UTSA Journal of Undergraduate Research and Scholarly Works
Volume 8 **December 2022**

Stem Cells used for Tissue Engineering of Articular Cartilage: Literature Review

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Abstract

Adult articular cartilage (AC) has a limited self-healing capacity. Cartilage defects lead to osteoarthritis (OA) characterized by severe pain and impaired mobility. Currently, there are no approved treatments for OA that successfully reverse or heal structural defects permanently. Although techniques such as microfracture, arthroplasty and subchondral drilling have been effective at treating small to intermediate sized AC defects over the short term, a long-term solution for OA is still necessary. In recent years, research has focused on tissue engineering of articular cartilage (TEAC) as a potential treatment option for OA. TEAC therapies utilizing chondrocytes such as autologous chondrocyte implantation (ACI) are promising but are limited by their complexity, high cost and inability to promote the formation of healthy hyaline AC. Due to the limitations of ACI, stem cells have been investigated as an alternative cell source for TEAC. To engineer AC, stem cells are allowed to differentiate on/in a scaffold in a bioreactor that controls chemical, physical and biological cues to support the chondrogenic potential of the stem cells. The use of stem cells provides numerous advantages as treatment costs can be lowered, the number of required surgeries can be reduced and high-quality AC can be formed. Mesenchymal stem cells (MSC) in particular are advantageous in that they are easily available and can be extracted from a diverse range of tissues including, bone marrow, adipose, and synovium. Each type of MSC have their own advantages and disadvantages but generally each of them possess high chondrogenic potential and immunosuppressive capacities. Induced pluripotent stem cells (iPSC) have also been recognized as a promising cell type for TEAC due to their unlimited proliferation and self-renewal capacities. Ultimately, each cell source has potential for use in TEAC therapies but further studies comparing cell sources are required before a gold standard can be determined. This review summarizes the pros and cons for potential use of each stem cell source in TEAC. The review is not meant to be comprehensive of the current literature.

Keywords: Articular cartilage, osteoarthritis, stem cells and tissue engineering.

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Introduction

Healthy hyaline articular cartilage (AC) serves as a cushion between joints where it exhibits high lubrication and resistance to wear.[1] The superior biomechanical properties of AC allows it to act as an effective shock absorber and to distribute easily forces exhibited by the joint.[2] The AC of a healthy adult has a limited self-healing capacity due to its lack of vasculature, neurons and lymphatics.[3, 4] Due to the limited healing potential of AC in adults, cartilage defects commonly lead to osteoarthritis (OA).[4] OA is known to be the most prevalent human joint disease, affecting an estimated 240 million people worldwide.[5, 6] OA results in the progressive degeneration of AC due to an anabolic/catabolic imbalance.[4] The gradual loss of an AC tissue commonly leads to full-thickness lesions and osteophytes that promote direct bone-to-bone contact causing severe pain, impaired mobility, swelling and joint stiffness.[6]

Currently, there are no approved treatments for OA that successfully reverse structural defects.[7] Clinicians generally prescribe medications, such as anti-inflammatory drugs (non-steroidal anti-inflammatory drugs (NSAIDs)), corticosteroid injections and hyaluronic acid injections to help manage pain associated with OA.[8] Lifestyle changes such as an increase in exercise and physiotherapy may also reduce the symptoms of OA.[6] In order to reduce pain and treat symptoms, total joint replacement has been identified as the most effective method of improving quality of life for patients suffering from OA. However, joint replacement procedures carry the risk of perioperative mortality and implanted joints in younger patients generally need to be replaced after about 10 years.[9] In recent years, the treatment of OA has become an interesting topic to researchers. Surgical attempts at solving the problem of OA include abrasion arthroplasty, microfracture, mosaicplasty and arthroscopic subchondral drilling; each of which has its own respective limitations as listed in Table 1.

