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Polyurethane scaffold with *in situ* swelling capacity for nucleus pulposus replacement

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ABSTRACT

Nucleus pulposus (NP) replacement offers a minimally invasive alternative to spinal fusion or total disc replacement for the treatment of intervertebral disc (IVD) degeneration. This study aimed to develop a cytocompatible NP replacement material, which is feasible for non-invasive delivery and tunable design, and allows immediate mechanical restoration of the IVD. A bi-phasic polyurethane scaffold was fabricated consisting of a core material with rapid swelling property and a flexible electrospun envelope. The scaffold was assessed in a bovine whole IVD organ culture model under dynamic load for 14 days. Nucleotomy was achieved by incision through the endplate without damaging the annulus fibrosus. After implantation of the scaffold and *in situ* swelling, the dynamic compressive stiffness and disc height were restored immediately. The scaffold also showed favorable cytocompatibility for native disc cells. Implantation of the scaffold in a partially nucleotomized IVD down-regulated catabolic gene expression, increased proteoglycan and type II collagen intensity and decreased type I collagen intensity in remaining NP tissue, indicating potential to retard degeneration and preserve the IVD cell phenotype. The scaffold can be delivered in a minimally invasive manner, and the geometry of the scaffold post-hydration is tunable by adjusting the core material, which allows individualized design.

1. Introduction

The intervertebral disc (IVD) is a complex tissue structure with important mechanical functions. It consists of a central part, the nucleus pulposus (NP), the surrounding annulus fibrosus (AF), and the cartilaginous endplates which provide the permeable connections to the bony vertebrae. The main function of the NP is to absorb and distribute mechanical load exerted on the spinal column. To fulfil this function, the NP contains specific extracellular matrix components rich in proteoglycans that allow the maintenance of a highly hydrated state and high swelling pressure [1].

IVD degeneration is associated with changes in extracellular matrix composition that often have biomechanical consequences and can lead to painful and debilitating conditions. The resulting neck and low back pain are major determinants of discomfort and disability and are the origin of enormous socio-economic health care problems worldwide [2–4]. Treatment methods range from physical therapies and pain relieving medication to highly invasive surgical procedures. Discectomy is often performed in cases of disc bulging or herniation to relieve the painful pressure on neural elements. Spinal fusion is another standard treatment intended to relieve pain by reducing motion across the joint. However, removal of disc tissue and fusion alter the biomechanical function and can accelerate IVD degeneration at adjacent levels [5]. Due to the limited healing potential and harsh nutritional conditions of adult IVDs, loss or failure of the extracellular matrix is virtually irreversible and requires restoration. Implants for total IVD replacement have been developed in order to preserve the motion at the operated level. However, the procedure is highly invasive and may

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not be appropriate at early stages of degeneration; in addition the long term effects are still uncertain [6].

Functionally, during IVD degeneration the NP becomes dehydrated, is replaced by fibrous tissue, and loses its high osmotic pressure [2,7,8]. The severe loss of water content in the NP also causes a reduction in disc height. NP replacement therapy has therefore been proposed as a less invasive alternative method to spinal fusion or total IVD replacement [9]. An ideal NP replacement material would allow immediate redistribution of loads and restoration of disc height, while maintaining the flexibility of the spine. Various synthetic polymeric materials have been described as potential NP substitutes that may be delivered as injectable hydrogel or pre-formed material [10–13]. However, several concerns still exist in their application. While conventional injectable hydrogels can be delivered through the AF in a minimally-invasive manner, their generally weak mechanical properties do not allow direct restoration of the disc height and mechanical function; in addition, their three-dimensional structure is difficult to control and there is a substantial risk of material leakage with adverse effects. Pre-formed polymers and scaffolds have the advantage of improved structural control and faster response to external mechanical forces. Nevertheless, their application requires a more invasive procedure, and unwanted outcomes such as material displacement may occur [14–16].

The polyurethane (PU) family of biomaterials has been introduced for medical use because of their elastomeric properties. By varying the composition of the monomer units and the size of the blocks of the dissimilar monomers within the polymer chain, these properties can be tailored. As such, PU has been used in applications in which an elastomeric material is likely to enhance the success or longevity of the implant. Specifically, PU has been used in cardiovascular applications, where material flexibility is important, such as for catheters, insulations, vascular prostheses, heart valves or assist devices [17]. Furthermore, a range of tissue replacement or augmentation materials are based on PU, especially as wound dressing for skin regeneration [18]. Among the PU family, polycarbonate urethanes have demonstrated improved biostability and are therefore preferred for many applications [19,20]. The function of the intervertebral disc relies on the elastomeric nature of the matrix components and fluid of which they are constituted; hence, PU is an attractive material for IVD restoration. In fact PU based materials have been used in spinal surgery and the overall mechanical and biological properties indicate no long-term problems to date; though information on their application as pure NP replacement is scarce [21].

