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Intervertebral disc and stem cells cocultured in biomimetic extracellular matrix stimulated by cyclic compression in perfusion bioreactor

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Abstract

BACKGROUND CONTEXT: Intervertebral disc (IVD) degeneration often causes back pain. Current treatments for disc degeneration, including both surgical and nonsurgical approaches, tend to compromise the disc movement and cannot fully restore functions of the IVD. Instead, cell-based IVD tissue engineering seems promising as an ultimate therapy for IVD degeneration.

PURPOSE: To tissue-engineer an IVD ex vivo as a biological substitute to replace degenerative IVD.

STUDY DESIGN: An extracellular matrix (ECM) structure-mimetic scaffold, cocultured human IVD cells and human mesenchymal stem cells (hMSCs), and mechanical stimulation were used to biofabricate a tissue-engineered IVD.

METHODS: An optimal ratio of human annulus fibrosus (hAF) cells to hMSCs for AF generation within aligned nanofibers, and that of human nucleus pulposus (hNP) cells to hMSCs for NP generation within hydrogels were first determined after comparing different coculture ratios of hAF or hNP cells to hMSCs. Nanofibrous strips seeded with cocultured hAF cells/hMSCs were constructed into multilayer concentric rings, enclosing an inner core of hydrogel seeded with hNP cells/hMSCs. A piece of nonwoven nanofibrous mat seeded with hMSC-derived osteoblasts was assembled on the top of the cellular nanofiber/hydrogel assembly, as an interface layer between the cartilagenous end plate and vertebral body. The final assembled construct was then maintained in an osteochondral cocktail medium and stimulated with compressive loading to further enhance the hAF and hNP cells differentiation and increase the IVD ECM production.

RESULTS: Among all cocultured groups, hAF cells and hMSCs in the ratio of 2:1 cultured in nanofibers showed the closest mRNA expression levels of AF-related markers to positive control hAF cells, whereas hNP cells and hMSCs in the ratio of 1:2 cultured in hydrogels showed the closest expression levels of NP-related markers to positive control hNP cells. The effects of compressive loading on chondrogenesis of hAF or hNP cell and hMSC coculture were dependent on the scaffold structure; the expression of cartilage-related markers in AF nanofibers was downregulated, whereas that in NP hydrogel was upregulated. Interestingly, we found that hMSC-derived osteogenic cells in the interface layer were turned into chondrogenic lineage cells, with decreased expression of osteogenic markers and increased expression of chondrogenic markers.

CONCLUSIONS: We demonstrate a unique approach using a biomimetic scaffold, IVD and stem cell coculture, and mechanical stimulation to tissue-engineer a biological IVD substitute. The

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results show that our approach provides both favorable physical and chemical cues through cell-matrix and cell-cell interactions and mechanobiological induction to enhance IVD generation ex vivo. Our findings may lead to viable tissue engineering applications of generating a functional biological IVD for the treatment of disc degeneration.

Key Words: Intervertebral disc; Tissue engineering; Mesenchymal stem cell; Electrospinning; Biomimetic scaffold; Bioreactor

Introduction

Degeneration of the intervertebral disc (IVD) often results in debilitating neck and/or back pain [1]. Current treatments for IVD degeneration include palliative therapies and surgical intervention, such as spinal fusion. However, these procedures only restore a part of IVD functions and often lead to secondary IVD degeneration adjacent to the surgical site [2]. Tissue engineering-based therapies promise to provide a viable option for the treatment of IVD degeneration. Specifically, a tissue engineering approach involves the use of cells, biomaterial scaffolds, growth factors, and bioreactor to generate a functional IVD substitute for implantation to restore functions of the IVD.

A biomaterial scaffold plays a critical role in guiding ex vivo tissue morphogenesis, as it also acts as a vehicle to deliver cells and provides a three-dimensional structure for cell attachment, proliferation, and differentiation. Therefore, biological activities of cells cultured within a biomaterial scaffold are highly dependent on the chemical [3] and physical [4] properties of a scaffold. In many previous studies, researchers often used hydrogels to culture annulus fibrosus (AF) or nucleus pulposus (NP) cells [5,6] for IVD tissue engineering. However, given that the morphology and properties of the native extracellular matrix (ECM) of AF and NP are different, and cell behavior is influenced by ECM properties, it is not desired to use the same type of scaffold for both AF and NP generation. Therefore, biomaterial scaffolds that imitate the ECM structure of AF or NP have recently been developed by several research groups [7–9] for enhanced tissue generation.

Previous studies have shown that nanofibrous scaffolds fabricated by electrospinning have unique physical properties, providing favorable cell-matrix cues to enhance cell activities [10,11]. With the aid of a high-speed rotator, aligned nanofibrous scaffolds have been fabricated for engineering tissues that have anisotropic ECM alignment and mechanical properties [12,13]. For example, patterning aligned nanofibers in the way of alternating the orientation of fibers with respect to the spinning direction of a rotator can create a nanofibrous scaffold with multilayered anisotropic fibers. Nerurkar et al. [9,14,15] have demonstrated a fabrication method by which they electrospin nanofibers to form a multilaminated structure to imitate the unique structure of AF. They report that the structure can direct mesenchymal stem cells (MSCs) or AF cells to deposit organized, collagen-rich ECM along with oriented nanofibers, suggesting the feasibility of using aligned nanofibers for IVD tissue engineering.

Annulus fibrosus and NP cells are appropriate cell choices for IVD tissue engineering because they are terminally differentiated and able to produce tissue-specific ECM. However, the key challenge with the use of AF and NP cells is associated with the hypocellularity of the IVD. Because cells make up only 1% of the total tissue volume [16], it is unlikely to isolate a sufficient amount of cells from AF or NP for tissue generation. Moreover, with the inherently limited capacity for cell proliferation, it is challenging to yield adequate amounts of AF and NP cells during in vitro culture for cell seeding in scaffolds. In contrast, multipotent human MSCs (hMSCs) isolated from various adult tissues can be expanded extensively ex vivo for IVD tissue engineering [17,18]. Sakai et al. [19,20] have reported a promising result, demonstrating the therapeutic potential of MSCs for the treatment of NP degeneration in a rabbit model. Although much progress has been made, the complete differentiation of MSCs into IVD cells in vitro still remains challenging. The current protocol is to induce MSCs to differentiate along the chondrogenic lineage into AF or NP cells. However, MSC-derived chondrocytes are phenotypically and genetically different from AF or NP cells [21,22]. For example, AF cells produce more collagen type V, and both AF and NP cells have a greater level of HtrA1 expression than articular chondrocytes. To increase the efficacy of differentiation induction, several groups have cocultured MSCs with IVD cells and demonstrated that the coculture enhances MSC differentiation [23–25]. Moreover, although how the ratio of cocultured chondrocytes to MSCs affects the MSC activities has been demonstrated [26,27], it has not been comprehensively studied whether different ratios of cocultured IVD cells to MSCs regulate MSC differentiation differently.

An IVD sandwiched between vertebral bodies is constantly subjected to mechanical loading. A previous study by Handa et al. [28] has demonstrated that hydrostatic pressure increases the proteoglycan production in human IVD explants. Although the magnitude of static force is an important parameter for spinal loading, it has been demonstrated that stimulation by cyclical loading is critical to the regulation of cellular metabolism and tissue homeostasis in the IVD. For instance, Kasra et al. [29] have shown that dynamic loading increases the collagen production in AF, stimulates protein synthesis, and inhibits ECM degradation