Table 1: Limitations of common surgical treatment approaches for AC defects

Treatment Method	Limitations
Arthroplasty	• Formation of fibrocartilage that is mechanically inferior to hyaline AC. [10, 11] • Only short-term functional improvements.[11, 12]
Microfracture	• High-inter patient variability.[13] • Formation of fibrocartilage that is mechanically inferior to hyaline AC.[11, 14] • Only short-term functional improvements.[11, 12] • Only suitable for small AC defects.[11, 14]
Mosaicplasty	• Donor site soreness.[11] • Limited donor tissue availability.[11] • Only suitable for small and intermediate sized defects.[15]
Arthroscopic Subchondral Drilling	○ Formation of fibrocartilage that is mechanically inferior to hyaline AC.[11] ○ Only short-term functional improvements.[12]

Autologous Chondrocyte Implantation	• Long Recovery Time.[16] • High cost.[17] • Multiple invasive surgeries.[16] • Dedifferentiation of chondrocytes leads to formation of fibrocartilage which is mechanically inferior to hyaline AC.[18]

An interesting approach for the potential treatment of OA is the use of autologous chondrocyte implantation (ACI). In this technique, chondrocytes are extracted from a low weight-bearing region of a patient's joint by removing a full thickness sample of AC *via* biopsy.[19] Chondrocytes from the sample are then isolated and expanded *in vivo*, which yields 12-48 million cells. A second operation is then performed in which a surgeon implants the expanded chondrocyte population into the AC defect. Chondrocytes are either covered by a cap (1st and 2nd generated) or are loaded onto a membrane prior to implantation (3rd generation, currently off label in USA[19]).[20] Compared to microfracture, ACI is one of the most popular alternative techniques proposed for the treatment of OA. It proves to be more cost effective and was shown to provide a significantly better clinical outcome in the treatment of AC defects in femoral condyles at 36 months than microfracture. [20-22]Additionally, as chondrocytes are extracted from the patient's own tissue, there is a limited risk of immune rejection which provides a major advantage compared to the implantation of allogenic cells.[19] Although ACI is a promising method for the repair of AC in OA defects, it has several limitations. First, ACI is a complex procedure that requires two operations and therefore a long recovery time of 6-12 months. Second, the number of chondrocytes that can be harvested from the patient's joint are limited and therefore expansion is necessary in order to obtain a suitable number of chondrocytes for cartilage repair. During expansion, dedifferentiation of chondrocytes into fibrochondrocytes or hypertrophic cartilage commonly occurs and thereby reduces the chondrogenic potential of the extracted cells and makes them unsuitable for ACI.[23] Third, ACI can also lead to complications such as periosteal graft hypertrophy if a periosteal cap is used to seal implanted chondrocytes in the AC defect. This adverse effect can be avoided using an allogenic scaffold however, these scaffolds increase likelihood of an unwanted immune response.[24]

The limitations of ACI call for an alternative option for the repair of AC and OA defects. Tissue engineering of AC *in vitro* using stem cells provides a promising option. To engineer AC, stem cells are allowed to differentiate on/in a scaffold in a bioreactor that controls chemical, physical and biological cues to support their chondrogenic potential. Several types of adult-stem cells such as: bone marrow derived mesenchymal stem cells (BM-MSCs), adipose derived mesenchymal stem cells (AD-MSCs), synovium derived mesenchymal stem cells (SD-MSCs) and human dermal stem cells have been found to have chondrogenic potential when cultured under certain conditions and treated with chondroinductive agents.[25] Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) can also be induced to differentiate into chondrocytes when exposed to certain growth factors and environmental conditions.[26] In this treatment method, the selected cell type is expanded *in vitro* and seeded onto a 3D scaffold or cell sheet. Alternatively, cells may be expanded into a cell dense scaffold-free architecture. The construct, or scaffold-free cells, are then implanted into the tissue defect and the construct is

slowly degraded while neocartilage formation occurs.[2] Stem cell therapies may be less invasive than ACI and therefore may be more appropriate for the treatment of AC defects.[27]

In this review, different stem cell types used in tissue engineering of articular cartilage (TEAC) strategies for the treatment of AC defects will be discussed. Current procedures for stem cell-based TEAC will be presented and the advantages and disadvantages of each cell source used for treatments will be evaluated.

Mesenchymal Stem Cells

In recent years, stem cell therapy has become a promising approach for the repair of AC injuries. Stem cells have the distinct ability to generate hyaline-like cartilage under certain conditions.[28] Mesenchymal Stem Cells (MSCs) are one promising cell source for the tissue engineering of AC. The international cellular society for cellular therapy defines MSCs using minimal criteria. These are: MSCs need to adhere to plastic in culture dishes; express certain cell surface markers such as cluster of differentiation (CD) 10, 73 and 90[29]; and must possess osteogenic, adipogenic, chondrogenic and myogenic differentiation potential. Although MSCs are referred to as stem cells, they are more akin to stem cell containing populations;the number of stem cells each MSC contains depends on the tissue from which they were extracted.

