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REVIEW ARTICLE

Cell sources for nucleus pulposus regeneration

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Abstract
Purpose There is increasing interest in the development of cell therapy as a possible approach for the treatment of degenerative disc disease. To regenerate nucleus pulposus tissue, the cells must produce an appropriate proteoglycan-rich matrix, as this is essential for the functioning of the intervertebral disc. The natural environment within the disc is very challenging to implanted cells, particularly if they have been subcultured in normal laboratory conditions. The purpose of this work is to discuss parameters relevant to translating different proposed cell therapies of IVD into clinical use.
Results Several sources of cells have been proposed, including nucleus pulposus cells, chondrocytes and mesenchymal stem cells derived from bone marrow or adipose tissue. There are some clinical trials and reports of attempts to regenerate nucleus pulposus utilising either autologous or allogenic cells. While the published results of clinical applications of these cell therapies do not indicate any safety issues, additional evidence will be needed to prove their long-term efficacy.
Conclusion This article discusses parameters relevant for successful translation of research on different cell sources into clinically applicable cell therapies: the influence of the intervertebral disc microenvironment on the cell phenotype, issues associated with cell culture and technical preparation of cell products, as well as discussing current regulatory requirements. There are advantages and disadvantages of each proposed cell type, but no strong evidence to favour any one particular cell source at the moment.

Keywords Intervertebral disc regeneration · Cell implantation · Differentiation · Microenvironmental factors · Cell products · Regulatory and governance issues

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Introduction

There is increasing interest in the development of cell therapy as a possible approach for the treatment of degenerative disc disease with the aim of repairing or regenerating disc tissue. This follows the use of cell therapy for treating articular cartilage defects in the clinic for nearly 2 decades. There are many studies attempting to identify an appropriate cell population to be implanted into the degenerate disc to repair or replace the degraded matrix. Most work has focussed on the nucleus pulposus as the target for disc regeneration or preventing further height

loss of the herniated intervertebral disc (IVD). Cells investigated include disc cells or other cell types with discogenic potential, such as chondrocytes or mesenchymal stem cells (MSCs) [1–7]. Cell therapies utilising MSCs have also been proposed for inducing ossification of the IVD space to facilitate spinal fusion [8, 9] and indeed are being trialled (ClinicalTrials.gov identifiers NCT01552707, NCT01513694, NCT01603836, NCT00549913).

Choosing the appropriate cell source is necessary for the successful outcome of disc cell therapy [10]. Implanted cells need to be able to survive and produce tissue of a desired quality. As well as the appropriate cell source, other factors also have to be considered. The microenvironmental conditions in the disc, especially of the degenerated disc, often differ from the native environments of the available cell sources and, more particularly, normal laboratory culture conditions. The way in which cell populations are expanded prior to implantation to the disc could potentially impair cell function significantly, even leading to the death of the implanted cells [11, 12]. To assure the safety and effectiveness of the cell-based therapies, it is crucial, therefore, to evaluate the effects of specific IVD conditions on both cell viability and matrix production.

To obtain sufficient numbers of cells for preparation of a therapeutic product, cell processing usually requires a proliferation stage. This step should be considered carefully with respect to cell de-differentiation, re-differentiation capacity and also cellular senescence. The protocols developed will have to be approved by the relevant regulatory bodies, such as the European Medicines Agency.

Cell sources for nucleus pulposus regeneration

Cell populations of the native nucleus pulposus

Nucleus pulposus (NP) cells of adult humans produce abundant extracellular matrix, rich in aggrecan and type II collagen [13]. The nucleus pulposus of all mammals is initially occupied by cells derived from the notochord [14, 15]; these notochordal cells are large, contain vacuoles and aggregate (Fig. 1a) [16] and produce a very fluid matrix of low collagen content but high levels of proteoglycan and hyaluronan [13]. In humans, these cells are no longer apparent morphologically after approximately 10 years of age [14, 15, 17] and the adult nucleus is populated by cells with a more chondrocyte-like appearance [18]. The fate of the original population of notochordal cells in the human disc is not known as to whether they die and are replaced by inwardly migrating mesenchymal cells [19] or whether they differentiate into the NP cells (reviewed [20]). Cells extracted from human and canine discs express notochordal markers thus apparently supporting this differentiation pathway [21, 22]. These notochordal markers, however, appear to be expressed by only a small fraction of NP cells in humans, suggesting that, although they may arise from the notochordal cell population, they are indistinguishable from other NP cells morphologically [23, 24]. Hence, it is still unclear whether most human NP cells are derived from the notochord or are mesenchymal in origin.

