Hepatocyte Spheroid Cultures in Galactosylated Cellulosic Sponge for Drug DMPK and Efficacy Testing

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INTRODUCTION

Spheroids are multicellular aggregates, that exhibit a high degree of cell-cell contact. This allows for preservation of *in vivo* phenotype that are otherwise absent in conventional 2D cultures. 3D cultures have been widely used in cancer biology and anti-cancer drug screening but find parallels in hepatocyte cultures for improving hepatocellular function and for hepatotoxicity / DMPK and modeling infectious diseases. Growing spheroids using methods such as the conventional hanging drop and centrifugation techniques are limited by their inability to constrain the spheroids physically for long term culture thereby leading to inability to determine spheroid size and difficulty in ease of operation for compound screening applications *(Nugraha et al, 2011)*. To address these difficulties, we have developed a thin soft macroporous galactosylated cellulosic sponge. The macroporosity helps control spheroid size and the galactose ligands provide chemical cues for improved hepatocyte functions.

We evaluated the utility of the system to culture primary hepatocytes and human hepatoma spheroids. The cellulosic sponge allows for culture of uniformly sized spheroids over prolonged culture and maintained and elevated hepatocyte function over prolonged culture. The cellulosic sponge also allows for increased sensitivity to drug responses for acute toxicity, long term toxicity and CYP induction over monolayer cultures. We have also utilized the sponge for studying pathogen infections by Hepatitis C virus and are currently exploring the utility for other infectious diseases such as malaria and HBV. The spheroids allowed for increased infection rates over monolayer cultures and increased production of viral titers over monolayer cultures.

The sponge supports high throughput applications and ease of use similar to 2D cultures.

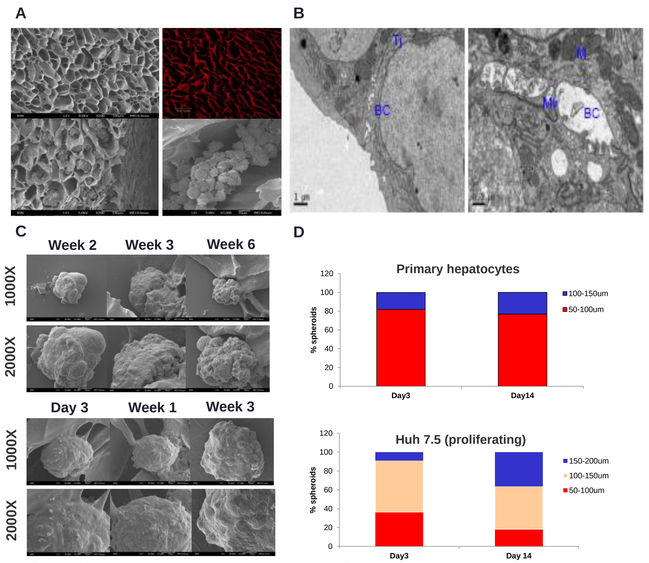


Figure 1. *Characterization of sponge and spheroids in the cellulosic sponge A) Pore size and macroporosity of the cellulosic sponge under dry and hydrated conditions B) Transmission electron microscopy images 48 hours post seeding. Tj: Tight junctions, Mv: Microvilli, BC: Bile Canaliculi, M: Mitochondria. C) Scanning electron microscopy images of human hepatocytes (Top) and Huh 7.5 cells over prolonged culture (Bottom). D) Size distribution of spheroids in human hepatocytes and Huh 7.5 cells over 2 weeks in culture.*

Collagen monolayer and sandwich cultured primary hepatocytes have been the mainstay of hepatotoxicity and pharmacokinetic testing in the pharmaceutical industry *(Hewitt et al, 2007)*. However owing to the loss of hepatocyte function and inability to culture the hepatocytes for prolonged culture periods in these models, newer cell models are being developed and are under evaluation for various end points that these current models cannot fulfill. We have developed a scalable cellular model that works with both primary hepatocytes and cell lines; forms uniformly sized organoids and exhibits polarity similar to *in vivo* hepatocytes in the liver. The organoids demonstrate elevated liver specific functions, are inducible for CYP 3A and demonstrate increased induction with increased time of dosage with prototypical inducers of CYP 3A. They also demonstrate increased sensitivity compared to monolayer controls and demonstrate dose response shifts in IC_{50} with prolonged treatment of 15 days. The organoids also support infection with Hepatitis C virus at higher rates than observed in traditional monolayer cultures. Similar use of these technologies and principles can be applied for the study of other infectious diseases.

