The state of cartilage regeneration: current and future technologies

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Abstract It is clear that mature human articular cartilage does not have the innate ability to regenerate. Due to this, much effort has been put forth to work on bestowing this ability. While early data focused on more basic outcomes such as percentage of defect fill, the tissue formed was a “cartilage scar” or “hyaline-like” tissue. Even with more advanced technologies, it is clear that no current procedure is able to reconstitute the native structure and function of true hyaline cartilage. As research advancement has somewhat plateaued in this regard, it is crucial that future work focuses on a multifactorial approach, treating the joint as an organ system. The purpose of this review is to update readers on the most recent literature and controversies surrounding articular cartilage regeneration. Specific focus will be placed on current technologies available in the USA and the basic science to support them.

Keywords Cartilage regeneration · ACI · DeNovo · Biocartilage · PRP · ACS · Stem cells · Growth factors

Introduction

When working to develop the ideal cartilage regeneration technology, there is a constant struggle between “ideal science” and what is practically achievable. The International Cartilage Repair Society (ICRS) has begun a registry of all available cartilage technologies located at <http://www.cartilage.org/?pid=313>. When reviewing this non-exhaustive list (entries are voluntary by the developing company), one notes that treatments typically involve a cell source, growth factors, a scaffold, or some combination therein. While a cellular component is arguably the most important aspect of successful cartilage regeneration, of the 12 ongoing clinical stem cell trials, 84 % are performed outside of the Americas (Asia 42 %, Europe 42 %, North America 8 %, South America 8 %). This is largely due to the regulatory burden that is present in the USA. Specifically, the Food and Drug Administration (FDA) formed the Tissue Reference Group (TRG) in 1997, which oversees the use of human cells, tissue, and cellular and tissue-based products (HCT/Ps) under section 361 of the Public Health Service (PHS) act. Understanding this process helps delineate the dichotomy of technologies that have and will continue to be developed in the USA and abroad. Essentially, if HCT/P guidelines deem that the implant is more than “minimally manipulated” or does not meet “homologous use” guidelines, the FDA market approval pathway is required. Examples on the FDA TRG website are available to help illustrate prior decisions and the rationale (<http://www.fda.gov/biologicsbloodvaccines/tissuetissueproducts/regulationoftissues/ucm152857.htm>). The aftermath has resulted in a significant increase in two main categories of technology: microfracture augmentation products and homologous use allogeneic tissue. Due to the vast amount of technologies available worldwide and wide scope of this topic, this review will focus on the state of cartilage regeneration in the USA along with future directions.

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Cartilage regeneration theory

Evidence is mounting that mature articular hyaline cartilage was formed because of *everything* that it was exposed to throughout development. In this light, it becomes clear that multiple factors are required such as weight bearing [1], motion [2], an orchestration of growth and differentiation factors [3], integration into the subchondral bone [4], and many more. The other difficulty is that true hyaline cartilage was formed over a period of many years in vivo, which is not practical in the clinical setting and likely too complex to recapitulate in the lab. Therefore, utilizing a structure/scaffold/graft that already contains the appropriate structure may be beneficial along with a cell source capable of coordinating its maintenance and integration. This integration will always be in two main directions: peripheral and deep. Peripheral integration always involves cartilage-to-cartilage healing; however, deep integration can either be cartilage to bone (microfracture or autologous chondrocyte implantation) or bone to bone (osteochondral allograft or autograft). The later is likely more reliable (fracture healing) versus the former which requires recapitulation of the calcified cartilage layer and can be subject to delamination. While structurally, hyaline cartilage would be ideal for long-term benefit in preventing further deterioration, restoration of true hyaline cartilage may not be necessary to impart short-term or symptomatic benefits.

Competing views would argue that restoration of true native articular structure and function is not necessary for *symptomatic improvement*. Just as a scar in the skin serves the same role as native skin to provide a barrier, a scar that is large enough may limit the skin's ability to excrete electrolytes, grow hair follicles, and other important factors. Therefore, some critical value may be present in cartilage by which cartilage repair opposed to restoration may be adequate. This is a difficult problem to address and the pain generator in the setting of a focal articular defect is unclear. As cartilage is aneural, some potential sources are synovial inflammatory cytokines, synovitis, and subchondral bone collapse.

