REVIEW

Role of cartilage-forming cells in regenerative medicine for cartilage repair

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Abstract: Currently, cartilage repair remains a major challenge for researchers and physicians due to its limited healing capacity. Cartilage regeneration requires suitable cells; these must be easily obtained and expanded, able to produce hyaline matrix with proper mechanical properties, and demonstrate sustained integration with native tissue. At present, there is a wide variety of possible cell sources for cartilage regeneration. This review explores the diversity of sources for cartilage-forming cells and the distinctive characteristics, advantages, limitations, and potential applications of each cell source. We place emphasis on cell sources used for in vitro and clinical studies.

Keywords: cell sources, cartilage regeneration, stem cells, chondrocytes

Introduction

Due to its avascular structure, cartilage tissue has limited innate regenerative ability. In 1743, William Hunter described "ulcerating cartilage" by explaining "From Hippocrates to the present age…when cartilage is destroyed, it is never recovered".[1] Since then, various techniques including debridement and lavage, marrow stimulation, autologous chondrocyte implantation (ACI), the osteochondral autograft transfer system (OATS), tissue engineering, and other techniques have been explored to improve cartilage regeneration, some of which have found their way to the clinic.[2,3] However, till date, no technique has reliably regenerated the biological composition and biomechanical properties of native cartilage, leaving unresolved pain and loss of joint function for millions of patients with defective cartilage from aging, injury, or disease.

In general, surgical intervention for cartilage lesions can be divided into marrow-stimulating (reparative) techniques and reconstructive techniques. Abrasion chondroplasty, Pridie drilling, and microfracture are reparative surgical techniques for bone marrow stimulation, which may stimulate healing by breaking bone around the defect but can result in the production of an inferior quality of newly formed fibro-cartilage tissue.[3] Reconstructive techniques include ACI, characterized chondrocyte implantation (CCI), and other variations of tissue engineering.[4] ACI and CCI involve the removal of healthy chondrocytes from a patient and the implantation of these into a defective cartilage site, stimulating the growth of new hyaline cartilage while avoiding graft rejection. However, the drawbacks of ACI and CCI include limited cell sources, difficulty in phenotype retention, and donor-site morbidity, all of which challenge autologous cell transfer procedures.[5,6] Thus, new strategies rely upon cell therapies that explore the use of stem cells rather than primary chondrocytes for cartilage regeneration. Since

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the range of potential cell sources for cartilage regeneration is so extensive, the following criteria may help identify the best candidate cell sources for cartilage regeneration: 1) easy to isolate and collect, 2) easy to expand in vitro to yield a large number of cells, 3) capable of expressing and synthesizing cartilage-specific molecules, 4) capable of producing neo-cartilage with comparable mechanical properties to native cartilage, 5) capable of integrating into the surrounding recipient site, and 6) immunocompatible.[2,7,8] With regard to these design goals, we have reviewed the cell sources used for cartilage lesion treatment and the trajectory of the field. We have compared potential cell sources, discussed their function in cartilage regeneration, and assessed their success in in vitro models and clinical experiences.

Chondrocyte cell sources

Cartilage mainly consists of avascular extracellular matrix (ECM) and has a low density of cartilage matrix-depositing cells namely chondrocytes. Mature chondrocytes are characterized by a round morphology and are located in lacuna structures. These cells are logical candidates for cartilage repair because they produce type II collagen, sulfated glycosaminoglycans (GAGs), and other ECM molecules that constitute functional cartilage.[9] Chondrocytes are mainly isolated from articular cartilage for reparative procedures, but they can also be isolated from other cartilage structures (ie, nasal, auricle, and costal cartilage), as discussed below.

