Biomaterials 34 (2013) 4259–4265

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

The effect of scaffold-cell entrapment capacity and physico-chemical properties on cartilage regeneration

J.A.A. Hendriks [a,b,1], L. Moroni [a,*,1], J. Riesle [a,b], J.R. de Wijn [a], C.A. van Blitterswijk [a]

[a] Institute for BioMedical Technology (MIRA), University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
[b] CellcoTec N.V., Prof. Bronkhorstlaan 10D, 3723 MB Bilthoven, The Netherlands

ARTICLE INFO

Article history:
Received 28 November 2012
Accepted 20 February 2013
Available online 13 March 2013

Keywords:
Rapid prototyping
Pore volume
Physico-chemical properties
Dynamic stiffness
Cartilage

ABSTRACT

An important tenet in designing scaffolds for regenerative medicine consists in mimicking the dynamic mechanical properties of the tissues to be replaced to facilitate patient rehabilitation and restore daily activities. In addition, it is important to determine the contribution of the forming tissue to the mechanical properties of the scaffold during culture to optimize the pore network architecture. Depending on the biomaterial and scaffold fabrication technology, matching the scaffolds mechanical properties to articular cartilage can compromise the porosity, which hampers tissue formation. Here, we show that scaffolds with controlled and interconnected pore volume and matching articular cartilage dynamic mechanical properties, are indeed effective to support tissue regeneration by co-cultured primary and expanded chondrocyte (1:4). Cells were cultured on scaffolds *in vitro* for 4 weeks. A higher amount of cartilage specific matrix (ECM) was formed on mechanically matching (M) scaffolds after 28 days. A less protein adhesive composition supported chondrocytes rounded morphology, which contributed to cartilaginous differentiation. Interestingly, the dynamic stiffness of matching constructs remained approximately at the same value after culture, suggesting a comparable kinetics of tissue formation and scaffold degradation. Cartilage regeneration in matching scaffolds was confirmed subcutaneously *in vivo*. These results imply that mechanically matching scaffolds with appropriate physico-chemical properties support chondrocyte differentiation.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

In tissue engineering, scaffolds have been shown to deliver an essential contribution to treatments of chondral defects. These constructs have to possess sufficient biocompatibility and biodegradability, provide mechanical stability and display favorable surface physico-chemical properties to direct cells into the proper lineage [1–5]. They can also act as a delivery vehicle for cells and it has been shown that they can be designed to deliver instructive signals to enhance chondrogenesis [6–8]. Copolymers of poly(ethylene oxide-terephtalate) (PEOT) and poly(butylene-terephtalate) (PBT) are interesting candidates to make scaffolds for tissue engineering as they comply with the above mentioned criteria. These polyether–ester multi-block copolymers are thermoplastic elastomers which exhibit satisfactory physical properties like elasticity, toughness and strength in combination with easy processability [9–12].

Previous results from our group showed that both architecture and composition of *a*PEOT*b*PBT*c* (*a*/*b*/*c*, where *a* is the molecular weight of PEG units used in the copolymerization, while *b* and *c* are the PEOT and PBT weight fractions in the final copolymer) scaffolds influence chondrocyte-driven cartilage specific matrix formation [13–16]. On two-dimensional surfaces supporting more protein adhesion like 300/55/45 a direct relationship between a spread chondrocyte morphology and reduced differentiation capacity has been shown [15]. In contrast, on less protein adhesive surfaces (e.g. 1000/70/30) chondrocytes take a spherical morphology, resulting in enhanced differentiation [16]. When three-dimensional (3D) scaffolds are fabricated with conventional techniques, like compression molding (CM) and salt leaching, the achievement of structures mechanically matching cartilage infers a low pore volume. The pore network of these scaffolds is often tortuous and not completely interconnected, resulting in a scarce nutrient perfusion for constructs thicker than 1–2 mm. This can be overcome by a rapid prototyping technique such as 3D fiber deposition (3DF), as the resulting scaffolds have a completely interconnected

* Corresponding author. Department of Tissue Regeneration, Institute for Biomedical Technology, University of Twente, Drienerlolaan 5, Zuidhorst 147, 7522 NB Enschede, The Netherlands. Tel.: +31 53 489 3502.
E-mail addresses: Jeanine.Hendriks@cellcotec.com (J.A.A. Hendriks), l.moroni@utwente.nl (L. Moroni).
[1] Authors contributed equally to this work.

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.02.060

pore network and scaffold's pore architecture and volume can be controlled [13,17].

