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Regenerative treatment strategies in spinal surgery

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1. ABSTRACT

Intervertebral disc degeneration is considered a major source of low back pain. Recent advances in regenerative medicine have led to promising new approaches for the biological treatment of disc degeneration. Treatment modalities include the administration of growth factors, the application of autologous or allogenic cells, gene therapy, *in situ* therapy and the introduction of biomaterials or a combination thereof. Promising experimental results *in vitro* and in animal studies support the potential feasibility of these treatment modalities in clinical studies. We will review the current literature on regenerative treatment strategies and discuss potential drawbacks as well as opportunities in translating current knowledge into clinical practice. Major obstacles to regenerative treatment strategies might be insufficient nutritional supply, pain mediating factors and functionally impaired donor cells. Therefore, for clinical application, patient selection will be essential. Molecular, cellular and radiological diagnostic tools to evaluate the eligibility of patients for particular treatment strategies need to be developed. In spinal surgery, two approaches are conceivable. Patients operated on lumbar disc herniations often develop back pain due to disc degeneration months to years after surgery. Here, additional regenerative interventions would have a preventive intention, whereas interventions for painful degenerative disc disease as an alternative to spinal fusion or disc arthroplasty would be a curative approach.

2. INTRODUCTION

For the last decade, spinal surgery has been one of the fastest growing disciplines in the field of musculoskeletal surgery (1-3). From the Medicare data for 2003, presented by Weinstein *et al.*, it can be estimated that in the United States of America approximately 320 out of 100,000 people receive surgical procedures of the lumbar spine per year (4). Approximately 65% of these procedures were lumbar discectomies and laminectomies, the remainder lumbar fusions. While lumbar discectomies increased only slightly since 1992, lumbar fusions almost quadrupled. This development has been not without controversy (1, 5). It reflects the emphasis of the scientific community and the industry in the last decade on the development and implementation of spinal instrumentation and arthroplasty. However, a critical look at the literature reveals that despite all technological advances in spinal surgery, the clinical outcome has not improved as expected (1, 6, 7).

Whereas spinal fusion and arthroplasty are symptomatic therapies, regenerative treatment strategies might offer preventive and curative treatment options. These approaches are mostly in the *in vitro* or small animal model phase. However, the first clinical trials have been launched with promising preliminary results. In our opinion, regenerative treatment strategies will be of high relevance in the next years.

With this review we intend to provide the reader with information to identify clinical problems accompanying intervertebral disc degeneration and to understand current surgical solution strategies. Within that context, we would like to discuss where and how regenerative treatment strategies might fit in and be of benefit to patients. We will report on recent advances in regenerative medicine approaches and present our view of possible future developments.

3. INTERVERTEBRAL DISC DEGENERATION

3.1. Normal Disc Function

The intervertebral discs (IVDs) are located in between the vertebral bodies, parted from them by cartilaginous vertebral endplates (8). Two regions are distinguished: the inner, soft, highly hydrated nucleus pulposus und the outer, more firm, collagenous anulus fibrosus (9). Their function is to resist spinal compression and permit limited movements in which loading forces are spread evenly on the vertebral bodies. In the adult disc, the anulus fibrosus consists primarily of collagen type I fibers, passing obliquely in alternating directions between vertebral bodies (10). This formation is ideal for resisting tensile forces. The nucleus pulposus, however, consists mainly of loosely assembled collagen type II fibers, containing large proportions of hyaluronan and proteoglycan, mostly aggrecan (11). Due to the hydration properties of these large molecules, disc tissue swells until equilibrium is reached. This results in the biomechanical properties to absorb compression forces and maintain segmental stability. Cell density in the adult nucleus pulposus and anulus fibrosus is considerably low: 4×10^6 cells/cm^3 and 9×10^6 cells/cm^3, respectively (12). Cells in each of those compartments have characteristic profiles of matrix production (11) and of secretion of bioactive factors (13). These profiles are influenced by age and grade of degeneration. Disc cells maintain homeostasis by balancing complex anabolic and catabolic processes. Important anabolic regulators that stimulate matrix production are bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-beta) and insulin-like growth factor (IGF) (13), but also tissue inhibitors of matrix metalloproteinases (TIMPs), which limit catabolic activities (14). Matrix degradation is mediated by catabolic enzymes, such as matrix metalloproteinases (MMPs), aggrecanases and inflammatory cytokines like interleukin 1 (IL-1) (15-18) and tumor necrosis factor-alpha (TNF-alpha) (19). The avascular nature of the adult IVD determines its metabolic functions (20, 21). Metabolite transport is mainly achieved by diffusion through the vertebral endplates and the anulus fibrosus (22, 23). Due to low oxygen tension, disc cells are specialized in anaerobic metabolism, resulting in high concentrations of lactic acid and low pH (20, 21). Although disc cells are very resistant to low oxygen tension (24, 25), they still are sensitive to low pH (26, 25) and especially to low glucose concentrations in terms of matrix production and cell viability (27, 24).

3.2. Aging and Degeneration

In their comprehensive review on IVD degeneration, Adams and Roughley suggest the following definitions (28): (a) a degenerated disc is characterized by structural failure combined with accelerated or advanced signs of aging. (b) The process of disc degeneration is an aberrant, cell-mediated response to progressive structural failure. (c) Early degenerative changes refer to accelerated age-related changes in a structurally intact disc. (d) Degenerative disc disease is applied to a degenerated disc that is also painful. In the same paper, tissue weakening is identified as the underlying cause for disc degeneration, occurring primarily from genetic inheritance (29, 30), aging (11), nutritional compromise (21) and loading history (31).

Major age-related changes in the IVD and the cartilaginous vertebral endplates occur in the first two and in the fifth to seventh decade of life (32, 33). In the third and fourth decade, progression occurs very slowly. Changes advancing primarily in the first two decades include anular disorganization and fibrous transformation of the nucleus pulposus, which starts in its periphery. This fibrous transformation accelerates in the sixth decade, resulting in fibrous tissue, indistinguishable from anulus fibrosus in more than 50% of this age group studied by Haefeli *et al.*. These processes may be the results of a marked reduction of nutritional supply, which is caused by the early regression of blood vessels in the osseous vertebral endplates (32), as well as by the age-related calcification of the cartilaginous vertebral endplates (34). Histologically and biochemically, these changes correspond especially to: (a) an early decay of cell density and function (35, 9, 17), (b) an increase of collagen content, particularly type I, which builds up matrix impairing cross links among the collagen fibers and (c) degradation and loss of proteoglycans (36, 11). Matrix degradation processes can be accelerated by different gene polymorphisms affecting matrix proteins, catabolic enzymes and signaling molecules (29, 30). Altogether, the IVD starts to lose its hydrostatic properties and gel-like appearance of the nucleus pulposus and its functional integrity in the anulus fibrosus (37). Moreover, mechanobiological studies have revealed that proteoglycan synthesis is negatively influenced by static compressive loading, as well as by the absence of loading (38-42). This suggests that static overloading, but also underloading of the spine may contribute to tissue weakening within the IVD.

