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Mesenchymal stem cells in regenerative medicine: Focus on articular cartilage and intervertebral disc regeneration

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ABSTRACT

Musculoskeletal disorders represent a major cause of disability and morbidity globally and result in enormous costs for health and social care systems. Development of cell-based therapies is rapidly proliferating in a number of disease areas, including musculoskeletal disorders. Novel biological therapies that can effectively treat joint and spine degeneration are high priorities in regenerative medicine. Mesenchymal stem cells (MSCs) isolated from bone marrow (BM-MSCs), adipose tissue (AD-MSCs) and umbilical cord (UC-MSCs) show considerable promise for use in cartilage and intervertebral disc (IVD) repair. This review article focuses on stem cell-based therapeutics for cartilage and IVD repair in the context of the rising global burden of musculoskeletal disorders. We discuss the biology MSCs and chondroprogenitor cells and specifically focus on umbilical cord/Wharton's jelly derived MSCs and examine their potential for regenerative applications. We also summarize key components of the molecular machinery and signaling pathways responsible for the control of chondrogenesis and explore biomimetic scaffolds and biomaterials for articular cartilage and IVD regeneration. This review explores the exciting opportunities afforded by MSCs and discusses the challenges associated with cartilage and IVD repair and regeneration. There are still many technical challenges associated with isolating, expanding, differentiating, and pre-conditioning MSCs for subsequent implantation into degenerate joints and the spine. However, the prospect of combining biomaterials and cell-based therapies that incorporate chondrocytes, chondroprogenitors and MSCs leads to the optimistic view that interdisciplinary approaches will lead to significant breakthroughs in regenerating musculoskeletal tissues, such as the joint and the spine in the near future.

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Contents

1. Introduction

Age-related musculoskeletal disorders represent a major cause of morbidity globally and result in enormous costs for health and social care systems. Chronic and inflammatory diseases of joints and the spine, including osteoarthritis (OA) and low back pain (LBP) caused by intervertebral disc (IVD) degeneration respectively, are major causes of disability in the elderly. With increases in life expectancy, the burden of musculoskeletal disorders will unavoidably and progressively grow. The increase in musculoskeletal disability among the ageing population highlights an acute and urgent need for a radical shift in healthcare strategies that involve lifestyle interventions that can prevent these disorders and novel pharmacological and biological therapies that can effectively treat them. Development of cell-based therapies is rapidly proliferating in a number of disease areas, including musculoskeletal disorders. Autologous chondrocyte implantation (ACI) has been used for treatment of osteoarticular lesions for over two decades. Although chondrocyte-based therapy has the capacity to slow down the progression of OA and delay partial or total joint replacement surgery, currently used procedures are associated with the risk of serious adverse events. Therefore there is significant interest in improving the success rate of ACI by improving surgical techniques and preserving the phenotype of the primary chondrocytes used in the procedure. Likewise, disc cell re-implantation has been trialed for the treatment of IVD degeneration and LBP, as has allogeneic juvenile chondrocyte implantation [1,2]; however while these therapies showed promising outcomes a number of hurdles prevent their widespread clinical adoption. As a result of the limitations of chondrocyte re-implantation-based therapies, experimental therapies using stem cells are receiving an increasing amount of scientific and public interest. Mesenchymal stem cells (MSCs) show considerable promise for use in cartilage and IVD repair and are being clinically explored as a new therapeutic for treating a variety of other immune mediated diseases. MSCs have potential applications in tissue engineering and regenerative medicine and may represent an attractive option for repairing focal lesions in cartilage and IVD degeneration [3]. Future tissue-engineering approaches for cartilage and IVD repair will benefit from advances in MSC-based repair strategies. This review article focuses on stem cell-based therapeutics for cartilage and IVD repair in the context of the rising global burden of musculoskeletal disorders. We explore the exciting opportunities afforded by MSCs and discuss the challenges associated with cartilage and IVD repair and regeneration by combining biomaterials and cell-based therapies with chondrocytes and MSCs. We also highlight several new areas for future investigation.

2. Stem cells in regenerative medicine

Stem cell-based therapies that integrate tissue-engineering technologies and biomaterials science are fundamental pillars of the science of regenerative medicine. Clinical success with hematopoietic stem cell transplantation for leukemia/lymphoma is very well established and has been clinically validated, thus providing a strong foundation for the establishment of stem cell-based therapeutics. However, the clinical outcomes of stem cell transplantation for other diseases remain poor and this prompts us to debate whether we should use stem cells or their biological derivatives. Therefore, there is always a continuous search for cells/stem cells with better safety and effective differentiation capacity to replace or restore function to damaged tissues and organs. Researchers today have access to a plethora of stem cells with varying potencies, viz. pluripotent (tri-lineage – ESCs, iPSCs) [4,5], multipotent (more than one lineage – adult and fetal tissue specific MSCs including bone-marrow, adipose tissue, amnion, umbilical cord), and unipotent (single lineage – hematopoietic stem cells) [6–10]. Choosing the right stem cell is imperative for obtaining favorable results in regenerative medicine. Since many recent reviews including some of our own papers [3,11,12] have already discussed the potential of BM-MSCs and AD-MSCs in regenerative medicine, in this review we have focused specifically on umbilical cord/Wharton's jelly MSCs (UC-MSCs/WJMSCs).

