

Unconfined compression properties of a porous poly(vinyl alcohol)–chitosan-based hydrogel after hydration

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Abstract

A poly(vinyl alcohol) (PVA) hydrogel composite scaffold containing *N,O*-carboxymethylated chitosan (NOCC) was tested to assess its potential as a scaffold for cartilage tissue engineering in a weight-bearing environment. The mechanical properties under unconfined compression for different hydration periods were investigated. The effect of supplementing PVA with NOCC (20 wt.% PVA:5 vol.% NOCC) produced a porosity of 43.3% and this was compared against a non-porous PVA hydrogel (20 g PVA: 100 ml of water, control). Under non-hydrated conditions, the porous PVA–NOCC hydrogel behaved in a similar way to the control non-porous PVA hydrogel, with similar non-linear stress–strain response under unconfined compression (0–30% strain). After 7 days' hydration, the porous hydrogel demonstrated a reduced stiffness (0.002 kPa, at 25% strain), resulting in a more linear stiffness relationship over a range of 0–30% strain. Poisson's ratio for the hydrated non-porous and porous hydrogels ranged between 0.73 and 1.18, and 0.76 and 1.33, respectively, suggesting a greater fluid flow when loaded. The stress relaxation function for the porous hydrogel was affected by the hydration period (from 0 to 600 s); however the percentage stress relaxation regained by about 95%, after 1200 s for all hydration periods assessed. No significant differences were found between the different hydration periods between the porous hydrogels and control. The calculated aggregate modulus, H_A , for the porous hydrogel reduced drastically from 10.99 kPa in its non-hydrated state to about 0.001 kPa after 7 days' hydration, with the calculated shear modulus reducing from 30.92 to 0.14 kPa, respectively. The porous PVA–NOCC hydrogel conformed to a biphasic, viscoelastic model, which has the desired properties required for any scaffold in cartilage tissue engineering. © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Keywords: Mechanical properties; Poly(vinyl alcohol)–chitosan-based hydrogel; Unconfined compression; Hydration effect; Cartilage regeneration

1. Introduction

Damaged articular cartilage has a limited ability to undergo self-repair and regenerate [1]. Recent studies have focused on the transplantation of a hybrid “scaffold cells” construct to encourage the cartilage regenerative process.

Several novel biomaterial scaffolds for the incorporation of cultured cells have since been proposed, including medical-grade polycaprolactone (mPCL) [2], bilayer type I/III collagen membrane [3], atelocollagen sponge/poly-L-lactic acid (PLLA) mesh composite [4], poly(D,L)-lactide-co-glycolide (PLGA)-based biomaterials [5] and fibrin glue [6,8]. Apart from ensuring that cultured cells incorporate into suitable biodegradable scaffolds, another important issue is to ensure that the scaffold mimics the structural properties of the native tissue and are able to withstand

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the loading environment of the recipient site. Recently, the use of hydrogels as a scaffold for articular cartilage regeneration has offered an attractive and promising approach. Their ability to maintain a high water content, biocompatibility, permeability, hydrophilicity, tissue-like elasticity and low coefficient of friction are among of the advantages of hydrogels. This enables the hydrogels to resemble the structural properties of cartilage extracellular matrix [7–15].

The choice of using an implanted material to replace a biological tissue-like the articular cartilage depends on the material's ability to behave in a way that is similar to the tissue it is replacing. Although, hydrogels have been used in a wide range of biomedical applications including contact lenses [7], corneal implants [8], drug delivery [9,10], adhesives [11], artificial menisci [12] and substitutes for skin, ligament, tendon, cartilage and bone [13–15]; their major drawback is the limited use under weight-bearing conditions. Current work has progressed to develop hydrogel composite scaffolds to be used as a construct for seeding cells. This is done by blending two or more polymers with the aim of obtaining a viscoelastic and interconnecting porous structure that will provide a more compliant biphasic material when hydrated. The key criterion is that the replacement biomaterial is expected to maintain the structural integrity of the tissue it is to replace, and this is during the course of the repair and regeneration phases. The mechanical properties of hydrogels are generally poor; although these biomaterials possess many attractive features for biomedical applications [16].

