



A Comprehensive Review of Stem Cells for Cartilage Regeneration in Osteoarthritis

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Abstract

Osteoarthritis (OA) is an age related joint disease associated with degeneration and loss of articular cartilage. Consequently, OA patients suffer from chronic joint pain and disability. Weight bearing joints and joints that undergo repetitive stress and excessive ‘wear and tear’ are particularly prone to developing OA. Cartilage has a poor regenerative capacity and current pharmacological agents only provide symptomatic pain relief. OA patients that respond poorly to conventional therapies are

ultimately treated with surgical procedures to promote cartilage repair by implantation of artificial joint structures (arthroplasty) or total joint replacement (TJR). In the last two decades, stem cells derived from various tissues with varying differentiation and tissue regeneration potential have been used for the treatment of OA either alone or in combination with natural or synthetic scaffolds to aid cartilage repair. Although stem cells can be differentiated into chondrocytes *in vitro* or aid cartilage regeneration *in vivo*, their

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potential for OA management remains limited as cartilage regenerated by stem cells fails to fully recapitulate the structural and biomechanical properties of the native tissue. Efficient tissue regeneration remains elusive despite the simple design of cartilage, which unlike most other tissues is avascular and aneural, consisting of a single cell type. In this article, we have comprehensively reviewed the types of stem cells that have been proposed or tested for the management of OA, their potential efficacy as well as their limitations. We also touch on the role of biomaterials in cartilage tissue engineering and examine the prospects for their use in cell-based therapies.

Keywords

Chondrocytes · *In vitro* · *In vivo* · Osteoarthritis · Regenerative medicine · Stem cells

Abbreviations

2D	Two dimensional
3D	Three dimensional
ACI	Autologous chondrocyte implantation
ACT	Autologous chondrocyte transplantation
BM	Bone marrow
BMAC	Bone marrow aspirate concentrate
BMP	Bone morphogenetic protein
CD	Cluster of Differentiation
COX-2	Cyclooxygenase-2
CP	Cartilage pellet
EBs	Embryoid bodies
ESCs	Embryonic stem cells
FDA	Food and drug administration
HA	Hyaluronic acid
HSCs	Haematopoietic stem cells
iPSCs	Induced pluripotent stem cells
ISCT	International Society for Cellular Therapy
MMP-13	Matrix metallo-proteinase-13
MSCs	Mesenchymal stem cells
NSAID	Nonsteroidal anti-inflammatory drug

OA	Osteoarthritis
PRP	Platelet rich plasma
SF	Synovial Fluid
SNRIs	Serotonin-norepinephrine reuptake inhibitors
TGF- β	Transforming growth factor beta
TKA	Total knee arthroplasty

1 Introduction

Ageing is inevitable and so is the decline in function of the various organ systems, particularly connective load-bearing tissues in synovial joints of the musculoskeletal system. Articular cartilage is prone to ‘wear and tear’ and the articular cartilage undergoes persistent degeneration. The poor regenerative ability of articular cartilage combined with continued cartilage degeneration leads to the development of the most common joint disease, osteoarthritis (OA). OA is a painful multifactorial degenerative joint disease characterized by low-grade inflammation in cartilage and synovium, which is associated with the loss of joint structure and progressive deterioration of cartilage (Fig. 1) (Musumeci et al. 2015). Traumatic injuries and some sequelae of anti-inflammatory events activate immune cells as well as inducing the secretion of pro-inflammatory cytokines which leads to development of subchondral bone lesions (Shabestari et al. 2016; Zhu et al. 2017). Aberrant metabolism of the synovial joint tissue and cell function is also implicated in the onset of OA (Mobasheri et al. 2017). Irrespective of the aetiology, OA is associated with potentially crippling symptoms such as pain, swelling, stiffness and limited mobility. The incidence of OA increases with age, and the associated altered molecular pathways causes progressive deterioration of the biomechanical properties of cartilage (Lotz and Loeser 2012).