3. Cartilage degeneration and current management strategies for OA

The ageing population (>60 years) is predicted to expand significantly by the year 2050, reaching well over 2 billion people globally [13,14]. This growing geriatric population will lead to an increase in all age related diseases, including OA. Articular lesions either due to OA or traumatic injuries is associated with

progressive degeneration of articular cartilage, osteophyte formation, pain, joint effusion and disability and this clinical problem still remains unresolved and poses a major challenge [15–17]. Age related 'wear and tear', chondrocytes' poor response to growth factors, altered bio-mechanical properties of articular cartilage, mitochondrial dysfunction, oxidative stress and inflammation are all implicated in the pathogenesis of OA, highlighting the multifactorial and complex nature of this degenerative joint disease [18]. Eventual decreases in the number of chondrocytes with age results in impaired production of extracellular matrix proteins. In addition, while progenitor cells have been identified in articular cartilage [19,20], the tissue displays an extremely limited natural healing capacity [18], due in part to its hypocellularity and to a lack of vasculature. An articular lesion can be either a focal defect in the cartilage surface or extensive cartilage degradation and therefore the treatment needs to be tailored. Pharmacological management with disease modifying osteoarthritis drugs (DMOADs), natural remedies, weight reduction and mild exercises, help relieve pain to some degree, but does not offer a disease cure [21]. Various surgical methods have been attempted to restore the damaged cartilage and improve joint function, such as microfracture, subchondral drilling and abrasion arthroplasty. These techniques are aimed to promote intrinsic healing by promoting vascular invasion, fibrin clot formation and recruitment of stem cells [16]. However, poor biomechanical properties of the tissue in microfracture, as well as donor site morbidity (which may result in substantial impairment for patients), low cellularity and surrounding cartilage damage limit their uses and moreover, long term efficacy is not known [16,22,23].

When pharmacological and surgical management strategies fail (which they often do) the disease progresses to end stage OA, where joint arthroplasty may become the only definitive and unavoidable option. However, the limited lifespan of currently available prostheses cannot cope with the demands of younger and more active patients. These harsh surgical realities present new opportunities for the development of future therapeutics including stem cell-based therapies. Stem cells have been used to restore damaged myocardium, spinal cord, brain, liver, retina and skin [24–27]. Regenerative medicine thus offers a significant therapeutic potential and could provide an excellent alternative to arthroplasty.

4. Cell-based therapies for cartilage regeneration

As discussed earlier, the use of autologous chondrocyte implantation (ACI), autologous matrix induced chondrogenesis (AMIC) and intra-articular injection of meniscus stem/progenitors cells [16,23,28–30] represent the current state-of-the-art in this area. MSC-based cell therapy is beginning to show some promising results. Considerable pain relief and improvement in the pain on visual analog scale were reported in four patients with severe OA, following injection of autologous BM-MSCs ($8–9 \times 10^6$ cells/-patient) into the knee joint that was most affected [31]. Likewise, administration of allogeneic MSCs (40×10^6 cells) in OA patients showed improvement in the articular cartilage quality as assessed by magnetic resonance imaging (T2 mapping) compared to those patients that received single intra-articular injection hyaluronic acid (60 mg) injections indicating that allogeneic MSCs could be an alternative source [32]. Thus, resident progenitor cells, as well as both autologous and allogeneic stem cells derived from various sources (viz. bone marrow, synovium, adipose tissue etc.) have been used for the treatment of OA with variable success either as direct injections into the damaged site or following differentiation into cartilage together with tissue engineered scaffolds or following treatment with growth factors [33,34]. Growth factors, cytokines, bioactive lipids, micro-vesicles that are released from implanted stem cells may also exert beneficial effects including anti-inflammatory, angiopoietic and apoptotic effects. Therefore the observed improvements in pain relief and function may be in fact due to paracrine effects of injected MSCs, indicating that biological products secreted by stem cells rather than the cells themselves could potentially be used as therapeutic agents.

4.1. Molecular control of chondrogenesis

Understanding the regulation of normal skeletogenesis is of great importance in the context of cartilage regenerative medicine because cell-based regeneration techniques recapitulate, at least in part, the main developmental steps that occur *in vivo*. Understanding the mechanisms and the machinery responsible for regulating chondrogenesis will enhance regenerative medicine. Chondrogenesis commences with the recruitment, migration, and proliferation of chondroprogenitors during the early phase of embryonic skeletogenesis [35]. At this stage, the chondroprogenitor cells produce an extracellular matrix (ECM) rich in hyaluronan, collagen type I, and the alternatively spliced long form of collagen type IIA containing an amino-propeptide encoded by exon 2 [36]. Aggregation into precartilage condensations is mediated by the appearance of cell–cell interactions (via N-cadherin), gap junctions, and cell adhesion molecules (N-CAM) [37]. These interactions, along with fibronectin, tenascins and thrombospondins deposited in the ECM, trigger intracellular signaling cascades that allow the differentiating chondroprogenitor cells to acquire the typical spherical morphology of chondrocytes and initiate synthesis of cartilage-specific ECM molecules such as collagen types IIB, IX, and XI, and aggrecan [37].

The HMG-box transcription factor Sox9, one of earliest markers of chondrogenic cells, is essential for the expression of collagen type II (*Col2a1*) and other ECM proteins such as *Col11a2* and CD-RAP [38]. Sox9 acts in cooperation with two additional Sox family members, L-Sox5 and Sox6, which contain no transcriptional activation domain, and are required for the expression of *Col9a1*, aggrecan, and link protein [38]. One of the upstream mediators of Sox9 is hypoxia-inducible factor-2α (HIF-2α) which promotes the upregulation of cartilage ECM genes [39]. Besides Sox proteins, the Runt-related transcription factor Runx2 is also expressed in chondrogenic cells; furthermore, other transcription factors, including Barx2, Nkx3.2/Bapx1, Msx1 and 2, β-catenin, Smads, Lef1, AP-1 and AP-2, are also known to control chondrogenic differentiation (reviewed in [40–42]).

A number of extracellular signaling molecules and growth factors, including various members of the fibroblast growth factor (FGF), hedgehog, transforming growth factor-β (TGF-β) and bone morphogenic protein (BMP), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EGF) families; retinoic acid (RA), as well as wingless/Int (Wnt) glycoproteins are all important regulators of prechondrogenic cell condensation and chondrogenic differentiation (reviewed in [37,42]). The proliferation rate of progenitor cells during chondrogenesis is determined by the balance of signaling by BMPs and FGFs; the mitogenic stimuli converge on the cyclin D1 gene [43]. The glucocorticoid dexamethasone is known to enhance cartilage-specific gene expression in TGF-β3 induced MSC cultures [40].

