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A NOVEL AUTOMATION SYSTEM FOR MICROPLASMA SURFACE PATTERNING AND BIOLOGICS PRINTING

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ABSTRACT

In the field of tissue engineering, regenerative medicine, and life sciences, the topological biochemical cues regulate cell attachment and alignment within the construct. In a native biological system, these cues are inherent. However, most of the biological materials utilized in the fabrication of tissue construct do not possess the appropriate cues required to develop an architecture to support the cell attachment and growth of a functional tissue Therefore the ability to manipulate structural and biochemical cues plays an important role in biofabrication process, and it is a key element to evaluate a engineered cellular model. Plasma surface functionalization and biologics printing have been investigated and validated as two effective techniques to guide cell functions by creating microenvironments. The objective of this work is to develop a novel dual functional platform for freeform microplasma surface patterning and biologics printing process as well as to study the underlying process science and the process induced cellular functions. The microplasma jet system was assembled by two parts. The upper part is a plastic NPT connector surrounding an extending high voltage copper electrode. The lower part is a dielectric Pasteur pipette connected with a capillary micro-scale nozzle tip. The lower part is interchangeable and the diameter of the tip ranges from 50 μm to 1 mm. With up to 20 kV output capability, a high-voltage power supply was connected to the copper electrode through the NPT connector which also served as gas inlet. A high-voltage probe linked to an oscilloscope is used to monitor the real time voltage. The whole microplasma jet system was set up on automation platform, which allows X-Y-Z motion control and switch control. This integrated system operates at atmospheric pressured environment. All tissue constructs could be fabricated at room temperature without the use of a mask. Clear polystyrene microplates were used as plasma treatment substrates. After O_2-He mixed microplasma treatment, 7F2 mouse osteoblastic cells were cultured in the microplates for cell biology studies. We demonstrated the capability of our dual functional platform by applying microplasma in the polystyrene wells and control group (without any treatment) in other wells of the same microplate substrate. The results show that the microplasma treatment changed the surface properties and improved cell attachment. This dual functional freeform system allows for surface patterning and printing of cells, proteins, growth factors, etc. to fabricate three-dimensional tissue constructs.

Keywords: biofabrication, cell printing, microplasma, surface patterning, tissue engineering

1. INTRODUCTION

Biologics printing has been widely acknowledged as an effective way to build two-dimensional (2D) or three dimensional (3D) bioengineered structures from assembling living and non-living materials [1]. The advantages of using biologics printing lie in three ways: (1) the integration of cells, biomaterials and other biologics, e.g. proteins; (2) the spatial control of cell distribution; (3) the automated, consistent and reproducible process [2]. However cell behavior varies on different biomaterial due to the material's inherent physical properties, especially surface properties. The modification of physical, chemical and biological properties of a biomaterial surface could enhance cell attachment, proliferation and differentiation. Microplasma surface functionalization is a novel technique to guild cell functions by changing surface energy and surface chemistry, to create microenvironments [3][4]. In this study we present a novel dual functional platform for freeform microplasma surface patterning and biologics printing process as well as to study the underlying process science and the process induced cellular functions. The goal is to design a novel plasma-based surface treatment device applied to the current cell printer, to develop an engineering model to predict the degree of microplasma surface functionalization and also to study the effects of surface functionalization on cell biology.

2. MATERIALS AND METHODS

Following principles of rapid prototyping technology, a Multi-nozzle Deposition Manufacturing (MDM) System is set up and introduced in this study. This system is able to perform both micron-scale spatial and temporal control on generating cell-laden constructs. Cell-laden material is loaded into a material reservoir mounted to a stage on a 3-axis motion arm. Pneumatic pressure extrudes the fluid through a nozzle tip onto a substrate. The system supports up to four separate chambers for printing biopolymer gel, cell aggregates, protein, and other biological factors. A microplasma jet system is added to the MDM system for a surface functionalization process before or after printing. The microplasma jet system was assembled by two parts. The upper part is a plastic NPT connector surrounding an extending high voltage copper electrode. The lower part is a dielectric Pasteur pipette connected with a capillary micro-scale nozzle tip. The lower part is interchangeable and the diameter of the tip ranges from 50 μm to 1 mm. With up to 20 kV output capability, a high-voltage power supply was connected to the copper electrode through the NPT connector which also served as gas inlet. A high-voltage probe linked to an oscilloscope is used to monitor the real time voltage. A schematic diagram of the overview of the automation system with microplasma nozzle configuration is shown in Figure 1.

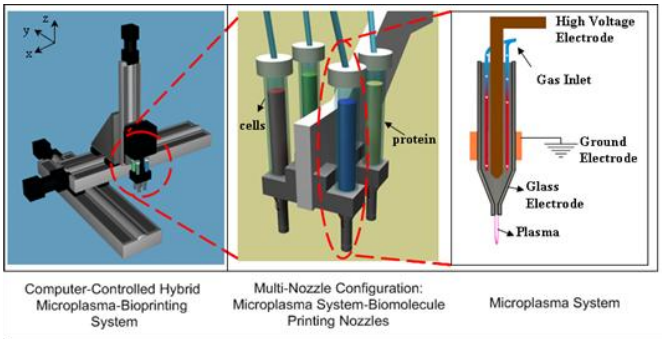

Fig. 1 Overview of System Configuration of 3D MDM System

For the process operation of the motion system, a user-friendly CAD/CAM development platform is integrated with solid freeform automation to assemble biologics in three-dimensional space. Rather than coding for pattern, one can import a STL file which is created in any common CAD/CAM software into the system and automatically receives a trajectory preview. The process flowchart can be found in Figure 2.

