Journal of Biomechanics 48 (2015) 1310–1317

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

An experimental fatigue study of a porous scaffold for the regeneration of articular cartilage ☆

L. Vikingsson [a,*], J.A. Gómez-Tejedor [a], G. Gallego Ferrer [a,b], J.L. Gómez Ribelles [a,b]

[a] Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, 46022 Valencia, Spain.
[b] Ciber en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Valencia, Spain

ARTICLE INFO

Article history:
Accepted 15 February 2015

Keywords:
Tissue engineering
Scaffolds
Mechanical properties of biomaterials
Fatigue prediction
Cartilage regeneration

ABSTRACT

The aim of this experimental study is to predict the long-term mechanical behavior of a porous scaffold implanted in a cartilage defect for tissue engineering purpose. Fatigue studies were performed by up to 100,000 unconfined compression cycles in a polycaprolactone (PCL) scaffold with highly interconnected pores architecture. The scaffold compliance, stress–strain response and hysteresis energy have been measured after different number of fatigue cycles, while the morphology has been observed by scanning electron microscopy at the same fatigue times. To simulate the growing tissue in the scaffold/tissue construct, the scaffold was filled with an aqueous solution of polyvinyl alcohol (PVA) and subjected to repeating cycles of freezing and thawing that increase the hydrogel stiffness. Fatigue studies show that the mechanical loading provokes failure of the dry scaffold at a smaller number of deformation cycles than when it is immersed in water, and also that 100,000 compressive dynamic cycles do not affect the scaffold/gel construct. This shows the stability of the scaffold implanted in a chondral defect and gives a realistic simulation of the mechanical performance from implantation of the empty scaffold to regeneration of the new tissue inside the scaffold's pores.

1. Introduction

Defects in the articular cartilage surface can lead to loss of cartilaginous tissue and osteoarthritis. Only in United Kingdom a third of the population over 45 years have sought treatment for osteoarthritis and in the United States over 27 million people are affected (Osteoarthritis in General Practice, 2013). Spontaneous regeneration of articular cartilage is very limited and current clinical treatments are focused on regeneration methods by bone marrow stem cells (bMSC) through microfracture (Steadman et al., 1999), abrasion chondroplasty, drilling (Insall, 1974) or mature chondrocyte implantation (Brittberg, 1999). The results are promising but the tissue generated is fibro-cartilaginous with limited mechanical properties (Hunziker, 2002) (Pelttari et al., 2008).

Hyaline cartilage is a specialized tissue containing around 75% water, and its extra cellular matrix (ECM) is composed of mainly collagen type II fibers and proteoglycan aggregates. The tissue is hard and able to sustain compression loading due to the combination of the collagen fibers stiffness and the high water sorption capacity of the glycosaminoglycans (GAGs) (Roughley and Lee, 1994). When a scaffold is implanted in a cartilage defect, either seeded with chondrocytes or pluripotential cells or invaded "in vivo" by bMSC, a cartilaginous tissue is expected to grow inside the pores of the scaffold. The mechanical behavior of the implant will result from the original stiffness of the scaffold and the water permeability through the newly formed tissue. The scaffold must be able to diminish the differences in stress in relation to neighboring tissue(Hutmacher, 2000) and at the same time give the cells proper mechanotransduction signals to create their ECM (Chiquet et al., 2003). The mechanical performance of the scaffold is therefore important, and due to the great influence of the newly formed tissue, "in vitro" essays of the empty scaffold are not representative for the "in vivo" situation.

Poly vinyl alcohol (PVA) aqueous solution has the interesting property to crystallize with repeated cycles of freezing and thawing (Hassan and Peppas, 2000) and the resulting hydrogel is proposed by other authors as a cartilage substitute due to its high equilibrium water content (80–90%), swelling capacities and good mechanical properties (Li et al., 2010)(Tamura et al., 1986). Other authors try to develop artificial cartilage combining different materials, such as PCL and alginate/polyacrylamide (Liao et al., 2013) or chitosan/agarose/gelatin (Bhat et al., 2011) or polylactic acid and agar (Gong et al., 2007). Without doubt, the combination of synthetic or natural scaffolding materials with hydrogels for cartilage regeneration is an arising and important field. In previous studies our group filled a porous PCL scaffold with a PVA gel physically cross-linked by different cycles of freezing and thawing

☆This work is submitted as original article.

