Biomaterials 45 (2015) 124–131

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Versatile fabrication of vascularizable scaffolds for large tissue engineering in bioreactor

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ARTICLE INFO

Article history:
Received 25 September 2014
Accepted 20 December 2014
Available online 29 January 2015

Keywords:
Sacrificial molding
Scaffolds
Vascularization
Tissue engineering
Bioreactor

ABSTRACT

Despite significant progresses were achieved in tissue engineering over the last 20 years, a number of unsolved problems still remain. One of the most relevant issues is the lack of a proper vascularization that is limiting the size of the engineered tissues to smaller than clinically relevant dimensions. Sacrificial molding holds great promise to engineered construct with perfusable vascular architectures, but there is still the need to develop more versatile approaches able to be independent of the nature and dimensions of the construct. In this work we developed a versatile sacrificial molding technique for fabricating bulk, cell-laden and porous scaffolds with embedded vascular fluidic networks. These branched fluidic architectures are created by highly resistant thermoplastic sacrificial templates, made of poly(vinyl alcohol), representing a remarkable progress in manufacturability and scalability. The obtained architecture, when perfused in bioreactor, has shown to prevent the formation of a necrotic core in thick cell-laden constructs and enabled the rapid fabrication of hierarchically branched endothelium. In conclusion we demonstrate a novel strategy towards the engineering of vascularized thick tissues through the integration of the PVA-based microfabrication sacrificial approach and perfusion bioreactors. This approach may be able to scale current engineered tissues to clinically relevant dimensions, opening the way to their widespread clinical applications.

1. Introduction

Tissue engineering aims at the creation of functional tissues and organs in vitro to be used for transplants [1,2]. Despite significant progresses were achieved over the last 20 years, a number of unsolved problems still remain, hampering the widespread application of engineered tissues and organs in clinics [3]. One of the most relevant issues is the lack of a proper vascularization of the engineered constructs [4]. To date, skin, cartilage and bladder grafts are successfully used in clinics [5–10], since their oxygen and nutrients demand is effectively satisfied by the host's blood vessel systems. This is not the case when large tissues are needed [11], since graft vascularization requires long time and the deficiency of oxygen and nutrients supply rapidly causes widespread cell death in large graft cores [12]. For this reason, engineered tissue constructs with dimensions larger than a few hundreds of microns, quickly become irreparably damaged without a perfusable vascular system [2,13]. Hence, the ability to engineer vascular architectures is fundamental to effectively transfer the in vitro fabrication of large tissues to clinical practice [11,14–16].

Several methods have been developed to engineer scaffolds and cellular constructs with perfusable microfluidic network [16–28]. One approach is layer-by-layer assembly [17–19], which is based on the accurate stacking and lamination of individual layers, thus imposing considerable design limitations both on the material choice and on the vascular architecture [20,21]. An emerging strategy for creating vascular networks is sacrificial templating

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http://dx.doi.org/10.1016/j.biomaterials.2014.12.031
0142-9612/

[22–29]. Molded gelatin was used as a sacrificial element to create microfluidic networks within collagen, fibrin, and matrigel [22]. 3D printed networks of sugar glass were also used to generate perfusable cylindrical networks in cell-laden gels [26]. Aqueous Pluronic fugitive ink was also employed for fabricating vascularized heterogeneous cell-laden tissue constructs [27]. More recently, to avoid cytotoxic reaction caused by sugar and Pluronic sacrificial elements [26,30–32], microchannel networks within hydrogel constructs were obtained by suction of bioprinted agarose template [29].

Nevertheless the state of art of 3D sacrificial molding techniques is still limited to biomaterials requiring mild and homogeneous reaction conditions, thus excluding biomaterials classes commonly prepared with aggressive solvents, monomers and high temperatures [4,33–36]. Moreover, the proposed sacrificial materials presents different drawbacks hindering their industrial applicability and scalability; for instance gelatin templates are not sufficiently rigid for precise handling and agarose removal requires cumbersome procedures [29]. Furthermore, despite the importance of hierarchical porosity in mimicking the complexity of the cellular milieu [33,36], and favoring cell colonization and viability in cellular constructs [37], so far the proposed solutions for the vascularization have not yet been applied to porous materials, hence hindering the fabrication and use of microfluidic scaffolds with an interconnected structure of pores. Therefore, there is still the need to develop novel sacrificial molding approach able to produce vascular architectures within large and porous scaffolds for production of 3D cellular constructs with different materials and fabrication approaches suitable for specific tissue engineering applications.