MSCs have the ability to be differentiated to chondrocytes in the presence of certain growth factors and environmental conditions which makes them suitable for the repair of AC.[28] Additionally, MSCs have relatively high expansion rates and have been shown to have many properties encouraging their use for AC repair such as self-renewal capacities and immunosuppressive characteristics.[30] MSCs are easily extracted from a diverse range of tissues, including bone marrow, adipose, synovium and human umbilical cord blood.[31] Given the chondrogenic potential of MSCs and their ease of availability, there have been several studies focusing on their use in the treatment of chondral defects.[32-37]Examples of these studies will be provided below.

Autologous MSCs vs. Allogenic MSCs

When harvesting MSCs from a human, it is important to recognize that the cell donor plays a role in treatment outcomes. MSCs can be extracted autologously from the patient being treated, or allogenically from healthy donors. It is still debated whether autologous MSCs or allogenic MSCs are more suitable for the treatment of AC defects, and therefore for use in TEAC. Autologous MSCs have been used in more clinical trials, likely due to their reduced risk of immune rejection and potential disease transmission when compared to allogenic MSCs.[38] Chahal et. al conducted a phase I/IIa clinical trial to investigate the treatment of 12 patients with late stage Kellgreen-Lawrence knee osteoarthritis using a single intra-articular injection of either 1, 10, or 50 million autologous bone marrow derived-MSCs. [39] It was found that, 12 months after injection, patients reported significantly improved outcome measures without any serious adverse effects suggesting that autologous MSCs may be a promising, safe cell source for the

treatment of AC defects. Interestingly, it was found that patients injected with 50 million BM-MSCs had significantly lower AC catabolic biomarkers and magnetic resonance imaging (MRI) synovitis than patients injected with 1 or 10 million BM-MSCs, which may suggest that the number of MSCs patients are treated with play a role in treatment outcomes. Several other studies have also demonstrated the therapeutic potential and long-term safety of autologous MSC therapy.[34, 40-42].

There has also been considerable interest in the use of allogenic MSCs due to their increased logistical convenience, in that they may be used "off the shelf" in a clinical environment without delays caused by MSC extraction procedures.[43] The use of allogenic MSCs can reduce or eliminate the need for cell expansion due to the surplus of cells from healthy donors, therefore decreasing treatment costs.[44]Allogenic therapy also only requires one implantation procedure, eliminating the need for MSC harvesting from OA patients and therefore decreasing the risk of donor site morbidity along with treatment costs.[45] Additionally, the self-renewal capacity and differentiation potential of MSCs is known to decrease with age; given that increasing age is a risk factor for OA, allogenic strategies for AC repair may offer higher quality MSCs and therefore more effective treatment for AC defects. [46, 47] Although limited in number, several clinical trials have been conducted using allogenic MSCs to treat AC defects. For example, Vega et. al conducted a randomized controlled trial on 30 patients suffering from chronic knee pain related to OA. [48] Patients were treated with an intra-articular injection of 40 million allogenic BM-MSCs obtained from healthy donors. A year after treatment, quantitative MRI T2 mapping indicated an increase in AC quality at the defect site, as illustrated by a decrease in T2 relaxation time, for patients treated with MSCs compared to patients in the control group. Although multiple clinical trials have demonstrated the utility and short-term safety of allogenic MSC therapy, long-term safety follow ups are still necessary before these cells can be widely utilized.[49]

Bone Marrow Derived MSCs (BM-MSCs)

BM-MSCs are a type of MSC isolated from the bone marrow and have several properties that make them suitable for use in AC repair. BM-MSCs have the capacity to undergo chondrogenesis when exposed to growth factors such as insulin growth factor 1 (IGF-1), bone morphogenic protein (BMP)-7 and transforming growth factor (TGF)-β2.[50-52] BM-MSCs have also been shown to inhibit inflammation *via* the stimulation of anti-inflammatory interleukin-1 (IL-1), which results to the subsequent generation of anti-inflammatory T-cells.[53] BM-MSCs have substantial immunosuppressive properties as well. They have been shown to inhibit T cell proliferation thereby reducing the chance of immune rejection when implanted into defects.[54] Due to the advantageous properties of BM-MSCs and their high chondrogenic potential, the use of BM-MSCs for the treatment of AC defects has been widely investigated. One of the most common techniques used for the treatment of small lesions of AC and early-stage AC defects is microfracture, in which BM-MSCs promote AC regeneration *in situ*. Microfracture surgery involves the drilling of several holes into subchondral bone to release BM-MSCs which then promote the regeneration of AC at the defect site. [55, 56] Steadman et. al found that microfracture

surgery significantly improved patient's joint function and reduced pain in patients with full thickness chondral defects. However, it was also found that compared to hyaline AC tissue, microfracture surgery produced unstable, mechanically inferior fibrocartilage at the defect site.[57] Microfracture is an example of the use of endogenous BM-MSCs for the treatment of cartilage defects, but due to its limitations, the use of exogenous BM-MSCs has become the subject of various investigations.

