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Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip

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Abstract

The objective of this paper is to introduce a novel cell printing and microfluidic system to serve as a portable ground model for the study of drug conversion and radiation protection of living liver tissue analogs. The system is applied to study behavior in ground models of space stress, particularly radiation. A microfluidic environment is engineered by two cell types to prepare an improved higher fidelity in vitro micro-liver tissue analog. Cell-laden Matrigel printing and microfluidic chips were used to test radiation shielding to liver cells by the pro-drug amifostine. In this work, the sealed microfluidic chip regulates three variables of interest: radiation exposure, anti-radiation drug treatment and single- or dual-tissue culture environments. This application is intended to obtain a scientific understanding of the response of the multi-cellular biological system for long-term manned space exploration, disease models and biosensors.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Bioactive microfluidic systems offer a promising platform for a small-scale study of pharmacokinetics/pharmacodynamics, drug absorption, elimination and toxicity on human biological material [1–3]. In addition, the liquid cell configuration enables real-time studies of bioaffinity reactions at predetermined flow rates and temperatures [4]. The fidelity of the in vitro test platform and reliability of the biological analog are directly related to the manufacturing and cell assembly capability [5]. Constituent components and their spatial arrangement are determined from the analysis of target biological systems, which is realized as physical prototypes. Manufacturing of those physical prototypes is limited by process resolution and material handling, meaning preserving cell viability and function.

In vitro drug discovery platforms and sophisticated cell assembly technology are independently recognized as bioadditive techniques with the potential for in vitro biomimicry and monitoring [6, 7]. One current trend in drug discovery and tissue engineering research is to use a combined three-dimensional cell assembly and microfluidic environment to probe and analyze the biological condition, response, and drug effectiveness [8, 9]. This becomes possible as appropriate architectural length scales and biological components are identified and relevant manufacturing capability is developed. In this work, the authors introduce a novel cell printing system and microfluidic chip to study dual-tissue drug
conversion and effectiveness of the radioprotective pro-drug amifostine against radiation of the magnitude found outside the Earth’s orbit [10]. This work is done in part to prepare a manufacturing and cell culture method to test pharmaceuticals in the space environment to make informed recommendations about medical preparedness for long-term manned space exploration [11].

The presented microfluidic system improves in vitro modeling beyond single cell activity by including multiple cell types [12, 13]. This works using two cell types: (1) epithelial cells as the cell type lining the body’s lumen, which the drug would pass through before moving from the blood stream to the target cell type and (2) hepatocytes as the target cell type we are studying for evidence of radioprotection. In the microfluidic environment the drug passes serially through the epithelial construct and then the hepatocytes, as this would be the path of the drug diffusing from the blood stream to the tissue. Each cell type is physically segregated into separate chambers of a microfluidic system and dynamically perfused by a syringe pump. The transient interaction between cell types is controlled by the flow rate of the pump. Soluble cues and experimental variables are introduced to cell by perfusion through the microfluidic chambers. Engineered devices, such as the direct cell writing process and microfluidic device, can be used to assemble biological materials to approximate in vivo pathogenesis, as presented in figure 1.

Macro and micro patternings of cells are part of mechanical cues to promote biomimetic function [14]. Cells, as dynamic spatial entities, rely on paracrine signaling and other environmental cues to define their own behavior. The cell culture environment is engineered to approximate in vivo conditions in vitro to elicit the biomimetic function. A motion-controlled bioprinting system and extracellular matrix provide structure and patterning control to leverage biologists characterization of known functional tissues for metabolism studies. A promising extracellular matrix is a gelatinous protein mixture Matrigel, which improved biomimetic cell function through bioactive factors and essential macromolecules [15–17]. However, existing printing techniques are unable to dispense cell-laden Matrigel because the devices operate at or above room temperature. Matrigel thermally cross-links at above 4 °C, and this occludes dispensing capillary and prevents further extrusion. A novel temperature-controlled printing system is presented in this work to pattern Matrigel and leverage both biological cues from the Matrigel matrix and physical cues from physiologically derived cell patterning to improve the biomimetic function of in vitro tissue analog [18, 19]. Printing technology allows researchers to leverage geometric positioning and proximity of specific biologics to bring functional abilities to cell aggregates [20–22]. In this work, cells are printed in a square wave form for nutrient and drug diffusion to the core of the extruded filament, to apply structural cues to cells, and for quality control over equal dispensing for each sample.

