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The effect of PEGT/PBT scaffold architecture on the composition of tissue engineered cartilage

J. Malda[a,b,c,*], T.B.F. Woodfield[a,b], F. van der Vloodt[a,c], C. Wilson[d], D.E. Martens[c], J. Tramper[c], C.A. van Blitterswijk[a,b], J. Riesle[a]

[a] *IsoTis S.A., Bilthoven, The Netherlands*
[b] *Institute for Biomedical Technology (BMTI), University of Twente, Enschede, The Netherlands*
[c] *Food and Bioprocess Engineering Group, Wageningen University, Wageningen, The Netherlands*
[d] *Department of Orthopaedics, University Medical Center, Utrecht, The Netherlands*

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Abstract

A highly interconnecting and accessible pore network has been suggested as one of a number of prerequisites in the design of scaffolds for tissue engineering. In the present study, two processing techniques, compression-molding/particulate-leaching (CM), and 3D fiber deposition (3DF), were used to develop porous scaffolds from biodegradable poly(ethylene glycol)-terephthalate/poly(butylene terephthalate) (PEGT/PBT) co-polymers with varying pore architectures. Three-dimensional micro-computed tomography (μCT) was used to characterize scaffold architectures and scaffolds were seeded with articular chondrocytes to evaluate tissue formation. Scaffold porosity ranged between 75% and 80%. Average pore size of tortuous CM scaffolds (182 μm) was lower than those of organized 3DF scaffolds (525 μm). The weight ratio of glycosaminoglycans (GAG)/DNA, as a measure of cartilage-like tissue formation, did not change after 14 days of culture whereas, following subcutaneous implantation, GAG/DNA increased significantly and was significantly higher in 3DF constructs than in CM constructs, whilst collagen type II was present within both constructs. In conclusion, 3DF PEGT/PBT scaffolds create an environment in vivo that enhances cartilaginous matrix deposition and hold particular promise for treatment of articular cartilage defects.

Keywords: Chondrocytes; Cartilage tissue engineering; Scaffold; Cell culture; In vitro; In vivo

1. Introduction

Tissue engineering holds promise for revolutionary advances in health care and considerable efforts have been directed towards the development of autologous substitutes to regenerate, maintain, or improve tissue and organ function. None more so than articular cartilage (AC), a connective tissue which, when damaged, exhibits limited intrinsic regenerative capacity [1]. In general, tissue-engineered constructs require a highly porous artificial extra-cellular matrix (ECM) or scaffold material to accommodate mammalian cells and to organize tissue regeneration in a three-dimensional (3D) environment. Nevertheless, limitation in the diffusion of nutrients has been suggested as a cause for the inhomogeneous neo-cartilage distribution observed in larger tissue-engineered cartilaginous constructs, whereby, the onset of chondrogenesis occurs solely within the peripheral boundaries [2–5]. Indeed, it has been demonstrated that nutrient gradients, such as oxygen in particular [5,6], can be measured and do occur within these tissue-engineered constructs. Therefore, in an effort to improve nutrient transport to cells, there has been considerable interest in the development of bioreactors in which medium flow is applied [7–9], or which mimic the periodic compressive stresses within articulating joints [10,11]. Although these dynamic culture conditions typically result in an improved quality of the neo-cartilage tissue formed, the 3D pore architecture present within scaffolds used for cartilage tissue engineering also likely has a large influence on tissue formation.

While several investigators [12–14] have evaluated the effect of scaffold pore size on cartilage tissue formation,

*Corresponding author. Department of Polymer Chemistry and Biomaterials, University of Twente/IsoTis S.A., P.O. Box 98, 3720 AB Bilthoven, Netherlands.
E-mail address: jos@malda.nl (J. Malda).

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to the best of our knowledge, pore architecture was not investigated systematically. In characterizing porous materials it is common practice to quote an average pore size or a pore-size distribution [12]. While these are important characteristics, particularly for controlling mechanical properties, pore accessibility and the pore tortuosity are, next to the porosity, of great significance for minimizing diffusional constraints and ultimately for successful tissue-engineering applications [15–19].

