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Manufacturing of meso-fluidic devices for life science application using biocompatible MJF process

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Multi Jet Fusion (MJF) is a recently developed industrial 3-dimensional (3D) printing process that produces functional prototypes and end-use production parts. This printing method is used for the production of biocompatible materials for research focusing on life science and invitro diagnostics. Polyamide - nylon 12 (PA-12) is the most tested material for additive manufacturing. The meso-fluidic device manufacturing are evaluated based on printability, surface condition, product design and development. The invitro biocompatibility of MJF-printed PA-12 was investigated as a preliminary step to verify its potential as a bioreactor. In addition, the printed PA-12 was also explored for developing assays such as for serological testing for the invitro detection of enzyme-linked immunosorbent assay (ELISA) coronavirus disease 2019 (COVID-19) antibodies. Depending on the application, several chip designs were developed and fabricated.

1. Introduction

Meso- and microfluidics or lab-on-a-chip is an interesting technology suitable for manufacturing devices characterized by chambers and fluidic channels geometrically restricted to tens micrometers to few millimeters. This interdisciplinary field emerged rapidly as a combined output of several areas such as engineering, microtechnology, chemistry, physics, biotechnology, and materials science. Meso- and microfluidics platforms develop miniaturized analytical models for a wide variety of applications [1-3]. The advantages of such miniaturized devices include decreased consumption of samples and reagents, reduced duration of the experiments, low cost, disposability, and reduction or elimination of cross contamination. Integrated meso- and microfluidics platforms have been adopted in scientific research labs for over two decades spanning a wide range of applications. The capability of these devices has increased dramatically mainly due to the invention of soft lithography and microfluidic large-scale integration and beginning to realize its immense potential for research in life science and medicine.

Three-dimensional (3D) printing is a fabrication method that has received considerable attention in the last few years. This technique deposits successive layer of materials on top of one another and constructs complicated pre-defined shapes from a computer-aided model, till the object is manufactured entirely. Different types of 3D printing techniques have been realised [4]. Powder based printing has come across as the most versatile method which uses raw material in powder form and employs wide range of materials . Multi-jet fusion (MJF) technology, a subset of powder bed fusion, was recently introduced into the market by Hewlett Packard (HP) Inc. In MJF, the printing process is carried out by multiple inkjet heads that independently recoats materials and distributes agent. Separate head arrays move across the print bed in different directions and thereby assist in heating and printing processes simultaneously. The resulting 3D-printed construct is then subjecting to bead blasting and other post processing methods for the material to be suitable for specific use [1, 2, 5-8].

One major challenge associated with a newly printed material is its ability to be biologically compatible with an appropriate host response that includes normal healing process, resistance to bacterial colonization, and preventing the formation of blood clots. Therefore, biocompatibility of a material is an important property to be considered and carefully assessed before being considered for real world biomedical use through in vitro and in vivo assessments [9-12]. Usually as an initial analysis, mammalian cells are cultured on the

3D-printed materials and cell response such as adherence, proliferation and differentiation studied in case the leachate or extractables exhibit a negative impact on the cells, in which case a material cannot be characterized as biologically compatible. Porosity is another key factor defined as an architecture with interconnecting open spaces. Porosity is taken into consideration in biomaterial development and characterization. Many properties are directly correlated to the porosity of a given material. For instance, the presence of material voids allows infiltration of cells, and outward diffusion of pharmaceutical agents. Consequently, cells and active agents fail to contact the material surface long enough to remain biocompatible or become incompatible. Biomaterial porosity also results in increased biofilm formation. 3D printing has emerged as a powerful technology in the past decade especially in the manufacturing sector. With rising trends in digitalized technologies, additive manufacturing remains to be of huge benefit to scientists, researchers, industrialists, and entrepreneurs. Many types of additive manufacturing processes have been introduced.

2. 3D printing manufacturing and preparation of meso-fluidic device

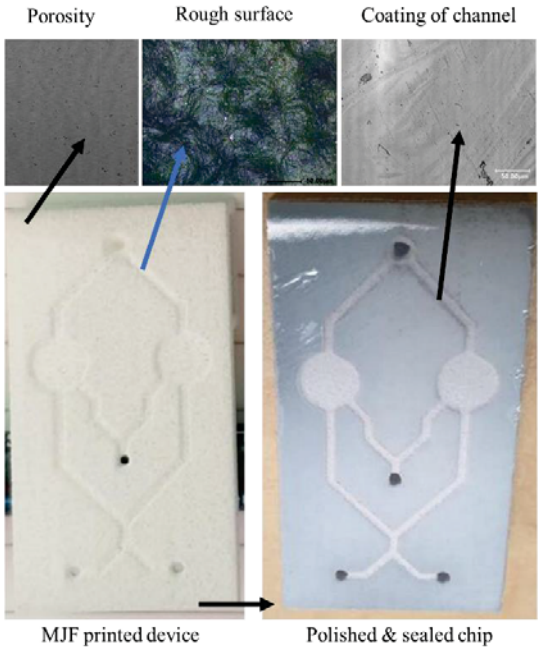

Fig. 1 MJF printed meso-fluidic device preparation and surface characteristic.

