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Review

Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions

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ABSTRACT

Osteoarthritis (OA) represents a final and common pathway for all major traumatic insults to synovial joints. OA is the most common form of degenerative joint disease and a major cause of pain and disability. Despite the global increase in the incidence of OA, there are no effective pharmacotherapies capable of restoring the original structure and function of damaged articular cartilage. Consequently cell-based and biological therapies for osteoarthritis (OA) and related orthopaedic disorders have become thriving areas of research and development. 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. Complications of ACI include hypertrophy, disturbed fusion, delamination, and graft failure. 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. Future tissue-engineering approaches for cartilage repair will also benefit from advances in chondrocyte-based repair strategies. This review article focuses on the structure and function of articular cartilage and the pathogenesis of OA in the context of the rising global burden of musculoskeletal disease. We explore the challenges associated with cartilage repair and regeneration using cell-based therapies that use chondrocytes and mesenchymal stem cells (MSCs). This paper also explores common misconceptions associated with cell-based therapy and highlights a few areas for future investigation.

Contents

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[1] http://cordis.europa.eu/projects/rcn/105314_en.html; http://ec.europa.eu/research/health/medical-research/severe-chronic-diseases/projects/d-board_en.html.

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1. Introduction

Cell-based therapy is a form of biological therapy. It involves the process of introducing new cells into tissues in order to treat a degenerative or age-related disease. The advent of cell-based therapies as a novel therapeutic platform has the potential to revolutionise the future of healthcare, driving a shift from the management of disease symptoms to their cure. Thus far, research in the area of cell therapy has mainly focused on the treatment of hereditary diseases, with or without the addition of gene therapy. However, cell therapy is also a form of regenerative medicine and is increasingly used in combination with tissue engineering and biomaterials. Although the combination of cell therapy, regenerative medicine and tissue engineering are relatively novel areas of therapeutic research, each individual element by itself is not novel and goes back several decades. Current cell therapy initiatives are deeply rooted in blood transfusion, bone marrow and organ transplantation, tissue banking and reproductive *in vitro* fertilisation. However, cell-based therapy is now an established component of modern healthcare and is predicted to grow exponentially as modern healthcare systems evolve and integrated knowledge of cell biology, biomaterials and regenerative medicine expands. The aim of this article is to provide an overview of chondrocyte-based therapies for the treatment of osteoarticular lesions, or "focal defects" in articular cartilage. It could be argued that these types of cell-based therapy are a contra-indication for OA due to the geometry of osteoarthritic lesions and the fact that inflammatory joint disease is rarely focal, unlike cartilage defects in younger patients and elite athletes. However, in the absence of effective pharmacological agents [1], novel biological [2] and cell-based therapies need to be developed for OA and related orthopaedic conditions. Therefore, in this review article we approach this problem from basic science viewpoint rather than a clinical perspective and refer readers to relevant clinical papers and trials instead of discussing them in significant detail. Our aim is to describe the significance of this topic in the context of the biology of the joint and the osteoarthritic disease process, discuss the current state-of-the-art and speculate on the impact of autologous and allogeneic chondrocyte-based therapies in orthopaedics, rheumatology and sports medicine. This paper also summarises key concepts and developments in the area of mesenchymal stem cell (MSC) based therapy.

2. The burden of musculoskeletal diseases and osteoarthritis

Age-related musculoskeletal and joint diseases are currently a major cause of morbidity globally and result in enormous costs for health and social care systems. Chronic and inflammatory diseases of joints are major causes of disability in the middle-aged and the elderly. With increasing life expectancy, the burden of musculoskeletal diseases is progressively growing, highlighting the need for a radical shift in healthcare strategies that involve interventions that can either prevent or significantly reduce the risk of development of these diseases.

Arthritic diseases are a group of conditions involving inflammatory damage to synovial joints. Arthritis literally means inflammation (*itis*) of the joints (*arthr*) and involves pain, redness, heat, swelling and other harmful effects of inflammation. Although there are over 200 different forms of arthritis, OA is the most prevalent and chronic form joint disease and a major cause of pain and disability affecting the ageing population with increasing prevalence as this population expands [3]. OA leads to joint pain, stiffness and loss of function predominantly in the knees, hips, hands and other weight-bearing joints. Although advancing age is a major risk factor for the development of OA, there are other significant contributing factors including obesity, a history of joint trauma and other co-morbidities such as diabetes, metabolic and endocrine diseases. OA is one of the top five causes of disability amongst non-hospitalised adults (source: Centers for Disease Control and Prevention (CDC, http://www.cdc.gov/), USA). According to estimates from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS, http://www.niams.nih.gov/) more than 20 million Americans currently suffer from OA. Conservative estimates suggest that 35–40 million Europeans have OA. Statistical data from epidemiological studies suggest that arthritis is the number one condition associated with functional limitation and physical disability among US population aged 65 and older and affects 30% of the population [4]. It is expected that by 2030, 20% of adults will have developed OA in Western Europe and North America. OA is an important cause of disability-adjusted-life years in both the developed and developing world [5]. Therefore, OA is expected to be a heavy economic burden on healthcare systems and community services in Europe, North America and the rest of the world as the population expands and the number of older people increases.

3. Cartilage degeneration in osteoarthritis

Classically OA has been considered a 'wear and tear' degenerative condition of joints. However, OA is a systemic disease that affects the whole joint, including cartilage, subchondral bone, synovium, tendons, and muscles [6–9]. The disease is characterised by degeneration of articular cartilage, low grade synovial inflammation (synovitis) [7], and alterations in peri-articular soft tissues and subchondral bone [10]. The synovitis that occurs in both the early and late phases of OA is associated with alterations in cartilage. Catabolic and pro-inflammatory mediators such as cytokines, nitric oxide, prostaglandin E_2 (PGE_2) and neuropeptides are produced by the inflamed synovium and alter the balance of cartilage matrix degradation and repair, leading to excess production of the proteolytic enzymes responsible for cartilage breakdown [7]. Cartilage alterations induce further synovial inflammation, creating a vicious circle and the progressing synovitis exacerbates clinical symptoms and stimulates further joint degradation in OA [7]. Fig. 1 outlines the major molecular and cellular changes that occur in the synovial joint in arthritis and synovitis.