Exogenous BM-MSCs are usually obtained *via* aspiration from the iliac spine or crest, but only 0.001%-0.01% of the cells harvested *via* this method can be classified as MSCs.[58] Due to the limited number of MSCs harvested, subsequent culture expansion is necessary in order to obtain an appropriate number of cells for articular cartilage repair.[59] This presents a challenge as although BM-MSCs are easily expanded in monolayer cultures, these cultures have been associated with cellular senescence and loss in multilineage differentiation capacity.[60] It has been shown that the chondrogenic potential of murine BM-MSCs decreased significantly after six cell passages.[61] Nevertheless, cell expansion remains a critical part of the standard procedures used in MSC therapy. Once expanded, BM-MSCs are implanted into AC defects either through intra-articular injection or surgically.

For surgical implantation, there are often two techniques used to improve cell engraftment in the targeted defect. The first method involves seeding BM-MSCs onto a biomaterial scaffold. A biomaterial scaffold provides a structurally supportive 3D environment that mimics the environment of the extracellular matrix (ECM) and therefore increases the surface area to volume ratio for seeded cells and boosts cell proliferation, attachment, migration and differentiation.[62 63] The material selected for the construction of the scaffold is important to determining it's efficacy. A wide range of synthetic and natural materials have been evaluated in lab studies and the therapeutic potential and safety of various scaffolds have been demonstrated in both animal models of OA and in clinical trials.[1, 35, 64-66] For example, Gobbi et. al implanted 25 patients suffering from large chondral knee defects with BM-MSCs and a collagen type I/III scaffold in a one-step surgery.[36] Three years post-surgery, patients noted significant improvements in knee injury and osteoarthritis outcome scores (KOOS), visual analog scale (VAS) for pain, and Lysholm, Marx and Tegner activity scores. In patients who underwent second-look arthroscopic surgery and tissue biopsies, hyaline AC was identified in biopsied tissues as evaluated *via* histological analysis. Interestingly, patients under the age of 45 had greater improvement in all scores evaluated compared to elderly patients suggesting that age affects the efficacy of the surgical treatment with BM-MSCs. Although 3D scaffolds have been beneficial in BM-MSC transplants, cell seeding often results in limited localization capacity and cells may not retain hyaline-like phenotypes when transplanted.[4, 67-69] For these reasons, scaffold-free cell sheets have been designed as an alternative approach. Cell sheets are a technology that utilize a thermosensitive surface. The technology constructs a cell-cell binding architecture depicting a spheroid type culture or a cell dense culture that supports self-assembly. This allows for immense control over the attachment and detachment of cells from the culture.[70, 71] Cell sheets are beneficial as they allow high density cell transplants to be implanted without the immune risks associated with scaffolds and their degradation products. [72] A preclinical trial expanded BM-MSCs *in vitro* forming a cell sheet that was transplanted into osteochondral

defects in nude rats. The Wakitani cartilage repair scores for these rats were significantly improved compared to the control group during a 12 week period.[73]

BM-MSCs are a promising cell source for TEAC due to their high chondrogenic potential and immunosuppressive properties. However, there is a need for more clinical trials to demonstrate the safety and efficacy of BM-MSC transplants before their use becomes common practice for clinicians.