Human articular cartilage thickness has been shown to range between 0.5 and 7.1 mm at various locations in the knee. For *in vitro* or *in vivo* studies in cartilage tissue engineering, scaffold thickness ranged typically between 1 and 5 mm [18]. In general, cells from an immature animal model are used to study correlation between limiting factors such as nutrient gradients, pore volume and interconnectivity of a scaffold and homogenous cartilage tissue formation [19]. However, matching scaffolds' thickness for cartilage repair with chondrocytes from adult animal models might not be just a matter of scaling up. Oxygen tension has shown to decrease throughout such clinically relevant size scaffolds [20,21]. Albeit no difference in oxygen tension was shown between 3DF and CM scaffolds with the same porosity (80%), tissue formation with chondrocytes from immature origin showed to be enhanced in 3DF scaffolds compared to CM scaffolds. Few studies showed that it is possible to regenerate articular cartilage in 3D scaffolds with matching cartilage static mechanical properties [22–24]. So far, it was not possible to manufacture scaffolds by conventional techniques with matching dynamic mechanical properties of articular cartilage without hampering porosity and pore interconnectivity to such an extent that cartilage tissue formation was obstructed.

Therefore, the aim of this study was to examine the influence of scaffold's pore volume and polymeric composition on matrix build up by mature articular chondrocytes. In addition, we examined the influence of extra-cellular matrix builds up in chorus with scaffold degradation on the mechanical stability of the engineered cartilage. Scaffolds with dynamic stiffness matching (M) or non-matching (NM) articular cartilage were created. Recently, co-culturing of primary chondrocytes with expanded chondrocytes was found to enhance cartilage tissue formation [25–28]. This co-culture model was applied to study the influence of cell–biomaterial interaction on cartilaginous tissue formation on PEOT/PBT scaffolds. PEOT/PBT 3DF scaffolds with 100% interconnectivity were fabricated with different porosity, pore volume, and copolymer composition. Specifically, 300/55/45 and 1000/70/30 were considered for their difference in physico-chemical properties and the influence that this showed to have on cartilaginous tissue formation.

2. Materials and methods

2.1. Scaffolds fabrication

Poly(ethylene oxide-terephtalate)/poly(butylene terephtalate) (PEOT/PBT) copolymers were obtained from IsoTis S.A. (Bilthoven, The Netherlands). The compositions used in this study were 300/55/45 and 1000/70/30 where, following an *a*PEOT*b*PBT*c* nomenclature, *a* is the molecular weight in g/mol of the starting PEG blocks used in the copolymerization, while *b* and *c* are the weight fractions of the PEOT and PBT blocks, respectively.

3DF scaffolds were produced with a Bioplotter device (Envisiontec GmbH, Germany), which is basically an *XYZ* plotting machine as previously described by Landers et al. [29,30] and by our group [31,32]. To extrude highly viscous PEOT/PBT fibers, few modifications were made. The polymer was placed in a stainless steel syringe and heated at $T = 200–210$ °C through a thermo-coupled cartridge unit, fixed on the "X"-mobile arm of the apparatus. When the polymer reached a molten phase, a nitrogen pressure of 4.5–5 bars was applied to the syringe through a pressurized cap. Rectangular block models were deposited, layer by layer, through the extrusion of the polymer on a stage as a fiber in a CAD/CAM controlled manner. Stainless steel Luer Lock needles with an internal diameter (ID) of 200 μm shortened to a length of 16 mm were used to extrude the polymeric filaments in this study. The deposition speed was set to 230 mm/min for 300/55/45 and to 280 mm/min for 1000/70/30. Three scaffold types were fabricated: 300/55/45 and 1000/70/30 (porosity = 74%) with similar, but not matching (NM) dynamic stiffness ($E' = 1$ MPa), as compared to articular bovine cartilage, and 1000/70/30 (porosity = 56%) mechanically matching (M) articular cartilage ($E' = 10$ MPa). For NM 300/55/45 and 1000/70/30 scaffolds the fiber spacing d_2 was set to 600 μm, the layer thickness d_3 was set to 150 μm, and the layer configuration was changed by 90° every two deposited layers (00–9090 configuration). For M 1000/70/30 scaffolds the fiber spacing d_2 was fixed to 370 μm, the layer thickness was fixed to 140 μm, while the layer configuration was changed by 90° every single deposited layer (0–90 configuration).