Recent studies have demonstrated that systemic factors regulate the metabolism of joint tissues, and that there is substantial

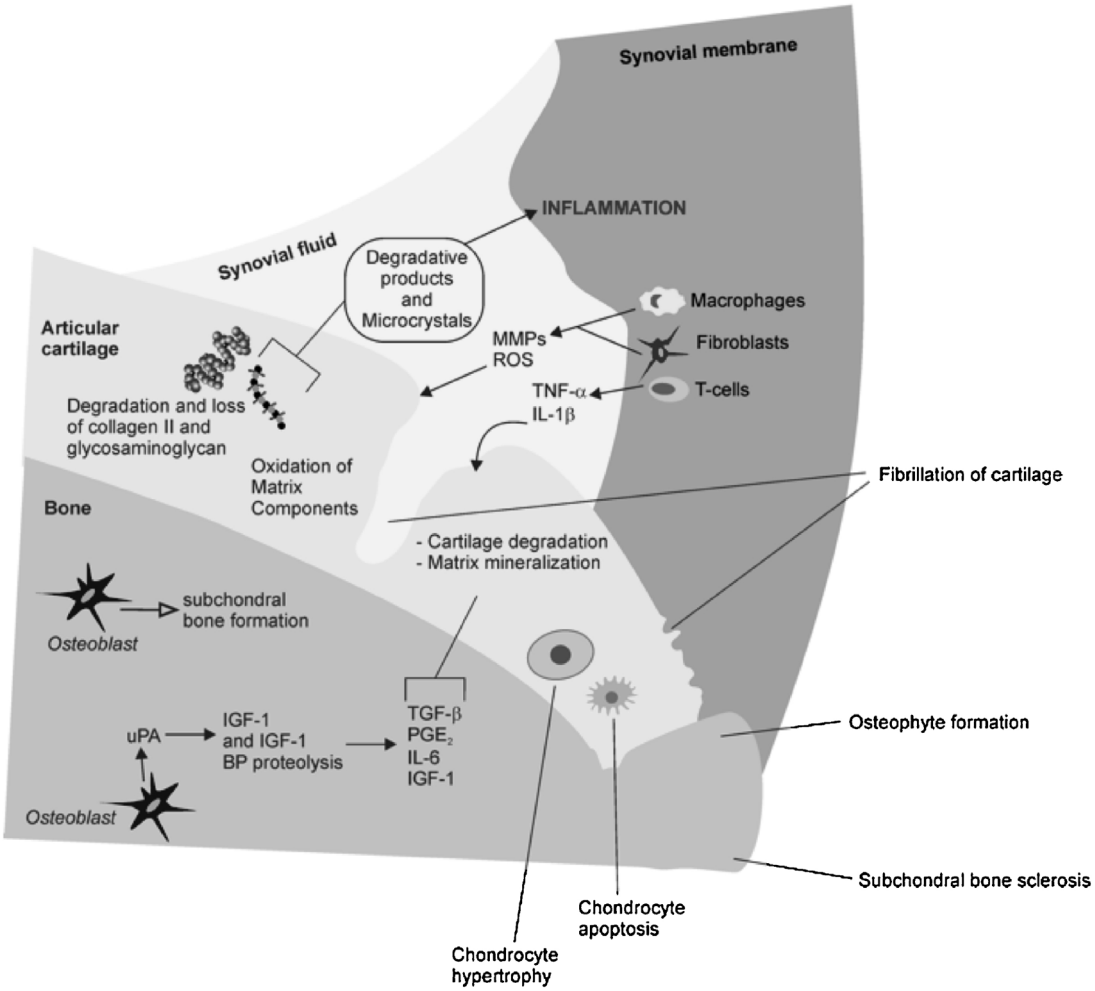

Fig. 1. The major molecular and cellular alterations that occur in the synovial joint in osteoarthritis and synovitis. This schematic highlights the actions of various inflammatory cells and mediators in OA. Chondral changes include cartilage fragmentation (fibrillation), cartilage degradation and loss of type II collagen and proteoglycans, chondrocyte apoptosis (hypocellularity) and matrix mineralisation. Synovial changes in OA include inflammation, synovial hypertrophy, recruitment and activation of T cells, macrophages and fibroblasts, production of matrix metalloproteinases (MMPs) and reactive oxygen species (ROS). Synovial fluid alterations in OA include accumulation of MMPs and ROS, release of IL-1β, TNF-α and other pro-inflammatory cytokines (IL-6, IL-8), release of inflammatory pain mediators such as prostaglandin E2 (PGE_2), formation of degradative products and microcrystals. Subchondral alterations in OA include subchondral sclerosis (*i.e.*, eburnation), osteoblast mediated subchondral bone formation, proteolysis (degradation) of IGF-I and IGF-I binding proteins (IGFBPs), increased production of some growth factors and cytokines including transforming growth factor β (TGF-β), PGE_2 and interleukin 6 (IL-6).

cross-talk occurring between different joint tissues [11]. Although OA is primarily associated with ageing, there are other key contributing factors, including obesity (which increases mechanical stress, and possibly inflammation), a history of joint trauma/injury or repetitive use, genetics, heritable and acquired metabolic disorders (see below), muscle weakness, underlying anatomical and orthopaedic disorders (*i.e.* congenital hip dislocation), joint infection, crystal deposition, previous rheumatoid arthritis, and various disorders of bone turnover and blood clotting. Many of these factors act to incite a cascade of pathophysiological events within the joint [12].

