strength Discussion 184

4.12 Vertebral Testing of Cements Results 185

4.12.1 Bone Mineral Density of Tested Vertebrae 185

4.12.2 Load to Failure Results 191

4.12.3 Vertebral Height Loss Analysis 194

4.12.4 Cement Penetration into Injected Vertebrae 200

4.13 Vertebral Testing Discussion 203

Chapter 5: Conclusion 205

5.1 Conclusion 206

5.2 Future work 208

Chapter 6: References 210

Chapter 7: Appendices 228
# List of Figures

| Figure 2.1: | Normal and Osteoporotic Bone Microstructure | 8 |
| Figure 2.2: | Cumulative Mortality Following Different Fractures | 10 |
| Figure 2.3: | Bone Mineral Density Chart Over Lifetime | 12 |
| Figure 2.4: | BMD T-score and fracture rates per 1000 person years | 16 |
| Figure 2.5: | T score of greater than +1 illustrated | 17 |
| Figure 2.6: | The Cutting Cone | 22 |
| Figure 2.7: | Atypical Subtrochanteric Fracture of Femur due to Bisphosphonate Use | 26 |
| Figure 2.8: | Osteoclast displaying many nuclei | 34 |
| Figure 2.9: | Haversian System | 36 |
| Figure 2.10: | The Intervertebral Disc and Inferior Vertebral Endplate | 43 |
| Figure 2.11: | Anatomy and Ligaments of the Spine | 44 |
| Figure 2.12: | Curves of the Spine | 47 |
| Figure 2.13: | Biomechanical Line of Gravity of the Body | 48 |
| Figure 2.14: | Vertebral Compression Fractures | 55 |
| Figure 2.15: | Trocar Placement into Right Pedicle under X-ray Guidance | 65 |
| Figure 2.16: | Bilateral Percutaneous Trocars and Pressurisers Attached Simultaneously for Kyphoplasty | 66 |
| Figure 2.17: | Lateral spinal screening film with bilateral kyphoplasty balloons being inflated in a vertebral body to restore vertebral height | 68 |
| Figure 2.18: | Lateral spinal screening film with cement being injected into vertebral body post balloon inflation and deflation | 69 |
| Figure 3.1: | GPC being fired at 1480°C for one hour in a crucible | 92 |
| Figure 3.2: | Shock quenching of GPC after being fired at 1480°C | 93 |
| Figure 3.3: | Simplex P® (Stryker Howmedica, Limerick) | 95 |
| Figure 3.4: | Stryker Mixevac III cement mixing bowl | 96 |
| Figure 3.5: | Dimensions (in millimetres) of mould used to determine cement setting time | 101 |
Figure 3.6: Vicat needle indenter with cement sample being indented

Figure 3.7: 13 Gauge Trocar and 5ml Luer lock tipped syringe used for vertebroplasty cement injection

Figure 3.8: Split ring moulds

Figure 3.9: Instron 4082 universal testing machine

Figure 3.10: Compression testing jig

Figure 3.11: Biaxial flexure testing jig with 3 support points inferiorly and a central compression point from superiorly

Figure 3.12: Cements samples were placed in premade circular rubber moulds

Figure 3.13: Clamp compressing the circular cement samples between two plates

Figure 3.14: Cement disc samples before and after removal from circular rubber moulds

Figure 3.15: Cement disc samples in water and in blood

Figure 3.16: Cement disc samples being tested for biaxial flexural strength

Figure 3.17: The three en bloc harvested cadaveric human spines 1, 2 and 3 respectively

Figure 3.18: Spine 1 antero-posterior and lateral X-ray views

Figure 3.19: Spine 2 antero-posterior and lateral X-ray views

Figure 3.20: Spine 3 antero-posterior and lateral X-ray views showing a lytic lesion

Figure 3.21: Phantom spine control

Figure 3.22: DEXA scanner calibration with Phantom spine control underneath water container

Figure 3.23: Spine being checked for BMD on DEXA scanner with a container of water mimicking body soft tissues as is used in DEXA scanner calibration

Figure 3.24: An example of a DEXA scan scout film with named segments and BMD and T-score result

Figure 3.25: DEXA scanner labelling spine levels for BMD
conversion to T-score

Figure 3.26: Lumbar FSU segment

Figure 3.27: Thoracic FSU segment

Figure 3.28: Transpedicular trocar placement lateral and antero-posterior X-rays

Figure 3.29: Injected GPC cement in superior vertebral body of FSU

Figure 3.30: Servohydraulic testing machine with mounted FSU for testing

Figure 3.31: Vertebral wedge compression fracture of inferior non-injected) vertebra affecting 2 columns of the spine

Figure 3.32: Vertebral coronal plane fracture of inferior non-injected vertebra affecting one spinal column

Figure 3.33: Crushed vertebral bodies with three column failure

Figure 3.34: Lateral vertebral X-ray measurements; heights and breadths

Figure 3.35: Antero-posterior vertebral X-ray measurements; heights and widths

Figure 3.36: Flow Chart for Cadaveric Spine Testing

Figure 4.1: GPC cement white pre-irradiation and colour change to grey post-irradiation

Figure 4.2: GPC Compressive Strength pre and post-irradiation

Figure 4.3: GPC Mean Compression Strength in Water and Blood

Figure 4.4: GPC Mean Compression Strength pre and post-gamma irradiation in water and post-irradiation in blood

Figure 4.5: GPC Biflexural Strength pre and post-irradiation

Figure 4.6: GPC Mean BFS in Water and Blood

Figure 4.7: Mean BFS pre and post-gamma irradiation in water and post-irradiation in blood

Figure 4.8: The distribution of the BMD of the superior vertebrae in the FSU by group

Figure 4.9: The distribution of the BMD of the inferior vertebrae in the FSU by group
Figure 4.10: The distribution of the average BMD in the FSU by group

Figure 4.11: The distribution of the failure load on the FSU by group

Figure 4.12: Mean vertebral width increases on AP radiographs post compression by cement type

Figure 4.13: Mean vertebral breadth increases on Lateral radiographs post compression by cement type

Figure 4.14: Cement Thickness on Lateral X-rays in different zones of a vertebral body by cement type

Figure 4.15: Cement Percentage fill on AP X-rays in different zones of a vertebral body by cement type on the right middle and left sides of the vertebrae
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>WHO T-score and Osteoporosis Definition</td>
<td>14</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Components of PMMA Cement</td>
<td>76</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>GPC pre-gamma irradiation rheology</td>
<td>158</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>GPC post-gamma irradiation rheology</td>
<td>159</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>PMMA (post-irradiated) rheology</td>
<td>160</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>GPC Cement Injectability</td>
<td>163</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>PMMA Cement Injectability</td>
<td>164</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Mean GPC Compressive Strength pre and post-gamma irradiation</td>
<td>168</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>GPC Compressive Strength samples in Water</td>
<td>171</td>
</tr>
<tr>
<td>Table 4.8</td>
<td>GPC Compressive Strength samples in Blood</td>
<td>172</td>
</tr>
<tr>
<td>Table 4.9</td>
<td>Mean GPC Biflexural Strength pre and post-gamma irradiation (in water)</td>
<td>176</td>
</tr>
<tr>
<td>Table 4.10</td>
<td>GPC Biflexural Strength Testing in Water</td>
<td>179</td>
</tr>
<tr>
<td>Table 4.11</td>
<td>GPC Biflexural Strength Testing in Blood</td>
<td>180</td>
</tr>
<tr>
<td>Table 4.12</td>
<td>PMMA mechanical properties (units of measurement MPa)</td>
<td>183</td>
</tr>
<tr>
<td>Table 4.13</td>
<td>Bone Mineral Density Means</td>
<td>186</td>
</tr>
<tr>
<td>Table 4.14</td>
<td>Mean Load to Failure in Each Cement Group</td>
<td>192</td>
</tr>
<tr>
<td>Table 4.15</td>
<td>Superior vertebral height loss post-compression compared to post cement injection height</td>
<td>195</td>
</tr>
<tr>
<td>Table 4.16</td>
<td>Inferior vertebral height loss post-compression</td>
<td>196</td>
</tr>
</tbody>
</table>
List of Appendices

Appendix A - Published Papers 229

Appendix B - Abstract Publications 238

Appendix C – Presentations 240

Appendix D – Posters 241
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>Al</td>
<td>Aluminium</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>Ba</td>
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<td>BaSO₄</td>
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<td>BCP</td>
<td>Biphasic Calcium Phosphate</td>
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<td>bFGF</td>
<td>Basic Fibroblast Growth Factor</td>
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<td>BFS</td>
<td>Biaxial Flexural Strength</td>
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<td>BMD</td>
<td>Bone Mineral Density</td>
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<tr>
<td>BMP</td>
<td>Bone Morphogenetic Protein</td>
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<tr>
<td>BPO</td>
<td>Benzyl Peroxide</td>
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<tr>
<td>BRU</td>
<td>Bone Remodelling Unit</td>
</tr>
<tr>
<td>BT101</td>
<td>Boyd Towler glass powder formula 101</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>C-spine</td>
<td>Cervical spine</td>
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<tr>
<td>Ca</td>
<td>Calcium</td>
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<tr>
<td>CaCO₃</td>
<td>Calcium Carbonate</td>
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<td>Calcium Oxide</td>
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<td>Calcium Phosphate</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>Cg</td>
<td>Centre of gravity</td>
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<tr>
<td>Cl</td>
<td>Chlorine</td>
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<td>COL1A1</td>
<td>Collagen 1α1</td>
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<td>CPC</td>
<td>Calcium Phosphate Cement</td>
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<td>CS</td>
<td>Compressive Strength</td>
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<td>Cerebrospinal Fluid</td>
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</tr>
<tr>
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</tr>
<tr>
<td>DCPD</td>
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</tr>
<tr>
<td>DEXA</td>
<td>Dual-Energy X-ray Absorptiometry</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DRESS</td>
<td>Drug Rash with Eosinophilia and Systemic Symptoms syndrome</td>
</tr>
<tr>
<td>DTA</td>
<td>Differential Thermal Analysis</td>
</tr>
<tr>
<td>E9</td>
<td>Polyacrylic Acid formula 9</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>EtO</td>
<td>Ethylene Oxide</td>
</tr>
<tr>
<td>et al</td>
<td>and others</td>
</tr>
<tr>
<td>F</td>
<td>Fluoride</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>FIRST</td>
<td>Fracture International Run-in for Strontium Ranelate Trial</td>
</tr>
<tr>
<td>FPPS</td>
<td>Farnesyl Diphosphate Synthase</td>
</tr>
<tr>
<td>FSU</td>
<td>Functional Spinal Unit</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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</table>
GIC Glass Ionomer Cement
GPC Glass Polyalkenoate Cement
GTPase Guanosine Triphosphatase
H Hydrogen
H2O water
H2O2 Hydrogen Peroxide
HA Hydroxyapatite
HCl Hydrochloric acid
HMG-CoA 3-Hydroxy-3-Methylglutaryl-CoA
HRT Hormone Replacement Therapy
IGF Insulin Growth Factor
in vitro occurring within a living organism
in vivo occurring in an artificial environment (not in a living organism)
ISO International Organisation for Standardisation
IU International Units
K Potassium
kGy kilo Grey unit of radiation
L Litre
L-spine Lumbar spine
LRP5 Low-Density Lipoprotein Receptor-Related Protein
mg milligrams
Mg Magnesium
ml millilitres
MMA Methyl methacrylate
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>Molecular number</td>
</tr>
<tr>
<td>MPa</td>
<td>Megapascal</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal Stem Cell</td>
</tr>
<tr>
<td>Mw</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>nm</td>
<td>nanometres</td>
</tr>
<tr>
<td>O2</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OH-</td>
<td>Hydroxyl</td>
</tr>
<tr>
<td>ONJ</td>
<td>Osteonecrosis of the Jaw</td>
</tr>
<tr>
<td>P</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PAA</td>
<td>Polyacrylic Acid</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>pH</td>
<td>Power of Hydrogen</td>
</tr>
<tr>
<td>PHA</td>
<td>Percipitated Hydroxyapatite</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear</td>
</tr>
<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
</tr>
<tr>
<td>PSA</td>
<td>Particle Size Analysis</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor Activator of Nuclear Factor Kappa-B</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor Activator of Nuclear Factor Kappa-B ligand</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Control Trial</td>
</tr>
</tbody>
</table>
RDA Recommended Daily Allowance
RES Reticulo-endothelial system
RMGIC Resin Modified Glass Ionomer Cement
S Spine Sacral spine
SBF Simulated Body Fluid
SD Standard Deviation
SEM Scanning Electron Microscope
Si Silicon
SiO₂ Silicon dioxide
SJS Stevens-Johnson Syndrome
SOTI Spinal Osteoporosis Therapeutic Intervention
SPSS Statistical Package for the Social Sciences
Sr Strontium
SR Strontium Ranelate
SrO Strontium Oxide
SSRI Selective Serotonin Reuptake Inhibitor
St Setting time
T-spine Thoracic spine
TCP Tricalcium Phosphate
TEG-DMA Triethylene glycol dimethacrylate
TEN Toxic Epidermal Necrolysis
Tg Glass transition temperature
TGF-β Transforming Growth Factor-Beta
TLICS Thoraco Lumbar Injury Classification and Severity score
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAP</td>
<td>Tartrate Resistant isoenzyme of Acid Phosphatase</td>
</tr>
<tr>
<td>TROPOS</td>
<td>Treatment of Peripheral Osteoporosis</td>
</tr>
<tr>
<td>TSC</td>
<td>Tri-sodium Citrate</td>
</tr>
<tr>
<td>TTCP</td>
<td>Tetracalcium Phosphate</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UL</td>
<td>University of Limerick</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>W</td>
<td>Weight</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>Wt.</td>
<td>Working time</td>
</tr>
<tr>
<td>wt. %</td>
<td>weight percent</td>
</tr>
<tr>
<td>X-RD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>Y</td>
<td>gamma irradiation</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
<tr>
<td>ZnO</td>
<td>Zinc Oxide</td>
</tr>
<tr>
<td>ZPC</td>
<td>Zinc Polycarboxylate Cement</td>
</tr>
<tr>
<td>ZrO2</td>
<td>Zirconium Dioxide</td>
</tr>
</tbody>
</table>
Chapter 1:

Introduction
1 - Introduction:

1.1 Rationale

The main aim of this research contained herein, is to build on the substantive research already carried out at University of Limerick (UL) by Professor Mark Towler and Dr Daniel Boyd and his team of researchers on dental based Glass Polyalkenoate Cements (GPC) which are also known as Glass Ionomer Cements (GIC) [1-11]. This project aimed to further test and assess the GPC cements with regards to orthopaedic applications with specific attention to vertebroplasty, with a view to making further recommendations in their development as deemed necessary.

The previous work undertaken at UL had developed a GPC cement from dental glass based cements that had the potential for use in orthopaedic applications [12]. This GPC cement is composed of a glass powder formula BT101 and a polyacrylic acid (PAA) called E9 (Allied Colloids, Bradford, UK) at 50 weight percentage (wt. %) with water [7, 9, 10, 13]. The BT101 glass powder consists of silicon in the form of silicon dioxide (SiO₂), zinc in the form of zinc oxide (ZnO), calcium in the form of calcium oxide (CaO) and strontium oxide (SrO) with the following mole fractions: 0.48 SiO₂-0.36 ZnO-0.12 CaO-0.04 SrO. The Polyacrylic acid E9 had a molecular weight (Mw) of 80,800 and a molecular number (Mn) of 26,100. This GPC cement combination of BT101 and E9 was chosen to be the best suited to development for orthopaedic application based on previous mechanical testing [2, 10, 13].
These GPC cements have shown many properties to date that lend themselves to orthopaedic application including good biocompatibility, antibacterial activity, short setting times and good mechanical properties [1, 4, 7, 14-16]. This study into the GPC cements as an alternative cement to PMMA for vertebroplasty uniquely assesses this in human cadaveric vertebrae for GPC cements.

1.2 Objectives

In order to develop any new GPC cement for orthopaedic application in vertebroplasty, testing in its practical application at the point of orthopaedic use was required. Thus, two sets of tests were deemed necessary; firstly, those required by ISO standards as per ISO 5833:2002 Implants for surgery – Acrylic resin cements, which specifies cement working time (dough time), bending and compression strength testing [17]. Secondly, tests that were felt necessary for orthopaedic application in vertebroplasty, including assessment post-irradiation for sterilisation, influence of setting in a blood-rich environment, injectability and cadaveric spinal testing.

The mechanical properties including its compressive strength and biaxial flexural strength were assessed pre and post-gamma irradiation, which is the method of choice in the sterilisation of medical products [18, 19]. As an end product, the GPC cement would have to be packaged and irradiated prior to medical use. Previous experience with other orthopaedic materials has shown that irradiation in air has compromised the mechanical properties of some materials such as the polyethylene used in total hip and knee arthroplasty due to excessive oxidation which resulted in unacceptable failure rates within a short period of time [20, 21]. The effect of the
gamma irradiation on both the GPC cement’s rheology (working and setting times) and its mechanical properties were also assessed.

Its compressive strength and biaxial flexural strength was also tested over time in water and in blood which will be the surrounding body fluid environment once it is injection into vertebral bodies. This was to assess if its mechanical properties were altered in the ion rich blood environment.

In addition, assessments were also carried out of the working and handling times of the GPC cement in order to assess its practicality with regards to handling and ultimately, injectability at the point of use. While short setting times are desirable to allow the patient to mobilise early following vertebroplasty, too short a working time will lead to difficulty in injecting the cement and thus make it unsuitable for this application.

Polymethylmethacrylate (PMMA) cement is the most commonly used cement in vertebroplasty [22]. It is associated with a high rate of neighbouring vertebral levels sustaining fractures post-vertebroplasty felt to be partly due to its higher compressive strength relative to that of bone, while also leading to an alteration in the load transfer to adjacent vertebral discs [23]. Adjacent and non-adjacent vertebral fractures can occur and are thought to be due to two different mechanisms. Adjacent fractures are thought to occur due to a direct pillar effect, that is caused by the difference in strength between the vertebrae from cement augmentation. While non-adjacent fractures are thought to occur due to a dynamic hammer effect, that occurs from the difference in
segmental spinal mobility from the stiffened cemented vertebra [24]. Thus, we also compared the incidence of neighbouring vertebral fractures and load to failure post-injection of PMMA and GPC cements into osteoporotic harvested human cadaveric lower thoracic and lumbar spinal segments. This was to assess if the GPC cement would be better than PMMA cement in vertebroplasty with regards to its compressive strength being closer to that of bone and thus leading to a lower “stress riser” effect and thereby a lower likelihood of neighbouring secondary vertebral compression fractures [25-27].

The above tests lead to a better understanding of the possible use of GPC cements in vertebroplasty in the future and lead to their further development.
Chapter 2:

Literature review
2.1 - Osteoporosis

2.1.1 Introduction to Osteoporosis:

The word osteoporosis comes from two Greek words; osteon meaning bone and poros meaning pore, thus osteoporosis literally means porous bone. This adequately describes the bone structure whereby the loss of trabecular plates and struts makes the bone grossly more porous due to the grossly enlarged pore structure of the bone (Figure 2.1) [28]. Osteoporosis is the most common metabolic bone disease worldwide affecting 75 million people in Europe, USA and Japan, and is thought to affect more than 200 million people worldwide [29]. It is characterised by both low bone mineral mass and microarchitectural deterioration of the bone tissue, but importantly it maintains a normal mineral to collagen ratio which distinguishes it from osteomalacia where the abnormality is that of low mineral content [30].

Between one in two and one in three women over the age of fifty years will experience osteoporotic fractures [31, 32]. It is much less common in men and is estimated to be one in five according to the National Osteoporosis Society in the United Kingdom [32, 33]. As little as a ten percent loss of bone mineral mass in a vertebra can double the risk of vertebral fractures [34]. In the United States of America (USA), the National Osteoporosis Foundation reported that by 2010, about 12 million people over the age of fifty years are expected to have osteoporosis and another 40 million to have low bone mass [35].

Osteoporosis is a silent and progressive problem affecting mainly
Figure 2.1: Normal and Osteoporotic Bone Microstructure (Adapted) [28]
postmenopausal women, which is often underdiagnosed and undertreated [36-38]. Thirty percent of postmenopausal women in Europe and the USA have osteoporosis [39, 40]. It is under-diagnosed, as often its first presentation is when a fragility fracture has already occurred, though the osteoporosis has been present many years prior.