Possible cell sources for regeneration of nucleus pulposus

In using cell therapy to treat damaged articular cartilage, autologous chondrocytes are sourced from healthy cartilage, culture expanded and used for autologous chondrocyte implantation. However, this approach has problems in the intervertebral disc for several reasons. The cell population itself is sparse and many cells in the adult disc are dysfunctional or dying. In addition, the procurement of cells from the disc would cause considerable morbidity as any procedure to obtain them necessitates damaging or invading the annulus and even a needle hole injury has been shown to lead to degenerative changes [25]. Finding a source of available cells which will produce a nucleus-like matrix is challenging. There have been a number of recent reviews which cover potential cell sources for use in autologous disc cell implantation therapies in detail [26–29]; the major sources which have been investigated are outlined below.

One way of assessing the potential effectiveness of cells for regenerative medicine is to determine how easily they express genes typical of the tissue to be repaired. In the case of the IVD, this poses some difficulties since NP cells have a similar gene expression profile to that of articular cartilage chondrocytes. However, some marker genes show significant differences between these tissues in terms of quantitative expression [30–33], one of these being the gene for cytokeratin 19, KRT 19 [31, 33]. As this marker was found only on subpopulation of nucleus pulposus cells of notochordal origin [23, 24], we cannot consider it as a specific marker which can distinguish the general nucleus pulposus cell population from chondrocytes. This lack of NP-specific markers hampers the search for differentiation protocols to produce NP cells. Hence, most in vitro studies for nucleus pulposus cell therapies define outcome in terms of expression of the matrix molecules typically produced by NP cells (although these are also mainly the same as chondrocytes), rather than in terms of a defined cell type (e.g. [34]).

Stem cells may be a suitable source of cells if appropriate differentiation protocols can be developed. Embryonic stem cells have been utilised in a small number of studies [35]. Due to the relative ease of access and the

