Cell-Based Therapies for Intervertebral Disc and Cartilage Regeneration - Current Concepts, Parallels and Perspectives

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Abstract

Lower back pain from degenerative disc disease represents a global health burden, and presents a prominent opportunity for regenerative therapeutics. While current regenerative therapies such as autologous disc chondrocyte transplantation (ADCT), allogeneic juvenile chondrocyte implantation (NuQu®) and immunoselected allogeneic adipose derived precursor cells (Mesoblast) show exciting clinical potential, limitations remain. The heterogeneity of preclinical approaches and the paucity of clinical guidance have limited translational outcomes in disc repair, lagging almost a decade behind cartilage repair. Advances in cartilage repair have evolved to single step approaches with improved orthopaedic repair and regeneration. Elements from cartilage regeneration endeavours could be adopted and applied to harness translatable approaches and deliver a clinically and economically feasible regenerative surgery for back pain.

In this article, we trace the developments behind the translational success of cartilage repair, examine elements to consider in achieving disc regeneration and the need for surgical redesign. We further discuss clinical parameters, objectives and coordination required to deliver improved regenerative surgery. Cell source, processing and delivery modalities are key issues to be addressed in considering surgical redesign. Advances in biomanufacturing, tissue cryobanking and point of care cell processing technology may enable intraoperative solutions for single step procedures. To maximise translational success a triad partnership between clinicians, industry and researchers will be critical in providing instructive clinical guidelines for design as well as practical and economic considerations. This will allow a consensus in research ventures and add regenerative surgery into the algorithm in managing and treating a debilitating condition such as back pain.

Key words: intervertebral disc; regeneration; cartilage; biomanufacturing; intraoperative

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Introduction

Lower back pain is a global health burden yet current surgical interventions are limited in addressing the sophisticated needs of an increasingly sedentary and heavily co-morbid population burdened with degenerative disc disease (DDD). '*Biological solutions to biological problems*', is a concept that has fueled the development of regenerative procedures and accelerated treatments, enhancing functional recovery.^{1, 2} Advances in applied engineering and design add to the clinical sphere with novel synthetics, scaffolds, cellular therapeutics and cell-infused matrices promising cost-effective technology, single-stage procedures, and creation of durable repair tissue.

Single stage repair is "*in vogue*" and, advancing into the orthopaedic sphere, with advances in biomanufacturing and biobanking, yielding high quality 'off the shelf' implants³ and intraoperative processing technology integrating autologous cell-based therapy in a point of care fashion.^{4, 5} These concepts have delivered tangible improved clinical approaches in knee cartilage regenerative surgery, promising better orthopaedic repair strategies for an increasingly active ageing population.

However, commercial translation remains significantly less apparent in many other areas of regenerative surgery with a struggle to provide adequate return on investment in regenerative therapies, as is the case with disc repair. In this article, we outline the approaches that have been adopted to refine and achieve the translational success of cartilage repair. We examine the need for surgical redesign in disc repair and discuss the clinical parameters and objectives that might be considered and required to deliver an improved regenerative surgery for DDD.

Evolution and progress in cartilage and disc repair strategies

Advances in cartilage regenerative therapeutics have been remarkable and may hold valuable lessons in translating technological advances and directing the evolution of disc regenerative surgery. Arthritis has been identified as the most prominent opportunity for regenerative therapies in musculoskeletal disease with the intervertebral disc (IVD) following as a close second.⁶ In the management of cartilage pathology, the three paradigms of regenerative surgery: microfracture, autologous chondrocyte implantation, osteochondral grafting as first generation (Figure 1A), bridge the gap between palliation of cartilage injury and resurfacing via arthroplasty.⁷ Wherein the early use of regenerative therapeutics in acute focal cartilage defects have been shown to delay the deterioration of the joint organ and the progression of osteoarthritis (OA),⁷ there is no such critical event in triggering disc degeneration which is a chronic disorder. As such the degenerative microenvironment with dysfunctional cells may also benefit from the early introduction of a vibrant cell population capable of matrix reconstitution.

Developed in the 1990's, Autologous Chondrocyte Implantation (ACI)⁸ is similar to Autologous Disc Chondrocyte Transplantation or ADCT (co.don® AG, Germany) in the treatment of disc degeneration.⁹ These two-step surgical procedures with time-consuming and expensive laboratory processing⁹ report subsequent diminished tissue forming capacity of these culture expanded cells,¹⁰ cell leakage, delamination and graft failure¹¹ as technical and economical obstacles. In ADCT, culture expansion is essential due to the paucity of cells that can be isolated from degenerated tissue.^{9, 12} Where emerging technologies seek to redesign these first generation techniques many parallels can be drawn between cartilage and disc repair strategies.

Much of the evolution of ACI has been based on well-designed clinical studies and subsequent meta-analyses,^{13, 14} interrogating and learning from current market approaches to address contemporary clinical needs and limitations. As such the leap from 1st to 2nd generation ACI addressed the inadequacy of cell support with the incorporation of biomaterial scaffolds into microfracture and Matrix-induced Autologous Chondrocyte Implantation (MACI) techniques (Figure 1A), serving as a biological ‘net’ to capture and nourish released marrow or implanted autologous chondrocytes.¹⁵⁻¹⁸ While this improved outcomes, the wider uptake of regenerative surgical approaches was still limited and the subsequent evolution in cartilage repair highlights the pertinent elements to address in achieving clinical translation; (i) cell source (ii) cell processing and (iii) delivery systems.

