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Disc Regeneration: Why, When, and How

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There is increasing acknowledgment that patients with back pain who are candidates for surgery will benefit over the long term from less invasive procedures that facilitate dynamic stabilization, rather than fusion [1]. The success of current fusion procedures is limited because of morbidity from the surgical technique or accelerated degeneration at adjacent segments [2].

Dynamic stabilization can be accomplished by providing assistance using mechanical devices (eg, partial disc replacement, posterior dynamic stabilization), or relying on biologic processes (tissue regeneration and repair). These lie on a spectrum of invasiveness or volume of tissue affected. Clearly, as treatments become more focused and less invasive, there needs to be a concomitant increase in the therapeutic precision based on a solid understanding of the source of the patient's symptoms (Fig. 1). Unfortunately, the specific cause of pain is unknown in most patients.

Tissue engineering is an evolving technology, in which living cells are used to reconstruct a variety of tissues and organs [3–6]. Several general approaches can be used. Host cells can be stimulated in an orchestrated fashion to achieve the desired outcome using techniques like local growth factor administration or gene therapy [7–10]. Guided tissue regeneration can also be facilitated by implanting acellular matrices that are repopulated by host cells; tissue engineering involves the implantation of in vitro seeded matrices [11].

The concept of biologic disc repair has grown in recent years because of improved understanding of the cellular and molecular events of disc aging and degeneration [12–16]. Realization that disc degeneration is a cell-driven process has raised the possibility of manipulating cellular content and behavior toward a beneficial outcome. Such manipulation is necessary as the disc has a limited vascularity and capacity for self-repair. Currently, various researchers are considering several approaches to biologic disc repair. One approach is to signal existing disc cells in situ to secrete increased quantities of proteoglycan in attempts to increase swelling pressure [17]. Growth factors transforming growth factor (TGF-β), osteogenic protein-1, fibroblast growth factor, growth and differentiation factor-5, or bone morphogenetic protein-2 (BMP-2) among others have been used directly [18,19,7,20–22], or their production in disc cells has been stimulated through gene therapy [9,23]. Although promising results have been reported using these techniques, the relative acellularity of human degenerated discs raises concerns that the patient's own disc cells may be in insufficient numbers to mount a therapeutic repair response. Therefore, the introduction of cells capable of surviving within the intervertebral disc and producing appropriate matrices is an important component of bioengineered regeneration.

Cell therapy seeks to restore a critical population of disc cells that will synthesize appropriate matrices in attempts to recover biomechanical properties. This approach was first attempted by transplantation of autologous nucleus pulposus tissue into denucleated rat discs, where it was shown to slow the progression of degeneration [24]. More recently, autologous disc cell implantation was shown to be technically feasible in animal models [25–27].

A commonality in each of these approaches is the use of disc chondrocytes as the cell type for regeneration. Ultimately, however, this cell source

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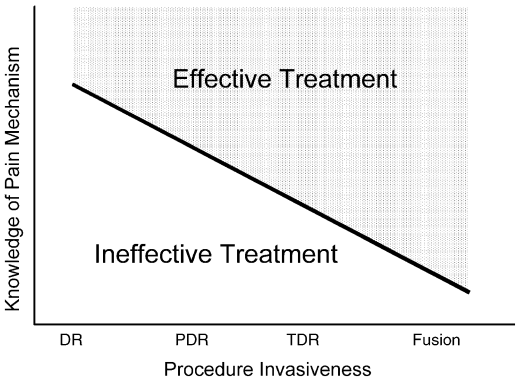

Fig. 1. To maximize probability of a positive clinical outcome, understanding of a patient's pain mechanism needs to increase as the procedure becomes less invasive. DR, disc regeneration; PDR, partial disc replacement, TDR, total disc replacement.