Articular cartilage chondrocytes

Mature articular cartilage is an anisotropic tissue organized into superficial, middle, and deep zones, and changes with depth reflect the tensile and compressive forces acting on the tissue. The superficial zone is at the articular surface, whereas the deep zone adjoins the calcified cartilage, which integrates into the subchondral bone. Chondrocytes from different zones have unique gene expression profiles of zonal markers (ie, proteoglycan (PG)-4, collagen type X, and clusterin) and matrix protein expression levels.[5,10,11] Chondrocytes from the deep zone are able to proliferate faster, produce more total ECM, and produce ECM with a greater ratio of GAGs to collagen than chondrocytes from the middle or superficial zones. Interestingly, engineered cartilage tissues grown in vitro from chondrocytes show different zonal properties corresponding to the zone from which the cells were isolated.[12] In a study by Waldman et al,[12] a mixture of full-thickness chondrocytes isolated from all zones demonstrated the highest collagen synthesis after being seeded and cultured in calcium phosphate substrates for 8 weeks, compared with

more homogenous deep zone only chondrocytes or middle zone only chondrocytes. The combination of middle zone and deep zone chondrocytes showed the highest accumulation of aggrecan and superior mechanical properties. However, culture conditions and cell–cell communication affect the retention of zonal-specific characteristics; all 3 zones of equine chondrocytes can restore their zonal matrix expression in alginate culture, but not in pellet culture.[13]

Articular chondrocytes have proven successful in surgical operations such as OATS or ACI. OATS is a procedure where an osteochondral plug of hyaline cartilage and bone is taken from a non-load-bearing area and packed into a prepared cavity. Mosaicplasty is similar but involves the use of multiple smaller plugs and the filling of smaller holes in the defect, which can produce a mosaic histological result. Although OATS and mosaicplasty can provide favorable results, complications have been reported including osteochondral plug fracture, postoperative hematoma, surface incongruity, donor-site morbidity, and insufficient stability of the graft.[14] Thus, although articular chondrocytes within osteochondral plugs, can enhance cartilage healing, the donor-site morbidity associated with removing large sections of bone and cartilage suggests that the use of articular chondrocytes alone, in a regenerative process, may serve the patient better.[15] The most common articular chondrocyte-based surgical procedure is ACI, which has demonstrated improved results compared with OATS.[16]

ACI was first applied to a knee cartilage defect in 1994 and since then, it has been widely studied in the field of cartilage repair.[9] In the ACI process, autologous articular chondrocytes are collected from a low-load-bearing area of a joint, expanded in vitro, resuspended, injected into a defect, and covered by a periosteal flap or collagen membrane. Generally, improved healing responses are reported with ACI treatment; neocartilage-like tissue is often formed, and defects are filled more completely with chondrocyte treatment than when left untreated or treated with cell-free therapies. Although the newly formed tissue is mainly fibrocartilaginous, some long-term follow-up studies suggest that the clinical functionality of ACI remains high even 10–20 years after the implantation.[17,18]

However, other studies have found the benefit of ACI more questionable. A randomized trial of 80 patients found no histological or clinical difference between microfracture and ACI treatment groups at 2 years after surgery, and the microfracture group showed better results according to a short form (SF)-36 physical component score.[19] A 5-year follow-up of the study reported the same findings.[20] A more comprehensive review of 9 trials with 626 patients found no advantage of ACI over

other treatments.[21] Hence, till date, there is no sufficient existing randomized trial to prove the superiority of ACI to other treatment strategies for full-thickness cartilage defects. This may be due to the fact that all articular chondrocytes, rather than exclusively those able to regenerate cartilage, are used in ACI, an issue which is addressed in CCI.

In a step toward more precisely selecting cells able to regenerate lost cartilage, CCI was investigated in 2007 in a trial with 118 patients presenting with symptomatic cartilage defects of the knee.[22] Across 13 orthopedic centers, articular chondrocytes were harvested from patients and cultured in vitro, similar to ACI. However, before implantation, cells were sorted using flow cytometry based on specific markers that predict a population more capable of producing hyaline-like cartilage, and the selected cells were used for implantation. One year after treatment, CCI resulted in superior structural regeneration compared with microfracture, although no significant clinical differences were found when measured by the Knee injury and Osteoarthritis Outcome Score (KOOS). The results at 36 months showed that for the treatment of articular cartilage defects of the femoral condyles of the knee, CCI resulted in a significantly better clinical outcome compared with microfracture based on the KOOS.[23] TiGenix (Leuven, Belgium) has further developed the technique of isolating a subpopulation of chondrocytes superior at hyaline production and formulated the product Chondro-Celect. The latest 5-year follow-up results of the cell-based product in a clinical study confirmed the durability of the product and demonstrated improvements over microfracture after 1 and 3 years.[24] However, as with ACI, the morbidity caused by the 2-stage procedure remains unresolved in CCI.[3] Out of the many surgical techniques for cartilage repair and regeneration, those that hold the greatest promise are cell-based procedures, and articular chondrocytes have proven to be one successful cell source in ACI and CCI.

