Strathprints Institutional Repository

CONFOCAL LASER SCANNING MICROSCOPY (CLSM) STUDY OF HEPATOCYTES CULTURED ON COLLAGEN FILMS AND GELS.

M. Kataropoulou, E. Goldie, G. Connel and M.H. Grant.

Bioengineering Unit, Strathclyde University, Wolfson Centre, Glasgow G4 0NW.

INTRODUCTION: Primary hepatocyte cultures form an integral part of many hybrid artificial liver designs, and the extracellular matrix environment of the cultures is an important factor for optimal expression of hepatocyte-specific phenotype. This study investigates the effect of incorporating 20% chondroitin-6-sulphate ($Ch6SO_4$), a glycosaminoglycan (GAG), into collagen films and gels, and crosslinking the films and the gels with 1,6-diaminohexane (DAH) on the viability of hepatocytes cultured for 48 hours.

METHODS: Hepatocytes were isolated from male Sprague-Dawley rats by collagenase perfusion and seeded at $3x10^6$ viable cells on 60mm Petri dishes in 2ml of Chee's medium containing 5% v/v foetal calf serum. Collagen films were prepared at a concentration of $25\mu g/cm^2$. Collagen gels were prepared as described by Osborne et al. [1]. Viability of the cell cultures was assessed by the fluorescence generated by de-esterification of 25μM carboxyfluorescein diacetate (CFDA) in viable cells measured by CLSM. Cell cultures were first incubated with 0.1% (w/v) ethidium bromide for 6 min in the dark at room temperature to locate dead cell nuclei. They were then washed three times with phosphate-buffered saline (PBS), before incubation with the CFDA solution at 4ºC in the dark for 15 min. After this time, cells were washed a further three times with PBS, and examined by CLSM immediately. CFDA is deacetylated by cytosolic esterases inside the cells to yield carboxyfluorescein, which stains the viable cells green. Ethidium bromide stains the nuclei of the dead cells red.

RESULTS: Cells cultured on plain collagen films were spread out and developed a flat and extended morphology (Fig.1a). This kind of morphology has been shown previously [2]. Hepatocytes on plain collagen gels had an entirely different shape and distribution throughout the surface of the plate; cells maintained a round shape after 48 hours in culture and they were scattered throughout the gel (Fig.1b). Crosslinking the gels with the GAG did not cause any improvement in the substrate (Fig.2b), however, the cells cultured on the films+GAG acquired a different shape compared with those on the plain films (Fig.2a). Cells were stained brighter with CFDA, suggesting an increase in viability, and they had a round shape, similar to that of the cells on the gels. The addition of DAH to the gels caused an increase in the number of cells stained with ethidium bromide, suggesting that there were more dead cells compared with the control (Fig.3b). However, cells on films+DAH remained viable generating green fluorescence from CFDA, and the shape was again more round compared with the plain films (Fig.3a). Finally, cells on gel crosslinked with the combination of the GAG and the DAH were only stained red (Fig.4b), whereas cells on the films+GAG+DAH were still bright green and appeared to be a lot more clustered together compared with the control (Fig.4a).

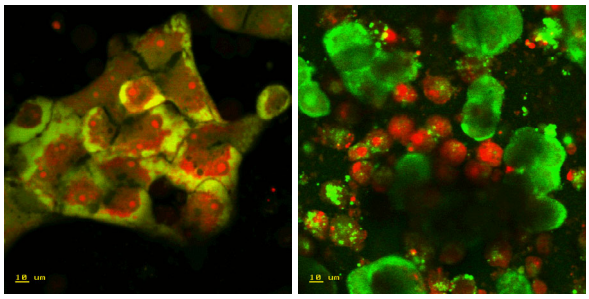

Fig. 1: Primary hepatocytes cultured for 48h on (a) plain collagen film and on (b) plain collagen gel.

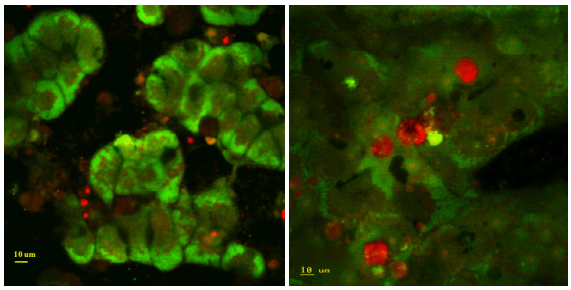

Fig. 2: Primary hepatocytes cultured for 48h on (a) collagen film+GAG and on (b) collagen gel+GAG.

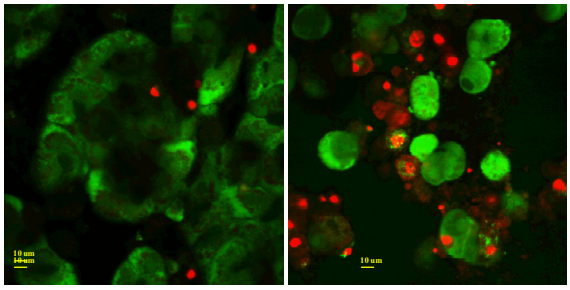

Fig. 3: Primary hepatocytes cultured for 48h on (a) collagen film+DAH and on (b) collagen gel+DAH.

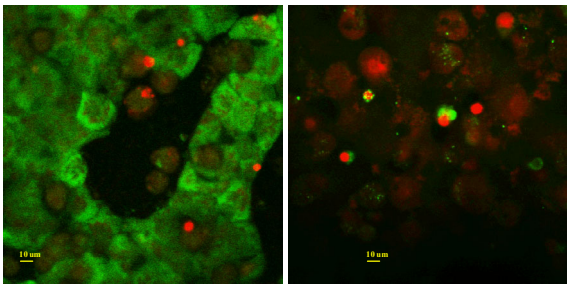

Fig. 4:Primary hepatocytes cultured for 48h on (a) collagen film+GAG+DAH and on (b) collagen gel+GAG+DAH.

DISCUSSION & CONCLUSIONS: When hepatocytes are cultured on a solid substrate, such as collagen-coated polystyrene, they spread out rapidly and develop a characteristic flattened morphology (Fig.1, left). On gel substrates hepatocytes do not flatten, and retain a rounded morphology more similar to the in vivo situation. This has been demonstrated by many previous studies, and the retention of round shape has been correlated with higher expression of in vivo hepatocyte functions [3]. In conclusion, the viability of primary hepatocytes in the crosslinked films appears to be higher compared with the plain film, and the cells retain their round shape. In contrast, crosslinking of collagen gels does not improve cell viability and in fact the presence of DAH in the gels appears to kill hepatocytes.

REFERENCES: [1] C. S. Osborne, W. H. Reid and M. H. Grant (1999) *Biomaterials* 20: 283-290. [2] A. Santhosh and P.R. Sudhakaran (1994) *Mol Cell Biochem* 137: 127-133. [3] W. J. Lindblad, E. G. Schuetz, K. S. Redford and P. S. Guzelian (1991) *Hepatology* 13: 282-288.