EPJ Web of Conferences **266**, 13005 (2022) https://doi.org/10.1051/epjconf/202226613005
EOSAM 2022

Multi-well platform manufacturing combining stereolithography and pulsed laser ablation for cellular studies

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Abstract. Novel cell culture platforms, with more physiological surface roughness, require different technologies capable of precisely micropattern substrates. 3D printing offers a considerable accuracy and user-friendly procedures. For its part, pulsed laser ablation proves to be a versatile technology to perform detailed surface micropatterning. In this work, both technologies were combined to easily fabricate a versatile PDMS multi-well platform for performing cellular studies on a micropatterned biocompatible surface.

1 Introduction

Surface micropatterning of culture platforms is of great interest to obtain novel properties. Recent studies have found out how different micropatterns can offer antibacterial properties, improve hydrophobicity for self-cleaning, and even control cell attachment [1]. In the latter case, superficial microstructures help to obtain more physiological platforms than the normally used in cellular studies (with polished surfaces), capable of mimic the roughness of tissues or bones [2] to perform cell cultures.

Optical technologies have been traditionally used to micropattern different materials and until now, the most used optical technology has been photolithography, characterised by complex time-consuming protocols. This technology allows the precise micropattern of photoresists in 2D but produces highly polluting chemical waste that can be harmful for the cell cultures. Therefore, in recent years, the rapid development in laser applications has favoured the appearance of different technologies that can be used to microstructure diverse substrates in a more user-friendly way. This is the case of Pulsed Laser Ablation (PLA), that allows to achieve outstanding resolutions (in the micron range) when micropatterning 2D surfaces. On the other hand, 3D printers based on the selective Stereolithography (SLA) of liquid resins have facilitated the production of 3D objects with great structural complexity (in the millimetre range).

In this work, a multi-well platform is manufactured combining both technologies in each of their most suitable dimensional range. This platform can be easily used to perform cell attachment studies mimicking different surface roughness and patterns.

2 Materials and Methods

A Formlabs Form 3B SLA printer was used in this work to manufacture the multi-well master. SLA printers have a tank that contains the liquid printing resin coupled with a laser that performs a selectively layer-by-layer photopolymerization. Commercial Model resin from Formlabs was selected as printing resin given the high precision it offers, its good response to laser ablation, and its good performance when replicating polymers.

PLA of the 3D printed substrate was performed using a Rofin Nd:YVO_4 laser. This laser operates in Q-switch regime with 20 ns pulse duration and 1064 nm fundamental wavelength. The laser setup was combined with a galvanometer system and a flat field lens (f=160 mm) that focus the laser with an homogeneous energy distribution in a 120x120 mm^2 area.

Polydimethylsiloxane (PDMS) was selected as soft lithography polymer for replication of the substrate given its biocompatibility and optical transparency to visible and UV light. The polymer was prepared from Sylgard 184 elastomer by mixing the monomer and the curing agent in a ratio 10:1. Once the mixture covered the substrate, it was cured at 60 ºC during 12h in an oven.

3 Results and Discussion

3.1 Achievable micropatterns

By PLA, it is possible to create a series of micropatterns in the resin, to be later replicated by PDMS soft lithography. Several ablation studies were performed

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on Model resin surface to find the proper laser parameters and the achievable geometries, using different arrays of straight lines that were designed using a 2D Computer-Aided Design (CAD) software and micropatterned on the surface of the resin. Then, 700x700 μm^2 confocal images were taken to perform surface inspection (Fig. 1).

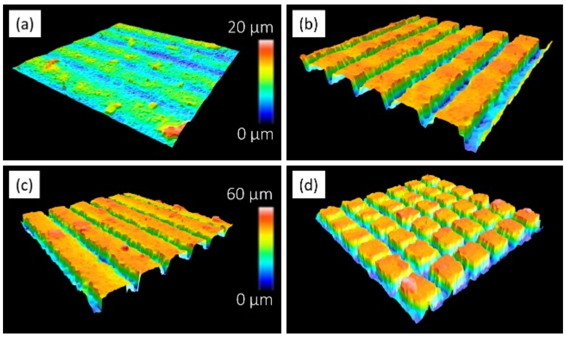

Fig. 1. Confocal images of a) unpatterned surface (control), b) and c) straight channels and d) matrix of square pillars micropatterned on the resin surface by PLA. The scale bar is the same in b), c) and d).

