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REVIEW ARTICLE

Disc cell therapies: critical issues

Marta Tibiletti · Nevenka Kregar Velikonja · Jill P. G. Urban · Jeremy C. T. Fairbank

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Abstract
Background Disc cell therapies, in which cells are injected into the degenerate disc in order to regenerate the matrix and restore function, appear to be an attractive, minimally invasive method of treatment. Interest in this area has stimulated research into disc cell biology in particular. However, other important issues, some of which are discussed here, need to be considered if cell-based therapies are to be brought to the clinic.
Purpose Firstly, a question which is barely addressed in the literature, is how to identify patients with 'degenerative disc disease' who would benefit from cell therapy. Pain not disc degeneration is the symptom which drives patients to the clinic. Even though there are associations between back pain and disc degeneration, many people with even severely degenerate discs, with herniated discs or with spinal stenosis, are pain-free. It is not possible using currently available techniques to identify whether disc repair or regeneration would remove symptoms or prevent symptoms from occurring in future. Moreover, the repair process in human discs is very slow (years) because of the low cell density which can be supported nutritionally even in healthy human discs. If repair is necessary for relief of symptoms, questions regarding quality of life and rehabilitation during this long process need consideration.
Also, some serious technical issues remain. Finding appropriate cell sources and scaffolds have received most attention, but these are not the only issues determining the feasibility of the procedure. There are questions regarding the safety of implanting cells by injection through the annulus whether the nutrient supply to the disc is sufficient to support implanted cells and whether, if cells are able to survive, conditions in a degenerate human disc will allow them to repair the damaged tissue.
Conclusions If cell therapy for treatment of disc-related disorders is to enter the clinic as a routine treatment, investigations must examine the questions related to patient selection and the feasibility of achieving the desired repair in an acceptable time frame. Few diagnostic tests that examine whether cell therapies are likely to succeed are available at present, but definite exclusion criteria would be evidence of major disc fissures, or disturbance of nutrient pathways as measured by post-contrast MRI.

Keywords Disc nutrition · Post-contrast MRI · Cell death · Rehabilitation

M. Tibiletti
IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Present Address:
M. Tibiletti
Department of Internal Medicine II-Cardiology, University of Ulm, Ulm, Germany

N. Kregar Velikonja
Educell Ltd., Ljubljana, Slovenija

J. P. G. Urban (✉)
Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, UK
e-mail: jpgu@physiol.ox.ac.uk

J. C. T. Fairbank
Nuffield Orthopaedic Centre, Oxford University Hospitals, Oxford, UK

Introduction

Autologous chondrocyte implantation for repairing articular cartilage defects has been used in the clinic since 1994 [1]. Here, autologous chondrocytes are implanted into a

defect where they produce cartilage matrix and repair the injured cartilage [2]. Following on from the success of cartilage repair, the idea of treating the degenerate disc by a relatively non-invasive injection of autologous cells [3] has captured the imagination of researchers and provided a tremendous stimulus for research into disc biology. For example, the number of papers published on intervertebral disc cells has increased exponentially from fewer than 250 papers in total before 1995 to over 2000 currently (PubMed search). From not knowing whether cells in different regions of the disc were phenotypically distinct or not even 15 years ago [4, 5], the different cell phenotypes populating the nucleus, cartilaginous endplate and annulus have been identified (reviewed [6]), differentiation pathways and progenitors identified [7–9] and specific markers differentiating cells of the nucleus from those of the annulus proposed [10–12]. Methods of culture have advanced from simple three-dimensional culture of primary cells in serum in alginate beads [13] to sophisticated matrices designed to promote differentiation of stem cells towards annulus or nucleus cell phenotypes [14–16]. Other areas of disc biology have also benefited from the interest in repair; knowledge of areas such as annulus structure and cell phenotype [17, 18], of disc-endplate organisation [19, 20], and of the intradiscal environment of degenerate discs (reviewed [21]) has increased substantially over the past decade.

However, in regard to disc repair, though much more is now known of disc tissue and of some of the technical challenges identified even 5 years ago [22, 23] such as identifying disc cell sources appropriate for disc tissue engineering [24–27] and of scaffolds for implanting and supporting such cells [28–32], other issues which have impact on clinical viability of disc repair tissue, such as long turnover times in human discs and nutritional impediments, have been comparatively neglected. Even though clinical implantation of cells into the human disc has been used on a limited number of patients [33–37] and a number of clinical trials are in progress, questions regarding the clinical aims and clinical feasibility of cell therapies for the disc are barely discussed.

