Effects of a three-dimensional polystyrene environment on HepG2 liver cells

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Statement of Purpose: Tissue engineering with three dimensional (3D) scaffolds represents a developing field in which a potential exists to create hepatic tissue to replace a weakening in vivo liver. The micro-environment HepG2 liver cells are cultured into is believed to dramatically affect growth and cell functionality. The importance of three dimensional (3D) environments lies in the fact that it has been utilized extensively to guide tissue/organ regeneration. To test 3D cultures, HepG2 liver cells grown in 3D environments, specifically porous polystyrene scaffolds, will be compared to those grown in two dimensional (2D) polystyrene surfaces. Such an experimental study will permit examination of the effects of 3D versus 2D environments, with polystyrene being employed as the material for both. Comparative data will be obtained by utilizing a quantitative fluorescent assay. The fluorescent assay results in the quantification of HepG2 liver cell proliferation.

The importance of 3D in vitro models stems from the understanding that engineering the micro-environment in which cells proliferate greatly influences cell behavior, along with the purpose of more closely mimicking in vivo behavior. Therefore, this study tests the hypothesis that 3D environments significantly enhance HepG2 liver cell growth as compared to traditional 2D cultures.

Methods: Human HepG2 hepatic carcinoma cells were purchased from American Type Culture Collection (ATCC, Manassas, VC, USA). HepG2 cells were cultured at 37°C in 5% CO_2 in growth medium [Eagle's Minimum Essential Medium (EMEM) supplemented with 10% (v/v) fetal bovine serum (FBS)]. Cells were passaged every 4-7 days. Polystyrene (PS) scaffolds were provided by 3D Biotek, Inc. Two models of PS scaffolds were utilized, PS 1520 and PS 3040, with fiber diameters (FD) of 150µm and 300µm respectively, and spacing (SP) of 200µm and 400µm respectively. The PS 1520 and PS 3040 exhibited surface areas of 16cm^2 and 30cm^2 respectively. The cell seeding density utilized was 10^5 cells per square centimeter (cells/cm^2). The rate of proliferation was determined using a commercially available Alamar Blue assay (AbD Serotec, NC, USA). The assay was performed using fresh media with alamar blue dye in a 1:10 ratio. Following the 4h incubation at 37°C, fluorescence was detected at excitation/emission wavelengths of 535/590nm respectively.

Results: The cell proliferation resulted in 33869 ± 339.37%, 29090.34 ± 655.76% and 32650.98 ± 245.87% fluorescence intensity units for the 2D wells, the PS 3040 scaffolds, and the PS 1520 scaffolds, respectively, in Day 10. Interestingly, on Day 1, PS 1520 and PS 3040 scaffold samples exhibited higher values of fluorescence intensity for the Alamar Blue dye compared to the 2D wells. This result is possibly due to efficient initial cell seeding, as well as minimal losses due to scaffold transfer between sampling points. In terms of the 10-day study, it is observed that the 2D well is better for cell proliferation in comparison to the 3D scaffolds, PS 3040 and PS 1520, considering the difference in fluorescence intensities. In terms of trends, the PS 3040 and the PS 1520 groups depict steady decreases over time, while the 2D group exhibits a steady increase over time. The difference between 2D and 3D group trends is most likely due to the sensitivity of 3D scaffolds to movement between measurement points. Below (Figure 1) is a visual representation of the Alamar Blue intensities:

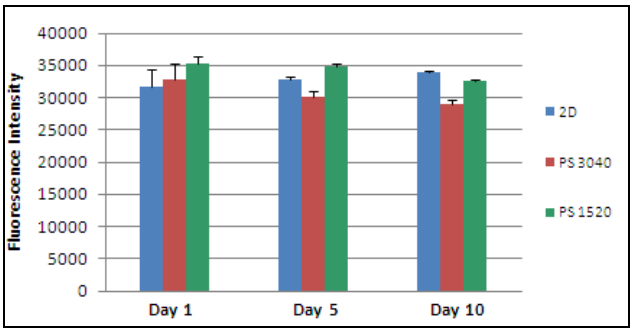

Figure 1: A bar-graph representing the Alamar Blue fluorescence intensities of HepG2 cells at three time points (Day 1, 5, 10) for a 10-day study. The blue bar represents the conventional 2D well, the green bar represents the polystyrene scaffold-PS 1520, and the red bar represents the polystyrene scaffold-PS 3040. Error bars are included to depict the standard deviation observed within each experimental group.

Conclusions: These results demonstrate that 3D PS scaffolds could potentially provide a better environment for *in vitro* proliferation of HepG2 as compared to conventional 2D PS tissue culture plates. Further experimentation must consider PS scaffolds with smaller values for fiber diameter and spacing since the PS 1520 scaffold more closely mimicked the cell proliferation exhibited by cells cultured on 2D PS wells. One factor that may contribute to increased cell proliferation on 3D microenvironments is improved cell-seeding efficiency. A more concrete comparison between 2D and 3D environments for HepG2 cells may be established by morphological analyses via optical light microscopes as well as drug metabolism studies to test cell functionality. The usefulness of drug metabolism analyses for future studies, specifically using urea, draws from the fact that it is one of liver's most vital functions, and is a subject of great interest in healthcare.

References:

Bokhari M. J Anat. 2007 (211:567-576)
Elkayam T. Tissue Eng. 2006 (12:1357-1368)
Dvir-Ginzberg M. Tissue Eng. 2003 (9:757-766)
Richert L. Toxicol In Vitro. 2002 (16:89-99)
Senoo H. Cell Biol Int. 1996 (20:501-512)
Buters J. Biochem Pharmacol. 1993 (46:1577-1584)