With increasing age, the IVD becomes stiffer and weaker, showing signs of microstructural defects. This mostly starts with alterations of the vertebral endplates and clefts in the nucleus pulposus before it extends to the anulus fibrosus (32, 33). The disc becomes increasingly susceptible to structural failures, defined as anular tears, disc herniations, damages to the vertebral endplates, internal disc disruptions, disc narrowing, radial bulging and vertebral osteophytes (28). Disc herniations and damages to the vertebral endplates decompress the nucleus pulposus, transferring most of the load to the anulus fibrosus, which bulges into the spinal canal and nucleus cavity (43, 44). As mentioned above, disc cells are sensitive to altered mechanical environments. Therefore, vital proteoglycan synthesis might be impaired by reduced pressure in the nucleus pulposus (38-40). These altered mechanobiological circumstances may also explain modified secretion profiles

of bioactive factors, typically found in degenerated discs. Masuda *et al.* describe this situation as the above-mentioned imbalance of anabolic and catabolic processes, which further accelerates disc degeneration (13).

3.3. Degeneration and Pain

Degeneration processes do not necessarily correlate with actual pain (45, 46). On the contrary, however, degenerative spinal segments can be found in most patients suffering from severe back and leg pain (47). Biomechanically, severe disc degeneration can create segmental instability. Thus, pain can result either from motion beyond physiologic constraints, causing compression or stretching of neural elements, or from abnormal deformation of ligaments, joint capsules, anular fibers or vertebral endplates, all of which harbor a significant number of nociceptors (48-50).

Additionally, ingrowth of nerves and blood vessels into the inner layers of a torn anulus fibrosus, sometimes even penetrating the nucleus pulposus, have been described (51), and an important role in pain pathogenesis has been suggested. This ingrowth is accompanied and possibly preceded by secretion of nerve growth factor (52), which was shown to be stimulated by the proinflammatory cytokines TNF-alpha and IL-1beta (53). Adams and Roughley suggest that ingrowth of nerves is facilitated by the loss of hydrostatic pressure that would normally collapse hollow capillaries (28).

Proteoglycan also plays an interesting role with regard to pain. Animal experiments have shown that nucleus pulposus material lowered the nerve stimulation thresholds in adjacent tissues (54) and that reduced proteoglycan content seemed to facilitate nerve ingrowth (55, 56).

At a molecular level, various studies, reviewed lately by Mulleman *et al.*, suggest that nerve root pain can be induced by inflammatory cytokines like TNF-alpha, secreted by nucleus pulposus cells in degenerated IVDs (57). Notably, Burke *et al.* were able to differentiate secretion patterns of inflammatory cytokines from patients suffering from degenerated disc disease and from patients with nerve root related pain (58).

4. BACK PAIN AND CURRENT TREATMENT OPTIONS

Back and leg pain is the main reason why patients with spinal problems consult a physician. As indicated above, back pain is a multifactorial problem. Since it is also strongly influenced by psychosocial factors, treatment decisions are a delicate issue (59, 60). From a surgical point of view, the origin of back and leg pain can be divided into two entities, often occurring in combination: (a) pain originating from compression of neural elements and (b) pain derived from pathological alterations of the musculoskeletal system (Figure 1).

4.1. Disc Herniation

Compression of neural elements like nerve roots can be caused by different pathologies, including spondylolisthesis (61), spinal stenosis (62) and disc herniation (63). Lumbar disc herniation is of particular interest for regenerative treatment options, as it is by far the most commonly treated disorder in spinal surgery (4), involving mostly a middle-aged population.

Apart from back pain, patients with an acute symptomatic disc herniation suffer from distinctive leg pain (64), which emphasizes the etiology of neural compression. Unless major neurological deficits also occur, these patients are first treated conservatively. Up to 70% recover from their acute symptoms within four weeks (65) due to spontaneous resorption of the herniated disc (66). With persistent intense pain or major neurological deficits, the neural elements are decompressed by removing the herniated material in microsurgical technique (63). In addition, many surgeons remove residual nucleus pulposus within the disc (nucleotomy), hoping to reduce the incidence of recurrent disc herniations (63, 67).

The initial results of surgical intervention are very satisfying (63) regarding leg pain caused by neural compression. But, according to the Maine Lumbar Spine Study (68), the long-term outcome is rather poor and has not significantly improved since the last major study by Weber *et al.* in 1983 (69). Ten years after surgical and conservative treatment respectively: 31% and 41% complain about the same or worse back pain, 30% and 45% are dissatisfied with the outcome and 25% in each group were re-operated (68).

Two problems complicating lumbar disc herniation can be identified: (a) re-herniation and (b) back pain due to painful degeneration of the spinal segment.

In a randomized controlled trial comparing microdiscectomy with simple sequestrectomy conducted by our group, 8% had to be re-operated within eighteen month for a re-herniation. In contrast to previous beliefs, removing residual nucleus pulposus from the disc, i.e. performing a nucleotomy or discectomy, did not lower the re-herniation rate (63). Other factors seem to influence the rate of re-herniations. Carragee *et al.* report a re-operation rate of 21%, when the defect in the anulus fibrosus is larger than 6 mm (70). Moreover, preserved disc height (71) and hyperintensity in T1-weighted magnetic resonance imaging (72) seem to be risk factors for re-herniation.

