Novel Temperature Controlled Printing System and Microfluidic Chip Coupled for In Vitro Radiation Study

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Purpose: Manned missions to Mars and other long term space missions require testing and development of novel pharmaceuticals for the microgravity environment. NASA is pursuing a high fidelity *in vitro* tissue models to simulate tissue-drug interaction for this purpose. This aim requires a printing method to incorporate digital biomodeling into physical cell pattering for enhanced biomimetic cell function and a sealed environment to observe and stimulate cells. We are proposing two systems, first a direct cell writing system to print cell-laden hydrogel and second a microfluidic chip to seal and control environmental stimuli as a portable research tool for drug studies. Presentation will include: 1) introduction of temperature/motion controlled printing sytem and 2) microfluidic device, and 3) application of this system to study anti-radiation drug.

Methods: Cell function is of primary concern for *in vitro* models. Matrigel hydrogel is shown to improve biomimetic function by bioactive factors and essential macromolecules. However, this material is thermally cross-linked at room temperature and requires a novel temperature controlled means of fabrication. In developing the fabrication method, we incorporate a CAD/CAM platform to reproducibly engineer biomodeling into cell-laden scaffold. A schematic representation and photograph of direct cell writing system is shown in Figure 1.

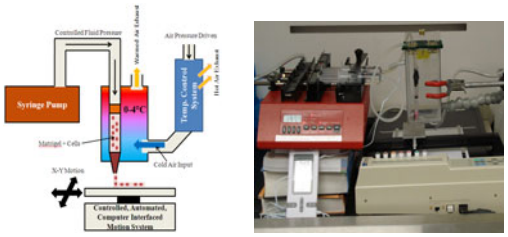

Figure 1. Bioprinter schematic for polymer depositions

A dispensing nozzle is positioned over a PDMS substrate mounted to printer head controlled by computer programming. Cell/Matrigel solution is loaded into a syringe and cooled to below 4°C. A syringe pump extrudes the material as the substrate is moved along a pre-programmed trajectory. Figure 2 is a photograph of a cell-laden Matrigel construct.

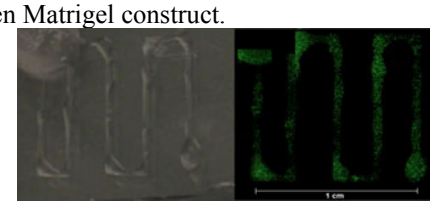

Fig. 2: Cell/Matrigel construct on PDMS substrate photograph (left) and cells labeled with Live/Dead Assay and viewed under fluorescent microscope (right).

Cellular constructs are sealed under glass in microfluidic chips and serially connected by hosing to create dual micro-organ microfluidic chips. The chips are connected to leverage melanoma's enzyme secretion for the hepatocyte's radiation protection. The anti-radiation drug amifostine is a prodrug, which means it is inactive and has no radiation shielding capability. Melanoma cells secrete alkaline phosphatase, which is capable of activating the drug by stripping the amifostine of its phosphate group. Hepatocytes secrete lesser levels of this enzyme and are therefore convert less drug to active form and have less shielding from radiation. In this study, we serially connect melanoma constructs and hepatocytes and introduce amifostine to observe cell function after printing and environmental control provided by the microfluidic platform. Figure 3 is a schematic of the dual micro-fluidic set-up.

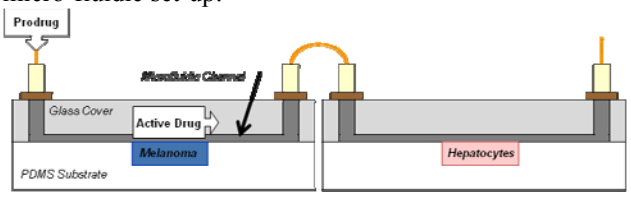

Figure 3. Side view of dual microfluidic set-up

Human hepatocytes and melanoma cells (ATCC, Manassas) are encapsulated in Matrigel (BD Bioscience, Rockville), printed using a novel direct cell writing system, sealed in microfluidic chips, then perfused with alpha Minimum Essential Medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Invitrogen) at 30 µL/hr. After 12 hours, 100 mM amifostine (Sigma) dissolved in medium is introduced. Samples are then irradiated with 2 gray of gamma radiation and subsequently perfused with amifostine for an additional 2 hours. A micronuclei count determined the amount of damage done to the cells.

Results: We observe micronuclei occur in small amounts during the experimental process (4% damaged cells), radiation causes elevated levels of micronuclei (26% damaged cells), amifostine shielded the cells from some radiation damage (8% damaged cells), and the dual organ improved the effectiveness of the drug to shield hepatocytes from 2 gray of radiation (3% damaged cells). We conclude amifostine perfuses through the melanoma cells, is converted to its active form, and then protects the hepatocytes from radiation.

Conclusion: Temperature controlled printing and the microfluidic chip serve as *in vitro* models of cell activity in response to environmental stimuli.

References: 1) Chang, R. Tissue Engineering Part C-Methods. 2008; 14: 157-166 2) Stetlersstevenson, WG. Annual Review of Cell Biology. 1993; 9: 541-573.