13

Despite almost 30 years’ experience, all commercial therapies and the majority of the clinical trials in cartilage repair are centered on primary cells. Superior to chondroprogenitor or stem cell populations, there is less of a requirement for exogenous supplementation nursing and supportive agents in differentiation and phenotypic maintenance, the delivery and application of which is hampered by regulatory hurdles.¹⁹ With the advent of new cell processing technologies²⁰⁻²² and novel bioactive materials, boasting chondroinductive properties,²³ stem cell populations are being trialled in both preclinical and clinical studies but the technology is still in its infancy, with early clinical trials such as CARTISTEM.²⁴

Current approaches and trials are exploiting novel intraoperative point of care cell and tissue processing. Technologies such as Carti-One™ (Orteq® LTD, United Kingdom) or minced cartilage approaches^{25, 26} allow for a single staged approach with scope for autologous tissue inserted onto a carrier to be delivered arthroscopically for improved

repair of cartilage defects.²⁰ Cartilage autograft implantation system (CAIS, DePuy Synthes, Mitek Sports Medicine, USA) is a simplified one step procedure which eliminates technical difficulties and high costs associated with conventional ACI.^{24, 27, 28}

Early success has also been demonstrated with DeNovo® ET (Engineered Tissue), renamed as RevaFlex™ in 2013 (ISTO Technologies Inc.) which is currently undergoing clinical trials as well as the commercially available DeNovo® NT (Natural Tissue) from Zimmer®.²³ NuQu® (ISTO Technologies Inc.) is a second generation equivalent for disc repair currently being clinically trialled and is a single step procedure using banked allogeneic juvenile chondrocytes from cadaveric donors delivered in a fibrin sealant.²⁹ Preliminary safety, efficacy and clinical results in disc repair are encouraging.³⁰ While this approach is following much in line with progress in cartilage, partly addressing time and costs involved in a single step approach to disc repair, there are still many elements to consider. The current expense and expertise involved in isolating, amplifying and banking disc cells under GMP (Good Manufacturing Practice) conditions may limit and confine the availability of these therapies to specialised spinal surgery centres.^{11, 31}

The need for surgical redesign

From ADCT to NuQu®, the evolution of disc regenerative therapeutics is reminiscent of cartilage repair, however restoration of the disc and cartilage, are two distinct pursuits. Nucleus pulposus (NP) disc cells reside in a pressurised gelatinous matrix, an avascular niche characterised by low concentrations of oxygen, glucose, a high concentration of lactic acid (low pH)^{32, 33} with steep nutrient gradients.³⁴ In DDD, calcification of the endplate limits nutrient supply and waste removal making this a challenging environment for cell survival.³² In the degenerating articular joint organ,

however, following acute injury, inflammatory signalling, neovascularisation alters joint microenvironment leading to progression of degenerative change.³⁵ These differences in microenvironment need to be considered carefully in cell based regeneration strategies as applied to each. The nutrient limitations in the disc for example can only support a critical cell number,³⁶ much less than the typical range (5×10^6 to 8×10^6 cells/ml) that has been reported at a diffusion distance comparable to a normal adult human disc (5-8mm).³⁴ Similarly wherein cellular implantation has been shown to modulate this degenerate microenvironment to a certain extent^{37, 38} through deposition of appropriate matrix, it may be necessary to concurrently supplement with anti-inflammatory or pH modifying agents to improve outcomes. Additionally, a study by Mwale et al.³⁹ reported that the proteoglycan to collagen ratio is distinctly higher in the NP compared to cartilage in individuals of the same age and this difference in tissue composition is important to consider in assessing successful tissue reconstitution. The fibrocartilaginous concentric annulus fibrosus maintains the integrity of the hydrostatically pressurized nucleus pulposus matrix in axial deformation and as such access for NP repair is limited to needle introduction through the AF unlike joint structures which can be accessed through a variety of arthroscopic approaches. While we can draw parallels to efforts in chondrocyte based regeneration in cartilage and disc, consideration of tissue architectural, microenvironmental differences and mode of delivery will be critical to success.

Cell-based therapeutics in disc are more recent than those for cartilage (Figure 1A). Only six clinical studies are identified in cell based regenerative approaches for back pain as outlined in Table 1 compared to cartilage where there is a five-fold higher number of studies (Figure 1B). While there is a clear paucity of clinical studies^{9, 29, 40-44}, the trials in disc repair as outlined in Table 2, are in the early phases seeking to validate preclinical

approaches with little critical evaluation or insight into relevant clinical considerations and parameters unlike trials in cartilage repair. Oeheme et al. report on the heterogeneity of approaches in the literature as a key limitation in the disc repair literature.⁴⁵ These range from basic science *in vitro* to preclinical *in vivo* modeling. Readers are referred to this review for a more comprehensive overview of the current status of literature in disc repair. The number of publications in regenerative approaches in disc repair is consistently below those in cartilage, almost a 6-9 fold difference in recent years (Figure 1C) despite the incidence in cartilage and disc pathologies being fairly similar.⁶ Additionally, there are differences in the proportionate application of multipotent stem cells (MSCs) and primary cells in disc compared to cartilage. There is a greater application of primary cells in cartilage repair (2 fold) compared to disc regeneration where there are more studies focusing on the use of MSCs (3 fold). Perhaps the heterogeneity in the literature highlights the need for greater consensus and clinical direction in approaching disc regeneration.

This is pertinent given the arsenal of emerging technologies with a scope to revise the treatment algorithm of degenerative disc disease within the next decade. In adopting single stage revision to surgical approaches in line with developments in cartilage, there are three key research elements to consider – cell source, processing and delivery.¹⁶ In line with clinical objectives (Figure 2A), novel intraoperative processing of autologous biologics (1), or ‘off the shelf’ tissue banked allogeneic cells (2) could be delivered in a single step. With advances in biomanufacturing technology and minimally invasive delivery, more versatile approaches using high quality processed allogeneic and autologous cells in two staged disc repair can be envisioned (2, 3) (Figure 2B). In any of these strategies obtaining an optimal cell source in the primary task (Figure 2C).

Cell source –primary or stem cells?

From a practical perspective, the ability to obtain primary cells from non-load bearing cartilage regions of the joint organ makes for an obvious cell source for autologous chondrocyte implantation. However, when attempting to apply this approach to the disc, the paucity of cells available, suboptimal regeneration from degenerated cell populations and morbidity in harvesting from adjacent sites are clear limitations. Over the last decade, the advances in the field of stem cell biology have enabled the pursuit of alternative cell sources for disc repair and has largely been the focus of the disc literature for cellular regeneration. The advent of alternative minimal morbidity chondrocyte sources and the application of donor banked allogeneic cells have expanded the scope of primary cells in disc regeneration much in line with cartilage repair where the lessons in translation can be applied.

The role of MSCs has been extensively investigated in preclinical studies and the proposed mechanism for regeneration is both trophic, rescuing the resident cellular population, as well as differentiating and functioning as tissue forming cells. In the latter approach, the concept of priming cells *in vitro* with growth factors for implantation has been successfully demonstrated as a translatable banked cell source for cartilage repair.⁴⁶ However, concerns remain with the maintenance of a stable phenotype⁴⁷⁻⁴⁹ and cell survival⁵⁰ *in vivo* in the absence of exogenous supplementation. While there may be a role for the former, elucidating the factors determining survival and function of implanted cells in the clinical setting would be crucial before successful translation.⁵¹

Similar to cartilage regeneration, while the safety and feasibility of MSC implantation has been demonstrated in various models of DDD, few human trials have been pursued. Yoshikawa et al.⁴² and Orozco et al.⁴¹ report promising results, however,

these findings are limited to non-controlled, non-randomized studies with a small, heterogeneous patient population. A phase 2 randomized controlled, double blind clinical trial by Mesoblast LTD (Australia) with a 100 patient enrolment is now complete. Percutaneous intradiscal injection of immunoselected, culture expanded, allogeneic adult adipose derived precursor cells with a hyaluronic acid carrier compared with standard microdisectomy showed promising 12 month preliminary results and phase 3 trials are currently underway. Under review by the US food and drug administration (FDA), follow-up results are awaited with this therapy on the road to commercial availability.⁵²

When using primary cells in disc repair, minimal morbidity harvest is an important consideration as harvesting autologous NP from adjacent healthy disc levels would be inappropriate, with the risk of initiating degeneration at the harvest site.⁴⁵ Similarly harvesting autologous articular cartilage for disc applications may be considered too invasive. Advances in scientific understanding of cell phenotype and microenvironment opens new horizons in considering alternative accessible, minimal morbidity cell sources for IVD repair.^{53, 54} Cells from the human ear,^{55, 56} nose^{53, 54, 57-62} and rib cartilage⁶³ have all been investigated as a clinically relevant source for cartilage engineering due to high tissue cellularity and regenerative capacity of the cells in both proliferative and synthetic capacities in biochemically distinct environments from their own such as joint, disc *etc.*⁶³⁻⁶⁵ Unlike autologous NP cells derived from diseased tissue,¹⁰ they retain better regenerative potential in forming cartilaginous-like tissues. Gorenssek et al.⁶⁶ demonstrated successful deposition of hyaline cartilage and tissue reconstitution on implantation in a rabbit IVD model and Rahmat et al.⁶⁷ reported autologous costal chondrocytes remained viable and able to deposit cartilaginous-like matrix in a sheep model. Given the inherent differences between non-disc sources and NP cells which has

been well documented,^{68, 69} whether it is truly possible to attain and maintain a native NP phenotype with these primary cells is unknown and perhaps unlikely. However, what is perhaps more pertinent is the ability of these cell populations to regenerate and deposit functional tissue capable of sustaining the mechanical and nutrient microenvironment thereby retarding the degenerative process. Hence, appropriate tissue reconstitution would need to be assessed for each of these cell types and ultimately the formation of functional tissue would have to be established both in the preclinical and clinical situation.

A number of studies have directly compared the potential of primary and stem cell populations for both disc and cartilage tissue repair. Specifically for disc, Allon et al.⁷⁰ report reduced MSC viability when compared with NP cells which retained viability at 12 months and Acosta et al.³⁷ report increased sGAG deposition and tissue formation of chondrocytes compared to MSCs. However, Feng et al.⁷¹ report comparable outcomes using MSCs and NP cells maintaining disc height, T2 signal and tissue formation. For cartilage regeneration, Pleumeekers et al. compared human chondrocytes obtained from the ear, nose, articular cartilage with bone marrow and adipose derived stem cells both in vitro and in vivo and reported articular chondrocytes to have the highest in vitro chondrogenic potential and auricular and nasal chondrocytes to be more highly potent in terms of in vivo tissue reconstitution.⁷²

Another study compared OA derived chondrocytes with bone marrow MSCs and found that chondrocytes expressed higher mRNA levels (COL2A1, Col 9) of hyaline extracellular matrix components.⁷³ Whether MSCs or primary cells are the optimal focus for cell based therapeutics is unclear, the advantages and disadvantages of each would need to be balanced with the specific aims of regenerative ventures in disc or cartilage and ultimately the clinical translatability.