Taking into account the requirements for an NP substitute, we have developed a new bi-phasic PU scaffold consisting of a highly hydrophilic core material with significant instant swelling capabilities and an electrospun envelope. The functions of the envelope are to assure the structural control of the implant after swelling *in situ* and to facilitate cell attachment and tissue integration to keep the implant stable also under mechanical load. Moreover, the unique flat geometry and flexible shape of the bi-phasic scaffold system allow minimally invasive administration into the NP of the IVD.

The aim of this work was to assess the mechanical, swelling properties and the cytocompatibility of the scaffold materials. A whole IVD organ culture model was then used to test the hypothesis that the implanted scaffold could restore the disc height and mechanical stiffness in a nucleotomized IVD. Histological and gene expression analyses were performed to evaluate the biological response of the disc cells and tissues to the implanted scaffold.

2. Materials and methods

2.1. Fabrication and characterization of PU scaffolds

2.1.1. Fabrication of PU scaffolds

PU scaffolds with swelling capability were manufactured to obtain implants with a flat discoid structure. The scaffolds are composed of a core film which swells to a hydrogel following contact with an aqueous medium, and an envelope composed of electrospun polymer mixture with a fibrous mat structure (Fig. 1A). The core solution was prepared with the ether-based hydrophilic urethane HydroMed™ (HM, AdvanSource Biomaterials), which was dissolved at a concentration of 20% (w/w) in 95% ethanol (Bio-Lab) by stirring overnight at room temperature. A predetermined solution volume was casted on a flat glass plate, dried for three days to ensure solvent residues evaporation and then cut into discs at a diameter of 3, 5 and 8 mm for manufacturing scaffold at external diameter of 6, 8 or 9, and 14 mm, respectively.

For production of the envelope a mixture was prepared by combining the polycarbonate urethanes Chronoflex™ (CF, AdvanSource Biomaterials) and HM. In order to select the optimal ratio of CF to HM in the envelope formulation, several ratios were tested. Wetting time was visually inspected until color change observed and mechanical property was evaluated by measurement of the tensile strength. As shown in Table 1, addition of HM to CF resulted in decrease in the mechanical strength and reduced the wetting time. Optimal balance between wetting, mechanical strength and manufacturing considerations by electrospinning resulted in the selection of CF:HM w/w ratio at 10:1. The polyurethane mixture was then dissolved at a concentration of 13% (w/w) in a solvent mixture of 1:2 w/w N–N-dimethylformamide (BioLab) and dioxane (BioLab). Electrospun envelope structures at a thickness of 200 μm were fabricated according to specifications for strong fibers at a range of 0.5–2 μm and porosity of ~70%. The following electrospinning parameters were applied at an environment of 40% relative humidity and 28 °C. A collector at diameter of 50 mm and length of 150 mm with rotating speed (ω) of 120 rpm was used. A five needles spinneret was used (22 G, 25 mm length) at linear speed of 57 mm/s and traverse shuttle of 170 mm. The spinneret was placed 260 mm from the collector. A potential of 60 kV, solution flow rate of 14 mL/h and consumption of 10 mL were applied.

Following electrospinning the envelope sheet was dried overnight at room temperature and then at 50 ± 5 °C for 48 h. The dried envelope sheets were cut into discs of 20 mm diameter before scaffold assembly.

Core discs at predetermined content (see 2.1.3) and size were wrapped by two envelope discs that were heat sealed using custom made tools (Fig. 1B). The sealing conditions were 4 s at pressure of 3 bar and temperature of 118 °C. The PU scaffold was then cut into its final size, at a diameter of 6, 8, 9 or 14 mm. The scaffolds were sterilized in a cold-cycle (37 °C) ethylene oxide process and subsequently evacuated at room temperature and 150 mbar for 7 days.

A delivery system for non-invasive injection of the PU scaffolds was developed by Melab Medizintechnik und Labor GmbH (Germany). The PU scaffolds at diameters of 6–14 mm can be rolled into a tube of 2.8–5 mm diameter and delivered through a needle with insertion guide. A demonstration of the delivery process is shown in Fig. 1C.

2.1.2. Structure characterization of the PU scaffold envelope

The electrospun envelope sheet material was examined using scanning electron microscopy (SEM). Electron micrographs were recorded for both sides and cross sectional areas of the electrospun envelope. Samples were snap-frozen in liquid nitrogen and fractured into 2 or more pieces to obtain a cross-sectional edge.