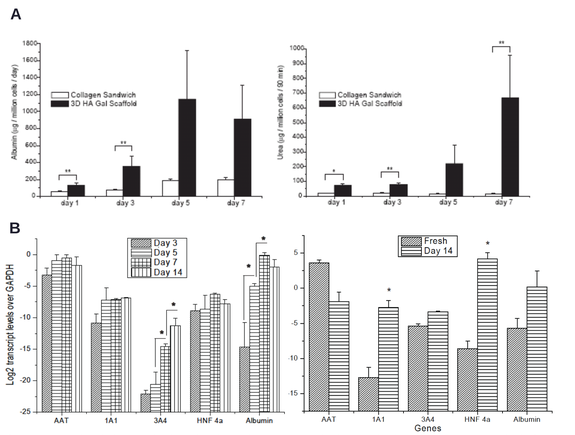
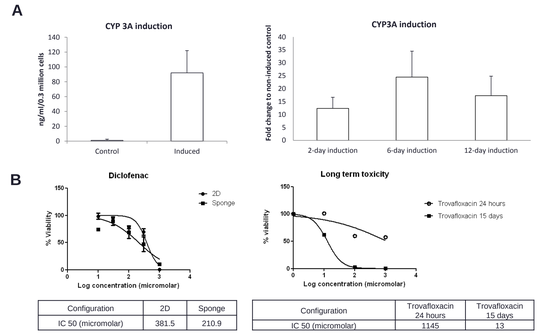


Figure 2. *Functional characterization of primary hepatocytes. A) Characterization of hepatocyte synthetic function. Spheroid culture maintains elevated levels of liver synthetic function. B) Characterization of gene expression of primary hepatocytes over 14 days in culture*



Configuration	2D	Sponge
IC 50 (micromolar)	381.5	210.9

Configuration	Trovafloxacin 24 hours	Trovafloxacin 15 days
IC 50 (micromolar)	1145	13

Figure 3. *Drug DMPK and toxicity characterization. A) CYP induction in rat hepatocytes after 48 hours of dosing (left) and upon repeated drug addition over 12 days in culture. B) IC_{50} evaluation of hepatotoxic compounds at 24 hours incubation and 15 days incubation. Spheroid cultures demonstrated a slight increase in sensitivity to diclofenac over monolayer cultures. Trovafloxacin shows increased toxicity at 15 days.*

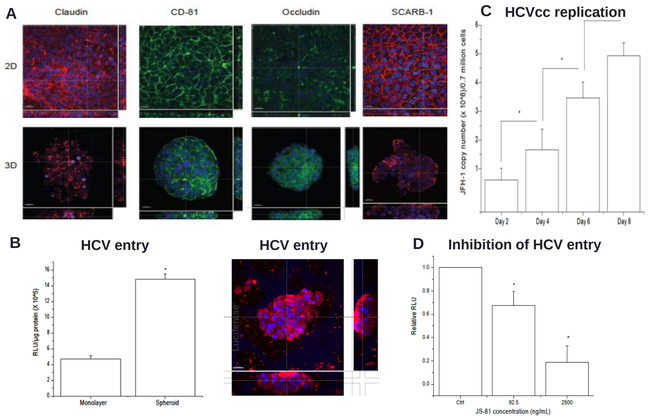


Figure 4. Characterization of cellulosic sponge for HCV infection. A) Expression and localization of HCV entry markers. Spheroid cultures demonstrate similar localization of receptors to in vivo liver B) HCV pseudoparticle entry into spheroid cultured hepatocytes. Spheroid cultures demonstrate higher rates of infectivity compared to monolayer cultures C) HCV replication in spheroids D) Inhibition of HCV entry upon addition of anti-CD81 antibody.

CONCLUSION

We have synthesized and fabricated a galactosylated macroporous cellulosic hydrogel sponge as a platform to culture hepatocytes as 3D spheroids for drug safety / efficacy testing applications. The soft macroporous cellulosic sponge with conjugated galactose facilitates the formation of hepatocyte spheroids by presenting both the mechanical cues (via matrix rigidity) and chemical cues for the hepatocytes to reorganize into 3D spheroids. The constrained hepatocyte spheroids maintain cell viability, cell polarity markers, and 3D cell morphology. These translate into maintained hepatocyte-specific functions and expression of drug metabolic enzymes for drug DMPK and efficacy testing.