The terms fragility or insufficiency fracture refer to fractures that occur in a weakened bone (in this case due to osteoporosis) by a force that would not usually cause a fracture in normal bone e.g. a fall from standing height or that from a lower height or lower force.

Osteoporosis results in an increased tendency for developing insufficiency fractures such as vertebral compression fractures. Other low energy fragility fractures include those of the wrist, hip, proximal humerus and ankle resulting in significant morbidity and loss of personal independence [41-45]. Other repercussions include an increased financial burden on the individual, their relatives and the state [46-48].

In addition, osteoporosis may also lead to higher mortality, as illustrated by hip fractures which are associated with approximately 12.7 to 27 percent mortality within the first year of the fracture occurring [49, 50]. But spinal fractures are also associated with increased mortality which is highest in the first year post fracture as illustrated in Figure 2.2. Men have a higher mortality rate than women in these osteoporotic fractures [51].
Figure 2.2: Cumulative Mortality Following Different Fractures [51]
2.1.2 Bone Mineral Density

Osteoporosis decreases bone mineral density (BMD) by resorption of bone through osteoclastic activity. Histologically, osteoporosis leads to decreased osteon size and enlargement of Haversian and bone marrow spaces. As trabecular bone has a greater surface area compared to that of cortical bone, it is affected earlier and to a much greater degree than cortical bone [52]. The loss of trabecular plates and struts results in a loss of support for the vertebral endplate, leading to eventual failure when an axial load is applied [53, 54].

Bone mineral mass increases between the age of two and thirty years of age, at which stage it peaks and after which it gradually decreases (Figure 2.3) [55]. The normal person’s estimated age-related bone loss is between one and three percent per year [56, 57]. After bilateral oophorectomy or during the first six to eight years after menopause (which on average is about fifty years of age), bone loss is much higher [58]. The cumulative effect of decades of bone loss leads to an osteoporotic fracture peak in the late seventies and early eighties [43].
Figure 2.3:  Bone Mineral Density Chart Over Lifetime [55]
2.1.3 The T-score

The World Health Organisation (WHO) definition of osteoporosis is based on the T-score [59]. The T-score is the number of standard deviations of measured BMD below the so-called normal mean peak BMD in a young (approximately thirty years old) healthy female cohort.

Osteoporosis is defined as a T-score of greater than 2.5 standard deviations (SD) below the normal BMD. Osteopenia is a precursor to osteoporosis and has a lesser degree of bone mineral density loss and is as such a grade between normal and osteoporotic bone. It is defined as a T-score between 1 and 2.5 standard deviations below the normal BMD. Normal bone can be up to 1 standard deviation below the normal BMD.

Severe or established osteoporosis is defined as having a T-score of 2.5 or greater associated with a fracture (Table 2.1). An illustration of a T-score of greater than +1 is shown in Figure 2.5 as would be displayed in a Dual-Energy X-ray Absorptiometry (DEXA) scan result. For each decrease in standard deviation in lumbar spinal BMD, there is an associated two-fold increase in spinal fracture risk [60]. This is illustrated in Figure 2.4 which is adapted from E. S. Siris et al [61].
### Table 2.1: WHO T-score and Osteoporosis Definition [62]

<table>
<thead>
<tr>
<th>Category</th>
<th>T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;1.0 standard deviation below young adult mean BMD</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>Between 1.0 and 2.5 standard deviations below young adult mean BMD</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>&gt;2.5 standard deviations below young adult mean BMD</td>
</tr>
<tr>
<td>Severe osteoporosis</td>
<td>&gt;2.5 standard deviations below young adult mean BMD, with a fracture</td>
</tr>
</tbody>
</table>
2.1.4 The Z-score

On the other hand, the Z-score is the number of standard deviations a patient's measured BMD is from the average BMD taking into account their age, race, and sex. This distinguishes it from the T-score which is only race and sex matched and not age matched. It is used with premenopausal women, men under the age of fifty and in children [63].

2.1.5 Dual Energy X-ray Absorptiometry

BMD is measured by a Dual Energy X-ray Absorptiometry (DEXA) scanner which utilises low dose X-rays with two distinct energy peaks. One energy peak is absorbed by bone and the other by soft tissue, hence the name. The soft tissue absorption is subtracted from the total absorption, thus giving the bone mineral component. The mass of bone mineral in the path of the beam is divided by the cross-sectional area of the beam giving the BMD and is measured in g/cm². The BMD is calculated by the DEXA scanner and the result is produced along with the T-score (Figure 2.5).
Figure 2.4: BMD T-score and fracture rates per 1000 person years [64]
Figure 2.5: T-score of greater than +1 illustrated [65]
2.1.6 Osteoporosis Non-Modifiable Risk Factors

There are a number of risk factors for osteoporosis which can be grouped into modifiable and non-modifiable risk factors. Non-modifiable risk factors include genetic polymorphisms in some of the thirty genes which are associated with osteoporosis. These include the COL1A1 (collagen 1α1), Vitamin D receptor, oestrogen receptor-1, calcitonin receptor and LRP5 (low-density lipoprotein receptor-related protein) [66, 67]. Other non-modifiable risk factors include increasing age, female sex, Asian race or White Caucasian race, early menopause i.e. before 45 years of age (whether natural or induced due to oophorectomy) as well as a parental history of hip fracture. In addition, a personal history of previous wrist fracture doubles the risk of future hip fracture and triples the risk of future vertebral fracture. The occurrence of one vertebral fracture, even one that is asymptomatic, results in an at least four-fold increased likelihood of additional fractures [60].

2.1.7 Osteoporosis Modifiable Risk Factors

Modifiable risk factors include lifestyle factors such as low body weight (less than 70kg) or a low body mass index (BMI) less than 19kg/m², cigarette smoking, excess alcohol and a sedentary lifestyle lacking in weight bearing activity. Other factors relate to drug use. The most well-known of which is steroids, with prednisolone being the most frequently used. Other drugs have been found to cause osteoporosis; anticonvulsants such as phenytoin, antidepressants such as selective serotonin reuptake inhibitors (SSRI) and more recently proton pump inhibitors (PPI) used in the treatment of indigestion and peptic ulcers [68-70]. Furthermore, there are a number of
diseases which are associated with osteoporosis independent of the drugs used to treat them. These include rheumatoid arthritis and diabetes mellitus [71].

**2.1.8 Treatment of Osteoporosis**

The treatment of osteoporosis consists of lifestyle modification and drug treatment to increase calcium in the diet and decrease bone loss. An adequate total dietary calcium of 1700 mg/day for postmenopausal women not on hormone replacement therapy (HRT) and 1000-1200 mg/day for premenopausal and postmenopausal women on HRT treatment is recommended [72]. This calcium requirement increases with age as do Vitamin D requirements. Recommended Vitamin D dose is 800 IU/day for postmenopausal not on HRT and 400 IU/day for premenopausal and postmenopausal women on HRT [73].

**2.1.9 Bisphosphonates**

The main drug class that is currently the most used in the treatment of osteoporosis is the bisphosphonate group which was developed for medical use in the 1990s [74-76]. The bisphosphonates have a very high affinity for calcium and so are concentrated in bone where they are ingested by osteoclasts by endocytosis. They are anti-resorptive agents that encourage osteoclasts to undergo apoptosis. This results in decreased bone turnover with a resulting increase in bone mineral density and as a consequence less osteoporotic fractures [77].

Their use not only includes the treatment of osteoporosis but also Paget's disease, fibrous dysplasia and osteogenesis imperfecta, as well as malignant conditions.
such as multiple myeloma and bony metastases from breast, lung and prostate cancer [78-81].

2.1.10 Non-Nitrogen Containing Bisphosphonates

The bisphosphonates can be sub-classified into two groups [82]. This is based on the presence or absence of a nitrogen atom attached to the alkyl chain of the bisphosphonate. Non-nitrogen containing bisphosphonates induce osteoclast apoptosis by forming a toxic adenosine triphosphate (ATP) analogue which interferes with mitochondrial function resulting in the osteoclast’s inability to utilise energy-dependent pathways within the cell.

2.1.11 Nitrogen-Containing Bisphosphonates

The newer and more potent nitrogen-containing bisphosphonates are structurally similar to pyrophosphate and work by inhibiting enzymes that utilise pyrophosphate, such as farnesyl diphosphate synthase (FPPS) in the 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) reductase pathway. This leads to an inhibition of guanosine triphosphatase (GTPase) formation that is necessary for protein biosynthesis, resulting in an alteration in cell membrane proteins that are required to maintain the ruffled cell membrane border necessary for bone resorption and eventually leads to apoptosis of osteoclasts [82]. They are up to a thousand times more potent in their anti-resorptive activity [83].

This drug class includes the orally administered alendronate (Fosamax®) and risedronate (Actonel®), and the intravenously administered zolendronic acid
(Zometa®). Their main side effect includes various gastrointestinal problems such as oesophagitis and gastritis [84]. A more serious side effect is bisphosphonate-associated osteonecrosis of the jaw (ONJ), the majority of which has been diagnosed after dental procedures such as tooth extraction while on these drugs especially the intravenous forms which are used for cancer patients [85, 86].

2.1.12 Atypical Subtrochanteric Femoral Shaft Fractures

A recognised side effect of long-term treatment with bisphosphonates, especially alendronate, is an increase in atypical subtrochanteric femoral shaft fractures due to impaired physiological bone remodelling from osteoclast suppression [87-91].

Osteoclasts are not only involved in decreasing bone mineral density but they are the leading cells of structures called cutting cones that are composed of osteoclasts and osteoblasts (Figure 2.6) [92]. The osteoclasts are at the leading edge of the cutting cone where they resorb bone and are followed by osteoblasts which lay down bone again. This bone is remodelled according to the stresses exerted upon it, which is the basis of Wolff’s law [93]. This prevents the formation of micro stress fractures and their propagation.
Figure 2.6: The Cutting Cone (Adapted) [92]
By inhibiting osteoclasts bone remodelling is also inhibited resulting in bone with a higher bone mineral density and sometimes even thicker cortical bone but this bone has not remodelled according to the stresses it is subjected to and is thus prone to micro stress fractures that accumulate and propagate as illustrated by Figure 2.7 [94]. This shows a thicker lateral cortical beak in the subtrochanteric femoral area that later develops a spontaneous stress (also known as insufficiency or fatigue) fracture resulting in an atypical subtrochanteric femoral fracture that is transverse and is characteristic of bisphosphonate use [95]. Lateral cortical beaking is recognised as a precursor to fracture [96].

This fracture occurs at the lateral cortex of the proximal femur as it is an area that is subject to high tension forces [97]. This type of fracture can occur from little or no trauma and its association with bisphosphonates was only first reported in 2005 [98]. The fracture can be preceded by groin or thigh pain [99]. In addition, once an atypical subtrochanteric fracture has occurred on one side there is an increased risk of developing such a fracture on the contralateral side [100]. It should also be noted that while the proximal subtrochanteric femur is the most common site for these atypical fractures, they do also occur in other regions of the femoral shaft [101].

In a study carried out in California, it was found that patients who continued bisphosphonate treatment for three or more years after the occurrence of an atypical subtrochanteric fracture, went on to develop a similar contralateral atypical fracture in almost fifty-four percent of cases [100]. In contrast, in patients who stopped
bisphosphonate treatment within months after their atypical femoral fracture, only approximately twenty percent went on to develop a contralateral fracture.

The risk of developing this atypical subtrochanteric fracture is low and is currently quantified as between one per one thousand to one per two thousand patients on treatment per year [102]. For every one atypical subtrochanteric fracture that occurs on bisphosphonate treatment, between fifty and a hundred osteoporotic fractures are prevented [91, 100]. Hence, the benefits of being on bisphosphonates to prevent fractures still outweigh their risk of causing fractures.

2.1.13 Strontium Ranelate

Another drug that is used in the treatment of osteoporosis is Strontium ranelate which is commercially available as Protelos®. It belongs to a class of drugs called dual action bone agents (DABA). It is unique in the sense that it has a dual mode of action.

Not only does Strontium ranelate inhibit osteoclastic activity but it also stimulates the differentiation of pre-osteoblasts to osteoblasts, thus inhibiting bone resorption and stimulating bone formation simultaneously [103]. Strontium ranelate has a high affinity for bone and is heavier than the calcium it replaces in the bone matrix. This leads to a disproportionate increase in BMD as measured by DEXA scanning.
Its dual mode of action, lack of gastrointestinal side effects and lack of association with osteonecrosis of the jaw and atypical subtrochanteric femoral fractures as side effects give it superiority over bisphosphonates. Strontium can be incorporated into GPC cements and so can leech out locally to the treated as well as neighbouring osteoporotic vertebrae [11, 104]. It is however not without its own side effects. Reported rare side effects include; drug rash with eosinophilia and systemic symptoms (DRESS) syndrome, Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) [105, 106]. In June 2013, the European Medicines Agency ‘s Pharmacovigilance Risk Assessment Committee (PRAC) published new safety warnings about Strontium ranelate’s increased risk of myocardial infarction.

FIRST (Fracture International Run-in for Strontium Ranelate Trial) was an open walk-in trial to recruit patients for one of two of the following major phase III clinical studies, which were assessing the reduction in the incidence of postmenopausal women developing new non-vertebral fractures in TROPOS (Treatment of Peripheral Osteoporosis) and new vertebral fractures in SOTI (Spinal Osteoporosis Therapeutic Intervention) which were started in 2000 and have shown that the daily oral dose of 2gm of Strontium ranelate can significantly reduce vertebral fracture and hip fracture risk over three years [107, 108].

The radio-opaqueness of Strontium which allows it to be visualised under X-ray screening, its affinity for bone, its effectiveness in the treatment of osteoporosis and its lack of serious side effects to date have all been factors which have encouraged us to incorporate it into our GPC cement.
Figure 2.7: Atypical Subtrochanteric Fracture of Femur Due to Bisphosphonate Use
2.1.14 Hormonal Treatment of Osteoporosis

There are other medical therapies for the treatment of osteoporosis and these include the following hormones: Parathyroid hormone (PTH) which is used in severe or refractory osteoporosis, hormone replacement therapy (HRT) which is oestrogen hormone replacement in the postmenopausal or post-oophorectomy woman, as well as calcitonin hormone [109].

Other agents include denosumab, an anti-resorptive agent, IgG2 monoclonal antibody for human receptor activator of nuclear factor-kappa B ligand (RANKL) [110]. The details of these treatments are beyond the scope of this thesis.
2.2 - The Structure of Bone

2.2.1 The Functions of Bone

Bone is a specialised connective tissue that has many functions as part of the skeletal system. Its solid structure allows it to play an important role in the mechanical support and protection of tissues and vital internal organs. Its resistance to compression especially in the spine allows the body to maintain an upright posture. Its hardness is second only to enamel and dentin contained in teeth within the human body [111]. It also provides sites of attachment for muscles and ligaments in order to facilitate the movement and stability of joints respectively. In addition, its light weight facilitates movement without compromising strength, as weight for weight bone is as strong, if not stronger than most steels [112].

But it also has other important functions including haematopoiesis where the bone marrow located within cancellous bone is involved in the generation of peripheral blood cells such as erythrocytes (red blood cells-RBC), leucocytes (white blood cells-WBC) and platelets.

In addition, it acts as the primary reservoir for calcium in the human body and is subject to hormonal control (such as parathyroid hormone, calcitonin, oestrogen and corticosteroids) in the regulation of calcium and phosphate metabolism and homoeostasis [113].
2.2.2 The Extracellular Matrix

Bone is a complex composite viscoelastic material that consists of cells which account for 10 percent and an extracellular matrix (ECM) which accounts for 90 percent of the bone structure [114]. The extracellular matrix consists of both organic (protein) and inorganic (mineral) components, both of which play important roles. The organic component accounts for approximately 25 percent of the ECM and confers tensile strength to bone, while the inorganic component accounts for 60 percent of the ECM and confers compressive strength to bone, with the balance made up of water. Bone's strength and elastic modulus are approximately proportional to the square of its density [115].

2.2.3 The Inorganic Matrix

The inorganic component consists mainly of calcium and phosphate in the form of calcium hydroxyapatite and tricalcium phosphate. The inorganic bone matrix contains 80 percent of the body’s phosphate and 99 percent of the body’s calcium. It is calcium hydroxyapatite (HA) that confers the compressive strength of bone, making bone hard and rigid [116]. Bone matrix mineralisation is the formation of calcium phosphate crystals which occurs as a result of a phase transformation of soluble calcium and phosphate at specific regions within the organic collagen bone matrix.

2.2.4 The Organic Matrix

The organic component gives bone its flexibility and resilience and consists mainly of type I collagen (90 percent) which is the main contributor to the tensile strength of the ECM [117]. Smaller amounts of type III and type IV collagen also exist
and their presence confers pliability to the ECM. It also contains non-collagenous proteins such as cell adhesive proteins, proteoglycans that provide tissue structure, biologically active proteins that are potent regulators of cell activation and differentiation such as bone morphogenetic proteins (BMP) and different types of growth factors such as insulin growth factor (IGF), basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β) [116].

Collagen is arranged in fibrils which have empty spaces called hole-zones between fibril ends, and pore-zones running longitudinally between collagen fibrils [118]. It is within these hole and pore zones that mineralisation of the extracellular collagen protein matrix occurs. The degree of mineralisation increases with age conferring more stiffness to bone mostly in the first three decades of life following which bone mineral density decreases. Hence, adult bones are more prone to fracture rather than plastic deformation as occurs in paediatric bone [119].

2.2.5 The Cellular Component of Bone

The cellular component which is involved in bone homoeostasis consists of osteoprogenitor cells, osteoblasts, osteocytes, osteoclasts as well as cells in the bone marrow and periosteum. In normal bone homoeostasis, bone production matches resorption resulting in a continuous turnover of bone [120]. In osteoporosis, there is decreased bone formation with age leading to a decrease in bone mass. Depending on the age of an individual bone mass is completely turned over every 4 to 20 years (faster in younger individuals) with the average adult rate of turnover decreasing for females by three to four percent per year [121]. Males on the other hand, decrease at a slower
rate of between 1.5 to 3 percent per year. This dynamic nature of bone allows it to repair and remodel potentially compromised or weakened regions over a period of time according to stress demands placed on the bone over that period.

2.2.6 Osteoprogenitor Cells

These cells are mesenchymal stem cells which are undifferentiated. When stimulated, they proliferate and differentiate into osteoblasts [122]. They are located within bone at the endosteum and on the outer surface of bone on the periosteum.

2.2.7 Osteoblasts

Osteoblasts are derived from undifferentiated osteoprogenitor stem cells in bone marrow and are the bone cells that synthesise bone matrix and regulate osteoclast activity. Osteoblasts have three fates: they can die by the process of apoptosis, they can become inactive and become bone lining cells or they can become osteocytes that are activated osteoblasts that have become embedded in the ECM [123].

2.2.8 Osteocytes

Osteocytes account for 90 percent of bone cells. They synthesise and secrete osteoid (unmineralized bone matrix) which then surrounds them and contains type I collagen. They have hormone receptors for parathyroid hormone and calcitonin. Interestingly, they respond to both mechanical and electrical potential stimuli too [124-126]. Thus, these cells are involved in Wolff’s law which states that the remodelling of bone is influenced and modulated by the mechanical stresses placed
upon it, essentially this means that bone is laid down where needed and resorbed where it’s not needed [93].

Osteocytes have cell processes which are in contact with other cell processes allowing cell to cell communication. Osteocyte cell membranes in combination with their cell processes cover 90 percent of the mineralised bone matrix, thus allowing access to this large amount of mineral in the control of calcium homoeostasis.

2.2.9 Osteoclasts

Osteoclasts on the other hand, are derived from the reticulo-endothelial system (RES) of the haematopoietic macrophage and monocyte stem-cell line [127]. These cells may be found in the bone marrow and circulating in the blood stream. When stimulated to activate they proliferate and fuse to form multinucleated osteoclasts with large amounts of mitochondria and lysosomes (Figure 2.8). When they have finished their bone resorbing activity, they may reform multiple mononuclear cells by cell division.

