Direct Cell Writing: Process, Viability and Function

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Statement of Purpose: In the new paradigm of tissue science and engineering, living cells are used as basic building blocks for biofabrication of cell-integrated medical therapeutic products and/or non-medical biological systems, such as tissue substitutes, cell/organ printing, microfluidic biochips and biosensors, drug delivery, pharmacokinetic testing and drug toxicity screening, microscale cell culture analogs, and "lab-on-a-chip" devices [1-3]. The objective of this paper is to report our recent work on a direct cell writing by freeform deposition of living cells to construct 3D cell assemble and tissue structures. Presentation will include: 1) introduction of direct cell writing process; 2) effect of the process parameters on cell survivability; 3) characterization of biological response of endothelial, fibroblast, liver, and muscle cells to the process; and 4) effect of nano-scale artifacts, for example, carbon nano-tubes, nano-particles, and magnetic nano-particles on cellular function for fabricated tissue structures.

Methods: A proprietary multi-nozzle direct cell writing system is applied for fabrication of 3D tissue constructs. Biomimetic design is engineered into the scaffold/cell environment using the system's enabling freeform and micron fabrication technology. Simultaneous or sequential deposition of cells, growth factors, and scaffolding biomaterials to form intricate heterogeneous or functionally gradient tissue constructs can be accomplished. A schematic representation of direct cell writing system is shown in Figure 1.

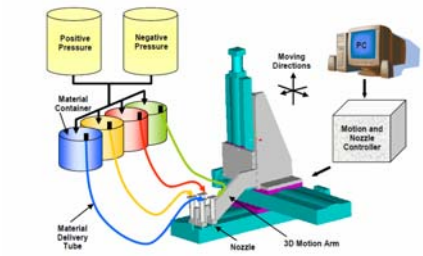

Figure 1. Bioprinter schematic for polymer depositions

Cell dispensing nozzles are mounted to a 3D motion arm and positioned over a substrate. Pneumatic pressure opens the nozzles for direct cell writing while computer programming control the movement of the arm over the stationary substrate to form the written patterns. Endothelial, fibroblast, liver, and muscle cells will be written through the micro-nozzle with the alginate as cell delivery medium or scaffolding materials. Cell basal metabolic recovery will be measured with Alamar blue and Alamar blue fluorescence will be measured using a microplate reader (GENios) at excitation and emission wavelengths of 520 and 590 nm, respectively. To determine cell proliferation, both for cell function recovery and to account for proliferation in the Alamar blue assay.

Results: Cells are encapsulated in a hydrogel and printed using selected combinations of process parameters. These experimental trials are to determine the viability of the process, the influence of process parameters (dispensing pressure and nozzle diameter) on cell survival rate and subsequent function. A florescent image of direct written 3D tissue construct made of endothelial cells with alginate is shown in Figure 2. Figure 3 presents fibroblast cell survivability after the direct writing and the viability after 7 days of the deposition.

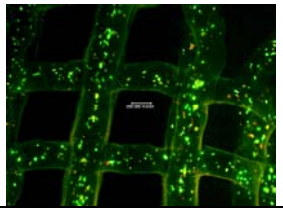

Fig.2: 3D tissue construct made of endothelial cells with alginate

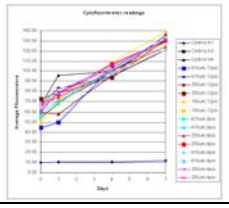

Fig.3: Fibroblast cell survivability and the viability after deposition

Liver cells are also encapsulated and deposited with nano carbon tubes, HA nano-particles and magnetic nano-particles for testing enhancement of cell viability, proliferation and cell labeling. Cell survival rate was higher when cells were written with nanospheres. Endothelial cells are encapsulated in an alginate/single wall carbon nanotube (SWCNT) and the composite constructs yielded increased cell proliferation and structural strength when compared to pure alginate controls. Both boost cell viability after direct writing. Endothelial cells are labeled with magnetic nanoparticles and encapsulated in hydrogel for direct writing. The macroscopic location and individual cell alignment are sensitive to and can be manipulated by magnetic forces.

Application: Direct cell writing can be applied for biofabrication of 3D novel and advanced cell-tissue models for applications as tissue constructs for regenerative medicine, disease models, drug models and new generation of biological systems. This presentation will report the direct cell writing technique feasibility, the effect of process to cellular function, the application to make 3D tissue constructs and a liver micro-organ for drug testing. Incorporation nano-artifacts with living cells in direct writing also shows enhanced cell survivability and proliferation for applications in tissue science and engineering.

References: 1) Griffith LG, Naughton G. Tissue engineering - Current challenges and expanding opportunities. Science 2002; 295(5557):1009-14; 2) Mironov, V., Trusk, T., Kasyanov, V., Little, S., Swaja, R. & Markwald, R., " Biofabrication: A Twenty-first Century Manufacturing Paradigm", Biofabrication, 1(2), 1-16; 3) "Advancing Tissue Science & Engineering: A multi-agency strategic plan". http://tissueengineering.gov/welcome-s.htm