A considerable amount of data has been published concerning cytoplasmic signaling pathways that relay extracellular stimuli to gene expression. Various members of the TGF-β superfamily are strong inducers of chondrogenesis; TGF-β1 and TGF-β3 are more potent mediators than TGF-β2 [40]. BMP signaling is essential for chondrogenic differentiation [44] as the expression of Sox proteins is dependent upon BMP signaling via the Ser/Thr kinase receptors ALK3 (BMPR1A) and ALK6 (BMPR1B) [45], mediated by the canonical Smad pathway; the phosphorylated Smads form complexes with Smad4, translocate to the nucleus, and bind to SMAD elements in the promoters of target genes such as *Sox9*, *Col2a1*, and *Runx2* [46,47]. Among BMPs, BMP-2 has been shown to possess

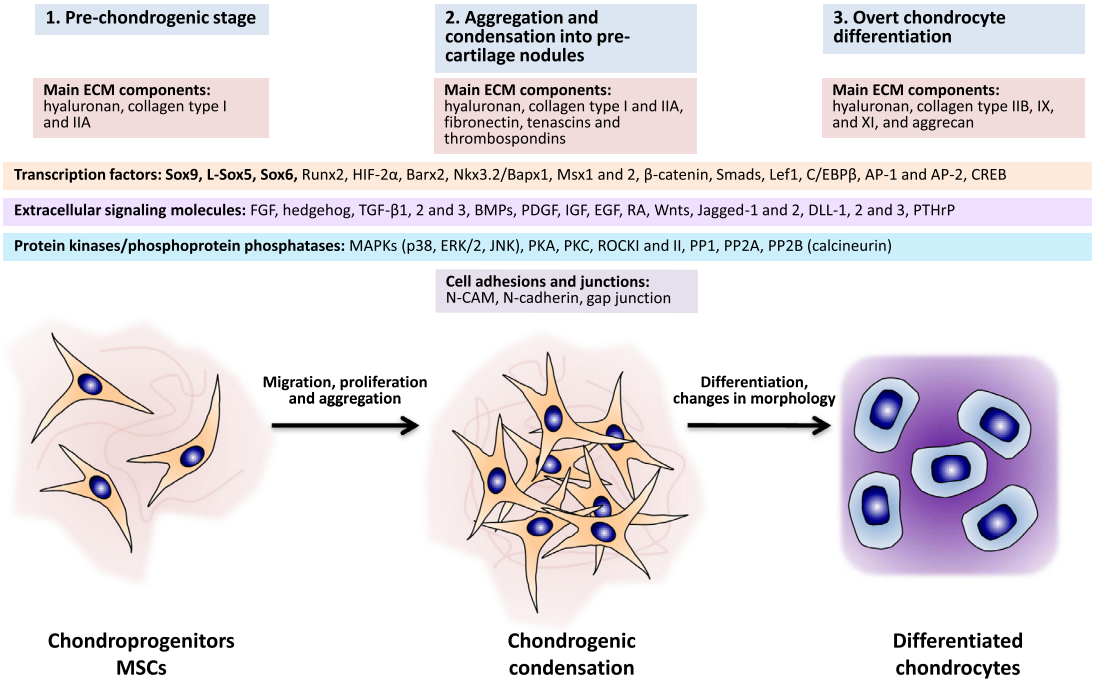

Fig. 1. Schematic summarizing the key stages of chondrogenic differentiation including the transcription factors, signaling molecules and protein kinases/phosphoprotein phosphatases involved.

the most potent chondro-stimulatory effects [40]; BMP-7 stimulates ECM synthesis and at the same time inhibits catabolic factors [48]. Through an alternative (Smad-independent) pathway, BMPs can also activate the mitogen-activated protein kinase (MAPK) signaling route by triggering MEKK1 and subsequently p38 and c-jun N-terminal kinase (JNK) cascades, or by activating the Ras/ERK1/2 or RhoA/ROCK axis [44,47]. Of the three main MAPK pathways, p38 and ERK1/2 have been described as key regulators of chondrogenic differentiation; the p38 pathway is primarily involved in the initiation of condensation, and ERK1/2 activation interacts with BMP-2-induced signaling to promote chondrogenesis [41]. Downstream MAPK signaling results in the activation of target transcription factors including AP-1, ETS, Runx2, HIF-2α, and C/EBPβ [39]. In BM-MSCs, all three MAPKs were found to be positive transducers in TGF-β1-induced chondrogenesis by promoting cell adhesion through elevated N-cadherin levels [41].

Besides MAPK cascades, virtually all major members of Ser/Thr protein kinases including protein kinase A (PKA) [49], PKC (reviewed by [50]) and Rho kinases (ROCKI and II) [40], as well as phosphoprotein phosphatases such as PP1, PP2A and calcineurin (reviewed by [51]) have been well documented as key regulators of chondrogenesis, with either stimulatory or inhibitory effects. The Notch pathway is also active during the early stages of chondrogenesis. The roles of Notch receptors (Notches 1–4) and the Notch ligands (Jagged-1, Jagged-2, DLL-1, DLL-3 and DLL-4) have all been investigated in this context. Interestingly, while a transient Notch signaling was found to be necessary at the beginning of chondrogenic differentiation of MSCs, it has to be switched off at subsequent stages [52]. The schematic shown in Fig. 1 summarizes the key stages of chondrogenic differentiation including the transcription factors, signaling molecules and protein kinases/phosphoprotein phosphatases involved.

While collagen type II is the major structural collagen in cartilage ECM, MSCs cultured in chondrogenic conditions maintain collagen type I secretion, accompanied by upregulation of *Col10a1* in parallel with gradual downregulation of *Col2a1* toward later stages, suggesting that they do not halt their initial programme and undergo hypertrophy [52]. To avoid hypertrophic differentiation, strategies based on the use of molecules with an inhibitory effect on growth plate development have been developed; indeed, PTHrP (parathyroid hormone related peptide) or FGF-2 are known to down-regulate *col10a1*, but also *col2a1*, during *in vitro* chondrogenesis of adult MSCs [53]. The main challenge of cartilage regeneration techniques, therefore, is to ensure appropriate differentiation and matrix synthesis by MSCs and thus avoid production of a fibrocartilage, or progression to hypertrophic cartilage which may eventually ossify.

