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DEVELOPMENT OF NOVEL SOLUBLE GLASSES FOR TISSUE ENGINEERING OF HARD TISSUE

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INTRODUCTION: Tissue engineering explores cell transplantation[1] using a variety of materials and matrices in order to regenerate fully functioning tissues. This study involves the *in vitro* seeding of bone cells onto a series of soluble phosphate based glasses. The physical properties of the glasses, such as solubility, are determined by their chemical composition, in particular the calcium content. The two main aims of this study are

Phenotypic identification of bone cells.

Identifying the most biocompatible range of glass compositions.

MATERIALS AND METHODS:

Cells: Primary cells were obtained from human alveolar bone explants[2]. MG-63, human osteogenic sarcoma derived[3], cell line was also used.

Glass for cell work: Various glass compositions were produced in the form of 1.5x13 mm discs based on the P_2O_5-CaO-Na_2O System[4]. With the amount of P_2O_5 fixed at 50 mol%, glasses of higher calcium proportion were less soluble when incubated in culture medium. Poly-L-lysine (Sigma) coated glass coverslips were also used as positive controls.

Immunocytochemistry: MG-63 cells were seeded onto the discs. In order to arrest extracellular matrix destined proteins within the cell boundary, cells were incubated in medium containing monensin (Sigma) for 24 h prior to fixing in ice-cold methanol at a 48 h. The primary antibodies used were bone sialoprotein, osteonectin and osteopontin (gift of Prof. L. Fisher). FITC-conjugated IgG were used as secondary antibodies (Stratech Scientific).

Cell adhesion and proliferation assays: Primary cells were seeded on discs placed in 24 opaque well plates, incubated at 37°C in 1 ml of medium per well. Adherent cells were later quantified using the CyQUANT® Cell Proliferation Assay kit at 1, 2, 3 and 7 days post seeding. Similarly, MG-63 cells were quantified after 3 h in culture.

RESULTS: Punctate staining for the glycoproteins osteonectin (Fig 1), osteopontin and bone sialoprotein (data not shown) was evident in the cytoplasm of cells cultured on both soluble glass discs and glass coverslips. Both primary and MG-63 cells adhered to (Fig 2) and survived when plated on phosphate based glasses. Early adhesion data, however, showed that cells have a clear preference to less soluble compositions (Fig 3).

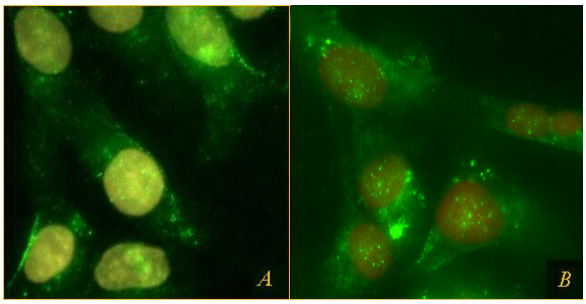

Figure 1. Leica® fluorescent microscope image of MG-63 cells cultured on A. glass coverslips and B. 40 mol% calcium containing glass showing positive staining for osteonectin.

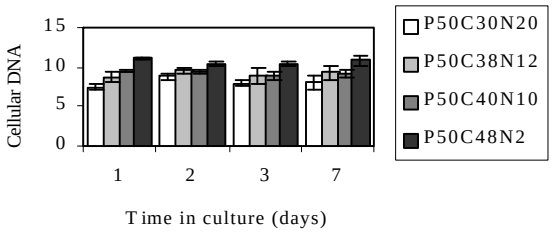

***Figure 2 P**rimary cells proliferation behavior on various compositions at different time points(error bars; ±SD).*

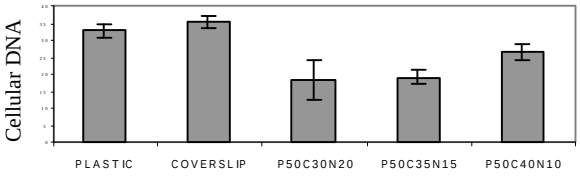

***Figure 3.** Graph showing MG-63 cells adhesion rates on various materials after 3 hours in culture (error bars; ±SD).*

DISCUSSION & CONCLUSIONS: Phosphate based biodegradable glasses support the adhesion and survival of primary and osteoblast-like cells. Whilst proliferation rates have shown no significant increase over time, less soluble glass compositions proved to accommodate higher numbers of

functioning cells. Phenotypic characteristics, of adherent cells, were also preserved on these glasses and observed as positive immunolabeling of several extracellular bone markers.

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ACKNOWLEDGEMENT LW Fisher, et al (1995) *Acta Orthop Scand* (Suppl 226) **66**:61-65.