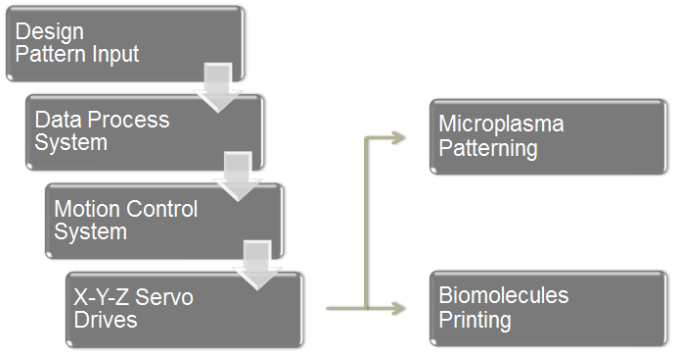

Fig. 2 Process Flowchart of 3D MDM System

In this study, clear polystyrene microplates (24 Well Cell Culture Microplates, Corning Life Sciences) are used as plasma treatment substrates. O_2 and He mixed gas was used as to generate plasma. After microplasma treatment, 7F2 mouse osteoblastic cells were cultured in the microplates for 6, 12, 18 and 24 hours separately.

3. RESULTS AND ANALYSIS

In previous studies we have determined that 1% O_2 (0.5 slpm He, 5 sccm O_2) was the optimal gas composition for plasma treatment. We demonstrated the capability of our dual functional platform by applying microplasma on the polystyrene substrate with nozzle moving speed of 0.1, 0.2, 0.5mm/s and a control group (without any treatment) in different wells of the same microplates. MarkerGene™Live:Dead/Cytoxicity Assay Kit was used to analyze the cytotoxicity of the plasma-treated area. Live/Dead solution was created from the propidium iodide according to Manufacture's protocols. The damaged cells would be marked red and the intact cells would be marked green, fluorescently by

carboxyfluorescein dye. Alamar Blue Kit (Serotec) was used to determine cell proliferation rate. Quantitative data for cell viability and proliferation were collected using the microplate reader (GENios, TECAN, North Carolina, USA). Figure 3 shows that in the treated wells cell growths can be observed but in the untreated wells very low density of cells can be found because most cells were eliminated during the regular cell culture process due to low attachment to the substrate surface. For example, changing cell culture media caused a very high cell loss, because the cells were in suspension in old culture media and were then replaced by new media. The results show that the microplasma treatment changed the surface properties and improved cell attachment. The minor difference between moving speeds did not affect the viability and proliferation of the cells due to the small scale. Fluorescent images were taken on 6, 12, 18 and 24 hours separately. Figure 4 further validates that cells are inclined to adhere to the plasma patterned area. The microplasma treatment on the polystyrene surfaces resulted in oxygen related functional groups such as C-O, C=O, O=C-O. With the increase of oxygen, the hydrophilicity and wettability on the surface was increasing, which caused the improved attachment of cells on the surface [5].

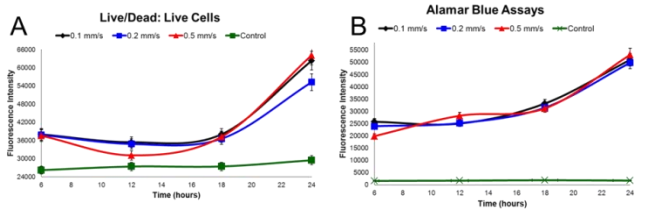

Fig. 3 (A) Cell viability results from LIVE/DEAD® assays and (B) cell proliferation results from alamarBlue® assays

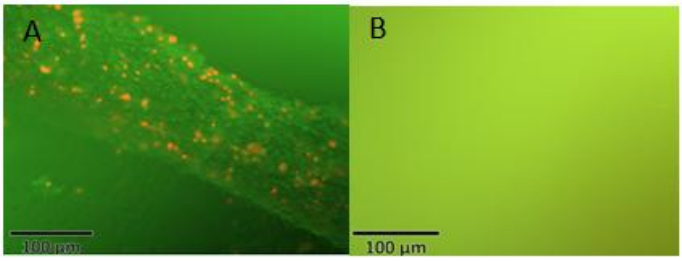

Fig. 4. Fluorescent images of (A) cells on microplasma patterned area and (B) control group on 24 hrs, magnification: 10X

Scanning Electron Microscopy (SEM) images were taken after 6 hours incubation. Figure 5 presents the SEM images of cells well spread and attached to the functionalized surface while no cells were found in the untreated area.

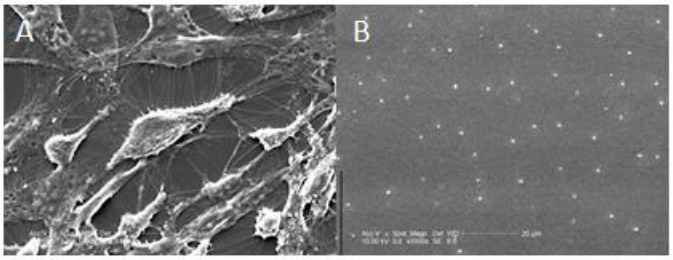

Fig. 5. Scanning Electron Microscopy (SEM) images of (A) cells on microplasma patterned area and (B) control group, magnification: 1000X

4. CONCLUSIONS

A dual functional freeform microplasma surface patterning and biologics printing device has been developed. Microplasma surface patterning has a positive effect on cell attachment and proliferation. Future work will include the characterization of the surface chemistry change and the direct effects on cell behaviour. The mathematical model of the printing process also is under development.

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