Micropatterned Agarose and Polyacrylamide Scaffolds for Canine Hepatocyte Culture

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

Statement of Purpose: Hepatocytes are liver cells capable of regeneration *in vivo*.[1,2] Although hepatocytes are capable of self renewal, their ability to proliferate is impaired by liver damage due to trauma or disease. *In vitro* culture models have been used to define the mechanisms involved in hepatocyte regeneration. However, propagation of hepatocytes has been difficult as they do not readily adhere or multiply on biomaterial substrates. Hepatocytes also lose their native liver phenotype upon extended culture. The rat-tail type I collagen commonly used as a substrate material for hepatocyte culture has had limited success.[3] Earlier studies indicated that three-dimensional (3D) scaffolds with certain micropatterns may facilitate cell growth and function. We tested the hypothesis that primary canine hepatocytes seeded on micropatterned materials will exhibit improved adhesion, proliferation, and maintenance of the hepatocyte phenotype. To test this hypothesis, we simulated the native hepatocyte microenvironment by culturing hepatocytes on 3D scaffolds patterned with microchannels using agarose and polyacrylamide (pAAm). These biocompatible biomaterials have low modulus similar to liver tissue.[4,5]

Methods: Agarose scaffolds (1 or 2%w/v) were prepared using UltraPure™ Agarose (Invitrogen) in HBSS (Gibco). Polyacrylamide (pAAm) scaffolds were prepared using a procedure modified from Yeung et al.[6] Polymerization of equal volumes of acrylamide (14%w/v; Sigma), bis-acrylamide (0.7%w/v; Sigma), and HBSS was initiated by ammonium persulfate (10%w/v; Sigma) and TEMED (Sigma). Solutions were poured over micropatterened silicon wafers with 15 μm channel width and 5 μm depth [Fig. 1]. Samples between 6 and 16 mm were prepared for each biomaterial. Polymerized samples were coated with 0.1% type I collagen (Sigma) overnight at 4ºC. Samples were sterilized in 100% ethanol and washed 3X with sterile HBSS. The hydrogel-like properties of pAAm led to increased sample thickness following swelling in aqueous solution compared to dry samples. Primary canine hepatocytes (Celsis IVT; Baltimore, MD) were seeded at a density of $5x10^4$ cells per scaffold and allowed to attach for 4 hrs prior to addition of media. Aliquots of supernatant at different timepoints were frozen for albumin analysis using canine-specific antibodies (Covance). MTT cytotoxicity assays (Promega) confirmed cell viability. SigmaStat software was used to perform statistical analysis using one-way ANOVA and Student-Neuman-Keuls post-hoc analysis. Significance was set at $p<0.05$.

Results: Differences in agarose concentration (1% and 2% w/v) did not affect cell adhesion. However, substrate thickness influenced hepatocyte adhesion to agarose and pAAm. Agarose samples between 8-9 mm best supported hepatocyte adhesion and cell proliferation. In comparison, the optimal swollen pAAm sample thicknesses that promoted hepatocyte adhesion were 8 and 10 mm. Both substrates promoted and maintained hepatocyte adhesion for up to 3 months in culture. Non-patterned agarose and pAAm samples supported cell aggregation and clustering. Hepatocytes cultured on the agarose grew into the channels while those on pAAM grew in the channels and on the ridges [Fig. 2]. Cell viability remained 100% over the course of culture. Initial evaluation following 1 week in culture showed continued production of albumin. Hepatocytes cultured on micropatterned substrates, regardless of material, showed significantly enhanced albumin levels relative to non-patterned controls.

Figure 1. Photomicrographs of micropatterned samples at (A) low and (B) high magnifications. (C) Schematic of microchannel pattern on silicon disks. Scalebar = 100 μm.

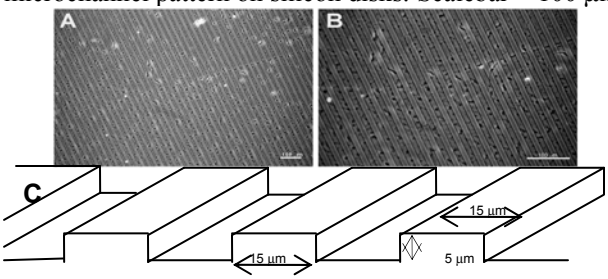
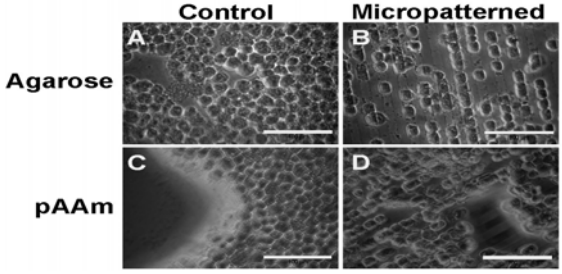


Figure 2. Photomicrographs of canine hepatocytes cultured on control or micropatterned 2%w/v agarose (A,B) or pAAm (C,D). Cells aggregated and clustered on non-patterned controls (A,C) but aligned in the agarose channels (B) and in both the pAAm channels and troughs (D) by 2 hours following cell seeding. Scalebar = 100 μm.



Discussion/Conclusions: Propagation of canine hepatocytes on patterned substrate materials facilitated adhesion, proliferation, and phenotype function. The observation that hepatocytes aligned in the microchannels on both agarose and pAAm but also on the ridges on the pAAm may be attributed to differences in mechanical properties between the materials (~10X higher modulus in agarose). Our study demonstrates that hepatocyte behavior is modulated by the material substrate, surface topology, and mechanical properties *in vitro*.

References:

1. Tanimizu N, et al. Int Rev Cytol. 2007;259:1-48.
2. Fausto N, et al. Hepatology. 2006;43:S45-53.
3. Hansen LK and Albrecht JH. J Cell Sci. 1999;112(17):2971-81.
4. Stokols S, et al. Biomaterials. 2006;27(3):443-51.
5. Hynd MR, et al. J Biomat Sci Polym Ed. 2007;18(10):1223-44.
6. Yeung T, et al. Cell Motil Cytoskeleton. 2005;60(1):24-34.