Osteoclasts resorb bone on both endosteal and periosteal surfaces. In addition, they are quite mobile and can move from one site of bone resorption to another. They have a ruffled brush border when lying on the surface of bone so as to increase the surface area for resorption. At this sealed brush border, the pH is lowered by the secretion of hydrogen ions (via the carbonic anhydrase system, the Na⁺/H⁺ exchange system as well as the adenosine triphosphate (ATP)-dependent proton pumps at the cell membranes) [128].
The inorganic apatite crystals are dissolved by the low pH while the organic matrix is hydrolysed by secreted lysosomal enzymes such as tartrate resistant isoenzyme of acid phosphatase (TRAP). When the HA is dissolved, its calcium and phosphate components are absorbed back into the bloodstream.

Osteoclast activity is regulated by osteoblasts via parathyroid hormone (PTH) [129]. Osteoblasts are stimulated to release receptor activator of nuclear factor kappa B ligand (RANKL) by intermittent pulsatile exposure to PTH. RANKL released by the osteoblasts binds to RANK receptors on osteoclast precursors stimulating them into becoming active mature osteoclasts which then cause bone resorption. A number of recent studies have shown that other cell sources of RANKL such as osteocytes exist [130, 131].

2.2.10 The Bone Remodelling Unit

As mentioned previously, osteoblasts and osteoclasts form the cutting cone which is the bone remodelling unit (BRU). Firstly, osteoclasts form the lead end of the cutting cone which resorbs bone resulting in a resorption pit called a Howship lacuna [132]. The osteoclasts are followed by osteoblasts that then form new bone matrix. On completion of bone formation, the osteoblasts form bone lining cells. If this occurs in cortical bone, then osteons and Haversian canals form around blood vessels within the bone.
Figure 2.8: Osteoclast displaying many nuclei [133]
2.2.11 The Microscopic Structure of Bone

Microscopically, the fundamental structural unit of bone is the osteon as illustrated in Figure 2.9. At the centre of each osteon is a Haversian canal through which passes blood vessels and nerves fibres [27]. Surrounding this Haversian canal is a series of layers of mineralized matrix called lamellae. At each of the lamellae, there are small cavities called lacunae, within each is an osteocyte. Osteocyte cell processes within canaliculi connecting lacunae and thus osteocytes, allowing cell to cell communication and also allowing nutrients from the Haversian canal blood vessels to reach the osteocytes.

Within a layer of each lamella, collagen fibres run in parallel. Collagen fibres in adjacent lamellae run at oblique angles to other lamellae. This intertwining of collagen fibre structure between lamellae within an osteon increases the bones resistance to torsion as well as conferring increased tensile strength and toughness [134]. Osteons are attached to each other via cement lines containing glycosaminoglycan (GAG) proteins which serve as a cementing substance between layers. Cement lines are not crossed by collagen fibres or canaliculi. This forms an area of relative weakness in the bones microstructure along which cracks may propagate [135].
Figure 2.9: Haversian System [136]
2.2.12 The Macroscopic Structure of Bone

Macroscopically, there are two very different types of bone: cortical (also known as compact) bone and cancellous (also known as trabecular) bone which differ greatly in both structure and function.

2.2.13 Cortical Bone

Cortical bone accounts for 80 percent of the adult skeleton [137]. It forms the diaphysis of long bones where it is much thicker in diameter and is load bearing. It is the outer casing of the metaphysis and epiphysis of long bones as well as that of cuboid bones but is much thinner in diameter in these regions. Its lamellae are laid down in a tubular pattern in contrast to cancellous bone whose lamellae are laid down in sheets. Cortical bone is quite dense, of low porosity and has a high modulus of elasticity. It is more resistant to torsion and bending stress than cancellous bone [138].

2.2.14 Cancellous Bone

Cancellous bone on the other hand, is found in the metaphysis and epiphysis of long bones as well as the core of cuboid bones (e.g. vertebrae) where it has a load bearing function. It also consists of lamellae which are laid down in parallel sheets to form trabeculae. These trabeculae form a three-dimensional interconnecting lattice of plates and struts (or rods). Trabeculae are aligned along axes of mechanical (tensile or compressive) stress [139, 140]. However, there are no Haversian systems in cancellous bone [141]. This is due to the high porosity of the bone which exposes a high surface area of the bone to its blood supply in the bone marrow.
As a result of this high surface area, cancellous bone has eight times the metabolic turnover of cortical bone [142]. The porous areas of cancellous bone are filled with red (haemopoetically active) bone marrow. It is less elastic and less brittle than cortical bone. Cancellous bone is always surrounded by cortical bone. In osteoporosis, there is thinning of the trabecular struts with increased porosity of the cancellous bone, which is why osteoporotic patients are more prone to metaphyseal (e.g. tibial plateau) and vertebral compression fractures. In addition, osteoporosis also affects cortical bone which decreases in thickness thus diminishing bone strength and stiffness [143].
2.3 - The Structure and Biomechanics of the Spine

While describing the structure of the spine, there will be a focus on the biomechanics of the thoracolumbar spine where the majority of vertebral compression fractures occur [144].

2.3.1 The Structure of the Spine

The spine is a complex segmented structure that has numerous functions. It supports the body in an upright position as well as being flexible enough to allow movement in multiple planes including flexion (forward bending), extension (backwards bending), lateral flexion (bending side to side) and rotation (turning) as well as a combination of the above. In addition, it acts as a shock absorber during weight bearing activities [145]. Finally, it helps protect the spinal cord and nerves.

As mentioned it is segmented and consists of 33 vertebrae consisting of 7 cervical (in the neck), 12 thoracic (in the chest), 5 lumbar (in the lower back) and 5 sacral as well as 4 coccygeal vertebrae (which make up the tail bone). The cervical, thoracic and lumbar vertebrae have intervertebral discs while the sacral and coccygeal segments do not and are fused together.

2.3.2 The Functional Spinal Unit

The functional structural unit of the spine is called the functional spinal unit (FSU) [146]. It consists of two sequential vertebrae and their intervening intervertebral disc as well the surrounding supportive soft tissues including ligaments and joint capsule. Each vertebra consists of an anterior vertebral body and a posterior vertebral
arch surrounding a vertebral (spinal) canal through which runs the spinal cord and nerves. The intervertebral discs, facet joint capsules and ligaments provide intrinsic spinal stability and the muscles provide extrinsic spinal stability.

2.3.3 The Vertebral Body

The vertebral body consists of an outer vertebral cortical shell and an inner cancellous bone core. As one moves from cervical to thoracic and onto lumbar vertebrae, the vertebral body (and its disc) gets larger both in diameter and in height, due to the increasing body weight and axial loads it must bear [147]. The posterior vertebral arch is made up of a pair of pedicles, a pair of laminae, and seven processes; 2 superior articular facets, 2 inferior articular facets, 2 transverse processes and 1 spinal process. It is the posterior elements, mainly the facet joints, that guide the movement of a motion segment and resist shear forces that occur in rotation [148].

The vertebral bodies increase in width in a craniocaudal (i.e. head to toe) direction with the exception of the T1 to T3 segment [149]. The lumbar vertebrae are thus the largest vertebrae. Their anterior height is normally greater than their posterior height, thereby resulting in a lumbar lordosis (posterior curvature in the sagittal plane) [147].

2.3.4 The Intervertebral Disc

The intervertebral discs account for approximately 25 percent of the total spinal height, which varies according to upright or recumbent posture [150]. The intervertebral disc is the largest avascular structure in the human body [151]. It
consists of mainly 2 parts; a gelatinous centre called the nucleus pulposus and a fibrous outer shell called the annulus fibrosus illustrated in Figure 2.10. The nucleus pulposus is a hydrated gelatinous structure consisting of 80 percent water and the other 20 percent of hydrophilic proteoglycans and collagen type 2. The proteoglycans form aggrecans whose main function is to trap up to 500 times their own weight in water [152]. It is the water content that gives the nucleus pulposus its strength via the hydrostatic water pressure that is able to bear the axial stress of the body load placed upon it. The collagen type 2 also allows it to resist compressive loads [153]. The intervertebral disc has the most stiffness in compression and this is increased in flexion. The intradiscal pressure is highest in the flexed sitting position and lowest in the prone positions [154]. Maintenance of the spines normal lordosis in the lower thoracic and lumbar spine helps to offload the disc.

The annulus fibrosis is the outer fibrocartilaginous shell whose function is to contain the nucleus pulposus. It too has a high water content of 65 percent and is made up of 15 to 20 sheets or lamellae of collagen type 1 which are arranged in a criss-cross pattern allowing it to endure high bending and torsional loads as well as tensile stresses [155]. The intervertebral disc nucleus pulposus bulges out circumferentially exerting tensile stresses on the annulus fibrosus as high as 5 times the axial load applied especially in the lower lumbar segments [156].

As the intervertebral disc ages, it becomes dehydrated due to a decrease in its proteoglycan content and thus less pliable and transfers the axial load to the peripheral annulus fibrosus, vertebral body and facet joints [157]. The intervertebral disc has
viscoelastic properties and is subject to creep (gradual deformation under a constant compressive force over an extended period of time) and hysteresis (a decreasing ability to absorb energy with time) [158]. These are important mechanisms that contribute to vertebral compression fractures in the elderly.

2.3.5 The Vertebral End Plate

In addition, the superior and inferior cortical surfaces of the vertebral bodies are capped by a cartilaginous pad that is attached to the intervertebral discs and is called the vertebral end plate (superior and inferior). Its biochemical make up is very similar to that of the intervertebral discs and plays a role in providing vascularity to the central intervertebral discs [159].

2.3.6 The Spinal Ligaments

The ligaments of the spine consist of two types; the intra-segmental and inter-segmental. The intra-segmental ligaments are those that connect 2 sequential vertebrae and include: the ligamentum flavum (between the laminae), the inter-spinous (between the bodies of each of the spinous processes) and the inter-transverse (between transverse processes) ligaments.

The inter-segmental ligaments on the other hand are those that connect several vertebral segments and include the supraspinous ligament (between the tips of spinous processes), the anterior longitudinal ligament (connecting the anterior vertebral bodies) which is strong and resists hyperextension and the posterior longitudinal ligament (connecting the posterior vertebral bodies and forming the anterior part of
Figure 2.10: The Intervertebral Disc and Inferior Vertebral Endplate [160]
Figure 2.11: Anatomy and Ligaments of the Spine [161]
the spinal canal) illustrated in Figure 2.11. The posterior longitudinal ligament is weaker than the anterior longitudinal ligament [162].

The above ligaments have a high collagen content limiting their extensibility, with the exception of ligamentum flavum which has a high percentage of elastin content [163]. This confers elastic properties on the ligament which is constantly under tension and is thought to contribute to keeping a sustained intradiscal pressure which aids the intrinsic support of the spine.

2.3.7.1 Facet Joint Orientation

There are a number of additional factors which contribute to the biomechanics of the spine. Firstly, the orientation of the facet joint in each of the segments of the spine determines the type and range of motion of that spinal region. In the thoracic region, the facet joint orientation allows lateral flexion and rotation but limited flexion and extension. While in the lumbar region the facet joint orientation allows for flexion and extension as well as lateral flexion but almost no rotation [164]. In contrast, the lumbosacral facet joints allow for appreciable rotation.

2.3.7.2 Curves of the Spine

Secondly, the spinal column is not a straight column. It consists of two different types of sagittal curves. Curves which are convex anteriorly are known as lordotic curves while those that are concave anteriorly are known as kyphotic curves. The cervical and lumbar curves are lordotic while the thoracic and sacral curves are kyphotic (Figure 2.12). This alternating curve pattern to the spine confers a spring-like
capacity to the spine allowing it to withstand higher loads when coupled with the shock absorbing capability of the intervertebral discs and the elastic ligamentum flavum while being stabilised by the other spinal ligaments. This allows the spine to withstand much higher loads than if it had been straight. In addition, the trunk muscles confer extrinsic support and stability on the spine, while also modifying static and dynamic loads exerted on the spine [164].

2.3.7.3 The Line of Gravity of the Body

Thirdly, the line of gravity of the body lies anterior to the spine, passing anterior to the body of L4. Thus, there is a constant forward flexing moment on the spine in the normal erect stance. The line of gravity of the body is the centre of gravity (cg) of the body (that portion of the body of weight W that exists above the lower lumbar spine L5/S1) and is under the influence of the gravitational force and is expressed as a vector. This is illustrated in Figure 2.13.

A moment force is mass (W) multiplied by the perpendicular distance from the fulcrum (L). In relation to the spine, the moment is a product of the weight of the upper body (which is constant) and the lever arm of the force (which is the distance of the line of gravity from the fulcrum that is usually at a lower lumbar intervertebral disc and moves according to posture). Thus, any force or situation that causes further flexion of the spine will result in an increasing magnitude of the moment force on the lower thoracic and lumbar spine as the line of the centre of gravity moves anteriorly and further away from the spine, thereby increasing the lever arm distance L.
Figure 2.12: Curves of The Spine [165]
Figure 2.13: Biomechanical Line of Gravity of the Body [52]
These anterior flexion forces are usually counterbalanced by the intrinsic and extrinsic spinal ligaments as well as the erector spinae muscles and other spinal extensor muscles such as the multifidus muscles and inter-transversarii muscle groups illustrated in Figure 2.13 as force S which act at a constant distance (I) from the fulcrum.

### 2.3.8 The Anterior Elements’ Disproportionate Load

Fourthly, 80 percent of the compressive force acting on the spine in the erect resting standing position is borne by the anterior elements of the lumbar vertebral bodies and their intervertebral discs [166]. This is illustrated by the fact that in the erect relaxed standing position the axial load on the L3 and L4 discs is almost twice the weight of the body above these levels. Furthermore, 40 percent of the vertebral body’s resistance to compression is borne by the cortical bone shell [167]. The posterior elements, specifically the articular facets at their maximal loading which occurs at hyperextension of the spine, only bear 30 percent of the axial load [168]. Thus, even in hyperextension most of the axial load is transmitted via the anterior elements of the spine.

In addition, previous testing by other investigators has shown that on compressive load testing of the lower spine, fracture point was reached in the spinal vertebral bodies between 5,000 and 10,000 Newtons before any intervertebral disc damaged occurred. Thus the intervertebral disc is more capable of resisting compressive forces than bone [169].
All of the above results in the lower lumbar spinal vertebral bodies having a high anterior compressive force which can result in vertebral wedge compression fractures in the weakened osteoporotic bone. As a result, vertebral fractures usually start as anterior wedge fractures and then progress to burst fractures depending on the magnitude of the load exerted on them.
2.4 - Vertebral Compression Fractures, Vertebroplasty and Kyphoplasty

2.4.1 Incidence of Vertebral Compression Fractures

A 50 years old white Caucasian woman has a 16 percent lifetime risk of experiencing a vertebral fracture and this risk is 5 percent in white men [170]. There are approximately 700,000 diagnosed vertebral compression fractures annually in the USA [171]. However, less than 33 percent of these become chronically painful. It’s estimated that only one-third of vertebral fractures come to clinical attention [172].

The majority of these fractures occur in the elderly population affecting 20 percent of people older than 70 years and 50 percent occurring in women greater than 80 years of age [173, 174]. A kyphotic deformity of the spine results from anterior wedging of the vertebrae and can lead to a decrease in pulmonary function especially in thoracic vertebral fractures and leads to an increased risk of further compression fractures [175-178]. The presence of at least one vertebral fracture results in a five-fold increased risk of developing another vertebral fracture.

2.4.2 Vertebral Compression Fractures

Vertebral compression fractures occur mostly about the thoracolumbar junction at T12/L1 vertebral levels where there is a change in the spinal curve from kyphotic to lordotic and there is a change in facet joint orientation which facilitates this spinal curve change [144]. More than 50 percent of the thoracolumbar spine fractures occur in the four transitional vertebrae between T11 to L2 inclusive [179].
This is also due to the change from the well supported thoracic vertebrae (T1 – T10) which are attached to the rib cage [180].

Vertebral compression fractures can lead to height loss with increasing kyphotic spinal deformity. In addition, with severe kyphosis there may be costo-iliac impingement, where the lowermost ribs start impinging on the iliac crests [181]. These can also lead to compromised pulmonary function. They are also associated with a higher mortality within 2 years than post hip fractures [51].

2.4.3 Two Column Theory of Holdsworth

The stability of the spine post-vertebral fracture is based on the column theories of the spine. There are two theories of the spine. The first is a two-column theory of the spine is based on work by Holdsworth in 1970 [182]. He splits the spine into two columns, an anterior column and a posterior column. The anterior column consists of the anterior longitudinal ligament, the whole vertebral body and the posterior longitudinal ligament along with the whole of the intervertebral disc. The posterior column consists of everything posterior to the posterior longitudinal ligament i.e. the pedicles, facet joint complex, lamina, spinous process and their associated ligaments. According to Holdsworth, a fracture affecting only the anterior column is stable, while a fracture which affects the posterior column as well is unstable.

2.4.4 Three Column Theory of Denis

The second column theory is the three-column theory of Denis which was introduced in 1983 [183]. Essentially, this theory further subdivides Holdsworth’s
anterior column into an anterior and middle column via an arbitrary line which divides the spinal vertebral body and disc in half in the coronal plane. The posterior column is the same as that for Holdsworth.

According to this theory, a fracture of more than one column (anterior and middle columns at least) confers instability to the spine. Essentially, Denis is stating that middle column fractures confer instability on the spine. This has not been borne out in more recent experience since then, as only fractures including the posterior column have been found to confer instability. As a result, there is a return to scoring systems that resemble the two-column theory of Holdsworth in terms of determining the stability of spinal vertebral fractures such as the Thoraco Lumbar Injury Classification and Severity score (TLICS) [184, 185].

2.4.5 Vertebral Compression Fracture Morphology

Vertebral compression fractures have several morphologies. As the anterior part of the vertebra fails, there is anterior wedging of the vertebral body due to the posterior part of the vertebral body remaining intact resulting in a relatively stable aptly named anterior wedge fracture. As the compressive force continues, the posterior vertebral body fails, leading to a burst fracture which is less stable. If the compressive force is large enough then failure of the posterior arch elements of the vertebra also occurs e.g. at the pedicles, laminae or facet joints. This results in a very unstable burst fracture.
The anterior part of the superior end plate and cortical bone of the vertebral body is usually the first to fail, resulting in wedge fractures. A biconcave vertebral fracture results from both the superior and the inferior end plates and their cortices failing. If the posterior part of the vertebral body fails first, then a crush fracture results. Posterior wedge fractures are rare and would suggest a pathological fracture from a more sinister neoplastic lesion [186]. The severity of the height loss is classified as mild (grade 1), moderate (grade 2) and severe (grade 3) depending on the percentage height loss as illustrated in Figure 2.14 [187].

2.4.6 Intervertebral Disc Pathology Influencing Vertebral Fractures

The intervertebral disc helps to redistribute compressive load to the endplate and vertebral body. In young adults, the discs distribute the compressive load evenly on the vertebral body. As patients get older, intervertebral disc degeneration occurs leading to a loss in disc fluid and height. This leads to uneven distribution of compressive forces. In the erect position, these compressive forces are mostly distributed to the posterior vertebral body, while in the forward flexed posture these forces are distributed anteriorly on the vertebral bodies [188].

As a result, the anterior vertebral body is stress shielded from these compressive forces in the erect position due to uneven load redistribution from degenerate discs [188]. Since bone is remodelled according to the direction and magnitude of the stresses exerted on it (as per Wolff’s law), this then results in the anterior vertebral bone becoming weakened while the posterior vertebral bone is made comparatively stronger [93]. In the flexed position the intervertebral disc concentrates
Figure 2.14: Vertebral Compression Fractures Classification [189]
the compressive stress on the anterior vertebra which is weakened resulting in gradual anterior wedging which accentuates the problem. This leads to a creep phenomenon, whereby there is a gradual progressive loss in anterior vertebral height over time under a constant compressive force. This in turn, leads to a progressive increase inkyphosis of the spine.

In addition, the weakened anterior vertebral bone deforms inwards by the nucleus pulposus under compressive forces, leaving mainly the annulus fibrosis of the disc to take the rest of the compressive forces. This effectively leads to a loss in disc height especially anteriorly leading to higher contact stress posteriorly which further strengthens the posterior elements and stress shields the anterior elements. This is in keeping with Hooke’s law which states that when two springs next to each other are loaded (the anterior and posterior halves of the vertebral bodies), more force is transmitted through the stiffer spring (posterior vertebral body) [190]. Thereby stress shielding the anterior half of the vertebral body according to Wolff’s law [93].

