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Mechanical Characterisation of UVA-Riboflavin Crosslinked Collagen Hydrogels

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INTRODUCTION: Collagen hydrogels have been investigated for growing numerous tissue equivalents in-vitro, including skin, cornea and vascular tissue. One of the major difficulties to be overcome in using collagen to engineer tissue equivalents is to replicate the native tissues mechanical strength. A UVA-riboflavin technique has been developed to crosslink collagen fibers in the cornea, which does not damage corneal cells [1]. We propose that this same technique can be modified to improve the mechanical strength of collagen hydrogels for use in tissue engineering applications. A non-destructive technique is also required to characterize the changes of mechanical properties in the hydrogels.

METHODS: Rat-tail collagen type 1 (BD Bioscience) was used to make collagen hydrogels of concentration 3.5 mg/ml. The hydrogels were submerged for 15 minutes in a 0.1% riboflavin photosensitizing solution. Hydrogels were then placed under a UVA light source with a maximum irradiance of 3 mW/cm^2 for 30 minutes.

The mechanical properties of the collagen hydrogels were obtained using a novel indentation system [2]. This consists of a sample holder and an image acquisition system (Fig. 1). The holder clamped the hydrogel around its outer edge while it was submerged in buffer solution at 37ºC. A ball of known weight and size was placed on top of the hydrogel causing it to deform. The image acquisition system, consisting of a long focal distance objective microscope linked to a CCD camera, recorded side-view images of the deformation profile from outside the incubator through a glass window. A theoretical model was derived to calculate the mechanical properties of the hydrogels from their deformation profiles.

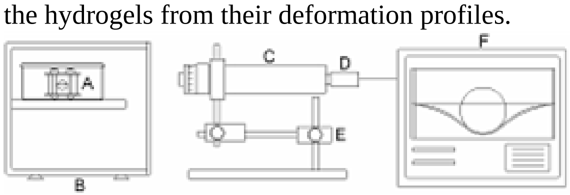

Fig. 1: Schematic of instrument system: (A) Sample holder and ball; (B) incubator at 37°C; (C) long focal microscope; (D) CCD camera; (E) X-Y translation stage; (F) image analysis system.

RESULTS: Our initial results demonstrate that the UVA-riboflavin crosslinking technique improved the mechanical strength of collagen hydrogels. The Young's modulus of the collagen hydrogels was measured before and after UVA-riboflavin crosslinking (Fig 2). A student T-test (95% confidence interval) was used to verify that there was a significant increase in the modulus of the hydrogels after crosslinking.

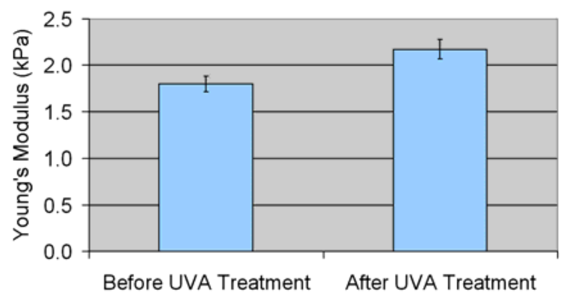

Fig. 2: Young's modulus of collagen hydrogels (n=4) before and after UVA-riboflavin crosslinking treatment.

DISCUSSION & CONCLUSIONS: This experimental technique potentially has many applications in tissue engineering, as collagen hydrogels are much weaker than the native tissues they are used to replicate. In addition to demonstrating a method of improving the mechanical strength of collagen hydrogels, this work also demonstrates the capabilities of the long focal indentation system for measuring the mechanical properties of hydrogels non-destructively, at evaluate temperatures and in biological solution. Work still needs to be carried out to optimise the crosslinking conditions and to determine the effect this technique has on cells when they are seeded to the collagen hydrogels.

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