The prevalence of OA is significantly higher in women compared to men. OA affects 10% of males and 18% of females over 45 years (especially after the menopause and in women with comorbid metabolic bone conditions such as osteoporosis (OP)) [13]. Although the underlying causes for the increased susceptibility of women to OA are not fully understood, research is beginning to focus on associations with sex hormones, obesity and physical activity to determine whether modifiable factors such as oestrogen, weight management, and protection during sport and physical exercise can be used as treatment options for postmenopausal women with OA and OP [11,14]. There is increasing evidence for a connection between metabolic dysfunction and OA [11,13]. Indeed, metabolic OA has recently been described as a subtype of OA [13].

OA has an important inflammatory component that includes increased activity of a number of cytokines and chemokines in affected joints [15]. These inflammatory cytokines and chemokines drive the production and secretion of enzymes that mediate the destruction of cartilage matrix [10]. Ageing is a major contributor that decreases the ability of chondrocytes to maintain and restore articular cartilage and thereby increases the risk of degeneration of the articular cartilage surface [16]. Cartilage ageing drives cellular alterations that result in a damage-induced, senescence-associated secretory phenotype characterised by the production and secretion of cytokines, chemokines, and proteases [17,18]. Oxidative stress and inappropriate mechanical signals can further promote the senescence-associated secretory phenotype of ageing chondrocytes [17,19] as has been shown in tumour cells [20].

OA is effectively the final common pathway for ageing and traumatic injuries of synovial joints, as well as being an active, inflammatory and insidiously progressive joint disease. There are no established disease-modifying pharmacological therapies for

OA and the use of existing symptom-modifying drugs with deleterious side effects highlight a genuine need for novel, safe and effective treatments for OA patients. Currently established therapies insufficiently address the enormous clinical needs and there are many cases where prevention is either too late or is impossible and existing pharmacological therapies is ineffective. Therefore, surgical techniques will continue to be used as the treatment of choice especially in cases where lack of intervention will have deleterious effects for long-term joint function. The aim of this article is to provide an overview of chondrocyte and stem cell based therapeutics for OA and related joint disorders.

4. Articular cartilage and chondrocytes

Articular cartilage is a tough yet flexible load-bearing connective tissue with unique biological and biomechanical characteristics. It covers the articulating surfaces of long bones in synovial joints. Cartilage is a smooth and translucent tissue that acts as a cushion to absorb shock and allows the bones to glide over each other with frictionless articulation. It is sub-classified into three different types: elastic, hyaline and fibrocartilage. These types of cartilage differ in the relative amounts of three principal components, namely collagen fibres, ground substance (proteoglycans) and elastin fibres. Anatomically, normal articular cartilage is composed of four main zones (Fig. 2A) and a tidemark that separates articular cartilage from subchondral bone [21,22].

The zones of cartilage are based on the shape of the chondrocytes, the composition of the extracellular matrix (ECM) and the orientation of the type II collagen with respect to the articulating surface and the subchondral bone. The superficial zone (tangential zone) makes up 10% of articular cartilage and is the thinnest layer. Type II collagen fibre orientation is parallel to the articulating surface in the joint. This zone has flattened chondrocytes, condensed collagen fibres, and sparse proteoglycans. In the intermediate zone (middle) zone the type II collagen matrix has a random organisation. This is the thickest layer with nearly spherical chondrocytes oriented in perpendicular or vertical columns paralleling the collagen fibres. In the deep zone (basal layer) type II collagen is perpendicular to joint and crosses the tidemark. Here chondrocytes are spherical and collagen has a random organisation. The tidemark separates uncalcified articular cartilage from the deeper calcified tissue that participated in the process of endochondral ossification during longitudinal bone growth during childhood and adolescence.

The biochemical properties of cartilage depend on the structural design of the tissue and the molecular composition of the ECM that makes up the bulk of the dry weight of the tissue. The tissue is neither vascularised nor innervated; it does not contain any lymphatic vessels either [23]. The ECM is hyper-hydrated and water accounts for more than 80% of the total wet weight of cartilage [23]. Cartilage hydration is crucial for load bearing wear resistance and joint lubrication. The function of cartilage is also controlled by the interactions between its resident cells and the ECM [24]. Chondrocytes are the main cell type found within cartilage [25] (Fig. 2B). They are responsible for the synthesis and maintenance of the ECM and have been referred to as 'architects' of cartilage [26]. Chondrocytes are isolated from each other by a large quantity of ECM [25]. Consequently, nutrient provision and metabolic waste removal must occur by diffusion through the ECM. Therefore, under normal and pathophysiological conditions, chondrocytes exist in a low oxygen tension environment [27]. These unique properties mean that cartilage has low reparative potential, further predisposing the tissue to degenerative conditions such as OA, which is a significant clinical problem [25].

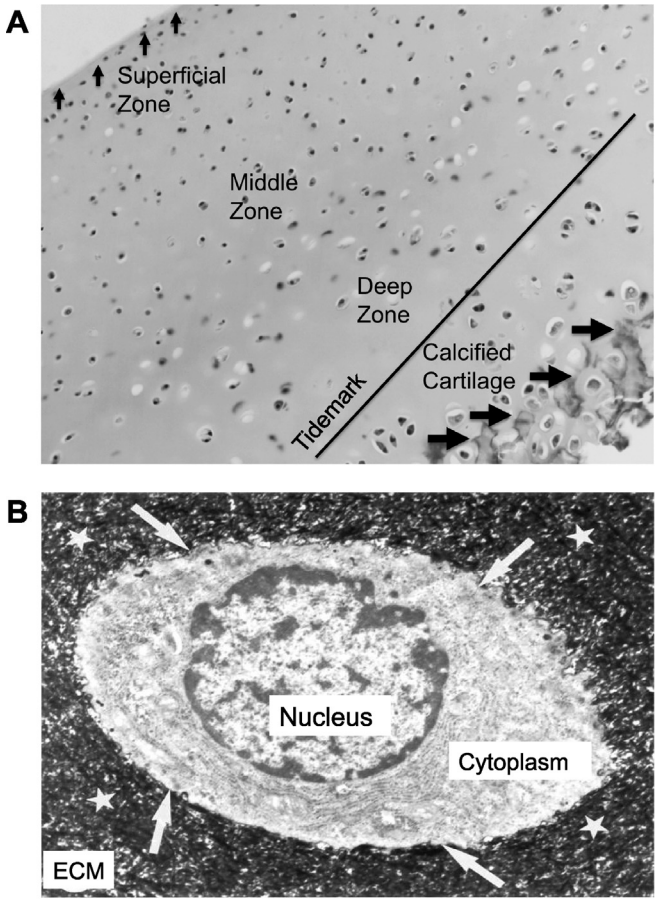

Fig. 2. (A) Section of articular cartilage stained with haematoxylin and eosin showing the major zones of articular cartilage including the superficial, middle, deep and calcified zone of porcine articular cartilage. Cartilage is predominantly an avascular, aneural and alymphatic load-bearing connective tissue. Blood vessels are only present in subchondral bone. Cartilage contains a single cell type known as the chondrocyte. (B) An electron micrograph of an articular chondrocyte. Chondrocytes are cytoplasmically isolated and their energy requirements are derived primarily through glycolysis. They possess a high matrix/cell volume ratio and do not divide after skeletal maturity unless the cartilage becomes diseased.

Chondrocytes build the macromolecular framework of the ECM from three distinct classes of macromolecules: collagens (type II collagen), proteoglycans (mainly aggrecan), and a variety of non-collagenous proteins. Cartilage ECM is continually remodelled as chondrocytes replace matrix macromolecules lost through degradation. ECM turnover depends on the ability of chondrocytes to detect alterations in the macromolecular composition and organisation of the matrix, such as the presence of degraded macromolecules, and to respond by synthesising appropriate types and amounts of new ECM components. It is known that mechanical loading of cartilage creates mechanical, electrical, and physicochemical signals that help to direct the synthesising and degrading activity of chondrocytes [28]. In addition, the ECM acts as a signal transducer for chondrocytes [29]. A prolonged and severe decrease in the use of the joint leads to alterations in the composition of the ECM and eventually to a loss of tissue structure and its specific biomechanical properties, whereas normal physical strain stimulates the biosynthetic activity of chondrocytes and possibly internal tissue remodelling [30,31].

Although articular cartilage can tolerate a tremendous amount of intensive and repetitive physical stress, it manifests a striking inability to heal even the most minor injury [30,32–34]. This makes joints particularly sensitive to degenerative processes [35]. Ageing leads to alterations in ECM composition and alters the activity

of the chondrocytes, including their ability to respond to a variety of stimuli such as growth factors [36–38]. All these alterations increase the likelihood of cartilage degeneration and impair repair responses [33,39–41].

5. Cartilage regeneration and repair

The capacity of articular cartilage for repair and regeneration is extremely poor [27,42]. Cartilage is largely avascular and does not contain the blood vessels, which are important for repair responses [43,44]. Since circulation is a critical part of the normal healing process, the absence of a blood supply in cartilage may suppress the normal responses associated with healing. Mature and aged cartilage face the additional problem of containing fewer cells and receiving a more restricted blood supply [43]. Chondrocytes themselves exists in an environment that does not support healing. They are trapped in lacunae and cannot migrate to damaged areas and initiate repair processes. Also, the synthesis of new ECM in damaged cartilage is very slow. When repair can take place, it is usually quite feeble. Damaged cartilage is usually replaced by a fibrocartilage-like scar tissue. To compound matters further, cartilage loses its limited capacity for repair with ageing. Chondrocytes gradually decline in number with age [45]. Chondrocyte apoptosis is important during normal skeletal growth and development but it is also thought to play an important role in cartilage ageing and disease [46]. Chondrocyte-derived apoptotic bodies express degradative properties that may contribute to pathologic processes in cartilage including ECM degradation and calcification [47,48]. The cells that survive in aged cartilage become senescent [49–51]. Therefore, over a period of time, chondrocyte senescence and death, and cartilage hypocellularity in ageing joints may contribute to the pathogenesis of OA [52]. All these changes mean that the restoration of articular surfaces becomes more and more difficult with age [53].

Thus far, no surgical technique has ever been completely successful in stimulating articular cartilage repair and regeneration. The success of cartilage repair depends largely on the size and physical dimension of the lesion. If surgery is indicated, there are several options for treatment. These include smoothing of the lesion and removing loose tissue by debridement, application of techniques to stimulate fibrocartilage scar cartilage to grow into the lesion (*i.e.* microfracture) and surgical techniques to replace the lesion with new cartilage (*i.e.* osteochondral autografts, or autologous chondrocyte implantation (ACI)). In terms of symptom modification most of these approaches are unsatisfactory. Arthroscopic lavage and debridement provide temporary relief of symptoms and are controversial. Bone marrow stimulation techniques such as microfracture and drilling produce mechanically inferior fibrocartilage and therefore do not typically offer a long-term solution. Detailed discussion of the surgical techniques for cartilage repair is beyond the scope of this article but the following section will briefly review current cell-based strategies for cartilage repair.