Intraoperative processing – towards a single step procedure

Tissue harvesting, cell isolation, laboratory processing with amplification, growth factor priming, and assembly into an implantable construct has been the research standard and approach to date. However, there has been a recent shift to point of care isolation and processing technology enabling intraoperative solutions for cartilage repair.⁷⁴ These strategies allow the use of fresh biological material without the risk of dedifferentiation in prolonged laboratory expansion. In the case that the material does not leave the operating theatre or is stored for any period of time, this alleviates much of the regulatory constraints in clinical translatability.⁷⁵

There are several cell processing devices on the market including Harvest SmartPrep[®] (TERUMOBCT), point of care preparation systems for platelet rich plasma (PRP),⁷⁶ bone marrow aspirate concentrate (BMAC) and stromal vascular fraction (SVF) of lipoaspirates⁷⁷ or Thermogenesis Res-Q System[™] 60 for BMAC.⁷⁸ These systems facilitate the enrichment or concentration of cells, growth factors and endogenous serum constituents,^{75, 79} representing a viable alternative to growth factor administration which is currently hampered by regulatory hurdles. However, depending on the method of preparation, the exact bioactive constituents vary and little is known with respect to the precise regenerative mechanisms of these agents.⁷⁵

Where PRP, bone marrow concentrates or similar have shown success in other areas of orthobiologics, they have been trialled both as a supplement to cell-based therapeutics or a standalone treatment to augment IVD repair in preclinical trials.⁸⁰⁻⁸² Intra-articular injection of PRP has shown promise in knee osteoarthritis in the short-term, with many studies demonstrating declines in efficacy after 1 year.⁸³⁻⁸⁶ Similarly for disc as outlined in a review by Wang et al.,⁸⁷ the use of PRP has been demonstrated to restore disc height

and improve MRI signal intensity in preclinical studies. Where there is an absence in a uniform standard for the optimal formulation of stable PRP production, quality assurance and dosing remain additional challenges to be addressed. Further clinical evaluation would be required to elucidate the exact mechanisms and implications of PRP for disc regeneration. Similarly, Pettine et al.⁴⁴ demonstrated the efficacy of BMAC in patients with DDD and report improvements in visual analogue scale (VAS) and Oswestry Disability Index (ODI) at 12 months following administration of 2,713 CFU-F/ml with greater improvements observed in younger (under 40) patients. However, in contrast, the administration of SVF has been reported by Detiger et al.⁷⁹ to cause an acute inflammatory reaction on injection into a goat disc degeneration model. Therefore, it is important to exercise caution in the free administration of such agents in the absence of sound scientific data and proposed regenerative interventions should be carefully considered and evaluated.

Alternatively, primary cells have been shown to adapt to microenvironmental cues in vivo to maintain phenotypic expression. The dual model of oxygen (HIF1a) and glucose sensing (GLUT 1/2) as proposed by Mobasheri et al.,⁸⁸ allows chondrocytes to adapt to this ischaemic nutrient limited niche and maintain regenerative potential in the absence of exogenous growth factor supplementation. Application, however, may be limited by low cell yields obtainable from cartilage biopsies. Where large populations of viable chondrocytes are required for optimal chondrogenesis, a key limitation in this regard is the development of intra-operative cell processing protocols and devices to optimize yield for delivery and regeneration.

The commercially available Carti-One™ (Orteq® LTD, United Kingdom) system is a rapid intraoperative cellular-based and technician enabled point of care approach for cartilage defect repair. This service enables point of care separation of harvested autologous bone marrow mononuclear cells (MNCs) and primary chondrocytes which can be subsequently combined with the INSTRUCT™ (CellCoTec B.V., Netherlands) copolymer scaffold or an alternative carrier depending on the defect type and surgeons preference. While this approach has its limitations, it highlights the role of such translatable protocols in facilitating single step regenerative ventures using primary cells. Success of such approaches further demonstrated by Bekkers et al.^{22, 74} could potentially be emulated in minimal manipulation options for IVD repair. They demonstrate cartilage regeneration in chondron coculture with allogeneic MSCs amenable to a single stage surgery which is currently in Phase 1/2 evaluation in the IMPACT trial. However progress in this area will be dictated by the regulatory landscape where FDA approval for the intraoperative use of enzymes is necessary to apply rapid isolation of cells for use in single step approaches. This will be a key factor into the pursuit and investment into single step autologous primary cell based ventures (Figure 2B) in the coming years.

The concept of using minced autologous donor cartilage in ‘tissue transplantation’, can overcome regulatory concerns associated with use of enzymes.²⁷ Loading minced cartilage with a carrier material allows for a chondroinductive implant and has shown good outcomes in preclinical and animal models for cartilage repair.^{20, 74} Adopted from reconstructive plastic surgery, further challenges lie in the automation of this process, which can reduce time and improve efficiency of intraoperative tissue mincing.²⁷ In the context of the disc where the access to the NP is limited to narrow bore needles, particle size of the tissue fragments would need to be appropriately sized to be compatible with

intradiscal delivery and poses a technical challenge to the adoption of this approach for disc repair. Additionally, the transpedicular approach as proposed by Vadalà et al.⁸⁹ is an alternative accessible route that could be pursued, bypassing the need for annulus fibrosus puncture and nucleotomy, in the delivery of therapeutics through the endplate.

Optimizing biomanufacturing – maintaining robust cells for regeneration

The application of tissue engineering approaches has become more feasible with the advent of GMP cell processing facilities. The ability to augment cell processing and deliver high quality cellular implant systems can enable the next generation of tissue-engineered therapeutics.