* Corresponding author. Tel.: +34 963877007x88939; fax: +34 963877276.
E-mail address: livi1@upv.es (L. Vikingsson).

http://dx.doi.org/10.1016/j.jbiomech.2015.02.013
0021-9290/

and evaluated its mechanical properties. It was possible to tailor the elastic modulus of the PCL-PVA construct to reach values characteristic of natural articular cartilage after six cycles of freezing and thawing (Vikingsson et al., 2014). The outcome is an cartilage model mimicking the behavior of the growing ECM inside the pores of the implanted scaffold in a cartilage defect.

In this work we have studied the long-term mechanical behavior of the PCL-PVA construct under compressive fatigue tests. The performance of the empty scaffold or filled with water has been compared to the behavior of the scaffold filled with hydrogel after six cycles of freezing and thawing, to mimic the characteristics of natural articular cartilage (Vikingsson et al., 2014). This experimental study aims to predict the mechanical performance of a macro and micro porous PCL scaffold in a cartilage defect. This is important for a better understanding of the process of surgery and implantation of the scaffold for cartilage regeneration without any animal sacrifice.

2. Materials and methods

PCL, number average molecular weight (Mn) 80 000 Da Mw/Mn < 2, was obtained from Sigma-Aldrich (Spain). Microspheres of Elvacite 2043 (a mixture of low molecular weight poly(ethyl methacrylate) (PEMA) and poly(methyl methacrylate) (PMMA)) with diameters ranging from 120 to 200 μm were purchased from Lucite International (USA). 1,4 dioxane from Sigma Aldrich (Spain) was used as solvent for PCL, and ethanol (99% pure) from Scharlab (Spain) was used to dissolve the dioxane in the freeze extraction process. Poly(vinyl alcohol), average molecular weight (Mw) 130 000 Da, and 99+% hydrolyzed was purchased from Sigma Aldrich (Spain). All the chemicals were used as received and with no further modification.

2.1. Scaffold preparation

The PCL scaffold was fabricated by mixing a 15 wt. % PCL in 1,4 dioxane solution with PEMA-PMMA microspheres at a weight ratio 1:1.25 in a freeze extraction method to produce both macro and micro pores with a total porosity of around 90% (Rodenas-Rochina et al., 2013)(Santamaría et al., 2012). The mixture was immediately frozen with liquid nitrogen, and then immersed in pre-cooled ethanol. It was kept at −20 °C with three changes of cold ethanol to eliminate the solvent. The scaffold was washed in ethanol at 38 °C for 8 days with daily changes of ethanol to wash the PEMA–PMMA microspheres. The resulting scaffold was dried at room temperature under vacuum until reaching constant weight. The scaffolds were cut with circular stamps and surgical scalpels of 5 mm diameter and 2 mm high. The water immersion of the hydrophobic scaffolds was done by solvent change ethanol to water. To make sure that all the pores were filled with water the scaffolds were put in water and subjected to continuous vacuum extraction. All samples were put in water 24 h before the PVA filling or mechanical testing.

2.2. Hydrogel preparation

A 10% aqueous solution of PVA was prepared by stirring at 90 °C for 1 h. The solution was left to cool at room temperature and then poured into custom made wells, with dimensions of 6 mm diameter and 4 mm height, and frozen for 12 h at −20 °C and then thawed back to room temperature in a chamber with controlled air humidity for 8 h. The freezing and thawing step was repeated six times. The water content in the gels was analyzed and calculated for six samples after 6 cycles of freezing and thawing and overnight immersed in water. The samples were freeze dried with −80 °C condensation and pressure < 100 mbar (Lyoquest, Telstar) and the mass before and after freeze drying was measured and the difference in mass was considered to be water. A Thermo Gravimetric Analysis (TGA/StarSystem, Mettler Toledo) to 400 °C was done to evaluate the resting amount of water in the gel. The mass loss until 180 °C was considered to be water.