Nasal and auricular cartilage chondrocytes

In addition to articular chondrocytes, auricular (ear), nasal septum (nose), and costal (rib) chondrocytes can be isolated and used for cartilaginous tissue regeneration. Chondrocytes from different anatomical sites have different gene expression profiles, proliferative rates, and redifferentiation characteristics. Clinical studies have demonstrated that the 3 cell sources mentioned above can be used for autologous cartilage graft generation with auricular and nasal chondrocytes showing superior postexpansion chondrogenic potential.[25–27] Many studies have demonstrated that both auricular and nasoseptal

chondrocytes display higher cell yield and offer greater expansion opportunities when compared with articular chondrocytes. When cultured in alginate beads, auricular chondrocyte proliferation rates are up to 4 times that of articular chondrocytes.[28] Kafienah et al[29] found that human adult nasal chondrocytes proliferated approximately 4 times faster in monolayer culture and had greater chondrogenic capacity than human articular chondrocytes, but these chondrocytes may not survive or produce the same results in an articulating joint in vivo. Nasal chondrocytes can also be cultured at a very low density such that an 838-fold expansion can be reached within one passage without differentiation.[30] In addition, removal of auricular and nasal chondrocytes is much less invasive than removal of cartilage from a joint and causes fewer functional defects for the patient. Hence, nasal and auricular sites appear to be superior for cartilage removal and hold potential as a source for highly proliferative chondrocytes.

In vivo animal studies demonstrate that, when implanted in radically polymerized hyaluronic acid (HA) hydrogels, auricular chondrocytes cause construct growth, neocartilage formation, and an increase in aggregate modulus and ECM accumulation, whereas articular cartilage chondrocytes do not result in construct growth and produce a minimal increase in the compressive modulus. However, in vitro, contradictory differences in gene expression after dynamic mechanical loading were found.[31] Since non-articular chondrocytes are not under mechanical loading in the natural state, they logically respond differently to mechanical loading compared with constantly loaded articular chondrocytes. This raises questions regarding the ability of nasal and auricular chondrocytes to withstand the mechanical forces experienced in the joint. It is also unclear how articular and non-articular chondrocytes will integrate into the surrounding tissue when implanted into a joint.[7]

Allogeneic chondrocytes

Although our understanding of the immune response to foreign cartilage and chondrocytes is limited, allogeneic chondrocytes may provide a solution to the low quantity of autologous chondrocytes typically obtained in ACI surgery and the associated donor-site morbidity. Large animal studies indicate that allogeneic chondrocytes from articular, auricular, and costal cartilage can support and encourage successful healing of lesions in the avascular zone of the meniscus.[32] An in vitro study demonstrated the utility of harvesting chondrocytes from younger patients, where it was found that allogeneic chondrocytes from juvenile donors had greater potential to restore articular cartilage compared with chondrocytes from adult donors.[33] Further, juvenile chondrocytes stimulated no

immunologic response based on a lymphocyte proliferation assay, which may be due to the lack of their surface molecules that induce a T-cell immune response.[34] In another study, allogeneic human chondrocytes in alginate beads were implanted into 21 patients for the treatment of symptomatic cartilage defects in the knee, and a significant clinical improvement in patients was found after 6 and 24 months of follow-up.[35] However, in this study, since the chondrocytes were encapsulated in an alginate gel, the alginate may have acted as an immune barrier and protected the chondrocytes from the immune system.[36] Thus, the immune response to allogeneic chondrocytes remains to be investigated, although an immune barrier system may be considered as a potential strategy to overcome immune reactions.