In the United states alone, people aged over 60 years (10% men and 13% women) are most commonly afflicted with knee OA (Zhang and Jordan 2010). A cross sectional study that

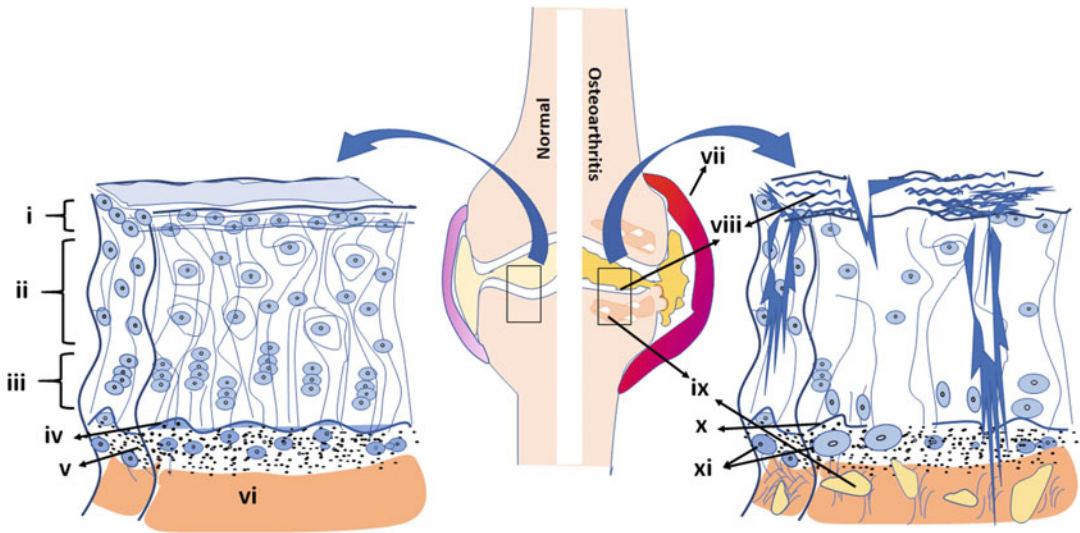


Fig. 1 A normal and osteoarthritic (OA) joint showing the major anatomical structures and the key pathological changes is diagrammatically represented. The enlarged boxed area from the normal and OA knee joint shows cartilage structure and the pattern of cellular arrangement or its derangement respectively. In the normal knee joint the cartilage surface is smooth and clear demarcation exists between the superficial, mid and deep zones. The tidemark, calcified cartilage and the underlying subchondral bone are intact. In the OA knee joint the changes include thickening of the joint capsule, inflammation, cartilage destruction and osteophyte formation. The changes in cartilage include fragmentation (fibrillation),

degradation and loss of chondrocytes (hypocellularity). The changes in the subchondral bone include degradation, cystic degeneration and minor fractures. (i) – Superficial zone (10%–20%) with tangential arrangement of chondrocytes; (ii) – middle zone (40% – 60%) with vertical arrangement of chondrocytes; (iii) – deep zone (30%) with compact and longitudinal arrangement of chondrocytes; (iv) – intact tide mark; (v) – calcified cartilage; (vi) – normal subchondral bone; (vii) – inflamed joint capsule in OA knee joint; (viii) – cartilage degradation and fibrillation; (ix) – subchondral bone cysts; (x) – damaged/absent tide mark; (xi) – hypertrophied chondrocytes

evaluated the prevalence of radiographic OA involving 300 patients from 14 different primary health centres in Saudi Arabia reported that 53.3% men and 60.9% women had clinical evidence of knee OA (Al-Arfaj and Al-Boukai 2002). Another study on 243 patients with radiographic evidence of OA from the Eastern province of Saudi Arabia reported that more than 90% of these patients were obese indicating a strong association between obesity and OA. The incidence of OA was higher in obese female patients (73.09%) than males (41.65%) (Ismail et al. 2006). Other independent risk factors of OA include gender, increased physical activity, trauma, genetic predisposition and lifestyle (Zhang and Jordan 2010). Existing treatment methods fail to cure OA, leading to active research initiatives and integration of multiple

disciplines to find a permanent cure. Regenerative medicine is a translational research solution to some of the protracted or incurable diseases which integrates both cells/stem cells and tissue engineering principles to regenerate tissues/organs and help functional restoration. It is hoped that regenerative medicine will provide strategies for effective regeneration of articular cartilage.

2 Current Treatment of Options for OA

The main methods of OA treatment involve non-pharmacological, pharmacological and surgical measures with the aim of reducing pain and improving tolerance for functional activity.

Non-pharmacological management includes moderate exercises to strengthen the muscles, weight loss and massage therapies (Christensen et al. 2005; Ernst and Posadzki 2011).

