Characterization of Hepatocytes Cultured on Micropatterned Agarose Scaffolds Crosslinked with Collagen

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Statement of Purpose: The success of propagating anchorage-dependent tissue cells on a biomaterial is determined by the chemical structure of the material as well as surface topography.[1] Hepatocytes are anchorage-dependent cells which need to first attach to the material surface for survival *in vitro*.[2,3] Once adequately anchored on the material surface, hepatocytes can remain viable and resume their metabolic activity. However, available biomaterials with surface topography suitable for propagation of hepatocytes are limited. In the present study, we tested the hypothesis that micropatterned agarose crosslinked with collagen will provide appropriate surface topography for hepatocyte adhesion. Agarose with similar modulus to that of liver tissue was used. The material design being evaluated has microchannel architecture that mimics the native liver microenvironment. We determined whether hepatocytes cultured on the agarose-collagen scaffolds will respond to pro-inflammatory and oxidative stress (OS) stimuli.

Methods: Agarose (2% w/v in HBSS; Invitrogen) was pipetted over micropatterened silicon wafer molds [Fig 1A]. Sulfo-SANPAH (Thermo Fisher; 1 mM in HEPES) was covalently attached to agarose by UV exposure for 10 mins. Samples were washed 2X with 50 mM HEPES and incubated overnight at 4ºC with 40 µg/ml type I rat-tail collagen. Samples were washed 3X with HBSS and sterilized by UV exposure. Primary canine hepatocytes (Celsis In Vitro Technologies) were seeded ($5x10^4$ cells/scaffold) and allowed to attach for 4 hrs before addition of media. Cells were evaluated by light microscopy, SEM, and TEM. Phenotype was confirmed by immunostaining while secreted albumin was measured by ELISA. Cells were cultured for 24 hrs on the substrates. Media was exchanged and cells were incubated for another 24 hrs with: (i) control media alone, (ii) the pro-inflammatory cytokine interleukin-1 beta (IL-1β; 10 ng/ml) or the OS-inducer hydrogen peroxide (H_2O_2; 400 µM) to induce the inflammatory mediator prostaglandin E2 (PGE_2) or (iii) with the potent antioxidant N-acetylcysteine (NAC; 10 mM) to induce the OS-marker reduced glutathione (GSH).

Results: Hepatocytes adhered to micropatterned surfaces by 4 hrs [Figs 1B-C]. SEM show hepatocytes aligned within the troughs and not on the ridges [Fig 2A-C]. Hepatocytes also aligned between the agarose microchannels as shown by TEM [Figs 2D-E]. Distinct hepatocyte organelles, large nuclei [Fig 2D] and cellular extensions typical of polarized hepatocytes [Fig 2F] were visualized by TEM. Hepatocytes continued to produce markers of liver phenotype: albumin and cytokeratin 8 (CK8) [Figs 3A and 3B, respectively]. Production of albumin decreased in a time-dependent manner over 7 days in culture [Fig 3C]. Hepatocytes responded to stimulation by IL-1β and H_2O_2 [Fig 4A] as indicated by significantly increased PGE_2 production ($p<0.05$). NAC also significantly induced ($p<0.05$) antioxidant GSH levels 20-fold [Fig 4B]. The response of hepatocytes to anti-inflammatory and antioxidant stimuli in the present study paralleled continued albumin production within 3 days of culture [Fig3C].

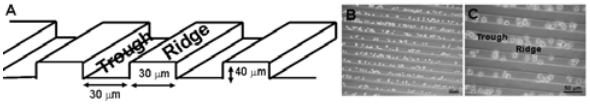


Figure 1. (A) Schematic of microchannel dimensions (not drawn to scale). (B) Low and (C) high magnification photomicrographs of hepatocytes cultured for 24 hrs.

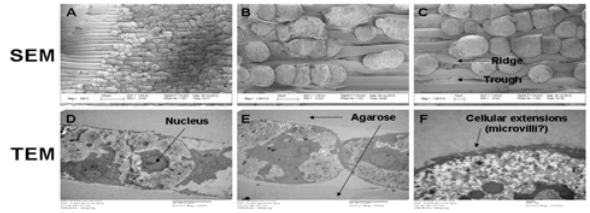


Figure 2. SEM (top panel) and TEM (bottom panel) images of canine hepatocytes cultured for 24 hrs on micropatterned agarose at different magnifications.

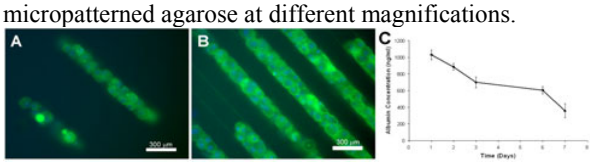
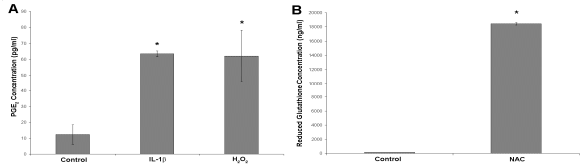


Figure 3. Immunostaining of liver phenotype markers (A) albumin and (B) CK8 in green following 3 days in culture. DAPI nuclear stain shown in blue. (C) Albumin production decreased in a time-dependent manner.



* = Significant difference where $p<0.05$ relative to Control.

Figure 4. Hepatocytes cultured for 24 hrs on the micropatterned agarose and then (A) activated with IL-1β and H_2O_2 for another 24 hrs produce increased amounts of PGE_2 while (B) cells treated with NAC for 24 hrs were shown to produce significant increases in GSH relative to non-treated control.

Discussion/Conclusions: The key finding of the present study is that collagen crosslinked micropatterned agarose facilitated attachment of viable and metabolically active hepatocytes. The model is useful in evaluation of hepatocyte response to stimuli with pharmacologic and biological agents.

References:

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