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DOI: 10.1557/adv.2017.21

Multi Material 3D Scaffold Printing with Maskless Photolithography

Joyce Tam[1] and Ozlem Yasar[1]

[1] Department of Mechanical Engineering Technology, New York City College of Technology, Brooklyn, NY 11201, USA.

ABSTRACT

In today's technology, organ transplantation is found very challenging as it is not easy to find the right donor organ in a short period of time. In the last several decades, tissue engineering was rapidly developed to be used as an alternative approach to the organ transplantation. Tissue engineering aims to regenerate the tissues and also organs to help patients who waits for the organ transplantation. Recent research showed that in order to regenerate the tissues, cells must be seeded onto the 3D artificial laboratory fabricated matrices called scaffolds. If cells show healthy growth within the scaffolds, they can be implanted to the injured tissue to do the regeneration. One of the biggest limitation that reduces the success rate of tissue regeneration is the fabrication of accurate thick 3D scaffolds. In this research "maskless photolithography" was used to fabricate the scaffolds. Experiment setup consist of digital micro-mirror devices (DMD) (Texas Instruments, DLi4120), optical lens sets, UV light source (DYMAX, BlueWave 200) and PEGDA which is a liquid form photo-curable solution. In this method, scaffolds are fabricated in layer-by-layer fashion to control the interior architecture of the scaffolds. Working principles of the maskless photolithography is, first layer shape is designed with AutoCAD and the designed image is uploaded to the DMD as a bitmap file. DMD consists of hundreds of tiny micro-mirrors. When the UV light is turned on and irradiated the DMD, depending on the micro-mirrors' angles, UV light is selectively reflected to the low percentage Polyethylene (glycol) Diacrylate (PEGDA) photo-curable solution. When UV light penetrates into the PEGDA, only the illuminated part is solidified and non-illuminated area still remains in the liquid phase. In this research, scaffolds were fabricated in three layers. First layer and the last layer are solid layers and y-shape open structure was sandwiched between these layers. After the first layer is fabricated with DMD, a "y-shape" structure was fabricated with the 3D printer by using the dissolvable filament. Then, it was placed onto the first solid layer and covered with fresh high percentage PEGDA solution. UV light was reflected to the PEGDA solution and solidified to make the second and third layers. After the scaffold was fabricated, it is dipped into the limonene solution to dissolve the y-shape away. Our results show that thick scaffolds can be fabricated in layer-by-layer fashion with "maskless photolithography". Since the UV light is stable and does not move onto the PEGDA, entire scaffold can be fabricated in one single UV shot which makes the process faster than the current fabrication techniques.

INTRODUCTION

In recent years, tissue engineering has been developed as an alternative approach to the organ transplantation. Primary objective of tissue engineering is to develop biological substitutes that restore, maintain, or improve function of tissue or whole organ [1-4]. In this promising field, firstly cell supportive structures called scaffolds are designed and fabricated. Once scaffolds are designed in the computer environment and fabricated in the laboratory environment, they are seeded with the cells. If the right bio-chemical environment are provided to cells that are seeded to the scaffolds, cells can expand and proliferate on the scaffolds to form a fully functional tissue [5].

Ideally engineered scaffolds must have an inbuilt oxygen and nutrients delivery networks to mimic the vasculature systems. They can be fabricated by using biocompatible materials. After the cell seeding process, if cells show healthy growth, cell seeded scaffold can be implanted to tissue to do tissue or organ regeneration. If biodegradable material is used for the fabrication process, after the scaffold implantation, scaffolds can degrade away without producing any kind of toxic materials [6, 7].

Currently; heat, light, mold and adhesives are being used to fabricate the 3-D scaffolds [8]. In this research, maskless photolithography, which is a light-based fabrication technique, was used to fabricate the scaffolds in layer-by-layer fashion. With this technique, complex structures that are as small as 25µm can easily be fabricated. This technique also does not depend on the intermediary file transfer format which enables to fabricate the complex patterns within seconds [9].

EXPERIMENT

Poly(ethylene glycol) diacrylate (PEGDA) preparation for maskless photolithography

In this study, photo-curable solution PEGDA with the molecular weight Mn 700; photo-initiator 2,2-dimethoxy-2-phenylacetophenone, and solvent chemical 1-vinyl-2-pyrrolidinone was purchased from Sigma-Aldrich, MO, US. First of all, photo-initiator was dissolved within the solvent to prepare 100mg/mL solution [10]. Then, 5ml of 20% PEGDA was prepared by mixing distilled water with PEGDA in 1:4 ratio. After that, final photo-curable chemical solution was prepared by adding 60µl of photo-initiator-solvent mixture to 5ml of 20% PEGDA.