Coculture approaches have been explored to exploit the beneficial effects of both trophic bone marrow or adipose derived stem cell populations and stable nucleus pulposus or articular chondrocytes cells in a laboratory setting.^{48, 90-93} In vitro coculture, priming and activation of NP-like phenotypes by trophic MSCs or predifferentiation of MSCs by NP cells alike, applied to biomanufacturing could improve health and maintain robusticity in laboratory processed cells for clinical application.⁹⁴ This has similarly been demonstrated for articular chondrocytes where preconditioning with stromal vascular fraction of adipose tissue and BMSCs has been shown to improve chondrogenesis⁹² and likewise cartilage extracts have been shown to promote chondrogenesis in human infrapatellar fat pad derived stem cells.⁹⁵ There are however, several technical considerations. Yamamoto et al. demonstrated the importance of direct cell-cell contact for activation of NP cells when cultured with BMSCs,⁹⁶ however, early human clinical trials by Mochida et al. (2015) demonstrated minimal efficacy and more study remains to be done in this area.⁴³ Inversely, contact dependent differentiation of BMSCs to an NP-

like phenotype has been demonstrated successfully in coculture by Richardson et al.⁴⁸ Similarly, the role of molecular agents secreted by notochordal cells (NCs) and NC conditioned media formulations in inducing NP-like phenotype in BMSCs and priming NP cells has demonstrated to be efficacious in vitro.⁹⁷⁻¹⁰⁰ NCs have also been shown to play a role in the differentiation of human induced pluripotent stem cells to NP like cells.

101

In this regard, NP/NC conditioned media could possibly substitute the need for recombinant growth factors in vitro, and potentially provide the means to initiate MSC differentiation for in vivo use for the disc. Whereas for cartilage repair, there may be a greater need for recombinant growth factor support. Other biochemical factors, such as glucose and oxygen have been shown to play an important role in regulating matrix synthesis of MSCs.¹⁰² As such, pre-conditioning cells during culture expansion to be able to adapt and sustain the respective biochemical microenvironments they will experience upon transplantation in vivo may be worth exploring further.

In cartilage, post-traumatic joint instability, incongruity or mal-alignment increases release of reactive oxygen species, and oxygen may not be able to support chondrogenic phenotype in MSC populations in the absence of trophic support.¹⁰³ However, further to understanding the maintenance of a stable *in vivo* phenotype^{104, 105} the nature of this two way interaction has yet to be decoupled.⁹³ These approaches would add significantly to the biomanufacturing arsenal for off the shelf, banked, high quality and characterised functional cells for disc repair.¹⁰⁶ Furthermore, the advances in 3D culture systems and our understanding of mechanobiology could facilitate mechanical priming of cellular systems¹⁰⁷⁻¹⁰⁹ thereby improving the quality of cellular implants.

Cryobanking of tissues and bioprocessed cells allows for storage for allogeneic or

autologous primed cells for clinical use. Retention of high cell viability, differentiation and tissue forming potential has been demonstrated by Pravaduyk et al.¹¹⁰ and Tanaka et al.¹¹¹ This is an important facet and addresses the logistical storage and transport challenges with applying biomanufacturing technology to translatable clinical ventures.

112

‘Structure modifying’ versus ‘Symptom modifying’

The progression of osteoarthritis and disc degeneration are distinct and implications for the success of cell based therapies in the context of the specific microenvironment should be considered. In DDD, the calcification of endplates, nutrient limitation and accumulation of waste results in an ischaemic niche with low pH poses a significant challenge.³² In contrast, cartilage degeneration results in subchondral bone thickening, increased vascular invasion and altered nutrient environment.³⁵ These differences in nutrient deprived versus nutrient altered microenvironments can have implications for the maintenance of cell populations in successful cell based therapeutics as ‘structure modifying’ strategies to restore tissue structure and mechanics. While in vitro studies have shown that low oxygen and glucose microenvironments can be beneficial for matrix formation by MSCs,¹⁰² the low pH conditions may inhibit the success of cell-based therapeutics for disc repair.^{50, 113} In contrast, the increased vascularity in cartilage injuries and increased oxygen tension has been shown to promote cartilage healing¹¹⁴ but this may require the need for additional trophic factors for the maintenance of a chondrogenic phenotype of MSCs for cartilage repair.¹¹⁵ The careful consideration of these niche microenvironments in assessing tissue reconstitution and identifying a target patient group will be important in the conception of therapies. The appropriate delivery of

‘symptom modifying’ approaches in addition to ‘structure modifying’ therapeutics may enhance tissue support and cell survival as proposed by Masuda.¹¹⁶ Equally, concurrent modulation of the harsh IVD microenvironment especially in the context of nutrient limitations might improve regenerative success with the chronic disorder or the use of anti-inflammatory supplements in acute chondral injuries.

The degenerative niche and microenvironment of the IVD may contribute to a ceiling effect above which increased cell numbers cannot be supported due to nutritional demands rendering treatment using a cell therapy approach futile and unethical.³⁶ While we outlined the potential for use of lower seeding densities of robust populations of cells amenable to intraoperative processing, microenvironmental optimisation can further improve regenerative outcomes.¹¹⁷

Further, increased expression of IL1, IL6, IL 12, IL17, TNF- α , IFN- γ and consequent upregulation of matrix metalloproteinases (MMPs) contribute to the inflammatory microenvironment with neo-innervation resulting in discogenic pain and acute cartilage injury.^{118, 119} The delivery of inflammatory modulators has been proposed as a possible strategy for improving pain outcomes. Cytokine and proteolytic enzyme production by NP cells can be potentially modulated using IL-1 receptor antagonist (IL-1RA)^{120, 121} and anti-TNF agents.¹²²

In addition, much in line with progress in cartilage, adaptable biomaterials for cell retention and support are required to improve cell retention¹²³ especially in the context of mechanically loaded and pressurized systems like the IVD given the high risk of extrusion relative to the articular joint.¹²⁴ In open joint procedures large membrane and porous scaffold supports can be appropriately sized and press-fit into the defect and the risk of

delamination is reduced in this case. However, limited physical access to the disc and higher pressures pose additional challenges in adapting biomaterials for injectable delivery and uniform dispersal through narrow gauge needles to minimise AF incision and fibre damage.

