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REVIEW

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A biological approach to treating disc degeneration: not for today, but maybe for tomorrow

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Abstract The intervertebral disc unites the vertebrae in the spine, providing the flexibility required for bending and twisting and resisting the compression inflicted by gravity when in an upright posture. The discs have a complex structure, with the outer annulus fibrosus having lamellae of organized collagen fibrils and the inner nucleus pulposus having a more random collagen organization and an abundance of aggregating proteoglycans. This composite nature endows the disc with both the tension-resisting properties of a ligament and the compression-resisting properties of articular cartilage. Unfortunately, disc structure and function does not remain optimal throughout life, but undergoes progressive degeneration, commencing in the young adult, and is particularly evident in the nucleus pulposus. With time, disc degeneration may result in clinical symptoms, such as low back pain, and require medical intervention. Such treatment may involve removal of the offending disc by surgery rather than its repair, which would be the preferred course of action. In the near future, current bioengineering techniques may offer the possibility of repairing the damaged disc, if an engineered tissue with the appropriate functional properties can be generated to augment the ailing disc. In this report, we summarized our recent results, in which disc cells were implanted into a scaffold of collagen and hyaluronan, or entrapped into a chitosan gel, and growth factors were used to modulate matrix synthesis in an attempt to produce a tissue with a similar molecular composition to native nucleus pulposus tissue.

Keywords Intervertebral disc · Degeneration · Repair · Scaffolds · Growth factors

Introduction

Intervertebral discs are characterized by their abundant extracellular matrix and low cell density, coupled with an absence of blood vessels, lymphatics, and nerves in all but the most peripheral annulus layers. In many respects, this absence leaves the disc prone to degeneration, because the cells have a large extracellular matrix to maintain without nociceptive feedback to limit and detect damage, and no source of repair through the vasculature.

Intervertebral discs are not uniform in composition, but consist of two clearly distinct regions. The outer annulus fibrosus is a fibrocartilage, and contains concentric lamellae rich in collagen, whereas the inner nucleus pulposus is a less structured gelatinous substance rich in proteoglycans. Degeneration and age-related changes in both the biochemical composition and structure of each component of the intervertebral disc have been widely reported [5, 14, 37, 40, 49]. As discs degenerate, the nucleus pulposus becomes more consolidated and fibrous, and is less clearly demarcated from the annulus fibrosus. Focal de-

fects appear in the cartilage endplate, and there is a decrease in the number of layers of the annulus with an increase in thickness and spacing of the collagen fibrils [38]. Degeneration causes decreased hydration, especially in the nucleus [5]. Water content in the nucleus pulposus drops from about 90% of the tissue wet-weight in the infant to less than 70% in the elderly [5, 21]. In the annulus fibrosus, the water content remains relatively constant with age, accounting for approximately 60–70% of the tissue wet-weight [5, 21].

Collagen represents about 15–20% of the nucleus, and 65–70% of the annulus dry-weight [5, 17, 18]. At least seven distinct collagen types have been identified in the intervertebral disc, types I, II, III, V, VI, IX and XI. The annulus fibrosus of the intervertebral disc has been reported to contain all these collagen types, whereas the nucleus pulposus contains only types I, II, VI and IX collagen [1, 6, 7, 8, 17, 18, 62]. In addition, type X collagen has been shown to be present in discs with histomorphological alterations consistent with disc degeneration [3, 11]. Types I and II collagen constitute about 80% of the collagens in the intervertebral disc [5, 17]. Although the other collagen types identified in the disc account for a smaller proportion of the total collagen, they may make a very significant contribution to the overall function of the tissue. Recent work has shown that type II collagen degradation in the human lumbar intervertebral disc is increased with age and degeneration, and in parallel, the cell synthetic capacity is strongly suppressed with aging and degeneration [5, 20].