6. Autologous chondrocyte implantation (ACI)

ACI is one of the most widely used cell based repair strategies for articular cartilage. It was introduced by a Swedish group following the general principles of tissue repair [54]. The idea in ACI is to fill up the cartilage defect with autologous chondrocytes (*i.e.* chondrocytes derived from the same patient). This approach combines surgical treatment with *in vitro* and cell culture methods. Many modifications of the ACI technique exist. The basic technique has been re-evaluated by Brittberg [55] who has also provided an update on the clinical results. A cartilage biopsy is surgically taken from a non-weight-bearing area of the affected joint and transferred to a sterile nutrient solution for transport and storage. In the cell culture laboratory, chondrocytes are isolated from the cartilage tissue by enzymatic digestion with collagenase. The chondrocytes are then expanded in monolayer culture. This expansion amplifies the total number of cells for implantation, allowing the surgeon to fill the cartilage defect. In a second surgical procedure, the *in vitro* expanded chondrocytes are injected into the defect. To secure the chondrocytes remaining at the implanted side and to prevent the mass from floating away, a periosteal flap is further sewed over the defect [54]. It is well documented that the periosteal flap alone can have chondrogenic capacities, and induce cartilage regeneration. However the precise role of the periosteum still remains to be elucidated.

In the first animal experiments performed on rabbits, the ACI technique was performed on chondral defects that had not penetrated the subchondral bone. These results were very encouraging; the rabbits showed new cartilage formation in 82% of the defect area [56]. In further studies, chondral defects of the patella in rabbits were either treated with chondrocytes or left empty with only the periosteal flap covering the defect [57] or scaffolds were used, with chondrocytes seeded into an agarose gel and then transplanted [58]. In both cases, the 1-year outcome showed significantly higher hyaline cartilage production in treatments with added chondrocytes compared to control treatments without cells (between 47 and 87%). To evaluate whether the implanted chondrocytes stayed at the implantation site or whether other cells performed tissue repair, chondrocytes were membrane labelled with a fluorescent dye to track them after implantation *in vivo*. A 6 week outcome in a goat model showed that cells persisted in the defect site [59]. In contrast to the rabbit studies, a canine model showed no significant difference between the ACI treated areas and the controls [60]. However, in a more recent canine study, a scaffold seeded with chondrocytes was used which showed significantly higher values of defect regeneration (42% of defect area filled with hyaline cartilage) [61].

ACI has been in clinical use in human patients since 1987 and has been performed on over 12,000 patients worldwide [62]. ACI has significantly reduced pain in patients – even the production of durable cartilage-like tissue has been observed [62]. In human patients, results after 3–9 years are very encouraging, although repair of the defect is not uniform in all areas of the joint [54,63]. Clinical results are encouraging and overall the patients are satisfied. There are published studies that have compared ACI and microfracture [64]. The randomised trial by Knutsen et al., compared ACI with microfracture and the findings at 5 years suggested that both methods provided satisfactory results in 77% of the patients. There was no significant difference in the clinical and radiographic results between the two treatment groups and no correlation found between the histological findings and the clinical outcome. The authors suggested that further long-term follow-up is needed to determine if one method is better than the other and to study disease progression [64]. A similar randomised controlled trial by Vanlauwe et al. [65], suggests that 5 years after treatment, clinical outcomes for CCI and MF are comparable. However, this study proposed that time since onset of symptoms is an essential variable that should be taken into account in future treatment strategies for the repair or knee cartilage [65]. In light of these clinical trials, there is still a lack of comparative, blinded long-term group studies in human subjects and it is sensible to propose longer-term studies on the efficacy of ACI.

Despite the encouraging clinical results there are still limitations to the use of ACI. These are mainly related to: (a) the complexity and cost of the two surgical procedures, (b) the biological response of the periosteal flap, and (c) the de-differentiation and consequent capacity loss associated with *in vitro* expansion of isolated chondrocytes [59,66–68]. Most clinical complications associated with ACI in fact are connected to the periosteal flap. These include periosteal

flap detachment, delamination and late periosteal hypertrophy [69]. Second generation ACI procedures using chondrocytes plus collagen sheets dramatically reduce adverse events, hypertrophy, delamination and third generation ACI with resorbable scaffolds are more promising. The use of scaffolds that "hold" the chondrocytes in place may offer a treatment option for OA cartilage defects that often lack intact cartilage rims. Detailed discussion of second and third generation ACI is a clinical topic that is beyond the scope of this and readers are referred to some relevant papers [70–75].

Despite all the progress with ACI over the last few decades, one of the main hurdles to successful cartilage repair is chondrocyte dedifferentiation during the monolayer expansion phase. The close interaction between chondrocytes and the ECM represents a major factor in the maintenance of chondrocyte function, vitality and its unique biosynthetic programme. Damaged ECM or its complete absence will result in a major shift in chondrocyte gene expression. Instead of producing cartilage specific proteoglycans and collagen type II, chondrocytes switch to making non-specific proteoglycans and collagen type I [76–78]. These matrix components lack the biomechanical properties and the resilience of articular cartilage. The monolayer culture conditions *in vitro*, where chondrocytes are forced to give up their round shape in order to adhere to the plastic in order to survive, are a key component of chondrocyte de-differentiation which becomes phenotypically evident after the cells adhere to tissue culture plastic and continue de-differentiating with prolonged culture. De-differentiated cells are no longer capable of re-differentiation when re-implanted in the cartilage defect. Re-differentiation in three-dimensional culture models can be achieved up to the fourth passage in monolayer [67]. Furthermore, growth factors are known to be involved in the re-differentiation of chondrocytes. Insulin like growth factor I (IGF-I) as well as transforming growth factor beta (TGF-β) influence and modulate the collagen network in cartilage and can prolong the re-differentiation capacity of monolayer-expanded chondrocytes [79–81]. Treatment of monolayer-cultured chondrocytes with IGF-I will prolong their re-differentiation potential. After a protracted monolayer expansion phase, IGF-I treated chondrocytes are still able to produce specific cartilage matrix components in three-dimensional conditions in comparison to chondrocytes not exposed to IGF-I [82].

7. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal cells that can be isolated from bone marrow, marrow aspirates, skeletal muscle, adipose tissue [83], synovium and many other connective tissues [84]. Adult mesenchymal stem cells were originally isolated from bone marrow in 1999 by Pittenger and co-workers [85], who demonstrated their multilineage differentiation potential or multipotency. Subsequent studies have identified the presence of stem cells in a number of adult tissues, including adipose, muscle, dermis, periosteum, synovial membrane, synovial fluid and articular cartilage. Due to their culture-dish adherence, they can be expanded in culture while maintaining their multipotency [86]. They can differentiate into cells of the mesodermal lineage, giving rise to a range of specialised connective tissues including bone [87–89], adipose tissue [90,91], cartilage [86,89,90], intervertebral disc [92–94], ligament [93–95] and muscle [90]. MSCs in culture can be induced to generate chondrocytes myocytes, adipocytes, osteoblasts and tenocytes (Fig. 3) [96] [85].

Recent studies have demonstrated that MSCs can interact with immune cells, leading to the modulation of a number of effector functions [96]. The immunomodulatory properties of MSCs may be exploited for the treatment of inflammatory and rheumatic conditions [97]. MSCs can migrate to injury sites, induce peripheral tolerance and inhibit the release of pro-inflammatory cytokines. They can also promote tissue repair and the survival of damaged cells [96]. Thus far, MSCs have been described as 'hypo-immunogenic' or 'immune privileged'. However, recent studies suggest that MSCs may not be 'immune privileged'. Although they possess the temporal capacity to exert therapeutic functions through 'hit and run' mechanisms, they may not be 'immune privileged'.

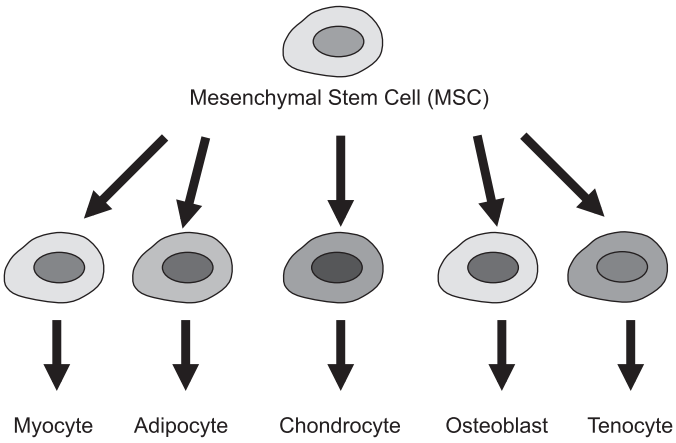

Fig. 3. The mesenchymal stem cell (MSC) lineage. MSCs are multipotent stem cells that can generate unipotent cells with the capacity to differentiate further to a variety of differentiated cells including myocytes, adipocytes, chondrocytes, osteoblasts and tenocytes.

After *in vivo* administration, MSCs can induce peripheral tolerance and migrate to injured tissues where they have the capacity to exert immunosuppressive properties [98] and inhibit the release of pro-inflammatory cytokines and promote the survival of existing cells and the repair of damaged tissue [96]. They are being clinically explored as a new therapeutic for treating a variety of immune-mediated diseases [99].

MSCs show considerable promise for use in repairing and rebuilding damaged or diseased mesenchymal tissues [86]. MSCs have potential applications in tissue engineering and regenerative medicine and may represent an attractive option for bone, cartilage, tendon and ligament regeneration. However, it is not clear which adult tissues MSCs should be sourced from. There are currently several different types of MSCs that have been proposed as potential sources of cells for cartilage repair: bone marrow-derived MSCs, adipose tissue derived MSCs, synovial-derived MSCs and Wharton's jelly/umbilical cord derived MSCs (Fig. 4).

The best choice of cell type for cartilage repair will depend on availability and chondrogenic differentiation potential. Unlike bone marrow, synovial and adipose derived MSCs Wharton's jelly/umbilical cord derived MSCs are advantageous for the following reasons: they can be easily harvested from discarded umbilical cords obtained at birth; they exhibit high proliferation rates; they can be expanded for many population doublings; they are hypo-immunogenic and non-tumorigenic. Wharton's jelly/umbilical cord derived MSCs have been differentiated into cartilage following culture in three-dimensional biodegradable nanoscaffolds [100].

Despite these advances, there are no published consensus statements relating to the optimum growth factor and culture conditions needed to drive differentiation of MSCs to a stable chondrocyte phenotype. Also, there is no consensus on how MSCs are isolated, identified and characterised. There is a paucity of standardised specific cell surface markers. Although MSCs cells have been isolated and expanded in culture, their use for therapeutic strategies requires protocols and technologies that have not yet undergone clinical trials [101]. Also, there are no established guidelines from governmental and intergovernmental agencies for their