2.3. Scaffold/hydrogel construct

The PVA solution was introduced into the already water immersed PCL scaffold by vacuum injection by syringe. The scaffold with the gel inside the pores was freeze and thawed during the same conditions as the pure PVA gel for six cycles. The effectiveness of the PVA filling was calculated through gravimetric analysis according to Eq. 1, by measuring the construct volume and weighing the empty PCL scaffold and the PCL scaffold filled with PVA. The density of the PVA solution was estimated from a 10% PVA aqueous solution with a pure PVA density of 1.30 g/cm^3 (Hassan and Peppas, 2000). The PCL density is 1.146 g/cm^3 (Labet and Thielemans, 2009).

$$\phi = \frac{V_{\text{pores}}}{V_{\text{total}}} = \frac{V_{\text{pores}}}{V_{\text{scaffolds}} + V_{\text{pores}}} \quad \text{(a)}$$

$$V_{\text{pores}} = \frac{m_{\text{with PVA}} - m_{\text{dry}}}{\rho_{\text{PVA solution}}} \quad \text{(b)}$$

$$V_{\text{scaffold}} = \frac{m_{\text{dry}}}{\rho_{\text{PCL}}} \quad \text{(c)} \qquad (1)$$

where Eq. 1(a) The equation for calculating the porosity in the PCL and PVA construct (b) The volume of the PVA is calculated as the differencse in mass of the filled and unfilled scaffold divided with the density of the hydrogel (c) The volume of the PCL is the weight of the dry scaffold divided by the density of the PCL.

2.4. Mechanical testing

Cylindrical scaffolds of (5.00 ± 0.04) mm diameter and (2.00 ± 0.01) mm height were cut with circular stamps and surgical scalpels. The pure PVA hydrogels were produced in costume made wells with a diameter of (6.00 ± 0.05) mm and (4.00 ± 0.01) mm height. 6 samples were tested in each mechanical assay, and each sample dimension was measured three times to ensure the accuracy of the sample geometry. The empty scaffolds, the water immersed scaffolds and the scaffold and gel constructs, so as the pure PVA gels, were subjected to 100, 3000, 10,000 and 100,000 cycles of 1 Hz sinusoidal compression of 15% strain in an Microtest Universal Fatigue machine with a 1500 N cell. The chosen strain of 15% ensures that the samples are subjected to a higher compressive fatigue strain than the samples would suffer inside the human body, which would be less than 6% strain (Eckstein et al., 2005). The number of cycles has been chosen to compare short-time with long-time effects inside the scaffolds. The frequency of 1 Hz tends to imitate the frequency of a normal human step (Eckstein et al., 2000) After each fatigue time the samples were measured in a Termo-Mechanical Assay machine (TMA) Seiko TMA/SS6000 (Japan), with two successive programs of loading and unloading to 100 g with a rate of 3000 g/min in room temperature. The experiments in the TMA machine were also made for the samples without fatigue, serving as control.

2.5. Scanning electron microscopy

The morphology of the scaffold and gel construct was observed by an Scanning Electron Microscope (JEOL JSM-5410, Japan, acceleration voltage of 15 kV, frozen at −80 °C, sublimated during 40 min) equipped with a cryogenic device. The samples were cut inside the cryogenic equipment to see the cross-section of the samples and covered with gold to be observed.

2.6. Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) heating scans were performed at 20 °C/min in a PYRYS-DSC 8000 equipment (Perkin Elmer) under flowing nitrogen atmosphere between −90 °C and 90 °C in 20 μL aluminum pans for the dry and water immersed (24 h) PCL scaffolds. The excess water was absorbed by filter paper immediately before introducing the sample in the equipment.

3. Results and discussion

3.1. Analysis of the water content of the PVA gels

The average water content of the hydrogels, obtained as the difference in mass of the hydrogel after 6 cycles of freezing and thawing and 24 h immersion in water and the dry samples after freeze drying, was (86.73 ± 1.85)%. The TGA curves show a mass loss of (4.12 ± 0.29)% up to 180 °C that was assigned to the remaining water, In this way the PVA hydrogels contain (86.73 ± 1.85)% and (4.12 ± 0.29)% water.

3.2. Morphology

Before mechanical testing the dry PCL scaffold has micro, (10 ± 5) μm, and macro, (180 ± 60) μm pores (Fig. 1a). These mean pores size were obtained by measuring the size of 10 micro and macro pores in 3 different SEM photos. The structure is similar to the ones obtained in previous works (Santamaría et al., 2012) (Lebourg et al., 2010) (Deplaine et al., 2013) (Lebourg et al., 2013). Without fatigue, the structure of the dry and immersed scaffold is very similar. Not until 10,000 cycles of fatigue a change is seen