Here, we report a versatile sacrificial molding technique that overcomes these limitations, enabling the fabrication of bulk, cell-laden and porous scaffolds with embedded branched fluidic networks. The presented approach is based on highly resistant thermoplastic poly(vinyl alcohol) (PVA) that has the unique capability of being chemically affine both to natural and synthetic polymers, fully biocompatible, easy machinable and processable for industrially significant requirements. The presented approach is shown to be independent of the nature and dimensions of the matrix in which the channels are embedded, allowing to engineering complex vascular geometries in synthetic, inhomogeneous and large size constructs/scaffolds. In addition, the fabricated microfluidic scaffolds were successfully integrated in a 3D cell culture bioreactor with the aim to demonstrate the effectiveness of the adopted approach toward the engineering of vascularized thick tissues.

2. Materials and methods

All starting materials were purchased from Sigma Aldrich and used as supplied, unless otherwise stated.

2.1. Sacrificial templates fabrication

The microfluidic pattern is designed and converted into STEP files using AutoCAD mechanical 2012. The pattern was reproduced on a 10 mm Plexiglas sheet using a computer numerical control machine (Mikron HSM 600) with the aid of open-mind hyperMILL cad/cam software, yielding an open microfluidic negative mold (Fig. 2a). The mold was treated with a solution of castor oil and ethanol (15% w/w) to make it hydrophobic and non-adherent. Compressed air blowing removed the excess hydrophobic solution. Then approximately 3 mm layer of aqueous PVA (Mowiol 4-88) solution (20% w/w) was casted on the mold and allowed to dry overnight (Fig. 1a). The dried PVA layer was leveled with a Teflon blade and a wet microfiber cloth repeatedly wiped on the damped mold. This iterative process removes the polymer excess out of the microfluidic track. The mold was then allowed to dry overnight and the sacrificial template was gently removed from the mold with tweezers (Fig. 1b). In order to remove dust and polymer burrs, the sacrificial templates were firstly washed by immersion for 1 min in ethanol/water a solution (70% v/v), and then in pure ethanol for 3 min, followed by rapid air blowing. In order to avoid the absorption of ambient moisture, PVA sacrificial templates were stored at 25 °C with calcium chloride until use.

2.2. Mold fabrication

The mold for microfluidic experiment was prepared using two preformed silicone spacers of 1 mm thickness, with a rectangular void area of 20 × 12 mm interposed between two silanized glass slides. The PVA sacrificial structure was placed in mold and fixed between the two silicone spacers. In this way, the sacrificial structure is suspended in the middle of the rectangular chamber (20 × 12 × 2 mm) created by the void parts of the silicone spacers (Supporting Fig. 1a). Different molds configurations were specifically prepared for porous materials and cellular experiments, as described in the supporting inormation.

2.3. Fabrication of microfluidic hydrogels

In order to form a microfluidic gel, the liquid gel precursors were injected into the mold around the sacrificial template and solidified, making sure that the sacrificial structure remains completely immersed except for its ends (Fig. 1b). Gel precursors used in the fabrication of microfluidic gels were 2-hydroxyethyl

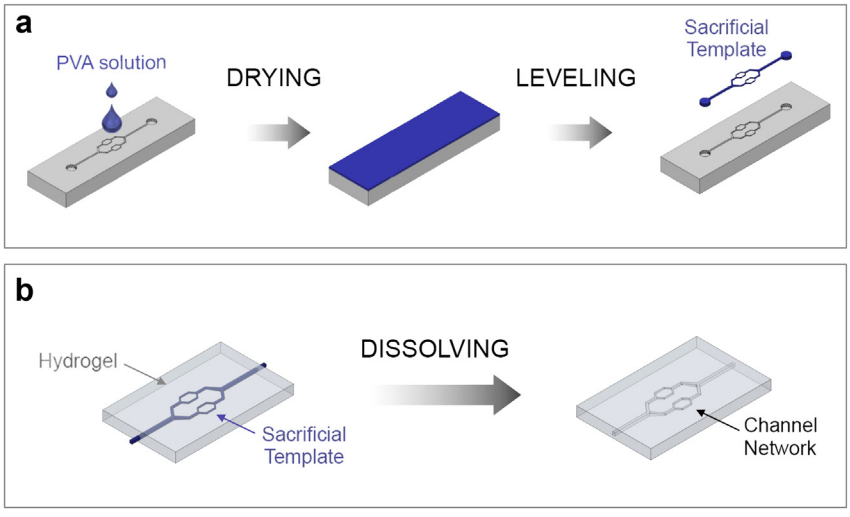

Fig. 1. (a) Sacrificial template fabrication diagram. Aqueous PVA solution is casted on the mold and allowed to dry. The dried PVA layer was leveled in order to remove the polymer excess out of the microfluidic track. The mold was then dried a second time and the sacrificial template was gently demolded. (b) Schematic fabrication diagram of microfluidic gels. The sacrificial template is encapsulated into a liquid matrix. After the matrix cross-linking, the template is dissolved with water, yielding a monolithic gel with embedded channel network.