To balance these two approaches, there is likely some role for hyaline-like tissue to provide short-term benefit, but this will ultimately succumb to any technology that is cost effective and available and can restore normal architecture and function.

Microfracture and augmentation

Though microfracture may not be viewed as an emerging technology, it is important to revisit as it is uniquely inexpensive, requires little expertise, and can be performed at the index operation. As mentioned earlier, methods that augment this cell source will continue to develop in light of FDA regulations. While the outcomes for microfracture alone have been varied, most agree that it produces inferior fibrocartilage fill that has limited long-term benefit [5]. As with any cartilage

procedure, patient selection is the key to successful outcomes. Based on a systematic review by Mithoefer et al., optimal outcomes are achieved in the following setting: age <40 years old, body mass index <30, no prior cartilage surgery, symptom duration <12 months, lesion size <4 cm², pre-operative Tegner score >4, and MRI evidence of lesion fill >66 % [6••]. Results of lesions located on the femoral condyle tend to be superior to the patellofemoral joint [7]. A review by Negrin et al. highlighted the importance of these strict indications as the study by Gudas et al. was removed from their analysis due to its significantly superior outcomes when compared to other studies [8]. The study population of the Gudas article was highly competitive sportsmen (40 %) or well-trained athletes (60 %) that were under 40 years old with lesions <4 cm², demonstrating the outcomes yielded by strict indications [9]. Though there has been concern that prior microfracture may decrease outcomes of subsequent autologous chondrocyte implantation through increased revision rate and recurrent pain, there has been no differences in function or return to play [10•, 11, 12].

Microfracture technique likely plays an important role in subsequent outcomes (Fig. 1). Specifically, research has focused on the differences between devices (awl, drill, and smooth wire) and the effect of the subchondral bone and its integration. Frisbie et al. demonstrated the importance of removing the calcified cartilage layer in a horse model for tissue integration [4]. However, the ability of the surgeon to adequately remove this 0.2-mm-thick undulating layer accurately has been drawn into question. Regarding technique, evidence suggests that creating marrow access with 4–6 mm of depth using a fluted drill may decrease osseous compaction and decrease thermal necrosis, leading to increased marrow access [13, 14].

Technologies that augment microfracture commonly involve a scaffold that maintains clot stability. These technologies may also attempt to leverage growth factors as well (e.g., platelet-rich plasma). Stanish et al. reported the first microfracture augmented randomized clinical trial comparing microfracture alone to BST-CarGel (Piramal Life Sciences, Bio-Orthopaedic Division) [15]. BST-CarGel is an example

Fig. 1 Arthroscopic image of microfracture after completion with the pump pressure let down, demonstrating access to mesenchymal stem cells

of a “clot stabilizer” that is an aqueous form of chitosan (glucosamine polysaccharide) which is mixed with fresh whole autologous blood and delivered through an arthrotomy. While the treatment group demonstrated improved fill and tissue quality at 12 months, hyaline cartilage was not formed and there was no difference in subjective outcomes. BioCartilage (Arthrex, Naples, FL) is a commercially available clot stabilizer that consists of hypothermic dehydrated allograft articular cartilage that is micronized to particles 100–300 μm in size. This is delivered as an injectable after being mixed with equal parts of an autologous blood solution that is typically covered with fibrin glue (Fig. 2). The benefit of this technology is the shelf life is 5 years and can be utilized at the time of the index arthroscopy. Clinical outcomes of BioCartilage have yet to be determined.