For minimally invasive delivery, injectable solutions such as microcapsules, microcarriers, shape-memory scaffolds and thermo-gelation hydrogels are all viable modes for the delivery and protection of cellular populations or trophic factors.¹²⁵ Examples of injectable bioactive cell carrier systems incorporating alginates, collagens, hyaluronic acid or fibrin have all shown to improve cell delivery for IVD repair. Blaquer et al., Malafaya et al. and Pereira et al. offer comprehensive reviews of these delivery systems and strategies.¹²⁶⁻¹²⁸

The delivery of biomaterial laden inflammatory modulators could provide pain relief and a more favourable microenvironment to improve survival and support of robust cell populations with high nutritional demands, promising superior tissue reconstitution. Lower numbers of cells required to achieve IVD repair in this regard, are more amenable to intraoperative processing and biomaterial systems can possibly provide the appropriate niche for *in vivo* proliferation and subsequent regeneration.¹²⁵ With novel biomimetic and bioactive ECM based biomaterials, these delivery systems could potentially be engineered to match the surface topography, stiffness for *in vivo* amplification as an alternative to GMP standard – laboratory based expansion. Through manipulation of the inflammatory microenvironment and taking advantage of biochemical and mechanical cues, these bioactive biomaterial systems could ‘instruct’ cellular populations to facilitate both ‘structural’ restoration and ‘symptomatic’ relief in DDD.¹²⁹

The clinical – researcher - industry triad – need for greater consensus

Despite strong preclinical research efforts, only six clinical trials have been reported for IVD repair while others are ongoing as outlined in Table 1. This is reflected in the limited commercial options on the market. This has been labelled as the ‘valley of death’¹³⁰ reflecting the hurdles in clinical translatability of current research approaches. The reality of translation and the regulatory constraints implies that not all possibilities will be equally feasible or reasonable. Eventually, a line of effort must focus on a consensus to meet the clinical objectives of disc regeneration and commercial requirements.¹³¹⁻¹³³ In addition to scientific freedom, practicality or commercial considerations there is a fundamental central role of the patient, a unique customer populace and the clinical demands of such must be met. Hence a transition from the traditional bench-to-bedside approach to a more applicable bedside-to-bench approach as proposed by Spindler and Dunn¹³⁴ where the clinician serves as the best advocate for the patient and close liaison can improve translatability of therapeutic approaches. A clear evidence base with both preclinical and clinical safety as well as efficacy in addition to economic considerations is crucial to reap the broader benefit of these promising approaches and adopt them into treatment algorithms.

A clearer perspective on target population can improve the connectivity between the researcher, surgeon and industry facilitating delivery of therapeutics to a broader population in a cost-effective, affordable manner. DDD affects an increasingly sedentary middle aged population. Lifestyle choices are important in this population, facing the evolution of chronic multifactorial illness, the likes of cardiovascular disease and diabetes. Painful, activity restricting orthopaedic pathologies such as back pain can have major impacts. In a European wide study comparing orthopaedic diagnoses and

treatments, spinal surgery has been noted to have the best improvement in quality of life compared to hip and knee replacement.¹³⁵

Where the presentation of cartilage injuries is acute, the early intervention with regenerative therapeutics can prevent the progression of degeneration and osteoarthritis. Given the experience in this area, a clear patient selection criterion includes a combination of osteochondral lesion size and location as determined by MRI, patient age and functional level.¹³⁶ With the disc however, more subtle presentations are common with patients presenting much later. This creates a challenge in identifying a suitable target population at an early stage of DDD as cell based therapeutics would perhaps be most successful in mild, moderate levels of degeneration as posited by Sakai and Andersson.⁴⁹

Given the degenerative cascade of disc disease,¹² current approaches recommend cell-based therapies should be limited to single-level, early DDD, which is reflected by Pfirrmann Grade III or IV on MRI. Patients with a Pfirrmann grade of I–II or V would be either too mild or too advanced in the disease process to show measurable improvement.²⁹ In practice however, the DDD process is slow and gradual and there is not always a direct correlation between back pain and disc degeneration. Current diagnostic methods lack the sophistication to adequately identify good surgical candidates and this is further complicated in regenerative surgery¹³⁷ where optimal cell dosage would depend on the stage of degeneration.¹³⁸ Measurement of local pH, a reliable marker of stage of degeneration has been proposed as a possible method to stratify the patient population.¹¹³ Advances in imaging modalities with pH mapping adjuncts to MRI make this a clinically feasible strategy for application in DDD.^{139, 140}

As with cartilage, there are limited options for repair in late stage presentation where microenvironmental modulation in addressing the inflammatory oxidative environment of the knee joint or the nutrient limited ischaemic niche of the IVD would need to be addressed with anti-inflammatory agents or pH regulators. Additionally, much like the joint organ, where consideration of meniscal and anterior cruciate ligament (ACL) integrity will impact on the success of cartilage regeneration,¹³⁶ facilitation of annulus fibrosus (AF) and endplate (EP) regeneration is clearly needed to synergistically improve the efficacy of cell-based therapy in the disc.¹²