In the first place, a surface was left unpatterned (Fig. 1a) to serve as a control, as is usual in cellular assays. A smooth waviness of the surface was observed, with an amplitude of approximately 5 µm. This periodic behaviour has its origin in the layer-by-layer photo-polymerization working principle of stereolithography, and will be present on all printed surfaces, with the same orientation and period.

In the second place, regular straight channels of 70 µm width, 45 µm of height and 120 µm of period can be manufactured using an average power of 4.5 W, a repetition rate of 10 kHz, a velocity scan of 50 mm/s and 3 repetitions. Two orientations were selected: perpendicular (Fig. 1b) and parallel (Fig. 1c) to the background waviness of the resin, with the intention of analysing its influence on the surface finish. No obvious differences were observed, which makes sense given the large difference in magnitude between the two.

Furthermore, a matrix of rectangular pillars (Fig. 1d) can be obtained by crossing the previous lines and using the same parameters. The same dimensions were obtained.

3.2 Multi-well platform manufacturing

Once the proper laser parameters are found, manufacturing process starts with the SLA 3D printing of the multi-well master. This master consists of 35 mm diameter disk that featured four cylindrical elevations of 8 mm in diameter and 2 mm in height (Fig. 2a). A circular wall was added around the master to avoid replication polymer losses. In the next step, PLA of the upper part of the elevations was performed, using the previous introduced parameters and geometries (keeping a unpatterned well as control). The PDMS was then deposited over the master structures forming a sheet of 3 mm (Fig 2b) and introduced in an oven. Next, the PDMS multi-well platform was peeled off from the master, replicating the four different patterns made by PLA (Fig. 2c). Finally, the platform was placed at the bottom of a 6 well plate (Fig. 2d) commonly used to perform cell attachment studies.

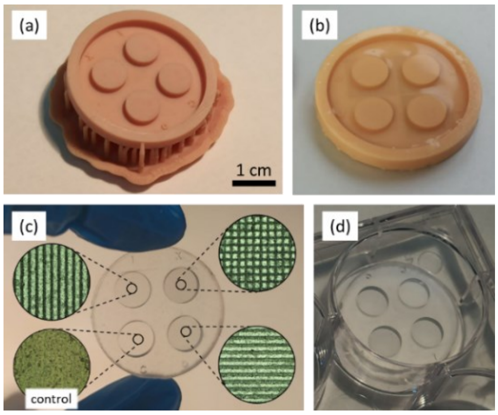

Fig. 2. a) Multi-well master 3D printed in Model resin. b) Soft lithography process in which the master is filled with liquid PDMS. c) PDMS multi-well platform with different replicated micropatterns in the bottom of each well. d) Multi-well platform coupled with a common 6 well plate ready to perform cellular studies.

4 Conclusions

Novel cell culture platforms, with more physiological surface roughness, require different technologies capable of manufacturing very precise structures reliably and accurately. 3D printing has the potential to revolutionize these areas, abridging manufacturing processes through simple procedures and very low waste production. For its part, laser ablation is a versatile and much easier technique than photolithography for making detailed surface micropatterns in the micron range.

In this work, both technologies were combined (taking advantage of each in the range of dimensions in which they show most suitable results) to easily fabricate a versatile PDMS multi-well platform for performing cellular studies on a micropatterned biocompatible surface. Some geometries were presented, but the proposed manufacturing process allows to obtain a wide range of different configurations in a simple way, depending only on the needs of the user.

This work has been supported by contracts: AEI RTI2018-097063-B-100 and Consellería de Educación Xunta de Galicia/FEDER ED431B 2020/29. B. Carnero acknowledges GAIN/Xunta de Galicia for contract 11-IN606D-2021-2604925. Ana I. Gómez-Varela acknowledges Consellería de Cultura, Educación e Ordenación Universitaria for ED481D-2021-019 postdoctoral fellowship.

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