MJF is a powder-based 3D printing technology unveiled by HP Inc. in 2016. As a leader in microfluidics, HP Inc. 2D (2 dimensional) and 3D technologies supports the ability to place billions of drops of ink or agents per second with accuracy. HP exploits the use of this technology in inkjet printers as well as for MJF 3D printing. In this study, PA-12 material printed (test substrate and meso-fluidic device) by MJF was used for biocompatibility and enzyme-linked immunosorbent assay (ELISA) detection. The biocompatibility of the MJF process and existing materials used in the process are examined. The MJF 3D printing process and post-processing are initially found biocompatible. Further, in the detailed biocompatible investigation is planned for PA-12 material. Figure 1 shows the MJF printed meso-fluidic device and its surface characterization. MJF 3D printed PA-12 showed a porosity of 0.5%, with surface roughness of inside microchannel being 23 to 25.4 µm whereas that of outside microchannel was approximately 15-18µm. The meso-fluidic device with open channels have been printed successfully with a minimum of 500 µm width and 500 µm depth.

2.1 Biocompatibility

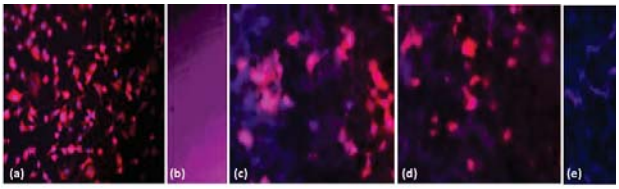

Fig. 2 Representative fluorescence images showing (a) L929 cells on pure cast PA 12 (b) Control pure PA 12 without cells and MC3T3E1 cells on 3D-printed PA 12 substrates (c) without coating (d) with polystyrene coating (e) Control 3D-printed PA12 without cells.

3D-printed PA-12 substrates were tested for cell adherence and viability under several coated and non-coated conditions (only PA-12 substrates and control plates were coated) using L929 fibroblasts and MC3T3E1 osteoblasts. Figure 2 shows the adherence of L929 and MC3T3e1 on 3D-printed PA12. These data proved that cell growth on PA-12 is reproducible. Cells can attach and grow on hydrophobic surface of PA-12 without the help of plasma-treatment, though to a marginally lesser extent than plasma-treated ones. Moreover, fluorescence microscopy is a feasible and reliable method for examination of cells cultured on opaque substrates (e.g 3D-printed PA-12) not observable by transillumination bright-field microscopy. Fluorescence images indicated cell growth on 3D-printed PA-12 substrates with/without plasma treatment, poly-d-lysine, collagen and polydopamine coating. All samples showed cell growth after Day 2 and day 4 respectively, however, collagen-coated samples exhibited a well-spread broad morphology in comparison to other substrates. Cells grown on substrates coated with polystyrene demonstrated very good attachment similar to conventional polystyrene dishes. A striking observation was that the bacterial viability on 3D-printed polyamide - nylon 12 (PA-12) substrates was slightly higher than that observed on conventional culture plates, that remarkably facilitated the subsequent bacterial adhesion. No inhibiting effect of PA-12 on the growth, adhesion, and viability of the bacterial cells was observed.

2.2 ELISA

Meso- and microfluidic devices have been used in point-of-care (POC) diagnostics due to its portability and undemanding requirement of sample quantity. Existing methods and materials used for making microfluidic chips range from conventional manufacturing processes using polydimethylsiloxane (PDMS) to 3D-printed materials.

Conventional production methods are slow and costly. While 3D-printing offers marked improvement on speed and cost, not all 3D-printing methods are the same with regard to the advantages they offer. Such differences offer an opportunity for us to leverage on the advantages of certain method, such as MJF, to address existing challenges faced by other production methods.

Our results indicated that MJF-printed PA-12 ELISA chips (see figure 1) when lacquer-painted, not only tackled the liquid seepage though the chip but also served as potential substrates for detection of human and mouse coronavirus disease 2019 (COVID-19) antibodies. However, several leakage issues and drop in liquid volume were encountered at different stages of running ELISA experiments. For the chip leakage issue, the vacuum channel has been redesigned. With lacquer coated top surface, the new batch of chip proved it has solved the first type of leakage. For leakage due to direct blunt needle injection, it is solved by using direct tubing connection method.

2.2 Other meso-fluidic device example

Throughout the development of different novel meso- and microfluidic devices, incorporating more novel functionalities in different types of meso- and microfluidic devices has been incorporated with the help of HP MJF 3D printer. Figure 3 shows few examples of meso-fluidic devices printed by MJF 3D printer.

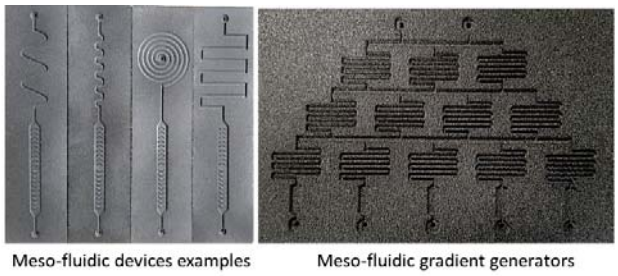

Fig. 3 MJF printed meso-fluidic device examples.

3. Conclusions

The MJF is capable of biocompatible component manufacturing with great reproducibility. The minimum feature size 500 μm is found best repeatability. MJF is found to be capable of printing various 2D and 3D meso-fluidic devices. The MJF printed meso-fluidic devices are able to seal with thermal table, transparent glass and PDMS. The surface of MJF printed microfluidic devices is possible to improve by polishing, chemical etching, plasma treatment and coating. Overall progress of MJF 3D-printed meso-fluidic devices show satisfactory improvement.

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