4.2. Wharton's Jelly stem cells (WJSCs): a promising source of MSCs for regenerative medicine

Despite the presence of many different types of stem cells, in recent years UC-MSCs have gained much attention as being a potential cell source for tissue engineering and regenerative medicine. These MSCs were first isolated in 1991 by McElreavey and colleagues [10]. Since then significant effort has been devoted to the refinement of methods for their isolation, characterization and functional evaluation in an attempt to find an alternative source of stem cells to keep pace with the growth and demands of regenerative medicine. MSCs have been isolated from various zones within the umbilical cord [54], including the subendothelial layer [55]; perivascular zone [56], Wharton's jelly [57], umbilical cord lining [58] and the whole umbilical cord [59]. MSCs isolated from within the various regions of the umbilical cord fulfill the stipulated minimum criteria of 'plastic adherence', 'immunological profile' and 'differentiation' as stated in the position paper of the International Society for Cellular Therapy [60]. The yield of MSCs from within the Wharton's jelly (WJSCs) is high compared to other zones in the umbilical cord (Fig. 2). In addition, WJSCs has several advantages that make them an attractive choice for use in tissue engineering and regenerative medicine. WJSCs (i) are a relatively young cell type compared to most other MSCs, (ii) have no ethical concerns unlike ESCs, (iii) can be harvested painlessly unlike bone-marrow MSCs, (iv) share few embryonic features, (v) have high cell proliferation, (vi) have wide differentiation potential, (vii) are hypo-immunogenic and (viii) are non-tumorigenic [61–67]. Developmentally, the umbilical cord and its contents are embryonic in

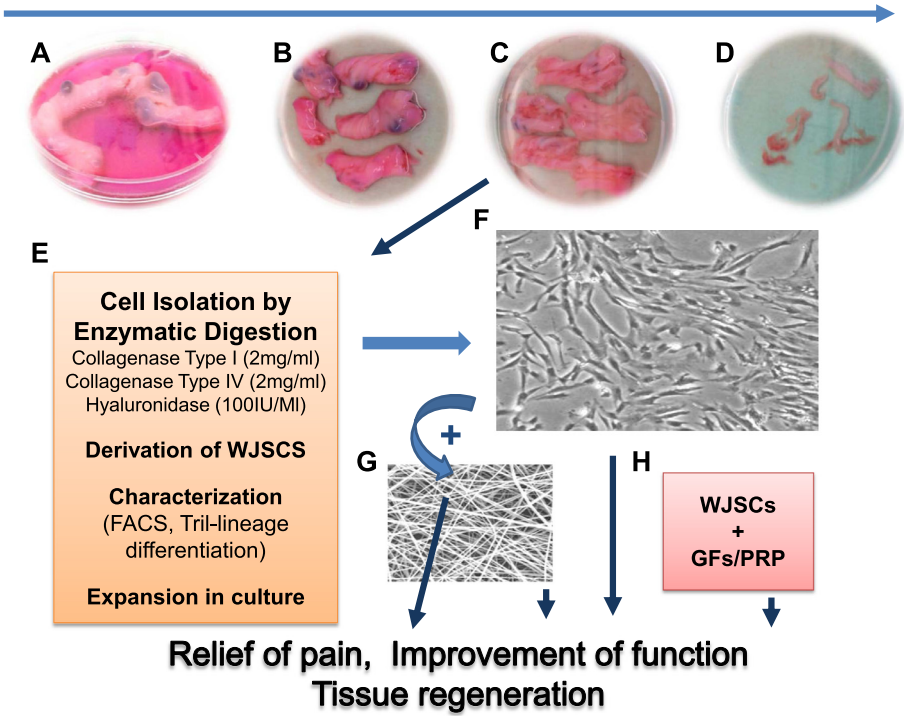

Fig. 2. Umbilical cord-Wharton's Jelly stem cells (WJSCs) for cartilage or IVD regeneration: (A) human umbilical cord (~10 cm); (B) sectioned pieces of umbilical cord (~2 cm each); (C) each piece opened longitudinally; (D) blood vessels removed from sections of the umbilical cord; (E) the sectioned pieces (with opened side down in contact with the Petri dish were treated with an enzymatic cocktail) for 45 min at 37 °C; loosened Wharton's Jelly was gently scraped into the medium, centrifuged at 300*g* × 5 min; pellet resuspended in culture medium comprising (DMEM high glucose, 20% fetal bovine serum, basic fibroblast growth factor 16 ng/ml, 2 mM L Glutamine, insulin-transferrin-selenium and antimycotic-antibiotic); derived WJSCs were characterized using FACS (presence of MSC related CD markers) and tri-lineage differentiation. (F) Phase contrast image of Wharton's Jelly stem cells showing characteristic short fibroblastic cells; (G) Electrospun biodegradable nanofibrous scaffold; (H) Cells together with growth factors or platelet rich plasma. Useful applications include either (i) use of WJSCs alone; (ii) following culture on scaffolds (with or without differentiation media) or (iii) cells together with growth factors or platelet rich plasma.

nature as it arises from the epiblast, which also give rise to the three primordial germ layers, the amnion and the allantois. Therefore, WJSCs come to occupy an intermediate position between the most versatile pluripotent ESCs/iPSCs and adult tissue specific MSCs, which might explain the presence of some embryonic stem features and increased stemness [63,68].

4.3. Articular cartilage tissue engineering: challenges and prospects

Cartilage is avascular and is plagued by slow repair processes following injury. Chondrocytes are the only cell type in the cartilage and with age the proportion of senescent cells within the tissue increases. These senescent cells have been shown to adopt a more catabolic senescence-associated secretory phenotype (SASP), which is characterized by increased secretion of catabolic cytokines and matrix degrading enzymes [12,69], potentially limiting the number of cells suitable for use regenerative therapies. Therefore, there exists a great dependence on other sources of cells/stem cells that can efficiently undergo chondrogenic differentiation. Cartilage tissue engineering is rapidly emerging as a promising potential cure for articular cartilage lesions and this in turn has intensified the screening of many different types of stem cells. Irrespective of the cell source, MSCs have been demonstrated to undergo chondrogenic differentiation upon stimulation with inductive agents including transforming growth factor beta and bone morphogenetic proteins, insulin-like growth factors, insulin-transferrin-selenium and hepatocyte growth factor [70]. As ageing MSCs have reduced chondrogenic potential [71], use of adult tissue specific MSCs in cartilage regeneration will have limited benefits. Lack of significant cell numbers has always been a limiting factor in tissue engineering and regenerative medicine. Cartilage is mainly composed of collagen and proteoglycans that mainly help maintain the biomechanical properties. Maintaining phenotypic stability and biomechanical properties following differentiation is a great challenge, as these characteristics tend to change over time. With most MSCs there could occur a transition in production of type I collagen rather than the desired type II collagen and this might lead to development of a fibrocartilage [72] with a poor therapeutic outcome.

