A Digital Micro-mirror Device-Based System for the Fabrication of Microfluidic Tissue Array

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***Abstract*—Micro-Electro-Mechanical Systems (MEMS) technologies demonstrate the potential for applications in the field of tissue engineering, regenerative medicine, and life sciences. The development of a successful model would require investigations of the targeted organ. Computer tomography (CT) and magnetic resonance imaging (MRI) allow for biomodeling of the targeted organ. The Digital Micro-mirroring Microfabrication system (DMM) would utilize its dynamic micro mirrors to fabricate the developed tissue construct according to its desire design and characteristics. The DMM has the capabilities to fabricate many advantageous devices, amongst them; microfluidics systems have the most tissue engineering, regenerative medicine, and life sciences applications to develop *in vitro* tissue models. Unlike many microfabrication techniques and or devices available to fabricate tissue model, this system eliminates the need for mask by incorporating a dynamic mask-less fabrication technique. Since the system can develop models on a micro-scale level, this would make many investigation more economic; requiring less reagents, cells, and above all; it will allow for consistency in experimental analysis to due limited interactions with the end user.**

I. INTRODUCTION

Tissue Engineering, Regenerative Medicine, and Life Sciences brings together the multidisciplinary field of engineering and integrated sciences to develop models that aides the exploration, generation or regeneration of organic tissues and organs. Micro-Electro-Mechanical Systems (MEMS) were first introduced on conventional semiconductor materials, which were used to develop integrated circuits (ICs). Since the development of the first integrated circuit (IC) with MEMS technologies, MEMS have been utilized in many other fields with great success. This technology is expensive and has many limitations [1].

Scientists and researchers are constantly working around the clock to develop pharmaceuticals and tissue replacements that would allow humans to live longer lives. However, many of these developments require tremendous investigation on its affects on humans. Quite often, the use of animal and human models is limited by the feasibility of testing protocols, availability, and ethical anxieties [2]. The development of the dynamic digital micro-mirroring microfabrication system (DMM) gives scientists the capabilities to develop models that can be utilized to characterize new pharmaceutical, tissue replacements, and even develop models to study fatal disease. This paper investigates the DMM capabilities to fabricate a microfluidic construct that has the capabilities to support cell attachment and proliferation within its channels.

II. MATERIALS AND METHODS

A. Digital Micro-mirroring Microfabrication System

This system consists of three major components: 1) digital micro-mirroring projection system, 2) photolithographic substrate alignment system, and 3) mask modeling system. The micro-mirroring projection system will be connected to a computer interface, in which the computer system will activate and deactivate the projection of the mask onto the substrate. The photolithographic substrate alignment system consists of a digital microscopic device that will allow for alignment of the substrate's features to that of the projected mask from the micro-mirroring projection system. The mask modeling system will utilize computer-aided design (CAD) technologies to design mask for projection. Fig. 1 illustrates a model of the proposed system, where the ultraviolet light source is projected through a series of optical lens onto the micro-mirroring system, which will then project the mask onto the substrate.

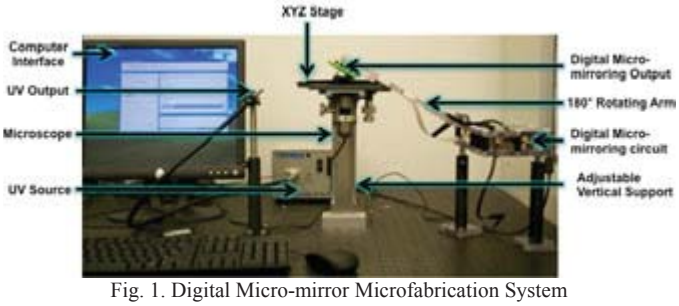

Fig. 1. Digital Micro-mirror Microfabrication System

B. Fabrication Protocol

The DMM utilizes a systematic layer by layer approach to fabricate three-dimensional models using photosensitive a polymer. The digital micro-mirroring microfabrication system would project an image of the desired layer onto the photosensitive polymer; once the polymer is exposed to the UV projection, it would manipulation the material's chemical properties and mimics the projected pattern. Prior to exposing the first layer, a Polydimethylsiloxane (PDMS) substrate is developed. This substrate enables the end user to fill the slot with *n* μm of photosensitive polymer, where *n* represents the depth of the layer/PDMS slot. The PDMS with the photosensitive polymer goes through a soft bake process prior to exposure. After exposure, it is baked once more then developed. After the developing phase, the develop layer will maintain the desired pattern. This process is repeated until the desire architecture is fabricated. Baking and developing time is provided by the manufacture of the photosensitive material.

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The photosensitive material used in this paper is SU-2050 (MicroCHEM, USA). Fig. 2 shows the design of the PDMS susbtrate (left) and the square architecture design (right) used for the fabrication of the microfluidic construct within the PDMS slot.