2.1 Conventional Pharmacological Agents

Current commonly prescribed pharmacological agents in the management of OA include (i) acetaminophen; (ii) non-steroidal anti-inflammatory agents (NSAIDs); (iii) opioid analgesics; (iv) serotonin-norepinephrine reuptake inhibitors (SNRIs) and (v) intra-articular injections (Zhang et al. 2016). Acetaminophen which is normally used to control fever, headache and muscle aches is also used in the treatment of mild OA. However, acetaminophen cannot be used long term due to its hepatotoxic side-effects. NSAIDs are used in mild to moderate cases of OA and appear to be more effective than acetaminophen as they possess anti-inflammatory effects in addition to analgesic properties. Prolonged treatment with NSAIDs is not desirable due to the associated incidence of gastritis and gastrointestinal bleeding. Selective cyclooxygenase-2 (COX-2) inhibitors such as celecoxib, etoricoxib and pamacoxib are effective and relatively safe than less selective NSAIDs (Lee et al. 2017; Huang and Tso 2018). Opioid agents are used in moderate to severe OA, when acetaminophen and NSAIDs fail to provide symptomatic relief. However, the associated side effects such as nausea, vomiting, drowsiness, constipation and addiction risks limit their potential use (DeLemos et al. 2011). Placebo controlled randomized clinical trials have identified duloxetine an SNRI agent observed to be effective in the management of OA, but studies on its long-term safety and recommendation for use in OA are currently lacking (Chappell et al. 2011). Intra-articular injections of hyaluronic acid (HA) or corticosteroids are used for the treatment of moderate to severe OA, however, they have variable acceptance and efficacy (Zhang et al. 2016).

2.2 New Biological and Pharmacological Agents

Lack of sustained benefits with conventional pharmacological agents have led to the development of novel agents that can limit OA disease progression at the molecular level and broadly these agents are known to stimulate chondrogenesis, inhibit apoptosis, inhibit matrix degradation and reduce inflammation. Biological recombinant agents [bone morphogenetic protein-7 (OP-1); fibroblast growth factor 18 (Sprifermin)] and autologous platelet rich plasma (PRP) act as inducers of chondrogenesis (Zhang et al. 2016). Novel pharmacological agents include the disintegrin and metalloproteinase with thrombospondin motifs 5 - (ADAMTS-5) inhibitor (compound 114,810) and matrix metalloproteinase 13 (MMP-13) inhibitor (compound CL82198) which inhibit apoptosis and matrix degradation respectively. Interleukin-1 beta receptor antibody (AMG108) and ultrafiltrate of human serum albumin (Ampion) are known to exert their effects by modulation of inflammatory cytokines (Zhang et al. 2016).

2.3 Surgical Management of OA

Surgical intervention is indicated in severe OA with full thickness cartilage loss, concomitant to failures with non-pharmacological and pharmacological methods leading to disease progression. Knee realignment or joint replacement surgeries are common in patients with end stage OA, which helps in the relief of arthritic pain and improvement of function. Approximately 700,000 total knee arthroplasties (TKAs) were performed in the united states in the year 2010, of which nearly 50% were reported to be in individuals who were less than 65 years of age (Nguyen et al. 2016). The number of TKA procedures continues to rise irrespective of the aetiology of OA, despite the excessive cost involved. Recent advancements in regenerative medicine using cell-based therapies may provide an alternative and efficient treatment strategy thereby reducing the need for TKA.

Regenerative therapy uses various cell types to enable both structural and functional recovery. Cell therapies using both mature cells and stem cells have been practised for nearly three decades. Cell therapy may alleviate the symptoms associated with OA and offer a long term solution (Jiang et al. 2011).

2.4 Autologous Chondrocyte Implantation (ACI)

In the early 90s, autologous chondrocytes were used for autologous implantation/transplantation (ACI/ACT) which induced hyaline cartilage regeneration. Chondrocytes removed from adjacent healthy cartilage using arthroscopy were expanded in culture and subsequently implanted at the site of lesion and covered with a periosteal flap (First generation ACI) (Brittberg et al. 1994). However, the procedure utilising periosteal flap for ACI was associated with cartilage hypertrophy. Subsequent changes to the procedure were made including the use of a bilayer collagen membrane instead of the periosteal flap (Second generation ACI) (Gooding et al. 2006; Niemeyer et al. 2014) or a composite chondrocyte laden scaffold in which chondrocytes were previously cultured upon biodegradable matrix prior to implantation (Third generation ACI) (Brittberg 2008; Mistry et al. 2017). Unlike the first and second generation ACI methods whereby the membranes need to be sutured, the matrix-induced autologous chondrocyte transplantation/implantation (MACT, third generation ACI) utilized fibrin glue to hold the cell laden matrix in place. Although the clinical outcome with ACI was effective their utilization was limited due to insufficient numbers of chondrocytes as well as the tendency of chondrocytes to dedifferentiate upon *in vitro* culture.

