Lower back pain (LBP) is one of the most common causes of morbidity worldwide, found in approximately 80% of the population in their lifetime [1]. One of the major causative factors of LBP is the damaged intervertebral disc (IVD) which leads to degenerative disc disease (DDD). This disease is associated with a substantial economic and societal burden, accounting for about 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 about 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 % of water content which is progressively decreased with age, provides shock absorption, and is required of 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 to 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 such as embryonic stem cell (ESCs), bone marrow (BM) derived adult stem cells (ASCs), and umbilical cord (UC) stem cells (SCs). Although ESCs derived from early embryo are the most primitive and promising for regenerative medicine, their therapeutic use faces 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 regenerative field, the use of MSCs remain 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. In contrast, isolation of multipotent cells can be derived using non-invasive procedures from perinatal sources such as cord blood, cord tissue, and 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 to cord blood banking [7]. Recent studies suggest that SCs derived from cord and placentas 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, NANO2, SOX2, and LIN28, although to a lesser extent to 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 increasing investigated for their potential to treat various degenerative diseases.

Similar to BM-MSCs, in vitro studies with the 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 rabbit model have demonstrated that transplanted cells not only survived but also engrafted and dispersed into NP of the damaged IVD. Furthermore, a significant improvement in the histology, cellularity, extracellular matrix protein, and in water and glycosaminoglycan contents in IVDs recipients 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 implicated in NP biosynthesis. These studies suggested involvement of TGFβ1 pathway in regulating NP regeneration. While these preclinical studies are encouraging, in a recent clinical study, UC cells transplanted to 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 should proceed with well-planned studies and thorough knowledge of regulation of degeneration and regeneration processes, using well characterized and analyzed UC cells and 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 and 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 molecular regulation of the processes involved. Use of advanced molecular biology techniques such as microarray and RNAseq analyses of the normal vs degenerated IVD could help delineate molecular regulators of degenerative and regenerative processes.
Quality control and 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; 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. Use of biomaterials along with the augmentation of cells could be one of the minimally invasive approaches. Another approach could involve 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 and 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 of cells under hypoxic conditions greatly improve 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 stops growing eventually. 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 MSCs 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 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 higher number of subjects/patients are needed for determining the safety and efficacy of UC cell applications for treatment of DDD.

References: