Biomaterials xxx (2013) 1–9

Contents lists available at ScienceDirect

Biomaterials

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

An annulus fibrosus closure device based on a biodegradable shape-memory polymer network

Shahriar Sharifi [a], Theo G. van Kooten [a], Hendrik-Jan C. Kranenburg [b], Björn P. Meij [b], Marc Behl [c], Andreas Lendlein [c], Dirk W. Grijpma [a,d,*]

[a] University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, W.J. Kolff Institute, PO Box 196, 9700 AD Groningen, The Netherlands
[b] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, PO Box 80154, 3508 TD Utrecht, The Netherlands
[c] Institute of Biomaterial Science, Helmholtz-Zentrum Geesthacht, Kantstrasse 55, 14513 Teltow, Germany
[d] MIRA Institute for Biomedical Technology and Technical Medicine, Department of Biomaterials Science and Technology, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands.

ARTICLE INFO

Article history:
Received 6 May 2013
Accepted 18 July 2013
Available online xxx

Keywords:
D,L-lactide and trimethylene carbonate-based networks
Photo-crosslinking
Shape-memory
Thermo-mechanical analysis
Intervertebral disk
Annulus fibrosus

ABSTRACT

Injuries to the intervertebral disc caused by degeneration or trauma often lead to tearing of the annulus fibrosus (AF) and extrusion of the nucleus pulposus (NP). This can compress nerves and cause lower back pain. In this study, the characteristics of poly(D,L-lactide-*co*-trimethylene carbonate) networks with shape-memory properties have been evaluated in order to prepare biodegradable AF closure devices that can be implanted minimally invasively. Four different macromers with (D,L-lactide) to trimethylene carbonate (DLLA:TMC) molar ratios of 80:20, 70:30, 60:40 and 40:60 with terminal methacrylate groups and molecular weights of approximately 30 kg mol^{-1} were used to prepare the networks by photo-crosslinking. The mechanical properties of the samples and their shape-memory properties were determined at temperatures of 0 °C and 40 °C by tensile tests- and cyclic, thermo-mechanical measurements. At 40 °C all networks showed rubber-like behavior and were flexible with elastic modulus values of 1.7–2.5 MPa, which is in the range of the modulus values of human annulus fibrosus tissue. The shape-memory characteristics of the networks were excellent with values of the shape-fixity and the shape-recovery ratio higher than 98 and 95%, respectively. The switching temperatures were between 10 and 39 °C. *In vitro* culture and qualitative immunocytochemistry of human annulus fibrosus cells on shape-memory films with DLLA:TMC molar ratios of 60:40 showed very good ability of the networks to support the adhesion and growth of human AF cells. When the polymer network films were coated by adsorption of fibronectin, cell attachment, cell spreading, and extracellular matrix production was further improved. Annulus fibrosus closure devices were prepared from these AF cell-compatible materials by photo-polymerizing the reactive precursors in a mold. Insertion of the multifunctional implant in the disc of a cadaveric canine spine showed that these shape-memory devices could be implanted through a small slit and to some extent deploy self-sufficiently within the disc cavity.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Intervertebral disc (IVD) abnormalities resulting from metabolic disorders, aging processes, trauma, or degenerative discs diseases are common in the human population and can cause considerable pain, particularly if they affect adjacent nerves. When the results of conservative treatments are insufficient, and the neurological problems that lead to pain need to be resolved, surgical intervention is required [1]. Currently, a standard surgical procedure for the reduction of pressure on the surrounding (nerve) tissue is partial- or total nucleotomy followed by implantation of a nucleus pulposus prosthesis (NPP) to restore the biomechanical function of the disc [2].

Among the several hundred thousand patients that undergo disc operations each year, recurrent disc herniation is a complication that often occurs [3]. The defect that remains in the outer

* Corresponding author. MIRA Institute for Biomedical Technology and Technical Medicine, Department of Biomaterials Science and Technology, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands.
E-mail addresses: D.W.Grijpma@utwente.nl, d.w.grijpma@umcg.nl (D.W. Grijpma).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.07.061

Please cite this article in press as: Sharifi S, et al., An annulus fibrosus closure device based on a biodegradable shape-memory polymer network, Biomaterials (2013), http://dx.doi.org/10.1016/j.biomaterials.2013.07.061

layers of the disc can allow re-herniation of residual nuclear material to occur [4,5]. More importantly, correct performance of NP implants is very much dependent on proper confinement by the annulus fibrosus (AF). For example, when the intradiscal pressure was restored to physiological levels upon insertion of an NPP, the surrounding AF was generally too weak to prevent its extrusion [6]. Maintaining the NPP centrically in situ is one of the major challenges in nucleus prosthesis technologies [7–9].