Minced cartilage products

The benefit of allogeneic tissue is the presence of native collagen architecture; however, this comes at a great expense and decreased availability. Again, while osteochondral grafts are not new technology, it is important to note that this is the only method that delivers native hyaline articular cartilage to the defect site and simultaneously addresses the subchondral bone (Fig. 3). Several technologies have emerged in the allograft space that attempt to circumvent the shortcoming of fresh osteochondral allografts. One category is particulated articular cartilage [16]. DeNovo NT (Zimmer, Warsaw, IN) is minced juvenile cartilage allograft delivered with fibrin glue used to treat chondral defects without significant bone loss (Fig. 4). Juvenile chondrocytes (<13 years old) have 100-fold increased ability to produce proteoglycans and have the potential to increase the ability of adult cartilage fragments to

Fig. 2 Gross photograph of BioCartilage placed into a trochlear defect in a cadaver. The BioCartilage has been combined with equal parts of platelet rich plasma and has not been covered with fibrin glue at this stage

Fig. 3 Arthroscopic image of an osteochondral allograft used to resurface the femoral condyle

produce matrix [17, 18]. These cells also do not stimulate an immunogenic response in vivo [17]. While this is ideally a single-stage procedure, unlike autologous chondrocyte implantation, DeNovo NT has a ~40-day shelf life similar to a fresh osteochondral allograft. Therefore, many surgeons only utilize this after performing a diagnostic arthroscopy. Cells within a given package are from a single donor and contain 30–200 unites from the donor’s knee. As outcomes of autologous chondrocyte implantation is superior in the patellofemoral joint, DeNovo NT has been applied most commonly to this location, as osseous defects are also less common. Tompkins et al. reported its use in the patella at an average of 28 months in defects $2.4 \pm 1.2 \text{ cm}^2$ [19]. The density of DeNovo utilized was 1 unit per 1.3 cm^2 , opposed to the manufacturer recommended 1 unit per 2.5 cm^2 . Outcomes demonstrated mean fill on MRI of 89 % with 2/15 grafts developing hypertrophy that required debridement. More recent data from Farr et al. in a similar patient population demonstrated a favorable type II/I collagen ratio on immunohistochemistry biopsies with no reoperations in 25 patients at 2 years [20•]. Whether DeNovo NT provides sustainable

Fig. 4 Gross photograph of DeNovo NT placed into a trochlear defect with fibrin glue. Note the density and relative size of the particulated cartilage

cartilage repair and symptom relief at long-term follow-up remains to be seen. A number of potential concerns with this technology have been identified in our laboratory during *in vitro* testing [21]. First, minced juvenile cartilage represented relatively heterogeneous preparations, in which cartilage pieces were subjectively discolored and contained dead cells. Second, their metabolism, the ability to migrate out of the tissue onto fibrin glue and to form new cartilage, was not superior to mature adult cartilage from donors of 50 years or younger. Third, there are no compelling data to suggest that the use of juvenile cartilage in a form of minced tiny pieces is an absolute need since adult chondrocytes from larger explants (3 mm in diameter or larger) performed as good, if not better, at least in culture. Finally, the source of juvenile cartilage will always remain an issue.

One competing autograft equivalent is the Cartilage Autograft Implantation System (CAIS, DePuy Mitek and DePuy Biologics). The technique involves a single-stage procedure where cartilage is harvested from a non-weight-bearing portion of the knee, is minced, and then seeded onto a resorbable copolymer foam with fibrin glue which is implanted with polydioxanone (PDS) staples [22]. Equine data from Frisbie et al. set the stage for CAIS as they demonstrated similar outcomes to a modified ACI procedure with regard to type II collagen and proteoglycan content [23]. Further, data from a randomized control study (clinical safety trial) by Cole et al. demonstrated in 29 patients at 2 years improved IKDC and KOOS scoring with decreased intralesional osteophyte formation in CAIS compared to microfracture [24]. While CAIS showed promise with regard to a cost-efficient single-stage procedure, it has stalled in phase 3 due to lack of enrollment. As such, this is a common fate for such technologies and has further discouraged technologies that require such approval.

Cell-based products

Just as microfracture and osteochondral grafts are the standard by which new technologies are compared, autologous chondrocyte implantation (ACI) deserves the same recognition within cell-based treatments. Autologous chondrocyte implantation requires a first stage consisting of cartilage biopsy, after which the cells are expanded in culture. During a second stage procedure, the cells are then implanted to the defect site, which is covered with a periosteal patch. This first-generation ACI unfortunately had a 15–28 % reoperation rate due to hypertrophy [12, 25]. Second generations have used a type I/III collagen membrane to avoid patch hypertrophy (Fig. 5). Long-term outcomes regarding first-generation ACI have demonstrated 71 % survival and 75 % of patients with continued symptomatic improvement at 10 years [26, 27]. However, this technology requires two procedures and must be combined with grafting in the setting of significant bone loss [28].

