

FABRICATION OF THREE DIMENSIONAL TISSUE ENGINEERING POLYDIMETHYLSILOXANE (PDMS) MICROPOROUS SCAFFOLDS INTEGRATED IN A BIOREACTOR USING A 3D PRINTED WATER DISSOLVABLE SACRIFICIAL MOULD.

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ABSTRACT

We present a new scalable and general approach for manufacturing structured pores/channels in 3D polymer based scaffolds. The method involves 3D printing of a sacrificial polyvinyl alcohol (PVA) mould whose geometrical features are designed according to the required vascular channel network. Polydimethylsiloxane (PDMS) polymer is cast around the PVA mould, cross-linked and then the mould is dissolved, leaving behind a structured porous PDMS scaffold. The fabrication method described here is demonstrated with silicone elastomer but various other natural and synthetic polymers are compatible with this fabrication technique

KEYWORDS: Scaffold, 3D printing, Bioreactor, Elastomeric

INTRODUCTION

In tissue engineering scaffolds play a major role to provide structural support for cell attachment and subsequent tissue development. For long-term culture, the scaffold material with attached cells has to be continuously perfused for supplying nutrient and oxygen as well as waste removal. It is therefore really crucial to design a proper porous scaffold with desired mechanical properties and with mass transport function to facilitate tissue regeneration¹.

Recently there has been a move towards employing 3D printing as a rapid prototyping technique to fabricate micro-scale porous structures of desired complexities, allowing a true engineering of the scaffold^{2,3}. Processes combining 3D printing and moulding have already been used for making structured 3D scaffolds^{3,4}. Different sacrificial printing materials such as wax⁴ and sugar glass lattice³ have been utilized to form vascular networks. However the use of wax limits the materials that can be cast around the mould to form the scaffold since polymers, requiring higher temperatures for cross-linking, cannot be employed. Sugar glass is difficult to print into large 3D structures as the sugar glass is very brittle, and the inter filament distance (defined by the printing process) is limited to a minimum distance of 1mm. It may therefore not be feasible to use this technique for creating dense vascular channels in large-scale structures for engineering large organs.

In this contribution, we focus on developing a method of fabrication enabling to control the physical, chemical and morphological characteristics of the porous scaffold material by indirect 3D printing of sacrificial polyvinyl alcohol (PVA)⁵.

EXPERIMENTAL

The method involves 3D printing of a PVA mould using a commercial 3D filament printer (Makerbot, USA)⁵. Geometrical features of the mould are designed according to the required vascular channel network. PDMS pre-polymer is cast around the PVA mould, cross-linked and then the mould is dissolved, leaving behind a structured porous PDMS scaffold. The fabrication method is shown schematically in figure 1.I. To render them hydrophilic, the scaffolds were modified with oxygen plasma using a 13.56 MHz RF generator equipped Atto Plasma System (Diener Electronic GmbH, Ebhausen, Germany). The plasma chamber was evacuated to a pressure below 15 Pa, after which oxygen was introduced (pressure stabilization at 30 Pa) and the plasma was ignited (power 50 W) for 2 min on each side of the scaffold.

Human hepatoblastoma (HepG2) cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 growth medium supplemented with 10% foetal bovine serum (FBS, Sigma-Aldrich Chemie GmbH, Switzerland) and 100 µg/ml penicillin and 10 µg/ml streptomycin in a humidified incubator at 37 °C and 5% CO₂. Scaffolds were loaded with a suspension containing 250,000 cells in 20 µl of media according to the procedure described previously⁵ and incubated in a 24 well plate with 1 ml culture media in each well. Medium was refreshed every 2 days.

