

EDITORIAL



Umbilical cord derivatives for intervertebral disc regeneration: advances and challenges

Sasha Mikhael, Naimisha Beeravolu & G Rasul Chaudhry

Recent advances in stem cell research provide promising opportunities to explore cell therapy as an alternative to traditional medicine to treat degenerative diseases such as DDD.

Lower back pain (LBP) is one of the most common causes of morbidity worldwide, found in approximately 80% of the population in their life time [1]. One of the major causative factors of LBP is a damaged intervertebral disc (IVD), which leads to degenerative disc disease (DDD). This disease is associated with a substantial economic and societal burden, accounting for approximately 25–30% of direct and indirect healthcare expenses in North America and Europe.

The IVD, located between the vertebrae of the spinal column, is an avascular, aneural tissue with low cell density. It is composed of an inner gelatinous structure, the nucleus pulposus (NP), which is surrounded by a ring of fibrous laminated collagen, the annulus fibrosus (AF) and the end plates. A healthy disc contains approximately 1% of NP tissue by volume and is predominantly composed of extracellular matrix (ECM) rich in

proteoglycans, in particular sulfated glycosaminoglycans (sGAG) and collagen II. The NP permits the retention of 80–88% water content, which is progressively decreased with age, provides shock absorption and is required for IVD homeostasis.

In humans, NP is derived from the notochord during development and it transitions from highly vacuolated notochordal cells into a fibrocartilaginous tissue by the second

decade of life, whereas the AF is made of 15–25 concentric rings of collagen fibrils arranged parallel to each other in a lamellar fashion. The lamellar structure of the AF allows the IVD to withstand tensile stresses encountered by the spine. Damage to AF can predispose the disc to herniation and bulging, leading to the loss of NP. Unfortunately, there is no cure for the debilitating DDD.

Currently, available treatments only provide short-term relief, and often lead to loss of function, morbidity and altered spinal biomechanics resulting in further aggravation of lumbar spinal vertebrae and in turn, increased pain. In some cases, spinal fusion, allografts and metal implants have been used with modest success [2]. In addition, commonly practiced pain management modalities can only provide limited relief. Recent advances in stem cell research provide promising opportunities to explore cell therapy as an alternative to traditional medicine to treat degenerative diseases such as DDD. As a result, a significant effort is being devoted to develop regenerative cell therapies to repair, regenerate or prevent IVD degeneration.

The core of this research lies in the use of different types of stem cells (SCs) such as embryonic stem cells (ESCs), bone marrow (BM)-derived adult stem cells (ASCs) and umbilical cord (UC) stem cells. Although ESCs derived from early embryo are the most primitive and promising for regenerative medicine, their therapeutic uses face significant ethical and moral concerns along with their potential to form teratomas [3]. ASCs found in various tissues and organs play a major role in maintaining the homeostasis. Among ASCs, multipotent

BM-SCs have shown substantial clinical applications for leukemic patients and those with blood dyscrasias. Not only BM-derived mesenchymal stem cells (MSCs) but also MSCs from other sources, such as adipose tissues, have also been investigated for their potential to regenerate IVD [4]. While these studies carried out *in vitro* or using animal model systems have helped to advance the regenerative field, the use of MSCs remains restricted due to limited availability and requiring invasive procedures for isolation. Furthermore, MSCs procured from adult sources have limited proliferation and differentiation potential. They are likely to have undergone genetic changes due to aging and exposure to environmental stressors. By contrast, the isolation of multipotent cells can be derived using non-invasive procedures from perinatal sources such as cord blood, cord tissue and the placenta. Moreover, these cells are more primitive and display a lower risk of graft-versus-host disease (GVHD) compared to BM-MSCs [5]. In fact, studies have shown that treatment of patients with leukemia complicated by GVHD with UC-MSCs showed notable improvements in symptomatology [6]. Furthermore, UC-MSCs do not require HLA compatibility and have been suggested to be safe and feasible for transplantation even without complete HLA compatibility. Because of their abundance and easy availability, UC tissues are being increasingly stored by public and private biobanks much like cord blood banking [7]. Recent studies suggest that SCs derived from cord and placenta grow robustly, and exhibit greater differentiation potential as compared to ASCs [8–10]. These

cells express not only MSC markers, CD29, CD44, CD73, CD90 and CD105, but also pluripotent markers, such as OCT4, NANOG, SOX2 and LIN28, although to a lesser extent than those found in ESCs. While more work is warranted to accurately characterize and classify the UC and placenta SCs, nonetheless, it is abundantly clear that these cells are unique and are more suitable for cell therapy, regenerative medicine and pharmaceutical applications. Therefore, it is not surprising that UC derivatives have been increasingly investigated for their potential to treat various degenerative diseases.

