OPINION

Intervertebral disc regeneration: do nutrients lead the way?

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Abstract | Strategies for the biological repair of intervertebral discs derive from the premise that disc degeneration results from impaired cellular activity and, therefore, that these structures can be induced to regenerate by implanting active cells or providing factors that restore normal cellular activity. *In vitro* and animal studies using this approach have had some success, but whether this success can be reproduced in degenerate human lumbar discs is unknown. Successful repair requires that the disc cells remain viable and active; they therefore need an adequate supply of nutrients. However, as the disc degenerates, the nutrient supply decreases, thereby limiting cell activity and viability. Current biologic approaches might place additional demands on an already precarious nutrient supply. Here, we discuss whether the loss of nutrients associated with disc degeneration limits the effectiveness of biologic approaches, and indicate that this neglected problem requires investigation if clinical application of such therapies is to succeed.

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Introduction

Low back pain is the main cause of disability worldwide and imposes an enormous clinical and socioeconomic burden on society.[1] Although numerous potential causes are recognized, the condition is strongly associated with degeneration of intervertebral disc tissue. Currently, the only medical interventions available are surgical measures aimed at removing possible sources of pain and restoring the biomechanical function of discs. However, interest in developing alternative methods of treatment—in particular, biological methods for repairing or regenerating intervertebral disc tissue—is growing.

To date, disc tissue regeneration strategies have focused on cellular approaches, with or without the use of specialized biomaterials or growth factors. Although these approaches seem effective *in vitro* and in animal studies, disc degeneration in humans is typically accompanied by a decreasing nutrient supply; hence, the loss of nutrients might limit the effectiveness of these therapies. In this Perspectives article, we discuss whether the limited nutrient supply to degenerate discs might adversely affect various biological repair approaches. We provide an overview of the nutrient supply to normal discs and summarize how it is impeded during disc degeneration, as well as outline current approaches to the biological repair of degenerate discs and highlight the importance of nutrient balance. Future research that is required to understand how nutrient supply affects disc regeneration is also discussed. We also emphasize the importance of developing diagnostic criteria for determining whether or not biological repair in a patient is feasible (and, indeed—although not discussed—whether it will be clinically beneficial).

Competing interests
The authors declare no competing interests.

Intervertebral discs

The structure of healthy discs

Intervertebral discs comprise a fibrocartilaginous structure that lies between the vertebrae, providing the spinal column with stability and flexibility. In each disc, a collagenous annulus fibrosus encloses a central, highly hydrated nucleus pulposus; these structures are separated from the adjacent vertebral bodies by a thin layer of hyaline cartilage, the cartilaginous endplates (Figure 1a).[2] The biomechanical role of the disc is governed by the organization and properties of its macromolecular components, which are synthesized and maintained by a small population of resident cells; accordingly, cellular activity is essential for continued disc health.

Disc degeneration

Intervertebral discs degenerate before any other tissue in the body, and the term 'disc degeneration' encompasses a variable range of morphological and biochemical changes.[3] The extent of degenerative changes can be classified by various grading schemes, such as the Thompson grade,[4] which is derived from the *in vitro* assessment of gross morphological changes seen in sagittal sections of the disc, and the Pfirrmann grading system,[5] which is commonly used clinically to classify the severity of lumbar disc degeneration based on changes in signal intensity and disc height from MRI scans.

The causes of disc degeneration are poorly understood; however, twin studies have shown there to be a major genetic component to this condition.[6] Regardless of the initial trigger, degeneration is thought to be driven by the disc cells.[3] In a normal disc, the rates of macromolecular synthesis and degradation are balanced, but when degradation predominates, the tissue loses organization and biomechanical function.[7]

Nutrient supply to normal discs

Maintaining an optimal nutrient–metabolite milieu for the survival and function of disc cells is a particular problem for intervertebral discs, which comprise the largest avascular tissue in the body.[8] Although the cells of the outer annulus fibrosus receive their nutrients (and eliminate their metabolites) from capillaries in the soft tissues that surround the disc, the only contact with the blood supply for most of the remaining cells within the disc is via capillaries that arise in the vertebral bodies, penetrate the subchondral plate through marrow spaces and terminate in loops adjacent to the cartilaginous endplate (Figure 1a).[9] Nutrients move, mainly by diffusion,[10] from the capillaries through the cartilaginous endplate and dense disc matrix to the cells of the disc; metabolites move in the reverse

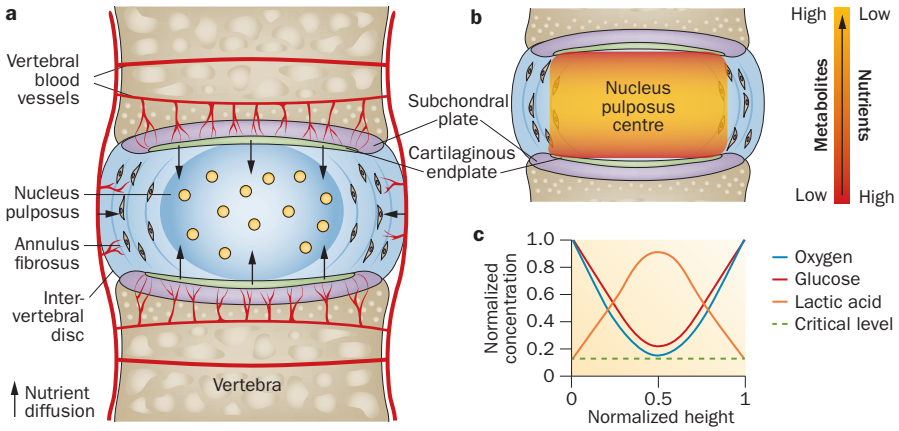

Figure 1 | Pathways of nutrient supply in a normal intervertebral disc. **a** | Cells of the avascular disc nucleus pulposus and inner annulus fibrosus are supplied by vertebral blood vessels. Capillaries penetrate the subchondral plate through marrow spaces and terminate in loops at the junction of the subchondral plate and cartilaginous endplate. Nutrients (e.g. oxygen and glucose) diffuse from the capillary bed through the cartilaginous endplate under gradients arising from metabolic demands of disc cells, while metabolic wastes (e.g. lactic acid) diffuse in the reverse direction. Cells of the outer annulus fibrosus are supplied by capillaries from blood vessels in the surrounding soft tissues that penetrate a few millimetres into the disc. **b** | The centre of the disc has the lowest levels of nutrients and highest concentration of metabolites. **c** | Schematic showing normalized concentration gradients of glucose, oxygen and lactic acid across the nucleus, endplate–endplate. Nutrient concentrations must remain above the critical levels to maintain cell viability and activity.

direction. The ensuing concentration gradients are therefore determined by the balance between the rates of nutrient supply and consumption by the cells (Figure 1b).[8] Consequently, the concentrations of glucose, oxygen and other nutrients are at their lowest at the centre of the disc. These levels must exceed a critical threshold for the cells to remain viable and active, (Figure 1c); hence, factors that negatively influence the nutrient concentration levels also limit the number of cells that can be supported in this avascular tissue.[11,12]

Disc nutrition and degeneration

The balance between nutrient supply and nutrient consumption is precarious, and if either parameter is disturbed, the concentration of nutrients and the pH (as a consequence of metabolite accumulation) in the discs can decrease to levels that adversely affect cellular activity and even cell viability (Figure 2).[12–15]

Surprisingly few studies have investigated how changes in the nutrient–metabolite milieu influence disc cell behaviour. Moreover, most *in vitro* studies investigate only one variable, such as oxygen levels, at a time, whereas the levels of oxygen, glucose and lactic acid *in vivo* are necessarily coupled.[8] However, *in vitro* experiments that simulate the adverse nutrient environment seen in degenerate discs have confirmed that this leads to a reduction in the production of stem and progenitor cells (and their chondrogenic potential) and in matrix molecules, as well as an increase in cell death.[12,16–18]

Defective nutrient supply

The majority of disc cells depend on the capillaries emerging from the vertebral bodies to supply their nutrients; however, this route seems to fail in various ways during degeneration, as described below.

Impaired vertebral blood supply

Atherosclerosis of the arteries that feed the lumbar spine is associated with disc degeneration,[19,20] as are disorders such as Gaucher disease, sickle cell anaemia and Caisson disease, which negatively affect the microcirculation and hence reduce nutrient supply to discs.[8] As the capillaries also express muscarinic receptors, factors that affect the peripheral microcirculation (such as exposure to vibration and vasoactive substances) can also restrict nutrient transport into discs.[21–23]

Impaired endplate blood supply

The nutrient supply to most of the disc cells must pass through the vertebral endplate (both subchondral plate and cartilaginous endplate), and in degenerate discs this route can be impeded. Occlusion of the marrow spaces, resulting in a loss of contact between the capillaries and the cartilaginous endplate, increases with increasing disc degeneration, as does calcification of the cartilaginous endplate,[24–26] which thereby inhibits the diffusion of solutes from the capillaries to the disc.[27] Other degenerative features of the endplate, such as endplate sclerosis, Modic changes, Schmorl's nodes and endplate lesions,[28] might also adversely affect nutrient supply (Figure 3a,b).

The importance of the endplate route has been demonstrated *in vivo* in animal experiments in which blockage of the endplate drastically reduced the rate of diffusion of a tracer dye into the disc.[29] MRI studies in humans[30] have also revealed that the rate and extent of enhancement of the contrast agent gadodiamide (Omniscan® [GE Healthcare, Oslo, Norway]) in discs were noticeably lower in mildly and moderately degenerate discs than in nondegenerate discs. Gadodiamide accumulated mainly at the endplate, demonstrating the importance of this region in regulating the movement of nutrients into the disc. In severely degenerate discs, gadodiamide enhancement was higher than in normal discs, probably because the cartilaginous endplate loses integrity and the blood vessels are able to invade the disc at this stage of degeneration,[2,3,9,30] enabling rapid transport of the contrast agent into the degenerate disc matrix.

Increased cellular demand

As well as a decrease in the rate of transport into disc cells, an increase in cellular demand, resulting from a higher cell density[12,15,27] or a rise in the rate of nutrient consumption per cell,[31] can reduce the nutrient concentrations to below a critical level. Growth factors and cytokines, such as IL-1β, which are expressed at higher levels during disc degeneration than in a normal nondegenerated disc,[32] can also induce marked increases in the rates of glucose consumption and lactic acid production by cartilaginous cells.[31] Mathematical models show that the presence of factors that increase nutrient demand, in addition to an increase in cell numbers, can cause nutrient concentrations to drop below critical levels.[33]

Thus, current evidence indicates that decreased nutrient availability to disc cells—whether through defective supply or increased consumption—is a common finding in intervertebral disc degeneration, with consequent adverse effects on cellular activity and viability.

Disc regenerative approaches

Strategies for the biological repair or regeneration of intervertebral discs are based on the premise that degeneration results from inappropriate cellular behaviour.

Implanting new disc cells

Many approaches for disc repair involve implanting active cells (both disc cells and stem cells) into the damaged disc, either alone or embedded within an appropriate biomaterial as a carrier (Figure 3c), the aim being that the implanted cells would produce matrix macromolecules to replace those lost during the process of degeneration. A number of different cell types have been tested to examine the conditions under which cells survive and produce matrix or retard disc degeneration.[17,34,35] Various different biomaterial formulations, some of them injectable, seem to support and stimulate cell activity.[36,37] There are even reports of a limited number of small clinical studies in which cells have been implanted into patients with back pain, with mixed outcomes.[38–41] Eight clinical trials using mesenchymal stem cells (MSCs) and cartilage cell types to rescue disc degeneration are in progress according to the ClinicalTrials.gov database, although no results are yet available.[42,43]

Altering the activity of disc cells

Another option envisages altering the activity of the remaining resident cells of a degenerate disc by intradiscal injection of appropriate factors[44] or by using gene therapy.[45] A number of growth factors have been shown to increase matrix production by disc cells *in vitro* and *in vivo*, as have small molecules such as link protein peptide and link-N,[46,47] and clinical trials for treating disc degeneration using injections of growth factors (such as growth differentiation factor 5) shown to be effective in animal studies are currently underway.[48] Alternatively, intradiscal injection or gene therapy techniques have also been used to retard disc degeneration by inhibiting the production of inflammatory cytokines by disc cells.[44,45]

Whole disc transplantation

Additionally, it has been proposed that the function of a degenerate disc could be restored by transplanting an entire replacement disc. On the one hand, this structure could be produced by tissue engineering and, consistent with this notion, studies on methods to produce nucleus pulposus, annulus fibrosus and endplate tissues, and to integrate and fix them into the disc, are ongoing.[37] A disc-like structure comprising nucleus pulposus and annulus fibrosus cells seeded into an artificial scaffold has been successfully implanted into the rat caudal spine, where it seems to remain viable and functional.[49] However, attempts to generate an entire tissue-engineered disc have not yet succeeded in regenerating the intricacies of the disc annulus[50] or the complex vascularized interface between each disc and vertebral body.[51,52]