Some of the issues which we believe require attention are summarised (Fig. 1). If cell therapy is to become a routine clinical treatment, research into cell therapy for treatment of disc-related disorders must go beyond research into cells and scaffolds and examine questions related to cell therapy practice. These questions are both technical, such as how to implant cells safely into the disc, and how to identify when conditions in a patient's disc are permissive for successful survival and activity of disc cells, but also relate to the important issue of whether a patient's symptoms might benefit from cell therapies. Here we discuss some of these clinical and technical issues relating to biological therapies for disc repair, concentrating on approaches which seek to repair the disc by implanting cells, but some of the issues raised are also relevant to developing methods that would repair the disc by protein injection or gene therapy.

1. **Is disc degeneration the cause of symptoms?**
   a. Will repair of the disc remove symptoms or prevent progression leading to symptoms?
   b. Is an appropriate rehabilitation scheme in place for the long repair process"
2. **Is an adequate cell implantation procedure available?**
   a. Can the cells be introduced into the disc without causing damage?
   b. Can the cells be retained in the desired area of the disc?
   c. Can the cells remain in the disc without being expelled?
3. **Is nutrient supply sufficient to maintain viability of any implanted cells?**
4. **Can the implanted cells produce an appropriate load-bearing matrix?**
   a. Will the implanted cells find conditions in the disc appropriate for production of matrix and tissue repair?

Fig. 1 Neglected issues. Some technical and clinical issues which are poorly researched and discussed and yet are important for the clinical success of disc cell therapies

Which patients will benefit from cell therapies?

The basic assumption, explicitly stated in most studies on disc repair, is that disc degeneration leads to low back pain which is an enormous clinical problem, and regenerating or repairing the disc will provide symptomatic relief [26, 38, 39]. Disc degeneration itself is, however, universal and in many cases non-symptomatic [40, 41] and there is no means at present of identifying whether anyone with a degenerate disc is likely to develop symptoms [42, 43].

As far as back pain patients are concerned, low back pain and degenerative disc disease are widely used terms, but are not specific; degenerative disc disease, for example, has been used to refer to patients with disc degeneration and non-specific pain, with herniated discs with radiculopathy, with lumbar spinal stenosis and with degenerative spondylolisthesis (reviewed [44]). These patients in general have degenerative or pathological changes to the disc which can be seen on MRI. And while there are studies which indicate that patients with severe disc degeneration are more likely to suffer from back pain [45, 46], it is not evident that the disc is the source of symptoms as severely degenerate discs with pathological features such as disc herniations, annular tears, Modic changes and severe lumbar spinal stenosis are also present in a significant fraction of the symptom-free population [40, 47–50].

There is also no direct evidence that the process of degeneration is responsible for symptoms [42, 51]; the idea that the pain can arise from the disc itself is controversial [52, 53]. Moreover it is not clear whether, in a significant

proportion of patients, regenerating or repairing the disc will provide any relief of pain, the symptom which drives most back pain patients to seek clinical help. Indeed, in many cases, by the time the patient gets to the clinic, epidemiological studies find that 20–35 % of back pain patients suffer from neuropathic pain (reviewed [54]). There is also evidence for augmented pain transmission (central sensitisation) and referred pain in a significant proportion of chronic back pain patients [55]. Thus, for these patients, the Cartesian view of pain, i.e., that a pathological change leads directly to pain and that removal of the pathology will remove the pain, no longer holds [56]. Is disc repair the appropriate treatment for such patients?

Cell therapy has been used on patients to prevent loss of disc height after herniation and to prevent recurrent herniation [34, 57]; interim results found that disc height was not regained after 2 years in comparison with untreated patients though water content was higher [34, 57]. In addition, cell therapies have been used in small pilot studies to alleviate back pain by injecting chondrocytes or stem cells into the disc; in some studies, but not all, patients report some pain benefit and in one case loss of high intensity zones on imaging [33, 35–37, 58]. However, none of these pilot studies have controls. It should be noted that needle injection alone may give pain relief, possibly through a placebo effect, even in cases of chronic back pain [59], so effectiveness of cell-based therapies can only be assessed by well-designed clinical trials. Eight Phase 1 or Phase II clinical trials on use of cell therapies for treatment of chronic low back pain or of herniated discs are now listed in http://clinicaltrials.gov/ but no outcomes are yet available.

