Biomaterials 31 (2010) 4322–4329

Contents lists available at ScienceDirect

Biomaterials

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

The role of three-dimensional polymeric scaffold configuration on the uniformity of connective tissue formation by adipose stromal cells

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ARTICLE INFO

Article history:
Received 23 December 2009
Accepted 3 February 2010
Available online 2 March 2010

Keywords:
Uniform tissue formation
Tissue engineering
Scaffold with repeating units
Dynamic culture
Microenvironment

ABSTRACT

To form tissues with uniform cell distribution and extracellular matrix arrangement is of great relevance to obtain the desirable function and maintain structural integrity. Scaffold configuration is believed to play a critical role in regulating cell spatial distribution and consequently tissue formation. In this study, three types of poly(ethyleneglycol-terephthalate)–poly (butylenes terephthalate) (PEGT/PBT) scaffolds [compression molded scaffold (CM), compression molded scaffold after chloroform/isopropanol reticulation (CMR), 3D rapid prototyped fibrous scaffold (RP)] with various configurations were used to support the tissue formation of adipose stromal cells for up to 21 days. Characterization of the scaffolds with μCT revealed that RP scaffolds were composed of repeating structural units with well controlled interconnected pores, in contrast to the irregular pore morphology in CM or CMR. Cell seeding efficacy onto various scaffolds was comparable (from 67 ± 4% to 82 ± 3%), while only RP scaffold led to even cell attachment onto the inner fibers of the scaffolds. Continuous cell proliferation and deposition of new collagen and glycosaminoglycans (GAG) were measured for all three scaffolds, while with a significant amount measured in RP at 21 days. By 21 days, complete uniform tissue formation was only achieved in RP scaffolds under a dynamic cell culture in spinner flasks. The present study successfully demonstrates the feasibility of controlling uniform tissue formation at a microscale by manipulating the structural configuration of the scaffold.

1. Introduction

Tissue engineered substitutes have been considered as a promising alternative to repair the diseased or damaged tissues in clinical practice [1] and as a well defined in vitro model for drug screening or tissue related studies [2]. With continuous interdisciplinary efforts, tremendous progress has been made in tissue engineering from cell isolation and culture, scaffold fabrication to bioreactor design. However, it remains a big challenge to completely restore the lost function of diseased tissues with the implants prepared through tissue engineering approach, i.e., seeding and culture of cells in a preformatted porous scaffold [3]. This can be partly ascribed to the lack of structural integrity and non-uniformity of newly formed tissue in the implants.

Increasing evidences suggest that initial cell distribution throughout the scaffold is a premise for uniform tissue formation in the scaffold [4–8]. This has inspired extensive efforts to increase cell adhesion by choosing the scaffold materials or modifying the scaffolds with cell adhesive molecules like RGD, and to improve cell infiltration into the scaffolds by employing dynamic cell seeding or optimizing the scaffold porosity, pore size and interconnectivity [8–16]. It has been successful to form integral tissues in small porous scaffolds with several millimeters in dimension [14,17], readily allowing the cell migration and tissue ingrowth to the interior void space of the scaffold. While in the case of large scaffolds, the cell migration and tissue ingrowth are often limited to the peripheral region [4,18,19], which inevitably results in a poor structural integrity and localized tissue formation. Our findings indicate that the scaffolds with well-connected pores can significantly increase the interior tissue formation under a dynamic cell culture in spinner flasks [4]. However, large variation in tissue formation even in the same constructs is constantly observed in terms of varied cell density and spatial arrangement of new ECM. In general, comparable cell distribution and extracellular matrix (ECM) arrangement is preferred in the same tissue to provide a uniform function and control the quality of formed tissues.

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doi:10.1016/j.biomaterials.2010.02.008

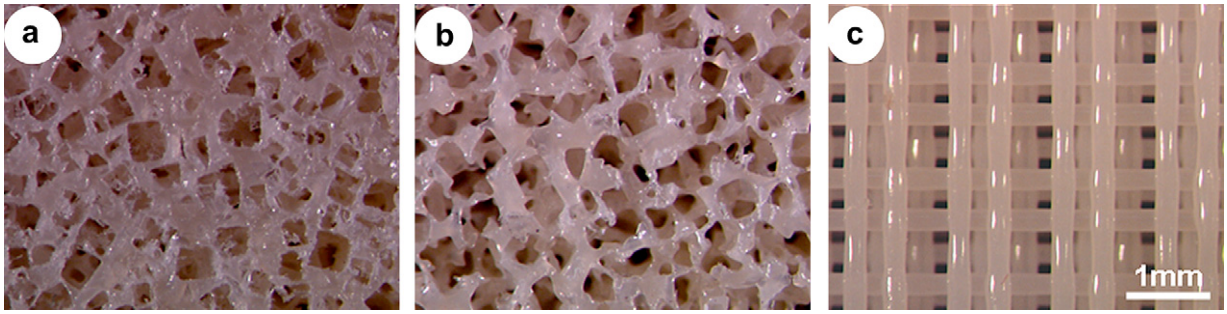

Fig. 1. Optical images of various porous PEGT/PBT scaffolds. (a) Compression molded scaffold (CM); (b) compression molded scaffold after chloroform/isopropanol reticulation treatment (CMR); (c) 3D rapid-printing fibrous scaffold (RP).