Adipose Derived MSCs

Adipose Derived MSCs are present in the subcutaneous adipose tissue and can be extracted *via* a liposuction technique requiring only local anesthesia and causing minimal discomfort. [74] Lipoaspirate processing is then used to extract the stromal vascular fraction (SVF) of the fat tissue which consists of large quantities of AD-MSCs among other cell types. [75] AD-MSCs can then be isolated from the SVF and expanded through cell culture if necessary.[76] The primary benefit of AD-MSCs is their ability to be obtained in large quantities. In fact, compared to bone marrow, an adipose tissue contains 100-150 times more stem cells. [77] Adipose tissues are found throughout the body and the extraction techniques used have limited risk of donor site morbidity making AD-MSCs suitable for autologous use. AD-MSCs have many of the same characteristics as BM-MSCs, including trilineage differentiation potential (osteocytes, adipocytes and chondrocytes), plasticity and immunosuppressive characteristics.[78] In fact, AD-MSCs have been shown to have a stronger immunomodulatory capacity than BM-MSCs.[79] Additionally, the effects of age and OA's progression have been found to be less pronounced in AD-MSCs as compared to BM-MSCs.[80] Although AD-MSCs have numerous advantages, they are not without limitations.

Although AD-MSCs do possess chondrogenic potential, studies have shown that their capacity for chondrogenesis is limited compared to BM-MSCs.[81, 82] The quality of AD-MSCs also vary depending on the health condition of the patient from which they are extracted. AD-MSCs derived from obese patients have been shown to have lower differentiation and angiogenic potential when compared to those derived from non-obese patients.[83] There is no standard methodology for the culturing of AD-MSCs either.AD-MSC phenotypes are dependent on culture conditions, which has led to difficulty creating standardized AD-MSC therapies.[84, 85] Despite the limitations of AD-MSCs, numerous clinical trials have demonstrated their potential for use in TEAC. AD-MSC therapies are similar to BM-MSC therapies with delivery methods including intraarticular injection and surgical implantation. One study attempted to treat OA knee defects *via* intra-articular injection of SVF cells. Three Months post-operation, patients showed significant improvements in Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index and VAS scores as compared to pre-treatment without any significant adverse effects reported.[86] The efficacy of intra-articular injections of cultured AD-MSCs has also been demonstrated in several studies.[87, 88] In these studies, the VAS and WOMAC scores also showed significant improvements and it should be noted that there was a reported increase in AC volume after injection.[89] Interestingly, a review evaluating 8 studies on the treatment of knee OA using

intra-articular injections of AD-MSCs reported that 50% of the studies noted adverse effects in patients. Only one patient out of 115 experienced a severe adverse effect which was unstable angina pectoris. Other studies reported adverse effects such as joint discomfort and ecchymosis which were resolved without any intervention.[89] No clinical trials have yet been conducted utilizing AD-MSC seeded scaffolds. However, their potential has been demonstrated in several studies.[33, 90] Zubillaga et. al cultured human AD-MSCs in spheroids under hypoxic conditions and seeded them onto a porous chitosan/chitin nanocrystal scaffold.[33] It was found that the seeded AD-MSCs were capable of chondrogenic differentiation and produced an ECM high in sulfated glycosaminoglycan (GAG) content. Additionally, the group found that cells cultured in hypoxic conditions adhered better to the scaffold and showed greater chondrogenic differentiation potential than cells cultured under normoxia. The 3D scaffold utilized during the study had also limited oxygen tension which may have increased chondrogenic differentiation[33]. Although the therapeutic potential of AD-MSC seeded scaffolds is high, further preclinical and clinical trials evaluating their safety and efficacy are required.

Synovium Derived MSCs

The synovial membrane (SM) is a soft mesenchymal tissue that lines the spaces of the diarthrodial joints, bursae and tendon sheaths.[91, 92] The membrane can be divided into the intima, the upper layer, and the subintima. Cells present in the intima secrete synovial fluid (SF) which lubricates the cartilage and plays a role in chondrocyte activity. It has been shown that MSCs can be isolated from the SM. These SM-MSCs have similar phenotypic profiles to BM-MSCs and possess chondrogenic potential.[93] It has also been shown that a distinct, highly clonogenic population of MSCs are present in the SF.[94] Morito et. al found that SF-MSCs are genetically similar to SM-MSCs but it has been suggested that SM-MSCs are less clonogenic than SF-MSCs.[95, 96] SD-MSCs are obtained through minimally invasive arthroscopic surgery that is performed to extract portions of the SM This operation is advantageous as it allows for the avoidance of donor site morbidity. MSCs can then either be isolated from the SM itself and processed to collect SM-MSCs or SF-MSCs may be extracted from the intra-articular SF.