Stem cell sources
Mesenchymal stem cells
Mesenchymal stem cells (MSCs) are pluripotent stromal cells resident in mesenchymal tissues that can be isolated using glass or tissue culture plastic adhesion, expanded in vitro, and differentiated into many lineages (osteogenic, adipogenic, and chondrogenic).[37] Although there is no single MSC marker, and the expression of MSC markers varies depending on donor, passage number, and source tissue type, MSCs are often characterized by the following surface marker profile: $CD73^+$, $CD90^+$, $CD105^+$, $CD166^+$, $CD34^-$, and $CD45^-$. However, even these markers are not always sufficient to identify MSCs, nor are their morphology able to specifically identify them.[38] In contrast to primary chondrocytes, primary MSCs are isolated from different tissues and are more abundant within certain tissues, making them easier to isolate. Bone marrow-derived stem cells (BMSCs), adipose-derived stem cells (ADSCs), synovium-derived progenitor cells, and stem cells from skeletal muscle and umbilical cord blood are all capable of chondrogenic differentiation in appropriate culture environments. The ability for MSCs to differentiate down bone, cartilage, tendon and ligament lineages suggests the possibility of constructing a biphasic osteochondral graft, or even a more complex joint tissue from a single cell source or type.[37]

Limitations and potential risks in applying MSCs to cartilage repair remain. Expression of type X collagen, a major hypertrophy marker that can lead to calcification and vascularization, was found in BMSCs, ADSCs, and periosteum-derived progenitor cells (PDPCs) during chondrogenesis, whereas articular chondrocytes resist calcification.[39,40] However, it is likely that hypertrophy can be prevented by improving differentiation parameters. For example, a recent study suggested that hypoxia may inhibit the expression of type X collagen

during chondrogenesis of ADSCs.[41] Another concern regarding MSCs is that BMSC-generated matrix is mechanically inferior to that generated by articular chondrocytes, perhaps because the in vitro differentiation of MSCs does not perfectly mimic normal chondrogenic differentiation in the body.[42–44]

Bone marrow-derived stem cells
BMSCs are one of the most common human adult MSCs used in tissue engineering and have been well studied for their ability to form cartilage-like structures in vitro and stimulate cartilage repair in animal models. Surgical techniques utilizing BMSCs include Pridie drilling and microfracture. With these procedures, the repair of fibrocartilage by bone stimulation is done by drilling small holes (a few mm for Pridie drilling or 0.5–1.0 mm for microfracture) into the subchondral bone plate after surgical debridement of cartilage defects. These are some of the most frequently used techniques for treating lesions of the articular cartilage in the knee, which work by stimulating bone marrow and encouraging marrow progenitor cells to migrate to the lesion. BMSCs, growth factors, and cytokines are released into the defect and penetrate into subchondral bone and articular cartilage where progenitor cells can differentiate into chondrocytes to begin to form new fibrocartilage or hyaline-like cartilage.[45] These methods have proven safe and effective in many studies of articular knee cartilage defects, and microfracture was successful in the treatment of full-thickness chondral lesions of the knee in National Football League players.[46,47] However, a recent report from Vasiliadis et al[21] pointed out that microfracture provides good results in the short term (1–2 years), whereas ACI provides delayed but prolonged healing. Dozin et al[48] compared the performance of ACI and mosaicplasty at resurfacing local full-thickness chondral defect of the knees of 47 patients and found that ACI and mosaicplasty were clinically equivalent, perhaps due to the trauma caused by mosaicplasty despite the mobilization of BMSCs. Moreover, bone marrow stimulation often generates fibrocartilage tissue of an inferior quality with less type II collagen and little resemblance to surrounding tissue.[49] In addition, clinical results of drilling procedures and ACI are age dependent. This may be due to the general decrease in MSC populations in older patients, a decrease in older patients' healing potential, or the decreased ability of aged chondrocytes to produce collagen-rich, mechanically functional cartilage ECM, as demonstrated using aged bovine chondrocytes.[20,50,51] Clinical findings also demonstrate that patient's compliance is essential for healing in all mentioned procedures, and excessive activity too soon after surgery can cause great harm to the joint.[52,53] Since many studies have

found surgical fracture techniques unsuccessful at restoring normal hyaline cartilage and ineffectual in the long term, scientists have begun to explore more regenerative, stem cell-based tissue engineering options.[54]

