MEDICAL HYPOTHESES

Novel Cartilage-derived Biomimetic Scaffold for Human Nucleus Pulposus Regeneration: a Promising Therapeutic Strategy for Symptomatic Degenerative Disc Diseases

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Because current therapies have not always been successful and effective, the possibility of regenerating the nucleus pulposus (NP) through a tissue-engineered construct offers a novel therapeutic possibility for symptomatic degenerative disc diseases (DDD). However, more research is necessary to identify the optimal scaffold, cell type and mixture of signal factors needed for NP regeneration. Numerous possible scaffolds for NP regeneration have been investigated; they have many shortcomings in common. Various biological scaffolds derived from decellularized tissue and organs have been successfully used in tissue engineering and received approval for use in humans. Regrettably, harvesting of human NP is difficult and only small amounts can be obtained. The macromolecules of cartilage, which include collagen and proteoglycan aggrecan, are similar to those of the extracellular matrix of immature NP. Recent studies have shown that adipose-derived stem cells (ADSC) can be induced to develop NP-like phenotypes when stimulated by appropriate signals. We thus reasonably postulated that an ideal NP scaffold for tissue engineering could be fabricated from decellularized cartilage matrix (DCM). Furthermore, a combination of ADSCs and DCM-derived biomimetic scaffolds would be advantageous in NP tissue engineering and, in the long run, could become an effective treatment option for symptomatic DDD.

Key words: Degenerative disc diseases; Nucleus pulposus; Stem cells; Tissue engineering

Introduction

Seventy-five percent of all adults will experience low back pain (LBP) at some point in their lives^{1,2}, with an annual total cost exceeding \$100 billion in the USA alone^{3,4}. The main cause of LBP is degenerative changes in intervertebral discs (IVDs)^{5,6}. IVD degeneration is believed to begin with degeneration of the nucleus pulposus (NP), which is avascular and cannot self-repair. Although considerable attempts have been made to reverse NP degeneration, this field remains difficult and challenging. Although various surgical techniques and interventional therapies have been developed

to improve the management of IVD degeneration, they have had limited success and do not radically alleviate the underlying degenerative process. Recently some advanced biological technologies, including gene therapy, molecular therapy, cell therapy and tissue engineering methods have emerged; these offer potential treatment strategies for degenerative disc diseases (DDD)⁷⁻¹¹. Among them, tissue-engineered healthy NP is becoming a promising alternative to conventional approaches^{12,13}. However, several problems still need to be addressed before this strategy can be used clinically^{9-11,14}.

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The scaffold is one of the major components of tissue-engineering strategies. A broad band of scaffolds, both natural and synthetic, have been used for NP tissue engineering both *in vitro* and/or *in vivo*^{15–20}. In summary, these scaffolds have mainly focused on mimicking the NP extracellular matrix (ECM) by incorporating various purified components, including collagen II, collagen I, hyaluronic acid and/or proteoglycan aggrecan. These scaffolds have some shortcomings in common, for example, the inability to retain sGAG produced by seeded NP cells and omission of the minor collagen components (collagen types IX and XI) typically found in the NP ECM.

Because decellularization removes xenogenic or allogeneic cellular antigens and preserves most of the structural and functional proteins that constitute the ECM, biologic scaffolds derived from decellularized tissue and organs have been successfully used in tissue engineering. It is believed that scaffold fabricated using such techniques may prove advantageous biologically. Furthermore, many kinds of decellularized scaffolds, including human dermis (Alloderm; LifeCell, Branchburg, NJ, USA), porcine SIS (Surgi SIS; Cook Biotech, West Lafayette, IN, USA), porcine heart valves (Synergraft; Cryolife, Atlanta, GA, USA) and porcine urinary bladder (ACell, Columbia, MD, USA) have received approval for use in humans²¹. Regrettably, human NP harvesting is difficult and only small amounts can be obtained. NP is a type of cartilage tissue and is similar to articular cartilage in many respects²². For example, the macromolecules in the extracellular matrixes of cartilage and immature NP are similar; these include collagen, hyaluronic acid and proteoglycan aggrecan. To the best of our knowledge, little research has been done to investigate use of decellularized cartilage matrix-derived biomaterials as scaffolds in NP tissue engineering.

The source of cells is another important component of tissue-engineering NP. Because they are easy to procure, proliferate vigorously and have a strong capacity for self-renewal and the potential to differentiate into NP-like cells, adipose-derived stem cells (ADSC) have attracted immense attention as a cell source for NP regeneration^{12,23–30}.

Our Hypothesis

Based on the above considerations, we reasonably hypothesized that an ideal NP scaffold for tissue engineering could be fabricated from decellularized cartilage matrix (DCM). Furthermore, a combination of ADSCs and DCM-derived biomimetic scaffold could be advantageous in NP tissue engineering and might provide an effective treatment for symptomatic DDD.

The Rationale for our Hypothesis

Emerging Tissue Engineering Method for Treatment of Symptomatic Degenerative Disc Diseases

The surgical interventions for DDDs that are currently practiced (discectomy, spinal fusion, total disc replacement, etc.) are directed towards removing the damaged or altered NP

tissue. There is growing consensus that these strategies are not always effective³¹. Accordingly, there is a need to develop entirely new treatment modalities to treat this disorder.

