Regenerative Therapy 3 (2016) 45–57

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Review

Hydrogel microfabrication technology toward three dimensional tissue engineering

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form
15 February 2016
Accepted 18 February 2016

Keywords:
Hydrogels
Microfabrication
Tissue engineering
Microfluidics
Perfusion

ABSTRACT

The development of biologically relevant three-dimensional (3D) tissue constructs is essential for the alternative methods of organ transplantation in regenerative medicine, as well as the development of improved drug discovery assays. Recent technological advances in hydrogel microfabrication, such as micromolding, 3D bioprinting, photolithography, and stereolithography, have led to the production of 3D tissue constructs that exhibit biological functions with precise 3D microstructures. Furthermore, microfluidics technology has enabled the development of the perfusion culture of 3D tissue constructs with vascular networks. In this review, we present these hydrogel microfabrication technologies for the *in vitro* reconstruction and cultivation of 3D tissues. Additionally, we discuss current challenges and future perspectives of 3D tissue engineering.

Contents

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Peer review under responsibility of the Japanese Society for Regenerative Medicine.

http://dx.doi.org/10.1016/j.reth.2016.02.007

1. Introduction

A multicellular three-dimensional (3D) cell culture model in a collagen hydrogel prior to implantation was constructed in the 1990s [1], with the aim of repairing vascular tissues using hydrogels with encapsulated cells. Over the past several decades, *in vitro* tissue model reconstruction in tissue engineering relied on hydrogels to mimic native tissue, owing to the biocompatibility of hydrogels, their ability to encapsulate bioactive molecules and cells, and the efficient mass transfer by diffusion [2]. The hydrogels composed of natural materials, including collagen, alginate, gelatin, hyaluronic acid, chitosan, and fibrin, are useful for the investigations of cell–cell and cell–extracellular matrix (ECM) interactions as well [3,4]. Although these hydrogels provide a microenvironment that chemically mimics cell–cell and cell–ECM interactions, they may lack an appropriate mechanical strength. In order to improve the mechanical properties of hydrogels, synthetic polymers, including poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), and poly(lactic-co-glycolic acid) (PLGA), have been widely used [2,3]. Numerous strategies have been developed in order to alter the biochemical and mechanical properties of the hydrogels. For example, ECM proteins (*e.g.*, collagen, fibronectin, and laminin) and/or their functional peptide sequences, may be chemically incorporated into hydrogels to prompt the cells to adhere to the surface of a hydrogel [5]. The mechanical strength of hydrogels is often adjusted by controlling the cross-linking density.

A key requirement for the replication of functional organs and tissues is a comprehensive knowledge of the organization and composition of their components, based on the *in vivo* model, and the desirable 3D microstructure in the reconstructed tissue. Recent advances in the field of tissue engineering have been based on the precise 3D microfabrication technologies, such as micromolding, 3D bioprinting, photolithography, and stereolithography [6]. These technologies allow the fabrication of precise 3D architectures at the micron scale. Additionally, microfl-uidics technology has been used for the fabrication of building blocks for 3D tissue engineering, while the medical imaging technologies are attractive systems for the design of 3D tissue constructs, and they include X-ray computed tomography (CT), and magnetic resonance imaging (MRI). The architectural parameters can be designed by the application of computer-aided design (CAD), using the captured 3D image of the normal tissue. Furthermore, microfluidics technologies [7] offer an attractive platform for the enhancement of the biological functions of 3D tissues. The combination of the existing biomaterial [8], microfabrication, and microfluidics approaches has an excellent potential for the reconstruction of large organ models in the future. Here, we provide an overview of these microfabrication and microfluidics technologies using hydrogels, in 3D tissue model engineering.

2. Hydrogel microfabrication in tissue engineering

Hydrogel microfabrication technologies in tissue engineering have been extensively reviewed [9]. These technologies include micromolding, 3D bioprinting [10,11], photolithography [12], and microfluidics [13,14]. Here, we focus on hydrogel microfabrication, and highlight the abundance of recent studies in the field of tissue engineering. These approaches provide different advantages or disadvantages in the selection of material, complexity of the 3D architecture, resolution, damage to the cells, and fabrication speed, and we have taken into consideration these properties and compared them.

2.1. Micromolding

Various micromolding approaches for the fabrication of 3D tissue constructs have been reported. Most of the other microfabrication technologies are limited by the selection of suitable materials for each fabrication process, and this suitability depends on their physicochemical properties. The micromolding approach allows this limitation to be overcome, while offering the advantages of short processing time and easy-to-use procedures. In this technique, elastomers, such as polydimethylsiloxane (PDMS) and poly(methyl methacrylate) (PMMA), have been employed as templates for the creation of tissue constructs. Although alginate and poly L-lactic acid based polymers are often used as sacrificial hydrogels for the fabrication of complex structures [15], there are no technical limitations for the use of other materials as templates,

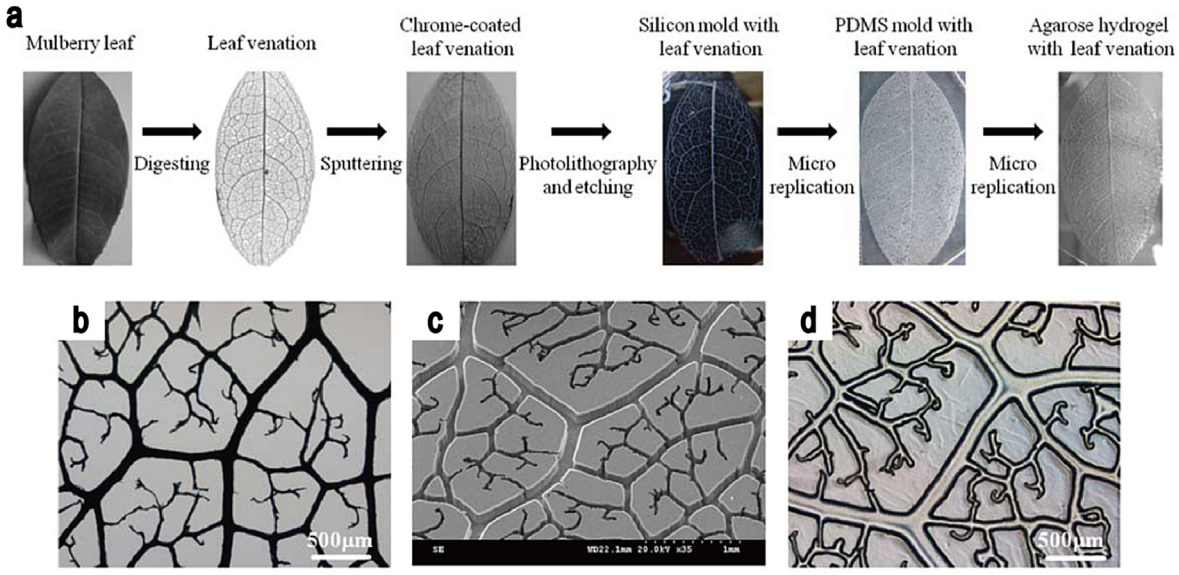

Fig. 1. The production of nature-inspired perfusable microfluidic network in the hydrogels, using micromolding technique. (a–c) Fabrication of agarose gel micromold using leaves. (d) The fabricated 3D perfusable structure in the hydrogel.
Source: He et al. [18], copyright (2013) with permission from John Wiley & Sons, Inc.