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Three-dimensional biodegradable microscaffolding: Scaffold characterization and cell population at single cell resolution

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ABSTRACT

Engineering artificial tissue scaffolds with a similar organization to that of the natural tissue is a key element to the successful recapitulation of function. However, three-dimensional (3-D) fabrication of tissue scaffolds containing complex microarchitectures still remains a challenge. In addition, little attention has been paid to the issue of how to incorporate cells within 3-D tissue scaffolds that contain precisely engineered architectures. Here we report a 3-D biodegradable microscaffolding (3D-BMS) technology and its process characterization as well as a microscale cellular loading technology as an efficient way to massively populate biodegradable polymers with cells at single cell resolution. In this study a particular emphasis was given to characterization of the material properties of the biodegradable polymers undergoing the 3D-BMS processes. Optimal process conditions were identified in order to avoid any unwanted change in material properties, such as crystallinity and scaffold strength, that have a direct impact on the degradation speed and physical integrity of the constructed scaffolds. For precise control of the cell distribution within the microstructured scaffolds a high precision microsieve structure was designed to localize rat hepatocytes and human articular chondrocytes in the biodegradable polymers. Cell suspensions were passed at a predetermined flow rate through biodegradable polymer layers that contained tapered microholes in a massively parallel process. This high resolution cell seeding method allows accurate manipulation of cell placement in thin layers of biodegradable polymers.

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1. Introduction

Development of three-dimensional (3-D) tissue scaffolds is of great interest in regenerative medicine [1]. In particular, various microfabrication technologies have been investigated to mimic the architecture and thus function of cartilage [2,3], bone [4,5], blood vessels [6,7], liver [8,9], nerve [10] and other natural tissues. These efforts have led to the successful development of novel scaffolding techniques that allow the building of more precise structures at the cellular scale (tens of microns) with a variety of biomaterials. In earlier investigations most microfabricated tissue scaffolds were constructed in two-dimensions, and mainly focused on the analysis of cell growth and function as influenced by two-dimensional (2-D) topological cues [11–13]. These 2-D microstructuring techniques have also been utilized to control chemical stimuli with microfluidic structures or micropatterning and have even been employed in the vascularization of artificially created tissues, a current challenge in regenerative medicine, in order to facilitate the transport of oxygen and nutrients to the inside of tissue scaffolds. Other work on building fluidic connections within engineered scaffolds was designed to improve the function and prolong the life of artificial tissues in vitro and in vivo [14–16].

More recently, three-dimensional (3-D) scaffolds containing precisely designed microgeometries have been created to more closely resemble the structure of natural tissues [17,18]. These 3-D scaffolding methods allow the creation of microchannels, pores, and cavities in multi-layered structures. In addition, textures or pores have been incorporated in the microstructures of 3-D scaffolds made of poly(dimethyl siloxane) (PDMS), using the surface topography to enhance cell proliferation or differentiation [19].

It is not only important for native tissues to have the ability to remodel implanted engineered tissues, but also to match the degradation rate of the supporting scaffold material to that of the infiltrating tissue. Careful selection of scaffold materials enables complete replacement of the temporary material with natural tissue ingrowth from the surrounding host tissue. Thus it is desirable to use biodegradable polymers rather than non-biodegradable polymers

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that will remain in the human body permanently. Previously we reported a 3-D microfabrication method for biodegradable polymers (3-D biodegradable microscaffolding, 3D-BMS) for tissue engineering [20]. The 3D-BMS was based on a combination of microstructuring of biodegradable polymer layers and the assembly of microstructured layers using a solvent vapor bonding method (Fig. 1).

Despite this development in 3-D microscaffolding for biodegradable polymers there still remain questions regarding the physical and chemical properties of tissue constructs built by 3D-BMS. First, during microstructuring biodegradable polymers are exposed to various thermal cycles [21]. This may cause unwanted changes in the properties of the polymer, such as their strength and degradation time. In addition, the use of solvents in the layer by layer assembly can leave residual solvents in the polymer scaffolds that could be toxic. The structural integrity of the multi-layer constructs produced by 3-D microscaffolding also needs be verified.

On the other hand, while much effort has been made to develop novel scaffolding methods on the cellular scale, much less attention has been paid to how to incorporate cells in the scaffolds with the same precision. Among the limited methodologies, microcontact printing (μCP) enables the patterning of cells, but its reliance on chemical modification of the surface limits its use in cell seeding within 3-D tissue scaffolds [22]. Capillary force lithography (CFL) has also been used to pattern cells on 2-D substrata followed by stacking of the patterned layers [23]. However, CFL also requires that the cells be mixed with the scaffolding materials in suspension, limiting use to only a small number of biocompatible materials. The Bio Flip Chip (BFC) method is free from both the chemical and material constraints that μCP and CFL have [24]. In the BFC method cells are placed in microwells and are then transferred to another substrate by inverting the culture plate, and are maintained in contact until the cells adhere to the substrate. However, the BFC process is time consuming and produces patterned cells on flat surfaces, which may not be suitable for 3-D microstructured tissue scaffolds.