Disc herniation is accompanied by partial loss of nucleus pulposus. Depending on the surgical technique, residual nucleus pulposus is further removed (63, 67). For the first time, we could show in the above-mentioned randomized controlled trial that the extensive loss of nucleus pulposus is associated with pronounced radiological signs of degenerative disc disease as early as two years after microdiscectomy (73). Among these signs, especially the degenerative alterations of the adjacent vertebral bodies, classified by Modic (74, 75), correlated closely with the incidence of back pain. In earlier long-term observational studies, loss of disc height after nucleotomy proceeded rapidly in the first two years and correlated significantly with the occurrence of back pain in an observation period of ten years (76, 71, 77). On the other

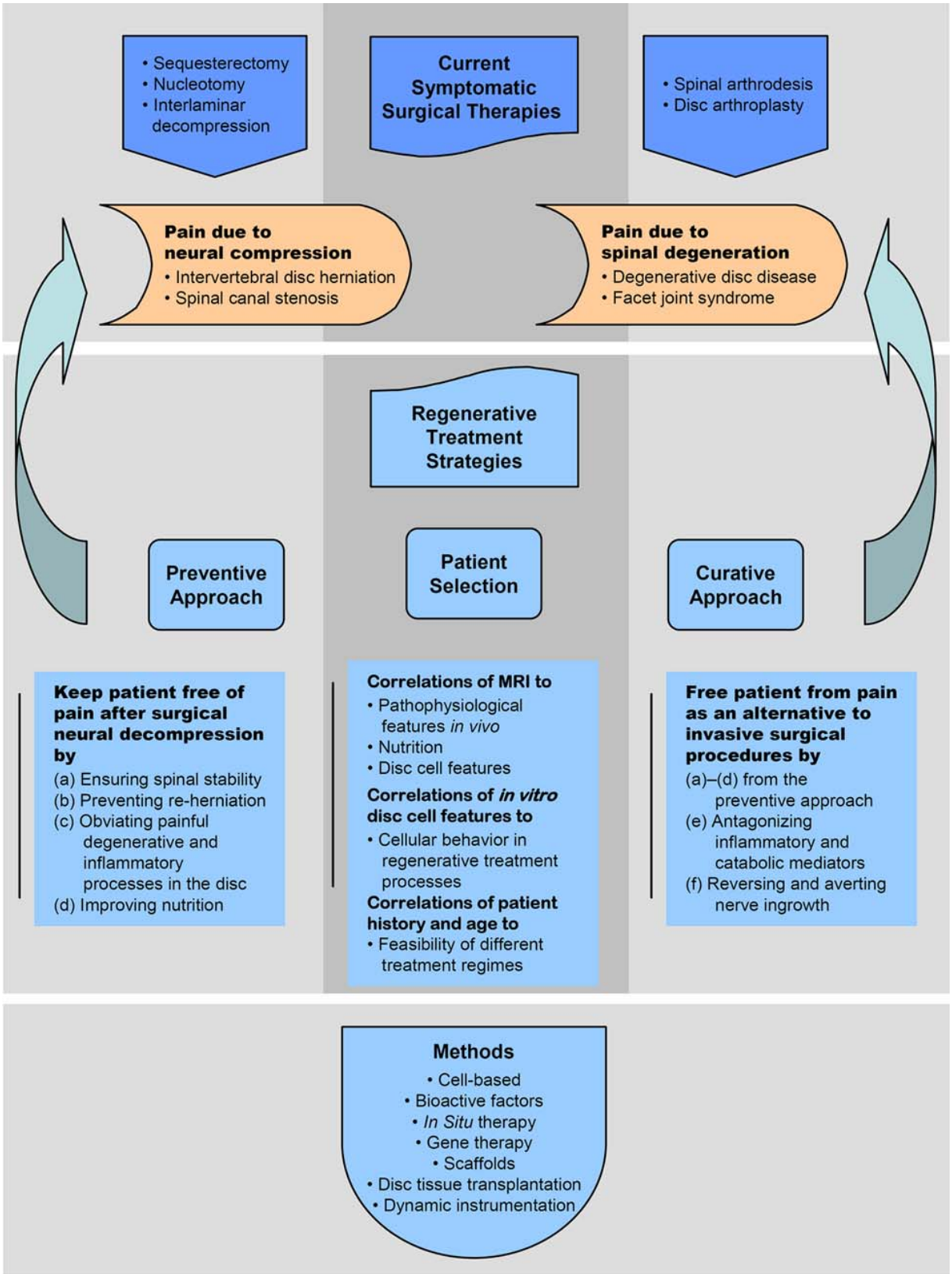

Figure 1. Overview of our current strategic approach to regenerative treatment in spinal surgery.

hand, however, loss of disc height occurs as a part of aging, thus especially in the elderly population (33), and is not necessarily accompanied by pain. Therefore, we think it is a legitimate hypothesis that the non-physiological, accelerated loss of disc height after disc herniation and nucleotomy can result in a painful decompensation of the spinal segment.

4.2. Degenerative Disc Disease

Painful pathological alterations of the spinal musculoskeletal system include a wide spectrum of pathologies, namely degenerative, neoplastic and infectious entities. Most commonly seen by physicians are degenerative entities that can be subdivided into (a) degenerative disc disease (6) and (b) painful facet joint degeneration (78).

Concepts on etiology and pathogenesis of degenerative disc disease were discussed above (chapter 3). In section 4.1, we reported that lumbar disc herniation and nucleotomy can accelerate the process from IVD degeneration to the painful state of degenerative disc disease.

Current treatment options are a matter of controversial discussion. For degenerative disc disease, uninstrumented and then instrumented spinal arthrodesis has been the standard surgical intervention for decades (79, 6). The aim is to immobilize the spinal segment, preferably by bony fusion, so as to avoid mechanical stimuli causing pain and to calm down inflammatory processes (see section 3.3). Up to now, however, several randomized controlled trials could show only a small, if any benefit compared to rigorous conservative treatment (80-84). In the last years, total disc replacement has become part of the clinical routine as an alternative to spinal fusion for some indications of degenerative disc disease (85). The widespread use of this new technology has been criticized by some authors (86, 87), as the accreditation studies did not show any relevant benefit compared to the spinal arthrodesis control groups (88, 89). Further objections are high complication rates (85), especially vascular complications, as well as an absence of long term experience and adequate explantation strategies. Other new technologies on the market include nucleus pulposus replacement (90, 91) and dynamic stabilization, either pedicle screw based (92, 93) or by interspinous devices (94). Long term clinical outcomes will disclose possible benefits of these new technologies, like preventing adjacent disc disease.

5. REGENERATIVE TREATMENT STRATEGIES

The objective of regenerative treatment strategies in surgery of the degenerated spine is to generate healthy disc tissue or functional surrogate tissue in order to avoid or to reverse painful degeneration processes. In this chapter, *in vitro* and animal studies will be discussed to illustrate the different approaches.

5.1. Cell-Based Strategies

One of the first regenerative approaches to IVD degeneration was the allogenic transplantation of whole intact discs, including vertebral endplates. In a dog model, results were disappointing because of subsequent progressive disc degeneration (95, 96). Better results were achieved in a rhesus monkey model, where only minimal disc degeneration and partial disc height reconstitution was observed after a 12-month follow-up (97).