Poly(vinyl alcohol) (PVA), one of the most widely used polymers, is known for its excellent weight-bearing properties and biocompatibility [8,12,17–19]. Stammen et al. [14] found that PVA hydrogels formed via freeze–thawing possessed compressive and tensile mechanical properties comparable to native articular cartilage in the knee. PVA can be prepared as a porous structure. Highly porous PVA hydrogels are prepared in a variety of ways including the addition of porogens such as sucrose, sodium chloride and polyethylene glycolic acid (PEG) [20]. Our group has synthesized porous PVA hydrogel by blending a low molecular weight of PVA with *N,O*-carboxymethylated chitosan (NOCC), and without the use of porogens. Chitosan has a similar structure to glycosaminoglycans (GAGs), a key component of cartilage extracellular matrix. The incorporation of natural polymer such as chitosan and gelatin can also improve the degradation of a biomaterial [19]. We hypothesized that PVA–NOCC scaffold with appropriate interconnecting pores will ensure a biphasic property when hydrated, thus qualifying this scaffold as a potential candidate for cartilage tissue engineering [21–23].

This study investigates the behavior of a highly porous, NOCC supplemented PVA hydrogel (PVA–NOCC) under compression and determines their viscoelastic properties and Poisson's ratio when hydrated under unconfined compression [24–32]. The PVA hydrogel without any additives under the same loading and hydration conditions will be

used as a control for comparison. The main aim of this study is to assess the feasibility of this porous PVA–NOCC hydrogel as a potential extracellular matrix scaffold for future cell implantation in cartilage tissue engineering to repair the damaged cartilage in load-bearing joints.

2. Materials and methods

2.1. Preparation of hydrogels

PVA-117 ($M_w = 74,000 \text{ g mol}^{-1}$) was obtained from Kuraray Co. Ltd, Japan. NOCC was obtained from the Standards and Industrial Research Institute (SIRIM), Malaysia. The porous hydrogel was prepared by blending PVA with NOCC in the ratio (w/v) of 20% PVA to 5% NOCC. The control PVA hydrogel was prepared as 20% PVA in distilled water. The polymer solutions were then cast into cylindrical molds and physically cross-linked by irradiation at 50 kGy. The hydrogels were frozen at -80°C for 24 h prior to lyophilization. Subsequently, the hydrogels were cut into disks approximately 2 mm in height, with a diameter of 5 mm. The porosity of the specimens was sampled and assessed using an electron microscope (FEI Quanta 400, USA) and was found to be on average 43.3% (SD, 13.9%). The average pore sizes ranged from small (with a mean relative diameter of 18.13 μm ; SD, 3.28 μm), medium (35.27 μm ; SD, 3.06 μm) and large (78.91 μm ; SD, 5.41 μm). The control PVA had no pores.

2.2. Unconfined compression test

The hydrogel composite samples ($n = 18$) were immersed in Dulbecco's Modified Eagle's Medium (DMEM) (pH 7.4) at 37°C . Unconfined compression tests were performed at 1, 3 and 7 days after immersion [24]. Samples at day 0 were also included, and this involved only the hydrogel composite without it being immersed in DMEM. The initial diameter and thickness of hydrogel composites at day 0 were measured using a vernier caliper and a micrometer, respectively. Samples were then loaded in between two impermeable, unlubricated compression platens using a universal testing machine (Instron Model 5543, MA, USA) with the top platen coupled to a 50 N capacity load cell and a machine actuator. Samples were compressed at a strain rate of $100\% \text{ strain min}^{-1}$ until a 30% compressive strain, before being unloaded. Both the tangent compressive modulus and compressive stress values were calculated at 15%, 20% and 25% compressive strain for each sample over the periods of hydration (1, 3 and 7 days). Poisson's ratio, ν , was obtained by directly measuring the lateral expansion in unconfined compression test at 30% compressive strain using an optical method as previously described by Jurvelin et al. [28–31]. Briefly, this involved taking a digital image at the end point of compression, against a calibrated grid. The grid was used to estimate the change in the height and width of the specimen. Potential optical distortions were minimized by using

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