has practical limitations in the clinical setting because of graft procurement and harvest site morbidity. For example, harvesting the patient's own cells requires damage to an adjacent disc, likely inducing degeneration in that level. Also, the relative acellularity of disc tissue will require culture expansion, which is a slow process for disc cells. Furthermore, the cells acquired from one level to treat another will be similarly aged and potentially limited as to the extent they may develop a therapeutic repair response. Alternatively, some have proposed the use of autologous articular chondrocytes [28]. This approach has similar limitations, in addition to the current uncertainty regarding functional differences between articular and disc chondrocytes, since articular chondrocytes are differentiated and adapted to the unique mechanical and biochemical environment of the diarthrodial joint. By contrast, adult mesenchymal stem cells (MSCs) are easily procured by bone marrow aspiration, readily expanded in culture, and may be more capable of adapting successfully to the environment of the intervertebral disc, and therefore, achieving a differentiated state appropriate for long-term matrix synthesis [29,30].

Perhaps most relevant to disc repair is the ability of human MSCs to differentiate into chondrocytes. In vitro culture conditions that promote chondrogenesis depend on: (1) a three-dimensional construct of cells to facilitate cell–cell interactions, and (2) a defined medium containing specific agents [31]. The pellet system is most commonly used to produce cells similar to chondrocyte condensations in vivo. Briefly, isolated MSCs expanded in monolayer culture are centrifuged in defined medium to form a pellet in the bottom of the tube. Agents alone or in combination that induce a chondrocytic phenotype include dexamethasone, TGF-β −β1, -β2, -β3, BMP-2, and BMP-9 [31–33]. For example, it has been demonstrated that both 10 ng/mL TGF-β2 or TGF- β3 readily induce a sequential expression of chondrocytic markers in adult human MSCs [32]. These include the early induction of fibromodulin and cartilage oligomeric matrix protein followed by an increase in aggrecan and versican core protein. Subsequently, type II collagen and chondroadherin are increased; type X collagen is then enhanced in the hypertrophic stages of cell differentiation. TGF-β3 is slightly more effective in producing these responses than TGF-β2, while the effect of TGF-β1 is minimal. The effects of TGF-β3 on chondrocytic differentiation of human MSCs have been described in recent publications [34,35].

Similar differentiation patterns have been observed for mesenchymal cells in gel culture. Majumdar and colleagues [33] have demonstrated that human MSCs cultured in alginate express type II collagen between day 8 and 14, which is consistent with the temporal pattern of collagen expression reported for primary cultures of dedifferentiated avian chondrocytes [36], and human MSCs in pellet culture [37]. Similarly, Bahrami and colleagues [38] have demonstrated that pluripotent cells from calf periosteum differentiate into chondrocytes and express elevated type II collagen after 2 weeks of culture in agarose.

In vivo, repair of articular cartilage defects treated with MSCs has been demonstrated in rabbits [39]. There are also reports of treating intervertebral discs with MSCs [14,40–43]. The disc is a potentially hostile environment to transplanted cells because it is an avascular structure with an age-related decrease in nutritional supply and a chronic exposure to mechanical stress. In normal discs, the physiochemical environment plays an important role in affecting resident cell activity. Consequently, consideration of the factors that affect disc cell differentiation and metabolism will be important to evaluating the potential of MSCs for disc repair. For example, the nucleus pulposus contains a high osmotic pressure that decreases with age as the proteoglycan content diminishes with decreases in synthesis rates [44]. Other important factors are a low pH and oxygen tension, both of which decrease the rate of matrix synthesis [45,46] and are

characteristic of human degenerated discs. Finally, diffusion-limited nutrition is another important characteristic of pathologic discs that leads to decreased cell viability [47]. A major concern of cell transplantation therapy is that the transplanted cells will not survive or differentiate properly in this harsh environment. Risbud and colleagues [48] recently demonstrated the ability to drive mesenchymal stem cells toward a nucleus pulposus-like phenotype. As genuine disc cells are capable of generating matrix and surviving adverse conditions within the disc, these results are a promising step toward overcoming transplant viability issues.