BMSCs have been cultured in a variety of 3D systems in an effort to generate cartilage-like tissue, including collagen,[55,56] gelatin,[57] silk,[58] alginate,[59] hyaluronan,[60] chitosan,[61] agarose,[62] polyethylene glycol (PEG),[63] polyglycolide (PGA),[64] poly(lactic-co-glycolic acid), and hybrids of synthesized or natural materials.[65–67] In general, regardless of scaffold structure, cartilage-like tissue can be induced using BMSCs, as evidenced by type II collagen and aggrecan expression and accumulation both in vivo and in vitro, and some studies have documented that chondrogenic differentiation and matrix deposition are superior in BMSCs compared with chondrocytes.[68] Addition of growth factors like transforming growth factor β1 (TGFβ1) and TGFβ3 can enhance chondrogenesis.[69,70]

Till date, only a handful of clinical studies using BMSCs have been published for cartilage applications. A series of clinical ACI studies were conducted where culture-expanded BMSCs were embedded in collagen gels and transplanted into cartilage defects covered with periosteum.[71–75] In 2002, this system was tested on patients with knee osteoarthritis who underwent a high tibial osteotomy. A total of 12 of 24 patients were treated with BMSCs in an articular cartilage defect in the medial femoral condyle, whereas the other 12 subjects served as cell-free controls. The results showed that after 42 weeks, arthroscopic and histological grading scores were better in the cell-transplanted group than in the cell-free control group, but no significant clinical improvement was found.[75] With this same system, 2 further studies were conducted in 2004 and 2007 on the repair of full-thickness articular cartilage defects in the patella and patellofemoral joints. Both studies found that defects were repaired with fibrocartilage at the study end points (1 or 2 years after transplantation), although the patient's clinical symptoms improved after only 6 months.[73,74] In 2010, a long-term follow-up was reported on BMSC transplantation for cartilage repair in 45 joints of 41 patients. The clinical results indicated that up to 11 years and 5 months after autologous transplantation of BMSCs, neither partial infections nor tumors appeared in these patients, which demonstrated the safety of using autologous MSC transplantation in cartilage repair. However, no functional repair outcomes were discussed.[72]

Adipose-derived stem cells

The chondrogenic potential of ADSCs has been validated in vitro using a variety of culture systems, growth factors, and differentiation culture conditions. Maintained in chondrogenic medium, chondrocyte-associated genes (type II collagen and aggrecan) can be induced in ADSCs, and substantial aggrecan protein can be transcribed and secreted. Challenges remain in stimulating the expression and accumulation of type II collagen in scaffolds,[76] despite the addition of TGFβ1.[77] Still, in vivo experiments have verified that ADSCs differentiated toward chondrogenesis can proliferate and form new cartilage after subcutaneous injection with fibrin glue.[78] In an in vitro pellet culture, ADSCs also demonstrate higher potential for chondrogenic differentiation compared with human umbilical cord matrix cells.[79]

ADSCs resemble BMSCs in their phenotype and their ability to differentiate into several mesenchymal lineages including the chondrocyte lineage, but they differ from BMSCs regarding their source tissue.[80] Both cell types are recognized as potential cell sources for cartilage repair, but ADSCs appear advantageous in a few ways. ADSCs can be obtained via less invasive methods from large volumes of waste tissue from common surgeries (abdominoplasties and lipoplasties) and have a yield similar to BMSCs per gram of source tissue.[81] However, some investigations have revealed that ADSCs have inferior chondrogenic potential compared with BMSCs and that pellet cultures of ADSCs show much weaker chondrogenesis in both cell morphology and matrix production.[76,82,83] Another study confirmed that under the same chondrogenesis culture condition, type II collagen and PGs were synthesized only by the growth factor-treated human BMSCs, but not by human ADSCs.[84] This reduced potential for chondrogenic differentiation may be a result of the lack of TGF-β receptor expression and reduced expression of mRNAs for bone morphogenetic proteins (BMPs) in ADSCs compared with BMSCs.[85]

In animal studies, conflicting results for cartilage repair by ADSCs are reported. With supporting matrices of fibrin glue or fibrous PGA/polylactic acid scaffolds, induced ADSCs can heal full-thickness cartilage defects and form a hyaline-like cartilage tissue.[86,87] In contrast, other reports found that the presence of ADSCs had no significant effect on cartilage repair compared with unseeded polycaprolactone/F127 scaffolds in a rabbit model.[88] Till date, no clinical studies on ADSCs in cartilage regeneration have been reported.

