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Basic Science

Creation of an injectable in situ gelling native extracellular matrix for nucleus pulposus tissue engineering

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Abstract

BACKGROUND CONTEXT: Disc degeneration is the leading cause of low back pain and is often characterized by a loss of disc height, resulting from cleavage of chondroitin sulfate proteoglycans (CSPGs) present in the nucleus pulposus. Intact CSPGs are critical to water retention and maintenance of the nucleus osmotic pressure. Decellularization of healthy nucleus pulposus tissue has the potential to serve as an ideal matrix for tissue engineering of the disc because of the presence of native disc proteins and CSPGs. Injectable in situ gelling matrices are the most viable therapeutic option to prevent damage to the anulus fibrosus and future disc degeneration.

PURPOSE: The purpose of this research was to create a gentle decellularization method for use on healthy nucleus pulposus tissue explants and to develop an injectable formulation of this matrix to enable therapeutic use without substantial tissue disruption.

STUDY DESIGN: Porcine nuclei pulposi were isolated, decellularized, and solubilized. Samples were assessed to determine the degree of cell removal, matrix maintenance, gelation ability, cytotoxic residuals, and native cell viability.

METHODS: Nuclei pulposi were decellularized using serial detergent, buffer, and enzyme treatments. Decellularized nuclei pulposi were solubilized, neutralized, and buffered. The efficacy of decellularization was assessed by quantifying DNA removal and matrix preservation. An elution study was performed to confirm removal of cytotoxic residuals. Gelation kinetics and injectability were quantified. Long-term in vitro experiments were performed with nucleus pulposus cells to ensure cell viability and native matrix production within the injectable decellularized nucleus pulposus matrices.

RESULTS: This work resulted in the creation of a robust acellular matrix (>96% DNA removal) with highly preserved sulfated glycosaminoglycans (>47%), and collagen content and microstructure similar to native nucleus pulposus, indicating preservation of disc components. Furthermore, it was possible to create an injectable formulation that gelled in situ within 45 minutes and formed fibrillar collagen with similar diameters to native nucleus pulposus. The processing did not result in any remaining cytotoxic residuals. Solubilized decellularized nucleus pulposus samples seeded with nucleus pulposus cells maintained robust viability (>89%) up to 21 days of culture in vitro, with morphology similar to native nucleus pulposus cells, and exhibited significantly enhanced sulfated glycosaminoglycans production over 21 days.

FDA device/drug status: Not applicable.

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CONCLUSIONS: A gentle decellularization of porcine nucleus pulposus followed by solubilization enabled the creation of an injectable tissue-specific matrix that is well tolerated in vitro by nucleus pulposus cells. These matrices have the potential to be used as a minimally invasive nucleus pulposus therapeutic to restore disc height.

Keywords: Decellularized matrix; Disc degeneration; Injectable scaffold; Intervertebral disc; Nucleus pulposus; Tissue engineering

Introduction

Low back pain will affect 70% to 80% of the population at some point in their lifetime, and disc degeneration is the leading cause of low back pain [1]. Disc degeneration is characterized by a loss of intervertebral disc (IVD) height, reduced water content, end plate thickening, and annular fissures [2–7]. Breakdown of the nucleus pulposus, a gelatinous matrix rich in collagen type II and chondroitin sulfate proteoglycans (CSPGs) [8,9], has been implicated in disc degeneration [10]. The CSPGs have sulfated glycosaminoglycan (sGAG) side chains that attract water, enabling the nucleus to withstand substantial compressive loads and prevent nerve ingrowth [8,9,11]. Cleavage of the CSPGs and consequent loss of sGAG side chains have been implicated in disease progression by resulting in the loss of the ability of the nucleus to hold water and therefore bear compressive loads [10]. The loss of CSPGs may additionally enable innervation of the disc and subsequent pain [11,12]. Discogenic pain is one source of low back pain that is thought to arise from increased nociceptive fiber innervation in the IVD [13,14].

A variety of approaches are currently being investigated to repair the nucleus pulposus and prevent low back pain from occurring. Tissue engineering approaches often combine growth factors and cells to attempt to repair the nucleus pulposus; however, in the harsh catabolic environment of the degenerating disc, growth factors and cells cannot survive [15–20]. The addition of a biomaterial carrier has the potential to increase cell viability and enable long-term growth factor delivery. Various synthetic and natural materials are currently being investigated for nucleus pulposus tissue engineering [21–26]. However, synthetic materials, such as chemically modified hyaluronan, can have toxic byproducts or result in a foreign body response [27–29].

An ideal solution for tissue engineering of the nucleus pulposus would (1) mimic the native disc composition and consist of a proteoglycan rich matrix to restore the hydrostatic compressive resistance of the disc, (2) serve as a cell carrier, (3) prevent innervation into the nucleus, and (4) be deliverable via injection and in situ gelation. Decellularized nucleus pulposus tissue has the potential to be this ideal scaffold because it is composed of native disc proteins and proteoglycans with similar structure to native disc. In addition, a decellularized scaffold has the potential to be developed into an injectable formulation that gels in situ.

Tissue-derived biomaterials can offer unique advantages over synthetic materials such as limited foreign body response, native cell response, and integration into surrounding tissue using endogenous enzymes [30–33]. However, to minimize immune rejection of the tissue and to prevent the need for systemic immunosuppression, it is essential to remove cells and antigens. Decellularization of tissue is a practice that has been used for decades to create naturally derived scaffolds. Removal of cells and antigens is essential to preventing an immune response; however, decellularization must also maintain the composition and structure of the native tissue [33–36]. Decellularization techniques have been optimized for the heart [37], lung [38], nerve [39], skin [40], brain [41], and spinal cord [42], among other tissues, and can involve physical, chemical, and biological methods to lyse cells, and subsequently remove cellular debris. Our laboratory has previously developed gentle decellularization techniques for nerve tissue to preserve essential proteins, proteoglycans, and microstructure [39,43], which would be ideal for the delicate nucleus pulposus. To date, some research has been performed to create decellularization protocols for the nucleus pulposus [44–47], anulus fibrosus [48,49], and the intact IVD [50]. These matrices have shown promise as viable scaffolds for IVD cells as well as drivers of stem cell fate [44–50], although they are not injectable or in situ gelling.

After a tissue has been decellularized, it is possible to turn the tissue into an injectable in situ gelling biomaterial [37,51], which would enable an easier therapeutic delivery with minimally invasive surgery. For IVD, an injectable in situ gelling material would support delivery through a fine gauge needle with no disruption of the anulus fibrosus, while also enabling formation of a gel capable of supplementing load bearing of the nucleus pulposus. Works by Freytes et al. and Singelyn et al. document the early processes to solubilize decellularized urinary bladder matrix and heart tissue, enabling in situ gelation by using pepsin in hydrochloric acid [37,51]. Kwon et al. used pepsin in acetic acid to solubilize cartilage [52]. Others have used modifications of these protocols to solubilize a variety of tissues, including brain [41], liver [53], and tendon [54]. However, these protocols have not yet been adapted to create injectable in situ gelling hydrogels from decellularized nucleus pulposus or IVD tissue.

The purpose of this research was to create a gentle nerve decellularization method for use on the nucleus pulposus tissue of the IVD, thereby creating a tissue-specific acellular matrix with increased maintenance of proteoglycans and sGAGs with the potential to prevent nerve ingrowth and drive stem cell fate. In addition, we also wanted to create an injectable