First attempts to reimplant cultivated nucleus pulposus cells in IVDs of rats and rabbits demonstrated a deceleration of degeneration processes in comparison to the control groups (98, 99). Nomura *et al.* examined the implantation of cultivated allogenic nucleus pulposus cells (50,000 cells) and the allogenic transplantation of intact nucleus pulposus tissue in a rabbit model (100). In comparison to the control group, degeneration was found to a lesser extent in both groups after 16 weeks. Best results were obtained with transplantation of intact nucleus pulposus tissue. Host versus graft reaction was not observed. The survival and integration of reimplanted cultivated nucleus pulposus cells (10,000 cells) could be demonstrated in a sand rat model (101): after eight weeks, the labeled cells were still detectable. Ganey *et al.* injected autologous cultivated nucleus pulposus cells (6×10^6 cells) into a degeneration model of canine IVDs (102). Cultivation of that cell number was achieved within six weeks. The labeled cells were still detected after six month. Compared to the control group, the cell reimplantation group demonstrated an increased disc height and an augmentation of proteoglycan content, but a complete restoration of normal disc architecture was not achieved. Bertram *et al.* demonstrated in a rabbit model that cell retention after injection into the disc can be considerably improved by matrix-assisted cell transfer, composed of cells in a liquid fibrinogen thrombin solution (103). Furthermore, they showed that transferred cell viability can be significantly increased by serum enrichment and by prior nucleotomy. The positive effect of serum supplementation is explained by the rapid shift from a high nutritional status in culture to the low nutritional environment in the disc that can be attenuated by adding serum, naturally containing nutritional and bioactive factors.

Mesenchymal stem cells (MSC) were tested for treating degenerated discs in several studies (104) and promising results were demonstrated in small animal models (105-108). By using MSC or other precursor cells, capable of appropriate tissue differentiation, potential problems of degeneration-related dysfunction of nucleus pulposus cells could be circumvented. Furthermore, harvesting MSC is a less invasive procedure than harvesting nucleus pulposus cells. Of interest for the use of MSC in cell-based therapies is the observation that these cells avoid allogenic rejection in animal models and in humans. Three main mechanisms contribute to this effect: (a) they are hypoimmunogenic; (b) they prevent T-cell responses; and (c) they induce a suppressive local environment. This was discussed in detail by Ryan *et al.* (109). MSC can be isolated from bone marrow (110, 111), expanded *in vitro* and differentiated into mesenchymal tissues (112, 113). To qualify for a regenerative approach, MSC have to be able to express ample quantities of

proteoglycan and collagen type II in an oxygen- and nutrition-deprived environment. Risbud *et al.* reported that MSC of rats show similar expression profiles to nucleus pulposus cells, when they are cultivated in a disc-like environment of low oxygenation (2%) and high osmolarity (114). Additional treatment with TGF-beta enforced gene expression of aggrecan and collagen type II (114). In contrast, Portier *et al.* report extensive cell loss of MSC under hypoxic conditions after three days (115). Particularly important are studies on interactions between MSC and nucleus pulposus cells. In co-culture, proliferation rate and proteoglycan synthesis of nucleus pulposus cells were enhanced (116, 117) and MSC demonstrated a similar expression profile as nucleus pulposus cells (118).

5.2. Bioactive Factors

In 1991, Thompson *et al.* elucidated how cell proliferation and proteoglycan synthesis of canine disc cells are influenced by different growth factors (119). Already at that time, the authors suggested possible treatment applications for injecting growth factors into degenerated IVDs. Since then, a vast amount of literature has been published on the subject, reviewed lately by Masuda *et al.* (13), An *et al.* (50) and by Yoon and Patel (120).

Fetal calf serum and plasma-derived equine serum were able to augment proteoglycan synthesis *in vitro* up to 300% (119). Also, platelet-rich plasma was reported to increase proteoglycan and collagen type II synthesis (121-123). With the following single growth factors, an increased *in vitro* proteoglycan synthesis could be observed: epidermal growth factor (50%), basic fibroblast growth factor (300%), TGF-beta (500%) (119) and growth differentiation factor-5 (GDF-5) (138%) (124). In human disc cells, 300 ng/ml BMP-2 enhanced proteoglycan synthesis by 67% and 1500 ng/ml enhanced it by 200% (125). Moreover, an increased gene expression of aggrecan and collagen type I and II was observed. In human nucleus pulposus cells from a degenerated IVD, BMP-2 (50 ng) raised proteoglycan synthesis up to 560%, but a stimulation of collagen synthesis was not achieved (126). For future clinical applications, it might be of interest that the anabolic effects of BMP-2 were neutralized by nicotine (127).

Most promising results have been reported for BMP-7, also called osteogenic protein-1. In comparison to other growth factors like IGF-1 (128), fetal and adult nucleus pulposus and anulus fibrosus cells were stimulated equally strong by BMP-7, resulting in an abundant proteoglycan synthesis (129). As initial proteoglycan synthesis in adult disc cells was significantly lower, they seem to be especially receptive to BMP-7 induction. Similar results were reported for BMP-2: it was shown to reverse the decline of anabolic capacity in aged rabbit disc cells (130). Another important study analyzed *in vitro* cultivation of rabbit discs with preserved endplates in different concentrations of fetal calf serum (5 %, 10 % and 20%) (131). In 5% fetal calf serum, a marked reduction of proteoglycan content was observed, caused by nutritional deprivation. This catabolic process was reversed by intradiscal injection of BMP-7 (200 µg/disc).

Following the first promising *in vitro* results, animal studies were conducted. Weekly TGF-beta injections (1.6 ng/disc) in a degeneration model of murine IVDs for 4 weeks resulted in an increased cell number in the anulus fibrosus (132). The same study reported significant disc heightening after a single intradiscal injection of GDF-5. Similarly, a single injection of GDF-5 in a rabbit model resulted in a restoration of disc height, improvement of magnetic resonance imaging scores and histological grading scores (124). Particularly noteworthy are the studies by Masuda *et al.* and Miyamoto *et al.* on BMP-7 in a degeneration model of rabbit IVD (133, 134). A single injection of BMP-7 (100 µg/disc) completely restored the initial height of the healthy disc after six weeks and this persisted until the end of the experiment after 24 weeks. Histological analysis revealed a higher proteoglycan content in the nucleus pulposus and anulus fibrosus as well as a higher collagen content in the nucleus pulposus than in the control group. Moreover, it was demonstrated that, after 8 weeks, biomechanical properties were restored and elastic and viscous moduli showed significant positive correlations with proteoglycan and collagen content. In a rat model, Kawakami *et al.* studied pain-related behavior caused by compression-induced degenerative disc disease (135) and reported an inhibition of pain-related behavior and an enhancement of disc matrix after intradiscal injection of BMP-7.

