Mapping of Tissue-Engineered Extracellular Matrices (ECMs) for Tissue Repair Using FT-IR spectroscopy

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The successful treatment of cartilage damage and lesions is still a major challenge despite many approaches being utilised including microfracture, autologous chondrocyte implantations, osteochondral autograft transfer and osteochondral allografting. Cartilage tissue engineering using naturally derived extracellular matrix (ECM) materials such as urinary bladder matrix (UBM) and small intestine submucosa (SIS) have potential. This is due to the fact that they have the capability to be actively remodelled by cells and contain essential growth factors such as transforming growth factor-beta1 (TGF-β1). This study investigates the 3D culture of chondrocytes in multi-layered sandwich models of UBM, SIS and synthetic based electrospun poly(L-lactic acid) (PLLA) for tissue engineered cartilage.

The PLLA scaffolds were fabricated by dissolving PLLA in HFIP at 8% w/v overnight and electrospun to generate randomly oriented fiber scaffolds (1 µm diameter). SIS and UBM materials were fabricated through a decellurisation process, resulting in removal of all the cellular components. The average thickness of all the scaffold was ~100 µm. Five individual scaffold layers were seeded with bovine chondrocytes (200K cells/layer), assembled into multi-layered scaffolds, and cultured for 4 weeks with and without TGF-β1 supplementation, to produce structurally bonded scaffolds of SIS, UBM and PLLA.

Characterisation of UBM, SIS and PLLA scaffolds sections was carried out using hyperspectral maps, collected using a Perkin Elmer AutoImage microscope connected to a Perkin Elmer Spectrum 100 FT-IR system. The sections were embedded in OCT and cryosections were cut and placed on MgF_2 slides for imaging. Spectra were collected using 10 integrations in the spectral region 1000-4000cm^{-1} and a spatial resolution of 20 µm. The maps were analysed using N-FINDR spectral unmixing and in each case found regions corresponding to the ECM and the scaffold. Spectra characterizing was compared using PLS-DA.

The findings identified differences in collagen content across the scaffold types. Also shown were significant changes in DNA/RNA and GAG content with and without TGF-β1 addition. Higher levels of DNA/RNA, Collagen and GAG were found in the UBM and SIS samples which may indicate that they have greater cartilage tissue engineering potential.