Multi material 3D scaffold printing with maskless photolithography

As shown in Figure 1., scaffold fabrication set-up consist of digital micro-mirror device (DMD), photo-curable solution and optical light correction elements. UV light source (UV-A) with the 365nm wavelength is collimated through the optical lens sets. UV light irradiance at 15 cm is 2.3mW/cm^2. In this technique, in order to control the scaffold architecture, scaffolds were fabricated in layer-by-layer fashion.

https://doi.org/10.1557/adv.2017.21

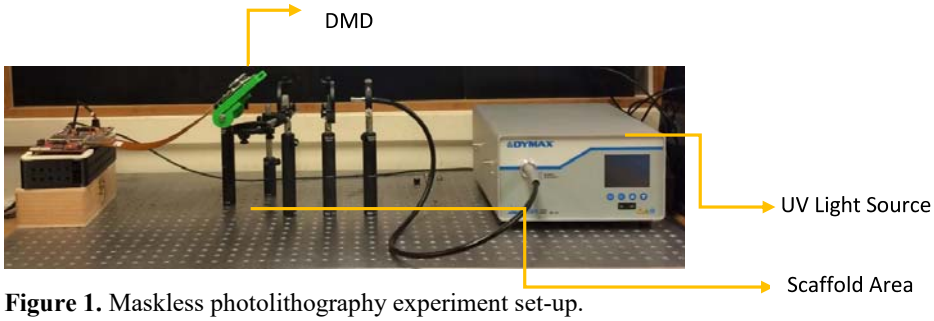
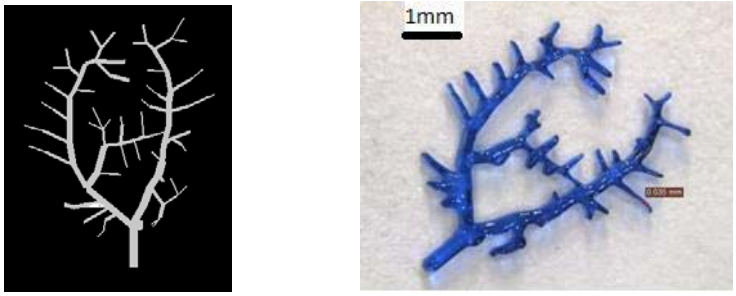


Figure 1. Maskless photolithography experiment set-up.

In this technique, layer architectures were designed with the computer and bitmap image of the designs are sent to the DMD. DMD consists of hundreds of individually tiltable and controllable tiny micro-mirrors. Each white pixel on the .bmp image represents the "on" state and each black pixel on the .bmp image represents the "off" state of the micro-mirrors [10]. Only the on mode micro-mirrors reflect the illumination from the UV light to the PEGDA photo-curable solution. Therefore, the bitmap images instruct the mirrors to selectively pass UV light based on their on or off modes [11]. As a result of interaction between the UV light and the PEGDA, illuminated area gets solidified and non-illuminated area remains liquid [12, 13]. One of the biggest advantages of this technique is, as shown in Figure 2., very complex shapes can be designed and accurately fabricated within seconds.

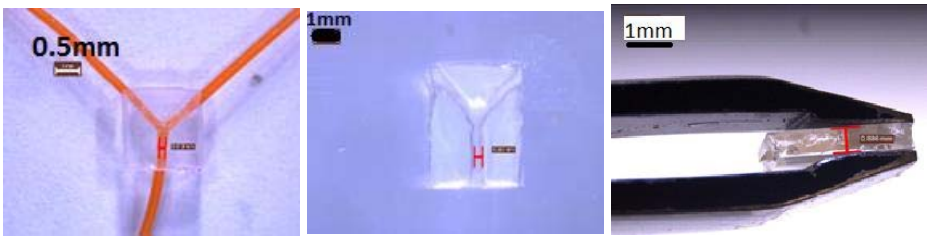


(a) (b)

Figure 2. (a) Computer design of branching networks (b) PEGDA based hydrogels fabricated with Maskless Photolithography

In this research, scaffolds were fabricated in three layers. First layer and the last layer are solid layers and y-shape structure was sandwiched between these layers. After the solid-first layer is fabricated with DMD, a "y-shape" structure was fabricated with the 3D printer by using the dissolvable filament. Then, it was placed onto the first solid layer and covered with fresh high percentage PEGDA solution. UV light was reflected to the PEGDA solution and solidified to make the second layer. In the last step, in order to fabricate the third layer, more fresh PEGDA solution was added to the second layer and it was also solidified to close up the y-shape. UV

exposure time was for each layer was 5 seconds. Figure 3. shows the three layers-fabricated scaffolds.