2.4.7 Vertebroplasty

Up until recently the treatment of vertebral compression fractures consisted of bed rest, bracing and analgesics until the advent of vertebroplasty [191]. Vertebroplasty is the minimally invasive, percutaneous trans-pedicle injection of cement into vertebral bodies.

Vertebroplasty was first successfully performed by Galibert in France in 1984 for the treatment of a painful cervical vertebral haemangioma [192]. Vertebroplasty
has now been used in the treatment of painful osteoporotic fractures since 1993 [193]. Its indications for treatment include: preventing further collapse of osteoporotic vertebral fractures, to provide pain relief for refractory pain (present for greater than 6 weeks) from vertebral fractures initially treated conservatively (with analgesia and bracing) and for prophylactic stabilisation of diseased vertebra due to primary or secondary cancer [194].

Pain relief following vertebroplasty has been shown to be immediate (with the majority occurring within 24 hours of the procedure), long lasting (up to 12 months) and statistically significantly greater than that achieved by conservative measures, as evidenced by the largest randomised control study comparing vertebroplasty to conservative management VERTOS II [195, 196]. VERTOS II was an unmasked but controlled Randomised Control Trial (RCT) that found effective pain relief at acceptable costs in patients with acute vertebral compression fractures. In addition, conservative measures do not address kyphotic spinal deformity and have complications of their own including deep venous thrombosis, pulmonary embolism, decubitus ulcer, pneumonia, and disuse osteoporosis [197]. VERTOS III looked at conservative treatment for vertebral compression fractures and found that half of the patients still had disabling pain beyond 3 months, while 46% of patients still felt that pain relief was insufficient even at 12 months [198].

Vertebroplasty may work by either mechanically stabilising the vertebral segment or by simply burning the nerve endings from the high temperatures created
by the cement polymerisation exothermic reaction which can reach up to 100 degrees Celsius [199].

Vertebroplasty of wedge fractures can be carried out if there is no neurological deficit, no spinal canal compromise and the spine is stable. Spinal fractures that do not meet these criteria may need formal open operative stabilisation and decompression surgery [194].

2.4.8 Vertebroplasty Trials

There are only two double-blinded multi-centre randomised control studies which looked at vertebroplasty versus sham vertebroplasty procedures published in 2009. One study compared vertebroplasty to a sham vertebroplasty procedure which was a facet joint injection of local anaesthetic, while the other compared vertebroplasty to subcutaneous local anaesthetic injection in the back [200, 201]. Both found no significant difference between vertebroplasty and sham local anaesthetic procedures in terms of pain relief at one and three months. These studies were small (78 and 131 patients respectively), and the use of local anaesthetic agents may be a confounder, although this does not explain the lack of a difference in pain relief on longer term follow-up.

On the other hand, this may be explained by the fact that the vertebral fractures in these studies were of different ages including both acute (less than 6 weeks) and subacute/chronic fractures of up to 12 months old. A significant proportion of the patients in these studies had fractures that were older than 6 weeks, 68 percent in the
Buchbinder study and 56 percent in the Kallmes study. It is logical that a fracture that is up to 12 months old is unlikely to have a significant improvement in pain compared to a fracture that is less than 6 weeks old as fracture healing will be at different stages in the two groups, keeping in mind that vertebral fractures usually heal between 6 to 12 weeks. Thus, these studies focused on patients who had fractured vertebrae that had likely healed but had some residual pain and as such were likely to get very little benefit from vertebroplasty, thus both diluting and skewing the results. Another criticism of these studies is that there was a high rate of exclusion. In one of the studies, there were 390 patients excluded of the 468 who initially were eligible for treatment [200].

A recent study published in the Lancet, vertebroplasty for acute painful osteoporotic fractures (VAPOUR): a multicentre, randomised, double-blind, placebo-controlled trial, has shown that vertebroplasty offers adequate pain relief for vertebral wedge fractures that are less than 6 weeks old with a Numeric Rated Scale (NRS) back pain greater than or equal to 7 out of 10 [202]. It is the first study to look at vertebroplasty within this time period of six weeks, compared to a sham procedure and concludes that vertebroplasty is a safe and effective alternative management for patients.

### 2.4.9 Future Vertebroplasty Trials

Further studies are still underway including VERTOS IV (since January 2011 which had an expected date of completion sometime in 2014 but remains unreported to date), which is currently being carried out as a prospective, multicentre, randomised
control trial in Holland comparing vertebroplasty to a sham procedure in acute vertebral osteoporotic fractures less than 6 weeks old. [203]. This is to help clarify the benefits of vertebroplasty despite conflicting evidence from currently available randomised control trials. In addition, subgroups that would benefit the most from vertebroplasty / kyphoplasty will hopefully be identified from this on-going research in order to help improve patient selection for these procedures. This may well identify a group of patients who have an acute or subacute fracture that is causing severe uncontrolled pain that is affecting their mobility or activities of daily living, who are likely to have the most benefit from these procedures.

2.4.10 Post-Vertebroplasty Fractures

Vertebroplasty has also been shown to reduce the creep phenomenon already described earlier, in augmented vertebrae thus helping to reduce progressive kyphotic spinal deformity [204]. On the other hand, the risk of developing subsequent vertebral compression fractures in patients who have already had vertebroplasty procedures is quite high with figures quoted varying between 23 percent and 63 percent [196]. Augmenting a vertebral fracture level with cement changes the biomechanics of the spine, resulting in increased strain at both the superior and inferior neighbouring vertebrae but the majority is redistributed to the inferior vertebra which is more likely to fail [178]. In one study, eighty percent of the patients who sustained an adjacent fracture had these occur within two months of the vertebroplasty procedure [205].

One study looked into the prophylactic vertebroplasty of unfractured adjacent vertebrae at the same time as carrying out the index vertebroplasty for the fractured
vertebra to decrease the risk adjacent fractures [206]. It found that the incidence of adjacent fractures at three months and one year was 16.8 percent and 22.4 percent in the index vertebroplasty without prophylaxis group, compared to 4.5 percent and 9.7 percent in the prophylaxis group.

Of course, given the already likely osteoporotic state of the neighbouring vertebrae, some of these fractures would have occurred even if the fractured vertebra had not been cemented. Studies have shown that there is a 19% incidence of sustaining a neighbouring vertebral fracture in the first year following the index vertebral fracture even without surgical intervention [205].

In addition, refracture of the cemented vertebra itself has also been reported especially in those patients where the anterior vertebral height has been restored [207]. In this case, it was found that 63 percent of kyphoplasty vertebrae refractured with the loss of anterior vertebral height and this was directly related to the vertebra that had a greater restoration of height.

The causes of refracture are multifactorial in nature, partially due to the stress riser effect of the cemented, augmented vertebra being reinforced to a compressive strength much higher than that of the adjacent vertebrae and partially due to the underlying disease problem of osteoporosis-i.e. their bones are already osteoporotic and thus they have a naturally higher predisposition to fracture in any case. Thus management of patients with osteoporotic fractures needs to include adequate mineral nutrition including calcium and Vitamin D, anti-resorptive medication and lifestyle
modification as well as treating the painful fracture with vertebroplasty. A recent study, looking at risk factors for adjacent vertebral fractures has identified increasing age over 85 years and BMD of less than 0.7 at lumbar spine or hips as conferring increased adjacent vertebral fractures [208]. Thus a patient tailored approach based on these characteristics has the potential to decrease these adjacent fractures.

2.4.11 Young’s Modulus of Elasticity and the Stress Riser Effect

Stress in mechanical terms is a measure of internal forces acting within a deformable material body or object. These internal forces between particles are produced by external forces acting upon the object. Stress is a form of pressure and is measured in force per unit area, thus its unit of measurement is Newtons per metre squared (N/m²) which is the equivalent of a Pascal (Pa).

A stress riser occurs in an area where stress is concentrated. This can be due to geometric shapes such as sharp corners and sudden changes in the cross-sectional area of an object or due to areas of weakness such as cracks, holes or sudden changes in a material’s strength or composition [209].

If in biomechanical terms, one considers the spine as one deformable material body, then where one particular vertebra becomes more osteoporotic relative to other vertebrae, it then has a relatively lower compressive strength and thus becomes a stress riser within the spine and will be the most likely to fracture in the event of axial loading.
Conversely, by augmenting a fractured vertebra with cement and increasing its compressive strength relative to the other (likely also osteoporotic) adjacent vertebrae, then stress risers are created in the vertebrae above and below the augmented vertebra. But the lower or more distal vertebra has a higher proportion of the body above it and is thus subject to higher compressive loading for the same axial force, and so the distal vertebra is more likely to fracture provided the proximal and the distal vertebrae are of the same bone mineral density [178].

The augmented vertebrae have an altered Young’s modulus of elasticity which is a measure of a material’s stiffness as well as its ability to resist deformation under tension. When a material is subjected to a stress (a force per unit area), it undergoes strain (a change in length relative to its original length). According to Hooke’s Law, stress is proportional to strain up to a limit [210]. The graph of stress to strain results in a characteristic curve with a slope specific to the material being tested, with the slope being the Young’s modulus of elasticity of the material [141]. The higher the Young’s modulus of elasticity is, the stiffer the material.

Normal cortical bone has a modulus of elasticity higher than PMMA which in turn is higher than that of cancellous bone [211]. Normal bone has a higher modulus of elasticity than osteoporotic bone and is dependent on the degree of osteoporosis [212, 213]. Where two materials in contact have a different Young’s modulus then there is a modulus mismatch between them, which results in a stress riser between the two resulting in the fracture of the weaker (lower modulus of elasticity) material [214].
Part of the mechanism that vertebroplasty causes a stress riser is thought to be due to the augmented vertebral body becoming stiffer with the injected cement, which in turn increases the intradiscal pressure in the adjacent discs. This increased intradiscal pressure is then transferred to the adjacent vertebral bodies resulting in their subsequent fracture [215].

2.4.12 Vertebroplasty and Kyphoplasty Methods

Vertebroplasty and kyphoplasty are two minimally invasive percutaneous techniques of treating vertebral body fractures which are refractive to conservative management [216]. These techniques are either carried out by spinal surgeons or interventional radiologists with the patient positioned prone (belly down). They can be carried out under local anaesthesia with or without sedation or under general anaesthesia. Only two small (right and left) approximately 1 cm incisions are required for each treated vertebral level. Vertebroplasty involves injecting cement into the fractured vertebra in order to prevent further collapse of the vertebral body, via right and left transpedicular placement of a 10–15-gauge trocar using X-ray screening.

Kyphoplasty uses the same percutaneous transpedicular technique, also guided by X-ray imaging to insert a trocar via right and left pedicles into the fractured vertebral body which has collapsed down. Both right and left balloons are then inflated simultaneously to the required pressure, in order to convert the wedge-shaped vertebra to a more square shape that restores it to its original, or close to original vertebral height. Each balloon is then deflated and removed and the resultant cavity is subsequently filled with cement. This is then repeated through the other pedicle for
Figure 2.15: Trocar Placement into Right Pedicle under X-ray Guidance
Figure 2.16: Bilateral Percutaneous Trocars and Pressurizers Attached Simultaneously for Kyphoplasty [217]
the same vertebral level although they are usually done simultaneously (Figures 2.15 and 2.16).

This is the essential difference between vertebroplasty and kyphoplasty. Vertebroplasty cements the fractured vertebra in situ to further stabilise it, while kyphoplasty tries to correct the kyphotic forward flexion deformity of the vertebra and spine, then cements it to maintain the corrected position. Hence kyphoplasty makes use of pressurised balloons to create cavities for the injected cement as well as leading to partial restoration of the vertebral height while vertebroplasty does not. Vertebroplasty requires higher cement injection pressures due to a lack of a preformed cavity and thus has higher rates of cement extravasation [218, 219].

These procedures are usually carried out as day procedures and patients usually remain flat on their backs for a number of hours to allow the PMMA cement to set and are then discharged home [216]. PMMA cement reaches its maximal compressive strength within 24 hours, but the majority of this is reached within one hour [220]. As with any surgical procedure, these operations are not without risk although complications are rare. The main risks associated with these procedures are some cement leakage (which can be into neighbouring discs or rarely into the vertebral canal where it can cause nerve injury or paralysis), adjacent vertebral fractures, pulmonary embolism and even death [221]. The benefits are that the patient gets immediate pain relief reducing the need for analgesics and is also able to mobilise without the need for bracing [196].
Figure 2.17: Lateral spinal screening film with bilateral kyphoplasty balloons being inflated in the vertebral body to restore vertebral height. The adjacent vertebral levels above and below, in contrast, have only had vertebroplasties and were cemented in their collapsed state.
Figure 2.18: Lateral spinal screening film with cement being injected into vertebral body post-balloon inflation and deflation.
2.4.13 Vertebroplasty versus Kyphoplasty

There have been very few studies which have compared the risks and benefits of kyphoplasty versus vertebroplasty. Most studies have looked at either vertebroplasty or kyphoplasty compared to conservative treatment or placebo [200-202].

In a meta-analysis of 168 studies looking at either kyphoplasty or vertebroplasty, it was found that there was a statistically more significant decrease in pain scores with vertebroplasty over kyphoplasty (p<.001) [222]. In addition, the risk of cement leakage was found to be almost 20 percent with vertebroplasty in comparison to 7 percent with kyphoplasty, which was also confirmed by another comparative review of the two procedures [223]. In this study, there was no significant difference in associated vertebral fractures.

In the only prospective, randomised, comparative study of the two procedures to date with fifty patients in each group and six months of follow-up, there was no significant difference in the clinical outcome between the two [224]. The only difference was the higher amounts of PMMA cement used and cost of the balloon kyphoplasty. As would be expected balloon kyphoplasty was better at restoring vertebral height and shape. On the other hand, another study has shown that this restoration of height is temporary and that balloon kyphoplasty treated vertebrae lose the restored height after cyclical loading [225]. This has been reflected in clinical studies which have shown that augmented vertebrae often lose their vertebral height [226].
One of the causes of re-collapse of the vertebral height is felt to be due to underfilling of the vertebral body with cement in an effort to prevent cement leakage. A new technique of repeat needle placement and repeat cement injection has been suggested, where there is a greater than twenty-five percent area of uncemented vertebral body volume on lateral X-ray screening to help prevent recollapse [227].

The biomechanical changes that occur after vertebroplasty or kyphoplasty are dependent on a number of variables. These variables can be divided into patient-related variables and procedure-related variables [228].

2.4.14 Patient-Related Variables

Patient-related variables include the BMD of the index fractured vertebra, the BMD of the other vertebrae especially the adjacent vertebrae, the overall spinal alignment, the level of the fractured vertebra, the number of fractured vertebrae, the severity and number of disc degeneration as well as the patient’s weight and activity level. These factors have already been alluded to previously.

It has been shown that vertebrae of lower BMD sustain more severe vertebral fractures in terms of vertebral height loss but have been found to also have the most benefit from vertebroplasty in terms of restoring vertebral biomechanical properties such as stiffness and load sharing [229].
2.4.15 Procedure-Related Variables

Procedure-related variables include the type, elastic modulus and compressive strength of the cement used, the volume of the cement used, the percentage of cement volume to vertebral volume, the symmetrical cement distribution and location.

The type of cement used will determine the elastic modulus and compressive strength of the cement and thus the stiffness of the augmented vertebra and the magnitude of the stress riser. The volume of cement used will also affect the strength and stiffness of the augmented vertebra. Studies have shown that a cement volume of between 4mls to 8mls for thoracic and lumbar spine respectively restores vertebral stiffness, but as little as 2mls is required to restore vertebral strength to pre-fracture levels [230]. Larger cement volumes result in a higher required injection pressure and cause higher rates of cement leakage [222].

A finite element analysis model of an L1 vertebra was used in a study to determine the influence of cement volume and vertebral fill rates on vertebral stiffness [231]. It found that a volume of 3.5ml was enough to restore pre-fracture stiffness. This was approximately a 15 percent volume fraction. In contrast, a 30 percent volume fraction was found to lead to an increased stiffness, 50 percent greater than the original pre-fracture vertebral stiffness. They also found that an uneven distribution of cement to one side results in medial to lateral instability or toggle due to uneven stress distribution [232].
Endplate to endplate cementing technique in some studies has been shown to restore vertebral strength better than partial augmentation with PMMA cement, in cadaveric spine compressed to 35 percent height loss [233]. It was noted that this did also lead to some vertebrae exceeding pre-fracture vertebral strength.
2.5 - Cements

2.5.1 PMMA Cement

The word cement is derived from architecture whereby a liquid and a powder are mixed to form a paste which then sets and hardens. The same term has been transferred to orthopaedic materials used in the same way. PMMA cements are currently the most commonly used cements for vertebroplasty and kyphoplasty and are considered the current standard.

2.5.2 PMMA Cement Uses

PMMA consists of a powder and a liquid which are mixed together to form a hard cement which has found much use, firstly in dentistry where it was used to make dentures and orthodontics and in neurosurgery for repairing skull defects which started in the late 1930’s and early 1940’s during World War II [234]. PMMA was first chemically discovered in 1843 [235]. In 1902 a German chemist Otto Röhm first synthesised PMMA and went on to patent a formulation of PMMA in 1933 [236]. The Judet brothers were the first to use a femoral prosthesis made entirely of PMMA in 1946 [237]. In 1951 the New York surgeon Edward Haboush, inspired by the Judet brothers, used bone cement for fixation of a total hip prosthesis [238]. The PMMA orthopaedic application was popularised since its use by the famous English orthopaedic surgeon Sir John Charnley, for fixation of his total hip Low Friction Arthroplasty acetabular cup and femoral stem since 1957 [239].
2.5.3 PMMA Liquid Components

There are many different commercial formulations of PMMA cement. But the basic constituents of PMMA are the same. The liquid components consist of the monomer Methyl methacrylate, the stabiliser or inhibitor hydroquinone which prevents polymerization of the monomer until it comes into contact with the initiator in the powder. The liquid also contains the activator or accelerator N, N-dimethyl-p-toluidine (DMPT) which offsets the inhibitor and promotes the polymerization reaction once it has started (Table 2.2).

2.5.4 PMMA Powder Components

The powder components consist of polymer granules of PMMA and an initiator of the polymerization reaction, benzoyl peroxide. It also contains a radio-opacifier which can be Barium sulphate (BaSO₄) or Zirconium dioxide (ZrO₂) depending on the formulation. It also can contain a dye such as chlorophyll to give it a colour to make it easy to distinguish it from bone intraoperatively. It may also contain a heat stable antibiotic such as Gentamycin or Tobramycin that are chemically stable at temperatures of approximately 80°C [240].

The liquid to powder ratio can affect the viscosity and compressive strength of the cement while the initiator to activator ratio can affect the setting time, the polymerization temperature and the ultimate strength of the cement [241-244]. The inclusion of radio-opacifying materials can decrease overall cement strength by increasing the porosity of the cement [243].
Table 2.2: Components of PMMA Cement

<table>
<thead>
<tr>
<th>Powder Constituents:</th>
<th>Role:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymethylmethacrylate (PMMA)</td>
<td>Polymer</td>
</tr>
<tr>
<td>Methacrylate-methylmethacrylate (MA-MMA)</td>
<td>Co-polymer</td>
</tr>
<tr>
<td>Benzoyl peroxide</td>
<td>Initiator of polymerisation</td>
</tr>
<tr>
<td>Barium sulphate / Zirconium dioxide</td>
<td>Radio-opacifier for viewing on X-ray</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>Colourant</td>
</tr>
<tr>
<td>Gentamycin or Tobramycin.</td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liquid Constituents:</th>
<th>Role:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmethacrylate (MMA),</td>
<td>Monomer</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>Stabiliser / Inhibitor</td>
</tr>
<tr>
<td>N, N-dimethyl-p-toluidine (DMPT)</td>
<td>Activator / Accelerator of curing</td>
</tr>
</tbody>
</table>
2.5.5 PMMA Porosity

As the powder and liquid are mixed air particles can get trapped in the cement creating holes or pores in the cement. These pores can be of different sizes from 0.1mm to 1mm in diameter. Larger pore sizes confer areas of weakness in the cement resulting in lower ultimate compressive and flexural strength of the cement. Vacuum mixing of PMMA cement decreases the ultimate porosity of the cement and can increase the tensile strength by 40 percent [245, 246].