3 Stem Cells for Articular Cartilage Regeneration

Cartilage by nature has poor regeneration capacity. Cartilage tissue being avascular does not

incite an immediate inflammatory response when there is an injury/lesion, unlike other tissues with vascular supply (Poulet and Staines 2016). As such the key players of tissue regeneration namely stem cells are not recruited to the site of lesion and hence it becomes a necessity to use stem cells isolated from other sources to aid cartilage regeneration. Stem cells are undifferentiated cells that are capable of self-renewal and differentiation towards a specific cell lineage. Stem cells have variable differentiation potential and accordingly they can be classified as pluripotent (ESCs, iPSCs) as these cells can give rise to almost all the tissues of the human body or multipotent (eg. adult and foetal MSCs) as these cells can be differentiated only into specific cell lineages. Stem cells are therefore an attractive choice for regenerative medicine applications and it is essential to understand the different types of stem cells that have the potential to be used for cartilage regeneration.

3.1 Types of Stem Cells

Embryonic stem cells (ESCs) are derived from day 5 blastocyst stage embryos that have a clearly demarcated inner cell mass, a trophectoderm and a blastocoelic cavity (Bongso 2006; Thomson et al. 1998). To qualify as *bona fide* ESCs, ESCs must be (i) derived from a pluripotent cell population, (ii) maintain normal karyotype, (iii) propagated indefinitely in the embryonic state, (iv) and capable of spontaneous differentiation into cells representing all three embryonic germ layers namely ectoderm, mesoderm and endoderm in teratomas or *in vitro* (Pera et al. 2000). Mesenchymal stem cells refer to hypothetical common progenitors originating from a wide range of mesenchymal (mesodermal, non-epithelial, non-haematopoietic) tissues (Budd et al. 2017) and have been recorded to have been isolated from the niches of various tissues including bone marrow, brain, liver, bone, retina, pancreas, adipose tissue, epidermis, synovial fluid, amniotic fluid, umbilical cord and placenta (Bianco et al. 2008; De Coppi et al. 2007; Kern et al. 2006). Not to be confused with

the heterogeneous population of cultured plastic adherent cells isolated from bone, referred to as bone marrow stromal cells (BMSCs) from which a diminutive population of bone marrow-derived stem cells exist, often also referred to as MSCs. Due to the controversy surrounding the MSC identity and differentiation potential, some research groups utilise the term skeletal stem cell (SSC) or bone marrow-derived stem cell (BMSC) to refer to this stem cell population isolated from bone marrow which possess specific differentiation potential towards skeletal tissues inclusive of cartilage, bone and marrow adipocytes (Bianco and Robey 2015). An alternative to adult tissue as a source for stem cells is foetal tissue, which has been shown to contain stem cell populations with enhanced plasticity, proliferation and expansion potential compared to stem cells derived from adult sources (Guillot et al. 2007). Stem cells with similar potential to that of ESCs are induced pluripotent stem cells (iPSCs) (Takahashi et al. 2007). However, unlike ESCs, iPSCs possess multipotency which has been induced artificially through the introduction of Oct3/4, c-Myc, Klf4 and Sox2, genes which encode transcription factors involved with maintaining pluripotency (Takahashi and Yamanaka 2006). The generation of iPSCs from human somatic cells (Takahashi et al. 2007) was a major step towards tissue personalization, opening up the potential to use autologous stem cells in applications which may be otherwise impeded by cell rejection following transplantation. In recent years the use of stem cells in orthopaedics has gained significant attention as a potential application for cartilage repair. To achieve efficient cartilage regeneration different types of stem cells have been explored using both *in vitro* and *in vivo* studies.

3.2 Pluripotent Stem Cells and Cartilage Repair/Regeneration

Human ESCs (BresaGen variant cell line, BG01V) were differentiated into chondrocytes *in vitro* using either embryoid bodies (EBs),

