Durotactic Control within a 3D Collagen Matrix

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INTRODUCTION: While matrix stiffness has been implicated in cell adhesion and migration, most studies have focused on the effects of substrate stiffness in 2D. This work describes a novel continuous stiffness gradient model for studying such processes in 3D.

METHODS: Collagen scaffolds with a gradient of biomaterial matrix stiffness were prepared by casting 6 ml of collagen solution (embedded with agarose marker beads to mimic cell seeding) in moulds which were inclined at 15º. After setting, the wedge-shaped scaffolds were compressed vertically to produce sheets of (0.1mm) uniform thickness, but with increasing density along the length of the sheet. Dynamic mechanical analysis was carried out on 1 mm wide strips obtained from the two ends and the middle of each sheet, to measure changes in elastic modulus and mean agarose bead density was quantified in each region as a measure of the density gradient formed. Collagen scaffolds were seeded with growth-arrested HDFs and cultured for 3 and 6 days. Mean cell density in each of the three regions was measured to assess the effect of the matrix stiffness gradient on cell migration[1].

RESULTS: The elastic moduli, 1057±487 KPa and 2305±693KPa at the soft and stiff end respectively and 1835±31 KPa in the middle, represented a near linear increase in modulus along the construct. Mean agarose bead density along the same gradient rose from 10±1 to 71±12 at the soft and stiff end respectively and was 19±5 in the middle. This indicates successful engineering of a density gradient (4% to 20% collagen from soft to stiff end respectively), corresponding to the stiffness gradient. Using agarose beads as an exemplar we have also successfully modelled the proposed formation of a cellular density gradient, that would be established in the direction of the stiffness gradient, if the construct was cell-seeded. Growth-arrested HDFs cultured within such constructs for 3 and 6 days, accumulated preferentially towards the stiff part of the gradient. Durotactic migration was significant after 6 days.

DISCUSSION & CONCLUSIONS: The ability to engineer a continuous 3D stiffness gradient together with precise control of both its absolute (by controlling the level of compression) and relative (by controlling the angle of inclination) properties, provides an effective model for studying cellular mechanotaxis, such as nerve or endothelial guidance in vitro and designing mechanically stable biomimetic material structures such as muscle-tendon interfaces for implantation.

REFERENCES: [1] Gray DS, Tien J, Chen CS. (2003)*Journal of Biomedical Materials Research* Part A 1;66A(3):605-14.

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