MIMICKING THE BODY- A NOVEL METHOD OF CROSS-LINKING COLLAGEN TO CONTROL SCAFFOLD DESIGN- IMPLICATIONS ON HUMAN TISSUE ENGINEERING

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INTRODUCTION: Scaffold design is an essential part of tissue engineering. Cross-linked collagen matrices are often used to increase the mechanical strength of scaffolds. The ability to create and control different patterns of cross-linking within a collagen matrix would allow manipulation of scaffold characteristics (e.g. anisotropy, strength and shape) in order to tailor the design of the scaffold more appropriately to different applications. Photochemical crosslinking using Riboflavin and blue light[1] has the potential to create patterns of crosslinking throughout a matrix. If successful, it can have applications in more sophisticated scaffold creation such as crosslinked tubular structures for nerve and vascular engineering. This study will assess the suitability of this method for such an application.

METHODS: Collagen (type 1) gels were made using standard techniques and plastically compressed for five minutes. Some of the gels were treated with riboflavin before setting, to be crosslinked after compression. Using a masking technique which only permitted blue light (436nm) to enter and crosslink those gels in certain directions, varying patterns of crosslinked gels were produced- fully crosslinked, vertically crosslinked, horizontally crosslinked and native. The extent of crosslinking was measured in dry and hydrated states through thermal analysis (DSC and Mettler FP Hot stage analysis) and anisotropy was measured using dynamic mechanical analysis (DMA) and tensile testing[1]. Further investigation using micro thermal analysis (MicroTA)[2] on the individual characteristics of collagen crosslinked in this manner were used to create a melting 'finger print'- which can be used to recognise such cross-linked areas later on.

RESULTS: There were differences in the break stresses of vertically crosslinked collagen- 0.25MPa (± 0.01) compared to a fully crosslinked - 0.16MPa(±0.01) (table1.). The melting temperature of photochemically crosslinked collagen compared with native collagen was unremarkable in the DSC and Hot Stage data. However MicroTA data showed marked differences in the sensor derivative data (fig.1).

Table 1. DMA results for relative break stresses of collagen under varying treatments- different patterns of cross-linking

Collagen samples	Stress at Failure(Mpa)
Fully Cross-linked	0.32 (± 0.04)
Vertical anisotropy	0.25 (± 0.01)
Horizontal anisotropy	0.37 (± 0.04)
Native	0.14 (± 0.01)

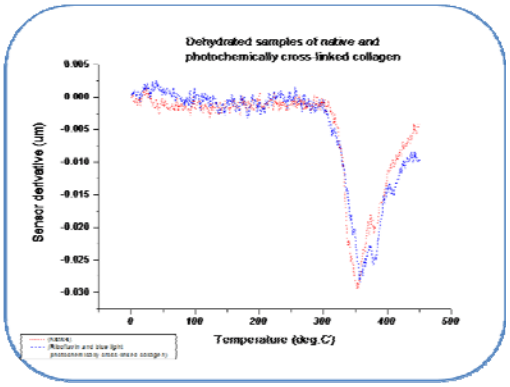

Fig.1: Sensor derivative data for dehydrated collagen. Native collagen red, photochemically cross-linked collagen blue

DISCUSSION & CONCLUSIONS: This study has shown that photochemically cross-linking collagen can be successfully manipulated to re-create anisotropy. However, there is controversy over the nature of the anisotropy, as unlike our findings, the gels should have a higher break stress in the vertical direction- the direction in which the DMA was assessing strength. This could be due to the masking technique allowing less light in the vertical direction therefore allowing less area of those samples to be cross-linked. The different denaturing characteristics highlighted by the MicroTA are promising in identifying individual 'fingerprints'. This is another desirable trait of photochemical cross-linking which can be used as a basis for recognition in more complex future scaffolds.

REFERENCES: 1. Torres-Giner, S. et al. Comparative Performance of Electrospun Collagen Nanofibres Cross-linked by Means of Different Methods. ACS Appl. Mater. Interfaces, 2009 1(1) pp 218-223. 2 Nguyen A et al (1974) The Dynamic Mechanical, Dielectric, and Melting Behavior of Reconstituted Collagen. May;13(5):1023-37.