micromass or pellet cultures with defined xenofree media containing Knockout serum replacement and growth factors including transforming growth factors (TGF- β 3, TGF- β 1) and bone morphogenetic protein (BMP-2) for up to 21 days (Suchorska et al. 2017). The authors reported that differentiation into chondrocytes was more efficient with EBs followed by pellet and micromass cultures. However, chondrocytic differentiation *via* EBs involved an additional step of EBs generation prior to their differentiation, unlike pellet or micromass cultures which can be directly differentiated (Suchorska et al. 2017). Transplantation of undifferentiated ESCs embedded in fibrin glue into the knee joint in an ovine osteochondral defect model resulted in successful hyaline cartilage regeneration and integration compared to sham controls after 24 months (Manunta et al. 2016). iPSCs derived from cord blood mononuclear cells were also demonstrated to be successfully differentiated into cartilage upon pellet culture for 30 days. Embryoid bodies (EBs) were generated from the iPSCs and the outgrowth cells from EBs were used in a pellet culture system for cartilage differentiation. The differentiated cells expressed cartilage associated genes and extracellular matrix indicating efficient cartilage differentiation (Nam et al. 2017). *In vitro* differentiation of human iPSCs and transplantation into articular joints of immunodeficient mice resulted in hyaline cartilage differentiation at 8 weeks but there was teratoma formation at 16 weeks (Saito et al. 2015). Undifferentiated ESCs produce teratomas upon transplantation to immunocompromised mice, thus making it mandatory to ensure complete differentiation of ESCs into target tissues or removal of any undifferentiated ESCs prior to transplantation, to render them safe for use in cell-based therapies (Aldahmash et al. 2013).

3.3 Multipotent Stem Cells and Cartilage Differentiation

MSCs have long been considered as an attractive choice of stem cell for cartilage repair and regeneration as they are multi-potent and have anti-

inflammatory and immunomodulatory effects. Adult MSCs have been used for cartilage regeneration as early as 1990s, however, there are no *bona fide* cell surface markers to identify or isolate these MSCs. Their identification is mainly dependent upon the minimal essential criteria stipulated by the International Society for Cellular Therapy (ISCT), namely plastic adherence; differentiation into three different mesodermal lineages; osteoblasts, chondrocytes, adipocytes and expression of CD73, CD90, CD105 and lack expression of CD34 as well as being HLA-DR negative (Dominici et al. 2006). MSC progenitors are reported to reside within articular cartilage, however, their contribution to cartilage regeneration following disease/damage appears insufficient (Grogan et al. 2009). Human BM-MSCs cultured as micromass pellets in the presence of transforming growth factor-beta 3 (TGF- β 3) and dexamethasone differentiated into chondrocytes *in vitro* and expressed chondrocyte associated genes and collagen (Mackay et al. 1998). The use of nanoscaffolds, which mimic the ECM, in combination with human BM-MSCs in the presence of growth factors was observed to achieve successful chondrocyte differentiation (Wise et al. 2008).

In addition to BM-MSCs, MSCs derived from other sources such as adipose derived MSCs (Diekman et al. 2009), synovial fluid MSCs (Lee et al. 2012) and umbilical cord derived Wharton's jelly MSCs (Fong et al. 2012), have also been successfully differentiated into chondrocytes. Intra-articular injection of BM-MSCs in sheep resulted in the regeneration of cartilage with normal histoarchitecture, especially when supplemented with growth factors such as TGF- β 3 and insulin-like growth factor-1 (Al Faqeh et al. 2012). However, the use of adult derived MSCs can be limiting in that isolation of total MSCs from human tissue varies in number as a result of variability amongst individuals from which MSCs are derived and subculture of MSCs for expansion purposes induces morphological and phenotypic changes and may impact upon MSC senescence. MSCs derived from ESCs provide an additional stem cell source with great proliferation capabilities unlike adult MSCs and

hence offer enormous potential for biomedical research. Chondrogenic differentiation of MSCs derived from ESCs with a combination of TGF- β 1 and BMP-7 led to increased mRNA expression of collagen type 2, aggrecan and SOX9 compared to differentiation of MSCs with TGF- β 1 alone. The enhanced chondrogenesis with a combination of TGF- β 1 and BMP-7 was due to increased expression of TGF β R2 and the production of endogenous TGF- β (Lee and Li 2017).

3.4 Stem Cell Initiative for Cartilage Differentiation at King Abdulaziz University

Our stem cell unit in the department of orthopaedics at King Abdulaziz University hospital Jeddah Saudi Arabia, is a nascent facility and given our interests in this emerging field we have derived MSCs from bone marrow (BM) and synovial fluid (SF) samples obtained from both healthy and OA patients. Although, both BM-MSCs and SF-MSCs fulfilled the minimal criteria stipulated by ISCT (Dominici et al. 2006) their expansion *in vitro* was limited and demonstrated cellular senescence after subpassages (Fig. 2a). This could be attributed to the age of the patients (50–75 years) from which cells were derived. As microenvironmental cues are reported to play an essential role in effective chondrocyte differentiation (Watt and Huck 2013), we evaluated an explant culture model (Fig. 2b) where the osteochondral plugs obtained from OA patients were subjected to microdrill defect and the cells (BM-MSCs, cartilage pellet or a combination of both) obtained from the same patient were used for chondrocyte differentiation (Abbas 2017). Combination of both BM-MSCs and cartilage pellet demonstrated good differentiation into chondrocytes with increased cartilage matrix proteins secretions indicating that micro-environmental cues effectively contribute to the differentiation process (Abbas 2017). With insights obtained from both *in vitro* studies we then evaluated the combined effects of BM-MSCs and cartilage pellet

