ENGINEERED CRANIOFACIAL MUSCLE CONSTRUCTS EXPRESS MARKERS OF MUSCLE DIFFERENTIATION

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INTRODUCTION: Tissue engineering has the potential to serve as an alternative to surgical tissue transfer for the management of soft tissue defects. The perceived advantages include reduced donor site morbidity and restoration of function and aesthetics to ideal. Degradable scaffolds are utilised in the early stages of cell growth and development with the advantage of eventual elimination to leave space for the engineered tissue and a reduced chance of rejection.

METHODS: Cells were extracted and characterised from human masseter muscle biopsies obtained from healthy consented adult patients. Ethical approval had been obtained. Immunofluorescent markers to desmin and α-sarcomeric actin confirmed myogenicity. Degradable iron-phosphate glasses were produced in disk and fibre form. The disks were coated with collagen and the fibres were aligned and coated with a thin layer of collagen or encased within a collagen gel. Cells were seeded on these scaffold combinations and a collagen gel was used as a control. Cell attachment, survival, alignment, differentiation and maturation over time were assessed using the CyQUANT assay, light and modulation contrast microscopy, immunofluorescence and RT-PCR.

RESULTS:

Disk Biocompatibility: There was good cell attachment and survival on collagen-coated disks. myoD and myogenin gene expression increased over the 14-day experimental period. Furthermore, there was an increase in the embryonic and neonatal myosin heavy chain gene expression by day 14 and variable expression of all other myosin heavy chains.

Biomimetic Scaffold Response: Interestingly, cell orientation was unidirectional on the glass fibre and glass fibre-collagen scaffolds analogous to native skeletal muscle; this was in contrast to the collagen gel controls where the cell direction was random. The gene expression of myoD and myogenin was highest on the glass fibre scaffolds initially, however by day 21, the other scaffolds had greater expression ($p<0.05$). This was also the finding for all myosin heavy chains gene expression ($p<0.05$).

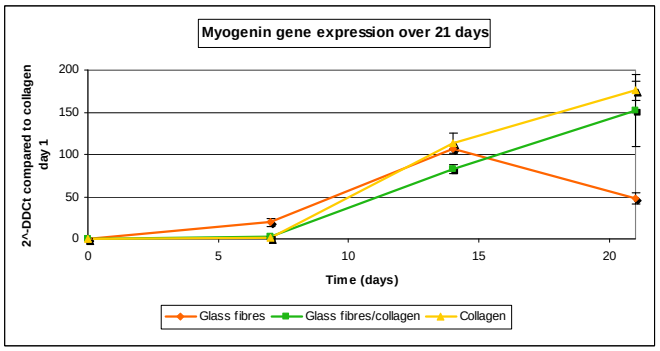

FIGURE ONE: Expression of myogenin transcripts by skeletal jaw muscle constructs (determined by quantitative PCR). After 21 days in culture, the glass fibre-collagen composites show comparative performance to collagen gels alone. Both are superior to glass fibres alone.

DISCUSSION & CONCLUSIONS: There was good cell biocompatibility with degradable iron-phosphate glass disks. The creation of a biomimetic scaffold enabled orientation of the cells into the same direction to produce architecture similar to native skeletal muscle. Although the control collagen gels demonstrated evidence of muscle cell differentiation and maturation, as did the glass fibre scaffolds, these scaffolds may not be ideal for implantation: in particular, the collagen gels contracted uncontrollably. The glass fibre-collagen gel scaffolds provided a much more manipulable system and have the potential to provide a suitable hard-soft tissue interface.

REFERENCES: Lewis,M.P., Mudera,V., Cheema,U, Shah,R. (2009). Muscle Tissue Engineering. in Meyer,U., Meyer, Th., Handschel,J., Wiesmann,H.P., (ed.) Fundamentals of Tissue Engineering and Regenerative Medicine. Heidelberg: Springer. pp. 243-254; Shah, R., Sinanan, A.C.M., Knowles, J.C., Hunt, N.P and Lewis, M.P. (2005) Craniofacial muscle engineering using a 3-dimensional phosphate glass fibre construct. Biomaterials. 26: 1497-1505.

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