

Investigation of Tenocytes Proliferation under Perfusion in Microchannels Chitosan Scaffolds by Optical Coherence Tomography

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INTRODUCTION: We produced a three-dimensional environment suitable for tenocyte proliferation and subsequent production of a well organized uniaxially orientated collagen matrix, one of the key issues in tendon engineering. To encourage this production, tenocytes were stimulated by continuous fluid flow in a perfusion bioreactor. To this specific purpose, chitosan microchannels in scaffolds were designed to provide micro tubular structures where cells were in contact with a continuous laminar flow. We expected that the flow would have helped aligning cells and extracellular matrix along the channel. The structural changes occurring in the scaffolds were monitored by optical coherence tomography (OCT) given its ability to acquire 3D image in a non-destructive manner¹.

METHODS: Primary pig tenocytes were grown from pig explants obtained from the local abattoir and cultured in DMEM, supplemented with 10% FCS and 1% L-Glutamine. Chitosan gel (2%) was cast inside moulds containing needles arrays, which formed the template for the microchannels. After freeze-drying, the resulting scaffolds were microporous and possessed several microchannel parallelly orientated and regularly spaced. Different channel sizes (250 μm to 500 μm) were prepared by varying the type of needles used. 1×10^6 cells were seeded into each scaffold. Two weeks of static culture in growth medium allowed cell proliferation on the inner surface of the microchannels. The scaffolds were then cultured in a differentiation medium (5 %FCS, 100 mg/mL acid ascorbic) and split into two groups. One group was in static culture, whereas group 2 was cultured in a perfusion bioreactor² with a 0.1 ml/min flow rate for one week. A bench-top OCT system equipped with a broadband light source centred at 1300 nm providing an imaging resolution at $\sim 10 \mu\text{m}$ was utilised in this study.

RESULTS: OCT revealed clearly the microchannel structure in the chitosan scaffold (Figure 1). Cell proliferation and ECM generation changed the size and morphology of the microchannels. These changes were not only shown in OCT images, but were also verified

directly by scattering intensity profile (Figure 2). The empty channel had a low intensity response due to background noise, whilst cell plus extra cellular matrix (ECM) filled channel produced a distinct scattering signal from that of chitosan. The channels subjected to perfusion exhibited broader and higher intensity peak.

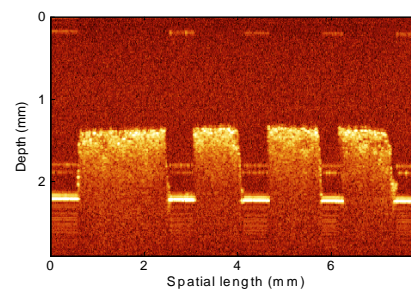


Fig. 1: OCT scan of a blank chitosan scaffold with microchannels.

DISCUSSION & CONCLUSIONS: Tenocytes proliferation and ECM formation have occurred in the microchannels, and these features were increased by continuous perfusion. The resulting microstructural variations were displayed adequately by OCT.

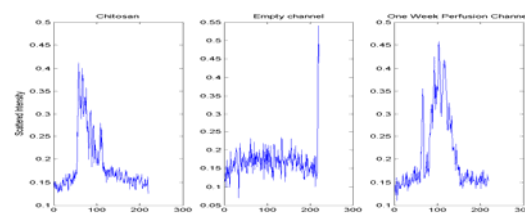


Fig. 2: Mean scattering intensity profile of chitosan scaffold (left), blank channel (middle) and cultured channel (right).

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