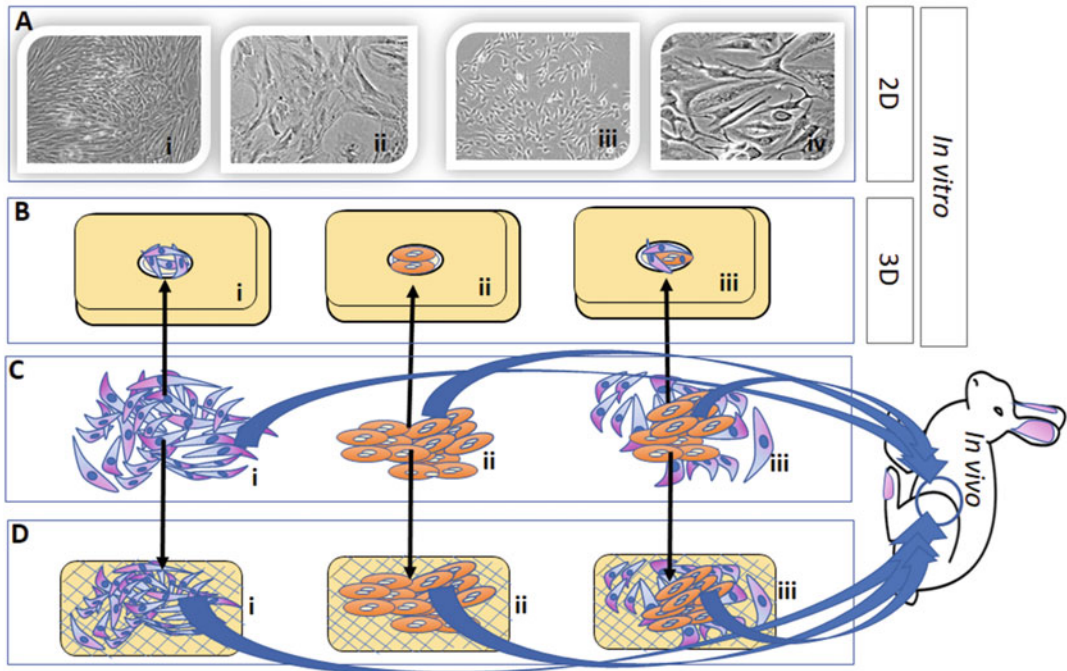


Fig. 2 Diagrammatic representation of the *in vitro* and *in vivo* models to evaluate cartilage regeneration. **Ai and Aii:** Phase contrast images of the bone marrow mesenchymal stem cells (BM-MSCs) at early (P0) and late (P6) passages. **Aiii and Aiv:** Phase contrast images of the synovial fluid mesenchymal stem cells (SF-MSCs) at early (P0) and late (P5) passages. The cells in late passages of both BM-MSCs and SF-MSCs showed broad flattened morphology that were not actively dividing in culture which was indicative of cellular senescence. **B:** Diagram representing the osteochondral plugs obtained from OA patients undergoing total knee arthroplasty (TKA). A

central drill defect of 2 mm was made and filled with **(Bi)** – pelleted BM-MSCs; **(Bii)** – homogenized cartilage pellet (CP); or **(Biii)** – a combination of BM-MSCs and cartilage pellet to evaluate cartilage repair *in vitro*. The *In vivo* cartilage repair was evaluated following full thickness surgical defect of the articular cartilage over the Tibial surface in NZW rabbits. The defects were filled with either **(Ci)** – BM-MSCs; **(Di)** – BM-MSCs and Hyalofast™; **(Cii)** – CP; **(Dii)** – CP and Hyalofast™; **(Ciii)** – combination of BM-MSCs and CP; or **(Diii)** – Combination of BM-MSCs, CP and Hyalofast™

(CP) *in vivo* in rabbits (Fig. 2c), which also demonstrated improved cartilage regeneration. In addition, we also evaluated the efficiency of Hyalofast™, a biodegradable scaffold, together with BM-MSCs and (CP) in repair/regeneration of full-thickness cartilage defect *in vivo* in rabbits (Fig. 2d). Efficient and accelerated regeneration/repair of the defective cartilage was observed with combinations of Hyalofast™, BM-MSCs and CP, rather when used separately [unpublished results].