Biological repair of human discs: a slow process

Because of its size and avascular nature, the human disc can only support a small number of viable cells; the cell density of the adult nucleus pulposus (NP) is reported to be around 1–5 million cells/ml [60, 61], i.e., <0.5 % tissue volume. Biological repair of human discs would be expected to be slow. Repair in animal models of disc degeneration may thus in general provide an over-optimistic view of success in the human lumbar disc [62], as, apart from other issues concerning the relevance of many of the acute models of degeneration, discs of even large animals, such as pigs, dogs or sheep, are very much smaller than human lumbar discs and support a very much greater cell density [63, 64]. Restoration of disc height has been reported to occur relatively fast in small animals after acutely induced disc degeneration—within 6 weeks in rats [65] and 18 weeks in rabbits [66]. Studies on discs of larger animals such as dogs, pigs and sheep, which are still considerably smaller than human discs [64, 67] have found that even in young healthy animals with degeneration induced acutely, disc properties were not effectively restored to control values by 3, 6 or even 12 months [24, 68–71].

Repair in human discs, including restoration of disc height, is unlikely to be faster and could be considerably slower than seen in animals—the half life of aggrecan in human discs is 3–6 years and is many decades for fibrillar proteins such as collagen and elastin (reviewed [72]) reflecting the low rate of matrix synthesis and degradation in this tissue. Appropriate clinical rehabilitation regimes, which allow healing without overloading the repair tissue or other structures, have been developed for human knee cartilage [73, 74] This cartilage is much thinner (2–3 mm) and more cellular than the lumbar discs (around 10 million cells/ml [75]), but has still not regained its mechanical properties 12 months after cellular implantation and 3 years is required for the repair tissue to mature [76, 77]. Healing would be considerably slower in the disc which is less cellular and considerably thicker; until it regains stiffness and height, its biomechanical behaviour will not return to normal and other structures such as facet joints will continue to experience inappropriate loads [78]. As far as we can determine the problems arising because of slow healing have not been discussed in relation to cellular repair of discs nor have any rehabilitation regimes been considered in the 8 clinical trials on cell therapy approaches for treating back pain now under way.

Can cells be safely implanted into the disc?

One advantage put forward for using cell therapies is that they are relatively non-invasive, as cells can be implanted directly into the degraded nucleus by injection. There are, however, factors which need to be considered regarding this technique.

Firstly, after a recent 10-year follow-up study, Carragee et al. [79, 80] reported that, in patients who had undergone discography, painful disc degeneration was accelerated significantly compared to matched controls who had not undergone discography. The discography cohort underwent four times as many lumbar surgeries as the control group over this period. The cause of the damage is not known; it could arise from the contrast medium (CM) used, from needle damage or from pressure damage arising from fluid injection into the disc. While high injection pressures could injure the disc, this has not been discussed directly; only a small volume of fluid can be injected into a normal disc [81] with fluid volume increasing and required injection pressure decreasing as the degree of degeneration increases. Contrast medium could have adverse effects on cell

viability as seen in vitro cell culture tests [82, 83]; however, cells in these tests have been exposed to culture medium for considerably longer durations and higher concentrations than would result from discography in vivo. Of the three proposed modes of damage arising from discography, needle puncture damage of the annulus has been most studied. There are some indications from animal studies that use of very fine needles can obviate damage to the annulus [84, 85]. However, other studies report that needle puncture with a 25G or 26G into the relatively large bovine disc can cause damage to the annulus fibres, changes in disc biomechanical responses and leads to degenerative changes [86, 87]. Until the causes of discography-induced disc damage are identified, implanting cells into the disc via injection through the annulus is potentially risky and risk-benefits have to be considered [88]; therapeutic injection of cells, or indeed any other agent, into the disc for the purpose of reversing the process of disc degeneration could instead actually accelerate it. Some studies are now investigating alternative routes into the disc via the endplate [89], but endplate damage itself can induce disc degeneration [90]. Whether this route is viable in the long term has not yet been proven.