WJSCs, by their inherent nature have high hyaluronic acid, sulfated glycosaminoglycans (GAGs) and collagen expression [73], which to some extent reflect native cartilage tissue. Moreover, uses of WJSCs following their differentiation into multiple cell types as reported by many different research groups, with some progressing on to clinical trials is encouraging [74–76] and justify the use of WJSCs in cartilage regeneration procedures. Persistence of B7 family co-stimulator immune molecule B7-H3 (CD276) in the undifferentiated and chondrogenic differentiated cells, indicate that WJSCs will continue to have immune privilege [77] which is another added advantage. High density WJSC cultures using rotary cell culture system enabled development of soft, opaque non-scaffold cartilage-like tissue which was larger than the conventional pellet cultures and also showed high expression of GAG and collagen II [78]. However, compared to two-dimensional (2D) cultures, three-dimensional (3D) systems that closely mimic the native tissue are hoped to improve transplant outcomes. Marked chondrogenic differentiation is reported following culture of WJSCs in 3D electrospun nanofibrous scaffolds [79], collagen hydrogels [65] and poly-ε-caprolactone (PCL)/collagen nanoscaffolds [63]. Expression of collagen and GAGs were much higher than BM-MSCs and genes related to chondrogenic differentiation including SOX9, collagen type II and cartilage oligomeric matrix protein (COMP) were also highly expressed following culture of WJSCs on nanoscaffolds [63]. The fact that articular cartilage has

a very poor intrinsic healing capacity and is involved in weight bearing function, it is pertinent that suitable tissue engineering material and an appropriate cell type be used in cartilage regeneration. Synthetic polymers are widely used in tissue engineering as they have excellent three dimensionality, porosity, biomechanical and biodegradable properties. Poly (lactide-co-glycolide) (PLGA) is approved by the FDA and it has been extensively used in ligament, tendon, cartilage and bone regeneration. WJSCs seeded on electrospun scaffold prepared by combination of PLGA, hydroxyapatite and zein facilitated cartilage regeneration in an *in vivo* rabbit model with a chondral defect [80]. The cross talk between cartilage and the subchondral bone indicate that for effective cartilage repair it would be best to consider scaffolds that would aid cartilage-bone differentiation. The use of human telomerase reverse transcriptase (hTERT) to prolong the life span of stem cells and prevent replicative senescence is yet another strategy [81] to meet the desired cell numbers. However, preclinical studies with long-term follow-up are necessary to rule out development of any tumors before moving on to human applications.

In summary, WJSCs could be derived in abundance to meet the growing demand of tissue engineering and regenerative medicine that would help cure many diseases including chondral or osteochondral defects. They have several advantages over other existing MSCs and could be used effectively in translational research. Emerging developments and improvisations in tissue engineering technologies combined with use of the right stem cells/stem cell derivatives as WJSCS, will hopefully lead to the much-anticipated advancements in regenerative medicine in the near future.

5. Back pain and intervertebral disc (IVD) degeneration

Low back pain (LBP) is one of the leading causes of disability in the developed world, with lifetime prevalence estimated at over 80% [82]. As with many other musculoskeletal disorders, including OA, the prevalence of LBP increases with age [83], suggesting incidence is likely to increase due to a global aging population, changes in lifestyle and occupational stresses [84]. The global economic burden of LBP is significant and alarming. The total cost of back pain in the UK is estimated to be between 1% and 2% of gross domestic product (GDP), equating to between £14 and £28 billion lost per annum [http://www.backcare.org.uk/factsandfigures]; [85], while in the United States costs have been estimated at around $85.9 billion [86].

LBP is a complex and multifactorial entity, encompassing mechanical, physiological and psychosocial dimensions [87]. Genetic predisposition and environmental factors, including smoking, obesity and abnormal mechanical loading, have been implicated in the pathogenesis of LBP [82,88–92]. Stress has also been implicated as a modifiable risk factor for persistent and non-persistent LBP [93]. However, there is increasing evidence, obtained through imaging studies, to suggest that a significant proportion of LBP is associated with degeneration of the intervertebral disc (IVD) [94,95], and direct clinical evidence implicating disc space narrowing (which develops with progression of IVD degeneration) with chronic LBP [96–98]. Studies have also demonstrated a potential direct mechanistic association between degeneration and LBP, with increased nociceptive nerve ingrowth occurring in the painful degenerate IVD [99].

Magnetic resonance imaging (MRI) scanning is a non-invasive commonly used diagnostic modality for the assessment of degenerative disc disease. This sensitive tool provides accurate morphologic information regarding the disc and can influence clinical making decisions [87,100,101]. Conventional T2 weighted sagittal MRI sequences have been utilized to create a subjective grading scale for disc health based on morphological features [102].

IVD degeneration may initially start as a silent and therefore sub-clinical process. There is a spectrum of degeneration occurring over many years, which starts with a healthy disc and which can end with a bone-on-bone appearance with near total obliteration of the disc space. For clinicians investigating and treating LBP, the challenge has been to ascertain the duration and degree of symptoms and loss of function a patient has, before embarking on treatment.

Once symptomatic degenerative disc disease has been diagnosed, a multitude of treatment strategies exist ranging from conservative treatment and physical/behavioral therapy, through to spinal corticosteroid injections and more invasive options such as minimally invasive and open surgeries with or without insertion of implants to either replace the disc or restrict motion or fuse the motion segment. Treatments ranging from physiotherapy to osteopathy may be provided by different healthcare professionals including physicians, pain specialists and spinal surgeons. There is certainly no universal agreement regarding the management of degenerative spine conditions.