Embryonic stem cells

Similar to the adult stem cells mentioned above, embryonic stem cells (ESCs) comprise an appealing prospective cell source for regenerative medicine due to their extensive in vitro expansion capability and multilineage differentiation potential.[89] However, ethical issues and the heterogeneous and

uncontrolled differentiation of ESCs currently limit clinical applications. Still, the use of ESCs for cartilage regeneration is an active area of study. One recent study demonstrated the ability of ESCs to differentiate in response to paracrine signaling from mature chondrocytes. A layer of feeder-free human ESCs were cocultured with primary chondrocytes and, with no further addition of growth factors, developed into an 85% positive chondrogenic-committed ESC culture. When the differentiated ESCs were embedded in PEG-based hydrogels and implanted subcutaneously into mice, they formed pellets of robust cartilage-like tissue and maintained their phenotype and tissue quality for 24 weeks.[90] MSCs isolated from human ESCs (hESCd-MSCs) have also shown multilineage differentiation potential in vitro and have demonstrated an ability to form neocartilage in mice when expanded in chondrocyte-conditioned medium and embedded in PEG-based hydrogels. Chondrocyte-conditioned medium expanded hESCd-MSC pellets were also found to contribute to the full repair of cartilage defects in a rat model, suggesting a new, simple, and efficient strategy of using ESCs in cartilage repair.[91]

Perhaps the most promising result for ESCs in cartilage repair is from a study where hESCs were differentiated into chondrocytes using the growth factors BMP7 and TGFβ1 for 4 weeks and embedded into HA-based hydrogel constructs.[92] The constructs were then examined in a rat model for their ability to repair critical-sized osteochondral defects. A hyaline-like neocartilage layer resulted with surface regularity and complete integration with adjacent host cartilage, and no signs of teratoma formation were found by 12 weeks. More importantly, an orderly remodeling process was observed resulting in complete osteochondral regeneration over a 12 week period.[92] Progress is being made in developing the use of ESCs as a cartilage repair cell source; however, the technology has yet to reach a practical level.

Synovium-derived stem cells

MSCs were first successfully isolated from synovial membrane tissue by de Bari et al[93] in 2001 and since then, researchers have probed these cells for their possible utility in musculoskeletal therapies, particularly for cartilage regeneration. Synovium-derived MSCs possess chondrogenic superiority compared with other MSC types.[4] In terms of biological similarity, 1 study on synovial joint development revealed that articular chondrocytes and synovial cells originate from the same progenitor cell pool, and synovium-derived cells have higher hyaluronan receptor (CD44) expression and uridine diphosphoglucose dehydrogenase levels, key chondrocyte markers, compared with expression in other MSC types. Moreover, in synovial chondromatosis

tumors, pathological cartilage is formed in the synovial tissue, indicating the chondrogenesis potential of synovial cells.[94,95] In vitro investigations have shown that cartilage pellets formed by synovial MSCs are significantly larger and heavier than those formed from BMSCs.[96] Other investigators compared human MSCs derived from 5 different tissue sources, including bone marrow, adipose, synovium, periosteum, and muscle in terms of yield, expansion, and chondrogenesis potential in pellet culture. Synovium-derived cells were found to have rapid expansion ability and the greatest ability for chondrogenesis.[97] In 1 in vivo study, MSCs isolated from bone marrow, synovium, adipose tissue, and muscle of adult rabbits were embedded in collagen gels and transplanted to full-thickness cartilage defects. Synovium- and bone marrow-derived cells demonstrated higher chondrogenic potential than adipose- and muscle-derived cells, and synovium-derived cells possessed the greatest proliferation ability in vivo.[98] Numerous studies have been conducted on the effects of chondrogenic inducers, including TGFβ family members, BMP2, and BMP7, in the chondrogenesis of synovium-derived MSCs. The results remain inconclusive, since different culture systems and combinations of growth factors have led to contradictory findings.[98–101]