The second issue is whether the implanted cells can be retained in the desired region of the disc. The view of a degenerate nucleus enclosed by an intact annulus which can thus contain any implanted cells does not apply to most adult human discs. Many discs, even at a young age (under 20 years), have small cracks and even major fissures that may not be visible by routine MRI [91–94]. Anterior or posterior radial tears on histological examination, some extending to the disc margins, were found in 47–68 % of young (10–30 years) L4–5 discs, the proportion of discs with such tears increased with age [93]. Virtually all discs examined had concentric treats and many other lesions were reported. There is, thus, a danger that cell suspensions injected into the disc nucleus could be forced into the annulus once the disc is loaded, or indeed out of the disc itself, as seen also in nuclear implants where the annulus is breached [95]. Leakage of cells is potentially harmful, has occurred in animal experiments [96] and could lead to inappropriate osteophyte formation [97]. An intact annulus thus seems a requirement for nucleus cell therapy [98] unless some kind of method of finding and sealing annulus cracks is developed. The requirement of repairing the annulus as well as the nucleus is now becoming recognised [17, 18, 99–102]; however, the necessity for annulus repair moves disc cell therapies away from a simple minimally invasive procedure.

Thus, understanding how to implant cells into the disc safely and maintain them in the required region are areas in urgent need of further study if simple relatively non-invasive disc cell therapies are to be introduced routinely to the clinic.

Limitations arising from the nutrient supply

A fall in nutrient supply in degenerate discs is one of the main impediments to the success of any form of cell therapy. If the original cells failed to function appropriately, or died because of lack of nutrient supply, the implanted cells will suffer the same fate.

Disc cells, like all others in the body, must have an adequate nutrient supply to survive and function (reviewed [103]); they consume glucose and oxygen and produce metabolic products, particularly lactic acid which acidifies the matrix and must be removed from the tissue to maintain cell viability [104]. The disc is large and avascular. Nutrients are supplied to the cells of the nucleus and inner annulus by capillaries which arise in the vertebral bodies and penetrate the subchondral plate through marrow spaces, terminating in loops at the junction of the subchondral plate and cartilaginous endplate [100]. Nutrients then diffuse through the disc, under concentration gradients governed by the balance between the rate of transport and the rate of cellular demand. Concentrations of glucose and oxygen, which are consumed by the cells, fall with distance from the blood supply and reach low levels in the disc centre while lactic acid concentrations follow a reverse concentration profile [105]. If glucose concentrations fall below 0.2 mM or pH levels fall below pH 6.8, cell survival and activity are compromised [104]. These levels can be disturbed by a fall in the rate of transport. The nutritional pathway from the blood supply to the disc cells is disturbed in degenerate discs through changes such as calcification of the cartilaginous endplate which inhibits diffusion of solutes into the disc [106–108], loss of marrow contacts with the cartilage endplate [109] and atherosclerosis of the vertebral arteries [110]. Disturbances of the nutrient pathway into the discs have long been associated with disc degeneration [109, 111].

Recent developments in MRI have now been able to show this association in vivo. Sequential MR images of the lumbar spine following intravenous injection of a CM are able to follow diffusion of the CM into the disc [112–115]. Post injection, the CM reaches the subchondral plate within 5 min after injection and diffuses into the disc, but is only seen in the central part of the lumbar disc at around 4 h post injection while enhancement peaks in the central part of the (NP) only 6–7 h post injection [114, 116]. In some, but not all studies, peak enhancement is considerably delayed and diminished in intensity in mild or moderately degenerate discs [115, 117] with most hold-up seen in the endplate region. Enhancement into very degenerate discs is rapid, possibly because of endplate breach and vascular ingrowth [114, 115, 117] following from proteoglycan loss [118–120].

This post-contrast method of monitoring transport does not provide direct information on nutrient levels as the transport of CM into the disc is purely diffusive while the gradients of nutrients such as oxygen or glucose are governed by both diffusive transport of these species into the disc and by cellular activity [103]. Therefore, at the moment it is not possible to draw direct conclusions on nutrient levels necessary for cell activity and survival from this methodology. It should be noted that direct measurements of nutrient levels in discs of patients show very varied levels [121], pointing to the complexity of the relationship between the changes in transport in degeneration and cellular activity; such interactions will complicate any assessments. Measurement of both nutrient pathways and viable cell density in a disc would allow assessment of whether nutrient supply is adequate to support the survival of newly implanted cells. The number of viable cells can be assessed in situ using two-photon microscope probes introduced through injection needles to measure cell autofluorescence [122]. The potential dangers arising from needle puncture, as noted above, prevent any further development of such measurements at present.