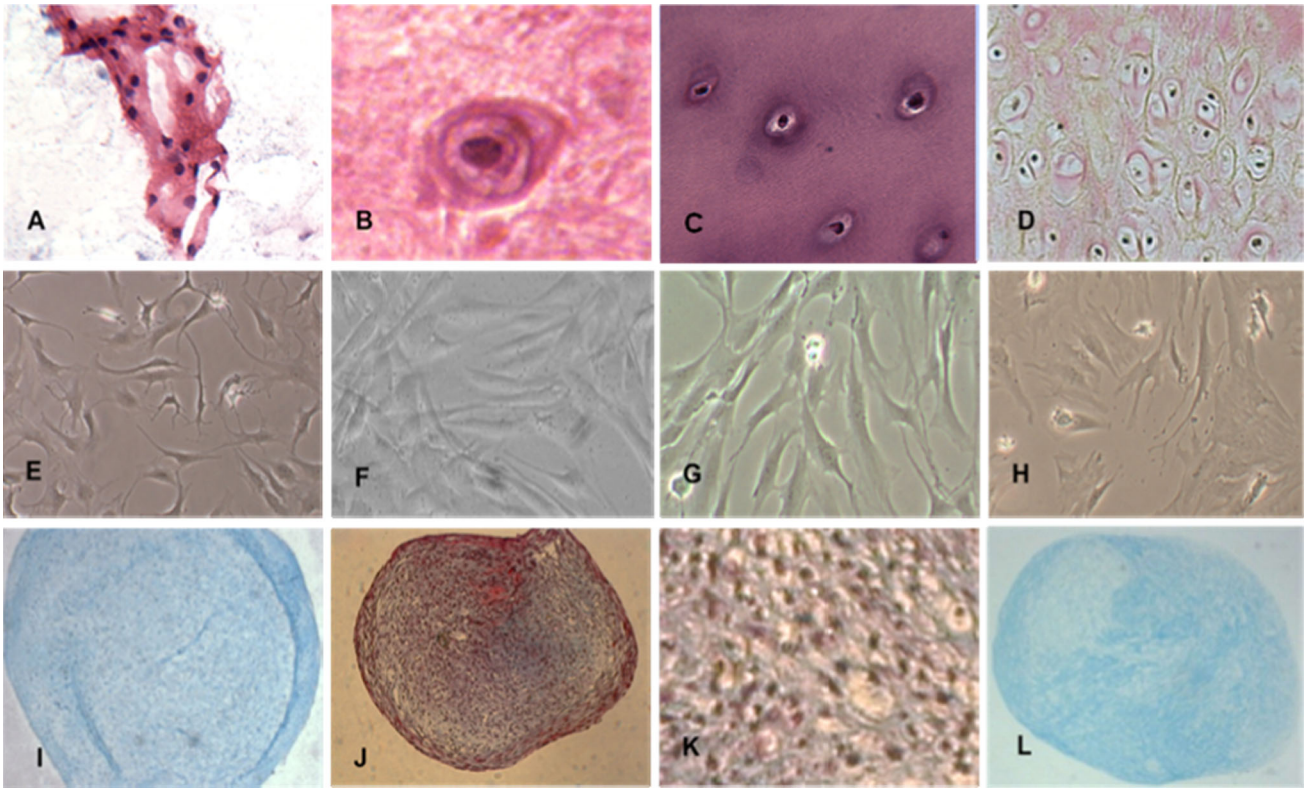

Fig. 1 The cells within the human NP are notochordal early in life (**a**; 3 months old) but replaced by more rounded, usually single, nucleus pulposus cells (**b**) often surrounded by a capsule. These resemble cells in other cartilaginous tissues, e.g. chondrocytes in articular cartilage (**c**) or auricular cartilage (**d**). In monolayer culture, all these cells, which are rounded in vivo (**b**–**d**), flatten out in a similar manner [nucleus pulposus cells (**e**), articular (**f**) and auricular chondrocytes (**g**)], as do MSCs, whether bone marrow derived or adipose derived (**h**). All these cells can also take on a more rounded and discogenic/chondrogenic appearance, if grown in 3-dimensional pellet cultures [MSC (**i**–**k**) and ASC (**l**)]. **k** is a higher power magnification of **j** showing lacunae around rounded cells but with cells at a much higher density than in native cartilaginous tissues. (**a**–**c** Haematoxylin and eosin stain; **d** Weigert van Gieson stain; **e**–**h** light inverted microscopy, phase contrast; **i**, **l** Alcian blue stain; **j**, **k** Masson stain)

multilineage differential potential, the MSCs present a more attractive cell source for disc cell therapies. They can be obtained successfully from both bone marrow and adipose tissue (ASC) (reviewed [28]); MSC progenitors are also reported to be present in degenerate discs and cartilage endplates, but numbers are sparse [36–39]. Minogue et al. [21] reported increased expression of chondrogenic markers when ASCs and MSCs were grown in 3D cultures compared to monolayer, with ASCs appearing to express a more nucleus pulposus-like phenotype than MSCs. However, it remains to be seen whether any of these cells truly differentiate into disc cells (Fig. 1i–l) (e.g. [34]), but reports show that MSC implantation stimulate regenerative changes in degenerative discs in animal models [40, 41]. With stem cells, there is also the issue of them differentiating inappropriately, for example bone marrow-derived MSCs might follow an osteogenic pathway. Indeed, Vadala et al. [42] observed the presence of injected MSCs in osteophytes which had developed subsequently, suggesting a potential unwanted side-effect with this approach. Survival of the implanted MSCs is another issue and an in vivo study of Acosta in a porcine model did not confirm the ability of MSCs to survive in the IVD space [43].

Cells isolated from degenerated/herniated nucleus pulposus have been used clinically [1] and disc chondrocytes are also now being used in a phase I/II clinical trial (see Table 1). However, their use is questionable as the state of the cells within such discs appears to be affected by the degeneration process. Kluba et al. [44] observed that NP cells from patients with degenerative disc disease expressed lower levels of type II collagen compared to those from patients with idiopathic scoliosis. Cells in the nucleus of degenerate discs form clusters which often contain apoptotic and senescent cells [45, 46]. In addition, tissue from herniated discs, removed at surgery, frequently contains fragments from the annulus fibrosus, the cartilage endplate, as well as the nucleus pulposus and may also contain blood vessels and inflammatory cells [47–49]. Hence, isolating a pure population of nucleus pulposus cells (Fig. 1b) from such fragments may be technically difficult.