Alternatively, an intact disc could be transplanted *ex vivo*. Entire intervertebral discs have been successfully transplanted into animals[53] as well as into the cervical spine of 13 patients, in whom they have provided acceptable clinical outcomes for up to 10 years.[54,55]

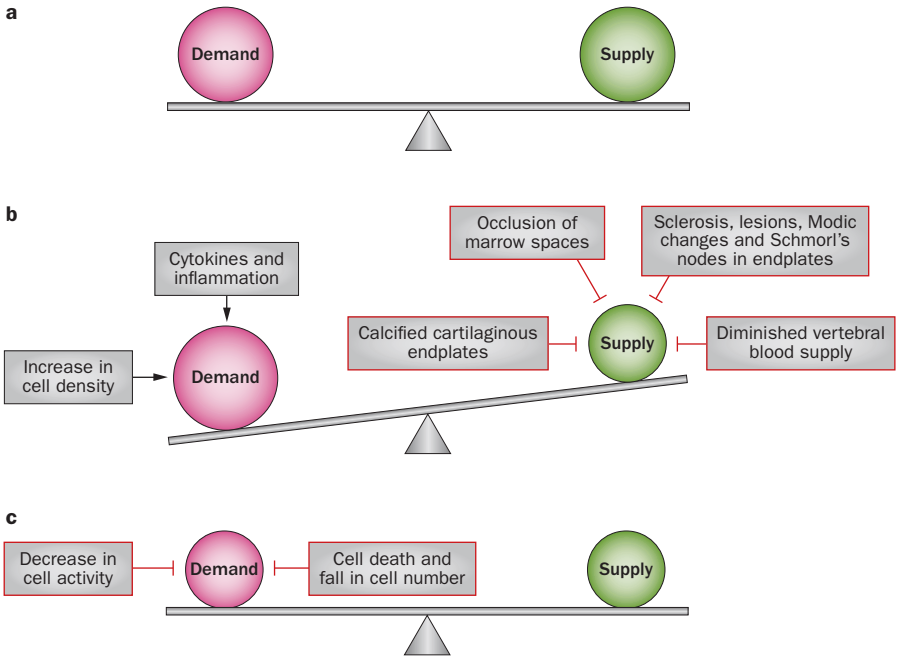

Figure 2 | Schematic showing factors influencing the balance between the rates of nutrient supply and demand. **a** | In normal discs, the rates of cellular demand and nutrient supply are in balance. **b** | In degenerating discs, demand exceeds supply. Demand increases because cytokines stimulate the rate of cellular energy metabolism or because cell density increases;[31–33] supply falls owing to decreased blood supply through such changes as endplate degeneration (by calcification, sclerosis, lesions, Modic changes and Schmorl's nodes), occlusion of marrow spaces and atherosclerosis diminishing flow through vertebral arteries.[8,16,20,24–27] **c** | Degenerate discs establish a new balance in which the demand falls below that seen in normal discs through decreased cellular activity and/or cell death to balance the reduced nutrient supply.

The importance of nutrients

All these different approaches to disc regeneration have one essential requirement: successful long-term repair requires that the endogenous or implanted cells remain alive and active. Although this approach can be readily achieved *in vitro* and in animal studies, to what extent this is possible in human degenerate lumbar discs is still unknown.

Considering the decreased availability of nutrients as the disc degenerates, and that adequate nutrition is crucial for cell survival, we propose that this parameter should be the first to be addressed in the design of any disc regeneration strategy.

Animal models of disc degeneration

As described earlier, several approaches to regenerate the disc have shown apparent success in animal tests. However, it should be acknowledged that although animal models serve as invaluable tools to elucidate the pathology of disc degeneration and to examine the regenerative potential of biological strategies, an approach that is successful in animal models might not necessarily extrapolate to humans.

Is the nutrient supply affected?

For one thing, the animal models used in almost all disc repair studies to date do not mimic many of the features present in the human condition: the animals are mostly young and healthy, and the degeneration, induced by injury, is acute, resulting in changes to animal discs that do not simulate

