The Analysis of Lactate-Pyruvate Levels and Growth Proliferation Rates of Endothelial Cells on Urinary Bladder Matrix: A Potential Vascular Graft.

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Introduction

Arterial diseases are a common cause of death in the western world. The development of arterial substitute materials for the improved treatment of cardiovascular diseases is vital. One such material is urinary bladder matrix (UBM) an extracellular matrix material derived from porcine urinary bladder. Studies have reported that UBM contains a highly important factor for vessel and endothelium formation, an intact basement membrane (1). Cellular performance and growth are also important characteristics of an arterial substitute. Lactate is a respiratory metabolite which under optimal aerobic conditions is converted to pyruvate and can be used to measure cellular performance of cells. Thus the lactate/pyruvate (L/P) ratio indicates the respiratory status of a cell (2). The main aim of this research is the investigation of the (L/P) ratios and proliferation rates of human endothelial cells on UBM, to demonstrate its suitability for vascular applications.

Materials and Methods

Human aortic endothelial cells (HAEC) were seeded in a flat sheet of UBM on the luminal and abluminal side and on a tissue culture plastic substrate at a cell seeding density of 60,000. Growth rates were measured on the samples at 1 and 2 days after seeding using Alamar blue proliferation assay. Media samples were extracted for analysis at 3 days from the UBM luminal samples. Lactate and pyruvate analysis was achieved using ion exchange chromatography using a sulphuric acid mobile phase. Quantification of pyruvate and lactate was performed using ultra violet (UV) detection at 214 nm.

Results

The results showed that lactate levels were lower on the UBM substrates compared to those seeded on the tissue culture plastic substrate (Fig. 1). The pyruvate levels were similar for both kinds of substrate.

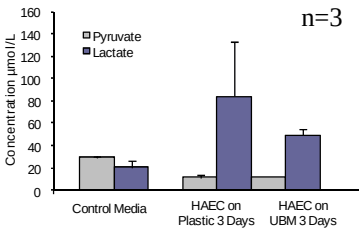

Fig.1. Basal extracellular lactate and pyruvate levels (µm/L) recovered from HAEC samples seeded for three days on either plastic or UBM substrate. Unseeded media acted as control. Each data point represents the mean ± SEM of three samples.

The cell attachment and growth rates differed between the luminal and abluminal surfaces, with the highest cell attachment on the abluminal surface (Fig. 2).

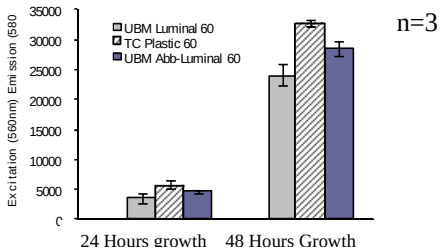

Fig.2. Proliferation rates of HEAC on UBM luminal and abluminal surface for 1 and 3 days. HAECs on tissue culture plastic acted as the control. Each data point represents the mean ± SEM of three samples.

Discussion and Conclusions

This study has shown endothelial cell attachment to the basement membrane with good cell viability which is an important factor in the development of vascular grafts. The lower L/P ratio observed in the UBM-seeded samples appears to indicate optimal oxygen infiltration for cells seeded on this matrix material.

References

1. Brown, B., Lindberg, K., (2006) *Tissue Engineering*, ***12*** (*3*), 519-526.
2. Guihen, E., O'Connor, W.T., (2009) *Electrophoresis*, **30**, 2062-2075.

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Disclosures None