Table 1 Overview of clinical applications of cell therapies for regeneration of the nucleus pulposus

Cell source	Clinical indication	No. of patients	References	Product	Producer
Autologous cells from herniated tissue	Disc herniation	NN	http://www.codon.de/patients/spinal-disc-defects.html?L=1	Chondro-transplant DISC	Co.don
Autologous mesenchymal stem cells	Lumbar herniated discs; degenerative disc disease;	NN	http://www.regenadisc.com/	*Regena*DISC	Celling Treatment Centers
Allogeneic adult mesenchymal progenitor cells	Degenerated disc disease	100	ClinicalTrials.gov Identifier: NCT01290367		Mesoblast Ltd.
Allogeneic juvenile chondrocytes	Discogenic back pain	NN	http://www.istotech.com/nuqu-phase-i-clinical-study.html	NuQu®	Isto Technologies
Autologous marrow mesenchymal cells	Degenerated disc disease	2	[91]		
Autologous mesenchymal bone marrow cells	Lumbar disc degeneration	10	[92]		
Hematopoietic precursor stem cells (HSCs)	Disc degeneration	10	[93]		
Autologous disc chondrocytes	Intervertebral disc displacement Intervertebral disc degeneration	120 planned	www.clinicaltrials.gov; identifier: NCT01640457	NOVOCART®Disc plus	Tetec AG

NN number not known

Notochordal cells when co-cultured with MSCs or NP cells appear to stimulate matrix production [50, 51] and these cells have consequently been suggested as a source for disc cell therapies. However, at present, no differentiation protocol for such cells has been developed and whether notochordal cells as such (Fig. 1a) could survive in adult human discs is questionable [19].

Chondrocytes are phenotypically the most similar cells to nucleus pulposus cells. Both types reside in load-bearing tissues that are hypocellular, avascular and non-innervated [4]; they have similar patterns of gene expression, including sox 9 [52] and MMPs [53], and they produce abundant extracellular matrix which is rich in aggrecan and type II collagen [13]. However, the matrix produced by these two different cell types is clearly different in its mechanical properties, in line with the differing biomechanical requirements of the tissues [43, 54]. Nevertheless, both articular [43] and auricular [7] chondrocytes (Fig 1c, d) have been investigated as sources for cell therapy of the IVD. Gorensek et al. [7] used auricular chondrocytes for regeneration of nucleus pulposus in a rabbit model. Histological analysis of new cartilage produced after implantation in this study confirmed the hypothesis that the implanted cells survive and produce a hyaline-like cartilage. Allogeneic juvenile articular chondrocytes in a porcine model produced nucleus pulposus-like tissue regeneration with viable cells in the newly formed tissue [43].

In vitro processing of cell sources intended for nucleus pulposus regeneration

Cell proliferation and senescence of cultured cells

The cell density in the human disc is extremely low, with 2,000–5,000 cells/mm^3 in the adult human nucleus pulposus [55, 56]. Nevertheless, despite the low cell densities required, for replacement of 1 ml of tissue about 2–5 $\times$ 10^6 of cells are needed, so cell proliferation and population expansion are still required to obtain sufficient cells for implantation. For example, approximately 10^6 cells can be obtained from an average ear cartilage biopsy (Educell, unpublished data). Likewise, MSC from readily available sources also require expansion; on average, 3 $\times$ 10^6 cells can be obtained after the 1st passage of cells isolated from typical volumes obtained in the clinic of either bone marrow (20 ml) or lipoaspirate (10 ml) (Educell, unpublished data), which corresponds to reported isolation yields that are in the range of 6 $\times$ 10^3–6 $\times$ 10^4 per ml of bone marrow aspirate and 5 $\times$ 10^3–2 $\times$ 10^5 per gram of adipose tissue (reviewed in [57]). However, there is high variability in yield of MSC among different donors [58].