The trends in molecular abundance observed for collagen are reversed for proteoglycans, which represent approximately 50% of the dry-weight in the nucleus, but only 10–20% in the annulus [5, 17, 18]. The ability of the discs to resist compressive forces is largely due to their high content of the proteoglycan aggrecan and its ability to interact with hyaluronan [23, 25, 44]. Versican, another proteoglycan with the ability to interact with hyaluronan, has also been shown to be present within the intervertebral disc [58]. In addition to aggregating proteoglycans, the discs also contain decorin, biglycan, fibromodulin and lumican [29, 53, 57], which belong to the family of leucine-rich repeat proteoglycans. Ageing and degeneration of the discs are accompanied by a marked decrease in proteoglycan content in the nucleus and major alterations in proteoglycan structure [5, 12, 37].

The process of disc degeneration involves the destruction of structural proteins, including collagens and proteoglycans, within the extracellular matrix. It is generally agreed that proteinases play a major role in this process. One group of proteinases thought to be involved in the destruction of the disc matrix includes members of the matrix metalloproteinases (MMPs) [13, 19, 41, 43, 46, 47], particularly the collagenases and gelatinases. Once activated, collagenases can degrade types I and II collagen by cleavage in their helical domains, thus making these collagens susceptible to further enzymatic degradation by gelatinases. A second group of proteinases involved in matrix degradation includes members of the ADAM family [55], particularly those members with thrombospondin repeat motifs (ADAMTS) [59]. Two members of this subfamily are of particular importance because of their ability to specifically degrade aggrecan – aggrecanase-1 (ADAMTS4) and aggrecanase-2 (ADAMTS5). It has been shown that aggrecan cleavage products due to degradation by both the matrix metalloproteinases and aggrecanases are present in the intervertebral disc, suggesting that these enzymes are active in this tissue [56]. Unlike most other connective tissue cell types, little is known about the ability of disc cells to produce the different metalloproteinases. The only proteinase extracted directly from intervertebral disc appears to be a serine proteinase rather than a metalloproteinase, and it has properties similar to plasmin [15, 39]. However, human disc in organ culture has been shown to synthesize stromelysin (MMP3), which can become activated within the matrix [34]. MMP1, 2, 3, 7, 8 and 9 have also been shown to be present in degenerated human discs, suggesting a role for these metalloproteinases in disc degeneration [16, 52].

Mechanisms that may contribute to the age-related and/or degenerative changes of the disc include reduction in nutrient supply, diminished cell viability, loss of notochordal cells, cell senescence, cell apoptosis and genetic factors, which lead to biochemical alterations in the composition and structure of the extracellular matrix [2, 9, 10, 22, 24, 26, 48, 50, 51]. In addition, alterations in intervertebral disc structure are associated with, or aggravated by, mechanical factors [27, 28, 30, 31, 32, 36, 42, 45].

The degenerative disorders of the lumbar spine that require surgical intervention include herniated discs, spinal stenosis, degenerative spondylolisthesis, degenerative scoliosis, and degenerative disc disease. Among these, it is the treatment of idiopathic low back pain associated with lumbar degenerative disc disease that is the most controversial, and remains a challenge for the orthopaedic surgeon. Although, surgical procedures involving vertebral fusion produce a relatively good short-term clinical result in relieving pain, they alter the biomechanics of the spine and can lead to further degeneration of the discs at adjacent levels. In fact, the failure rate for lumbar fusions is estimated to be in the 20–40% range [60], and there is clinical and radiological evidence that spinal fusion leads to accelerated degeneration of the adjacent motion segment [33, 54].

Biological approach to repair disc damage

In general, surgical procedures try to remove rather than repair the problems associated with the degenerate intervertebral disc. Repair is, however, the ideal therapeutic approach, as it restores the normal structure and function

of the intervertebral disc. We believe that future treatments will be able to effect biological repair of the damaged tissue by restoring it to a tissue of similar functional competence to the healthy native one. These are the dreams of all investigators involved in tissue engineering research, which extend from the "simple" injection of cells into the defect, to the futuristic aspiration of implanting an in-vitro-generated intact motion segment.

However, unless one is a true optimist, only two biological approaches to the treatment of disc degeneration are likely to become clinically available within the next 10 years. At the earlier stage of disc degeneration, injection of inhibitors of proteolytic enzymes or biological factors that stimulate cell metabolic activity (i.e. growth factors) can be foreseen, in order to slow down the degenerative process. Alternatively, when disc degeneration is confined to the nucleus, it is not unreasonable to propose that implantation or injection of a biomatrix embedded with cells will have the potential to restore functionality, and to retard further disc degeneration. In both cases, several problems need to be addressed before the two potential treatment modalities can be turned into clinical realities.