Mechanical factors are also important [49]. Mechanical pressure on disc explants alters matrix synthesis and matrix degradation. Hydrostatic pressures above or below normal physiologic levels decrease proteoglycan and collagen II synthesis and increase expression of matrix metalloproteinases [44,50]. In vivo models of compression demonstrate alterations of cell viability, matrix synthesis, and regional phenotype in response to mechanical loads [51].

The primary philosophy underlying disc tissue regeneration is that by guiding matrix synthesis, one can achieve a self-sustaining beneficial outcome with the minimum of detrimental side effects. However, as approaches like those described above are being developed and refined, a set of guiding principles are needed against which success can be measured. That is, what is the specific desired outcome(s)? It won't necessarily be sufficient to just reconstruct anatomy, as degenerative change by itself doesn't explain back pain or disability incidence [52–55]. Rather, pain mechanisms must be identified, and novel diagnostic techniques developed to customize patient-specific treatment.

Discogenic pain is multifactoral. For a disc to hurt, there is a combination of three factors acting simultaneously. First, is the presence of nociceptors. Normally, the disc is avascular and only sparsely innervated at the margins [56]. These unmyelinated, substance P-containing fibers, are typically unresponsive and termed silent nociceptors [57–59]. As degeneration proceeds, nerves can follow microvessels and grow deeper into discs, either peripherally [60,61] or via the endplate [59,62]. This nerve and vessel ingrowth is facilitated by degeneration-related decreases in disc pressure and proteoglycan content [63].

Second, intradiscal nociceptors need to be sensitized. This can occur via cytokines, which are small, secreted proteins that mediate and regulate inflammation. Elevated levels of certain cytokines have been measured in human discs, and are associated with degeneration and pain [64]. The major cytokines found are interleukin-1, -6, and -8, tissue necrosis factor-α, and prostaglandin E_2 [48,65–67]. The source of cytokines can be circulating inflammatory cells in the case of herniated discs [68,69], or disc cells in the case of contained disc degeneration [70]. These pro-inflammatory stimuli can trigger cells to initiate a number of catabolic programs meant to stimulate tissue repair and remodeling that includes production of matrix metalloproteinases 1, 9, and 13 [71]. During this wound-healing process, cytokines are also involved in stimulating angiogenesis and granulation tissue formation [72].

Third, disc depressurization leads to mechanical instability as pre-stress in the annulus and interspinal ligaments is diminished [73,74]. Depressurization and instability, in turn, lead to abnormal internal disc stress that may stimulate nerves in disc tissue, leading to discogenic pain [75,76]. Abnormal disc stress may also cause disc cells to be proinflammatory [77,78], compounding the adverse effects of an abnormal mechanical environment.

For tissue regeneration strategies to be clinically successful, they should influence all three components of discogenic pain: innervation, inflammation, and instability (Fig. 2). Because these factors are not independent, affecting only one or two may not be clinically effective. For example, inflammation may be addressed acutely with pharmacologic means, but if mechanical instability persists, disc cells may continually be stimulated to be proinflammatory, potentially overcoming pharmacologic interventions in the long term.

Other important constraints on disc regeneration strategies are the factors that initiated

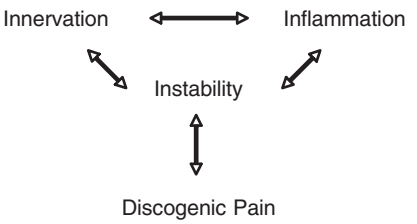

Fig. 2. Triad of factors contributing to discogenic pain. These factors are not independent, but synergistically act to compound their effect.