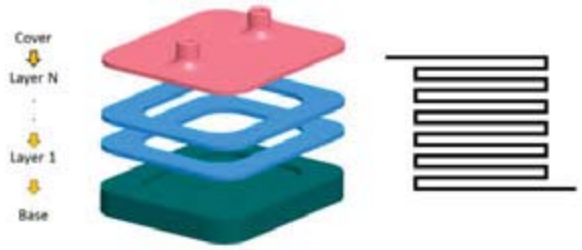

Fig. 2. (left) PDMS Substrate, (right) square wave architecture.

C. Cell Cultures

7F2 (osteoblast) cell line (source: ATCC, Manassas, VA, USA) were seeded onto 75cm^2 vented flasks and incubated. 6 hours after the cells were seeded, the culture medium was changed to remove any dead cells in the flask; culture medium was also changed every 2-3 days until flask are ready to be harvested. Confluent flasks were harvested and counted using hemocytometer. The Harvested cells were seeded into the channels of the develop microfluidic construct at a cell density of 1x10^6 cells/ml. Culture media was change every 2-3 days and after every characterization point. This paper investigates two sample types; the first samples are the microfluidic construct without any surface treatment. The second samples investigated are with air plasma surface treatment. Plasma treatment is global where developed chips were place into Plasma Cleaner PDC-32G (Harrick Plasma) and vacuumed for 30 seconds and air plasma was applied for 30 seconds. The results of the biological investigations can be viewed in section III.

III. RESULTS AND DISCUSSIONS

A. Cell Interaction

It is important that cells maintain interaction within the micro channels; without the cell–cell interaction, cells cannot proliferate and differentiate into mature cells that are essential for functional tissues. A fluorometric indicator (Alamar Blue, Serotec) of cell metabolic activity was utilized to determine the cell proliferation within the channels. Cell laden samples (both treated and untreated) were removed from the culture plates, washed twice, placed into a new culture plate where 10% (v/v) alamar blue was added to micro channels and incubated for 4 h. The resulting solution was removed from the culture plate and placed into the microplate reader (GENios, TECAN, North Caroline, USA) whose excitation and emission wavelengths were 535 and 590 nm, respectively. This study was conducted on days 0, 3, 5, 7, 10, and 14. The results of this study are shown in Fig. 3 where the untreated samples showed no signs of cell proliferation. However, the plasma treated samples showed a linear progression of proliferation up to day 7. After day 7, this progression subdued. The decline of proliferation of the plasma treated samples on day 7 is believed to be due to limited space for cells to continue to grow.

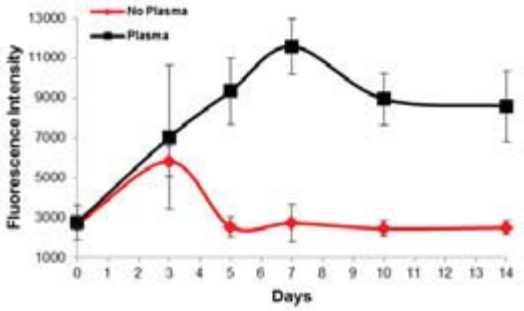

Fig. 3. Cell proliferation on treated and untreated microfluidic constructs

B. Cytotoxicity Analysis

MarkerGene™ Live:Dead/ Cytoxicity Assay Kit was used to analyze the cytotoxicity of the scaffolds. Manufacture's protocols were followed to create the working live:dead solution from the propidium iodide (PI) solution and the carboxyfluorescein di-acetate (CFDA) solution. The carboxyfluorescein dye is retained within live cells, producing a green fluorescence, while cells with damaged membranes allow the entrance of PI, which undergoes a fluorescence enhancement upon binding to nucleic acids promoting a red fluorescence in dead cells. Qualitative data were collected on both microfluidic constructs on days 7 and 14 after cells were seeded. Fig. 4 Illustrates live (green) and dead (red) cells within the micro channels of the construct. As seen in Fig. 4, there are more cells within the plasma treated channels in comparison to the untreated channels.

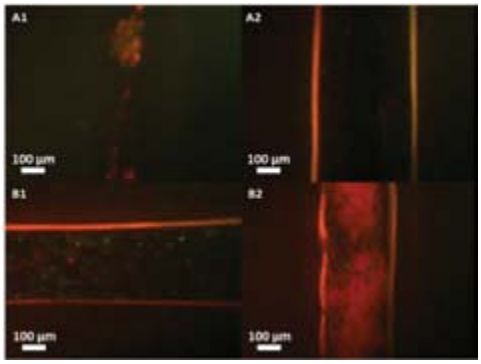

Fig. 4.Live: Dead Characterization: Images A1 and A2 are fluorescence images of untreated microfluidic samples on days 7 and 14, respectively; images B1 and B2 are fluorescence images of plasma treated microfluidic samples on days 7 and 14, respectively

IV. CONCLUSIONS

According to the experimental characterization presented in this paper, it is clear that the DMM system has the capabilities of develop biological microfluidic system that can promote cell attachment and proliferation. This preliminary investigation gives way for complex micro-architectural design for biological applications.

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