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Biphasic scaffold for annulus fibrosus tissue regeneration

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Abstract

Intervertebral disc (IVD) degeneration is the major cause of lower back pain, while the currently available treatments are symptomatic rather than curative. Tissue engineering is a powerful therapeutic strategy that can restore the normal biomechanical motion of the human spine. The ability of a biphasic elastic scaffold to structurally and elastically simulate the annulus fibrosus (AF) tissue of the IVD was explored. The outer phase of the scaffold was a ring-shaped demineralized bone matrix gelatin (BMG) extracted from cortical bone, which mimicks the type I collagen structure and ligamentous properties of outer AF. The inner phase of the scaffold was a bio-biomaterial poly(polycaprolactone triol malate) (PPCLM) orientated in concentric sheets and seeded with chondrocytes to recapitulate the inner layer of the AF, which is rich in type II collagen and proteoglycan. The mechanical properties and degradation of PPCLM could be adjusted by controlling the post-polymerization time of the pre-polymer. PPCLM also demonstrated good biocompatibility in a foreign body response *in vivo* assay. Incorporation of BMG into the scaffold enhanced the compressive strength compared with PPCLM alone. In addition, the tensile stress of the BMG/PPCLM scaffold was 50-fold greater than that of PPCLM alone, and close to that of normal rabbit AF. Finally, the biphasic scaffold supported the growth of rabbit chondrocytes, as confirmed by Safranin-O and type II collagen immunostaining. The excellent mechanical properties and biocompatibility of the BMG/PPCLM scaffold make it a promising candidate for AF repair.
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1. Introduction

The intervertebral discs (IVD) separate the vertebrae of the spine and account for approximately one-third of the height of the entire spinal column. The discs have a unique structure that enables the bending and twisting motion of the spine. While degeneration of the IVD is associated with the majority of cases of lower back pain, current treatments are symptomatic rather than curative, and rely on a limited repertoire of medical and surgical interventions depending on the stage of degeneration that has been reached. In particular, medical intervention can only relieve the symptomatic pain. The current surgical interventions, such as discectomy and spinal fusion, although fairly successful in relieving back pain in the short term, fail to restore the normal biological and mechanical properties of the human spine, and further sacrifice the flexibility of the spine. New approaches, such as artificial total disc replacement and nucleus replacement, aim to relieve back pain and restore the normal biomechanical motion of the human spine.

The IVD is comprised of a central, gelatinous nucleus pulposus (NP), surrounded by a fibrous annulus fibrosus (AF), with cartilaginous end plates on the superior and inferior surfaces (Fig. 1(a)). The AF consists of an extracellular matrix (ECM) composed of both type I and type II collagen oriented in a lamellar structure. The outermost part of the AF is ligamentous, and is rich in type I collagen, whereas the inner part is fibrocartilage-like, and is rich in type II collagen and proteoglycan. Thus far, the

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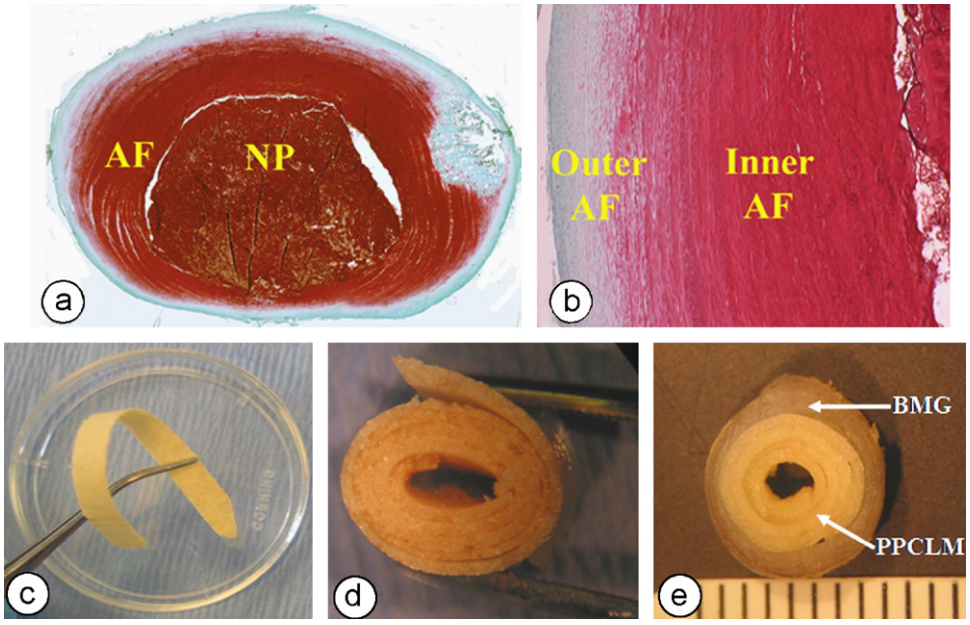