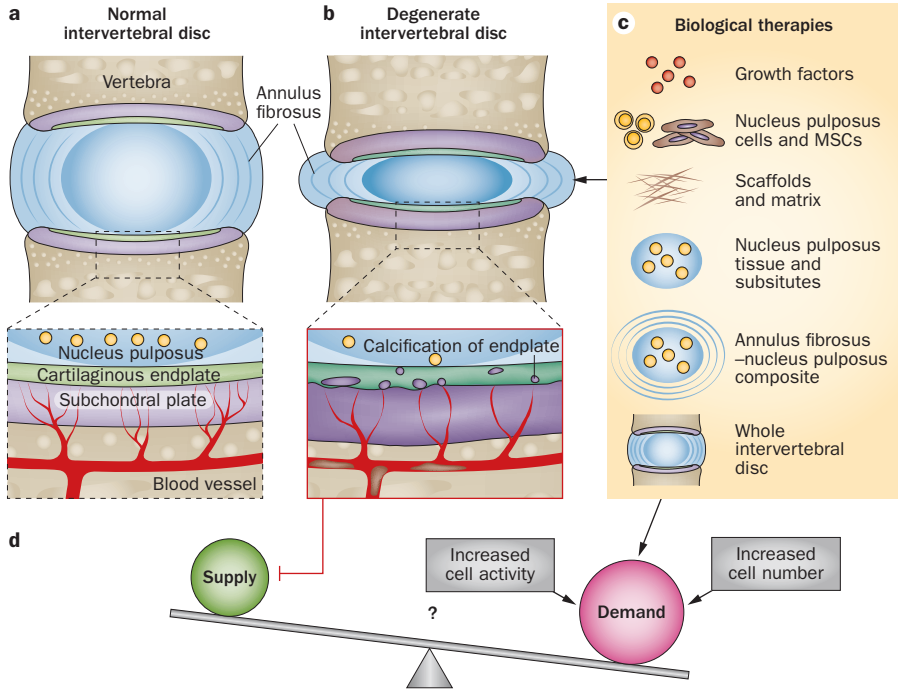

Figure 3 | Schematic showing the potential influence of biological therapies on nutrient balance. **a** | Nutrient pathways in normal disc. **b** | Nutrient pathways in a degenerate disc with changes such as calcification of cartilaginous endplate, occlusion of marrow spaces (so that they are no longer in contact with cartilage surface), atherosclerosis of vertebral arteries, reduced capillary density, all of which limit nutrient transport, leading to decreased viable cell density. **c** | Different forms of biological therapies for disc repair: growth factor injection, implantation of nucleus pulposus disc cells and mesenchymal stem cells alone or in conjunction with scaffolds and tissues, implantation of annulus fibrosis–nucleus pulposus composites, and whole intervertebral disc transplantation. All approaches require cells to remain viable and active for successful repair. **d** | Current therapies increase the cell number and/or cellular activity causing nutrient demand to exceed nutrient supply, which is already diminished in degenerate discs. A balance can only be achieved by reducing demands—that is, by cell death or decreased cellular activity.

features seen in the chronic degenerative situation in human discs.[56] Notably, no observable changes in the nutrient supply between animals with degenerate discs and control animals have been observed in the few studies carried out so far.[22,57] For instance, the decrease in vertebral blood flow seen in a stab-induced disc degeneration model in mini-pigs was reversible by implantation of gels with or without MSCs.[57] However, no detectable influence on the influx of a contrast agent into the disc was seen by MRI, indicating that endplate permeability and overall nutrient supply seemed to be unaffected in this model. There is thus no evidence to date that repair studies in animal discs have been carried out under conditions in which the nutrient supply to the disc is adversely affected.

The importance of size

Another important feature of the animal discs used for research is that they are much smaller than those of humans—even discs of relatively large animals such as goats and pigs.[56] As the density of cells that can be supported in avascular tissues and constructs is inversely related to the thickness or height of the tissue,[12] rat or rabbit discs, or even pig and sheep discs, can support a much greater cell density than human discs. Thus, whereas it took only weeks to observe increases in disc height after growth factor injection in rats,[44] it would take many months or even years to produce a similar change in humans, even if there was no impediment to the nutrient supply.

Can human discs support repair?

As mentioned previously, the number of cells that can be supported is governed by the nutrient supply.[8,12,33] Consequently, implanted exogenous stem cells, disc cells or tissue constructs (Figure 3c) will compete with resident disc cells for nutrients, and degenerate discs, in particular, might not be able to support the nutrient demands arising from the increase in cell number. Similarly, using growth factors and biomaterials to enhance the proliferation and activity of disc cells *in vitro* also tends to increase the rates of energy metabolism[31] and hence increases the demand for nutrients when supply is already restricted (Figure 3c,d). Strategies that aim to prevent further degradation by inhibiting proteolytic activity or cytokine production might be the most promising in the context of energy balance;[45,58] theoretically, such approaches might even reduce nutrient demand.

