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MICRO THERMAL ANALYSIS: A NOVEL METHOD OF DETERMINING THE EXTENT OF COLLAGEN CROSS-LINKING WITHIN A COLLAGEN CONSTRUCT- APPLICATIONS FOR TISSUE ENGINEERING

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INTRODUCTION: Increasingly complex designs of collagen scaffolds used in tissue engineering require more accurate and sensitive methods of testing. A new proposal to create cross-linked tunnels within a collagen scaffold for use in vascular and nerve studies will require a novel method of assessing the extent of cross-linking within the walls of the tube- to ensure maximal strength for matrix viability. To date this method has not yet been investigated.
Aim: this study will investigate ways to differentiate between cross-linked and native collagen using melting characteristics found by Micro thermal analysis at point regions throughout the construct to give a detailed intra-matrix map.

METHODS: Three samples were made for testing: native (control), cross-linked with glutaraldehyde (control) and photochemically cross-linked with riboflavin and blue light (436nm wavelength)- the method under test. Differential scanning calorimetry (DSC) and a Mettler FP Hot Stage (heating rate 10C/min from 20-80˚C) investigations were conducted for macro-level testing. Comparison studies were then performed using MicroThermal Analysis (Micro TA) (heating rate 25˚C/sec). The MicroTA combines the imaging ability of the Atomic Force Microscope and the thermal characterization of temperature modulated DSC to estimate the point deterioration of collagen when exposed to heat[1]. Data collected included temperature of onset (change in slope of sensor corresponding to melting temperature) and sensor derivative peaks.

RESULTS: DSC and Hot stage thermal analysis (fig.1) showed the controls (native and glutaraldehyde cross-linked collagen) to have expected melting temperatures[2] in hydrated (58˚C and 78˚C respectively) and dehydrated states as did the Micro TA. However, the macro analysis did not accurately differentiate between the native and test collagen using melting temperatures. The MicroTA data showed marked differences in the sensor derivative peaks of all collagen forms (fig. 2) as well as identifying other characteristics such as swelling temperatures in cross-linked samples.

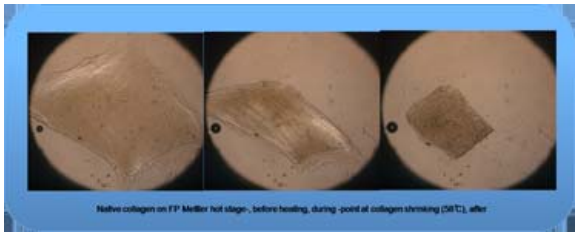

Fig. 1:Hydrated native collagen- hot stage, before heating (left), during heating- shrinking at 58˚C (centre) and after heating (left).

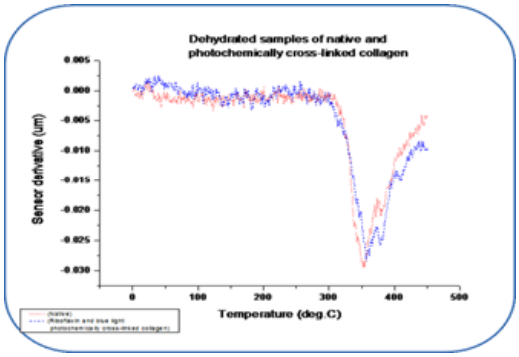

Fig.2: Sensor derivative data for dehydrated collagen. Native collagen red, photochemically cross-linked collagen blue.

DISCUSSION & CONCLUSIONS: This study has found that MicroTA can successfully identify the individual denaturing characteristics of collagen after each treatment, allocating a 'fingerprint' to each as a means of recognition when analysing collagen. This is not only useful for differentiating between cross-linked and noncross-linked locations in collagen but also to further assess the extent of cross-linking by each treatment.

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