RESULTS AND DISCUSSION

Scaffolds were fabricated by casting PDMS around sacrificial moulds printed using two different infill patterns (fig.1.II) and four different infill densities to achieve different porosity (not shown here). Photographs and SEM images of the printed moulds and resulting PDMS scaffolds of the two different infill patterns are shown in Fig. 1.II. The high scalability of the method is demonstrated in Fig. 1.II (i & j)

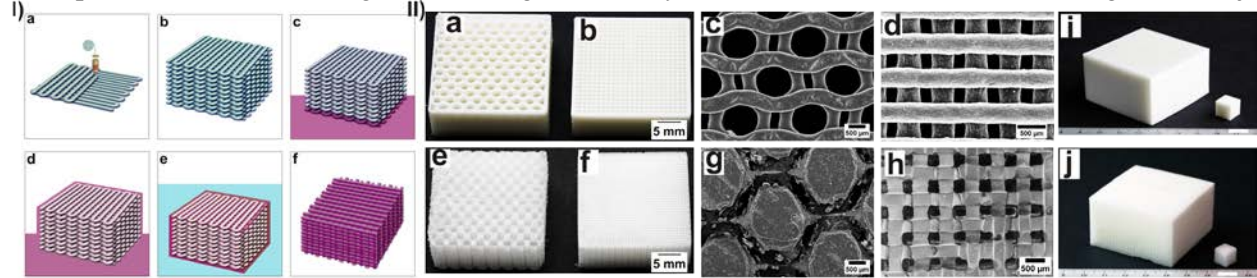


Figure 1: I) Schematic illustration of the steps involved in the fabrication of structured porous elastomeric scaffolds. A sacrificial 3D mould was printed in PVA (a, b). The printed PVA mould was transferred into a container containing pre-cured PDMS (c). Vacuum was applied to ensure complete filling of pre-cured PDMS into the pores of the mould (d). Following crosslinking of the PDMS, the sacrificial PVA mould was dissolved in water (e) leaving behind the structured PDMS scaffold (f). II) Photographs of moulds and scaffolds with hexagonal (a, e) and woodpile (b, f) infill patterns. SEM images of moulds and scaffolds with hexagonal (c, g) and woodpile (d, h) infill patterns. Photograph of: (i) a 50 layered (1 cm³ cube) and 150 layered (75 cm³ cube) 3D printed PVA mould, (j) 50 layered (1 cm³ cube) and 150 layered (75 cm³ cube) PDMS scaffolds replicated from the mould (i). Scale bar in (i) and (j): 1 cm⁵.