In general, the current cell-based repair strategies largely remain unsuccessful, especially when cartilage repair is undertaken in an autologous setting given the limitations in cell

expansion and the excessive cost involved with the use of human grade culture media and supplements. Therefore, other alternative avenues of research are also being explored for cartilage repair/regeneration.

4 Cartilage Tissue Engineering

4.1 Bioprinting of Articular Cartilage

Despite significant advances using various approaches including tissue engineering and regenerative medicine, repair of articular cartilage and the osteochondral interface specifically

remains a major challenge (Apelgren et al. 2017; Daly et al. 2016; Zhang et al. 2017). Most of the inefficiency is derived from artificial matrices currently available often leading to inadequate healing and tissue regeneration. Various classes of biomaterials, such as hydrogels, can be tuned to provide mechanical stability and bioactivity (Duarte Campos et al. 2012; Memic et al. 2015; Schon et al. 2017; Yang et al. 2017). Furthermore, current three-dimensional (3D) approaches with cell-laden biomaterials are a significant step forward from conventional two-dimensional (2D) methods. However, for clinical translation these tissue engineered constructs often require large numbers of cells with complex structural hierarchies (Mouser et al. 2017; Richardson et al. 2016; Yang et al. 2017). In this regard, technologies focusing on 3D bioprinting approaches have the potential to offer several advantages that could address the limitations of traditional biomanufacturing methods. Ultimately, these novel constructs could prove to be better mimics of the native environment and provide improved clinical outcomes.

To develop clinically relevant tissue mimics, several 3D bioprinting strategies exist (Apelgren et al. 2017; Mouser et al. 2017; Schon et al. 2017; Zhang et al. 2017). By combining several biomaterial sources and cell types we can develop better articular cartilage tissue mimics. Similarly, these bioprinting methods could be used to recapitulate and study the diseased state, having characteristics that better mimic *in vivo* conditions (Arslan-Yildiz et al. 2016; Kang et al. 2016; Mehrali et al. 2016; Memic et al. 2017; Murphy and Atala 2014; Ozbolat et al. 2016; Zhang et al. 2017). As such they could provide answers related to the underlying biology that plays a role in articular cartilage repair and regeneration. In addition to providing modifiable biophysical cues, mechanical stiffness and bioactivity, 3D bioprinting allows for assembly of complex native-like architectures (Duarte Campos et al. 2012; Mouser et al. 2017; Yang et al. 2017). This is one of the major advantages

of 3D bioprinting technologies whereby well-defined geometries with gradient composition of biomaterials and cells can be achieved during construct manufacturing with complex structural features (Jang et al. 2016). Ultimately, 3D bioprinting could be combined with patient derived cells, custom bioinks and biosensing technologies that could be the stepping stone for the development of truly personalized medicine applications (Bertassoni et al. 2014; Hasan et al. 2015; Park et al. 2016; Vaidya 2015).

A recent report showed that MSCs could be bioprinted within silk fibroin-gelatin hydrogel constructs (Das et al. 2015). The 3D bioprinted constructs were crosslinked *in situ* by tyrosinase or sonication maintaining good viability of encapsulated MSCs over 30 days during *in vitro* culture. Next, these MSCs could be guided into either chondrocyte and/or osteoblast lineage by using differentiation medium. Similarly, other studies have shown that bioprinting cell-laden alginate-based biomaterials, fabrication of cartilage-like tissue with a complex geometry could be possible (Markstedt et al. 2015). In this study, the bioprinted chondrocytes had long-time viability. Other strategies focus on bioprinting parameters that allow fabrication of gradient compositions and structures via active and efficient mixing of complex fluids. Such techniques have improved bioprinting efficiency and thus hold much potential towards the development of tissue-engineered zonal osteochondral/cartilage complex structures (Ober et al. 2015).

In future, once the convergence of several bioprinting technologies takes place (Daly et al. 2016; Duchi et al. 2017; Memic et al. 2017; Schon et al. 2017), we expect progress in the development of constructs with patient specific cells and custom bioinks that have improved structural resolution and mechanical integrity during bioprinting. Taken together advances in the field of articular cartilage repair and regeneration may be quickly translated into the clinic with significantly improved biological, physiological and personalized treatments and outcomes.

