Topographic Patterning of 3D Collagen Scaffolds: From Surface to Interface Engineering

E.Hadjipanayi,[1] T.Alekseeva[1], L.Bozec[2] and R.A.Brown[1]
[1] *UCL, Tissue Repair and Engineering Centre, Division of Surgical and Interventional Sciences, Institute of Orthopaedics and Musculoskeletal Sciences, London, HA7 4LP, UK*
[2]*UCL Eastman Dental Institute - 256 Gray's Inn Road - London - WC1X 8LD, UK*

INTRODUCTION: Topographic patterning provides a useful tool for regulating cell function, such as adhesion, proliferation, differentiation and contact-guidance. While current (e.g.lithographic) techniques allow precise control of topographic pattern (anisotropic vs isotropic) and scale (nano- vs micro-topography) [1], they are only applicable to 2D surface patterning, which compromises their relevance to 3D tissue engineering. In this study we developed a novel method for rapid fabrication of micro-textured 3D collagen scaffolds.

METHODS: The two-step fabrication process involved, firstly, plastic compression of collagen hydrogels to remove interstitial fluid and increase matrix stiffness (from 42.2±22 to 1805±214KPa) [2], followed by embossing a customizable template of parallel-aligned phosphate-based glass-fibers (35 or 55μm fiber diameter with 100μm inter-fiber spacing) on the scaffold's surface. Groove width and depth was measured by SEM and AFM, respectively. For 2D culture, HUVECs were cultured on smooth or micro-textured collagen scaffolds for 48hrs to test cell alignment and morphology. Cell attachment was tested by washing scaffolds with PBS 4hrs post-seeding and counting the number of adherent cells with a phase-contrast microscope. For 3D culture, human dermal fibroblasts (HDFs) were seeded within smooth or micro-textured scaffolds, with keratinocytes seeded on top. Constructs were cultured submerged for 2 days, before being raised to the air-liquid interface and cultured for an additional 12 days. Constructs were H&E stained at 2weeks and epidermal thickness was quantified by image analysis.

RESULTS: SEM showed a regular pattern of parallel grooves and ridges (Fig.1). Groove width was 30.5±3.3μm and 49.5±11.6μm for 35μm and 55μm diameter fibers, respectively. Groove depth was 0.95±0.49μm and 1.55±0.31μm, respectively. 2D culture of HUVECs on micro-textured collagen substrates showed preferential cell adhesion on 30μm-wide grooves compared to 50μm-wide grooves or smooth surfaces. Groove width also affected cell alignment and morphology, with 66% of cells aligning along groove direction on 30μm-wide grooves (cell elongation-index=3.1±1.9), compared to 7.3% of cells on 50μm-wide grooves (cell elongation-index=1.35±0.23) (Fig.2). 3D culture of HDFs within micro-textured scaffolds, with keratinocytes seeded on top, indicated that the micro-topography of the dermo-epidermal interface influenced the rate of keratinocyte stratification, since epidermal thickness decreased from 40±14μm for smooth interfaces to 14±6μm for grooved interfaces.

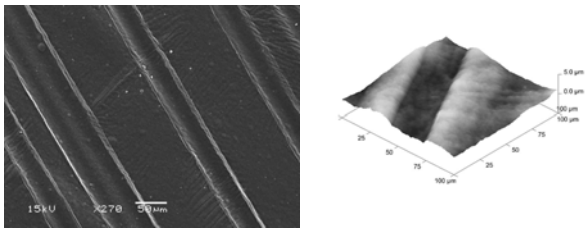

Fig. 1: SEM (left) and AFM (right) images of micro-textured collagen scaffolds

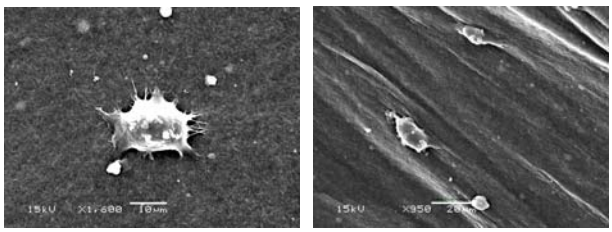

Fig. 2: Endothelial cells on smooth (left) and 30μm-wide grooved (right) collagen scaffolds.

DISCUSSION & CONCLUSIONS: We report on a novel method for topographic patterning of 3D biomimetic collagen scaffolds. The versatile applicability of this technique to 2D and 3D culture makes it a promising tool for investigating cell behavior on both 2D surfaces and 3D interfaces, where cell-cell interactions could critically influence cell function.

REFERENCES: [1]J.Y.Lim and H.J.Donahue (2007) *Tissue Engineering* **13**, 1879-1891. [2]R.A.Brown, M.Wiseman, C.B.Chuo, U.Cheema, S.N.Nazhat(2005)*AdvancedFunctionalMaterials***15** ,1762-1770.