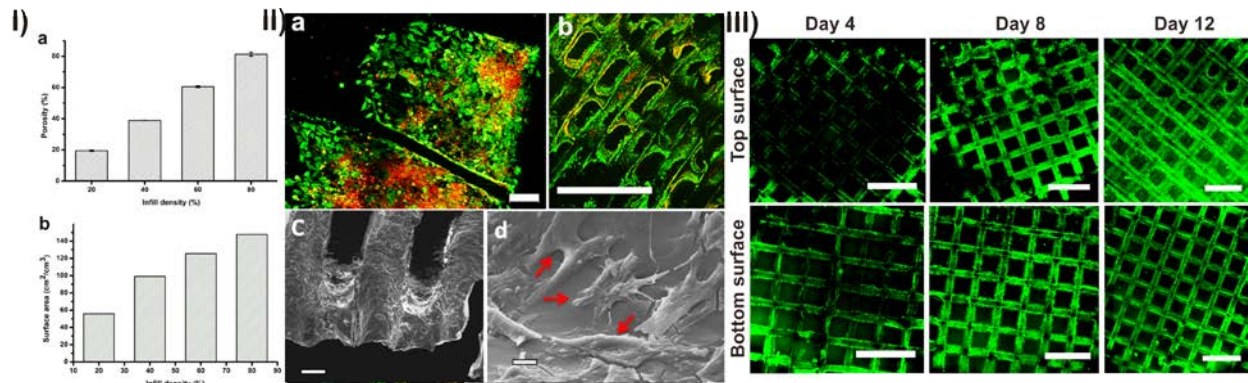


Figure 2: I) Measured porosity (a) and calculated surface to volume ratio (b) of scaffolds fabricated from mould with different infill density (standard deviation (n = 4)). II) HepG2 cell morphology and attachment to PDMS scaffold using immunostaining (a & b) and SEM (c & d). Cell cytoskeleton beta tubulin (green) and nucleus (red). (a) Top surface of the scaffold. (b) Longitudinal cross section of the scaffold. Low- (c) and high- (d) magnified SEM images of HepG2 cells cultured on PDMS scaffolds for 4 days. Scale bar of image (a) 100 µm, (b) 1 mm, (c) 50 µm and (d) 5 µm. III) Live/dead (green = live, red = dead) staining of HepG2 cells grown on top and bottom part of the PDMS scaffold for 4, 8 and 12 days. Scale bars represent 1 mm.

Figure 2. I (a & b) shows physical characterization of the PDMS scaffolds. The experimentally determined porosity of the scaffolds varied linearly as a function of the infill density of the printed mould and

the calculated surface areas of a 1 cm³ fabricated scaffolds of varying infill densities increases from 52.5 cm²/cm³ to 150.9 cm²/cm³. Live/dead staining of the HepG2 cell-scaffold construct on day 12 of culture, showed a confluent layer of live cells on the scaffolds and no dead cells were observed throughout the culture period (fig 2. III)). Immunostaining (fig 2. II a & b) and SEM images (fig 2. II c & e) of the scaffold show well-attached cell morphology with spread-out cell attachment onto the top as well in the centre of the scaffold surface.

As a proof of concept a single perusable bioreactor with an integrated scaffold was fabricated using a 40% porous/infilled PVA mould as shown in the figure 3. In short, after printing the mould was placed into a Petri dish and PDMS solution was poured into it, degassed and cured. The sacrificial PVA mould was dissolved from the cross-linked PDMS and the bioreactor with an integrated porous scaffold part was formed. The reactor was dried at 60° C overnight. The inlet and outlet of the reactor was punched vertically. For better visualization, the PDMS device was bonded to a glass slide using O₂-plasma activation. Before use, the device was degassed and cleaned using 100% ethanol and finally cleaning with water.

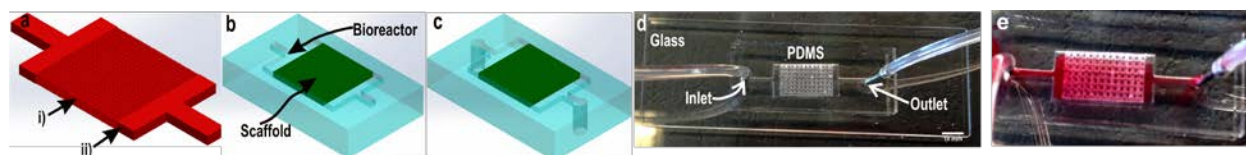


Figure 3. Schematics of fabrication of PDMS bioreactor with integrated scaffolds. a) 3D printed PVA mould using 3D filament printer: (i) indicates the part designed to obtain a porous scaffold and (ii) indicates the design of the bioreactor. The PVA mould was casted with uncured PDMS and cross-linked. b) The sacrificial PVA mould was dissolved, leaving behind a PDMS structure having a chamber with an integrated porous scaffold. c) The integrated device was punched from its inlet and outlet chamber. d) Photograph of the 3D PDMS bioreactor with integrated scaffold bonded to a glass slide and for visualization e) red food dye was loaded into it (E).

CONCLUSION

In this study, we have demonstrated a way for fabricating scaffolds and microfluidics chambers by indirect 3D printing of water dissolvable PVA, and the high scalability of the process is shown. Different designs of PDMS scaffolds with various porosities and surface areas were successfully fabricated and tested for in-vitro cell studies. High cellular viability and homogeneous cellular distribution across the scaffold was achieved for such PDMS scaffolds. Furthermore, the fabrication method can generate bioreactors with integrated porous scaffold materials from PDMS for perfusion. The fabrication method described here is demonstrated with silicone elastomer but various other natural and synthetic polymers can also be compatible with this fabrication technique. In conclusion, the presented process is scalable, bio-compatible, rapid, and inexpensive.

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