2.5.6 PMMA Polymerisation

The polymerization reaction for PMMA cement is an exothermic reaction. This process consists of breaking the covalent carbon to carbon double bonds (which releases energy at 52KJ/mole of monomer) and forming new long chain polymer carbon single bonds [235]. It is thought that part of the pain relief resulting from vertebroplasty may be due to the thermal damage to nerve endings from the exothermic polymerization of PMMA cement [247]. Protein damage occurs at approximately 45°C while PMMA cement polymerization is known to reach temperatures of 80°C and even up to 120°C [248]. It then cools as the polymerization process slows down and this can lead up to six percent shrinkage in the cement volume [249].

2.5.7 PMMA Cement Leakage

In vivo studies have shown that the temperatures reached at the intervertebral discs and the spinal canal do not exceed 41°C and 47°C [199]. The temperatures in these areas exceeded 40°C for less than one and a half minutes. An interesting study
showed that despite the core vertebral temperature reaching over 45°C for almost four minutes post PMMA cement injection into porcine vertebrae, the temperature of the vertebral anterior and posterior cortices never exceeded 45°C, although they did reach up to 44.6°C from a baseline temperature of 37°C [250].

This was provided that there was no cement leakage. In the event of a cement leakage into the vertebral canal, the posterior cortex temperatures reached 66.7°C and were above 45°C for at least five minutes [247]. Thus, indicating that cement leakage at the posterior cortex has a high chance of causing thermal damage to surrounding soft tissue including nerve roots and the spinal cord. This is worrying as cement leakage is the most common complication of this procedure and can occur in varying degrees in up to approximately 43% of patients [251]. In addition, this high polymerization temperature may result in bone necrosis and may further weaken the vertebra [252].

2.5.8 PMMA Cement Emboli

PMMA cement can also leak into the paravertebral veins and can result in cement pulmonary emboli. Cement in the lung fields was noted on the chest X-rays of approximately 5% of patients having a vertebroplasty or kyphoplasty procedure in one study and 10% in another study with only one of ten affected patients being symptomatic [253, 254]. It must be noted that these cases had a diagnosis of multiple myeloma and were asymptomatic from their cement pulmonary emboli.
2.5.9 PMMA Cement Phases

The polymerization process for cement also leads to a change in the physical consistency of the cement in phases and are defined by ISO standards [17]. The cement components are initially mixed and polymerization starts. The first phase is the mixing phase which starts with the mixing of the liquid and powder components and ends when the cement reaches dough time, which is defined as the time following mixing at which a freshly exposed cement surface fails to adhere to a powder-free latex surgical glove and has a doughy consistency. The setting time is the time when the temperature of the cement reaches halfway between ambient and the peak exothermic polymerisation temperature. The working time is the time that the cement is available to be used i.e. to be injected, and is between the dough time and setting time. Once the cement has hardened it starts to lose its temperature as the rate of the exothermic polymerisation reaction is markedly slowed down.

For PMMA the dough time occurs approximately 2 to 3 minutes after cement mixing starts. The working time usually lasts for about 5 to 10 minutes after the dough phase [255].

The polymerisation process is affected by a number of factors including the ambient temperature, whereby an increase in room temperature will lead to a decrease in the working and setting times by a factor of approximately 5% per temperature increase [255]. The percentage humidity will also affect setting and working times. Increasing humidity leads to a corresponding increase in these cement times [256].
2.5.10 PMMA and Young’s Modulus of Elasticity

PMMA has a much higher Young’s modulus of elasticity than either cortical or cancellous bone leading to a modulus mismatch and a stress riser effect. Young’s modulus is the measure of a material’s stiffness and its ability to resist deformation. It is the ratio of stress versus strain, where stress is the deforming force per unit area (measured in N/m$^2$ or Pascals) and strain is the measure of the deformation that occurs for a given stress force. The strain is the change in length that a material sustains divided by its original length. It is a ratio in its self and has no units. Different materials have a characteristic Young’s modulus of elasticity.

2.5.11 GPC cements

GPC cements are currently used in dental applications and it is the aim of this research to contribute to their modification for orthopaedic application. GPC cements were introduced in 1972 by Wilson and Kent to dentistry and are used as cavity fillers, liners and bases, core build-up agents, fissure sealants and as luting cements to release fluoride, as orthodontic cements as well as restorative agents [257].

They are quite biocompatible and have properties which lend themselves to their use as potential orthopaedic cementing agents. GPC cements may have zinc incorporated into them, which has been shown to have intrinsic antibacterial properties by inhibiting biofilm and is known to be beneficial in the immune process [1, 4, 14]. GPC cements may also have strontium which is radio-opaque aiding visualisation during injection under image intensification and as mentioned earlier is also used in the treatment of osteoporosis (Strontium ranelate - Protelos®-an oral agent used in
the treatment of osteoporosis). Unlike PMMA which is not adhesive and acts like a grout, GPC cements have the ability to adhere to bone and be osteoconductive [258]. In addition, they are not prone to volumetric shrinkage like PMMA cements. It is these properties that have led to their development for orthopaedic use.

GPC cements are hybrids of two types of dental cements, polycarboxylate and silicate cements [259]. They were developed to combine the characteristic of the two cement types—the silicate cements for their fluoride eluting properties while the polycarboxylate cements could chemically bond to tooth enamel.

2.5.12 Glass phase of GPC cements

Glasses are solid materials that exhibit properties of both liquids and crystalline solids [260]. Glasses are non-crystalline solids that have the liquid property of flow which they exhibit under very high shear stress. Glasses have a glass transition phase whereby they change from a solid brittle material to a more molten rubber-like form when heated, without undergoing a phase transition from a solid to a liquid. X-ray diffraction tests which use X-rays and measure the angles they are reflected to determine the atomic arrangement of a substance, show that glasses lack a lasting arrangement of their atoms thereby resembling liquids but in a solid state [261].

5.13 Formation of GPC cements

GPC cements are formed by acid-base reaction between a glass powder and an aqueous acid which is usually a polyacrylic acid. The alumina-silicate glasses consist of oxide forms of aluminium (Al) and silicate (Si) bonded to each other and calcium
(Ca) (and fluoride (F) if present) into two essential glass types: SiO$_2$ – Al$_2$O$_3$ – CaO or SiO$_2$ – Al$_2$O$_3$ – CaF$_2$.

2.5.14 Polyacrylic Acid

Polyacrylic acid (PAA) is the liquid component of glass ionomer cements (GIC). PAA can also be freeze-dried and made into a powder form. This freeze-dried PAA can then be mixed with glass powder and activated with the addition of water. The acid PAA degrades the glass releasing metal cations. These metal cations crosslink the polyacrylic chains forming metal polyacrylate salts. It has been shown in studies that the increased molecular weight (also known as molar mass) of the PAA is what significantly affects and increases both compressive and flexural strength of the GPC cements [262].

2.5.15 GPC Maturation Time

GPC cements continue to form crosslinks for up to a year, which increases their compressive strength with time. They reach most of their compressive strength within 24 hours but as aluminium cations continue to crosslink, their compressive strength continues to improve even up to a year [263, 264].

2.5.16 Osteogenic Properties of GPC Cements

GPC cements that contain fluoride have been found to be osteogenic in vivo studies. Thus, they are bioactive, encourage bone formation and are osteoconductive [265, 266]. They are also biocompatible with few adverse reactions in over 20 years of dental use [267].
2.5.17 GPC Cements and Ion Leaching

GPC cements had fluoride incorporated for their ability to leach fluoride to the surrounding teeth which was beneficial to tooth enamel. The ions are able to leach out due to the water content of the GPC cements which allows for ion interchange with the surrounding fluid environment [268]. Beneficial inorganic ions can thus be incorporated into the GPC cement and leach out over time. These ions can include calcium (Ca$^{2+}$), strontium (Sr$^{2+}$), silica (Si), zinc (Zn$^{2+}$) or fluoride (F$^{-}$). All of these ions have been found to be beneficial to bone. Zinc has also been found to have some antibacterial properties [1, 4, 14]. Strontium can be incorporated into the GPC cements instead of calcium and has the benefits mentioned previously. Aluminium (Al$^{3+}$) which had been previously incorporated in GPC cements has been found to be neurotoxic and been related to the development of Alzheimer’s disease and so it has not been incorporated into the GPC cement for testing in this thesis [269, 270]. In the GPC cement used, the Aluminium (Al) has been replaced by Zinc (Zn), as it can act as a network modifier in the glass structure.

2.5.18 Calcium Phosphate Cements

Other cements that are available for vertebroplasty include Calcium Phosphate Cements (CPC). These cements were first developed in the 1980s by Brown and Chow for remineralising dental caries [243]. This group of cements includes hydroxyapatite (HA), tricalcium phosphate (TCP) and tetracalcium phosphates (TTCP). These cements have been previously developed to coat dental and orthopaedic implants [271]. The new bioactive CPC self-setting cements consist of powder form calcium
and phosphorous reagents, mixed with an aqueous medium containing water and accelerators and retarders. These CPC cements form HA as the end product.

Compared to PMMA, these CPC cements mimic the natural mineral phase of bone and are thus potentially resorbable, and thus may promote bone ingrowth and with time may be completely replaced and remodelled by natural bone [272]. CPC cements cure via crystallization and have reduced curing temperatures, removing the risk for thermal necrosis [273]. Despite these advantages, there are reservations with traditional CPC cements due to deficiencies in their mechanical properties. As CPC cements are ceramics rather than polymers like PMMA, they have different fracture mechanisms and tend to be very brittle. One solution that is currently under investigation is fibre reinforcement of the CPC cements [274].

As CPC cements that are able to resist high compressive stresses were still being developed, they were not used for comparative testing in this research project.

2.5.19.1 Cement Sterilisation

Instruments, implants and any foreign material such as cements used on patients must be sterilised in order to prevent infection. There are a number of sterilisation techniques but each has its advantages and disadvantages [275]. Sterilisation techniques can also alter the mechanical properties of materials [276]. So the choice of sterilisation technique is important.
Sterilisation is a process that leads to the complete destruction of all microorganisms (viruses, bacteria, fungi and prions etc.) whether they are pathogenic or not, along with their spores. Sterilisation methods can be classified into heat dependent and heat independent (cold sterilisation).

### 2.5.19.2 Heat Dependent Sterilisation

Heat dependent sterilisation relies on the fact that micro-organisms will have their protein structures denatured or altered due to extreme heat which will lead to cell death. Heat dependent sterilisation can also be subdivided into dry heat or moist heat. Dry heat is used to sterilise instruments which may corrode if exposed to water (steam). It is used on glassware and other items were steam penetration may be difficult. Ideally, these items should have a high surface area to volume ratio. It is carried out in dry air ovens at a temperature of 160°C for two hours [275].

Moist heat sterilisation is carried out in autoclaves which use steam pressurised at 15 lbs/inch². This raises the temperature from 100°C to between 121°C and 135°C. Typically sterilisation is achieved at 121°C for fifteen minutes. However, in order to destroy prions, the temperature must be raised to 135°C or the time increased to one hour at 121°C [277]. To ensure that the autoclaving process was adequate enough for sterilisation there are tapes with chemicals that change colour when a certain temperature has been achieved for a certain period of time. These tapes are included for example within surgical instrument trays and indicate that there was adequate sterilisation of the instruments [275].
2.5.19.3 Heat Independent Sterilisation

Heat independent sterilisation is necessary for objects which are heat sensitive such as plastics e.g. polyethylene used in arthroplasty, fibre optics used in arthroscopes and endoscopes as well as electronic equipment. Heat independent sterilisation can be of two types, either by irradiation or toxic chemical substances.

2.5.19.4 Chemical Sterilisation

Chemical sterilisation can be with gases (e.g. ethylene oxide –EO or EtO) or liquids (e.g. hydrogen peroxide and glutaraldehyde). Ethylene oxide is commonly used to sterilise materials which are sensitive to irradiation and temperatures above 60°C [275]. Ethylene oxide sterilisation is carried out at temperatures between 30°C and 60°C for at least three hours with an EtO gas concentration between 200 and 800 mg/l. EtO can kill all types of micro-organisms as well as bacterial spores and has good penetration of materials [277]. Important disadvantages are that it is highly flammable, explosive and carcinogenic, making it quite hazardous to use.

Hydrogen peroxide is a liquid chemical sterilisation agent. Hydrogen peroxide is a strong oxidant which allows it to destroy a wide spectrum of pathogens. Hydrogen peroxide does not penetrate materials as well as ethylene oxide and can react with some materials due to its high oxidant state, which limits its use [277].
2.5.19.5 Radiation-Based Sterilisation

Radiation-based sterilisation includes the use of ultraviolet light, X-rays and gamma irradiation which damage microorganism DNA leading to cell death and sterilisation. They differ in their level of material penetration [277].

Ultraviolet waves at 260 nm result in the formation of pyrimidine dimers in cell DNA. This damages the genetic makeup of cells and ultimately leads to cell death. Ultraviolet irradiation does not penetrate glass and thus its use is limited to sterilising work surfaces and air. It is routinely used to sterilise the interiors of biological safety cabinets between uses.

X-rays are a form of ionising radiation allowing sterilisation of large packages and pallet loads of medical materials but their penetration is too unpredictable except in low density packages to allow sufficient sterilisation.

2.5.19.6 Gamma-Irradiation Sterilisation

Gamma-irradiation uses gamma waves emitted from a radioisotope usually Cobalt-60 [277]. They are very penetrating and are commonly used for sterilisation of disposable medical equipment, such as syringes, needles, cannulas and intravenous sets. It has the advantage of allowing sterilisation of products within its packaging.

Gamma irradiation can affect the material that is irradiated. This has come from past orthopaedic experience from other materials e.g. polyethylene (PE) used in total hip and knee replacements that were gamma irradiated in some oxygen rich
environment (e.g. air) and led to PE becoming oxidised which in turn led to early implant failure due to delamination and excessive wear [278, 279]. On the other hand, gamma irradiation of PE in an oxygen depleted environment such as a vacuum or PE packaged with an inert gas e.g. Argon leads to polyethylene cross-linkage improves resistance to adhesive and abrasive wear [279].

Gamma irradiation of cement is known to affect the molecular weight of the PMMA although the compression and bending strengths are not affected significantly [280]. It can also lead to a colour change and a 12°C drop in glass transition temperature (Tg) of PMMA [281, 282].

Since this GPC cement will have to be sterilised for orthopaedic use, we opted to gamma-irradiate the GPC cement and to assess that its mechanical properties were not affected by the sterilisation process. We opted to use gamma irradiation as the sterilisation process for this GPC cement as it is both an effective method for cement batch sterilisation and a cost efficient method that is widely used in the sterilisation of cements. In addition, the control PMMA cement Simplex P® that was being tested is also gamma-irradiation sterilised [283]. This is unlike Palacos R® PMMA cement which is ethylene oxide sterilised.
Chapter 3:

Materials and Methods
3.1 Cement Synthesis and Sterilisation
3.1.1 GPC Cement Synthesis

The GPC cement used was a mixture of 1gm of the BT101 glass powder consisting of silica, zinc, calcium and strontium oxides (0.48 SiO$_2$-0.36 ZnO-0.12 CaO-0.04 SrO) with 0.37gm of the E9 50 wt. % Polyacrylic acid (PAA) and 0.37gm of H$_2$O making up the other 50 wt. %.

3.1.2 GPC Glass Powder Synthesis

The GPC glass powder was prepared by weighing out the required amounts of analytical grade reagents (Sigma-Aldrich, Dublin, Ireland) and then subjected to ball milling for one hour. This mix was then dried in an oven at 100°C for one hour. This was then fired at 1480°C for one hour in a mullite crucible. The melts were then shock quenched into demineralized water. The resultant glass frit was then dried, ground in a gyro mill (Glen Creston, London, UK) and then sieved through a sieve of pore size 45µm producing a GPC glass powder of maximum particle size of 45µm.
Figure 3.1: GPC being fired at 1480°C for one hour in a crucible
Figure 3.2: Shock quenching of GPC after being fired at 1480°C
3.1.3 Polyacrylic Acid Syntheses

The Polyacrylic acid (PAA) E9 that was used in these experiments was purchased from Advanced Healthcare, U.K. The PAA had a molecular weight (Mw) of 80,800 with a peak molecular weight of 83,500 and a molecular number (Mn) of 26,100. The PAA was freeze dried, ground and sieved through a sieve with pore size 90µm. Thus due to the pore size, the PAA had a maximum particle size of 90µm.

3.1.4 PMMA Cement Preparation

A commercially available PMMA bone cement Simplex P® (Stryker Howmedica, Limerick) was used. Simplex P® contains 75% Methyl methacrylate-styrene copolymer, 15% Polymethylmethacrylate (PMMA), and 10% Barium Sulphate. Benzoyl peroxide is encapsulated within each methyl methacrylate-styrene-copolymer bead.

Cementing techniques have evolved over time. The first generation cementing techniques involved bowl mixing and finger packing of cement. Second generation techniques involved the use of a cement gun for pressurisation. Third generation techniques are the current standard with regards to vertebroplasty cement and involve the use of a vacuum during cement mixing in order to decrease porosity as well as pressurisation [284].
Figure 3.3: Simplex P® (Stryker Howmedica, Limerick)
Figure 3.4: Stryker Mixevac III cement mixing bowl
The PMMA cement was prepared according to third-generation cementing techniques and was mixed in a Stryker Mixevac III (Stryker Howmedica, Limerick) cement mixing bowl with a vacuum attachment in order to remove toxic monomer fumes and to decrease the porosity of the cement. The PMMA cement was then loaded into a 10ml syringe and injected into the moulds or into the vertebrae.

3.1.5 Differential Thermal Analysis

The glass transition temperature is the temperature at which the glass changes from a solid state where it is hard and brittle to a more viscous liquid and more soft, rubbery state. The glass transition occurs when there is enough thermal vibrational energy to create sufficient free volume within the material, to allow sequences of 6 to 10 main chain carbons to move as a unit. It represents the upper temperature that the glass can be used. The glass transition temperature is also an indication of the amount of network disruption that exists in the glass. The greater the glass network is disrupted, the lower the value of Tg. Thus, it is an indirect measure of the structure of glass.

A combined differential thermal analyser and thermal gravimetric analyser (DTA-TGA) machine (Stanton Redcroft STA 1640, Rheometric Scientific, Epsom, U.K.) was used to measure the GPC cement’s glass transition temperature (T_g). The sample’s temperature was measured every six seconds between temperatures of 110°C and 950°C. A heating rate of 10°C per minute was used in an air atmosphere while using alumina in a matched platinum crucible as a reference.
3.1.6 Gamma Sterilisation

The GPC cement was gamma irradiated according to the following ISO standards: ISO 11137:2006 sterilisation of Healthcare Products, MD76165; ISO 9001:2000 Quality Management System, FM76164 and ISO 13485:2003 Quality System – Medical Devices MD76165 [285-287]. The dose of gamma irradiation used was between a minimum and maximum dose of 31.5 kGy and 31.8 kGy. A differential thermal analysis was carried out post-irradiation to assess for any change in the glass transitional temperature (Tg).

The commercially available PMMA Simplex P® cement which has a molecular weight of 198000 came pre-sterilised by gamma radiation in its packaging.
3.2 Experiment 1:

Cement Working and Setting Times with Injectability Assessment.
3.2.1 Working and Setting Time

The working and setting times of the cements were determined using a stopwatch. Working time was measured from the time mixing started to the time that the cement became no longer pliable. The setting time was measured according to ISO 9917:2007 standards [288]. An empty mould with the dimensions shown in Figure 3.5 was placed on aluminium foil which formed its base.

The cement was mixed and was immediately used to fill the mould to a level surface. Sixty seconds after cement mixing had commenced the mould with its contained cement was placed on a metal plate (8mm x 75mm x 100mm) in an oven maintained at 37°C (body temperature). At ninety seconds after mixing had started, the mould was removed from the oven and a Vicat needle indenter of mass 400g (Figure) was gently lowered onto the cement surface and allowed to remain for five seconds.