The target population would ideally be treated using a minimally invasive surgery (MIS), reducing iatrogenic morbidity while maintaining multiple revision options.¹⁴¹ Procedures facilitated by small incisions and local anaesthetic allow treatment on an outpatient basis. Therefore, this provides favourable economics in regenerative disc repair with timely discharge, and reducing hospital stay costs.¹⁴² This is particularly the case with loaded orthopaedic tissues as it allows for faster mobilization of patients in ‘active, functional recovery’.¹⁴³ This ‘functional recovery’ will be crucial in optimizing the mechanical conditioning of repaired tissues and optimizing surgical outcomes in this regard.¹⁴⁴

Where the sophisticated technology in MIS and regenerative approaches are often more expensive upfront, the overall cost-effectiveness must be considered with improved clinical quality and outcomes, reduced mobilization time in addition to positive economics.¹⁴¹ To expand the scope of these regenerative surgical ventures to the broader population, cost minimization should be considered in the design. Logistical challenges relating to tissue retrieval, biologics storage, cryobanking and transport requires extensive

interdisciplinary support. These challenges need to be overcome and streamlined or the potential of bioprocessing approaches will be limited to specialised clinical centres.¹⁴²

Conclusions

Cell based therapies may hold significant promise but will need to be optimised and matched for efficacy that is commensurate with DDD and as such interdisciplinary input with guidance and interactions between clinicians, scientists and industry is critical. Whether through advances in biomanufacturing or intraoperative ventures, autologous or allogeneic approaches, reduced recovery and rehabilitation time has been documented in single stage procedures for cartilage repair.¹⁴³ Adopting some of these endeavors could perhaps harness a translatable, regenerative surgery for DDD with cell source, cell processing and delivery being key design considerations. In addition, to yield a widely applicable regenerative surgery, robust tissue regeneration and predictable clinical outcomes need to be achieved in a cost effective manner. Other key aspects to be addressed include target population identification and stratification, cell dosage as well as developing rehabilitation and functional recovery regimes as presented in the highlights of this article (Table 3).

Wherein disc repair remains behind cartilage in the delivery of feasible clinical options of regenerative repair, the disparity in the literature and consequently clinical trials can be attributed at least in part to the differences in identifying a suitable cell source. In addition, the challenges associated with overcoming the degenerating microenvironment of the disc as well as cell delivery strategies can account for the discrepancy and must be carefully considered. Wherein the disc literature focuses on MSCs, given the clinical limitations with NP cells, this remains a more challenging

population to adapt to tissue reconstitution and repair. This is predicated on our understanding of the exact role and interactions of MSCs 'in vivo', the possible need for trophic stimulation and the requirement for advanced processing and manufacturing facilities. In contrast, the use of robust, feasible autologous primary cells from the same articular joint in cartilage has facilitated the exploitation of single step procedures, point of care processing, allogeneic banking and delivery to expand the scope of applications. Lessons learnt in these ventures could be applied with the advent of alternative cell sources and banked allogeneic options for feasible clinical repair. In parallel, more sophisticated pre-clinical animal models will be required in order to assess these aspects in line with defined clinical objectives.¹⁴⁵

Given the current status of biomanufacturing, high associated costs and expertise to meet GMP standards limit wide scale clinical applications. Where safety is paramount, stringent but facilitative regulations are required to allow the continued endeavours and the cost-effective upscaling of bioprocessing approaches to expand possible clinical applications. However, given the extensive burden that chronic back pain imposes on quality of life and lost productivity in society, low cost, early recovery interventions are urgently required. As such single step approaches could perhaps reap significant rewards and facilitate the delivery of clinically feasible regenerative surgery in the immediate and short term until more sophisticated strategies are developed.

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Figure and Table Legends

Figure 1: Evolution and progress in Cartilage and Disc repair strategies. **(A)** Timeline showing the advances in regenerative therapeutics in cartilage and disc repair. **(B)** Clinical cell-based trials using stem cells or primary cells in cartilage and disc repair in PUBMED search with terms “regeneration” and “cartilage repair” or “disc repair”. **(C)** Research publications in PUBMED search with terms “regeneration” and “cartilage repair” or “disc repair” between 2006-2015. Abbreviations- ACI: Autologous Chondrocyte Implantation; OATS: Osteochondral Autograft Transfer System (Arthrex Inc., USA); MACI: Matrix-induced Autologous Chondrocyte Implantation; DeNovo® NT (Natural Tissue): particulated juvenile cartilage allograft implant with fibrin fixation (Zimmer®, USA); DeNovo® ET (Engineered Tissue), renamed as RevaFlex™ in 2013 (ISTO Technologies Inc.); scaffold-free tissue graft engineered from juvenile cartilage cells; CAIS: Cartilage Autograft Implantation System (DePuy Synthes, Mitek Sports Medicine, USA); ADCT: Autologous Disc Chondrocyte Transplantation (co.don® AG, Germany); NuQu®:

juvenile allogeneic chondrocyte implantation (ISTO Technologies Inc., USA); Mesoblast: immunoselected allogeneic adipose derived precursor cells (Mesoblast Ltd, Australia).