4.2 Cell Free Biomaterial Approaches for Articular Cartilage Repair

Although cell-based techniques using both MSCs and autologous chondrocytes have shown a lot of potential for clinical translation and application, challenges remain in developing novel clinical products. Often cell-based approaches are expensive and require a substantial amount of time for extraction, proliferation and differentiation of primary cells and/or MSCs (Foyt et al. 2018; Makris et al. 2015; Murphy et al. 2017). If these products are ultimately considered as biologicals, instead of as devices, by regulatory agencies it could further delay their approval and significantly increase the cost associated with their clinical translation. Considering these challenges, it appears that biomaterials built on cell-free platforms might provide substantial advantages for cartilage repair and regeneration (Calabrese et al. 2017; Makris et al. 2015; Mathis et al. 2017; Murphy et al. 2017).

Cell-free based biomaterial methods usually rely on controlled release of growth factors, serum or small bioactive molecules such as drugs that are able to modulate the local microenvironment in order to stimulate cartilage healing (Armiento et al. 2018; Duarte Campos et al. 2012; Makris et al. 2015). For example, biomaterials can rely on selective stimulation and recruitment of MSCs in order to exert therapeutic effects. If these biomaterials are further coupled with controlled release of tailored growth factors they could be able to activate chondrocytes in the local healthy microenvironment promoting tissue remodeling, repair and ultimately closure of the cartilage defect (Armiento et al. 2018).

One cell-free strategy is autologous matrix-induced chondrogenesis (AMIC) that aims to provide both early mechanical stability and long-term regeneration of cartilage. The procedures after the initial cleaning are based on inducing microfractures that release MSCs, blood, and serum, at the defect site that can then be sealed with a biomaterial (Patrascu et al. 2010). For example, a biomaterial implant that combines platelet-rich plasma or autologous serum with

hyaluronic acid is placed at the defect site after initial microfractures are generated (Patrascu et al. 2010). The role of this biomaterial combination is twofold; first, the platelet-rich plasma and/or autologous serum can aid in the recruitment of bone marrow MSCs found in the underlying subchondral bone, (Patel et al. 2013) and secondly hyaluronic acid might promote their differentiation into chondrocyte-like phenotype at the defect site (Patrascu et al. 2013). In a recent clinical trial based on this treatment procedure involving 52 patients 1-year post-surgery an improvement in patient-reported Knee injury and Osteoarthritis Outcome Scores (KOOS) was observed. At the defect site hyaline-like repair tissue appeared to be present when analyzed using histological methods (Siclari et al. 2012). However, to demonstrate long term effectiveness of this approach more controlled clinical trials are required with extended time periods to prove the durability of this new tissue. Another similar clinical trial looked at the placement of clot-like structures made from the combination of chitosan and autologous whole blood that was drawn immediately before placement into the defect site (Buschmann et al. 2007). One year follow up reported that the chitosan-based approach had increased lesion fillings and improved tissue repair compared to microfractures alone (Stanish et al. 2013). However, in terms of a functional outcome there was little difference in the two sets of patients. This is especially confounding when considering that microfractures begin to degenerate in as little as 2 years post-surgery requiring extended follow up.

Significant room remains for improvement of AMIC and other cell-free biomaterials-based approaches for repair and regeneration of articular cartilage. More in-depth studies are required in assessing how different concentrations, release parameters and combinations of chemoattractants and chemokines including bone morphogenetic proteins and growth factors incorporated within biomaterial platform affect the action of MSCs in cartilage repair (Filardo et al. 2012; Richter 2009). Perhaps, personalized strategies can be developed for a certain set of

conditions and/or anatomical locations. Although some of these approaches like AMIC are FDA-approved additional carefully designed pre-clinical studies are needed to investigate their efficacy.

5 Conclusions

Very few connective tissues have an inherent regenerative ability and if they do, this repair and regeneration potential decreases with age. Tissue engineering and regenerative medicine is intended to facilitate the restoration of many tissues including those of the musculoskeletal system. Effective articular cartilage regeneration with the use of stem cells has still not been achieved, and more fibrotic/hyperplastic changes tend to occur with time. In addition, the problems associated with cell expansion, culture induced phenotypic changes and the high cost of using of human grade culture media and supplements appear to delay the progress of research in this area. Harvest of multinucleated cells from within the bone-marrow and direct injection to the cartilage defect area using the bone-marrow aspirate concentrate (BMAC) system is gaining prominence amongst the clinicians as it helps to circumvent the labour-intensive protocols with cell culture and the associated high cost. However, the interaction of the transplanted cells with the host tissue, their differentiation into articular cartilage, fulfilment of the bio-mechanical properties and long-term benefits remains to be understood. Continued research involving cellular therapies and/or biomaterials therefore is mandatory for identifying a successful technique to regenerate cartilage.

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