In our study here, we present the integration of a microfluidic cell culture environment with the novel cell printing technique to create an in vitro analog using two different cell types to study pro-drug conversion in a dual-tissue microfluidic chip and resultant radioprotection to liver. Specifically, we are testing radiation protection afforded by pro-drug amifostine against space-like radiation exposure. The in vitro environment provides engineering control over the constituent components and spatial arrangement to focus the study of causality by isolating particular biologics of interest. The authors claim improved model fidelity, over single-cell-type models, by a combined microfluidic environment and cell assembly technique using a matrix Matrigel, based on the observation of radiation shielding.
2. Materials and methods

2.1. Cell culture and encapsulation

Two immortalized cell lines are co-cultured in the dual-tissue environment. Human hepatic carcinoma cells of the cell line HepG2 (ATCC) and human mammary epithelial of the cell line M10 (ATCC) are cultured in the alpha modification of Eagle’s medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin streptomycin. Half volume of the culture medium is changed every other day. Cultures are maintained at 37 °C and 5% carbon dioxide. The cell culture medium and supplements are purchased from Invitrogen unless otherwise noted.

Separate cell-laden solutions of epithelial and hepatocytes are created in an identical manner. Cells are rinsed with phosphate buffer solution (PBS), trypsinized and counted using exclusion dye Trypan Blue and a hemocytometer. Once counted, cells are mixed with the gelatinous protein mixture basement phenol red-free Matrigel (BD Bioscience) over ice to prevent gelation. Cells are homogenously distributed throughout the Matrigel using a gentle tapping technique with minimal pipetting to a concentration of 1.0 × 10^6 cells mL⁻¹. The final printing solution is 50% cells and 50% Matrigel (v/v) solution. The cell-laden solution is stored on ice for 5–10 min prior to printing to prevent gelation.

2.2. Temperature motion controlled printing

Cell printing is a powerful manufacturing tool to assemble biologics to bring structure and functional architecture to cell aggregates. Here we present a temperature-controlled cell printing system inspired by rapid prototyping technology. A CAD/CAM platform is integrated with solid freeform automation to assemble biologics in a three-dimensional space using layer-by-layer manufacturing techniques. This system executes micron-scale deposition and printing to repeatedly generate cell-laden constructs for scaffold-guided tissue engineering applications to include regenerative medicine, in vitro drug trials and disease analogs. A depiction of the subsystem components is presented in figure 2.

The system integrates a temperature-controlled enclosure, dispensing system and plotter to allow for the printing of thermally cross-linking materials, such as Matrigel or collagen. A polycarbonate enclosure is prepared to house the dispensing system due to the material’s low thermal conductivity of 0.23 W mK⁻¹, machinability and transparency [23]. The printing solution is completely housed in a thermal enclosure to prevent gelation prior to extrusion. Temperature enclosure is cooled by the vortex flow. Breathing quality compressed air (AirGas) is directed through a vortex tube (EXAIR) passively separated into a cool and a hot stream. The hot stream is vented to the ambient environment. The cool stream is directed to an enclosure containing the dispensing syringe. A digital thermometer (Thomas Scientific) inside the enclosure reports the temperature and the operator adjusts the flow of compressed air to achieve, and then maintain, enclosure temperature between 0 °C and 4 °C. Cell-laden material is extruded from a syringe onto a substrate positioned on a stage mounted to the print head of the Roland DXY-1100 plotter (Roland). The plotter is controlled via the QBASIC program run on a Windows NT Workstation 4.0 Intel Pentium 3 processor (Dell). The device is irradiated with a UV bulb for 120 min prior to the experiment and contains in laminar flow a positive pressure dissection hood (NuAire Laminar Flow Products) for sterility during the experiment. Figure 3 presents a schematic and as-built depiction of the temperature-controlled printing system.

A 150 μm diameter flexible precision dispensing tip (EFD) is fixed to the end of a 3 mL luer lock tip syringe (BD Syringe). Hepatocyte-laden and epithelial-laden Matrigel solutions are prepared as described previously and aseptically loaded into separate syringes under the culture hood. A rubber gasket is inserted behind the cell-laden Matrigel solution and the remainder of the syringe is filled with PBS (Gibco). Prepared syringes are incubated on ice until printing. All components of the printing system to come in contact with cell-laden material are flushed with 70% ethanol three times and dried overnight under UV light for sterilization in the laminar flow laboratory hood (NuAir). Vortex cooling from a compressed air cylinder cools the temperature-controlled enclosure to between 0 and 4 °C. Once the temperature is stable within the target range, the prepared syringes are positioned in the temperature-controlled enclosure and plumbed to a programmable syringe pump (New Era Pump Systems). Extrusion pressure is set to 12.3 Pa. A polydimethylsiloxane (PDMS) substrate is positioned directly below the dispensing tip stage mounted to the print head of the Roland DXY-1100.

Figure 2. Schematic representation of the subsystems of temperature-controlled printing.
plotter. A printing speed of $1.0 \text{ cm s}^{-1}$ and a printing trajectory were preprogrammed using QBASIC software. Extrusion of the cell-laden material and commencement of printing begin simultaneously by manually starting the syringe pump and running the QBASIC program. Hepatocyte-laden syringe is positioned and extruded first, and then the Epithelial-laden syringe is positioned and extruded. After all constructs have been printed, epithelial constructs are prepared. After each construct is printed, the PDMS substrate is set in the sterile petri dish and stored in a 37°C incubator to facilitate matrix polymerization.

2.3. Microfabrication and sealing of the microfluidic chip

The chip contains two components: a PDMS substrate and glass cover. Using photolithography and replica molding techniques, we created an indentation in the PDMS substrate to house a cell-laden construct. Micro-channels are etched on the glass cover as described in a previously published work [8]. Nanoport assemblies (Upchurch Scientific) are applied to the chip using an adhesive and connected to a programmable syringe pump (New Era Pump Systems) by 0.02 in inner diameter polyetheretherketone tubing (Upchurch Scientific) and luer lock fittings. The glass cover acts as a lid over the PDMS substrate and seals the chip. Cells are printed directly into the PDMS substrate. A 90 s treatment with air in the plasma cleaner (Harrick Plasma) and mechanical pressure constraints maintain a water tight seal. Chips are dynamically perfused with the cell culture medium and drug using a syringe pump, and microfluidic channels act as the plumbing system on the chip. The interaction of the pro-drug with multiple cell types is studied by serially connecting chips of various cell types. The microfluidic chip is incubated at 37°C and 5% CO$_2$ to maintain cell viability for the duration of the study. Figure 4 presents images of the assembled microfluidic chip with cell-laden material sealed inside.