While it is evident that cell therapy cannot succeed without an adequate nutrient supply, the relationship between supply, nutrient concentrations and viable cell density cannot yet be assessed directly. However, the link observed between fall in transport and degeneration [117, 123] suggests that unless the solute transport pathways as shown by post-contrast CM diffusion shows a normal pattern, cell viability or activity is certainly compromised [124]. Such assessment of nutrient supply is essential for determining if treatment by a cell therapy approach is feasible for any particular patient. Patients with abnormal post-contrast diffusion patterns should not be offered cell therapy treatments [124].

Are conditions in the treated disc permissive of matrix production by implanted cells?

The major aim of cell therapies is to introduce cells which will produce matrix to replace that degraded and lost and, hence, to restore the biomechanical properties and height of the disc. Even if implanted cells survive, disc repair cannot be regarded as successful if these cells are unable to produce sufficient matrix.

The environment of a degenerate disc is inimical to the production and retention of matrix by disc cells. Cells of degenerate discs produce inflammatory cytokines and enzymes which degrade the disc matrix [21, 125–128]; these inflammatory molecules would also tend to degrade matrix produced by implanted cells. Only discs in which this inflammatory process has been dampened down [129], possibly by gene therapy [130–132], would be appropriate for disc repair. In addition, while variable levels of oxygen, glucose, lactic acid and pH have been reported in degenerate discs, glucose and pH levels tend to be low [121, 133, 134] in agreement with results found in modelling studies [135, 136]. To produce matrix, cells require an extracellular environment with sufficient glucose and where the pH is not acidic (the optimal pH range is pH 7.0–7.2); a fall in pH to below pH 6.8 has disastrous consequences, as rates of matrix production and matrix metabolism fall, but not the rate of matrix degradation [137–140]. Levels of pH in degenerate discs can fall to well below this value [133, 141]. In addition, matrix production is very sensitive to extracellular osmolarity; the osmolarity, directly related to proteoglycan content [142], must be high enough to stimulate matrix production and retention [140, 143, 144]. As one of the first signs of disc degeneration is loss of proteoglycans [145], the low osmolarity found in degenerate discs will reduce rates of matrix production and, indeed, may stimulate expression of proteinases [140].

At present, even if nutrient supply can support implanted cells, there are no non-invasive means of assessing whether the intradiscal environment will support matrix production. However, many needle-based probes exist or are under development which would allow non-destructive assessment of the intradiscal environment. Needle microelectrodes can be developed to provide assessment of extracellular pH, oxygen and glucose [121, 146]. Needle probes can also assess cell viability [122], as discussed above. A recently developed needle micro-osmometer, based on a microdialysis probe, provides a rapid and sensitive way of measuring osmolarity in disc tissue [147]. If the potential damage arising from needle puncture can be discounted, development of such probes will help identify discs whose nutrient pathways and extracellular environment permit cell survival and matrix production and accumulation.

Conclusions

Cell therapy for the purpose of treating degenerate disc disease is a very attractive concept. Interest in this approach has stimulated research into the disc and led to a substantial increase in knowledge of disc biology. However, it is difficult to see how cell therapy can be introduced into routine clinical practice in the foreseeable future.

There are still many technical obstacles to be overcome. Injection of cells into the disc is the basis of current cellular repair approaches but needle puncture of the annulus may impose a risk to disc health and the risks need to be weighed against potential benefit. Alternative methods of cell implantation and the realisation that repair of the

annulus may be required in some cases move cell therapy towards a more invasive and less attractive approach. The potential that implanted cells will have to reverse the degeneration process and repair the intervertebral disc in human rather than animal discs remains to be determined. Few diagnostic tests to determine whether cell therapies are likely to succeed in an individual patient are available at present, but definite exclusion criteria would be evidence of major disc fissures or disturbance of nutrient pathways, as measured by post-contrast MRI.

On the clinical side, there is no acceptable diagnostic method at present, of deciding whether an individual patient might benefit from cell therapy. This needs major advances in understanding back pain that have defied serious investigations over the last century. Moreover, it should be realised that human disc regeneration and repair by cell therapies are likely to be very slow and thought should be given to appropriate rehabilitation protocols after implantation.

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Conflict of interest None.

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