Tissue formation in scaffolds is greatly regulated by the cell-residing microenvironment, which provides the cells with guidance for their proliferation, differentiation and production of new ECM [20–24]. In this regard, we hypothesize that uniform tissue formation can be achieved by providing identical microenvironment to the residing cells that distribute evenly in each repeating microenvironment. Multiple factors are involved in defining the cell growing environment [2], but at a given culture condition, for instance, the same medium, scaffold materials, and bioreactor, the scaffold configuration becomes critical to determine the interstitial flow through the scaffold. Based on our hypothesis, we expect that the scaffolds with repeating structure will most likely yield tissues with uniform composition.

In the present study, three types of scaffolds (compression molded scaffold, compression molded scaffold with chloroform/isopropanol reticulation, 3D rapid-printing fibrous scaffold with repeating structure) were prepared and evaluated for tissue formation. Human adipose stromal cells were dynamically seeded onto various scaffolds and their proliferation was quantitatively measured up to 21 days. The new ECM (collagen and glycosaminoglycans) deposited in the scaffolds was quantitatively determined. The spatial distribution of cells and new ECM was carefully characterized by scanning electron microscopy, histology and immunofluorescent staining.

2. Materials and methods

2.1. Materials

The segmented poly (ether ester) copolymer (PolyActive™, IsoTis NV, Bilthoven, The Netherlands) consisted of hydrophilic poly(ethyleneglycol-terephthalate) (PEGT) soft segments and hydrophobic poly (butylenes terephthalate) (PBT) hard segments and had a weight ratio of PEGT/PBT 55%/45% and PEG molecular weight of 300 Da (300PEGT55PBT45). All the other reagents and solution were obtained from Invitrogen (Carlsbad, CA) except as indicated.

2.2. Scaffold preparation

2.2.1. Compression molding

A homogeneous mixture of PolyActive™ (300PEGT55PBT45) granules (<400 μm) and NaCl salt particles (sieved to 250–300 μm or 400–600 μm) were placed in a designed mold (10 cm × 10 cm × 2 mm) and compressed with a pressure of 10,000 ± 500 pounds/inch^2. The mold was heated up to 210 ± 10 °C for 5 ± 0.5 min. After cooling, salt-leaching was performed by incubating in demineralized water. The porous blocks were dried at room temperature (RT) for 2 days and overnight in a 50 °C vacuum oven.

2.2.2. Reticulation-treatment

To increase the interconnectivity, compression molded scaffolds were reticulated with a mixture of chloroform (CHL) and isopropanol (IPA) to remove residual scaffold membranes as previously described [20]. Briefly, 10 mL of CHL/IPA solution was rapidly passed through the scaffold by applying a vacuum of approximately 600 mBar and the treated scaffolds were kept in 50 °C vacuum oven overnight to remove a trace of CHL/IPA. The content of residual CHL left in scaffolds was lower than the detection limit of 1 ppm using gas chromatography.

2.2.3. Rapid prototyping

3D deposited fibrous scaffolds laminated in perpendicular layers were constructed using a custom-made fiber deposition device by layering a 0°–90° pattern of molten polymer from a Ø280 μm nozzle onto a computer controlled *x-y-z* table. Fibers, which rapidly solidified, were laid down with the pre-defined patterns before lowering and changing the table 90° in the *z*-direction. Fiber layers were deposited up to a height of 1.8–2 mm. The actual distance between fibers was determined via scanning electron microscopy (SEM) and summarized in Table 1.

2.3. Scaffold characterization

2.3.1. Microscopy

Surface characterization of the porous scaffolds was performed by stereomicroscopy (Nikon) and SEM. For SEM analysis, the scaffolds were sputter-coated with gold and evaluated using a Philips XL30 ESEM-FEG SEM apparatus at an accelerating voltage of 10 kV (FEI & Philips, Eindhoven, NL).

2.3.2. Microcomputed tomography

Microcomputed tomography (μCT) was used to determine the scaffold porosity, average pore size, and interconnectivity expressed as mean connecting pore size [4]. Briefly, the measurement was performed on cylindrical scaffolds (Ø6 mm × 2 mm)

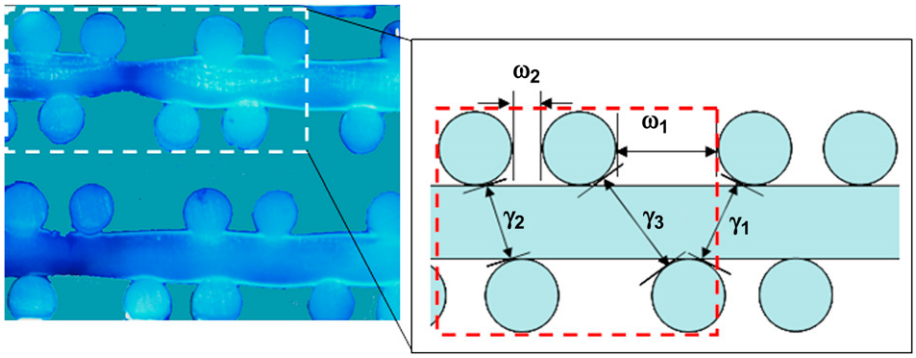

Fig. 2. Transverse section of the 3D printing fibrous scaffold (left) composed of repeating structural units (red dash line) (right), with controlled inter-fibre distances as shown in Table 1.