2.4. Dual- and single-tissue microfluidic culture environments

Cellular constructs are sealed under glass in microfluidic chips and serially connected to create dual-micro-tissue microfluidic chips. The chips are connected to capture multi-cellular interaction and downstream effects of metabolism on a target tissue. This works using two cell types, (1) epithelial cells as the cell type lining the body’s lumen, which the drug would...
pass through before moving from blood stream to the target cell type and (2) hepatocytes as the target cell type we are studying for evidence of radioprotection. In the microfluidic environment, the drug passes through the epithelial construct and then the hepatocytes, as this would be the path of the drug diffusing from the blood stream to the tissue. Each cell type culture is termed a 'tissue’ in this work. Transient interaction between the two cell types is regulated by the flow rate through the constructs. Real-time perfusion of the metabolized drug from the metabolizing tissue to the target tissue captures the kinetic interaction between cell types. In this work, the dual-tissue environment is leveraged to study amifostine conversion and its effectiveness as a radioprotective agent in a dual-cell-type environment. The anti-radiation drug amifostine is a pro-drug, which means it must be metabolized to an active form for any radiation shielding to occur.

2.5. Cell viability

Cell viability post-printing is qualitatively evaluated using a fluorescence-based Live/Dead Viability/Cytotoxicity kit for mammalian cells (molecular probes). Two-color discrimination by the fluorescent probes ethidium calcein AM and ethidium homodimer-1 labels live cells as green and dead cells as red. Samples were analyzed using a DM RIB inverted microscope (Leica) with UV source. Images of all cells were captured electronically by the Insight 4.0 Mp Monochrome digital camera (Spot Imaging software) and imaging software provided by the manufacturer. Samples were coded and scored blind.

2.6. Anti-radiation treatment by amifostine

Anti-radiation treatment is the perfusion of the radioprotective agent amifostine (Sigma) through the cellular construct both before and after radiation exposure. Powder amifostine is mixed with the culture medium to the final concentration of 1 mM amifostine. Amifostine is perfused through the constructs using a multi-channel programmable syringe pump (New Era Pump Systems) for 3 h prior to the radiation exposure and for 2 h after the exposure. The residence time of the drug in each chamber is 3 h. This calculation is based on the geometry of the chamber and flow rate, which is programmed to be 30 μL h⁻¹.

2.7. Radiation treatment

Radiation treatment was administered by the University of Pennsylvania School of Medicine Department of Radiation Oncology using a 137Cs gamma-ray source with a dose rate of 86 cGy min⁻¹. Irradiated samples were exposed to a 2 Gy dose over 1.72 min. Hepatocyte constructs remained sealed in the microfluidic chip during the exposure. Only select hepatocyte constructs are exposed to radiation; however all biological samples in this experiment are transported to treatment facility and remain outside the incubated environment for an equal amount of time.

2.8. Quantification of radiation damage by the micronuclei count

The micronuclei count quantified radiation damage of samples. After anti-radiation treatment is complete, cells are recovered from the Matrigel matrix using the BD MatriSperse cell recovery solution (BD Bioscience) and plated on tissue-treated polystyrene culture plates (Corning) for the in-culture medium with 3 μg mL⁻¹ permeable mycotoxin Cytochalasin B (Sigma) to block the cytoplasm division. After 72 h cells are fixed in a 50% (v/v) methanol solution, and genetic material is labeled with the fluorescent probe DAPI nuclear counter stain (Molecular Probes), and examined. Samples are analyzed on a Zeiss Axioplan fluorescence microscope. Images of all cells are captured electronically using a Sensys charge-coupled device (CCD) camera (Photometrics Ltd) and the MacProbe computer software developed by Applied Imaging. All samples are coded and scored blind. Cells are qualitatively sorted into three categories: (1) mononucleated, (2) binucleated without micronuclei and (3) binucleated with micronuclei. Figure 5 presents the categories of cells prepared for the micronuclei count. Only binucleated cells with micronuclei are considered damaged. The results of the count are presented as a percentage of radiation damage cells determined by the number of binucleated cells with micronuclei relative to the total number of binucleated cells.

