Structural and Physicochemical Characterisation of an Enzymatically Cross-linked Collagen Scaffold

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Introduction: Microbial transglutaminase (mTGase) catalyses a collagenase resistant bond [1] between collagen residues of glutamine and lysine [2]. We have in the past shown that this cross-linked scaffold exhibits cellular compatibility and can resist cell-mediated degradation. [3] The objective of this study was to deduce physico-chemical parameters for this cross-linked scaffold. Specifically, the effect of cross-linking on the porosity, thermal stability and collagen structure was investigated.

Materials and Methods: Collagen type I (2.9mg/ml) (BD Biosciences) was cross-linked with different concentrations of mTGase (0.025, 0.05 and 0.1 mg/ml) (Activa™ WM, Ajinomoto Corporation Inc. Japan).

Porosity: The scaffolds were freeze/dried and visualised on Scanning Electron Microscope (SEM). The images (n=3) were analysed using a image analysis software (IMAGE-PRO® PLUS). Additionally, SEM visualisation of viable cells within the native and cross-linked scaffold was performed.

Chemical Structure: Native and cross-linked collagen films were prepared on 13mm cover slips and the Fourier transform infrared (FTIR) spectra was obtained as an average of 40 scans.

Thermal Stability: The thermal stability of freeze/dried cross-linked scaffolds was compared with native collagen samples (n=3) by differential scanning calorimetry (DSC).

Intrafibrillar Morphology: The collagen fibrillar arrangement was analysed before and after cross-linking using atomic force microscopy (AFM).

Results: Area fraction measurements elucidated differences in the pore size of cross-linked and non-cross-linked scaffold (Fig.1 and 2).

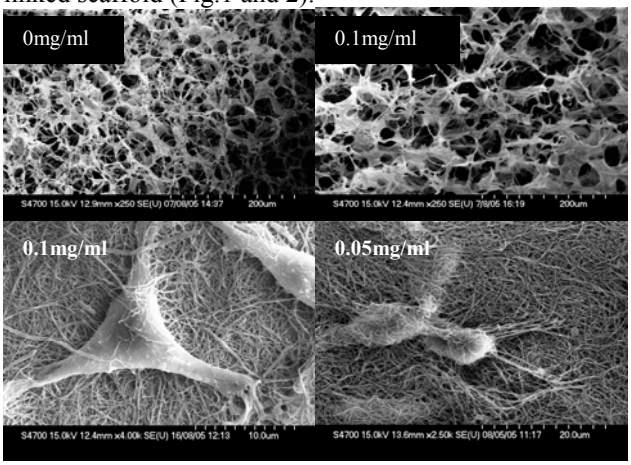
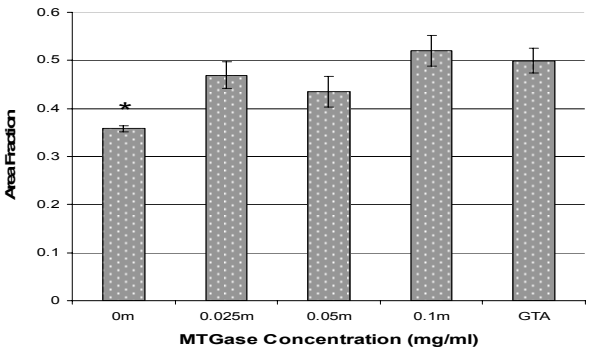
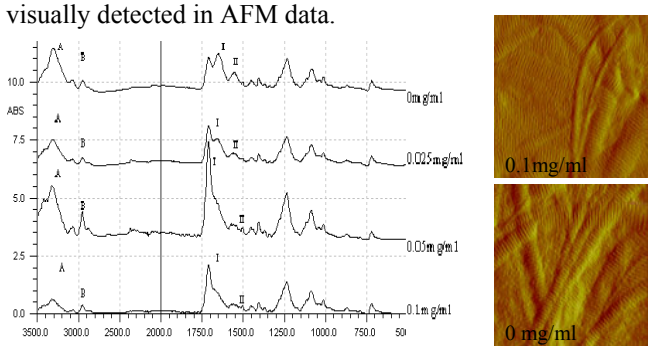


Fig. 1: SEM images of cross-linked and non-cross-linked collagen scaffolds with and without cells.

Fig.2: Pore area fraction in scaffolds with different concentrations of mTGase.*indicated statistical difference between control and cross-linked scaffolds, $p<0.05$, n=3.



Collagen structure as seen from FTIR spectra indicated increased covalent bond formation as the mTGase concentration increases. Increased intrafibrillar apposition proportional to the reduction in random distribution of fibrillar arrangement increased with cross-linking and was visually detected in AFM data.



(a) **(b)**

Fig. 3: (a) FTIR spectra obtained from collagen scaffolds treated with different concentrations of mTGase (0, 0.025, 0.05, 0.1mg/ml). (b) AFM topography of native collagen and cross-linked collagen scaffold.

Conclusions: Scaffold porosity and fibril apposition increases with the degree of cross-linking, as evidenced by pore area fraction analysis and AFM respectively. FTIR spectra show presence of covalent bonds as amide II band decreases and amide I band rises with increased concentrations of mTGase. These bonds enhance the thermal stability of the cross-linked scaffold as verified by DSC.

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References:

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