5.3. Gene Therapy

Since a single application of anabolic factors will probably have a time-limited effect and repeated applications of them are unpractical, different genetic strategies have been studied (136). The basic aim is to modify the genetic code, allowing cells within the IVD to (a) enhance their expression of matrix components, (b) express single or sets of anabolic growth factors, or (c) express inhibitory factors against catabolic enzymes and inflammatory cytokines, or (d) a combination thereof. This can be accomplished by direct *in vivo* gene therapy or by indirect *ex vivo* gene therapy. *In vivo*, disc cells are targeted directly with injections of vector-mediated genetic elements; whereas in the *ex vivo* approach, target cells, which can either be disc cells or precursor cells like MSC, are cultivated and transduced with the desired genetic elements *in vitro* and then introduced in the IVD.

Reports on the feasibility of *in vitro* gene transduction in IVD cells were published by Reinecke *et al.* and Wehling *et al.* (137, 138), while Nishida *et al.* performed the first *in vivo* experiments in a rabbit model (139). Using adenoviral vectors injected into the IVDs, they could incorporate the gene encoding for TGF-beta1 into disc cells. Increased levels of TGF-beta1 and proteoglycan were found and encouraged further studies. Using the same animal model, Yoon *et al.* transduced genes encoding for LMP-1 (LIM (LIM domain named after the three first described homeodomain proteins Lin-11, Isl-1, and Mec-3) mineralization protein-1). With this gene product, increased levels of messenger ribonucleic acid (mRNA) for BMP-2, BMP-7 and aggrecan were observed (140). To increase collagen type II secretion, Sox9 (SRY (sexdetermining region Y)-box 9) encoding genes were successfully

integrated *in vitro* in degenerated human disc cells, and *in vivo* in a degeneration model of a rabbit IVD (141). Both experiments resulted in increased collagen type II content and *in vivo* preservation of histological appearance was observed in comparison to the control group. Similarly, MSC were transduced with Sox9 and loaded on a biodegradable three-dimensional poly-l-lactic acid scaffold, where expression and deposition of collagen type II and aggrecan could be demonstrated (142). Zhang *et al.* compared the effect of adenovirus vectors that expressed 12 different BMPs and Sox9 on matrix metabolism of bovine nucleus pulposus cells *in vitro* (143). They reported that adenoviruses expressing BMP-4 and -14 were the most effective in stimulating collagen synthesis, while adenoviruses expressing BMP-2 and -7 were the most effective in stimulating proteoglycan accumulation. In an attempt to combine the effects of different growth factors, a combined gene transduction including TGF-beta1, IGF-1 and BMP-2 was undertaken (144). The transduced human disc cells demonstrated *in vitro* not only an additive, but also a synergistic effect on matrix synthesis. A different approach was followed by Wallach *et al.* in an *in vitro* setting with degenerated human disc cells (145). Instead of striving to enhance anabolic processes, they tried to slow down matrix degradation processes by transducing genes encoding for TIMP-1, a matrix metalloproteinase inhibitor. The TIMP-1 expressing cells exhibited a higher proteoglycan content than the control group.

5.4. Scaffolds

The combination of cells with different carrier materials is yet another interesting approach. Several *in vitro* studies demonstrated that nucleus pulposus cells can proliferate and express proteoglycans and collagen type II within carrier materials such as alginate beads (146, 147), collagen sponges and gels (148), collagen type I/hyaluronan composites (149, 150), gelatin/chondroitin-6-sulfate/hyaluronan tri-copolymers (151, 152), atelocollagen (153) and chitosan (149, 154). Others compared gelatin, demineralized bone matrix and polylactide scaffolds (155). Differences in expression profiles were observed. Electron microscopy revealed varying nucleus pulposus cell morphologies according to different surface textures of the scaffolds, presumably causing distinct cellular responses. A recent study compared six types of scaffolds loaded with rabbit disc cells: polylactide-co-glycolide (PLGA), small intestine submucusa (SIS)/PLGA (20:80), demineralized bone particle (DBP)/PLGA (20:80), SIS/DBP/PLGA (10:10:80), SIS sponge and nonwoven polyglycolide (PGA) mesh (156). Out of these, DBP (157, 158) and SIS (159-161) are considered as natural bioactive materials, facilitating cell proliferation and differentiation. Accordingly, proteoglycan and collagen type II content was found to be higher in DBP- and SIS-impregnated scaffolds, as well as in the SIS sponge.

Mizuno *et al.* attempted to grow a complete IVD *in vitro* by using a two-component carrier material (162, 163). Anulus fibrosus cells were seeded in a polyglycolic acid polymer and nucleus pulposus cells in alginate gel. Sixteen weeks after subcutaneous implantation in athymic mice, survival of the cells was verified and specific matrix expression profiles were demonstrated respectively. Proteoglycan and collagen accumulation reached more than 50% of the levels of native tissue except that the collagen content of nucleus pulposus reached only 15% of the native values. Moreover, hydraulic permeability and equilibrium modulus were similar to those in native tissue.

With regard to the problem of disc nutrition due to degenerated vertebral endplates (see section 3.2), Hamilton *et al.* demonstrated that it is possible to form a triphasic construct *in vitro*, which consisted of nucleus pulposus, cartilage endplate and a porous calcium polyphosphate bone substitute (164). First, bovine articular chondrocytes were seeded on the surface of the bone substitute. Then, nucleus pulposus cells were placed onto the *in vitro*-formed hyaline cartilage. After 8 weeks, histological analysis showed a continuous layer of nucleus pulposus tissue that was fused to the underlying cartilage tissue, which itself was integrated into the bone substitute. Similarly, it was shown that in a biphasic construct, nucleus pulposus cells could be seeded directly on the surface of porous calcium polyphosphate, where they formed a continuous layer of tissue with similar features to native tissue in proteoglycan content, tissue stiffness, viscosity and weight bearing capacities (165). Concordant with the results of Mizuno *et al.*, however, collagen content reached only 26% of the native tissue value.

In vivo, atelocollagen scaffolds were loaded with anulus fibrosus cells and implanted in a laser-induced anulus defect in a rabbit model (166, 167). Cells were still detectable 12 weeks after implantation and demonstrated good proteoglycan synthesis. In a rabbit model, autologous MSC embedded in atelocollagen were tested for their regenerative capacity (105, 107, 108). Atelocollagen proved to be an environment conducive for MSC proliferation, differentiation and matrix synthesis. Furthermore, radiological, macroscopic and histological evaluations revealed effective regeneration in comparison to control groups. Likewise, MSC embedded in a 15% hyaluronan gel were injected into rat coccygeal discs (168). Proliferation, viability and a trend of increased disc height suggested regenerative processes. In a pig model, injectable hyaluronan-derived polymers with and without MSC were able to prevent IVD degeneration following nucleotomy after six weeks follow-up (169). Interestingly, hyaluronan by itself seems to be able to prevent degeneration processes. This was also confirmed in a study with Cercopithecus monkeys (170), which received intradiscal applications of high-molecular-weight hyaluronan after nucleotomy.