3. Results

In this work a novel cell printing system and a microfluidic chip are used to study multi-cellular drug conversion and
effectiveness of the radiation shielding by pharmaceutical amifostine. Human hepatocytes and epithelial cells (ATCC) are encapsulated in Matrigel (BD Bioscience), printed using a novel temperature-controlled cell printing system, sealed in microfluidic chips and then perfused with cell culture medium at 30 μL h\(^{-1}\). After 12 h, 100 mM amifostine (Sigma) dissolved in the medium is introduced. Samples are then irradiated with 2 Gy of gamma radiation and subsequently perfused with amifostine for an additional 2 h. A micronuclei count determined the amount of damage done to the cells.

3.1. Cell viability after matrigel printing

Temperature-controlled printing is a physical process which requires low temperature incubation before printing and extrusion of living cells through pressurized micronozzle during printing. These environmental parameters introduce hydrodynamic and thermal stress, which may damage the cell. Prior to patterning cell-laden Matrigel and further study of printed structures, the authors used fluorescent probes to test for cell survival and viability 48 h after printing. Human mammary epithelial of M10 cell line was prepared in a Matrigel matrix as described previously and extruded using the temperature-controlled printing system through a 150 μm nozzle tip at 12.3 Pa. The viability of printed constructs is determined by the fluorescence-based Live/Dead Viability/Cytotoxicity kit for mammalian cells. Printed constructs were qualitatively screened using fluorometric indicators in commercially available Live/Dead kit for live (green) cells. Figure 6 presents the fluorescent images of cells less than 30 min and 48 h after printing. We observe viable cells embedded in the Matrigel after printing, as shown in figure 6. Temperature-controlled printing yielded constructs embedded with viable cells. Cells remained viable 48 h after printing.

3.2. Feasibility cell-laden matrigel patterning

After observing that cells survive the printing process, the authors designed patterns and coded trajectories to print cell-laden Matrigel. The structural form of the cell-laden construct is created using computer programming and an x–y plotter. A square wave pattern is desirable for gas and nutrient diffusion to the cells in the core of the printed filament. The pattern has a high surface area to volume ratio for the cell/drug contact during pharmacokinetic studies. Pattern coordinates were determined using Microsoft Excel 2007 and the print head trajectory is coded in QBASIC. Human mammary epithelial
of the M10 cell line was prepared in a Matrigel matrix as described previously and extruded using the temperature-controlled printing system through a 150 μm nozzle tip at 12.3 Pa. Figure 7 presents (A) the intended nozzle trajectory, (B) photograph of the printed construct and (C) fluorescent image of cells in the printed construct.

The plotter carried the substrate through the intended print head trajectory as the syringe pump extruded cell-laden Matrigel through a stationary capillary tip. Figure 7 presents a photograph of the material and viable cells in the intended geometric structure. The strut width of the printed filament is 250 μm, as presented in figure 7, by the identified manufacturing process parameters.

3.3. Radiation damage due to the radiation exposure

Temperature-controlled patterning of cell-laden Matrigel and dynamic perfusion of the microfluidic environment are used to create a dual-tissue microfluidic chip of epithelial and hepatocyte constructs serially connected and perfused with radioprotection treatment. Hepatocytes were irradiated and a micronuclei count conducted to determine radiation damage. The results of the micronuclei count to determine are presented in figure 8. Experimental variables include (1) radiation 0 or 2 Gy, (2) with and without the treatment of the tissue analog with anti-radiation drug amifostine and (3) the single- or dual-tissue microfluidic system. The purpose the 2 Gy radiation is to simulate radiation stress conditions outside the Earth’s atmosphere. The purpose of the drug is to examine its radioprotective effectiveness. The purpose of the dual microfluidic system is to use a multi-cellular system to better represent in vivo pathogenesis, as compared to a single cell model. Each experimental variable has one sample ($n = 1$).

We observe that micronuclei occur in small amounts during the experimental process (4% damaged cells), radiation causes elevated levels of micronuclei (26% damaged cells), amifostine shields the cells from some radiation damage (8% damaged cells), and the dual tissue improves the effectiveness of the drug by a twofold decrease in radiation damage to hepatocytes after 2 Gy of radiation (3% damaged cells).