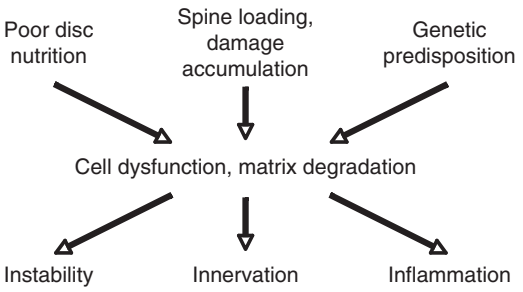

Fig. 3. Factors that influence the degeneration process and underlie discogenic pain.

degeneration in the first place. Disc degeneration is a normal consequence of aging and may be accelerated in some individuals because of nutritional, biomechanical, and familial factors [79] (Fig. 3). However, how these factors interact and contribute to degeneration for any particular patient is unknown. Yet, these stressors will likely be competing with regeneration strategies. Discogenic pain does not monotonically increase as degeneration progresses [80]. Rather, there are intermediate stages of degeneration where instability, innervation, and inflammation are maximally synergistic and give rise to pain. If the mechanisms of degeneration are not targeted specifically by the regeneration strategies, then the therapeutic persistence may be poor and the clinical benefits only short term (Fig. 4). Consequently, the relative rates of regeneration and degeneration need to be reconciled so that the long-term clinical benefits can be maximized.

Biologic strategies should be fine-tuned to address these competing factors. For example, studies have shown that fibrin gel can act as a drug delivery device by eluting growth factors and even adenoviruses for gene therapy over a period of time [81]. Nerve growth factor antibodies [81] or mast cell stabilizers could be incorporated into a fibrin gel carrier, to provide a coordinated therapy by: (1) targeting nerve and blood vessel ingrowth, (2) establishing early mechanical stability, while (3) delivering cells for matrix regeneration. As a result, a minimally invasive strategy could address multiple factors that influence degeneration and disc pain in a simple, single treatment.

Finally, to maximize clinical efficacy it is important to define which patients will benefit from disc regeneration and when it's optimal to intervene. The answer depends on the source of the patient's symptoms and the specifics of the intervention strategy. Biologic-only approaches will likely require a period of construct maturation and protection while the disc matrix is reconstituted. This approach is appropriate for patients with near-normal disc height and intact annulus and endplate. Hybrid strategies, with engineered constructs that provide stability and load-support in the early stages, may be more appropriate for intermediate stages of degeneration, where the annulus or endplate cannot sustain acute nuclear repressurization, or where acute restoration of disc height is desired. Advanced stages of degeneration where the biological and biomechanical challenges to regeneration are too severe may be best treated with total disc replacement or fusion [82,83].

In summary, tissue engineering approaches for disc regeneration and healing have significant clinical potential. Although significant progress toward developing these techniques is being made in vitro [41,84–88,89] it is unclear whether these approaches will naturally translate into positive clinical outcomes for back pain patients. What is clear, however, is that these approaches must

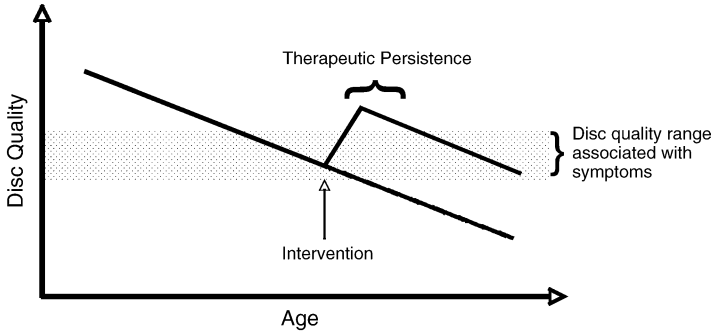

Fig. 4. Disc regeneration strategies will be competing with patent-specific degeneration stressors.

target specific pain mechanisms to optimize their potential. Equally important, degenerative mechanisms should be taken into account so that therapeutic persistence can be assured. Despite these concerns, disc tissue engineering holds significant promise as a minimally invasive and extensive treatment for millions of back pain sufferers.

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