In this study we wanted to more closely evaluate the effect of a pore architecture, and more specifically, pore accessibility, on the composition of tissue-engineered cartilage. Therefore, careful design and characterization of porous scaffolds was necessary. The two most commonly used scaffold architectures reported in the literature for cartilage repair are porous sponges and non-woven fiber meshes [20]. At present, we are evaluating a series of amphiphilic, biodegradable poly (ether ester) multiblock copolymers as carrier materials for AC repair. The co-polymers are based on hydrophilic poly(ethylene glycol)-terephthalate (PEGT) and hydrophobic poly(butylene terephthalate) (PBT) blocks. Varying the amount and the length of the PEGT and PBT blocks offers extensive possibilities in the design of polymer systems with tailor-made properties, such as swelling, degradability and mechanical strength, as reported previously [21–24].

Two porous PEGT/PBT scaffold architectures were evaluated herin; a compression-molded/particle-leached sponge (CM), and a novel 3D-deposited fiber (3DF) scaffold. By accurately controlling the two processing techniques, the aim was to produce scaffolds with the same bulk composition and overall porosity, but different pore geometries. Scaffold architecture was then comprehensively characterized and cartilage tissue formation was evaluated on cell-seeded constructs maintained in vitro and in vivo.

2. Materials and methods

2.1. Scaffold preparation

PEGT/PBT co-polymers were obtained from IsoTis S.A. (Bilthoven, The Netherlands). Two scaffolds with different architecture were produced from PEGT/PBT resin with a PEG molecular weight of 300 g/mol and a PEGT:PBT weight percentage ratio of 55:45.

CM scaffolds (Figs. 1A and C) were prepared using a compression molding and particle-leaching method as previously described [25]. Porous 3DF scaffolds (Figs. 1B and D) were produced using a novel 3DF technique also described previously [26]. Previous thermal analysis studies have demonstrated that the compression molding and 3DF processing techniques described here do not result in changes of PEG molecular weight or PEGT/PBT composition [26] and, therefore, any differences seen between scaffolds in this

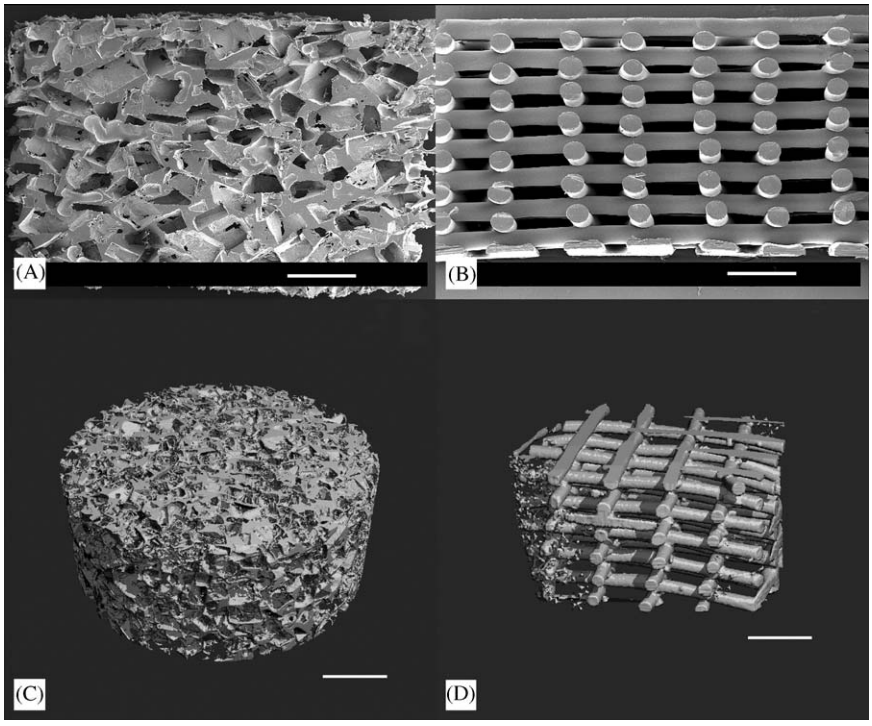

Fig. 1. Electron micrographs of CM (A) and 3DF (B) scaffolds. Three-dimensional reconstruction of CM (C) and 3DF (D) scaffolds from μCT scans. Scale bar represents 1 mm.