Proliferation of cells introduces two important features, de-differentiation and cellular senescence. Mesenchymal stem cells grown in monolayer tend to retain their differentiation capacity [62], but disc cells and articular cartilage

chondrocytes de-differentiate when grown in monolayer culture, losing their usual shape and typical metabolic profile after successive passages (Fig. 1e–g) [59, 60]. How the cells behave subsequent to this and their re-differentiation capacity are highly dependent on cell culture conditions (e.g. [61]). For example, by transferring monolayer-cultured chondrocytes or disc cells to three-dimensional culture systems, such as pellet cultures (Fig. 1i–l), and using a culture medium containing appropriate growth factors and other constituents, the cells can be driven towards a chondrogenic or discogenic phenotype. This 3-D pellet culture mimics the mesenchymal condensation that occurs during embryogenesis [63], but the high cell density is unphysiological when compared to the native cell density in adult NP tissue.

All cells normally perform a limited number of cell doublings when cultured in vitro, after which they become 'senescent' [64]. Senescent cells not only cease the ability to proliferate but also exhibit a distinct gene expression profile. They overexpress matrix metalloproteases, growth factors, cytokines and inflammatory molecules, and generally exhibit a catabolic and inflammatory phenotype, which will affect tissue homeostasis [65, 66]. This issue is an important aspect to be considered in culture expansion of NP cells for cell therapy. Indeed, 19.8 % of human NP cells from herniated disc exhibits signs of senescence as depicted by the presence of senescence-associated β-galactosamine (SAβGal) by the 4th passage (Fig. 2a) and 23.9 % by passage 5 (Turner, unpublished information); MSCs, in contrast, are more resistant to senescence with <1 % being SAβGal positive at passage 3 (Fig. 2b) [67]. As discussed above, MSCs require extensive proliferation for use in cell replacement therapies if millions of them are to be implanted; several studies have indicated that, even though MSCs are more resistant to senescence, proliferation can lead to a decreased differentiation capacity [68–70] [our unpublished observations]. It is, therefore, obvious that the control of the senescent status of the cells is necessary for their use in replacement therapies for IVD repair and NP regeneration.

Influence of IVD microenvironmental conditions on potential cell sources

Oxygen, pH and osmolarity

To produce matrix, disc cells require an extracellular environment with sufficient oxygen (>3 %) and glucose (>1 mM in the centre) and the pH must preferably not be acidic (optimally around pH 7.0–pH 7.2). In addition, the osmolarity must be high enough to stimulate matrix production and its retention (ideally >350 mOsm). These conditions are met in a normal disc [11, 71], but in degenerate discs, osmolarity falls as aggrecan is lost, oxygen levels are variable and the pH may be acidic [72].

The microenvironmental conditions in the disc, however, are very specific and differ from the native environments of other cells being considered as possible sources of cells for disc repair, for example, MSCs. The disc microenvironment could impair the functioning of any such implanted cells or even cause their death.

Studies of specific microenvironmental factors within the nucleus pulposus, such as pH and osmolarity, indicate limitations in the use of MSCs for NP regeneration [12]. Wuertz et al. [73], for example, reported that acidic conditions caused an inhibition of aggrecan, collagen-1 and TIMP-3 expression, as well as a decrease in cell proliferation and viability and altered cell morphology of bone marrow-derived MSCs. Adipose-derived stem cells (ASC) were also shown to respond to the environmental conditions found within intervertebral discs. For example, osmolarity, typical of the disc, reduced cell viability and proliferation [74] (Fröhlich, Potocar et al., manuscript in preparation); likewise, a reduced pH, such as might occur in degenerate discs, inhibited cell viability and proliferation, as well as aggrecan and collagen-1 expression [74].

