

## 3D Culture of Human Intervertebral Disc Cells Within PDLLA/Bioglass® Composite Scaffolds: Assessment of Extracellular Matrix Production

W. Helen & J.E. Gough

*Biomaterials and Tissue Engineering Group, School of Materials, University of Manchester, UK*

**INTRODUCTION:** With a view to the repair of damaged human intervertebral disc (IVD), we grew human annulus fibrosus cells (HAF) on 3D composite scaffolds made from the bioresorbable polymer PDLLA with different weight fractions of Bioglass®. These scaffolds were characterised in terms of their structural and biological properties including: glycosaminoglycan (GAG) production, HAF cell attachment, and collagen production.

**METHODS:** Scaffold fabrication: PDLLA composite scaffolds containing 0wt%, 5wt% and 30wt% Bioglass® were prepared by thermally induced phase separation (TIPS) [1].

Scanning electron microscopy: Cell attachment studies were performed using SEM. The AF cells were seeded within PDLLA/Bioglass® composite scaffolds at a density of  $5 \times 10^6$  cells/cm<sup>2</sup> and cultured. At pre-determined time points the cells were fixed in 1.5% glutaraldehyde at 4 °C for 30 min. They were then dehydrated through a series of increasing concentrations of ethanol and dried using hexamethyldisilazane. The samples were sputter coated with gold and viewed using a JEOL 6300 SEM operated at 10 kV.

DMMB assay: GAG production by the HAF cells was studied using the 1,9 dimethylmethylene blue (DMMB) method described by Farndale [2].

Collagen production: The amount of collagen type I and type II were determined using Western blotting [3].

**RESULTS:** SEM micrographs in Figure 1 show (left) HAF cells bridging pore walls in the interior of the scaffolds and (right) HAF cells on the surface of PDLLA/30wt% Bioglass® composite scaffold after 2 wks in culture. GAG assay in Figure 2 shows the increase of GAG production on the 5wt% Bioglass® scaffold from week 1 to week 4 and indicates the ability of HAF cells to secrete a GAG rich extracellular matrix. Western blotting in Figure 3 shows (left) collagen type I bands at approximately 300 KDa and 120 KDa and (right) collagen type II bands at approximately 130 KDa.

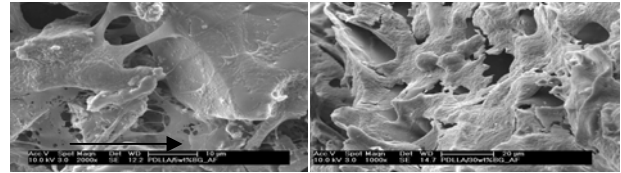


Fig. 1: SEM micrographs show the morphology of HAF cells within PDLLA/5wt% Bioglass® (left) and PDLLA/30wt% Bioglass® (right) composite scaffold..

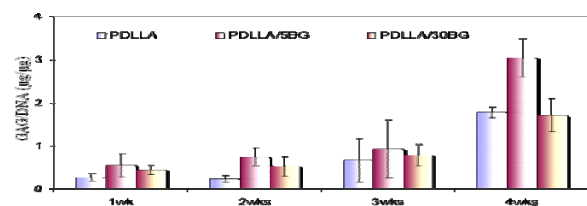


Fig.2: GAG production of HAF cells on PDLLA, PDLLA/5wt% Bioglass® and PDLLA/30wt% Bioglass® over 4weeks.

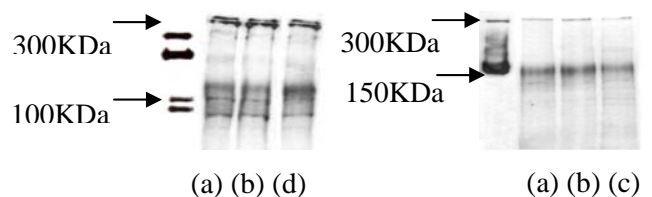


Fig.3: Type I collagen (left) and type II collagen (right) expression by HAF cells cultured on PDLLA foam (a) PDLLA/5wt% Bioglass® (b) and 30 wt% Bioglass® (c) composite scaffolds.

**DISCUSSION & CONCLUSIONS:** By adding Bioglass® particles to PDLLA scaffolds it is possible to tailor the scaffolds bioactive behaviour and promote extracellular matrix production. These scaffolds have potential for intervertebral disc tissue engineering.

**REFERENCES:** <sup>1</sup>V. Maquet (2004) *Biomaterials* **25**:4185-4194. <sup>2</sup>R.W. Farndale, et al (1982) *Connect Tissue Res* **9**:247-248. <sup>3</sup>J. Kumagai, et al (1994) *J Anat* **185**:279-284.

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