Recent advances in cellular biology, material technology and regenerative medicine (also referred to as tissue engineering) are beginning to influence the clinical practice of spine surgery and appear particularly promising in this regard^{10,11,32–36}. These tissue engineering techniques have proven safety and efficacy in functional reconstruction of tissues and organs such as cartilage, bone, bladder, heart valves, skin and so on^{37–40}. Theoretically, bioengineered NP tissue could be used for surgical reconstruction of the degenerated NP. Previous clinical studies have shown that this technique can be used to treat symptomatic DDD⁴¹. More research is needed to identify the optimal scaffold, cell type and mixture of signal factors that are required for NP regeneration.

Use of Decellularized Cartilage Matrix as a Nucleus Pulposus Scaffold

An ideal scaffold should help to retain cells in the desired location and provide appropriate biochemical signals in the same way as the natural ECM for which it substitutes. A variety of decellularized tissues including ligaments, heart valves^{42,43}, blood vessels⁴⁴, skin⁴⁵, nerves⁴⁶, and urinary bladder have been investigated for tissue engineering applications²¹. A DCM-derived scaffold has been successfully developed and used for cartilage tissue engineering⁴⁷. The reasons for preferring DCM scaffolds for NP tissue engineering are based on the following facts. Firstly, novel DCM scaffold retains most of the components of cartilage ECM (including collagen II, hyaluronic acid and proteoglycan aggrecan) with complete absence of resident cells, which means that it is very similar to NP ECM. Previous studies have reported that collagen type II, the predominant collagen in nucleus pulposus ECM, maintains the chondrogenic phenotype and can even induce a chondrogenic phenotype in marrow stroma cells³⁰. Secondly, the strong structure and biocompatibility of DCM scaffold make it a suitable candidate for a supportive structure that cells can repopulate⁴⁷. Thirdly, natural cartilage can easily be harvested from cadaveric donors and stored in tissue banks. The method of preparation is reproducible and easy to implement and the scaffolds can be custom designed into desired shapes and sizes by using molds. Finally, our preliminary *in vitro* study demonstrated that a DCM-derived biomimetic scaffold provides adequate three-dimensional support for attachment, proliferation and differentiation of bone marrow-derived stroma cell (BMSCs) into NP-like cells⁴⁸. Therefore, DCM-derived NP scaffolds might be a more suitable than other natural or synthetic biodegradable scaffolds.

Differentiation of Adipose-derived Stem Cells into Nucleus Pulposus Cells

To repair the ECM of degenerative NP tissue, seed cells must produce proteoglycan, collagens and other matrix proteins in large quantities⁴⁹. Various cell types are currently under investigation for their therapeutic potential in managing

Fig. 1 Principal components of nucleus pulposus tissue engineering for symptomatic degenerative disc diseases. ADSCs, adipose-derived stem cells; DCM, decellularized cartilage matrix; DDD, degenerative disc diseases; NP, nucleus pulposus.

intervertebral disc degeneration. Because there are few of these cells, they are difficult to harvest and culture and have low viability and metabolic activity, autologous NP cells are not a suitable source for clinical application. The rationale for preferring ADSCs as seed cells is based on the following facts. Firstly, because adipose tissue can easily be obtained in outpatient clinics and yields of ADSCs can reach up to 25,000/g of tissue⁵⁰, large numbers of ADSCs can be obtained for clinical application. A recent study demonstrated that ADSCs are a better stem cell source than BMSCs⁵¹; the yield of ADSCs is double that of BMSCs^{50,52}. Secondly, the ethical controversies surrounding embryonic stem cell do not apply to ADSCs. Thirdly, numerous recent studies have provided data that greatly encourages the use of ADSCs as candidates for NP seed cells^{12,23–30,53–55}. Scientists have found that under the influence of appropriate signals (e.g. treatment with transforming growth factor- β , growth differentiation factor-5 or adenoviral vectors expressing Sox-9), ADSCs can differentiate into the NP-like phenotype^{26,29,53} and produce proteoglycans, collagens and other matrix proteins, which can be used to repair the ECM of degenerative NP tissue. Some researchers have even reported that co-culture of ADSCs and NP cells results in

differentiation of ADSCs into NP cell-like phenotypes^{27,28} and improves the quality of the resultant *in vitro* tissue-engineered tissue in terms of matrix production and cell organization. Therefore, ADSCs are a suitable source of seed cells for clinical use in symptomatic DDD.

Our Novel Strategy for Enhancing Wound Healing

Our novel therapeutic strategy of NP regeneration for treatment of symptomatic DDD is schematically represented in Fig. 1. Although many factors need to be studied in more detail, application for intervertebral disc regeneration seems realistic.

Conclusions

Although current surgical therapies focus on removal of diseased NP after symptomatic DDD, the potential to regenerate it with tissue-engineered NP constructs offers a novel therapeutic possibility. Considering the data and arguments outlined above, it is reasonable to believe that use of ADSCs via DCM to produce NP tissue engineering scaffolds may represent a promising therapeutic approach to the treatment of symptomatic DDD.

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