European Cells and Materials Vol. 18. Suppl. 2, 2009 (page 21) ISSN 1473-2262

Influence of Cell Number and Collagen Concentration on the Mechanical Behaviour of Collagen Hydrogel Constructs

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INTRODUCTION: Collagen hydrogels have been under investigation for use in developing constructs for tissue engineering. Optimisation of initial cells seeding and collagen concentrations are important factors in successfully developing an engineered tissue. We have examined the effect of different collagen and cell concentrations on the elastic modulus of collagen hydrogels seeded with corneal fibroblasts and examined how the modulus changes over time in culture.

METHODS: Rat-tail collagen type 1 (BD Bioscience) and human corneal fibroblasts were used to make hydrogel constructs [1]. Hydrogels with collagen concentrations of 2.5, 3.5 and 4.5 mg/ml and $5x10^5$ cells per gel were manufactured. Hydrogels with cell concentrations of $5x10^5$, $3x10^5$ and $1x10^5$ cells per gel with 3.5mg/ml collagen concentration were also manufactured The hydrogels were cultured at 37°C 5% CO_2 over several weeks.

The elastic modulus of hydrogels was measured using a non-destructive spherical indentation technique [1]. Hydrogels were suspended around their outer edge and a ball was placed on top of them causing them to deform. An image acquisition system (Fig. 1), consisting of a long working distance objective microscope linked to a CCD camera, recorded the deformation profile. A theoretical model was used to calculate the elastic modulus of the hydrogels from their deformation every 3 to 4 days.

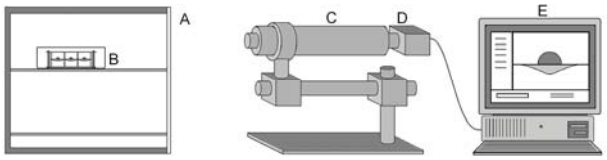

Fig. 1: Schematic of instrument system: (A) incubator at 37°C; (B) sample holder and balls; (C) long working distance microscope; (D) CCD camera; (E) image analysis system.

RESULTS: Hydrogels with lower initial collagen concentrations increased in modulus faster than those with higher collagen concentrations (Fig. 2A). These hydrogels contracted and became thinner more quickly than those with a higher collagen concentration. As hydrogels became thinner they increased the ratio of cells to total volume. Hydrogels with $5x10^5$ cells increased in modulus more quickly than those with lower cell concentrations (Fig. 2B).

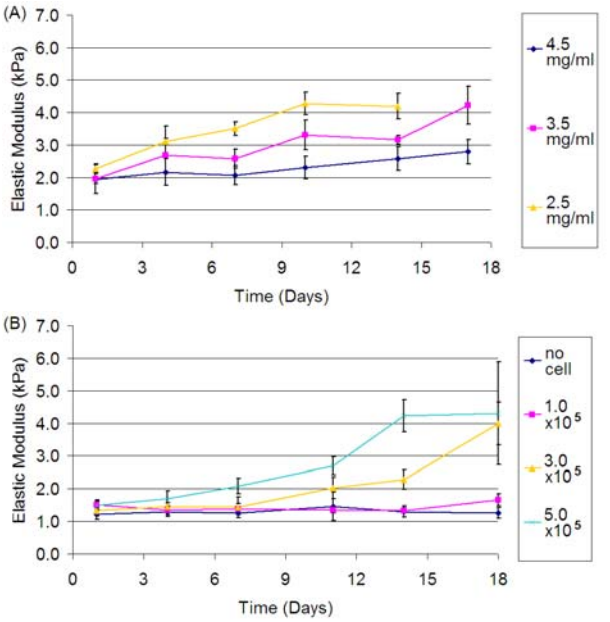

Fig. 2: Elastic modulus of collagen hydrogels with (A) different initial collagen concentrations and (B) different initial cell seeding concentrations.

DISCUSSION & CONCLUSIONS: The influence of collagen and cell concentration on the mechanical behaviour of collagen hydrogels has been demonstrated. Fibroblasts formed an integral part of the structure of these hydrogels and were able to manipulate the hydrogels overall mechanical properties. By using the spherical indentation technique, the effect of collagen and cell concentration can be optimized for tissue engineering applications.

REFERENCES: M. Ahearne, Y. Yang, K.Y. Then et al (2008) *Br J Ophthalmol* **92**:268-71.

ACKNOWLEDGEMENTS: This research was funded by BBSRC (BB/F002866/1)