Several materials currently being tested in cartilage tissue engineering might turn out to be useful for IVD approaches as well (171): minimally invasive injectable hydrogels (172, 173), thermosensitive hydroxybutyl chitosan (174) and fibrin-gel polymers (175). Also promising are molecularly defined bioscaffolds (176), silk biomaterials (177, 178) and, especially, nanostructured scaffolds, mimicking complex extracellular environments (179) and possibly incorporating peptide motives or bioactive factors that can interact with cell receptors and

might allow control over differentiation and expression profiles.

5.5. *In situ* Therapy

We would like to introduce the concept of a potential future generation of regenerative treatments. The hypothesis is that the application of supporting scaffolds combined with chemotactic molecules will allow the *in situ* recruitment of stem cells and progenitor cells, as well as resident disc cells, into the scaffolds. In them, regeneration of disc structures, guided by growth and differentiation factors, will be promoted. This approach is suggested by the fact that chondrocyte migration has been reported in isolated cell systems and in cartilage organ cultures. Also, recent studies indicate chondrocyte movements *in vivo* (180). In a rat model, migration of chondrocytes from fractured vertebral endplates into the nucleus pulposus was demonstrated (181). Besides markers for cell migration and cell proliferation, pericellular depositions of collagen type II were verified around the migrated chondrocytes.

MSC, inherent in bone marrow of vertebral bodies, (112, 182) are also promising candidates for possible *in situ* applications since they show potentials for homing, migration and engraftment (183). After penetration of subchondral bone by debridement or micro-fracture of articular hyaline cartilage, MSC migrate from bone marrow to injured sites and subsequently form fibrocartilage-like repair tissue (184). This implies a possible use of MSC for *in situ* IVD repair by applying micro-lesions at the cartilaginous vertebral endplates. For MSC, a dose-dependent migratory effect with chemokines (185) and BMPs (186) has been described. Resident disc cells may also be good targets for a chemotactic *in situ* approach. Our own preliminary studies indicate a migration potential of distinct disc tissue-derived cells *in vitro*.

5.6. Dynamic Instrumentation

Dynamic stabilization of a spinal segment, either by interspinous devices (94) or pedicle screw systems (92, 93), might be a possible, and in some cases, necessary supplement to IVD regeneration. For interspinous implants, biomechanical studies have demonstrated a reduced range of motion and intradiscal pressure in extension movements (187, 188). In an IVD degeneration model, instrumented distraction in rabbits resulted in IVD regeneration that was confirmed by disc rehydration, by stimulated gene expression for extracellular matrix and by increased numbers of protein-expressing cells (189, 190). Consequently, some authors argue that without restoring the physiological mechanical status of the affected spinal segment, it is unlikely that regeneration will occur (191).

6. TRANSLATION INTO CLINICAL PRACTICE

6.1. Preventive versus Curative Approach

In order to translate current regenerative treatment strategies into clinical practice, we feel it is of utmost importance to differentiate between preventive and curative approaches (Figure 1). We have to keep in mind that, ultimately, our main objective is not tissue regeneration, but the elimination of pain for the patient. The main difference between preventive and curative approaches obviously is, whether we intend to (a) keep a patient pain-free who is at high risk of developing severe back pain or (b) disburden a patient who already suffers from disabling back pain. Hence, each approach has its particular clinical indications and needs to address specific disease-related problems in order to be beneficial for the patient.

In spinal surgery, preventive procedures are conceivable in operations for disc herniation and spinal stenosis with predominant radicular leg pain. Here, surgery is done for neural decompression, potentially causing segmental instabilities as a side-effect (192-195). This adds to the degeneration-related prepotent risk of developing painful degenerative disc disease. Thus, it is reasonable to think about possible interventions during these surgeries to ensure spinal stability, prevent re-herniation and obviate painful degenerative processes in the disc.

Regenerative treatment attempts of already painful degenerative disc disease or facet joint syndrome are defined as curative procedures. They face especially complicated challenges. Besides biomechanical issues and attempts for matrix restoration, solutions will have to address inflammatory and catabolic disorders, as well as pathological nerve ingrowth, which are held responsible for causing pain (see section 3.3). First steps have been undertaken by testing different anti-metabolites such as anti-TNF-alpha (196) or IL-1beta antagonists (197). Curative procedures could be valuable alternatives to the currently applied strongly invasive surgical techniques, such as spinal arthrodesis and total disc replacement.

6.2. Patient Selection

A well-defined therapeutic concept needs a reasonable evidence-based plan for patient selection (Figure 1). Up to date, there is hardly any data on how to identify patients, for whom a regenerative treatment strategy would be a reasonable option, either as a preventive or as a curative measure. It would be desirable to predict, whether a patient's disc cells are capable to contribute adequately in a regenerative treatment strategy and to assess the nutritional status of the patient's IVD. Radiological assessment with magnetic resonance imaging (MRI), the gold standard in degenerative spinal surgery, will be of major importance. Different classifications for IVD degeneration have been suggested in previous works (198, 199). Postcontrast diffusion studies (200, 201) and other upcoming MRI techniques (202, 203) might help to elucidate questions of pathophysiology and nutrition to an extent that the success of a regenerative treatment strategy for a patient can be estimated. Additionally, parameters predicting cellular behavior in a regenerative treatment process need to be identified. Growth kinetics, expression profiles, the incidence of cell senescence (204, 205), genotypes and programmed cell death (206) might be appropriate characteristics to examine.