Unfortunately, while a wide range of treatments are available, they offer only short-term relief, and are often accompanied by loss of function, mobility, and altered spinal biomechanics leading to disc degeneration at adjacent levels and further pain [103–105]. Given these limitations, current research is now concentrating on the development of more biological/regenerative therapies to target the underlying pathogenesis to repair the IVD or prevent degeneration.

5.1. The biology of IVD degeneration

The IVD is vital to the flexibility and mechanical integrity of the spine by virtue of the opposing forces generated by its two main components; the central hydrophilic, proteoglycan (particularly aggrecan) and type II collagen-rich nucleus pulposus (NP), and the peripheral fibrous, type I collagen-rich annulus fibrosus (AF). In recent years the pathogenesis of IVD degeneration has been elucidated, with studies implicating aberrant disc cell function in its pathophysiology [106–114]. With degeneration, NP cells demonstrate increased expression of a range of pro-inflammatory/catabolic cytokines and inflammatory mediators, including IL-1, IL-6, IL-12 IL-17, TNF-α and IFN-γ [115]. Most notably, elevated expression of IL-1 by disc cells from degenerate tissue leads to the production of inappropriate matrix and increased matrix degrading enzyme expression by native disc cells [106,108]. Increased TNF-α expression in degenerate tissue [116,117] is also thought to be involved in upregulating matrix degrading enzymes [117] and stimulating nerve ingrowth, suggesting a potential role in innervation and development of discogenic pain [118]. IVD degeneration is thought to originate in the NP, where there is a loss of normal matrix, with increased Matrix Metalloproteinase (MMPs 1, 3, 7, 9, 10 and 13), and A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS 1, 4, 5, 9 and 15) activity being responsible for matrix catabolism [107,112–114]. There is also a shift from type II to type I collagen expression by NP cells and a decrease in aggrecan synthesis, leading to dehydration of the matrix in the NP [112]. Dehydration leads to the loss of swelling pressures responsible for maintaining mechanical integrity, ultimately leading to local spinal instability and mechanical trauma. In parallel, the diminished aggrecan and increased catabolic cytokine levels, allows the in-growth of neurites, resulting in pain [99,119,120].

5.2. Novel therapies for IVD degeneration

None of the current surgical methods address the aberrant cytokine-rich/pro-inflammatory milieu of the degenerate IVD, or

the inherent loss of functional native cells and tissue within the IVD. Consequently, research has focused on the development of biological therapies and regenerative approaches. Application of biological therapy is intended to inhibit the abnormal cytokine production, or stimulate matrix anabolism. Platelet-rich plasma contains growth factors and has been shown to induce annulus fibrosus cell proliferation and matrix production [121]. Furthermore injection of platelet-rich plasma into rat and rabbit IVD degeneration models has demonstrated that it may act to delay progression of early stage disease [122,123], although its ability to regenerate tissue is yet to be elucidated. Anabolic growth factors (including TGF-β, IGF-1, OP-1, GDF5 and GDF6) have been shown to promote matrix synthesis *in vivo* [124–128], and a clinical trial employing injection of recombinant BMP-7 into the IVD is currently underway. IL-1 receptor antagonist (IL-1RA) has also been shown to decrease both cytokine and proteolytic enzyme production by NP cells [129,130], with other studies demonstrating similar effects using anti-TNF therapies [131]. However, the limitation of such therapies is their short half-life, necessitating repeated injections [132]. The reduction in viable cells, particularly in late-stage degeneration, also limits the efficacy of a purely biologic therapy; thus cell-based tissue engineering/regenerative therapies have become the primary focus of current research in the field.

5.3. Cell-based therapy for IVD

Cell-based therapies aim to repopulate the IVD and restore functional tissue through matrix synthesis by implanted cells and, potentially, beneficial influences on native cells. Autologous NP cell re-implantation has been shown to retard degenerative changes in a dog model, and more importantly, a randomized human clinical trial using this approach demonstrated a clinically significant decrease in LBP score and retention of hydration and disc height compared to discectomy alone [2,133]. However, as the NP is relatively hypocellular, harvesting sufficient cells for re-implantation may result in complications [134,135] and NP cells from degenerate discs display increased/premature senescence [110] and a catabolic phenotype [107,111–113] which make them unsuitable for transplantation where normal cell function is required.

5.4. Native IVD progenitors

Increasing evidence suggests the presence of a native progenitor cell population within the IVD of animals and humans [136–139], although differences in isolation and characterization methodology mean it is difficult to compare between studies and a detailed phenotypic profile of any progenitor population(s) is yet to be established. While promising, work remains to determine the regenerative potential of any resident progenitor cell population. Furthermore Sakai et al. demonstrated that a population of Tie2+ progenitor cells identified within the human IVD decreased with both age and degeneration [136], suggesting isolation of sufficient progenitor cell numbers for (re)implantation may be an obstacle to clinical application.

5.5. MSCs for IVD regeneration

MSCs have been proposed as an ideal cell source for IVD regeneration, with an increasing number of studies demonstrating ability of both BM-MSCs and AD-MSCs to differentiate into an NP-like phenotype (discogenic differentiation) [140–147]. *In vivo* studies have also demonstrated the ability of implanted MSCs to enhance matrix production, particularly GAG synthesis, and increase disc height and hydration [148–153], while a small human clinical trial demonstrated improved pain and disability scores and an increase in water content in the disc 12 months after MSC implantation [154]. Over recent years the potential of UC-MSCs and WJSCs for IVD regeneration has also been demonstrated, with these cells showing differentiation capacity both *in vitro* and *in vivo* [155–160] and the ability to decrease pain scores when implanted in humans (albeit in a study of only two patients) [158]. Although these findings demonstrate the huge potential of MSCs for application in IVD regeneration, a wide range of questions remain to be addressed [161,162]. The most notable questions include: Do MSCs persist following implantation? Do they undergo discogenic differentiation? What is the effect of the microenvironmental niche on their survival and function? Are MSCs directly responsible for tissue regeneration? Or do they produce bioactive factors that influence resident cell function as has been shown in other systems?