The indent was then observed to see if a complete circular indent was made. The process was then repeated at least every five seconds at a different spot to the previous indents on the cement surface until the needle failed to make a complete circular indent when viewed at x2 magnification. The net setting time of three tests was recorded for each of the cements tested.
Figure 3.5: Dimensions (in millimetres) of mould used to determine cement setting time
Figure 3.6: Vicat needle indenter with cement sample being indented
3.2.2 Injectability

The assessment of the injectability of the cements was carried out at the time of injecting the cadaveric vertebrae. This assessment involved firstly mixing each of the cements and then loading it into the 5ml Luer lock tipped syringes attached to the 13-gauge trocar that would be used for injection. The Luer lock tip syringes allow the trocar to be screwed securely and not allow it to come off or cement to escape.

An assessment was then carried out to see if the cement could be injected out of the other end of the trocar. If this failed, then a new mix was then loaded into a 5ml syringe and an attempt was then made at injecting the cement directly from the syringe. The assessment was based on an all or nothing event i.e. if it was possible to inject the cement out of the syringe with or without the trocar attached or if it was not.
Figure 3.7: 13 Gauge Trocar and 5ml Luer lock tipped syringe used for vertebroplasty cement injection
3.3 Experiment 2:

Compressive Strength Testing
3.3 Experiment 2

3.3.1 Compressive Strength Testing

The compressive strength testing of the PMMA and GPC cements was carried out in accordance with the International Organisation for Standardisation ISO 9917:2007 standards [289]. Split ring moulds were made with sets of five cylindrical samples, each sample with a diameter of 4mm and a height of 6mm. These were filled to excess with freshly mixed PMMA and GPC cements and then covered with acetate sheets. The moulds were then sandwiched between two stainless steel plates, clamped and then incubated at 37°C (human body temperature) for at least 1 hour.

On completing incubation, the moulds were then removed from the clamps and the flash of cement around the moulds was removed using a grinding wheel (at 100 rpm) loaded with 1200 grit silicon carbide paper. This resulted in compression samples that had completely flat ends that were parallel to one another. The samples were then removed from the moulds and placed in either distilled water or human blood and labelled and then incubated at 37°C (human body temperature) for 1, 7 and 30 days before compression testing was carried out.

On completion of each of the above time periods, the samples for compression testing were then loaded on an Instron 4082 universal testing machine (Instron Ltd, High Wycombe, Bucks, U.K.) using a 5kN load cell at a crosshead speed of 1mm/min. There were five samples of each of the cement types, for each of the incubation fluids and each of the incubation periods.
Figure 3.8: Split ring moulds
Figure 3.9: Instron 4082 universal testing machine
Figure 3.10: Compression testing jig
The compression strength of each sample was then calculated according to the following equation:

\[ C = \frac{4\rho}{\pi d^2} \]

Where:

- \( C \) = Compressive strength (Megapascals)
- \( \rho \) = maximum applied load (Newtons) as measured by the Instron
- \( d \) = diameter of the sample (millimetres)

Five samples were chosen for testing at each of the time periods for each of the tests, as there was limited material available and previous research testing sample sets of \( n=5, 10, 20 \) and \( 30 \), had shown that for brittle materials such as glass polyalkenoate cements, it is acceptable to test only five samples of each material and the resultant 95% confidence intervals would encompass the corresponding Weibull characteristic strength of the material [290].

The data obtained was then entered into the Statistical Package for the Social Sciences (SPSS) 22 statistical analysis programme for processing of the results. The statistical approach adopted was for parametric data analysis, the details of which are discussed later (in paragraph 3.6).
3.4 Experiment 3:

Biaxial Flexural Strength Testing
3.4 Experiment 3

3.31 Biaxial Flexural Strength

Biaxial flexural strength testing of the cements was carried out in a similar method to that used by Williams et al. [291] using a testing jig with 3 support bearings on which rests a disk of the cement to be tested and onto which descends a bearing centred on the cement disk (figures 3.11 and 3.16).

After mixing the cements, samples were placed in premade circular rubber moulds of 12mm diameter and 2mm thickness and filled to excess (figure 3.12). These were then placed between two acetate films and the moulds were then sandwiched between two stainless steel plates, clamped and incubated at 37°C for at least one hour (figure 3.13). Following incubation, the samples were removed from the moulds and flash was removed from the edges of each disc (figure 3.14). Samples were then placed in either distilled water or human blood (figure 3.15) and labelled, then incubated at 37°C for 1, 7 and 30 days before biaxial flexural strength testing was carried out.
Figure 3.11: Biaxial flexure testing jig with 3 support points inferiorly and a central compression point from superiorly
Figure 3.12: Cement samples were placed in premade circular rubber moulds
Figure 3.13: Clamp compressing the circular cement samples between two plates
Figure 3.14: Cement disc samples before and after removal from circular rubber moulds
Figure 3.15: Cement disc samples in water and in human blood
Cement disc sample thickness was measured using a Vernier callipers for each sample immediately prior to testing. The test jig described above was fixed into an Instron 4082 universal testing machine (Instron Ltd, High Wycombe, Bucks, U.K.) using a 1kN load cell at a crosshead speed of 1mm/min. There were five samples of each of the cement types, for each of the incubation fluids and each of the incubation periods.

The biaxial flexural strength for each sample was then calculated according to the following equation:

\[
BFS = \frac{\rho(N)}{t^2} \left\{ 0.63 \times \ln \left( \frac{r}{t} \right) + 1.156 \right\}
\]

Where:

- BFS = Biaxial Flexural Strength (Megapascals)
- \(\rho\) = Fracture load (Newtons)
- \(t\) = Sample thickness (millimetres)
- \(r\) = Radius of the support diameter (millimetres)

Five samples were chosen for testing at each of the time periods for each of the tests, as there was limited material available and previous research testing sample sets of n=5, 10, 20 and 30, had shown that for brittle materials such as glass polyalkenoate cements, it is acceptable to test only five samples of each material and the resultant 95% confidence intervals would encompass the corresponding Weibull characteristic strength of the material [290].
Again, the data collected was entered into the SPSS 22 statistical analysis programme for further processing. The statistical approach adopted was for parametric data analysis, the details of which are discussed later (in paragraph 3.6).
Figure 3.16: Cement disc samples being tested for biaxial flexural strength
3.5 Experiment 4:

Cadaveric Vertebrae Compression Testing
3.5 Experiment 4

3.5.1 Preparation of Cadaveric Spines

Three human cadaveric spines were obtained from the University College Cork Anatomy Lab including lower thoracic and lumbar spinal segments (the most commonly affected spinal segments with regards to compression fractures). All cadaveric material used was bequeathed to the Medical School, National University of Ireland, Cork for the further advancement of medical knowledge. This is covered by the legislation governing the practice of Anatomy in the Republic of Ireland (Medical Practitioners Act 2007). The cadavers were previously embalmed using a standard mixture containing formalin, phenol, glycerine and methanol (12L water + 2.4L of a 37% – 41% formalin solution + 2L phenol + 6L glycerine + 6L methanol). The spines were of elderly cadavers. Each spine was allocated a number.

Spine 1 was that of a 69-year-old female and consisted of the lower thoraco-lumbar spine segment and had a mild kyphosis. Spine 2 was that of a 90-year-old male and consisted of the thoraco-lumbar spine segment with a lumbar lordosis and a thoracic kyphosis. Finally, Spine 3 was that of an 86-year-old female, consisting of the thoraco-lumbar spine segment showing a lumbar lordosis and thoracic kyphosis. No further background on the cadavers’ history was permissible. The spines were undamaged from any previous cadaveric dissection, were in excellent condition and were suitable for testing.

The spines were initially removed en bloc and then the soft tissues and ribs (except for intervertebral ligaments and facet joint capsules) were dissected from the
Figure 3.17: The three en bloc harvested cadaveric human spines 1, 2 and 3 respectively
specimens. Vertebrae above T5 level were excluded as these rarely get wedge compression fractures.

The steps in the preparation and testing of the 3 spines are illustrated in the flow chart in Figure 3.36.

3.5.2 Radiological Assessment of Cadaveric Spines

Radiographs in two planes – antero-posterior and lateral (taken at the Forensics Department in Cork University Hospital) were used to exclude specimens with lytic lesions or other bony abnormalities not related to osteoporosis which might affect testing results. Vertebrae that had been damaged or were incomplete due to the initial en bloc removal were also excluded. Single vertebrae that could not be paired up in situ and that were left after the spine was divided into sets of Functional Spinal Units (FSUs) were also excluded.

Spine 1 had eight vertebrae which yielded four FSUs suitable for testing. Spine 2 yielded five FSUs and Spine 3 yielded four FSUs amenable to testing. The vertebral FSUs were then numbered and labelled prior to further X-rays, injection and compression.

Spine 3 was found to have a diseased lower lumbar vertebral spine level which was removed and excluded from compression testing but left in place for DEXA scanning so as to allow correct labelling of the rest of vertebral spinal levels in the DEXA scan scout films for T-scoring.
Figure 3.18: Spine 1 antero-posterior and lateral X-ray views
Figure 3.19: Spine 2 antero-posterior and lateral X-ray views
Figure 3.20: Spine 3 antero-posterior and lateral X-ray views showing lytic lesion
3.5.3 DEXA scanning Cadaveric Spine

Bone Mineral Density was determined on each vertebral level using Dual-Energy X-ray Absorptiometry with a DEXA scanner (Lunar IDXA model, GE Healthcare) at Cork University Hospital Rheumatology Department.

The DEXA scanner is first calibrated using a phantom spine (a metal piece of known BMD) with an overlying body of water at room temperature mimicking abdominal and thoracic soft tissue.
Figure 3.21: Phantom spine control
Figure 3.22: DEXA scanner calibration with Phantom spine control underneath water container
Figure 3.23:  Spine being checked for BMD on DEXA scanner with container of water mimicking body soft tissues as is used in DEXA scanner calibration
It was noted that each spinal level is then named on the DEXA scan scout film after scanning a spine, as a different control BMD is assigned to each vertebral level. This leads to a different T-score for the same BMD measurement depending on the vertebral level assigned to it on the DEXA scan, e.g. an L5 level assigned to a vertebral level on the DEXA scan scout film for a specific BMD will give a completely different T-score compared to when an L2 level had been assigned to it for the same BMD value. This is because the comparative young female control BMD used in making up the T-score differs for each spinal level and is pre-programmed into the DEXA scanner.

The T-score was used to randomise the vertebrae but was excluded from analysis and the BMD was used instead, as this is a more accurate measure for the purposes of this experiment.

Once the DEXA scanner was calibrated, the spine phantom was then removed and the spinal specimens were then placed underneath the water mimicking the abdominal and thoracic soft tissue. The BMD for each of the vertebrae was then measured and recorded.
Figure 3.24: An example of a DEXA scan scout film with named segments and BMD and T-score result
Figure 3.25: DEXA scanner labelling spine levels for BMD conversion to T-score
Figure 3.26: Lumbar FSU segment
Figure 3.27: Thoracic FSU segment
3.5.4 Division of spine into FSU

Spines were then divided sequentially into sets of two vertebrae functional spinal units (FSUs) at the vertebral disc using a scalpel, leaving intact the intervertebral disc and ligaments between the two vertebrae. The various FSUs were then labelled with tissue markers. To minimise the effects of variability in bone density and the spinal level of treatment: specimen pairs were then sorted according to the T-score and then assigned in an alternating sequence to three groups for vertebroplasty treatment. The first acting as a control group with no cement injected, the second would be injected with the GPC cement and the third would be injected with the PMMA cement.

3.5.5 Cement Injection of Vertebrae

In order to be consistent and to allow for comparison between the two cement groups, only the superior vertebrae were injected. X-rays were taken to confirm the position of the 13-gauge trocar in the vertebral bodies prior to and following injection of cement to assess the location of cement injection.

A separate cement mix was used for each vertebral level injected using either PMMA or GPC cement so as to minimise the time from completion of cement mixing to injection and maximise the working time of the cement in order to optimise its injection capacity. In the case of the GPC cement, a separate cement mix was used per pedicle injected while it was possible to inject both pedicles per cement mix using PMMA.
The spines that were injected were left at room temperature to allow the cements to set for at least a period of twenty-four hours prior to further testing.
Figure 3.28: Transpedicular trocar placement lateral and antero-posterior X-rays
Figure 3.29: Injected GPC cement in superior vertebral body of FSU
3.5.6 Compression Testing of Cadaveric Vertebrae

The FSUs were then mounted into a servohydraulic testing machine ensuring parallel orientation of the outer surfaces of each FSU and perpendicular orientation of FSU with respect to the loading axis.

In order to achieve this, any parts of the superior articular facets that were protruding beyond the superior vertebral body that would compromise the plain of loading were removed. The lower spinal vertebrae’s spinal processes were also osteotomized so as to allow the vertebra to lie perpendicular to the loading axis.

Each FSU was then compressed at a constant displacement rate of 0.5 mm/s with a load of 0.2kN/mm²/min. The failure load of the FSU is defined as the peak compressive load measured. FSU specimen loading stopped two seconds after peak compressive load was reached and the specimens removed.

After testing, antero-posterior and lateral radiographs were taken to determine the presence and location of fractures and/or reduction in vertebral height.
Figure 3.30: Servohydraulic testing machine with mounted FSU for testing
Figure 3.31: Vertebral wedge compression fracture of inferior (non-injected) vertebra affecting 2 columns of the spine
Figure 3.32: Vertebral coronal plane fracture of inferior non-injected vertebra affecting one spinal column
Figure 3.33: Crushed vertebral bodies with three column failure
3.5.7 Analysis of Vertebral compression

The measurements were taken from X-rays which were all taken at the same standardised height with no alteration in magnification, i.e. true to size films. On taking the measurements osteophytes in non-load bearing areas were excluded. A note was also taken of the presence of visible vertebral fractures in either the superior or inferior vertebrae, as well as which spinal column was fractured and the number of spinal columns the vertebral fractures involved.

On the lateral radiograph illustrated in Figure 3.34, H1, H2 and H3 were the anterior, central and posterior heights of the superior vertebra. H4, H5 and H6 were the anterior, central and posterior heights of the inferior vertebra. B1, B2 and B3 were the superior, mid-level and inferior surface breadths respectively, of the superior vertebra, while B4, B5 and B6 were the superior, mid-level and inferior surface breadths respectively, of the inferior vertebra in the FSU.

On the AP radiograph illustrated in Figure 3.35, H7, H8 and H9 were the right side, central and left side heights of the superior vertebra. H10, H11 and H12 were the right side, central and left side heights of the inferior vertebra. W1, W2 and W3 were the superior, mid-level and inferior surface widths respectively, of the superior vertebra, while W4, W5 and W6 were the superior, mid-level and inferior surface widths respectively, of the inferior vertebra in the FSU.
Figure 3.34: Lateral vertebral X-ray measurements; heights and breadths of the FSU
Figure 3.35: Antero-posterior vertebral X-ray measurements; heights and widths of the FSU
The vertebral dimensions including height (H1-12), width (W1-6) and breadth (B1-6) of both the superior and inferior vertebrae were measured from the antero-posterior and lateral radiographs. This was done pre-injection of cement, post-injection of cement and post-compression of the FSU as shown in figures. Where cement injection led to an increase in height or width of the vertebra injected, the compression height (or width) was compared to the post-injection values rather than the pre-injection values.

The height of the injected cement was measured in each third of the vertebral body on antero-posterior and lateral X-rays. These cement height values were then divided by the vertebral height in each zone to assess the percentage cement fill of the vertebral body. The data obtained was entered into SPSS 22 for later statistical analysis and review. The statistical approach adopted was for parametric data analysis.

3.6 Statistical analysis

All the data obtained from the experiments was entered into SPSS 22 for later statistical analysis and review. Numeric variables were tested for normality and are presented as means (standard deviation). Graphical and numerical summaries are presented by time (Day 1, Day 7, and Day 31) and medium (blood, saline). A repeated measure analysis of variance (ANOVA) with time as the repeated measure and medium as the between samples factor was carried out to explore significant differences in outcome variables over time and by the medium. A 5 percent level of significance was used for statistical tests, with the level of significance adjusted for
multiple pairwise comparisons. Independent samples t-tests were used to compare means across the two groups (GPC, PMMA).
**Figure 3.36: Flow Chart for Cadaveric Spinal Testing:**

3 Cadaveric Thoraco-Lumbar spines harvested en bloc

Spines carefully dissected out from rib and muscle attachments while maintaining disc, ligamentous and bony integrity

DEXA scanning carried out and BMD noted for each vertebra

X-rays of the 3 spines in antero-posterior and lateral planes taken to rule out any pathological lesions

Pathological levels were removed and discarded

FSU paired vertebral units dissected out

FSUs arranged according to T-score and labelled, numbered and assigned to one of 3 groups
Group 1 = Control,

Group 2 = GPC cement to be injected into superior vertebrae of FSU

Group 3 = PMMA cement to be injected into superior vertebrae of FSU

X-rays to confirm placement of trocars transpedically bilaterally

and GPC or PMMA cement injected

X-rays to assess vertebrae post-cement injection

All 3 groups of FSUs loaded onto servohydraulic testing machine

and compression failure load testing carried out

X-rays post-compression taken of FSUs

Measurements taken from above tests and X-rays

and SPSS statistical analysis carried out
Chapter 4:

Results and Discussion
4.1 Transition Temperature Post-Gamma Irradiation of GPC Cement

Gamma irradiation of the GPC cement resulted in a colour change from white to a grey colour as illustrated in Figure 4.1. The PMMA cement colour was unaffected by gamma irradiation. A differential thermal analysis (DTA) was carried out on the samples pre and post-gamma irradiation to ensure that there was no change in the glass transition temperature (Tg). The colour change in the GPC cement was not of any significance.

The results of the glass transition temperature of the GPC cement pre and post-gamma irradiation were very similar and were 675.06°C and 672.64°C respectively, indicating no significant disruption to the glass network by the gamma irradiation at a dose of approximately 31.5 kGy.

The Simplex P glass transition temperature was 110°C pre-irradiation and was found to decrease by 12°C post-gamma irradiation at a dose of 23.5 kGy indicating very little structural change by gamma irradiation. This is in keeping with other studies [283]. The glass transition temperature declines as the molecular weight of a polymer declines [292, 293].
Figure 4.1: GPC cement white pre-irradiation and colour change to grey post-irradiation
Experiment 1:

4.2 Working and Setting Times Pre and Post-Gamma Irradiation Results

The setting times of the GPC cement pre and post-gamma irradiation and of the PMMA cement were measured and the results shown in Tables 4.1, 4.2 for the GPC cement and Table 4.3 for the PMMA cement.

These illustrate that there was no significant difference in the working times of the GPC cement pre and post-gamma irradiation. There was however, a difference in the setting times of the GPC cement pre and post-irradiation. There was also a significant difference in the working and setting times between the two GPC cements and the PMMA cement.

4.3 Working and Setting Times Pre and Post-Gamma Irradiation Discussion:

The significance of these findings is that the GPC cement is very hard to work with. It requires a very short mixing time of approximately 20 seconds within a working time of approximately 32 seconds. This is far too short a time period to mix and load the cement into syringes and then try to inject it through trocars placed in the vertebrae, before it becomes too viscous to pass through the trocars.

A potential solution might be to develop a syringe with the GPC cement powder preloaded that would allow water to be aspirated to the required predetermined amount and allow rapid mixing within the syringe prior to loading it for injection on to the trocars. But even with this solution, the short working time which is unaffected by gamma-irradiation will still need to be increased to be closer to that of the PMMA
cement’s, which was far easier to mix, handle and load into syringes as a result. This testing has identified that GPC cements must have their working time increased, in order to avoid costly failures of cement loading and to be more practical in its handling in a clinical setting.
**Table 4.1:** GPC pre-gamma irradiation rheology

<table>
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<tr>
<th>Sample</th>
<th>Working time (seconds)</th>
<th>Setting time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>37</td>
<td>71</td>
</tr>
<tr>
<td>Sample 2</td>
<td>30</td>
<td>73</td>
</tr>
<tr>
<td>Sample 3</td>
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<tr>
<td>Mean (SD)</td>
<td>31 (5.57)</td>
<td>72.67 (1.53)</td>
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<tr>
<td></td>
<td>Working time (seconds)</td>
<td>Setting time (seconds)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Sample 1</strong></td>
<td>35</td>
<td>95</td>
</tr>
<tr>
<td><strong>Sample 2</strong></td>
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<tr>
<td><strong>Mean (SD)</strong></td>
<td>32 (3.61)</td>
<td>91.67 (3.06)</td>
</tr>
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</table>

Table 4.2: GPC post-gamma irradiation rheology
Table 4.3: PMMA (post-irradiated) rheology

<table>
<thead>
<tr>
<th>Sample</th>
<th>Working time (seconds)</th>
<th>Setting time (seconds)</th>
</tr>
</thead>
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<tr>
<td>Sample 1</td>
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<td>Sample 3</td>
<td>510</td>
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<tr>
<td>Mean (SD)</td>
<td>484 (28.83)</td>
<td>602.67 (14.01)</td>
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</table>
4.4 Cement Injectability Results

Only the post-gamma irradiated GPC cement was used for injectability testing as the pre-gamma irradiated form would not be clinically relevant. The post gamma-irradiated PMMA cement was also tested.