Acidic conditions could also markedly affect the metabolic activities and biosynthetic ability of chondrocytes

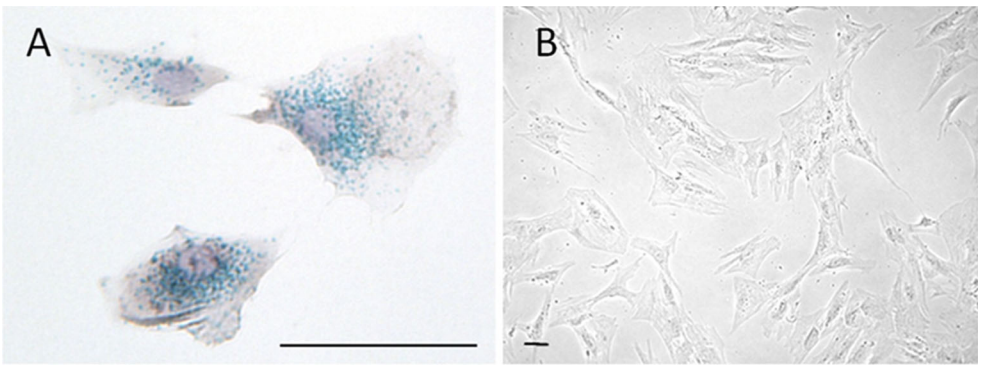

Fig. 2 A large proportion of human NP cells at passage 4 are senescent (as shown by *blue staining* of senescence-associated β-galactosidase within the cellular lysosomes) (**a**), whereas no bone marrow-derived MSCs are senescent at passage 3 (**b**). *Scale bar* 100 μm

[75, 76], and inhibit the disc cells from synthesising functionally important molecules such as the sulphated GAGs, whilst not altering the production of matrix-degrading proteases [77]. In contrast, nucleus pulposus and annulus fibrosus cells cultured in vitro respond to increasing osmolarity with an increased expression of aggrecan and collagen II [78].

Microenvironmental conditions found in degenerated discs can be highly variable [79], making the use of implanted cells even more challenging, since each cell population might need to be optimally selected or adapted for individual patients. Specific microenvironmental conditions could possibly be overcome with preconditioning of cultured cells prior to implantation to achieve better survival in the disc environment. This could include pre-culture of MSC in hypoxic conditions and supplementation of the culture medium with appropriate growth factors [80, 81].

Influence of biomechanical loading

Mechanical loading can influence behaviour of many cell types including those of the disc [82]. The cells in the disc in vivo experience high baseline loads, but there are also diurnal variations in hydrostatic pressure and strain, the degree of change depending on factors such as activity levels (and so loading due to muscles) and posture [83, 84].

There are some promising findings in the literature that have reported increased matrix biosynthesis when external dynamic loading, either hydrostatic pressuring or mechanical compression, is applied to tissues or tissue-engineered constructs containing MSCs, chondrocytes (reviewed by Huang et al. [85]) or disc cells [86]. Hydrostatic pressure is an important stimulus in cartilage as it can be both chondroprotective and can also drive chondrogenic differentiation via mechanotransduction (reviewed by Elder and Athanasiou [87]). Our studies have shown that mechanical loading has influence on gene expression of matrix protein (aggrecan, collagen II and collagen I) and also MMP2 and MMP3 expression, but the effects were quite load dependent and varied between annulus and nucleus cells [78]. In a more recent study, however, we were not able to obtain a significant increase in matrix production by applying hydrostatic pressure on cultured nucleus pulposus cells and found that the influence of medium composition was a more potent stimulus to chondrogenic differentiation [88]. Indeed, altering the biochemical environment (glucose, osmolarity, pH or oxygen) altered gene expression of aggrecan, collagen I, collagen II and also MMP-2, and MMP-3 when "degenerative" conditions were simulated. Compared to the effect of altering the biomechanical environment, the influences of mechanical loads (intermittent hydrostatic pressure) were quite low with differences only for aggrecan, collagen I and collagen II expression at different pH conditions [88]. There are many potential modifications that could be made to the loading protocols used; the optimal loading conditions that support differentiation towards the disc cell phenotype remain to be identified and are the subject of ongoing research activities. Nevertheless, application of external mechanical loads could be used in vitro to provide a stimulus to induce increased synthesis of matrix molecules by cells, precultured in suitable three-dimensional scaffolds.

Therapeutic approaches

Clinical applications utilising cells for regeneration of the nucleus pulposus

The use of cell therapy for regenerating the nucleus pulposus in human discs was first reported by Gerber [89] and subsequently by others [1, 90]. Their approach used autologous cells from herniated disc tissue or bone marrow-derived MSC that were expanded in vitro and injected into the area of the nucleus pulposus. More recently, other cell therapy applications based on the use of chondrocytes or stem cells from various adult tissues have emerged (Table 1).