Here we report the further characterization of the 3D-BMS fabrication process and introduce a novel technique to populate the scaffolds with cells at single cell resolution. This precise seeding achieves more effective organization of cells in the microfabricated 3-D scaffolds. The main focus of this characterization was the preservation of the material properties of biodegradable polymers after 3D-BMS. In addition, to provide more precise control over the cell population as well as the tissue architecture we propose a robust and simple method (called "microsieving") for populating microstructured scaffolds with cells at single cell resolution. Using this microsieving method cells are captured within microwells from circulating cell suspensions that are passed through a scaffold containing an array of wells configured with a larger inlet than outlet. This approach to organizing cells within tissue scaffolds at single cell resolution is independent of any chemical modification (as in μCP) or carrier matrix (as in CFL). Microsieving is also much simpler than BFC, which requires multiple steps to complete the patterning. Together with the material characterization, this microsieving method for highly precise cell organization complements the previous 3-D biodegradable microscaffolding as a promising tissue engineering technology.

2. Materials and methods

2.1. Three-dimensional biodegradable microscaffolding (3D-BMS)

25 μm thick films of 35/65PCGA (35/65 poly(ε-caprolcatone–co-glycolic acid)) (M_w = 98,000, M_w/M_n = 3.63) were generously donated by Ethicon, a Johnson & Johnson Company. Micromolds were fabricated by a combination of wet and reactive ion etching as previously described [25]. Thin films of 35/65PCGA were placed between a micromold and a silicone rubber sheet. The films were heated to 110 °C and 135 °C on silicon micromolds and compressed at 1 MPa for 5 min. The micromolded films were bonded together by a solvent vapor bonding process [20]. First, the micromolded films were exposed to hexafluorosoisopropanol (1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), VWR International) vapor at 50 Torr for 5 s in a vacuum chamber. Then the films were brought into contact with each other under a pressure of 0.21 MPa. After contact between the two surfaces the chamber was immediately purged of solvent vapor to rapidly remove residual solvent.

2.2. Characterization of biodegradable polymers after 3D-BMS processing

2.2.1. Differential scanning calorimetry (DSC)

DSC measurements were performed using a TA Instruments DSC Q1000 with a thermal analysis controller (TA Instruments, New Castle, DE), with cooling provided by a TA Instruments refrigerated cooling system. Data were analyzed by TA Q-series software. The samples were analyzed in standard aluminum pans under a nitrogen atmosphere. Approximately 5 mg of polymer film was heated from −50 to 120 °C at a heating rate of 10 °C min^{-1}.

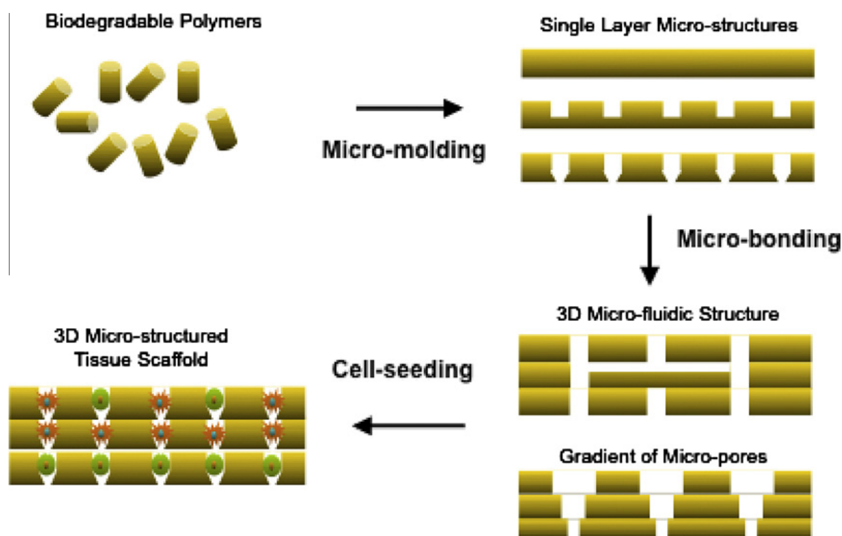

Fig. 1. Construction of 3-D tissue scaffolds by micromolding and solvent vapor bonding processes.

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