Early studies on discogenic differentiation of MSCs relied on the fact that NP cells are 'chondrocyte-like' and express chondrogenic markers such as SOX-9, type II collagen and aggrecan [163]. However, there are substantial differences in the ECM of the NP and articular cartilage [164], as well as the ontogeny of NP cells [165], suggesting a unique phenotype for NP cells compared to chondrocytes (or AF cells). In 2010 Minogue et al. published the first phenotypic profile of human NP cells, compared to articular chondrocytes, with many genes being confirmed by future studies [166–168]. A recent consensus paper has sought to define the NP cell phenotype and while the functional significance of many of the putative markers is unknown, their expression can be used to define discogenic differentiation of MSCs [169]. Such markers have allowed the optimization of discogenic differentiation strategies; most notably the comparison of growth factors to induce lineage-specific differentiation and the comparison of MSCs isolated from different anatomical locations. While TGFβ has conventionally been used to induce discogenic differentiation, growth differentiation factors 5 (GDF5) and 6 (GDF6) have both been shown to produce a more appropriate phenotype, with GDF6-stimulated MSCs demonstrating the largest increases in discogenic marker genes and secreting the most NP-like ECM in 3D-culture [140,143,144]. Of note, GDF6-stimulated AD-MSCs produced a more appropriate matrix than BM-MSCs obtained from the same donor [140], and given their relative ease of acquisition and high proliferation rate, AD-MSCs may thus offer the most appropriate cell source for IVD regeneration.

MSCs have also been shown to communicate with NP cells in a bidirectional manner during co-culture [147,170,171], suggesting that in addition to undergoing differentiation and de novo synthesis of ECM, implanted cells may influence NP cell function through secretion of bioactive factors, such as anabolic growth factors. Recent evidence suggests that in addition to the secretion of anabolic factors, MSCs possess anti-inflammatory and anti-catabolic properties, which could be used in the context of disc degeneration to reduce cytokine levels, thereby modulating the inflammatory niche, to produce a healthier, non-degenerate phenotype in native NP cells (Fig. 3). Indeed Tam recently demonstrated increased GAG content in an experimentally-induced IVD degeneration model following both intradiscal and intravenous UC-MSCs injection, without evidence of engraftment in the latter approach, suggesting paracrine signaling from the injected cells may be responsible for the effects demonstrated [160].

5.6. MSC implantation and the IVD niche

The IVD represents the largest avascular structure in the human body, with the resident cells relying on diffusion of oxygen and nutrients from blood vessels in adjacent vertebral bodies [172]. NP cells rely mainly on glycolysis to produce energy, with the resultant lactic acid also being removed by diffusion out of the disc. Calcification of the end plates, associated with age and degenera-

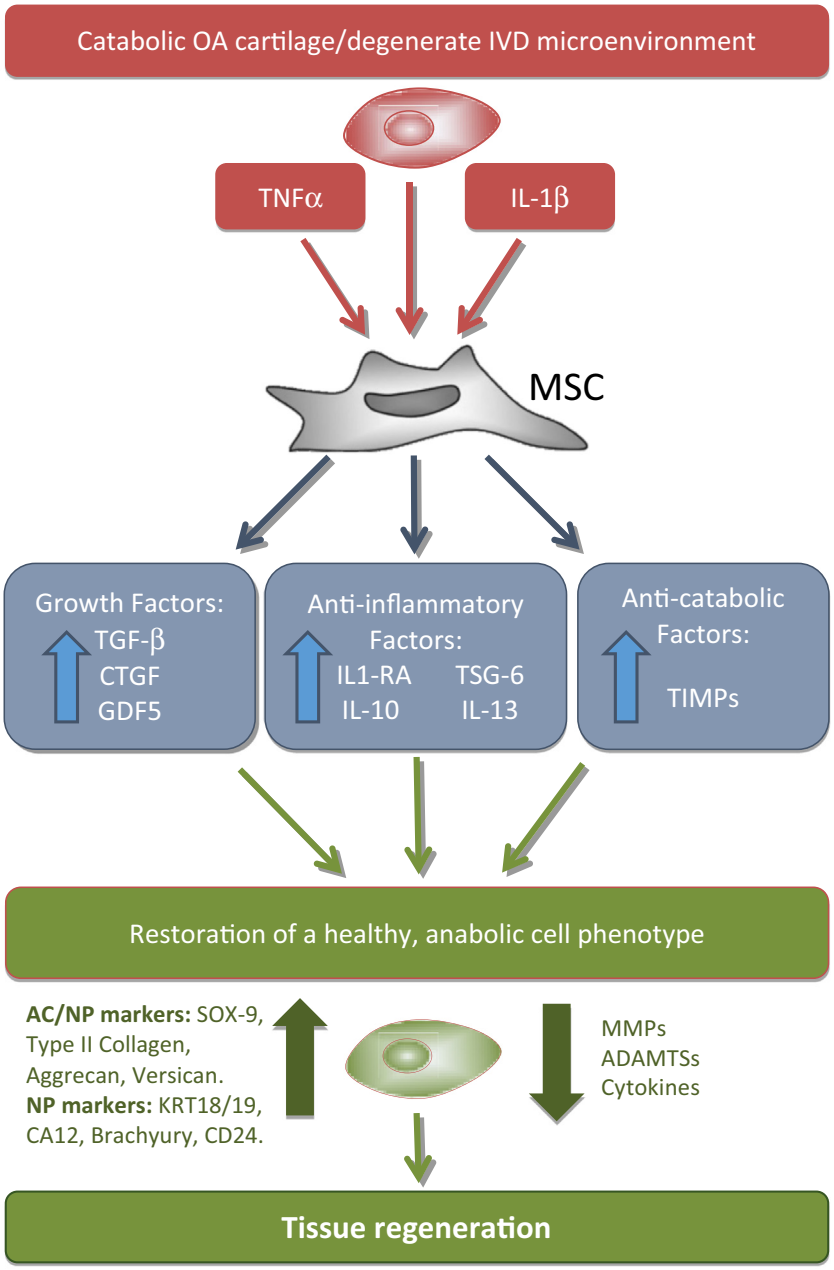

Fig. 3. Bi-directional cell interactions following MSC implantation. The degenerate IVD and OA cartilage represent harsh, catabolic microenvironments, with high levels of cytokine, most notably IL-1 and TNFα. Following implantation MSCs will respond in a paracrine manner by producing a range of growth factors, anti-inflammatory factors and anti-catabolic factors, which will influence resident articular chondrocytes (AC) or nucleus pulposus (NP) cells to produce a healthier, more anabolic phenotype and regenerate tissue.