Similar to BM-MSCs, *in vitro* studies with UC-MSCs have demonstrated that they can be differentiated into chondroprogenitors and chondrocytes capable of expressing markers such as ACAN, SOX9 and COL2. Studies have shown that differentiated cells produced extracellular matrix including GAG, an essential component of NP of the IVD and express NP cell markers [8,11]. *In vivo* studies have demonstrated that UC-MSCs improve histological and biomechanical properties of the degenerated IVD when injected into the NP region of rabbit IVDs, disc water content and also upregulate expression of markers involved in disc matrix formation [12,13]. Our studies with the differentiated UC-MSCs in a rabbit model have demonstrated that transplanted cells not only survived but also engrafted and dispersed into the NP of the damaged IVD. Furthermore, a significant improvement in the histology, cellularity, extracellular matrix protein and in water and glycosaminoglycan content in IVDs recipients

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of chondroprogenitors (CPCs) was observed. The IVDs receiving CPCs exhibited higher expression of NP-specific markers [14]. More recently, UC-MSCs differentiated into NP-like cells (NPCs) transplanted into the rabbit IVDs, aided in restoration of structure and cellularity of the NP. IVDs receiving NPCs also displayed higher sGAG and water content compared to the sham and degenerated IVDs. The transplanted cells were functionally active as they expressed human genes and proteins, SOX9, ACAN, COL2, FOXF1, KRT19, PAX6, CA12 and COMP, which are implicated in NP biosynthesis. These studies suggested involvement of the TGF β 1 pathway in regulating NP regeneration. While these pre-clinical studies are encouraging, in a recent clinical study, UC cells transplanted into the IVDs of two individuals with chronic discogenic LBP indeed appeared to alleviate pain and improved physical function, but showed no change in the disc MRI signal intensity [15]. However, this study was conducted on a limited number of patients and the transplanted cells were not well characterized. Thus, it is imperative that clinical trials proceed with well-planned studies and thorough knowledge of regulation of degeneration and regeneration processes, using well-characterized and analyzed UC cells and

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derivatives that are most efficacious to regenerate not only NP but also AF components of the IVD.

CHALLENGES

Although UC derivatives show great regenerative potential based on the encouraging results of basic, preclinical and limited clinical studies, developing treatments for DDD to regenerate IVD or stop the degeneration process still face a number of challenges. These challenges include:

Mechanism of degeneration & regeneration

Molecular events involved in the onset and progression of degeneration have been poorly understood. While changes in NP water content can be visualized using MRI, changes that progressively occur due to an altered balance between anabolic and catabolic processes in the IVD are difficult to determine. Therefore, studies are needed to develop a better understanding of structural and biochemical changes and the molecular regulation of the processes involved. Use of advanced molecular biology techniques such as microarray and RNAseq analyses of the normal versus degenerated IVD could help delineate molecular regulators of degenerative and regenerative processes.

Quality control & standardization of cells

Like many ASCs, UC cells often are given multiple names from fibroblastic to MSCs and are not well characterized. It is important to develop standardized nomenclature based on their *in vitro* and *in vivo* characterization.

Strategies for transplantation to regenerate IVD

It is imperative to investigate the options to be used for cell therapy. Direct injection versus cells encapsulated in hydrogels is an interesting area that requires further study. Biomaterial scaffolds and hydrogels have shown to be chondroinductive [16]. They can enhance differentiation of UC-MSCs and improve survival and integration of transplanted cells *in vivo*.

Strategies to restore disc height

Loss of disc height due to degeneration of NP is one of the major factors impacting spinal biomechanics leading to LBP. Restoration of the disc height would be one of the major challenges facing regeneration of IVD. The use of biomaterials along with the augmentation of cells could be one of the minimally invasive approaches. Another approach could involve the surgical adjustment of disc height using screw clamps at the time of cell therapy. However, careful studies are required to develop such approaches to regenerate IVD and restore disc height.

Simultaneous regeneration of AF & NP

In some cases, both AF and NP of the IVD could be damaged, which

may be corrected by using progenitor cells capable of differentiating and repairing or regenerating both of these tissues *in vivo*. Our preliminary studies have shown chondroprogenitors derived from UC-MSCs help in repairing both AF and NP in an animal model of DDD.

NEW OPPORTUNITIES

Preconditioning of cells

Culturing cells under hypoxic conditions greatly improves growth, multi-lineage differentiation capacity, genetic stability and chemokine receptor expression during *in vitro* expansion. This approach could yield more potent cell transplants for regeneration of IVD. Augmentation of growth factors such as bone morphogenetic proteins, which are known chondrogenic inducers, could support resident or transplanted cells to produce NP components *in vivo*.

Expansion of UC cells

Although UC derivatives are more proliferative for longer time periods than ASCs, they do slow down and eventually stop growing. Methods are needed to expand UC cells without the loss of their proliferation capabilities. Bioreactor and 3-D scaffold culturing may be helpful to promote long-term culturing with higher yields [16]. Alternately, non-viral iPSCs could be generated from the UC cells.

Gene therapy combined with cell therapy approach

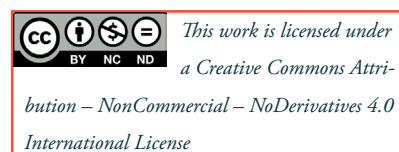
It has been shown that expression of SOX9 induced MSC differentiation into chondrogenic cells [17]. This approach could be used to develop

more potent cells for NP regeneration *in vivo*. Such studies could also be helpful in finding appropriate genetic markers and gene regulators of IVD regeneration. Use of CRISPR/CAS could be used to knock-in/out to edit or delete genes to correct defects or improve regenerative capabilities of cells.

Overall, recent advances in perinatal cells, specifically UC derivatives, have shown promise to regenerate/repair IVD. However, because of the unique anatomical characteristics, biological properties and avascular microenvironment of IVD, much remains unknown about the molecular mechanisms and interactions within the degenerated IVD, as well as the mechanisms controlling the regeneration upon augmentation of cells. Among the major challenges, a greater understanding of the beneficial effects of transplanted cells and their effects on the regulation of gene expression, including molecular signaling, would be helpful in devising strategies to regenerate/repair IVD. Finally, long-term clinical trials with a higher number of subjects/patients are needed for determining the safety and efficacy of UC cell applications for the treatment of DDD.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.



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