Figure 2: Translational cell based approaches for disc repair. **(A)** Clinical parameters to consider for patient centred research. **(B)** Regenerative surgical procedures envisioned could include novel intraoperative processing of autologous biologics (1), or ‘off the shelf’ tissue banked or GMP processed allogeneic cells (2) for use in single-step surgery. Alternatively, high quality autologous cells processed by GMP facilities could be delivered as part of a two-step procedure (3). GMP biomanufacturing may involve cell processing and manipulation to restore cellular integrity through priming with growth factors and/or biochemical factors for pre-conditioning, or by means of co-culture with notochordal cell populations or conditioned media to achieve successful tissue reconstitution. Logistical and supply chain challenges could be overcome through

cryopreservation and biobanking for “off-the-shelf” availability. (C) Cell based approaches can involve the use of stem cells or chondrocytes as part of intraoperative or GMP-biomanufacturing approaches, but key issues and technical challenges need to be addressed for successful clinical translation. Abbreviations- GMP: good manufacturing practice; PRP: platelet rich plasma; BMAC: bone marrow aspirate concentrate; SVF: stromal vascular fraction.

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Table 1. Published cell-based clinical studies for intervertebral disc repair

Clinical Details	Cell Type	Cell Number	Method of Administration	Observations and Outcomes	Population Age	Ref
10 patients with LBP	Autologous bone marrow hematopoietic precursor stem cells (HSCs)	1 cc of HSCs	Percutaneous injection with concurrent hyperbaric oxygen therapy	No improvement in back pain	Not provided	40
28 patients undergoing microdisectomy with LBP (EuroDISC study)	Autologous culture expanded disc derived chondrocytes	6×10^6	Percutaneous injection 12 weeks following microdisectomy	- reduced back pain at 2 years - increased MRI T2 signal of treated and adjacent discs.	18-60	9
10 patients with LBP with radiological evidence of DDD	Autologous bone marrow MSCs	Not provided	Percutaneous injection	- clinical improvement in back pain, leg pain and disability - increased MRI T2 signal - disc height not recovered	Not provided	41
2 patients with LBP and sciatica with radiological evidence of DDD	Autologous bone marrow MSCs	Not provided	Percutaneous injection with collagen sponge	- increased MRI T2 signal - less instability - clinical improvement in both patients	67 and 70	42
15 patients with degenerative disc disease and low back pain	Allogeneic juvenile chondrocytes	1×10^7 cells/ ml with mean injection of 1.3ml	Percutaneous injection with fibrin glue sealant	- clinical improvement in low back pain - increased MRI T2 signal	19-47	29
9 patients with Pfirrmann grade III disc degeneration at the level adjacent to the level scheduled for posterior lumbar intervertebral fusion	Autologous NP cells from fused disc were co-cultured with autologous BM -MSCs	1×10^6	Percutaneous injection 7 days after 1 st fusion surgery	Mild improvement in 1 case with minimal efficacy to retard further degeneration	20-29	43
26 patients with degenerative disc disease and chronic back pain (13 one level and 13 two level)	Autologous bone marrow concentrate (BMC) disc injections.	121×10^6 TNC/ml with 2,713 CFU-F/ml	Percutaneous injection	- Reduction in ODI and VAS up to 12 months - Patients receiving greater than 2000 CFU-F/ml faster and greater reduction in ODI/VAS. - Subjects older than 40 years less reduction in ODI/VAS compared with younger patients.	18-61	44

Table 2. Cell-based clinical trials currently being investigated for cartilage and intervertebral disc repair (Clinicaltrials.gov)

Cell Type	Trial Name	Phase	Sponsor	Clinicaltrials.gov ID
Cartilage				
Autologous chondrons and allogeneic MSCs	IMPACT	Phase 1/2	UMC Utrecht	NCT02037204
Autologous Nasal Chondrocytes	NosetoKnee	Phase 2	University Hospital, Basel, Switzerland	NCT02673905
Autologous Cartilaginous tissue implant	NeoCart	Phase 3	Histogenics Corporation	NCT01066702
3D spheroid culture of chondrocytes	Co.Don Chondrospheres	Phase 3	Co.Don AG	NCT01222559
Autologous BMSCs + collagen hydroxyapatite + PRP		Phase 0 - pilot	Dr Michel Assor	NCT01159899
ADSCs vs microfracture		Phase 1	Stanford University	NCT02090140
Allogenic MSCs	MSV_allo	Phase 1/2	Red de Terapia Celular	NCT01586312
Autologous BMSCs	ABM & LAM ST-OA Study	Phase 1/2	International StemCell Services	NCT01152125
HyloFAST+BMAC	FastTRACK	Phase 1	Anika Therapeutics	NCT02659215
Intervertebral Disc				
NovoCart disc gel + ADCT	NovoCart Disc	Phase 1/2	Tetec AG	NCT01640457
Allogeneic banked juvenile chondrocytes	NuQu	Phase 2	ISTO tech	NCT01771471
Autologous ADSCs		Phase 1	Inbo Han	NCT02338271
Allogenic MSCs	Disc.allo	Phase 1/2	Red de Terapia Celular	NCT01860417
Allogenic ADSCs		Phase 2	Mesoblast	NCT01290367
Autologous ADSCs		Phase 1	Bioheart Inc	NCT02097862

Table 3. Summary and highlights of key points

Summary and Highlights

- Degenerative Disc Disease (DDD) and Osteoarthritis (OA) are painful activity limiting conditions with global health consequences. As such the scope for regenerative therapeutics is high.
- Adopting some of the endeavors from cartilage repair strategies could perhaps harness a translatable, regenerative surgery for DDD.
- Cell source, cell processing and delivery are key considerations in the design of a cell based, single step regenerative surgery.
- Single or two step surgery, target population and stratification, cell dosage, rehabilitation and functional recovery are key areas to be addressed for clinical success to be realized.
- To yield a widely applicable regenerative surgery, robust tissue regeneration, predictable clinical outcome and a cost effective approach needs to be achieved.
- A multimodal, multidisciplinary approach with input from researchers, industry and clinicians is required.