2.2. Scaffolds characterization

Cylindrical samples of 4 mm in diameter by 4 mm in height were cored out in the "*Z*-direction" from the rectangular 3D fabricated blocks. Scaffolds geometry and architecture were characterized by environmental scanning electron microscopy (ESEM) analysis with a Philips XL 30 ESEM-FEG. The porosity of 3DF scaffolds was calculated as [29,32]:

$$P = 1 - \frac{V_{\text{scaffold}}}{V_{\text{cube}}} = 1 - \frac{\pi}{4} \cdot \frac{1}{\frac{d_2}{d_1}} \cdot \frac{1}{\frac{d_3}{d_1}} \quad (1)$$

where P is the scaffold porosity, d_1 the fiber diameter, d_2 the fiber spacing and d_3 the layer thickness, within each different structure.

2.3. Cell isolation and culture

Primary chondrocytes are referred to when chondrocytes were applied immediately after isolation. For chondrocytes isolation, full thickness articular cartilage was dissected from the patellar femoral groove of adult bovine. Dissected cartilage was incubated for 20–22 h in collagenase type II solution containing 0.15% collagenase (Worthington), Dulbecco's modified Eagle's medium (Gibco) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). The suspension was filtered through a 100 μm mesh nylon filter (cell strainer Nucleon) and cells were washed 2 times with PBS supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). For expansion chondrocytes were plated at a density of $3.5 * 10^4$ cells/cm^2 and cultured in proliferation medium (PM) containing DMEM supplemented with 10% fetal bovine serum, 1× non-essential amino acids (Sigma–Aldrich), 10 mM HEPES buffer (Biowhitaker), 0.2 mM ascorbic acid 2-phosphate (InVitrogen), 0.4 mM proline (Sigma–Aldrich), 100 U/ml penicillin (InVitrogen) and 100 μg/ml streptomycin (InVitrogen). Medium was refreshed every 3–5 days and cells were cultured in an incubator at 37 °C and 5% CO_2. After 2–3 passages (9–12 population doublings), expanded cells were mixed with primary chondrocytes for seeding onto a porous scaffold.

2.4. Cell seeding and culturing on 3DF scaffolds

Cylindrical scaffolds were sterilized in isopropanol for 4 h, rinsed in PBS extensively, incubated overnight in PM and blotted dry prior to seeding. Primary and expanded chondrocytes were mixed at a 20/80 ratio, centrifuged at 300 g for 5 min and re-suspended in 54 μl of PM containing 300 μg/ml fibronectin. Scaffolds were seeded with 54 μl of cell suspension containing 3×10^6 cells for 1 h, after which medium was carefully added. Construct were cultured statically in PM for 4 weeks in an incubator at 37 °C and 5% CO_2.

2.5. Biochemical analysis

Constructs ($n = 3$) for quantitative analysis of sulfated glycosaminoglycans (GAGs) and cell number were washed with PBS and frozen overnight at −80 °C. Subsequently they were digested with 1 mg/ml proteinase K (Sigma–Aldrich) in tris/EDTA buffer (pH 7.6) containing 18.5 μg/ml iodoacetamide and 1 μg/ml pepstatin A (Sigma–Aldrich) for >16 h at 56 °C. GAG content was spectrophotometrically determined with 9-dimethylmethylene blue chloride (DMMB) (Sigma–Aldrich) staining in PBE buffer (14.2 g/l Na_2HPO_4 and 3.72 g/l Na_2EDTA, pH 6.5) with a microplate reader (Bio-TEK instruments) at an absorbance of 520 nm. Cell number was determined via quantification of total DNA with CyQuant DNA kit according to the manufacturer's description (Molecular probes) on a fluorescent plate reader (Perkin–Elmer). Quantitative total GAG and total DNA were normalized for differences in wet weight of the scaffolds.

2.6. Scanning electron microscopy (SEM) analysis

Tissue constructs were also analyzed by SEM. Samples were fixed overnight in 0.14 M cacodylate buffer (pH = 7.2–7.4) containing 0.25% glutaraldehyde (Merck). Scaffolds were subsequently dehydrated in sequential ethanol series and critical point dried from liquid carbon dioxide using a Balzers CPD 030 machine. Specimens were then gold sputtered (Cressington) and studied under the SEM.

2.7. Mechanical characterization

A dynamic mechanical analysis (DMA) instrument (Perkin Elmer 7e) was used to measure the dynamic stiffness of the 3DF scaffolds before and after culturing ($n = 6$). Cylindrical fixtures were chosen to test the specimens and evaluate their behavior as a whole structure along their compression axis, in the "*Z*-direction".

Scaffolds were loaded with a dynamic force varying from 100 mN to 150 mN in a dynamic stress experiment. More specifically, a starting force of 100 mN was applied

Download English Version:

https://daneshyari.com/en/article/6419

Download Persian Version:

https://daneshyari.com/article/6419

Daneshyari.com