We therefore believe that cellular activities and viability are most likely diminished rather than enhanced by most of the current repair approaches and that blindly pursuing disc repair strategies that promote cellular proliferation and anabolic activities without considering the consequences on the nutrient–metabolite milieu *in vivo* might not be the correct direction for intervertebral disc regeneration.

Disc allografts need a blood supply

During the process of intervertebral disc transplantation, osteotomies are performed on the adjacent vertebral bodies,[53] rendering the disc allograft in an ischaemic state until the nutrient pathway is re-established during bone healing. During this period, many disc cells die as they are deprived of nutrients, but some survive, so it cannot be concluded that the disc allograft is a completely dead tissue-spacer that serves only to improve the mobility. Nevertheless, with current approaches, although degradation in the transplanted discs seems to be slow, probably because the rate at which degradative enzymes are produced in the tissue is limited, the discs do eventually show signs of degeneration. As long-term reparative strategies require the maintenance of cell viability, re-establishment of the nutritional supply into the disc allograft is required before any subsequent cell implantation or growth factor injection strategies are considered.

Conclusions

Over the past decade, interest in the cellular repair of the intervertebral discs has boosted research into disc biology and has increased our knowledge of this field enormously, as shown by the success of growth-factor-based, cell-based and biomaterial-based therapies in animals. This work has provided promising scenarios for intervertebral disc regeneration and shown that disc cells can survive *in vivo*, that stem cells can differentiate appropriately and that matrix and biomechanical properties can be restored to a large extent. However, whether the success of the achievements in the laboratory and

in animals can be reproduced clinically is not known. We believe that cellular activities and viability are most likely diminished rather than enhanced by most of the current repair approaches and that blindly pursuing disc repair strategies that promote cellular proliferation and anabolic activities without considering the consequences on the nutrient–metabolite milieu *in vivo* might not be the correct direction for intervertebral disc regeneration.

One of the factors that we feel requires further investigation—the role of nutrient insufficiency on repair—can really only be better understood by developing animal models that address this issue and take account of questions of size and scale. Animal models are also necessary for developing strategies to improve the nutrient supply by, for instance, enhancing vertebral blood flow by preventing calcification of endplate cartilage or through the long-term administration of vasoactive agents.

As far as information pertaining to humans is concerned, post-contrast MRI studies have demonstrated that the blood supply to the discs is impaired in line with the degree of disc degeneration.[30] However, further information is required to develop appropriate strategies for assessing nutrient demands in a patient's discs. How changes in the transport of nonmetabolized tracer dyes such as gadodiamide into the disc relate to nutrient profiles in either normal or degenerate discs has not been established. Furthermore, the profiles of gadolinium salts cannot offer information on the cellular demands for nutrients and other essential factors,[8] so little is known about cellular nutrient demands in either normal or degenerate discs, how these demands are affected by treatments such as growth factor injection, or indeed how the nutrient–metabolite milieu affects matrix turnover and hence tissue repair. Such work is difficult to carry out in humans, and information in this area will rely on *in vitro*, animal and modelling studies.

Commonly used classification systems for grading disc degeneration *in vivo*, such as the Pfirrmann grading scheme,[5] and quantitative T2 star MRI, a new classification scheme developed to predict altered kinematics,[59] cannot assess disc nutrition. Rajasekaran *et al.*[21] investigated the relationship between disc degeneration and the rate of gadolinium influx measured by MRI; they showed that the extent of the decrease in influx was associated with the increase in the disc degeneration grade, and particularly with changes in the endplate region. From this association, they developed a total endplate score (TEPS) and suggested that biologic therapies will only succeed in discs with a TEPS of <6 (on a scale of 1–12).[21] Those with a TEPS of 6 would be only mildly degenerate and have a near-normal pattern of gadolinium influx. However, this TEPS approach has not yet been independently validated. The TEPS also does not provide information on the total cell number that can be supported by a disc, nor on cellular activity—information that is also necessary for the rational design of biologic therapies, as discussed. There seems very little point in offering biological repair in a situation where, owing to poor or nonexistent nutrient flux into discs, for instance, this approach is likely to fail. The development of validated diagnostic criteria that can predict the likelihood of success of biological therapies in individual patients should be an aim of disc regenerative strategies.

Research into intervertebral disc regeneration has been ongoing for more than two decades, yet only a few treatments have progressed to clinical trials, and none are commercially available. For successful repair, we believe that it is essential for researchers to consider the nutritional balance of the disc as well as concentrating solely on reparative techniques.

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All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.