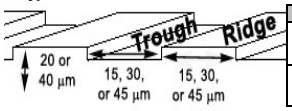
# Collagen Covalently Crosslinked to Micropatterned Agarose Scaffolds for Hepatocyte Culture

Angela Y. Au[1,2], Julie M. Hasenwinkel[2], Carmelita G. Frondoza[1,3,4]
[1]Nutramax Laboratories, Inc., Edgewood, MD, [2]Syracuse University, Syracuse, NY,
[3]Mississippi State University, Mississippi State, MS, [4]Johns Hopkins University, Baltimore, MD

**Statement of Purpose:** Material surface topography has been acknowledged to be critical for cellular adhesion and proliferation *in vitro*[1]. In particular, tissue cells such as hepatocytes need substrates that will promote attachment since they have difficulty adhering, and thus do not survive during propagation[2,3]. We previously evaluated type I collagen as a substrate material to facilitate hepatocyte attachment and growth in culture[4]. In the present study, we tested the hypothesis that collagen crosslinked to micropatterned agarose will promote hepatocyte adhesion and proliferation. To test this hypothesis, we prepared different sized microchannels in agarose and covalently attached collagen to the agarose surface to determine whether surface topography affects cell adhesion. The modulus of agarose is similar to liver tissue and the microchannels mimic the native hepatocyte sinusoid microenvironment where cells are aligned.

**Methods:** UltraPure™ agarose scaffolds (2%w/v in HBSS) 4-6 mm thick were pipetted over micropatterened silicon wafer molds with varying channel widths and depths [Fig 1]. The photactivated crosslinker Sulfo-SANPAH (SS; 1mM in HEPES) was covalently attached to agarose by UV exposure for 10 mins. SS-modified agarose samples were washed with 50mM HEPES 2X before overnight incubation at 4ºC with 40µg/ml type I rat-tail collagen. Collagen crosslinked to micropatterned agarose samples were washed 3X with HBSS and sterilized by UV exposure for 15 mins. Primary canine hepatocytes (Celsis IVT) or human HepG2 hepatocellular carcinoma cells (ATCC) were seeded ($1x10^5$ cells/scaffold) and allowed to attach for 4 hrs prior to the addition of media, which was exchanged daily.

**Figure 1.** Schematic of microchannel patterns.




| Pattern | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Width (µm) | 15 | 15 | 30 | 30 | 45 | 45 |
| Depth (µm) | 20 | 40 | 20 | 40 | 20 | 40 |

**Results:** Collagen coatings crosslinked to micropatterned agarose surfaces promoted hepatocyte adhesion by 4 hrs. Hepatocytes aligned within the microchannel troughs but did not adhere to the ridges for Patterns 2-6 [Fig 2]. However, hepatocytes aligned along both the troughs and ridges of Pattern 1, possibly due to width and depth constraints. HepG2 cells also aligned within the troughs [Patterns 1-4 and 6, Fig 3]. However, HepG2 cells attached to the troughs as well as ridges (Pattern 5). HepG2 tumor cells appeared larger than hepatocytes [Fig 3]. Both cell types appeared refractile, indicating viability. While hepatocytes showed rounded morphology, HepG2 cells were elongated and exhibited cytoplasmic extensions. Wider channels (30 and 45µm) promoted cell alignment in pairs instead of singles within the troughs, relative to thinner channels (15µm). Unlike hepatocytes, HepG2 cells formed aggregates which grew out of the troughs by day 7. Hepatocytes remained in the troughs.

**Figure 2.** Photomicrographs of hepatocytes cultured for 24 hrs on micropatterned agarose. Scalebar = 100µm (10X) and 50µm (40X).

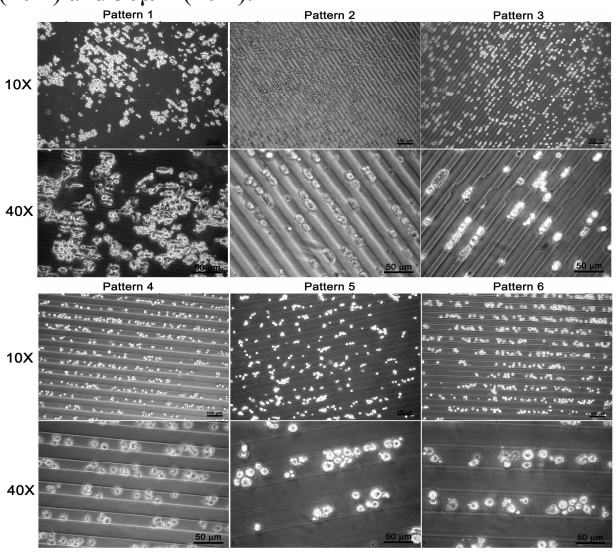
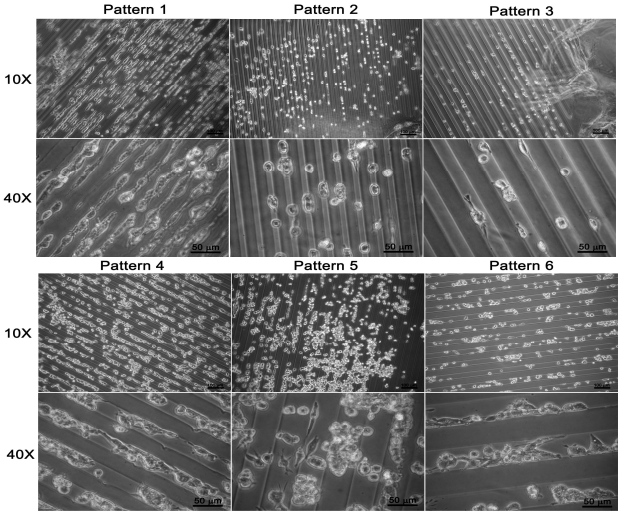



**Figure 3.** Photomicrographs of HepG2 cells cultured for 24 hrs on micropatterned agarose.




**Discussion/Conclusions:** The 6 designs of collagen coatings crosslinked to micropatterned agarose support adhesion of both hepatocytes and hepatocyte tumor cells. The observation that hepatocytes orient and align within the microchannel troughs and not on the ridges suggests that surface topography modulates adhesion, migration, and polarity. This modulation may involve alteration in cell signaling pathways. Our findings indicate that the surface modified collagen crosslinked to agarose may be useful to study hepatocyte function *in vitro*.

**References:**

1. De Silva MN, et al. Biomed Microdevices. 2004;6:219-22.
2. Davis MW and Vacanti JP. Biomaterials. 1996;17:365-72.
3. Lu HF, et al. Biomaterials. 2003;24:4893-903.
4. Au AY, et al. World Biomat Congress. 2008;Abstract 441.