(a) (b) (c)

Figure 3. (a) y-shape dissolvable filament is sandwiched between the solid layers (b) Top view of PEGDA hydrogel fabricated with Maskless Photolithography (c) Side view of 3 layers PEGDA hydrogel that is hold with the tweezers.

After the scaffold was fabricated, it is dipped into the limonene solution to dissolve the y-shape away. In order to hasten the dissolving process, fabricated scaffold was placed on a vibrating plate as shown in Figure 4. Vibration, enabled the interaction between limonene and dissolvable y-shape filament. The entire filament we dissolved away within 5 hours.

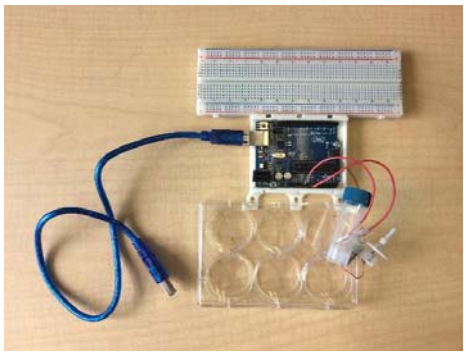


Figure 4. Test tube rotator used to expedites the dissolving process.

After the fabrication process is completed, measured channel with vs. percentage error graph was drawn to show the sample reproducibility as presented in Figure 5. Theoretical channel width is 200µm.

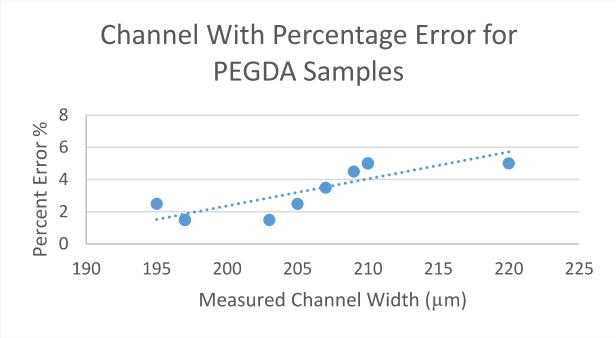


Figure 5. Percentage Error of channel width for PEGDA samples.

DISCUSSION

In today's technology, scaffolds are fabricated with using "light", "mold", "adhesives" or "heat". In this research, 5mm x5mm x 1mm cubic shaped scaffolds were fabricated with "maskless-photolithography" which is highly advantageous light-based fabrication method. Some of the benefits of using this technique are:

- In this technique UV light wavelength is 365nm it enables the cell encapsulation,
- Entire scaffold layer is obtained in one single UV light exposure. In this research fabrication time was 15 seconds which is relatively fast when it is compared with the other techniques,
- Complex shapes can be photopolymerized with the use of bitmap slices and
- No use of expensive masks. Channels with the width of 25µm can be fabricated with DMD only. In that case, since the 3D printer are not needed in the fabrication process, the major cost would be the only cost of the chemicals which is relatively low compared to mask or micro-scaled 3D printers.

Some limitations of this technique are, due to hydrogel swelling, feature stability and precise deposition of biomaterials are difficult to control [14]. Also, UV light may affect the cell viability but UV exposure time and UV light intensity can be controlled easily by changing the PEGDA and photo-initiator concentration. Interior structure dimensions can not be any smaller than the resolution of the 3D printer. The smallest dimension for the channels are in the order of 200µm. On the other hand, with this technique, any kind of networking channels that can be fabricated with the 3D printer can be used to generate the material transport routes.

CONCLUSIONS

In the past decades, "tissue engineering" has been studied to find an alternative solutions to the organ transplantation. However, design and 3-D fabrication of scaffold has been always a challenging part of this promising field. In order to help cells to show a healthy growth after the

cell seeding process, scaffolds must be fabricated precisely with the inbuilt material delivery networks. In this research, scaffolds were fabricated with "maskless photolithography" to shape and control the interior designs. Firstly, y-shapes were generated with the 3D printer, and then it was sandwiched between the first and third hydrogel layers. In the last step, 3D printed y-shapes were completely dissolved away within hours by using the limonene solution.

Future work will involve to test the effects of fabrication parameters, which are PEGDA concentration, photo-initiator concentration, UV light intensity and UV exposure time, on cell viability. Also, with the purpose of reducing the inner shape dimensions, automated elevator system will be designed to decrease the layer thicknesses.

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https://doi.org/10.1557/adv.2017.21