Multiple studies have found that SD-MSCs have a higher chondrogenic potential than BM-MSCs.[97-99] Koga et. al seeded collagen gels with BM-MSCs, SM-MSCs and AD-MSCs respectively and implanted the gels into rabbits with full thickness cartilage defects.[100] SM-MSCs and BM-MSCs were found to produce more AC ECM than AD-MSCs. SM-MSCs were also found to have a higher proliferation potential than BM-MSCs. However, a higher cell density was required for SM-MSCs to produce sufficient AC ECM in the defect. Nevertheless, the increased chondrogenic potential of SD-MSCs is controversial. Neybecker et. al evaluated the differences in stemness, immunophenotype and chondrogenic potential between MSCs isolated from human bone marrow (BM-MSCs), synovial membrane (SM-MSCs), and synovial fluid (SF-MSCs).[101] Each cell type was found to have the potential to differentiate into chondrocytes, osteocytes and adipocytes with use of appropriate media. Cell types expressed a similar immunophenotype with some variability. When induced using TGF-β1, it was found that chondrogenic genes were more strongly overexpressed in BM-MSCs than in SM-MSCs and SF-MSCs. Although the GAG's content remained similar regardless of cell type, Type II collagen, a

specific marker for AC, was more abundant in BM-MSCs than other cell types. Ultimately, although each cell type investigated was found to be appropriate for the repair of AC, BM-MSCs were found to be the superior cell type for use in hyaline TEAC. Further comparisons between the chondrogenic potentials of each cell source are required before a definitive answer can be provided with respect to their therapeutic potential *in vivo*. Other advantages of SD-MSCs include reduced hypertrophic differentiation as compared to BM-MSCs, high self-renewal capacity and multipotentiality.[102] [92]

SD-MSCs are limited in the fact that it is quite difficult to obtain a sufficient number of MSCs from the synovium and therefore *ex vivo* expansion is necessary in order to use these cells in AC repair.[103] Additionally, there are not many clinical studies reporting the use of SD-MSCs for the repair of AC defects, which limits their commercial use. Further trials investigating the safety and efficacy of SD-MSCs as a cell source for AC repair are required before they can be identified as a strong candidate for TEAC. [104]

Induced Pluripotent Stem Cells (iPSCs)

iPSCs are stem cells that have been derived from adult somatic cells and reprogrammed, using a variety of genes and transcription factors, to become pluripotent.[105] These cells resemble embryonic stem cells and subvert the ethical issues that the use of embryonic stem cells raise. These cells are prepared by genomic integration and forced expression of the four transcription factors (TF). Octamer binding TF 4 (Oct4), Sox2, c-Myc and Kruppel-like factor 4 (Klf4). The pluripotent nature of these cells is advantageous as it eliminates reduction in quality of generated AC as seen in MSCs[106, 107] Being pluripotent, these cells have multigerm layer differentiation potential and can differentiate into almost any adult somatic cell. iPSCs also have an unlimited proliferation and self-renewal capacity making their use highly attractive in TEAC.[108] [109] The proliferative qualities of iPSCs mean that they can theoretically provide an unlimited number of homogenous cells for TEAC, which makes autologous cell transplantation a feasible approach.[110] Through the use of various growth factors and feeder cells, iPSCs have been differentiated into chondrocytes.[110] Human iPSCs have also been differentiated into chondrocytes through the activation of the TGF-β pathway with an embryoid body stage.[111] Both feeder cells and the embryoid body stage limit the potential of iPSCs as they may introduce heterogeneity into the cell population. As the use of iPSCs for AC repair is relatively novel, there is not yet a standard protocol to induce the chondrogenic differentiation of iPSCs.

iPSCs are limited by their tumorigenicity. In addition, iPSCs that are induced to undergo chondrogenic differentiation *via* activation of the TGF- β pathway pose a risk as contaminant undifferentiated cells may form teratomas with embryoid body formation. Saito et. al implanted hiPSCs that underwent chondrogenesis into 36 mouse knee joints. Although a hyaline AC was generated after 8 weeks, one mouse developed an immature teratoma at 16 weeks post-transplant.[112] Another limitation of iPSCs is their high cost. Due to the highly complex technical procedures required to prepare these cells, their preparation cost is high and would place a large financial burden on patients undergoing treatment. Inducing chondrogenesis in iPSCs using

feeder cells also risks contamination as chondrocytes need to be separated from cocultured cells before transplantation.[113] Some iPSCs are also prepared using retroviruses, which poses several risks in patients such as permanent genetic alterations through retroviral mediated gene therapy, reactivation of silenced transgenes upon differentiation of iPSCs and stimulation of oncogenes. [105] More efficient and safe measures of iPSC reprogramming may be required before these cells can be utilized clinically. Finally, the long-term safety and survival outcomes of transplanted iPSC cells have not been evaluated.