The fact that there was a very short working time after mixing the GPC cements significantly influenced its ability to be loaded into the syringes and injected into the vertebra. In the initial stages, the cement would often harden in the syringes, or become very viscous and require increasing force to be applied to the syringe plunger, only to set within the trocars and block them.

Thus, there was a 100 percent failure of injection when the GPC cement was injected into a syringe with a trocar attached i.e. no cement came out the other end of the trocar. This was despite repeated practice attempts to speed up the adequate cement mixing time that comes from experience with handling this cement. The GPC cement was found to become quite viscous while being introduced into the syringes and sticky, thus making it difficult to load the syringes with it, even though it had not completely set.

The post-irradiated GPC cement was then rapidly mixed again and attempts made to inject it with the syringe alone were made. This aimed to shorten the dead space contained within the narrow bore 10cm length trocar, to the very minimum of no more than 1cm at the syringe tip. Five vertebrae were being injected and so ten separate successful injectable cement mixes were required. In order to achieve these
ten successful injections, there were five failed injections giving a 33 percent failure rate with the GPC cement injections when only syringes were used without a trocar (Table 4.4). The short syringe spout limited the ability to guide the injection of the GPC cement into the various regions of the vertebrae.

In contrast, no such failures occurred with the PMMA cement due to the long working and setting times. It was quite easy to mix in the bowl with its vacuum attachment, with a mixing time which allowed for up to 2 minutes, followed by easy loading of the cement into the syringes. There was no difficulty in injection from the syringes alone, four of which were carried out as a trial while not being injected into the vertebrae (Table 4.5). The PMMA cement was then loaded on syringes with trocars attached and injected into the four testing vertebrae. There were no problems injecting via the trocars which allowed the PMMA cement to be placed in the anterior vertebrae while slowly withdrawing the trocars to fill the central and posterior vertebral bodies.
<table>
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<th>Failed injection</th>
<th>Successful injection</th>
<th>Total</th>
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</thead>
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<td>5 (100% failure)</td>
</tr>
<tr>
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<td>10</td>
<td>15 (33% failure)</td>
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<tr>
<td>Total</td>
<td>10</td>
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<td>20</td>
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</table>
**Table 4.5:** PMMA Cement Injectability

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<th>Successful injection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe &amp; Trocar</td>
<td>0</td>
<td>8</td>
<td>8 (100% successful)</td>
</tr>
<tr>
<td>Syringe alone</td>
<td>0</td>
<td>4</td>
<td>4 (100% successful)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>
4.5 Cement Injectability Discussion

Both the working and setting times for the GPC cement were found to be very short and made the GPC cement quite difficult to work with. With working times averaging 32 seconds, this meant that there was extremely limited time left between the mixing of the cement and loading it into the 5ml syringe for injection in the spinal vertebrae. Enough time had to be spent mixing the BT101, the E9 polyacrylic acid and water evenly. If this exceeded 30 seconds it was no longer possible to easily load or inject the GPC cement as it started to exceed its working time. Once the GPC cement was in the vertebra it set quite quickly which is ideal. The problem was getting the GPC cement into the vertebrae.

Gamma irradiation is known to decrease the molecular weight of irradiated cements. This decrease in particle size in the GPC cement can result in the dissolution of the glass which in turn results in a lack of unreacted glass particles in the cement matrix. Decreasing particle size is also known to affect the cement rheology i.e. its working and setting times. In this case, there was no change in the working time but the setting time was prolonged by 26.1% compared to the pre-irradiated setting time. This may be explained by the fact that the dissolution of the glass does not take place quick enough to affect the relatively quick working time but the relatively slower setting time is thus affected and prolonged.

A second possible solution for the difficulty in injecting the GPC cement is to use larger bore syringes and trocars to accommodate the higher viscosity of the cement but this is impractical and is likely to cause more pain on injecting into patients. This
is because a larger bore trocar would cause more pain on introduction and risks difficulty in entering the pedicles, as well as causing too high a volume of cement to be introduced rapidly into the vertebra which in itself may be painful and also risks cement leakage and fat or cement embolus.

A third and more practical solution is to add tri-sodium citrate (TSC) to delay the polymerization reaction but the effect on the cement’s compression and biaxial flexure strength would also have to be assessed. Tri-sodium citrate works by chelating calcium ions and thus prolongs the reaction time and improves the GPC cement’s rheology [2]. Tri-sodium citrate is already used as an anticoagulant agent by chelating Ca ions in blood products, thereby blocking calcium-dependent clotting pathways. It has also been used in dialysis since 1914 [294].

Tri-sodium citrate has been found to at least double or triple the working and setting times of these GPC cements on previous testing. The tri-sodium citrate tended to increase compression strength at low concentrations of 5 weight percent (wt. %) but decreased the compression strength at higher concentrations of 15 wt. %. This was felt to be due to the gradual washout of the water soluble tri-sodium citrate resulting in residual cement porosity compromising the cement’s structure [2].

Further testing will need to be carried out to optimise the GPC cement’s rheology including working times and injectability, while minimising changes in its mechanical properties, which would then allow further clinical testing.
Experiment 2: Compressive Strength Testing Results

4.6 Pre and Post-Gamma Irradiation GPC Cement Compressive Strength

The compressive strengths of the GPC cement were compared pre and post-gamma irradiation (while incubated in water for 1, 7 and 31 days at 37°C). The results of the means of the five samples in each group, on each of the testing days, for compressive strength testing pre and post-irradiation, are shown in Table 4.6 and illustrated in Figure 4.2.
Table 4.6: Mean GPC Compressive Strength pre and post-gamma irradiation

<table>
<thead>
<tr>
<th></th>
<th>CS (MPa)</th>
<th>(SD)</th>
<th>CS (MPa)</th>
<th>(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-irradiation</td>
<td></td>
<td>Post-irradiation</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>63.38</td>
<td>(2.93)</td>
<td>32.39</td>
<td>(2.05)</td>
</tr>
<tr>
<td>Day 7</td>
<td>63.37</td>
<td>(4.20)</td>
<td>32.79</td>
<td>(4.70)</td>
</tr>
<tr>
<td>Day 31</td>
<td>62.54</td>
<td>(3.47)</td>
<td>48.9</td>
<td>(3.00)</td>
</tr>
</tbody>
</table>
Figure 4.2: GPC Compressive Strength (MPa) pre and post-gamma irradiation

![Graph showing GPC Compressive Strength (MPa) pre and post-gamma irradiation. The graph compares two lines: Pre irradiation (blue) and Post irradiation (red) across different days (Day 1, Day 7, Day 31). The strength values range from 50 to 70 MPa.](image)
4.7 Compressive Strength Testing in Water and Blood Results

Five samples of GPC cement were tested for compressive strength, after being incubated at 37°C in either blood or water for 1, 7 and 31 days. The means and standard deviations of the results measured are summarised in Tables 4.7 and 4.8 and illustrated in Figure 4.3. Again there is an overall increase in the compression strength of the GPC cement with time in both blood and water but more so in blood, most likely due to the higher ion availability in blood than in distilled water.

It is noted that the compression strength post-irradiation in blood improves with time towards that of its original strength pre-gamma irradiation, for the same reasons mentioned above, as illustrated in Figure 4.4.

Figure 4.2 illustrates a large decrease in mean compressive strength when comparing post-irradiation to pre-irradiation values of 48.9% on day 1 and 48.3% on day 7 but this decreases to only 21.8% strength loss on day 31.

In a repeated measures ANOVA, there is a statistically significant difference in compression strength over time (p < 0.001) with the measurements taken at day 1 significantly different from Day 31 (p<0.001). There is also a statistically significant difference between the measurements taken at Day 7 and Day 31 (p < 0.001) but no significant difference between Day 1 and Day 7 (p=0.74). Medium (blood, saline) is not a statistically significant factor in this analysis (p=0.12) but given the low p-value, this may be again due to the small sample size rather than no real differences.
**Table 4.7:** GPC Compressive Strength (MPa) samples in Water

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.56</td>
<td>36.21</td>
<td>43.66</td>
</tr>
<tr>
<td>2</td>
<td>29.65</td>
<td>31.82</td>
<td>49.47</td>
</tr>
<tr>
<td>3</td>
<td>31.27</td>
<td>34.74</td>
<td>50.11</td>
</tr>
<tr>
<td>4</td>
<td>32.50</td>
<td>25.03</td>
<td>50.10</td>
</tr>
<tr>
<td>5</td>
<td>34.98</td>
<td>36.18</td>
<td>51.19</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td><strong>32.39 (2.05)</strong></td>
<td><strong>32.80 (4.7)</strong></td>
<td><strong>48.91 (3.00)</strong></td>
</tr>
<tr>
<td>Sample</td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 31</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>44.47</td>
<td>42.55</td>
<td>49.24</td>
</tr>
<tr>
<td>2</td>
<td>47.94</td>
<td>40.36</td>
<td>54.45</td>
</tr>
<tr>
<td>3</td>
<td>26.90</td>
<td>28.18</td>
<td>45.96</td>
</tr>
<tr>
<td>4</td>
<td>39.06</td>
<td>38.74</td>
<td>50.28</td>
</tr>
<tr>
<td>5</td>
<td>33.74</td>
<td>35.90</td>
<td>58.65</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td><strong>38.42 (8.40)</strong></td>
<td><strong>37.15 (5.57)</strong></td>
<td><strong>51.72 (4.92)</strong></td>
</tr>
</tbody>
</table>
Figure 4.3: GPC Mean Compression Strength (MPa) in Water and Blood

![Graph showing GPC Mean Compression Strength (MPa) in Water and Blood over days 1, 7, and 31. The graph compares compression strength between Water and Blood conditions.](image-url)
**Figure 4.4:** GPC Mean Compression Strength (MPa) pre and post-irradiation in water and post-irradiation in blood

![Graph showing GPC Mean Compression Strength (MPa) pre and post-irradiation in water and post-irradiation in blood.](image)
4.8 Pre and Post-Gamma Irradiation GPC Cement Biflexural Strength

The biflexural strengths of the GPC cement were compared pre and post-gamma irradiation (while incubated in water for 1, 7 and 31 days at 37°C). The results for the mean of the five samples in each group on each of the testing days, for biflexural strength testing pre and post-irradiation, are shown in Table 4.9 and illustrated in Figure 4.5. These show a decreased biflexural strength post-irradiation on all days tested.

The biflexural strength is decreased post-gamma irradiation compared to pre-irradiation values, all be it to a lesser degree than compressive strength. The biflexural strength loss is 16.7% on day 1, 23.8% on day 7 and is 29.4% on day 31. This demonstrates a decrease in the mechanical properties post-gamma irradiation of the GPC cement which is altered with time. This is likely secondary to the decrease in molecular weight that is caused by gamma irradiation which causes a decrease in the mechanical properties of cements [295].
**Table 4.9:** Mean GPC Biflexural Strength pre and post-gamma irradiation (in water)

<table>
<thead>
<tr>
<th></th>
<th>BFS (MPa)</th>
<th>SD</th>
<th>BFS (MPa)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre- irradiation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>27.07</td>
<td>(4.15)</td>
<td>22.54</td>
<td>(2.92)</td>
</tr>
<tr>
<td>Day 7</td>
<td>31.84</td>
<td>(3.04)</td>
<td>24.25</td>
<td>(7.77)</td>
</tr>
<tr>
<td>Day 31</td>
<td>33.17</td>
<td>(3.90)</td>
<td>23.42</td>
<td>(2.79)</td>
</tr>
</tbody>
</table>
Figure 4.5: GPC Biflexural Strength (MPa) pre and post-irradiation
4.9 Biflexural strength testing in water and blood

Five samples of GPC cement were tested after being incubated at 37°C in either blood or water for 1, 7 and 31 days. The results measured for the mean biflexural strength and standard deviations are summarised in Tables 4.10 and 4.11 for water and blood and illustrated by Figure 4.6. This shows a more consistent increasing trend in the biflexural strength of the GPC cement in blood more so than in water. It also has consistently higher biflexural strength values in blood likely due to the high ion availability.

It is noted that the biflexural strength of the GPC cement in blood post-irradiation improves to close to that prior to irradiation, likely due to the initial small molecular weight glass washout secondary to irradiation being partially compensated for by the ions in the blood and increasingly so with time as illustrated in Figure 4.7.

There is no statistically significant difference in biflexural strength over time (p=0.25) or between media (p=0.11) in a repeated measures ANOVA with time as the repeated measure (Day 1, Day 7 and Day 31) and media (blood or saline) as the between samples factor. The low p-values would suggest, however, that a type II error cannot be excluded, due to the small sample size.
Table 4.10: GPC Biflexural Strength (MPa) Testing in Water

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.75</td>
<td>20.71</td>
<td>23.74</td>
</tr>
<tr>
<td>2</td>
<td>22.68</td>
<td>21.00</td>
<td>26.04</td>
</tr>
<tr>
<td>3</td>
<td>19.88</td>
<td>37.96</td>
<td>18.68</td>
</tr>
<tr>
<td>4</td>
<td>27.44</td>
<td>18.95</td>
<td>24.56</td>
</tr>
<tr>
<td>5</td>
<td>20.96</td>
<td>22.64</td>
<td>24.07</td>
</tr>
</tbody>
</table>

Mean (SD)  

|       | 22.54 (2.92) | 24.25 (7.77) | 23.42 (2.79) |
Table 4.11:  GPC Biflexural Strength (MPa) Testing in Blood

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.73</td>
<td>23.02</td>
<td>28.39</td>
</tr>
<tr>
<td>2</td>
<td>25.67</td>
<td>27.97</td>
<td>31.15</td>
</tr>
<tr>
<td>3</td>
<td>27.57</td>
<td>27.40</td>
<td>32.73</td>
</tr>
<tr>
<td>4</td>
<td>19.87</td>
<td>19.29</td>
<td>25.98</td>
</tr>
<tr>
<td>5</td>
<td>21.77</td>
<td>26.23</td>
<td>29.66</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td><strong>23.72</strong> (3.05)</td>
<td><strong>24.78</strong> (3.62)</td>
<td><strong>29.58</strong> (2.58)</td>
</tr>
</tbody>
</table>
Figure 4.6: GPC Mean BFS (MPa) in Water and Blood
Figure 4.7: Mean BFS (MPa) pre and post-gamma irradiation in water and post-irradiation in blood
**Table 4.12:** PMMA mechanical properties (units of measurement MPa)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>SD</th>
<th>Day 7</th>
<th>SD</th>
<th>Day 30</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>95.6</td>
<td>5.9</td>
<td>98.4</td>
<td>2.1</td>
<td>116.6</td>
<td>1.7</td>
</tr>
<tr>
<td>BFS</td>
<td>147.7</td>
<td>10.7</td>
<td>131.2</td>
<td>9.8</td>
<td>138.7</td>
<td>21.7</td>
</tr>
</tbody>
</table>
4.10 PMMA mechanical testing Results

Table 4.12 shows the compression strength and biflexural strength values for PMMA cement which are significantly higher than those for the GPC cement and also of bone. Cancellous trabecular bone (in vertebrae) has a compressive strength of 4 to 12 MPa. While normal non-osteoporotic cortical bone has a compressive strength of 131 MPa.

4.11 Compressive and Biflexural Strength Discussion

The testing of the GPC cement post gamma-irradiation has proved relevant as it has demonstrated that both compressive and biflexural strengths are decreased as a result. Thus, gamma-irradiation reduces the mechanical properties of the GPC cement. In both biflexural and compressive strength testing, there was an increase in strength with time and more so in blood than in water. This is a desirable effect as it shows that the GPC cement does not weaken over time and in fact, gains more strength in the vertebrae’s bone marrow blood environment. The fact that it does not weaken over time means that the injected vertebrae can continue to resist further compression and prevent refracture of the injected vertebrae. This will of course need to be verified with clinical testing but the results are encouraging.

Comparison of the GPC cement’s compressive strengths (32.39MPa) with that of the PMMA cement’s (95.6MPa), show that the GPC cement is much closer to that of cancellous bone (up to 12 MPa) which is desirable as the vertebroplasty cements aim to replace the fractured cancellous bone. This in turn likely will lead to lower stresses transferred to neighbouring vertebrae, as the GPC is closer to the patient’s
cancellous bone’s compressive strength and therefore creates less of a stress riser as it is less rigid than PMMA. This makes the GPC cement a potentially viable alternative to PMMA as a vertebroplasty cement.

Experiment 3:

4.12 Vertebral Testing of Cements Results

The analysis of the vertebral testing is based on several factors. This includes the bone mineral density of the tested vertebrae, the cement penetration of the injected vertebrae, the ultimate load failure on compression and the various severity and locations of the vertebral height loss and vertebral compression.

4.12.1 Bone Mineral Density of Tested Vertebrae

Analysis of the randomised functional spinal unit vertebral pair’s bone mineral density of the superior injected vertebrae and the inferior non-injected vertebrae, as well as the non-injected superior and inferior control group’s vertebrae was undertaken.

The results are tabulated in Table 4.13. Figure 4.8 shows a graph of the bone mineral density in g/cm² for the superior FSU vertebrae for the control, GPC cement injected and PMMA cement injected groups. There is no significant difference in the mean BMD of the superior vertebrae between the groups though there is a greater spread in the BMD for the GPC cement group.
**Table 4.13:** Bone Mineral Density Means (g/cm²)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>(SD)</th>
<th>GPC</th>
<th>(SD)</th>
<th>PMMA</th>
<th>(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMD superior</strong></td>
<td>0.777</td>
<td>0.055</td>
<td>0.767</td>
<td>0.089</td>
<td>0.796</td>
<td>0.072</td>
</tr>
<tr>
<td><strong>BMD inferior</strong></td>
<td>0.806</td>
<td>0.059</td>
<td>0.740</td>
<td>0.064</td>
<td>0.798</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>BMD average</strong></td>
<td>0.791</td>
<td>0.054</td>
<td>0.751</td>
<td>0.071</td>
<td>0.797</td>
<td>0.039</td>
</tr>
</tbody>
</table>
Figure 4.8: The distribution of the BMD of the superior vertebra in the FSU by group
Figure 4.9: The distribution of the BMD of the inferior vertebra in the FSU by group
There is no statistically significant difference in the mean BMD across all the groups for either the superior (p=0.85) or inferior (p=0.24) vertebrae, or the average of the two (p=0.45). It is noted that the GPC cement group has outliers but accounting for this using medians instead of means made no difference to the outcome. The lack of a statistically significant difference in the BMD between groups indicates that vertebral FSU randomization was effective and thus on average the cement groups were tested on similar vertebral FSU pairings in terms of the BMD.
Figure 4.10: The distribution of the average BMD in the FSU by group
4.12.2 Load to Failure Results

The failure load was also measured to assess if the cement augmented vertebral functional spinal units required a higher load prior to failure.