To seal the AF efficiently, the AF should ideally be closed from the inside, *i.e.* at the transition zone between the NP and AF, and not only at the outside of the annular ring as is the case when only suturing [10]. Inclose© and the Xclose© are two systems that have been developed to close a torn AF. In these examples permanent polyethylene terephthalate meshes are used in combination with sutures [11]. Despite an improved annulus fibrous sealing potential, their clinical efficacy has still to be addressed. As these methodologies are mechanical closure approaches that in the long-term cannot maintain the structure of the native AF or prevent the degeneration of the intervertebral disc, there is great need for an approach that not only seals the AF immediately, but in the longer term also allows regeneration of AF tissue to maintain its integrity.

Existing annulus fibrosus tissue regenerating approaches have focused on tissue engineering using biodegradable polymeric scaffolds such as those based on synthetic poly(ε-caprolactone) (PCL) [12] or poly(1,8-octanediol malate) [13] or based on natural polymers such as collagen and hyaluronan [14], chitosan [15] or porous silk [16]. In many cases the use of biodegradable scaffolds is combined with (biological) signal molecules and cells. Despite the success in preparing scaffolds that mimic the native AF structure and support AF cells *in vitro*, several major hurdles need to be taken before these constructs can be used *in vivo*: the scaffolds should be degradable, while adequate mechanical strength as well as long-term mechanical performance are required. The implants need to be fixed in or to the annular defect, for example by suturing or adhesive bonding [17–19]. The newly formed tissue will need to mimic the native annulus fibrosus tissue and provide a tight connection with the native tissue. The implantation of these tissue grafts or patches still requires invasive surgery.

In this study we describe the development of a multifunctional annular closing device designed to seal a ruptured annulus fibrous from within and simultaneously allow tissue regeneration using biodegradable shape-memory polymer networks. The shape-memory functionality enables the implant to be minimal-invasively inserted into the damaged intervertebral disk in a temporary shape and subsequent self-sufficient deployment to its permanent shape (sealing the defect) by exposure to physiological temperatures. As the implant degrades in time, fibrous- or annulus fibrosus tissue can form upon cellular infiltration.

An ideal shape-memory material for manufacturing the closure device should have excellent shape-recovery and fixity rates at physiological temperatures and conditions [20]. In addition, the tissue-forming implants should have mechanical properties that are close to those of AF tissue, thereby restoring the biomechanical properties of the disc. Furthermore, the material should support AF cell adhesion and proliferation allowing extracellular matrix production. For this purpose we prepared a series of biodegradable polymer networks from methacrylate-functionalized D,L-lactide and trimethylene carbonate oligomers with varying molar co-monomer contents. The polymer networks were characterized in terms of their shape-memory properties, elastic properties, and their ability to support AF cells in culture. Annular closure devices were made by preparing these networks in a designed mold, and the suitability of our approach was assessed in *ex vivo* experiments conducted using canine cadaveric spines.

2. Materials and methods

2.1. Materials

Trimethylene carbonate (1,3-dioxan-2-one, TMC) (Boehringer Ingelheim, Germany) and D,L-lactide (DLLA) obtained from Purac Biochem, The Netherlands were used as received. 1,6-Hexanediol (99%), stannous octoate, triethyl amine (TEA) and methacrylic anhydride (MAA) were purchased from Sigma–Aldrich, The Netherlands. The photo-initiator (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone) (Irgacure 2959) was a gift from Ciba Specialty Chemicals (Basel, Switzerland). Other reagents and solvents were of analytical grade and used as received. Human annulus fibrosus cells, cell culturing media and buffers were obtained from ScienCell, USA. Antibodies were from Sigma–Aldrich, the Netherlands (anti-fibronectin), Abcam, United Kingdom (anti-collagen type I) and Jackson Immunolab, United Kingdom (fluorescent labeled secondary antibodies). Other chemicals were also from Sigma–Aldrich.