Fig. 5 Gross operative photograph of autologous chondrocyte implantation (ACI) covered with a type I/III collagen membrane in the trochlea

Also, there is some concern that outcomes may be negatively affected by prior microfracture at the defect site [10, 11]. Furthermore, *in vitro* data accumulated in our laboratory suggest that the metabolic activity and survival rate of ACI chondrocytes might be lower than those of primary chondrocytes, which together might affect patient outcomes [29]. Similarly, the ACI cells had decreased phenotypic stability. ACI cells deposited fewer extracellular matrix proteins and the composition of the extracellular matrix was distinct from their primary counterparts. Growth factor dosing had to be increased twofold in the ACI cells to stimulate comparable levels of proteoglycan synthesis. Clinical results in randomized control trials comparing ACI to microfracture demonstrate similar results at 2 [30] and 5 years [31]. This highlights the importance of more Level I studies in this arena.

Cell sources for cartilage regeneration have included bone marrow mesenchymal stem cells (BM-MSCs, MSCs), umbilical cord cells, embryonic stem cells, human placental membrane, and adipose derived MSCs, to name a few. Several groups have investigated the chondrogenic potential of these cell sources. While adipose stem cells have the benefit of minimally invasive harvesting and being autologous, this is tempered by their decreased chondrogenic capacity. While synovial MSCs may have the most chondrogenic ability, they maintain a fibrocyte phenotype, which is not ideal. BM-MSCs are the most commonly utilized currently and though they have more chondrogenic capacity than adipose cells, the harvest is more invasive. Another benefit of MSCs is their anti-inflammatory and immunosuppressive properties [32, 33].

Several systems are available to obtain BM-MSCs both from the shoulder and knee for clinical use. As the cellular component includes platelets and MSCs, growth factors secreted include PDGF, TGF- β , and VEGF [34, 35]. Using the Harvest system (Harvest Technologies, Plymouth, Massachusetts), significant increases in platelet, leukocytes, and red

blood cells are obtained by concentrating the sample [36••]. Using this system in an equine model, treatment of an osteochondral defect with microfracture and BM-MSCs demonstrated increased defect fill and type II collagen compared to microfracture alone [36••]. Similarly, in a rabbit osteochondral defect model, Liu et al. reported improved repair with BM-MSC injection and even greater potential when embedded in a synthetic extracellular matrix [37]. Reports on clinical outcomes are limited and have not included level I or II data. Gigante et al. reported the use of BM-MSCs in conjunction with a fibrin carrier to arthroscopically treat cartilage lesions as a microfracture adjunct in a single patient [38]. Nejadnik et al. reviewed a cohort undergoing either first-generation ACI or cultured BM-MSCs [39•]. These cells were cultured as would be performed for ACI and demonstrated no difference in outcome at 2 years. Interestingly, outcomes were inferior for ACI patients older than 45 years old; however, this age dependence was not present for BM-MSCs. While this demonstrates the utility of BM-MSCs, this form is not available for use in the USA. Further studies are necessary to determine the clinical role of BM-MSCs in the setting of cartilage regeneration.

Our institution is involved in the first clinical stem cell trial in the USA, which utilizes Cartistem (Medipost, Seoul, Korea). Cartistem is obtained from allogeneic umbilical cord MSCs (UC-MSCs). Phase III trials are still enrolling in Korea and are currently in FDA phase I/IIa trials in the USA. The procedure is performed with microfracture and is delivered along with a hyaluronic acid scaffold (Fig. 6). Pre-clinical data has demonstrated that UC-MSCs are present in 23 % of samples and expansion can be up to 1000-fold [40]. Another advantage of these cells is their lack of major histocompatibility complex-II class molecules on their surface, unlike BM-MSCs [41]. This holds true even after differentiation into the chondrocyte lineage. While the combination of chondrogenic potential and lack of immunogenicity are attractive, the true role for UC-MSCs has yet to be defined.