Both Table 4.14 and Figure 4.11 show that surprisingly the uncemented control group had higher failure loads than the cemented groups. There was also a much greater spread in the failure loads for the GPC cement (with a much larger standard deviation value) compared to the PMMA cement which had the narrowest failure load spread. Overall there was a statistically significant difference in mean failure load across groups (p=0.01) with the control group significantly different from both the GPC (p=0.03) and PMMA group (p=0.02), but there is no significant difference between the GPC cement and PMMA cement (p=0.92). The GPC cement had a mean failure load which was only 5 percent higher than the PMMA cement.
Table 4.14:  Mean Load to Failure in Each Cement Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (Newtons)</th>
<th>Standard Deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6850</td>
<td>(645.5)</td>
</tr>
<tr>
<td>GPC</td>
<td>4900</td>
<td>(1278.7)</td>
</tr>
<tr>
<td>PMMA</td>
<td>4650</td>
<td>(191.5)</td>
</tr>
</tbody>
</table>
Figure 4.11: The distribution of the failure load on the FSU by group
The higher failure loads may well be due to a lack of a modulus mismatch between the two vertebrae in the control sample set of functional spinal units, especially since the mean BMD for both superior and inferior vertebrae were almost the same. In contrast, the cemented vertebrae would have a modulus mismatch possibly leading to earlier failure of the cemented functional spinal units. Unfortunately, it was not possible to assess if the failure occurred firstly in the cemented superior vertebrae or in the uncemented inferior vertebrae or simultaneously in both due to the speed at which the vertebral failure occurred. But it was possible to look at the severity of vertebral height loss and assess if this occurred in the superior or inferior vertebrae.

Thus, this illustrates that when is no modulus mismatch between vertebrae, higher failure loads are required prior to either vertebra failing as demonstrated in the control group. This highlights the need to get Young’s modulus of the GPC cement closer to that of bone (and be more patient BMD or vertebral BMD specific). This would require testing multiple samples of vertebrae of known BMD to assess their failure load, as well as testing these samples with a GPC cement with various Young’s moduli.

4.12.3 Vertebral Height Loss Analysis

By looking at whether there was more height loss in the superior or inferior vertebrae it is possible to extrapolate which was the weaker of the two and which would thus likely have failed first. Tables 4.15 and 4.16 show the mean percentage height loss sustained in the lateral radiograph in the superior and inferior vertebrae.
Table 4.15: Superior vertebral height loss post-compression compared to post cement injection height

<table>
<thead>
<tr>
<th></th>
<th>Anterior</th>
<th>Middle</th>
<th>Posterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.76%</td>
<td>0.00%</td>
<td>4.17%</td>
</tr>
<tr>
<td>2</td>
<td>5.60%</td>
<td>15.79%</td>
<td>13.64%</td>
</tr>
<tr>
<td>3</td>
<td>23.80%</td>
<td>35.00%</td>
<td>31.82%</td>
</tr>
<tr>
<td>4</td>
<td>27.80%</td>
<td>27.78%</td>
<td>40.00%</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.49% (12.02%)</td>
<td>19.64% (15.31%)</td>
<td>22.41% (16.41%)</td>
</tr>
<tr>
<td>GPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.29%</td>
<td>18.18%</td>
<td>20.00%</td>
</tr>
<tr>
<td>2</td>
<td>11.11%</td>
<td>18.75%</td>
<td>11.11%</td>
</tr>
<tr>
<td>3</td>
<td>52.38%</td>
<td>37.50%</td>
<td>55.56%</td>
</tr>
<tr>
<td>4</td>
<td>0.00%</td>
<td>11.11%</td>
<td>0.00%</td>
</tr>
<tr>
<td>5</td>
<td>0.00%</td>
<td>33.33%</td>
<td>21.88%</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.56% (21.57%)</td>
<td>23.77% (11.14%)</td>
<td>21.71% (20.81%)</td>
</tr>
<tr>
<td>PMMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.52%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2</td>
<td>5.56%</td>
<td>20.00%</td>
<td>18.52%</td>
</tr>
<tr>
<td>3</td>
<td>22.73%</td>
<td>36.36%</td>
<td>28.57%</td>
</tr>
<tr>
<td>4</td>
<td>9.52%</td>
<td>11.11%</td>
<td>28.00%</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.08% (7.91%)</td>
<td>16.87% (15.36%)</td>
<td>18.77% (13.34%)</td>
</tr>
</tbody>
</table>
Table 4.16: Inferior vertebral height loss post-compression

<table>
<thead>
<tr>
<th></th>
<th>Anterior</th>
<th>Middle</th>
<th>Posterior</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
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<td>4.76%</td>
<td>0.00%</td>
<td>12.00%</td>
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<td>21.05%</td>
<td>26.32%</td>
<td>17.39%</td>
</tr>
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<td>28.57%</td>
<td>30.00%</td>
<td>13.64%</td>
</tr>
<tr>
<td>4</td>
<td>42.86%</td>
<td>40.00%</td>
<td>31.82%</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>24.31% (15.86%)</td>
<td>24.08% (17.06%)</td>
<td>18.71% (9.02%)</td>
</tr>
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<th>Posterior</th>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10.71%</td>
<td>21.05%</td>
<td>0.00%</td>
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<td>2</td>
<td>15.79%</td>
<td>21.05%</td>
<td>21.43%</td>
</tr>
<tr>
<td>3</td>
<td>47.83%</td>
<td>40.00%</td>
<td>50.00%</td>
</tr>
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<td>0.00%</td>
<td>0.00%</td>
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<tr>
<td>5</td>
<td>21.21%</td>
<td>33.33%</td>
<td>11.76%</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>19.11% (17.86%)</td>
<td>23.09% (15.27%)</td>
<td>16.64% (20.70%)</td>
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<th>Anterior</th>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.00%</td>
<td>13.33%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2</td>
<td>0.00%</td>
<td>4.17%</td>
<td>0.00%</td>
</tr>
<tr>
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<td>0.00%</td>
<td>36.36%</td>
<td>37.04%</td>
</tr>
<tr>
<td>4</td>
<td>9.09%</td>
<td>31.82%</td>
<td>20.00%</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>7.27% (9.51%)</td>
<td>21.42% (15.21%)</td>
<td>14.26% (17.88%)</td>
</tr>
</tbody>
</table>
In the superior vertebrae, there is a trend to posterior and central vertebral body height loss more so than anterior vertebral body across all 3 groups even in the cemented groups. Interestingly, when it came to the inferior vertebral height loss, in the control group it was more so on the anterior and middle section of the vertebra than the posterior. In contrast, in the cemented groups the height loss was more at the central segments likely directly underneath the more cemented and higher elastic modulus (and thus stiffer) areas of the superior vertebrae. In the PMMA group, there was less anterior height loss and thus less anterior compression of the inferior vertebrae compared to the GPC cement group. The compression pattern of the GPC cement was more similar to that of the control than the PMMA cement group which is encouraging.

The height loss sustained by the vertebrae is converted to width and breadth increases on the AP and lateral X-rays respectively as illustrated in Figures 4.12 and 4.13. Overall the width and breadth increase of the superior vertebrae was greatest with both the control and GPC cement groups showing this. This may be explained by the poor penetrance of the GPC cement into the superior vertebrae resulting in a lack of GPC cement support to the injected bone.
Figure 4.12: Mean vertebral width (mm) increases on AP radiographs post-compression by cement type
Figure 4.13: Mean vertebral breadth increases (mm) on lateral radiographs post-compression by cement type
4.12.4 Cement Penetration into Injected Vertebrae

Lastly, we analysed the penetration of the cements into the injected superior vertebrae in the lateral and AP radiographs as illustrated by Figures 4.14 and 4.15 respectively.

As Figure 4.14 shows the GPC cement did not penetrate into the anterior segment of the vertebrae at all. This was due to the high viscosity and short working times. With the PMMA cement there was anterior vertebral penetration but to a lesser degree to that of the mid segment and posterior part of the vertebrae illustrating that cement penetration was not uniform. Cement underfilling is known to occur in vertebroplasty. More PMMA cement would reach the anterior cortex if balloon kyphoplasty was used to create a cavity for injection and lessen the cement injection pressures required. This may also be related to the fact that the cadaveric tissues are more rigid and less pliable compared to the more viscoelastic living tissue.
**Figure 4.14:** Cement thickness on lateral X-rays in different zones of vertebral body by cement type
Figure 4.15: Cement Percentage fill on AP X-rays in different zones of the vertebral body by cement type on the right, middle and left sides of the vertebrae.
In contrast, there was a more uniform penetration of both types of cement in the coronal plane as illustrated in Figure 4.15. As the high GPC cement viscosity limited its penetration anteriorly in the sagittal plane, it resulted in a greater penetrance or filling of the segments it did penetrate, averaging over 40 percent compared to over 20 percent for PMMA. There was a significant difference in the mean penetration for GPC and PMMA in the right and middle sides (both p=0.02). Overall the PMMA was able to fill more regions of the vertebrae than the GPC cement due to its longer working time and ease of injection.

4.13 Vertebral Testing Discussion

This aspect of the testing is unique as it is the first and only such experiment that we are aware of, which has tested GPC cements in human cadaveric vertebrae. The results of this testing have shown that the GPC cement is promising as an alternative vertebroplasty cement to PMMA, but it has also shown some shortcomings of the GPC cement which can be improved upon.

It is noted that the randomisation of the vertebrae according to T-score did not result in any statistical difference in the BMD of the vertebrae tested between all three groups. On the other hand, the inferior vertebrae’s BMD was noted to have a lower mean BMD which may have influenced the load to failure results, as illustrated in Figures 4.9 and 4.10.

In addition, the GPC cement’s poor injectability which led to poor anterior vertebral body penetrance is concerning, as it thus does not stabilise the most
weakened and fractured part of the vertebrae, when vertebroplasty is in fact indicated for refractory vertebral fracture pain. There may have been better penetrance of the cement if it had been possible to use a trocar and if the GPC cement had longer working times. This lack of anterior vertebral penetrance may have also influenced the load to failure results, as the anterior vertebral body was left unsupported by the GPC cement. This would have potentially allowed the anterior part of the vertebrae to fail much earlier than likely expected had there been better cement penetrance, as demonstrated by less anterior vertebral height loss in the PMMA cement group which had better anterior penetrance.

In essence, these tests have identified and illustrated the major weakness of this GPC cement which has likely influenced its performance on vertebral testing, and that is its very short working times. This must be addressed in future testing in order to improve the GPC cement’s performance and make it a truly viable as an alternative or possibly superior cement to PMMA in vertebroplasty application.
Chapter 5:

Conclusion
5.1 Conclusion

In conclusion, this research aimed to further test the GPC cement BT101 with regards to developing it further for orthopaedic applications including vertebroplasty and kyphoplasty. Tests were undertaken that were necessary to bring it closer to its end application in a theatre setting. As gamma irradiation would likely be used in its sterilisation, its effect on the rheology and mechanical properties was assessed. This showed that though gamma irradiation did not affect the glass transition temperature (Tg), it did however, cause a colour change in the GPC cement from white to grey. It also caused a change in the cement rheology by increasing the setting times but not the working times. More importantly, it was demonstrated that the mechanical properties of the cement were also altered with a decrease in both compression strength and biflexural strength of the cement post-irradiation on all tested days.

The effect of the GPC cement’s target bloody vertebral environment was also investigated and showed that the GPC cement had both increased biflexural and compression strength when setting in blood compared to distilled water on all days tested. This progressively compensated for the decrease sustained in these mechanical properties as a result of the irradiation. This is the first time that this has been shown although, there was not a statistically significant difference between media, which may be a consequence of the small sample size. This merits further investigation.

The GPC cement’s injectability and handling were assessed and found to be quite poor relative to those of PMMA. It had very short working times which made loading the cement into syringes quite difficult and resulted in a 100 percent failure of
injection via the trocars necessary to deliver cements in vertebroplasty through the skin and via the pedicles into the vertebral body. While it was possible to deliver the cement directly with a syringe in a fully dissected cadaveric spine bereft of soft tissue including skin, fat and muscle covering, this would not be possible in a clinical setting - at least not percutaneously. In spite of this, there was still a high failure rate of injection of 33 percent when directly injecting with syringes without trocars. By virtue of this quality alone, it fails to be suitable for clinical application in its current state.

Furthermore, due to its short working times and difficulty in injection, it was found to have no penetrance into the anterior vertebrae, the very area that vertebroplasty seeks to support and stabilise, since this is the area that is prone to fracture due to various biological, anatomical and mechanical reasons discussed in this research. If its delivery via trocars is improved this may also alter its anterior penetrance of vertebrae.

On vertebral testing post GPC cement injection of the superior vertebrae in the randomised functional spinal units, it was found that there was no significant difference in the bone mineral density between the 3 groups. There was a trend to a higher failure load required for the GPC cement group compared to the current gold standard PMMA injected group, but this was not statistically significant. In the PMMA group, there was significantly less anterior height loss and thus less anterior compression of the inferior vertebrae compared to the GPC cement group. Lastly, the compression pattern of the GPC cement group was more similar to that of the control group than the PMMA cement group which is encouraging as it would seem that the
GPC’s compression was closer to that of natural bone which may prevent stress risers and neighbouring vertebral fractures as a result.

Weaknesses of this research include a small vertebral sample size due to limited available human cadaver numbers and access restriction to allow for the DEXA scanning, radiology, cementing and compression testing. The small sample size decreases the statistical power to demonstrate a significant difference between the cements on vertebral testing. But it allows for this research to act as a pilot to guide further research on these cements. Another weakness is the DEXA scanning for bone mineral density determination of the vertebrae. Only the dissected out vertebrae were available and so an intact body DEXA scan was not possible. However, this confounder was controlled for by standardising the determination of the bone mineral density using the same calibration method used in DEXA scanners with the phantom spine, by simulating for the body tissue with a standardised volume of water. In addition, the gross value of the bone mineral density was not as important as the relative values for the vertebrae being tested. This is illustrated by the lack of a significant difference in the BMD across the three groups due to the randomization process.

5.2 Future Work

Future research work will need to include testing this GPC cement with various amounts of tri-sodium citrate to increase the working times (and thus make it more practical in its handling and injectability characteristics during vertebroplasty procedures as well as altering its vertebral delivery and penetrance), while also
analysing its effects on its mechanical properties. The combined effects of the gamma irradiation and the tri-sodium citrate, as well as cement setting in blood that has been demonstrated in this research will need to be further investigated. An alternative sterilisation method such as that using ethylene oxide may have to be used if radiation is found to detrimentally decrease the mechanical properties when used in combination with tri-sodium citrate.

In testing the use of radiation and the addition of tri-sodium citrate on the GPC cement, there can be an added advantage of the compression and flexural strength of the GPC cement being adjusted to be a lot closer to that of the vertebral bone of different bone mineral densities or T-scores while simultaneously improving its rheology. Thus, it may be possible to have a patient-tailored GPC cement to more closely match the bone mineral density of the neighbouring vertebrae thereby minimising the modulus mismatch and the stress riser effects. And in so doing, create a more patient-specific cementing solution for the orthopaedic surgeon when carrying out vertebroplasty or kyphoplasty.

This research has identified several areas mentioned above where the GPC cement can be further developed in order for it to reach clinical application.
Chapter 6:

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Chapter 7: Appendices

Publications, Presentations and Posters
Appendix B - Abstract Publications

A new cement for use in vertebroplasty; Comparison of a novel Zinc-Strontium Glass Polyalkenoate Cement with PMMA

O Abouazza, M Towler, D Boyd, F Condon,
Irish Journal of Medical Science, Volume 178, Supplement 2 / Feb 2009

Comparison of a Novel Zinc-Strontium Glass Polyalkenoate Cement with Current Cements Used in Vertebroplasty

O Abouazza, M Towler, D Boyd, F Condon,
Irish Journal of Medical Science, Volume 177, Supplement 7 / August 2008

Articles awaiting Publication:
A new cement for use in vertebroplasty; Comparison of a novel Glass Polyalkenoate Cement (GPC) with PMMA regarding compressive strength and risk of adjacent vertebral fractures following vertebroplasty

O Abouazza, M Towler, D Boyd, F Condon
JBJS supplement of BOA meeting 2009

A new cement for use in vertebroplasty; Comparison of a novel Glass Polyalkenoate Cement (GPC) with PMMA regarding compressive strength and risk of adjacent vertebral fractures following vertebroplasty

O Abouazza, M Towler, D Boyd, F Condon
A new cement for use in vertebroplasty; comparison of a novel Glass

O Abouazza, M Towler, D Boyd, F Condon

JBJS supplement of BORS meeting 2009
Appendix C - Presentations

National:

20\textsuperscript{th} June 2009
Irish Orthopaedic Association (IOA), Killenard, Portlaoise

\textit{A new cement for use in vertebroplasty; Comparison of a novel Glass Polyalkenoate Cement (GPC) with PMMA regarding compressive strength and risk of adjacent vertebral fractures following vertebroplasty}

7\textsuperscript{th} March 2009
17\textsuperscript{th} Sylvester O’Halloran Meeting (Limerick)

\textit{New cement for use in vertebroplasty; Comparison of a novel Zinc-Strontium Glass Polyalkenoate Cement with PMMA}

5\textsuperscript{th} September 2008
33\textsuperscript{rd} Sir Peter Freyer Surgical Symposium (Galway)

\textit{Comparison of a Novel Zinc-Strontium Glass Polyalkenoate Cement with Current Cements Used in Vertebroplasty}
Appendix D - Posters

A New Cement For Use In Vertebroplasty; Comparison Of A Novel Glass With PMMA

O. Abozouza, M. Towler, D. Boyd, F. Conlon,
Mid-Western Regional Hospital &
Materials & Surface Science Institute (MSSI), University of Limerick

Introduction
Vertebroplasty was first successfully performed in France in 1984 for treatment of post-traumatic vertebral hemangioma. It is the percutaneous transpedicular injection of cement into vertebral bodies.

Indications for treatment are:
- Meniscal further collapse of osteoporotic fracture
- Pain relief for refractory pain from vertebral fracture
- Hypothermic stabilisation of diseased vertebral due to primary or secondary cancer.

PMMA cements are currently the most commonly used while GPC cements are currently used in dental applications & are being modified for orthopaedic application.

Aims
To assess if GPC cements were better than PMMA cements in vertebroplasty regarding:
- GPC's compressive strength (129MPa) being closer to that of bone (14-132MPa) compared to PMMA (29MPa).
- Stress leading to lower 'stress rise' effect less stress concentration secondary vertebral compression fractures at lower compressive loads (known complication of 10-15% of vertebroplasties).

Methods & Materials

3 human cadaveric spine were obtained including lower thoracic and lumbar spinal segments. All soft tissues & disc except for intervertebral ligaments & facet joint capsules were dissected from the specimen.

Radiographs in 2 planes (AP and Lateral) were used to exclude specimens with spondylosis or other bony abnormality. Spondylosis were then divided into sets of two-vertebra functional spine units (FSUs) with intact intervertebral disc and ligaments.

Bone Mineral Density (BMD) was determined on each vertebra using the Dual-Energy X-ray Absorptiometry (DEXA) - Lunar iDXA model, GE Healthcare.

To minimise the effects of variability in BMD & the level of treatment, specimen pairs were sorted according to T-score and then assigned in an alternating sequence to three groups for vertebroplasty treatment:
- The first acted as a Control
- The second was injected with GPC cement
- The third was injected with PMMA cement

Results

In the treated FSUs, failure always occurred in the non-augmented, caudal vertebral body of which the majority were wedge compression fractures.

In the untreated control FSUs, failure occurred in both cranial & caudal vertebral bodies at higher compression loads.

Total volume of cement injected was 4.8 ± 1.2 ml vertebral body for both groups which was assigned T4 ± 8.6% of the total volume of the vertebral body.

For all FSUs, there was a correlation between increasing BMD & increasing failure load. There was a trend towards a lower failure load with increased degree of filling with cement. Failure strength in compression of the FSUs treated by augmentation of caudal vertebrae with either PMMA or GPC cement was lower than that of untreated FSUs.

Conclusions

GPC had vertebrae with significantly lower BMD for that level compared to the other groups.

GPC lead to similar vertebral fractures at lower compressive loads compared to PMMA but GPC had vertebrae with much lower BMD.

Further testing is required with a larger sample size and allocation according to BMD & not T-score.

In vivo testing is required to assess the benefit of cement on local vertebral osteoporosis & its effect on compressive strength.
BOA meeting 2009:

A new cement for use in vertebroplasty; Comparison of a novel Glass Polyalkenoate Cement (GPC) with PMMA regarding compressive strength and risk of adjacent vertebral fractures following vertebroplasty

*O Abouazza, M Towler, D Boyd, F Condon*

BORS meeting 2009:

A new cement for use in vertebroplasty; comparison of a novel Glass

*O Abouazza, M Towler, D Boyd, F Condon*