2.2. Synthesis and characterization of shape-memory networks

A series of poly(DL-lactide-*co*-trimethylene carbonate) diols with DLLA:TMC molar ratios of 80:20, 70:30, 60:40 and 40:60 was synthesized by ring-opening polymerization of the corresponding monomers under nitrogen at 130 °C for 3 days using 1,6-hexanediol as initiator and stannous octoate (2×10^{-4} mol per mol of monomer) as catalyst. The monomer: initiator molar ratio was adjusted to yield oligomers with a targeted number average molecular weight of 30 kg mol^{-1}. The prepared oligomeric diols were dissolved in dry dichloromethane (Sigma–Aldrich, Netherlands) and functionalized with methacrylate groups using a 4 times excess of MAA in the presence of TEA. The reaction was allowed to proceed for 7 d at room temperature. Subsequent purification was performed by precipitation in excess of cold ethanol. The precipitated dimethacrylate macromer was then vacuum-dried for 2 days at room temperature and stored at −20 °C until used.

Monomer conversion, copolymer composition and degree of methacrylation were determined by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (300 MHz, Varian Innova, USA) using $CDCl_3$ as a solvent.

To prepare photo-crosslinked films, the photoinitiator (2 wt% with respect to the macromer) was dissolved in chloroform (1 ml g^{-1} macromer) and homogeneously mixed into the dimethacrylate macromer followed by evaporation of the solvent by applying vacuum. The mixtures were then shaped into films measuring $100 \times 25 \times 0.5$ mm^3 by molding at 100 °C using a laboratory press (Fontijne THB008, The Netherlands). After cooling, the films were again heated to 75 °C and irradiated in a UV cabinet (Spectroline, USA at 365 nm and 3–5 mW cm^{-2}) for 500 s under a blanket of nitrogen gas. The distance between the films and the UV lamps was 10 cm.

The equilibrium swelling ratios and gel contents of the obtained photo-crosslinked films were determined by conducting swelling experiments in dichloromethane.

Glass transition temperatures (T_g) of the copolymer networks were determined by differential scanning calorimetry (Pyris 1, PerkinElmer, USA). Samples weighing 5–10 mg were heated to 100 °C at a rate of 10 °C min^{-1} and then cooled to −50 °C at a rate of 5 °C min^{-1}. After 5 min at this temperature, a second scan was recorded from −50 °C to 100 °C at 10 °C min^{-1}. The data presented are the data obtained in the second scan.

2.3. Investigation of tensile- and shape-memory properties of networks

Tensile tests and cyclic thermo-mechanical tensile tests were conducted with standard samples (ISO 527–2/1BB) cut from films in a Z1.0 (Zwick, Germany) equipped with a 200 N load cell and a thermo-chamber (Climatic Systems LTD, model 091250) controlled by a Eurotherm control 2408 unit (Eurotherm Regler, Germany). The deformation rate was 5 mm min^{-1}, the preload was 30 mN. Tensile test results are averages of 10 measurements. Tensile tests were conducted at 0 °C and at 40 °C, unless mentioned otherwise.

The cyclic thermo-mechanical tensile tests were performed as follows: the sample was stretched at a temperature of 50 °C to an elongation ε_m of 100% at an elongation rate of 5 mm min^{-1} (step 1). The stretched sample was kept at this elongation for 5 min, and cooled to 0 °C at a cooling rate of 5 °C min^{-1} (step 2) and equilibrated at this temperature for 10 min. The sample, which is now in its temporary fixed state, was unloaded to almost $\sigma = 0$ MPa (the offset zero force was 200 mN) (step 3) and the temperature was raised to 50 °C at a heating rate of 2 °C min^{-1} (step 4) and held there for 10 min. This program was repeated five times with the same sample ($N = 5$).

The strain fixity ratio R_f and the strain recovery ratio R_r were calculated from Eqs. (1) and (2), respectively.

$$R_f(N) = \frac{\varepsilon_u(N)}{\varepsilon_l(N)} \tag{1}$$

$$R_r(N) = \frac{\varepsilon_l(N) - \varepsilon_p(N)}{\varepsilon_l(N) - \varepsilon_p(N-1)} \tag{2}$$

where $\varepsilon_l(N)$ is the tensile strain of a loaded sample after cooling in a cyclic, thermo-mechanical experiment in the Nth cycle, $\varepsilon_u(N)$ is the strain in the stress-free state

Please cite this article in press as: Sharifi S, et al., An annulus fibrosus closure device based on a biodegradable shape-memory polymer network, Biomaterials (2013), http://dx.doi.org/10.1016/j.biomaterials.2013.07.061

Download English Version:

https://daneshyari.com/en/article/10228473

Download Persian Version:

https://daneshyari.com/article/10228473

Daneshyari.com