Although there are some commercially available products and clinical trials going on in the field, there are only a few reports on the outcome of such treatments and they are mostly based on low patient numbers. There are claims of promising outcomes following treatment with autologous cells from herniated disc tissue or autologous MSCs [1, 91, 92], whereas no significant benefit has been reported following treatment with hematopoietic stem cells [93].

Autologous vs. allogeneic approach

Most products trialled to date have been autologous cell therapies. Although such an approach has advantages (for example, being unlikely to ellicit an immune reaction and ethically acceptable to most religions), autologous therapies do have limitations. Sourcing the cells necessitates an invasive procedure which is an additional cost for the health care provider and patient. Also, it will inevitably cause some level of donor site morbidity. If there is a genetic problem with the patient causing the disc degeneration in the first instance, it may be repeated or perpetuated by treating with cells from any location in that individual. The use of allogeneic cells, in contrast, overcomes some of these problems. In addition, it could render the technology more attractive commercially, if 'banks' of cells were available for treating patients in a single procedure. The exceptional healing capabilities of the injured foetus, with virtual complete regeneration of the damaged

tissues and without forming any scar tissue [94], has focussed much attention on embryonic or foetal cells for tissue engineering. Proof of principle of being able to 'bank' these cells and their use for allogeneic cell therapy have been performed with the cells used for treating chronic skin wounds or burns [95]. Foetal cells do not need a feeder layer (often animal cells), as is often necessary for culturing embryonic stem cells, or external growth factors applied to establish a fully defined consistent cell bank [96]. Cells isolated from human foetal spine tissues at 12–16 weeks of gestation have been shown to have chondrogenic and discogenic potential in vitro, producing aggrecan and type II collagen when cultured in alginate beads [97]. Cells that are at a later stage of development are also a possible allogeneic source. Juvenile chondrocytes, obtained from individuals <10 years of age, are being trialled in patients with discogenic back pain; they are being injected with a protein-based carrier into patients who have not responded to other conservative treatments in a phase I trial (NuQu®, ISTO Technologies). An allogeneic approach, using adult MSCs, produced by Mesoblast Company, is also being trialled alone or with hyaluronan (Table 1) to regenerate the IVD.

Regulatory issues relating to cell therapy for intervertebral disc regeneration

Within Europe, there are strict standards and regulations governing the safety and quality of cells being used for cell therapies. All cells are governed by the Directive 2004/23/EC as this covers donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells for therapeutic purposes. Ethical issues related to donation for cell therapy applications are especially pertinent for allogeneic foetal tissues and continue to be discussed with institutional and national medical ethical committees. There are further regulations which apply if cell therapy products undergo any other manipulation, or 'engineering', prior to being inserted into the patient. This includes all processes which might alter their biological characteristics, physiological functions or structural properties or where the cells are not intended to be used for the same essential function(s) in the recipient as in the donor. In these cases, the cells are considered to be Advanced Therapy Medicinal Products (ATMP) and they are governed by Regulation (EC) No 1394/2007. All the processes involved with isolating cells, such as enzymatic tissue digestion and any form of culture in the laboratory, are considered substantial manipulations. Whilst these procedures are usually necessary to achieve enough cells for implantation (Table 2), their use and hence the implementation of Regulation No 1394/2007 bring with it a great deal of additional governance to comply with the regulation. This, in turn, adds significant cost, both logistically and financially, which should not be underestimated. It can raise the cost of development of a product significantly (in the level of millions of euros). In addition, regulatory controls apply to every additional stage or procedure which is included in the cell processing and application of the product, for example, the use of growth factors and/or scaffolds. For obvious reasons, the development of a 'minimally manipulated' cell source for cell therapies of tissue disorders, including disc degeneration, is increasingly attractive.

It could be possible for MSCs (either from bone marrow or adipose tissue) to be prepared with minimal manipulation, for example, bone marrow is isolated and cell fractions are separated in the operating theatre, perhaps by centrifugation or by other means, and then returned to the patient with no culture step. This is only possible if sufficient volumes of source tissues were obtained and the cells will be applied to undertake the same essential function as they had in the donor tissue. It is reasonable to consider such cell therapy approach in terms of homologous use, since MSCs have a natural capacity to migrate within the body [98–100].

