INVESTIGATING CELL-SUBSTRATE MECHANICAL INTERACTION: A TISSUE ENGINEERING APPROACH

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Introduction

As one of the major components of bone, collagen is a candidate scaffold material for orthopaedic tissue engineering. While the low stiffness of collagen is a significant drawback for implanted scaffolds – investigation into ways to ameliorate this is ongoing – the high compliance of porous collagen matrices (since the deformations are large) suggests their use in the investigation of how osteoblasts interact mechanically with a substrate. In particular, evidence for contraction of the underlying matrix by bone cells has been observed in newly-forming bone [1] and in fracture sites [2]. This suggests that there may be a relationship between mineralization and contraction of bone cells. Preliminary work on this model, using osteoblast-like MC3T3 cells, is presented here.

Materials and Methods

Cell contraction: The short-term contractile ability of MC3T3 cells was assessed. The cells were cultured under standard conditions (α-MEM with 1% antibiotic-antimycotic and 10% FBS). At confluence, the cells were trypsinized and a suspension in α-MEM was prepared. *Collagenous scaffold:* The substrate for this study was a porous collagen-glycosaminoglycan (GAG) scaffold, prepared as described in [3]. A template was used to cut rectangular samples for the contraction test. Collagen-only matrices were also prepared (Figure 1) using a slightly modified protocol; image analysis and mechanical testing of these matrices is ongoing. *Contraction of cells:* Rectangular samples of collagen-GAG matrix were seeded dropwise with $7x10^6$ MC3T3 cells in suspension, or with medium alone. Contractile testing was performed by mounting the samples in a cell-force monitoring apparatus, described in detail in [4]. Briefly, the matrix was held between polymer grips suspended in a well of culture medium. One grip was attached to a Cu-Be cantilever beam, mounted in front of a proximity sensor. Matrix contraction results in beam deflection and therefore an increase in measured voltage. The apparatus was placed in an incubator for 22h, and the proximity sensor voltage was recorded.

Results

These preliminary results indicate that MC3T3 cells do contract the collagenous scaffold in the short term, as evidenced by the increase in voltage between the medium-only and the cell-seeded samples (Figure 2).

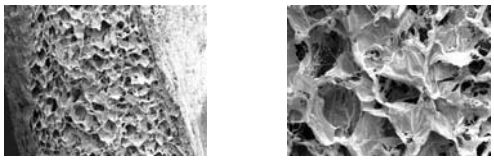

Figure 1: Collagen-only matrices (original magnification: left, 45x; right, 150x)

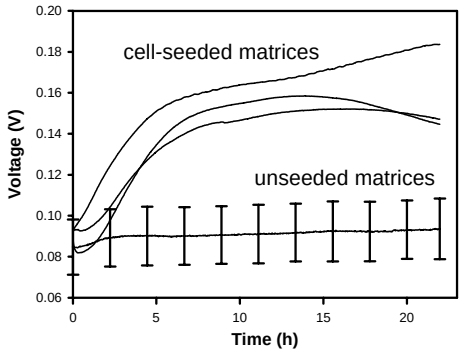

Figure 2: Proximity sensor voltage (contraction) of MC3T3 cell-seeded collagenous matrices.

Discussion and Conclusions

The data presented here, confirming that MC3T3 cells contract collagen matrices in the short-term, are the results of preliminary work in the development of a model to investigate mineralization and contraction by osteoblasts, utilizing the techniques of tissue engineering. Ongoing research includes the further characterization of the collagenous substrates, as well as the continuing development of techniques to assess the contraction and mineralization of osteoblast-like cells and to relate this force to cell number and behaviour.

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