3D Transdifferentiation of Human Mesenchymal Stem Cells into Hepatocyte-Like Cells Using Bioprinted Scaffolds

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Statement of Purpose: Human mesenchymal stem cells (hMSCs) are an ideal cell source for liver tissue engineering since they can be easily isolated from a patient's own bone marrow and have the capacity to transdifferentiate into hepatocyte-like cells in the presence of specific growth factors [1,2]. Previous studies have demonstrated that conditioned media produced by HepG2, an immortal cell line derived from hepatocellular carcinoma, can promote hepatocyte transdifferentiation of hMSCs in 2D monolayer [3]. Transdifferentiating hMSCs directly on 3D scaffolds, however, has not been attempted and may lead to a more efficient transdifferentiation strategy. This can also be advantageous for clinical applications since the step of having to differentiate the cells separately before seeding can be bypassed, decreasing the overall culture time. The main objective of this study, therefore, was to compare the transdifferentiation capacity of hMSCs seeded on collagen-infused 3D printed PLLA scaffolds to that of cells transdifferentiated in 2D monolayer.

Methods: ***3D Scaffold Fabrication:*** Poly-L-lactide (PLLA) was printed at 220 °C into 5-mm diameter, 2-mm thick scaffolds using a 3D-Bioplotter (EnvisionTEC GmbH, Germany). The material was printed through a 300 µm needle, with inter-strut spacing of 400 µm. Every other layer was rotated 90°, with a 1-mm X-Y shift every 4 layers. 12 µL of 0.05 wt% bovine type I collagen in 0.05 M acetic acid was infused into the scaffold pores and immediately lyophilized. ***Conditioned Media Preparation:*** HepG2 (ATCC) were plated ($1.75x10^4$ cells/cm^2) and cultured in MEM with 10% FBS. The conditioned media was collected and set aside every 2 days. The media for the transdifferentiation cultures was prepared by mixing 1:1 volume ratio of fresh MEM with 10% FBS/2 mM L-glutamine/1% pent/strep. ***Cell Culture:*** Commercially available hMSCs (Lonza) were expanded to passage 5 and seeded onto the PLLA scaffolds ($5x10^5$ cells/scaffold) or onto 2D treated tissue culture plates (TCP, $5x10^5$ cells/well in a 6-well plate). Cultures were maintained in conditioned media for up to 28 days. Proliferation was measured using a PicoGreen DNA quantification kit (n=5), and cell viability/morphology was assessed using laser scanning confocal microscopy with Live/Dead staining and scanning electron microscopy (SEM). Quantitative real-time PCR was performed to evaluate expression of albumin and other hepatocyte-specific detoxification genes. Data were analyzed using unpaired Student's t-test and reported as mean ± standard error.

Results: Representative confocal Live/Dead images revealed attached cells spread on the PLLA struts and into the collagen network at all 3 timepoints (**Fig. 1A-C**). An SEM cross-section of the scaffold showed cells penetrating the thickness of the entire scaffold (**Fig. 1D**). DNA amount increased 3-fold between 1 to 28 days in culture (**Fig. 1E**). Albumin gene expression was upregulated on PLLA scaffolds over time, and expression of detoxification genes was significantly higher compared to 2D transdifferentiated cultures after 28 days (**Fig. 2**).

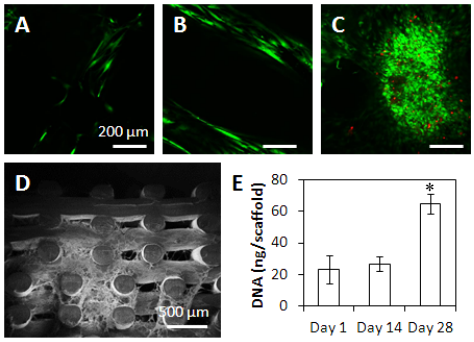

Figure 1. (A-C) Representative Confocal Live/Dead images of cells seeded on PLLA scaffolds at various timepoints. Scale bar = 200 µm. Green: Live cells. Red: Dead cells. (A) Day 1. (B) Day 14. (C) Day 28. (D) SEM image of a representative cross-section of PLLA scaffold at day 28 (orientation: top to bottom). (E) Picogreen results of cell-seeded PLLA scaffolds over 28 days. *: P≤0.005.

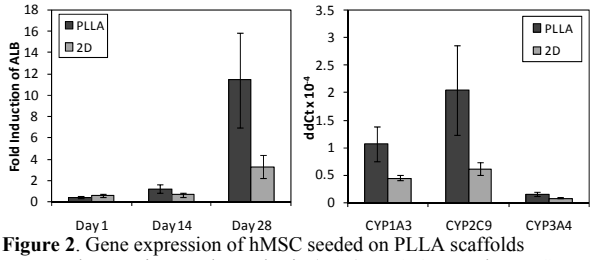

Figure 2. Gene expression of hMSC seeded on PLLA scaffolds compared to 2D tissue culture plastic (TCP). N=3-4. Error bars = S.E.M. (A) Fold induction of albumin (ALB) normalized to expression levels of non-differentiated P5 hMSC up to 28 days in vitro. (B) Expression of cytochrome genes at day 28.

Conclusions: These results demonstrated that 3D printed collagen-infused PLLA scaffolds enhanced hMSC transdifferentiation into hepatocyte-like cells compared to cells transdifferentiated in 2D culture. Even with increased expression of hepatocyte-specific genes, cell proliferation was maintained. This work shows the advantage of using 3D scaffolds to more effectively control stem cell fate. Future work will explore the use of more defined media conditions (i.e. with specific growth factors) for controlling hMSC transdifferentiation in 3D.

References: [1] Snykers S *et al*. Stem Cells. 2009; 27:577-605. [2] Ong SY *et al*. Tissue Eng. 2006;12:3477-85. [3] Tai BCU *et al*. Biomaterials. 2010; 31:48-57.

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