Our attempt to initiate biological repair of disc degeneration is based on the second of the above described approaches, namely the supplementation of the degenerated nucleus pulposus with cells seeded or embedded within a biomatrix. In principle, two types of biomatrices can be envisaged as scaffolds for use in disc repair. The first involves a preformed matrix into which isolated disc cells can be seeded, the composite maintained in vitro to attain an optimal composition, and the product then implanted into the degenerate disc. A second approach is to use a soluble polymer to which cells can be added and the mixture then injected into the disc where the scaffold can polymerize in situ. The advantages of the second approach are that the polymerized construct will conform precisely to the disc defect and that its clinical application is more straightforward, avoiding extensive surgical disruption of the annulus. However, the latter system is not readily compatible with prior in vitro culture of the construct, and requires that the cells produce an appropriate extracellular matrix in the nutritionally deprived disc milieu.

Our initial work focused on selecting a biomatrix that was able to support disc cell viability and phenotype, and allow the accumulation of an extracellular matrix rich in proteoglycans. Two biomatrices were selected: a collagen-hyaluronan scaffold from Orquest Inc. (Mountain View, Calif., USA), and a chitosan gel developed by Biosyntech (Laval, Quebec, Canada).

The first scaffold, composed of collagen and hyaluronan, was chosen because it mimicked the matrix by which disc cells are normally surrounded in their native environment [35]. Cells were isolated from the nucleus pulposus of coccygeal discs from mature bovine tails, and then imbibed by surface tension into the dry biomolecular scaffold consisting of cross-linked type I collagen and hyaluronic acid in a 9:1 ratio [61]. The cell-seeded matrices were maintained in culture on an orbital shaker in the presence of fetal calf serum (FCS) or a variety of growth factors (TGF-β1, bFGF and IGF-1). FCS was able to induce proteoglycan synthesis and produce a matrix that exhibited Safranin O staining. TGF-β1 produced similar levels of proteoglycan as FCS, whereas proteoglycan levels were reduced with either bFGF or IGF-1 (Fig. 1). Combinations of the growth factors did not greatly influence the effect of TGF-β1 alone. In contrast, cell division was stimulated to the greatest extent by the presence of IGF-1. It was also shown that by day 20 of culture the matrices not only contained aggrecan, but also the members of the small leucine-rich repeat proteoglycan family (decorin, biglycan, fibromodulin and lumican), as are found in normal disc, and that both type I and II collagen were being synthesized. However, while all the proteoglycans of a normal disc were present, it was evident that the construct was not able to retain the majority of the proteoglycans that the cells synthesized (Table 1). This resulted in a tissue that was biomechanically unable to counteract the compressive loads to which the disc is normally subjected. In future work in this area it will be important to establish conditions under

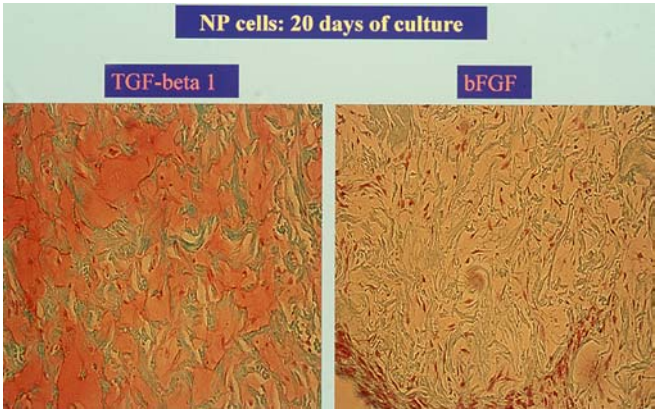

Fig. 1 Safranin O staining of nucleus pulposus cells seeded into collagen-hyaluronan scaffolds after 20 days of culture. Cells were stimulated with 10 ng/ml of TGF-β1 or 10 ng/ml of bFGF during the 20 days of culture