6.3. Clinical Trials

In their pioneering work for autologous disc cell transplantation, Meisel and co-workers conducted a clinical

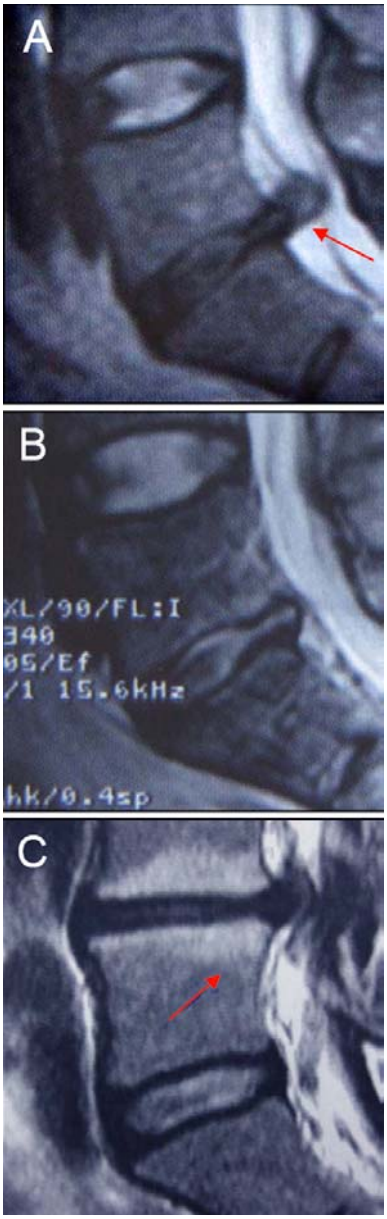

Figure 2. A case example for autologous disc cell transplantation, reproduced with permission from © Springer-Verlag Berlin Heidelberg 2006 (209): (A) 34-year-old man with lumbar disc herniation at L5/S1 (red arrow); (B) MRI one year post-transplantation with increased hyperintensity of the IVD in T2-weighted imaging, indicating good recovery of the disc. (C) 41-year old woman suffering from degenerated disc disease L4/5 (red arrow). Note reduced disc height, hypointensity in the IVD and inflammatory processes in the adjacent vertebral bodies.

feasibility and safety trial with 14 patients (207). Disc cells were harvested during a microsurgical discectomy procedure in young patients with nerve root compression due to a herniated disc. *In vitro*, the disc cells were isolated, expanded and, percutaneously reimplanted after 12 weeks. No negative effects were observed. This trial was followed by a multicenter, prospective, randomized, controlled trial (EuroDISC) comparing discectomy plus autologous disc cell transplantation with discectomy alone in 112 patients (208). Important exclusion criteria were Modic changes of grade II or III, as well as spondylolisthesis and chronic facet joint syndrome. Final analysis will be completed with a four-year follow-up. Interim analysis of 28 EuroDISC patients, after 2 years follow-up, implies a clinically significant reduction of back pain in the disc cell transplantation group (208). Reduced disability scores correlated with less low back pain. Loss of disc height over time was only found in the control group. Moreover, transplanted discs, as well as their adjacent discs, appear to retain hydration better than control discs. Using the same protocol, Grochulla *et al.* report on 22 patients, out of whom 8 were randomized to receive autologous disc cell transplantation (Figure 2) (209). All transplanted patients were satisfied and scored better in Visual Analog Scale (VAS) and Oswestry Score as compared to preoperatively. So far, however, no statistical analysis has been carried out due to the small number of patients, so that no conclusions can be drawn at the present time. The study's final results will provide us with a better understanding of the benefits of this procedure, which can be clearly classified as a preventive approach.

In a curative approach addressing painful degenerative disc disease, Haufe and Mork conducted a preliminary trial with 10 patients suffering from back pain not responsive to conservative treatment (210). Following discography to identify the affected discs, bone marrow aspirate from the iliac crest was percutaneously inserted into the painful discs. At one-year follow-up, no improvement of pain could be observed. Consequently, most patients received spinal fusion or total disc replacement. In contrast to the authors' assumption, we believe that hematopoietic stem cells and MSC are not comparable precursor cells, as specific distinguishing characteristics have been described for each group of cells (211, 212). Therefore, this study does not validate any conclusion as to whether MSC are a useful therapeutic option for degenerative disc disease. As discussed above, curative approaches for IVD disease will face the problem of the multifactorial etiology of pain. Successful curative therapies will have to address issues such as biomechanics, tissue regeneration, metabolic alterations and pathological nerve ingrowth.

Dynamic instrumentation has also been used in a curative attempt to regenerate painful IVDs. Specchia histologically compared samples of human disc tissue taken first during implantation and later during removal of the stabilization system. *In vivo* disc regeneration and reduced content of inflammatory cytokines could be demonstrated (213). Furthermore, rehydration of the disc could be observed on MRI after shielding by dynamic instrumentation. Accordingly, a pedicle screw-based dynamic stabilization system applied after nucleotomy in 35 patients protected against radiological signs of segmental degeneration in comparison to a control group (214).

Klein *et al.* report interesting results using biochemical treatment for long-standing chronic degenerative disc disease (215). In this pilot study in 30 patients, painful lumbar IVDs were injected with a solution of glucosamine and chondroitin sulfate combined with hypertonic dextrose and dimethylsulfoxide. After 12 months, significant improvements in disability and pain were observed. This and other studies (170, 216) indicate that different substances could be helpful in making curative treatment approaches more effective.

Recently Ruan *et al.* reported on their positive results of IVD transplantation (217). Five patients with cervical disc herniation underwent transplantation of fresh-frozen composite disc allografts after disc excision. At 5-year follow-up, motion and stability of the spinal segment were preserved with only mild signs of degeneration. No adjacent-level degeneration was found, and cervical lordosis was well preserved. None of the patients had any persistent or clinically significant neck pain, at rest or during neck movement. Signs of immunological incompatibility were also not observed.

7. POTENTIAL DRAWBACKS AND PERSPECTIVES

The ideal therapeutic solution for a degenerated IVD would (1) restore, maintain or at least slow down disc height loss, allowing a physiological pain-free aging of the IVD and shielding the facet joints from abnormal loading, (2) preserve adjacent vertebral bodies by providing even loading distributions, (3) prevent disc herniation or re-herniation by enforcing the anulus fibrosus, (4) reduce and prevent catabolic and inflammatory environments, (5) reverse and avert nerve ingrowth and (6) improve IVD nutrition (Figure 1). These requirements emphasize the importance of function. Regeneration of disc-specific tissues is one possible way to regain function. Other options could be surrogate tissues, anti-inflammatory agents and combinations with dynamic instrumentations.

IVD nutrition seems to be one of the main challenges for regenerative treatment strategies, since its deprivation is an age-related process (see section 3.2). Although adequate nutrition is thought to be a prerequisite of regenerative strategies, the definite contribution of inadequate supply to disc degeneration is still a matter of debate, especially since Hutton *et al.* demonstrated that blocking large parts of the nutritional pathways by injecting bone cement just below the vertebral endplates of dogs did not produce macroscopically visible degeneration after 70 weeks (218). Nevertheless, it is a valid concern that when nutrition is hardly sufficient for resident disc cells, stimulating these cells, or adding new cells, will not necessarily result in a higher matrix production and provide a regenerative effect. However, findings in the first clinical trials described by Meisel *et al.* (208) and by Grochulla *et al.* (209) indicate that radiologically assessed regeneration with autologous disc cell transplantation is possible and seems to correlate with a positive clinical outcome. Also Specchia´s curative approach (213) suggests the possibility of regeneration in degenerative disc disease with putative nutritional deprivation.