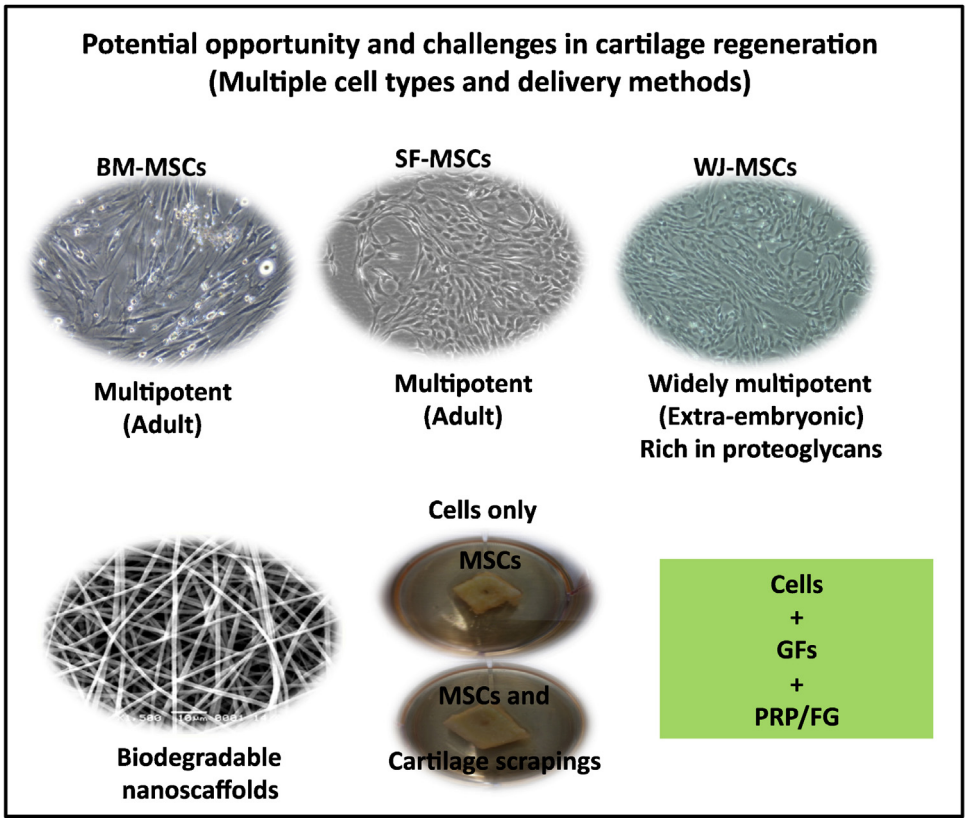

Fig. 4. BNM-MSCs: bone marrow mesenchymal stem cells; SF-MSCs: synovial fluid mesenchymal stem cells; WJ-MSCs: Wharton's jelly mesenchymal stem cells; GF: growth factors; PRP: platelet rich plasma; FG: fibrin glue.

use in clinical applications. Even if these guidelines existed, more research is needed to help understand their basic biology since the therapeutic effects afforded by MSC transplantation are likely to be short-lived and related to dynamic, paracrine interactions between MSCs and host cells [99]. MSCs possess a fibroblastic morphology but the published literature suggests that there is no well-defined phenotype for these cells. More work is needed to characterise the phenotype of these cells.

The International Society for Cellular Therapies has recently proposed a definition for MSCs [102]. While there are no definitive markers of MSCs a range of cell surface markers are routinely used. These include immunopositivity for STRO-1, CD73, CD105, CD106 CD145 and CD166, combined with negative immunoreactivity for CD11b, CD31, CD34, CD45 and CD117. These markers can also be used to identify a more homogeneous population of cells than previous methods utilising either density-gradient centrifugation, or even simple plastic adherence. The general heterogeneity of bone marrow cell populations can lead to variable results; however MSCs are generally regarded to be capable of differentiation along the chondrogenic, osteogenic and adipogenic pathways. A better understanding of the biology of MSCs is likely to improve future cell-based therapies and tissue engineering strategies.

8. Perspectives

Articular cartilage is an avascular load-bearing connective tissue and, as a consequence, it has a very limited capacity for intrinsic repair [103]. It is also highly prone to structural degradation, making it particularly difficult to restore once it is damaged or lost. Full-thickness defects of articular cartilage in the knee have a particularly poor capacity for repair [54]. Therefore, cartilage defects and OA remain major clinical challenges. According to the International Cartilage Repair Society (ICRS; http://www.cartilage.org/) there are a number of new therapy options and many of these have become available for human patients in the last 5 years. A recent a systematic review suggests that microfracture, ACI and osteochondral autografts all have the capacity to achieve a certain degree of short-term success. They can stimulate cartilage repair and restoration (especially in the knee) but the results are highly variable and there are patient-specific and defect-specific factors that influence clinical outcomes [104]. The ultimate outcome of any type of surgical intervention aimed at cartilage repair remains heavily reliant on indication and the surgeon's proficiency in the technical aspects of the chosen surgical procedure [105].

Tissue engineering with chondrocytes and MSCs is now considered to be a promising way of repairing articular cartilage lesions. Cartilage is the ideal tissue for engineering and regeneration. It is avascular, aneural and alymphatic. It contains just one cell type – the chondrocyte [25]. The aim of cell-based therapies for cartilage defects is to repair damaged joint surfaces with a functional tissue capable of withstanding the stresses and strains of joint loading. Chondrocytes have been used clinically in ACI for over two decades. Although ACI is the current 'gold standard' and treatment of choice for the biological repair of chondral defects [104,106], it has shown very mixed results in clinical and experimental studies [107–109]. In general, symptomatic cartilage defects that need surgical intervention are in the range of 1–5 cm^2. Such defects can be treated with ACI or microfracture without restriction although they will require large numbers of undifferentiated cells. This is a major obstacle in the clinic, particularly when sufficient numbers of phenotypically competent cells are not at hand. Currently, chondrocytes removed from a healthy region of the cartilage are used but they are unable to retain their phenotype in expanded culture – they simply dedifferentiate into fibroblastic cells. Some of the published literature suggests that the repair tissue formed by these cells is fibrocartilaginous rather than hyaline, which compromises

long-term repair [103]. In contrast, there are a number of clinical papers that have used histological evaluation to show that the majority of ACI repair tissue is of a hyaline-like to hyaline appearance [110]. Interestingly, the repair tissue had biomechanical properties comparable to surrounding cartilage and superior to those associated with fibrocartilage repair tissue [111].