Fig. 6 Image of defect treated with microfracture and Cartistem treatment

Growth factor treatments

Joint injections such as corticosteroids or hyaluronic acid aim to decrease inflammation and viscosity, leading to decreased symptoms without being disease modifying. However, these changes are transient and may require multiple injections and lose effectiveness. Newer injectables have included platelet-rich plasma (PRP) and allogeneic or autogenous stem cells, along with other growth factors. These factors play an important role in altering the environment that the cartilage regeneration technology is exposed to, attempting to improve symptom relief and ideally improve the quality of tissue fill. To date, no isolated growth factor injections are available for clinic use in the USA. Phase 1 safety trials of Osteogenic Protein-1 (Stryker) demonstrated both safety and some level of effectiveness when used as an intra-articular injection for knee osteoarthritis [42]. However, further trials were unable to be sustained and have been discontinued. FGF-18 has demonstrated the ability to repair cartilage in animal osteoarthritis models and clinical reports are now being published [43, 44]. Merk Serono (Darmstadt, Germany) is sponsoring the phase 2 trial utilizing FGF-18 injections to augment microfracture in the USA and is currently enrolling patients.

While recombinant growth factor delivery requires FDA approval, usage of autologous factors with minimal manipulation does not. Clinically, this has manifested as platelet-rich plasma. This contains both a cellular and growth factor component with a predominance of platelets, red blood cells, and leukocytes, depending on the formulation. Growth factors involved include TGF- β 1, thrombospondin-1, VEGF, PDGF, and insulin-like growth factor [45, 46•]. In vivo studies reported addition of PRP to an agarose gel scaffold increases chondrocyte-related gene expression and integration strength in a porcine model [47].

While this basic science data is promising, a recent systematic review highlighted the paucity of data on the use of PRP in the setting of an acute traumatic osteochondral defect [48]. Mei-Dan et al. reported on osteochondral lesions of the talus with improved outcomes at 7 months after PRP as compared to hyaluronic acid [49].

As PRP has garnered a significant following in the USA, the use of Orthokine (Orthogen, Düsseldorf, Germany) has a similar following in Europe. This was largely spurred by reports of professional US athletes traveling for these injections. Orthokine is now available in the USA as Regenokine; however, independent publications of clinical results have not been reported from this experience. Orthokine/Regenokine is autologous conditioned serum (ACS), which is procured from autologous blood that is incubated with borosilicate glass spheres, leading to increased levels of IL-1 receptor antagonist (IL-1ra) [50]. An equine model of induced OA demonstrated no adverse events along with improvements in lameness and histology compared to the placebo group [51]. Baltzer et al. reported improved outcomes in patients with Kellgren and

Lawrence grade 2–3 arthritis as compared to hyaluronic acid (HA) and placebo (saline) injections [52•]. While this study is listed as double blind, the ACS group received six injections whereas the HA and saline group only received one, which may induce significant bias. More recent work by Rutgers et al. highlighted that the increased IL-1ra content of ACS is accompanied by increased TNF-alpha amounts leading to a net zero effect on cartilage metabolism [53]. The largest detriment of these technologies is their short half-life in the synovial fluid. While further data is necessary to determine the appropriate role of autologous factor application, there is no doubt that altering the environment in which cartilage repair technology is placed is paramount.

Summary

Successful cartilage treatment hinges on appropriate indications, regardless of the method utilized. Long-term outcomes may benefit from true hyaline articular cartilage structure and function; however, no current treatments are able to impart this. The landscape of cartilage regeneration in the USA continues to change at a lumbering pace due to FDA restrictions, limiting the advancement of current methods. In this light, while many options may be on the horizon, osteochondral grafts, ACI, microfracture, and minced cartilage are the current options. Combining a metabolically active cell source along with treatment of the inflammatory environment will likely synergistically affect cartilage treatment in the future.

Compliance with Ethics Guidelines

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