Discussion

With the development of cell therapies, the safety of the treated patient and the patient's quality of life are the major issues. All cells being considered as appropriate sources have the potential to differentiate into cells capable of producing a disc-like matrix with high aggrecan content. However, there is little evidence about their response to the extreme physiological conditions of the IVD, especially in the case of DDD. The low number of such studies is probably due to the fact that many of these parameters are difficult to control in vitro.

Various animal models have been used to study different regeneration approaches in several species, including rat [101, 102], rabbit [2, 3, 6, 7, 35, 103], dog [4, 104] pig [43, 105] and sheep [41]. Reproducibility and control of different animal models for disc degeneration is an important issue [106], as is the clinical relevance of the animal model; this may have limitations in terms of the biomechanics or the size of their discs, which are important from the point of nutrition and osmotic conditions. These studies list potential cell sources to regenerate IVD tissue, including NP cells [3, 4, 103], MSCs [2, 6, 43, 105], adipose-derived stem cells [101], progenitor cells (mesenchymal [41] or chondroprogenitors [35]) and chondrocytes [7, 43]. However, the experimental conditions vary between these studies, for example, using different culture conditions or scaffolds.

Table 2 Overview of the technical steps needed to obtain enough cells for implantation into the IVD

Cell source	Cell isolation	Cell proliferation	Cell differentiation	Homologous/heterologous use	Cell product classification
Nucleus pulposus	Enzymatic	Necessary	Not needed	Homologous	ATMP
Cartilage	Enzymatic	Necessary	Not needed	Heterologous use	ATMP
Bone marrow	Cell separation	Optional	Optional	Heterologous/homologous use[a]	ATMP/cell therapy
Adipose tissue	Enzymatic Cell separation	Optional	Optional	Heterologous/homologous use[a]	ATMP/cell therapy

ATMP advanced therapy medicinal product

[a] The recipient site is not the same as the donor site, but according to the function of stem cells in the body, they could be considered as used for the same essential function as in the donor tissue

There is no strong evidence to favour any one particular cell source at the moment. Disc cells and MSCs which have been used in patients are reported to result in pain relief and prevention of further degeneration [1, 91, 92]. These trials predominantly address the safety issues as no serious adverse events have been reported in any of them.

How the cells are prepared prior to application to the patient has a major impact on the regulatory bodies' classification of the cell product. Performing a clinical trial, such as is required for registration of an ATMP, requires investment in the range of millions of euros. There are considerable advantages if protocols are restricted to minimal cell manipulation, both in reducing the cost of development and also in attracting greatly reduced regulatory control. Alternatively, the use of an allogeneic source of cells, with lower production costs per batch than for autologous cells, is commercially more attractive compared to the classic autologous approach.

It is likely that several different cell product formulations will be developed for applying to degenerate discs. Selection of the most appropriate solution should be based on an understanding of specific pathological conditions as a major issue will be whether microenvironmental conditions will allow the cells to perform their function. The clinical application of cell therapy for disc disorders is a complex issue in terms of patient selection and the choice of appropriate outcome measures to assess success; it is discussed in more detail elsewhere (Tibiletti et al., submitted).

Finally, there is an alternative approach which could be used if the resident cells within the disc could be stimulated to be more productive, either in terms of 'switching on' proliferation in a controlled and appropriate manner, or by being stimulated to synthesise more matrix molecules such as aggrecan. Growth factor therapies, such as Growth and Differentiation Factor (GDF)-5 being trialled by DePuy Spine [107], utilise this approach, while Henriksson et al. [27] and others (e.g. [37, 38, 108]) report the likelihood of there being disc progenitor or stem cells within the intervertebral disc, which could perhaps have the potential to aid regeneration.

Much of the work in the area of nucleus pulposus regeneration has concentrated on cell sources; however, there are clinical questions about patient selection and other technicalities that also require investigation. Hence, many questions still require answers before cell therapies can be introduced widely into the clinic, following approval from the relevant regulatory bodies.

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Conflict of interest None.

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