There is a need for novel methods and procedures that can provide sufficiently large numbers of phenotypically stable chondrocytes or chondroprogenitors capable of effective cartilage repair. The discovery of MSCs and the recognition of their ability for cartilage regeneration have revolutionised the way cartilage problems are viewed. Since they were first identified by Pittenger and co-workers [85] research on adult stem cells has proliferated at a staggering pace. Work using adult stem cells does not involve many of the ethical challenges associated with using embryonic stem cells. Another reason for the rapid expansion in this area is the availability of a range of adult tissues from humans and animals. MSCs also posses neuroprotective [112] and cardioregenerative [113] properties as well as the potential for musculoskeletal regeneration [114]. The use of MSCs may potentially make cartilage repair more widely available.

Therapies using stem cells and chondroprogenitors are currently receiving a huge amount of interest. MSCs and chondroprogenitor cells show considerable promise for use in cartilage repair [115] and are being clinically explored as a new therapeutic for treating a variety of immune mediated diseases. They have potential applications in tissue engineering and regenerative medicine and may represent an attractive option for cartilage repair [116]. Mesenchymal and stromal cells have also been used for equine cell-based cartilage repair. However, at the present time it is unclear which cell type is most suitable for cartilage repair. A distinct population of chondrocyte progenitor cells exists in the superficial (surface) zone of articular cartilage [103,117]. These superficial progenitor-like cells are able to form colonies from an initially low seeding density and can expand in culture without losing their chondrogenic phenotype. They can also maintain the ability to form cartilage when transferred into 3D pellet cultures. This population of cells may be a suitable source for cell-based cartilage repair and perhaps superior to equine bone marrow-derived stromal cells. Chondroprogenitors from articular cartilage itself clearly have major advantages over bone marrow-derived stromal cells. The development of clinical applications that use these cells will be an important step forward in the clinical field of cartilage repair. It makes sense both 'biologically' and 'physiologically' to use a cartilage-derived progenitor cell for reparative purposes rather than the 'one size fits all' paradigm that applies to bone marrow stromal cells. The future is looking bright for cartilage repair especially if intuitive approaches such as this are employed. Refinement of tissue-engineering and cartilage repair approaches promises to further improve surgical outcome for OA patients [118].

One of the fundamental weaknesses of all the tissue engineered and *in vitro* models available to date is that none of them possess the normal zonal organisation of chondrocytes that is seen *in vivo* (*i.e.* superficial, middle, deep and calcified zones) and the local composition of extracellular matrix in each zone. This structural organisation is a pre-requisite for normal cartilage function and the success of any future clinical applications. The currently available 3D models produce fairly homogeneous populations of cells without the ability to achieve any zonal organisation *in vitro* [119]. The ability to produce a construct that replicates the zonal and structural architecture of the original tissue is currently lacking. Even the mechanically stable scaffolds that have been created so far do not allow regeneration of a sufficiently large mass of structurally and functionally competent cartilage construct especially if they were constructed and seeded with 2D passaged (monolayer) chondrocytes in combination with a biomimetic carrier or scaffold [120]. Nevertheless, there are a number of preclinical studies that have shown that 2D passaged articular chondrocytes derived from hyaline cartilage of the knee seeded in biomimetic carriers or scaffolds may form adequate cartilage repair tissue.

Future studies must therefore begin with 3D cultured chondrocytes maintained in a physiologically relevant microenvironment that replicates the ionic, osmotic and biomechanical milieu of cartilage. The 3D and microenvironmental impact on cell phenotype is a significant factor creating cartilage constructs within biomimetic scaffold constructs [120].

Regenerative medicine researchers today must rely on the optimistic view that stem cells, allogeneic tissue transplantation, patient derived MSCs and biomaterials may eventually be used for repairing and regenerating tissues and organs in the future. Although researchers should maintain this optimistic view, it would be prudent to consider the numerous hurdles and complicating factors that need to be overcome as research progresses in this exciting and rapidly expanding field. There are still many technical challenges associated with isolating, expanding, differentiating, and pre-conditioning MSCs for subsequent implantation into degenerate joints. The physiological microenvironment of the degenerate joint 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 joints that were biomechanically compromised to begin with. Future regenerative medicine strategies will need to address these remaining concerns.

9. Conclusions

Chondrocyte and stem cell-based therapies are primarily targeted towards focal cartilage defects. It is important to bear in mind that in many cases joints with such defects may not be afflicted with OA. In the majority of OA cases in elderly patients the disease is much more widespread and inflammatory in nature. Therefore, cell-based therapies may be unrealistic options due to the complex geometry of the osteoarthritic lesion in contrast to the more local focal defects that may be seen in younger (*i.e.* athletic patients). As such, there is a major disconnect in the published literature in term of appropriate uses for cell-based therapies. Clearly, in many cases cell-based therapies may not be suitable or effective for end-stage OA. Nevertheless, further basic research is needed to better inform clinical studies and trials of stem cell-based products.

Contributors

Ali Mobasheri: Conceived, drafted and submitted the paper.

Gauthaman Kalamegam: Read, edited and approved the submission; contributed Fig. 4 and its legend; made a significant intellectual contribution to the manuscript.

Giuseppe Musumeci: Read, edited, and contributed new text and citations; made a significant intellectual contribution to the manuscript.

Mark E. Batt: Read, edited and approved the manuscript for submission; contributed clinical input and strategic overview; made a significant intellectual contribution to the manuscript.

Competing interests

The authors declare no competing interests.

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