Periosteum-derived progenitor cells

Periosteum, as a whole tissue, satisfies the following three requirements of a tissue-engineered construct: it contains cells, a scaffold, and correct signaling factors for the target tissue, in this case, cartilage. Periosteum contains pluripotent MSCs (among other cell types),[93] an ECM or "scaffold" to mechanically support the cells, and many chondrogenic growth factors.[102] It is not surprising, therefore, that when tested as a patch in cartilage defect repair, periosteum tissue guided the organization of chondrocytes and facilitated tissue formation in ACI procedure. One study compared the chondrogenic response of primary bovine chondrocytes and whole periosteum rabbit explants when mixed in alginate gels and cultured with TGFβ1. The cell–alginate constructs lacked the cellular and matrix organization of native hyaline cartilage after 42 days of in vitro culture, posing a significant barrier to utility. However, the periosteum–alginate samples showed extensive development of cartilage-like tissue with 54% ± 8% of the total explant area staining positive with safranin-O, a cartilage-specific stain.[103] Further studies of this system demonstrated that addition of FGF2 to the culture can increase proliferation of cells within the periosteum, increasing overall chondrogenesis, and that TGFβ1 was necessary for chondrogenesis to occur.[104]

The periosteum contains a fibrous layer and a cambium layer; PDPCs with chondrogenesis potential are resident in the cambium layer and are currently being examined for their potential as a cell source for cartilage engineering.[105,106] A combination of positive and negative surface markers can be used for PDPC isolation, and similar to MSCs, CD34 and CD45 are not expressed on PDPCs.[107] After sorting for CD105, SH2, SH3, and SH4, sorted PDPCs maintained chondrogenic potential over 15 passages in vitro. PDPCs show spontaneous chondrogenic activity at early passage numbers from young donors, but their chondrogenic differentiation capacity diminishes with age and passage number due to a decrease in stem cell number.[39,108] Regardless of age or passage, the phenotype of PDPCs is stable during cell expansion within 15 passages, and chondrogenesis remains inducible via stimulation with the growth factors TGFβ1 and TGFβ3.[108] The chondrogenic potential of PDPCs is similar to other MSCs in vitro and in vivo, but further cell sorting may be used to improve the chondrogenic potential of PDPCs. In animal studies, periosteum- and bone marrow-derived cells showed similar results of chondrogenic differentiation in the repair of large, full-thickness defects of articular cartilage in rabbits.[71,109] However, other research revealed that PDPCs do not survive for long periods within the repair tissue, and results are highly variable in animal experiments.[4,110]

Conclusion

In many cases, surgical methods for articular cartilage repair are successful in stimulating regrowth of damaged cartilage. Unfortunately, in many cases, repair may not be possible, and regeneration may be the only method for healing a defect and providing the patient pain-free and full-range mobility. Regeneration appears most successful when cell-based therapies are used, especially when the appropriate cells, matrices, and chemical signals are combined. Identification of the correct cell source is a key aspect in cartilage regeneration, as the source must provide a large reservoir of cells that are easily isolated and have strong chondrogenic potential, even after expansion. Primary chondrocytes and stem cells have advantages and disadvantages regarding these requirements, leaving the most useful cell source still debatable. However, during cartilage regeneration, the role of cell sources can be modified by other factors, including the environment in which they are cultured, chemical and biological factors, matrices, and other surgical procedures. This suggests that rather than hunting for an ideal cell source, combinations of the above features should be explored to improve cartilage regeneration. More in-depth analyses of

the combinatorial effects of tissue engineering variables are needed to develop more durable, immunocompatible, integrative tissue-engineered articular cartilage. Steps in this direction suggest stem cells or subpopulations of primary chondrocytes may be the best current cell sources for regenerative and tissue engineering applications.

Disclosure

The authors report no conflicts of interest in this work.

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