For successful regenerative treatment, we think that it is necessary to differentiate different stages of degeneration and make efforts to assess the nutritional status of the affected discs. It also remains to be seen how anticatabolic approaches (see section 5.3) will help to minimize the nutritional problem. Moreover, strategies need to be developed for improving disc nutrition. Lessons could possibly be learned from cartilage repair in the knee joint (219, 184, 220, 221). There, penetration of subchondral bone by micro-fracture facilitates cartilage repair through bone marrow cells and cytokines. It might turn out that micro-lesions, providing a direct contact in between vertebral bone marrow and disc space, are useful in vertebral endplate regeneration and enhancement of disc nutrition. Moreover, these techniques could facilitate *in situ* therapy approaches using chemotactical molecules to attract bone marrow progenitor cells for IVD regeneration. Up to now, there is no data on the feasibility of these techniques in spinal surgery. Most importantly, however, these techniques could also be strongly detrimental, since macro-lesions to the endplates, as done historically in scraping the endplates in radical discectomies, often resulted in acceleration of painful degenerative processes. It remains to be seen, whether micro-lesions can promote regenerative processes.

Allogenic transplantation of IVDs with their vertebral endplates, as recently reported by Ruan *et al.*, is an attractive approach for cervical disc herniation (217). Technically, it could be easily performed via the standard anterior surgical approach to the cervical spine, but would nevertheless represent a rather invasive procedure. A transplanted healthy vertebral endplate might improve the nutritional environment, although sclerotic changes of the vertebral body could still be an obstacle.

So far, many regenerative treatment strategies have only been tested *in vitro* or in animal studies. Unfortunately, findings from animal studies can not always be transferred to a human setting, since there are major differences in disc cell composition, weight-bearing properties, degeneration and age.

Despite very promising results for the application of bioactive factors in animal studies, some concerns, like the interaction with proinflammatory cytokines present in IVD degeneration, have to be taken into consideration. IL-1, for example, was shown to partially obliterate the stimulating effect of BMP-7 on matrix production (222). Moreover, an adequate number of functional disc cells are required for the successful application of bioactive factors, and even then, this strategy would only offer a time-limited regenerative effect. As mentioned in section 5.3, gene therapy seems to be a possible solution. It remains questionable, however, whether gene therapy will gain acceptance in patients with spinal diseases and in the health care community because of controversies concerning ethics, adverse side effects and high costs (223-225). Technically, direct *in vivo* gene therapy targeting resident cells will face two major problems: a limited number of cells in the IVD and an ineffective distribution of inserted genes due to fibrous transformation (see section 3.2).

Therefore, indirect *ex vivo* gene therapy seems more reasonable, even though target cells need to be removed.

Another concern is whether stimulated or implanted cells will be able to synthesize sufficient amounts of biomechanical functional matrix in a reasonable amount of time. Scaffold studies with disc cells have shown adequate and lasting proteoglycan synthesis, but meager collagen synthesis (see section 5.4). In an intradiscal, nutritionally deprived environment, the pace of matrix restitution might be delayed even further and, in fact, become a problem. Consequently, many scaffolds are made of or incorporate matrix components (see section 5.4). Some authors suggest substitution with a mixture of matrix components, combined with bioactive factors, to modulate and improve the intradiscal environment and prolong the effects of bioactive factors by embedding them in a slow release matrix (13, 103, 123). MSC were shown to produce nucleus pulposus-like expression profiles under certain circumstances (114, 113), but the quality of the produced matrix is unclear and requires further biomechanical evaluation. It is also important how stable the phenotype and corresponding expression profile develop over long periods of time *in vivo*.

In order to regain initial segmental stability, some scaffold materials offer suitable biomechanical characteristics (226), but also the combination with dynamic instrumentation might be a solution. Therefore, we have been observing with special interest the development of percutaneous implantable and removable interspinous devices that supposedly leave the dorsal structures of a spinal segment biomechanically intact (227). In the future, interspinous devices could possibly also open a way to facet joint regeneration by reestablishing physiologic loading properties. In addition to unloading, it is conceivable that injections of hyaluronan alone, or in combination with MSC, might endorse facet joint regeneration. At least in degenerative knee joint diseases, these injections have been used in animal and clinical trials with promising results (228-230).

Herniation of scaffold material has been described in biomechanical studies (103, 226) and poses a serious threat, since it could cause neural compression. We believe that proper scaffold choice and implantation technique will avoid this problem in the future and will even be useful to strengthen the anulus fibrosus and help to avoid nucleus pulposus herniation. Furthermore, some anulus sealing techniques have been recently introduced to the market (231, 232), with interesting first clinical results (232). In combination with scaffold materials, these techniques might prove to be useful for regenerative treatment strategies.

In summary, there have been great advances in understanding the physiology and pathophysiology of IVDs. Different promising regenerative strategies have evolved and have been tested *in vitro* and in animal models; new approaches are continuously being developed. The results of first clinical trials encourage further studies on regenerative treatment strategies. To successfully synergize and translate current knowledge into clinical practice, we feel it is of major importance to follow an interdisciplinary approach with a clinical problem-oriented research philosophy, including specialists from basic sciences, engineering, biotechnology, biomechanics, physiatry, radiology and spinal surgery.

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Abbreviations: IVD: intervertebral disc, BMP: bone morphogenetic protein, TGF-beta: transforming growth factor-beta, IGF: insulin-like growth factor, TIMPs: tissue inhibitors of matrix metalloproteinases, MMPs: matrix metalloproteinases, IL: interleukin, TNF-alpha: tumor necrosis factor-alpha, MSC: mesenchymal stem cells, GDF: growth differentiation factor, LMP: LIM (LIM domain named after the three first described homeodomain proteins Lin-11, Isl-1, and Mec-3) mineralization protein, mRNA: messenger ribonucleic acid, Sox9: SRY (sexdetermining region Y)-box 9, PLGA: polylactide-co-glycolide, SIS: small intestine submucusa, DBP: demineralized bone particle, PGA: polyglycolide, MRI: magnetic resonance imaging, VAS: Visual Analog Scale

Key Words: Back pain, Spinal surgery, Intervertebral disc, Nucleus pulposus, Anulus fibrosus, Intervertebral disc degeneration, Regenerative medicine, Tissue engineering, Cellular therapy, Mesenchymal stem cells, Growth factors, Gene therapy, *In situ* therapy, Scaffold materials, Patient selection, Preventive approach, Curative approach, Review

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