4. Discussion

The closed microfluidic system gives researchers engineering control over two principle design variables: (1) constituent biological and soluble components and (2) prescriptive exogenous stimulation to mimic high stress or disease conditions. In this work, the authors use the sealed microfluidic chip to regulate three variables of interest; radiation, drug and single or dual-tissue-culture environments. Cells were encapsulated and spatially patterned in a matrix and then linked in a microfluidic environment to elevate the behavior of cells to biomimetic tissue level function. Then, the system is probed with a prescriptive amount of environmental stress. In this work the stress was radiation. However, the
portable microfluidic platform is not limited to this magnitude or type of exogenous stress.

Microfluidics were used in this work to control the transient interactions between two cell types and residence time of the drug. Two tissues were serially connected in the microfluidic chip and dynamically perfused with the anti-radiation drug amifostine. According to the micronuclei count, the target tissue (hepatocytes) expressed less radiation damage due to drug treatment, when compared to the no drug control. The radiation damage was further decreased in dual tissue with the drug group, when compared to the single tissue with the drug group. The authors believe that the dual-tissue-culture environment was more effective radiation protection because the epithelial tissue converts the drug to an active form by producing the enzyme alkaline phosphatase. The active form of the drug then perfuses the target tissue and shields the tissue from radiation damage. The micronuclei count demonstrates that the active form of the drug is an effective form of radioprotection, as the percentage of radiation-damaged cells for the dual tissue is 3% compared to 4% for nonirradiated controls.

The interaction between the two cell types caused the observed radiation shielding in the dual-tissue microfluidic system. Amifostine enters the microfluidic system through a nanoport above the epithelial tissue in its inactive WR-2721 form [24]. Epithelial cells present alkaline phosphatase, which is capable of converting the drug to the active form. The membrane-bound enzyme alkaline phosphatase de-phosphorylated the amifostine and converted the drug to the active WR-1065 form. Dynamic perfusion forces the converted drug to the target tissue, which is the hepatocyte construct. The level of alkaline phosphatase present in the hepatocyte is much less than that of the epithelial construct. The drug metabolized to an active form by the metabolizing construct protects the target tissue from radiation damage. The single tissue model is only the target tissue and does not include the epithelial tissue, which presents more alkaline phosphatase activity. Without the increased level of alkaline phosphatase, less of the drug is converted to an active form and less radiation-protection is observed. The percentage of radiation damaged cells for the single tissue is more than twice that of the epithelial construct. The drug group, when compared to the single tissue model are twice that for the nonirradiated controls.

The dual tissue is a more authentic representation of in vivo pathogenesis and tissue function. The single tissue model gives the false conclusion that amifostine is not an effective radioprotective agent. The instances of radiation damage for the single tissue model are twice that for the nonirradiated control. However, this is because the drug is not converted to an active form. The dual-tissue model demonstrates the radioprotective benefits of amifostine by metabolizing the drug as it would occur in multi-cellular environments, which is more similar to in vivo conditions than the single cell model.

5. Conclusions

A new method of bioprinting and dual-tissue bioreactor design is offered in this paper. The solution is tailored to accommodate pharmaceutical testing using human-derived cellular material in relatively inaccessible environments, like space. Specifically, in this work we present the integration of a microfluidic cell culture environment with a novel cell printing technique to create an in vitro analog using two different cell types to study pro-drug conversion in a dual-tissue microfluidic chip and resultant radioprotection to liver. The radioprotective benefits and pro-drug conversion by multiple cell types are more fully realized by incorporating the dual-tissue model. Future work using the printing system and microfluidic environment is not limited to the cell type, drug or environmental stress presented here.

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References

[4] Huang J D et al 2008 Comparison of a resonant mirror biosensor (IAsys) and a quartz crystal microbalance (QCM) for the study on interaction between paenoniae radix 801 and endothelin-1 *Sensors* 8 8275–90