Although limited in number, studies utilizing iPSCs in TEAC have showed promising results. Diekman et. al differentiated murine iPSCs into chondrocytes by exposing them to BMP-4 in micromass cultures. These cells were then seeded them onto an agarose gel and implanted into an *in vitro* AC defect model.[114] After 21 days, it was shown that iPSC derived chondrocytes produced an ECM high in GAG and type 2 collagen. Atomic force microscopy showed that cartilage produced by these cells mimicked the zonal architecture of native cartilage. Uto et. al utilized minimally treated human iPS (hiPSC) cells and porcine iPSCs (PiPSCs) to treat an osteochondral defect in syngeneic miniature pigs.[115] The advantage of using minimally treated cells is that the risk of tumorgenicity is reduced by reducing pluripotency. iPSCs were precultured in a 3D beta-tricalcium phosphate/poly L lactic acid scaffold and embedded into the porcine osteochondral defect to induce cartilage regeneration. Histological analysis showed that both hiPSCs and PiPSCs formed regenerative AC within 8 weeks without tumor formation. Interestingly, hiPSCs formed cartilage within 4 weeks post-transplant. However, when compared using a macroscopic view, there was less cartilage regeneration in the hIPSCs than in the PiPSCs. Additionally, histological analysis showed that PiPSCs had slightly more AC regeneration than porcine MSCs implanted into the defect. It is important to note that in this study a synthetic CLAWN pig was used to avoid the risk of immunoreactions and thus immunologic mismatch was not necessary. However, in a clinical setting, matching human leukocyte antigen (HLA) haplotype is essential in reducing risk of rejection of transplanted allogenic iPSCs. Ultimately iPSCs are a promising cell source for TEAC but their clinical safety is yet to be determined.

Summary

Given the prevalence of OA and lack of treatments preventing AC degeneration, cell therapies for the treatment of AC defects are being thoroughly investigated. Although ACI is currently used in standard clinical practice to treat OA and fill AC defects, its limitations have led researchers to consider stem cells as a cell source for the repair of AC as they are less likely to dedifferentiate and are more abundant. The cell choice used for AC repair is important as it can have a large impact on the phenotype and structural integrity of regenerative AC. Either autologous or allogenic cells may be utilized for AC repair. The use of autologous cells limits the risk of immune rejection and disease transmission but reduces the number of cells that can be obtained. Furthermore, cell quality has been shown to decrease with age and disease progression and therefore autologous cells may not be suitable for the treatment of patients with late-stage OA. Allogenic cells on the other hand, offer higher quality cells, reduce the need for cell

expansion and decrease cost of treatment as well as donor site morbidity by eliminating the need for extraction procedures. However, allogenic cells increase risk of immune rejection and their long-term safety outcomes in AC repair have not yet been evaluated. Various types of autologous and allogenic MSCs have been investigated as they possess high expansion rates, immunosuppressive characteristics and self-renewal capacity. BM-MSCs are the most widely used MSCs owing to their high chondrogenic potential and immunosuppressive capacity. BM-MSCs have been implanted into AC defects in various studies utilizing 3D scaffolds, cell sheets or through intra-articular injection. These cells are limited in that they require an invasive bone marrow aspiration procedure to obtain, which limits cell number. AD-MSCs on the other hand, are abundant and can be extracted through minimally invasive liposuction but have limited chondrogenic potential compared to BM-MSCs. SD-MSCs are thought to have higher chondrogenic potential than BM-MSCs, although this is controversial. SD-MSCs also have a lower hypertrophic differentiation potential than BM-MSCs and a high self-renewal capacity but are limited by the number of cells that can be extracted from the synovium, therefore they require expansion *ex vivo*. Although each MSC type has undergone clinical testing, a gold standard has not been determined and more studies comparing the different sources of MSCs are needed. iPSCs are an alternative to MSCs and offer the advantages of unlimited cell numbers, proliferation and self-renewal capacity. They can be directed to chondrogenic differentiation through several different protocols but are limited by their high cost and potential to cause teratoma formation. Ultimately, both MSCs and iPSCs are capable of producing AC but there are still several obstacles that need to be overcome before the most suitable cell source for TEAC can be determined and TEAC can be utilized as a widespread treatment for AC defects.

Conflict of Interest

The authors have no conflict of interest to declare.

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