Enhancing the mechanical properties of collagen by photo-chemical cross-linking

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INTRODUCTION: Cell survival within mechanically strong scaffolds is critical in the design of tissue engineered constructs. Collagen type I gels tend to be mechanically weak due to the low percentage of collagen with limited orientation and crosslinking. To enhance the properties of collagen type I gels we used the following approaches: a) plastically compress the collagen gel to increase the density and b) photochemically crosslink the gel using riboflavin as a photoinitiator and high intensity blue light. Following plastic compression both the collagen density and cell number increase 58-fold[1]. This study aims to assess mechanical properties and the degree of cell viability in different areas of the compressed gel following cross-linking. Patterns of cross-linking were also applied to induce anisotropic features to the gels (*fig.1*).

Figure 1*: The negative image created by the mask indicates where light penetrates the gel and thus where cross-linking is encouraged, creating gels that are cross-linked horizontally, vertically and with a grid pattern.*

METHODS: Collagen gels were manufactured through combining 4ml of type I collagen, 0.5ml of 0.25mM riboflavin, 0.5ml Dulbecco's MEM and 0.5ml HDFs cell suspension. Once set, the gel was compressed for five minutes using a 120g weight, 2 sheets of absorbent paper and nylon mesh[2]. Masks were used to encourage cross linking in specific areas of the gel when placed under high intensity blue light (465nm in wavelength and 4680mW intensity). Mechanical properties were assessed using a tensile tester and the dynamic mechanical analyser (DMA) and cell viability was visualised using calcein AM and ethidium homodimer (EthD-1) under a fluorescence microscope.

RESULTS: Tensile testing showed that the collagen sample with crosslinks running horizontally had a higher Force at Failure (mN) than that of the samples which had been cross linked without a mask, with a vertical mask and with a gridded mask ($p<0.05$). The horizontal mask was applied over a collagen gel containing $2x10^6$ HDFs, and after 3 days of incubation at 37°C, a visible pattern of live and dead cells could be seen under a fluorescence microscope (*fig.2*).

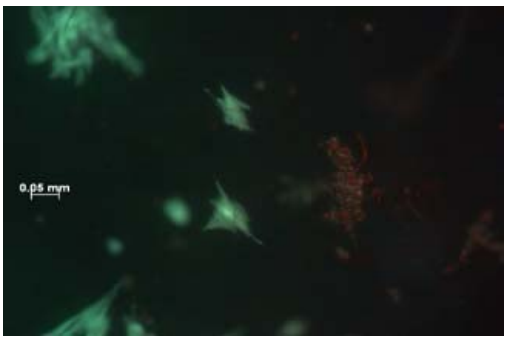

Figure 2*: Image of a cell seeded collagen gel at day 3 which had had a horizontal mask covering before being exposed to blue light. This border of live and dead cells indicates the masked and unmasked regions of the gel, respectively.*

DISCUSSION & CONCLUSIONS: Mechanical integrity and cell viability are both of great importance in the design of tissue engineered scaffolds. The ability to transform native collagen gels to increase their mechanical strength (using photoinitiated riboflavin) whilst maintaining cell viability (with the use of a mask) shows the exciting potential for designing mechanically stable biomimetic structures for implantation at muscle-tendon interfaces.

REFERENCES:

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