tion, results in a microenvironment within the NP, which is hypoxic, acidic and nutrient-deprived [173,174]. The disc is also exposed to regular dynamic loading, which influences both functional and phenotypic characteristics of the resident NP cells [175]. Abnormal over-loading or under-loading, as well as asymmetric loading have all been demonstrated to exert deleterious effects on NP cell viability and phenotype [176,177].

Despite preliminary results showing positive effects of cell-injection strategies for IVD regeneration, detailed basic research on IVD cells and their niche indicates that transplanted cells are unable to survive and adapt in the avascular niche of the IVD [178]. In particular the current evidence suggests that while hypoxia and load may be beneficial for discogenic differentiation, high osmolarity and low pH may be deleterious to MSC survival and function [143,179,180]. How a combination of factors influences MSC fate is yet to be fully elucidated, but may represent a major challenge for survival and function of implanted cells in a clinical setting.

5.7. Scaffolds and biomaterials for IVD tissue engineering

Newly transplanted cells are subjected to high mechanical loads, which may be detrimental to viability or function; however, such loads can potentially be minimized by temporarily placing a screw/rod construct that bridges the intervertebral disc. A more long-term approach that may enhance MSC survival and differentiation post-implantation is incorporation of cells into a biomaterial scaffold. Numerous biomaterials have been proposed, with many investigators focusing on injectable hydrogels in order to minimize damage to the surrounding AF (as reviewed in

[181,182]). However, studies have also proposed cell-seeded biphasic scaffolds to engineer whole IVD [183–186], biomaterials to regenerate the AF [187–189], and functionalized acellular biomaterials, for example with the chemoattractant SDF-1 to recruit resident progenitor cells or MSCs to the disc [190]. Production of mechanically robust, biodegradable, biocompatible and functionalized biomaterials, particularly hydrogels, has been a limitation within the field, although recent studies suggest that development of a suitable IVD-like biomaterial is an achievable goal [191,192].

5.8. Future perspectives

Like articular cartilage, the IVD presents a challenging and complex tissue to regenerate. While the origin of human NP cells is still debated and the transcriptional machinery underpinning discogenic differentiation remains relatively undefined, the elucidation of a defined NP phenotype has allowed development of methodologies to promote lineage-specific discogenic differentiation of MSCs, which may demonstrate clinical efficacies in future clinical trials. The fate of implanted cells in the harsh microenvironment of articular cartilage, and particularly in the degenerate IVD, remains to be elucidated. Likewise, the mechanism or mechanisms by which implanted cells induce regeneration, whether it be direct differentiation or paracrine stimulation of native cells, remains to be determined.

Such research in the IVD is hampered by the lack of an appropriate animal model, which accurately mimics the microenvironment of the human IVD [178,193]. However, novel *ex vivo* whole organ IVD model systems are being developed [194–196], in which microenvironmental parameters can theoretically be independently controlled, to allow testing of proposed therapies prior to clinical translation.

Whether cells require pre-conditioning or pre-differentiation prior to implantation into cartilage or IVD to enhance cell survival and matrix formation, also requires testing. The requirement for, and design of, suitable biomaterials which will withstand the enzyme-rich, mechanically load microenvironments of disease cartilage and disc also remains to be elucidated. Evidence suggests that cell leakage following MSC implantation into the IVD can cause peripheral osteophyte formation and this highlights the need for careful design of any cell implantation strategy [197]. Similarly, chondrogenic differentiation of MSCs for cartilage regeneration has the potential risk for hypertrophy and ossification and it remains to be seen whether such events occur in discogenic differentiation strategies. Such risks may be mitigated through careful selection of MSC source, with UC-MSCs or WJSCs offering one potential source, and differentiation strategy. Furthermore, patient selection will be critical for successful outcome and treatment modality will depend on stage of disease.

Cell-based therapies for advanced OA, where cartilage eburnation has occurred, remain a challenge and such patients may still require joint replacement or tissue engineered cartilage. Similarly, cell implantation approaches may not benefit those with multilevel disc disease or those with an advanced stage of disc degeneration, where the surrounding tissue may have degenerated. In such circumstances, a tissue engineered, whole IVD replacement may be more appropriate. However, the rate of advancement in the field of regenerative medicine suggests these problems may be overcome through a combination of functional biomaterials, identification of appropriate cell source and improved differentiation regimens.

6. Conclusions

MSC-based therapies offer huge potential to revolutionize the treatment of cartilage defects and IVD degeneration and the advances discussed in this manuscript highlight the progress being made toward clinical translation of such approaches. However, a wide range of technical hurdles and conceptual challenges must still be overcome as research progresses in this exciting and rapidly expanding field. There are still many technical challenges associated with isolating, expanding, differentiating, and preconditioning MSCs for subsequent implantation into degenerate joints and the spine. The physiological microenvironment of both diseased joints and intervertebral discs is likely to be hypoxic, acidic, deprived of nutrients, and exposed to higher than normal concentrations of pro-inflammatory cytokines and reactive oxygen species. Furthermore, MSCs may be exposed to abnormal physical loads in anatomical structures that have already been biomechanically compromised. Thus future regenerative medicine strategies will need to address these remaining concerns.

7. Contributors

The authors researched, discussed and approved the concept, drafted and submitted the commissioned paper. All co-authors made a significant intellectual contribution to the concept of the manuscript.

Conflict of interest statement

The authors wrote this paper within the scope of their academic and affiliated research positions. The authors declare no conflict of interests.

Competing interests

The authors declare no competing interests.

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