Table 1 Percentage of proteoglycans (PG) retained within the scaffolds and percentage of the total proteoglycans synthesized (medium + scaffolds) in vitro, compared to native mature bovine nucleus pulposus (NP) tissue. Disc cells were cultured in the presence of 10 ng/ml TGF-β1

Scaffolds	Results	
	% PG retained within the scaffold	% PG synthesized in vitro vs native NP tissue
Collagen-hyaluronan	25%	5%
Chitosan gel	75%	35%

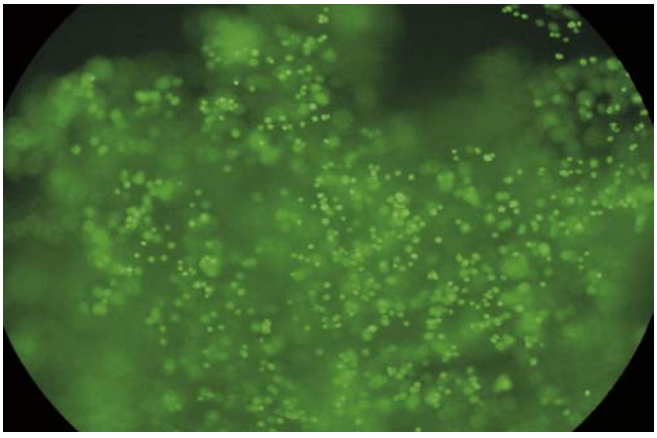

Fig. 2 Calcein AM and ethidium homodimer-1 staining of nucleus pulposus cells embedded into chitosan after 20 days of culture. Almost all the cells show a green fluorescence color, indicating excellent cell viability

which proteoglycan retention occurs efficiently, either by modifying the composition/structure of the scaffold or by treatment with more appropriate growth factors or biomechanical stimulation.

In a second approach, we used a chitosan-based polymer, which can be maintained as a soluble polymer at room temperature, and induced to gel at body temperature. Such a system might allow disc cells to be injected with the soluble polymer, which can then polymerize and entrap the cells in vivo (Fig. 2). Using identical experimental conditions to those described above for the collagen-hyaluronan scaffold, we found that the chitosan-based polymer was superior to the collagen-hyaluronan matrix, in both the synthesis and retention of proteoglycans (Table 1). This suggests that, at least in vitro, it should be feasible to generate a tissue with appropriate biochemical and mechanical properties similar to native nucleus pulposus. Thus, the disc cells can maintain their phenotype when cultured in a chitosan-based polymer, and over time are capable of producing a matrix with a proteoglycan content approaching that found in vivo [4].

Nevertheless, an important issue still requires to be addressed: the source of clinically useful cells. It is difficult to imagine that healthy nucleus cells could be obtained from the degenerated tissue that needs to be replaced. Two possible alternatives are conceivable: allogenic donor disc cells and/or autologous stem cells. While one can envisage the use of cells harvested from a donor, because of the immunologically privileged status of the nucleus pulposus, ethical considerations and the potential for spreading infectious diseases make the allogenic option less attractive. The use of stem cells as a source for generating nucleus pulposus cells would be the ideal choice, though at present there are no defined culture conditions where this differentiation process occurs. In addition, there are no well-defined cellular markers that can be used to identify disc cells and clearly distinguish them from other chondrocyte-like cells.

Furthermore, replacement of degenerate nucleus pulposus with a tissue engineered in vitro does not remove the reasons why the native tissue has degenerated. Indeed, one is attempting to repair without resolving the issue of what caused the original damage. So, what are the unidentified causes that lead to disc degeneration? Lack of an appropriate nutritional supply, and mechanical imbalance with excessive loading are possible explanatory mechanisms, together with genetic predisposition. Thus, more fundamental research investigating the possible mechanisms that lead to disc degeneration needs to be strongly supported by the scientific and industrial communities, if one is to be able to both initiate repair and prevent or retard subsequent degeneration.

Irrespective of these unresolved issues, our work does support the feasibility of generating a functional bioengineered disc matrix, and should provide optimism that biological therapy for disc degeneration may be a clinical reality in the not too distant future.

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