Fig. 1. (a) Horizontal section and (b) vertical section of normal rabbit IVD stained with Safranin-O. The outer layer (reduced red staining) and inner layer (abundant red staining) of AF can clearly be seen. (c) The elastic biomaterial PPCLM orientated in concentric sheets (d) and inserted into a BMG ring to mimic the structure of inner and outer AF (e), respectively.

majority of the research on AF tissue engineering has focused on recapitulating the inner layer of the AF while neglecting the outer layer, which plays a critical role in the biomechanical properties of IVD. Nonetheless, the goal of regenerating the AF tissue should not only be to achieve anatomic morphology, but also to restore its function. To achieve this, it is necessary to regenerate both the inner and outer layers of the AF, to reinstate both the histology and the mechanical function.

IVD has a very limited ability to regenerate, similar to that of articular cartilage, due to its avascular structure. When nucleotomy (a surgical treatment for IVD herniation) is carried out, little regeneration of the AF is observed, making degeneration of the IVD an inevitable consequence of this procedure. Tissue engineering of the AF would therefore be an ideal candidate for repair or substitution of the resulting degenerated discs with appropriate analogs. Recent advances in the treatment of disc degeneration involve nucleus prosthesis [1,2] and gene therapy [3]. Nevertheless, tissue engineering would offer a powerful strategy for the treatment of disc degeneration, as it integrates the principles of cell biology, molecular biology, chemistry, and engineering to restore tissue function. Several different types of materials have been explored as possible candidates for AF tissue replacement, e.g., PLGA [4], collagen-glycosaminoglycan [5], and PDLLA/45S5® [6]. However, none of them mimic the compositional structure and elastic properties of AF.

Herein, we propose a novel biphasic scaffold model to simulate both the outer and inner AF, structurally and elastically. The inner phase of the scaffold is composed of a biomaterial based on poly(polycaprolactone triol malate) (PPCLM, Fig. 1(c)), orientated in concentric sheets and seeded with chondrocytes (Fig. 1(d)), to mimic the lamella structured cartilage-like inner AF. The outer phase of the scaffold is composed of demineralized bone matrix gelatin (BMG) derived from rabbit femur, which mimics the high content of type I collagen in AF (Fig. 1(e)). The PPCLM and BMG were fabricated into a three-dimensional scaffold and then loaded with rabbit chondrocytes. To explore the utility of this scaffold for tissue engineering we characterized the cell growth by SEM observation, histo- and immunostaining. It is hoped that the results obtained in this investigation could offer a foundation for further study dedicated to replacement of the human AF with a material that will allow normal function of the spine.

2. Materials and methods

2.1. Synthesis of PPCLM

PPCLM was synthesized by polycondensation between PCL (Mn = 300, 900; Sigma, St. Louis, MO) and malic acid (Sigma). Briefly, 0.1 mol malic acid and 0.05 mol PCL were added to a 100 ml two-neck round-bottom flask fitted with an inlet and outlet adapter. The mixture was melted at 140 °C under a flow of nitrogen gas with constant stirring. Once the compounds had melted, the temperature of the system was lowered to 120 °C for 1 h under stirring to create a pre-polymer. The pre-polymer was post-polymerized at 120 °C for pre-determined times required by the experimental protocol, up to 5 days.

2.2. Fourier transform infrared (FTIR)

FTIR spectra were obtained at room temperature using a MIDAC Prospect Series (Model PRS) FTIR spectrometer. The PPCLM was shaved with a surgical scalpel to obtain a powder of polymer that was then ground along with KBr crystal into powder for FTIR analysis.