Viability and Function of Induced Pluripotent Stem (IPS) Cell-Derived Hepatocytes on Bioprinted Gelatin Scaffolds

Shah RN[1-3], Rutz AL[2,4], Jakus AE[1,2], Chien KB[1,2]
[1]Department of Materials Science and Engineering, Northwestern University, Evanston, IL USA
[2]Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, IL USA
[3]Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL USA
[4]Department of Biomedical Engineering, Northwestern University, Evanston, IL USA

Statement of Purpose: The lack of liver donors for patients with end stage liver disease is a major healthcare obstacle. Developing organ replacements using tissue engineering strategies as an alternative treatment is a promising possibility to alleviate this significant need. However, cell source is a challenge for such engineered tissues since primary liver cells (hepatocytes) are not readily available. Furthermore, hepatocyte viability and phenotype are difficult to maintain *in vitro* for prolonged times. This research evaluates the use of hepatocytes derived from induced pluripotent stem (IPS) cells cultured within 3D bioprinted scaffolds for liver tissue engineering. IPS cells are readily available adult somatic cells (i.e. skin or blood) that have been reprogrammed to become pluripotent, after which the cells can be differentiated into many cell types including hepatocytes. Since IPS technology is still in its infancy, differentiation and maintenance protocols are still being developed. Specifically, the optimal microenvironment necessary to support and enhance the mature hepatic phenotype of these cells still remains unknown. In this study, we evaluate the growth and function of IPS cell-derived hepatocytes within 3D bioprinted gelatin scaffolds and compare them to 2D culture to determine if a 3D culture environment can enhance viability and function.

Methods: 3D gelatin scaffolds were printed using a 3D-Bioplotter (EnvisionTEC GmbH, Germany) with a strut diameter of approximately 300 µm and with every other layer rotated 90°. While the first 4 layers had open pores, the bottom two layers were printed with no open pores to increase seeding efficiency. Scaffolds were cross-linked with N-Hydroxysuccinimide and N-(3-Dimethylamino-propyl)-N′-ethylcarbodiimide and punched into 6 mm diameter discs with a thickness of 2 mm. IPS cell-derived hepatocytes and media were obtained from Cellular Dynamics International (Madison, WI). Cells were seeded at a density of 500,000 cells per scaffold (**Fig. 1A**), as well as onto collagen coated 24-well plates for 2D comparison. Samples were collected for DNA quantification, live/dead confocal fluorescence imaging, SEM imaging, and albumin gene expression analysis after 1, 7, and 14 days of *in vitro* culture.

Results: Live/dead and SEM images (**Fig. 1B-D**) confirmed that cells were viable and present throughout the thickness of the gelatin scaffolds. Higher magnification images, especially at 2 weeks, indicated that the cells were depositing fibrous extracellular material (ECM) including collagen fibrils within the scaffolds (**Fig. 1D**). Cell proliferation was significantly higher within the 3D gelatin scaffolds compared to 2D culture where cells seemed to die over time (**Fig. 2A**). Gene expression analysis (**Fig. 2B**) showed an increase in albumin expression from day 1 to day 14 for both the 2D and 3D culture groups, with a significantly higher fold increase (~200-fold increase) of albumin expression from cells cultured within gelatin scaffolds compared to the 2D group (~20-fold increase).

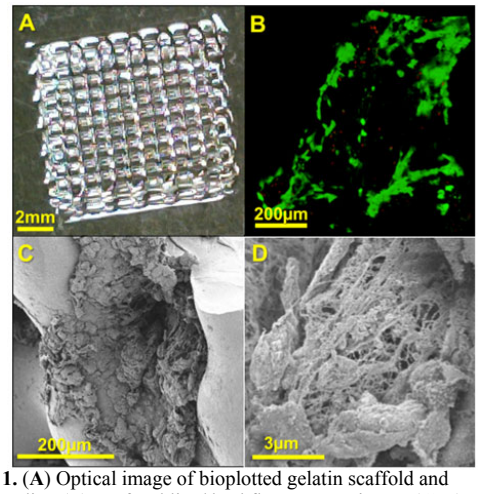

Figure 1. (**A**) Optical image of bioplotted gelatin scaffold and corresponding (**B**) confocal live/dead fluorescence image (**C-D**) scanning electron microscopy images of cell-seeded scaffolds after 1 week. **D** shows higher magnification of deposited fibrous ECM produced by seeded cells.

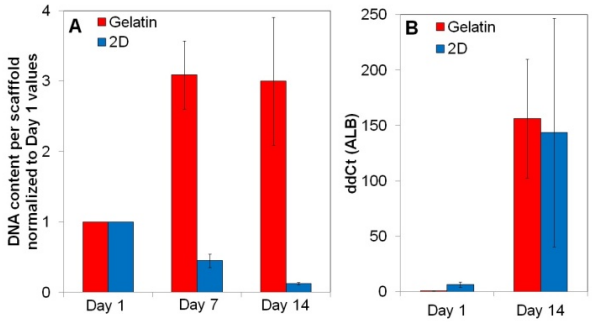

Figure 2. (**A**) DNA quantification per scaffold relative to day 1 values over 2 weeks compared to 2D culture. N=5. (**B**) Relative albumin gene expression of IPS cell-derived hepatocytes seeded on gelatin scaffolds over 2 weeks compared to 2D culture. N=3-4. Error bars = S.E.M.

Conclusions: These studies demonstrate that 3D bioprinted gelatin scaffolds can support IPS-cell derived hepatocyte viability and promote significantly higher proliferation and enhanced albumin expression *in vitro* up to 2 weeks compared to 2D culture conditions. Future work will evaluate the behavior of these cells at longer time points, as well as alter scaffold properties to further enhance hepatic function over time.

Acknowledgements: This work was supported by an NDSEG fellowship (Adam Jakus) and an NSF graduate research fellowship (Karen Chien).