ABSTRACT: The social impact of bone and cartilage pathologies entails high costs in terms of therapeutic treatments and loss of income. As a result, the current research trend includes preventive interventions and therapeutic solutions that can lead to an enhancement of tissue regeneration and the reduction of degenerative mechanisms.

Many options have been made available to address problems regarding cartilage damage, each with its own advantages and disadvantages. Several studies are currently in progress to clarify some of the questions that remain unanswered about the long-term durability of these procedures and the possible modifications that can be made to achieve better results.

Biotechnology is progressing at a rapid pace that allows the introductions of several products for clinical application; however, randomized, prospective studies for these innovations should be conducted to validate the safety and efficacy of cartilage regeneration.

INTRODUCTION

According to a study conducted by the World Health Organization, musculoskeletal injuries are the most common cause of severe long-term pain and disability, affecting millions of people worldwide. 2000 to 2010 has been called the “decade of bone and joints” to launch global awareness and promote further research in the prevention, diagnosis, and treatment of joint injuries.

The social impact of bone and cartilage pathologies entails high costs through therapeutic treatments and loss of income. In the United States, osteoarthritis medicines cost $5.31 billion in 2007, and musculoskeletal conditions including osteoarthritis cost nearly $128 billion per year in direct medical expenses (ie, total joint replacement procedures and loss of income and production in 2003). For these reasons, the trend of the research is now going toward preventive interventions and therapeutic solutions that can lead to an enhancement of tissue regeneration and the reduction of degenerative mechanisms.

CARTILAGE TREATMENT

Hyaline cartilage combines a smooth surface and the ability to withstand an extreme amount of pressure. It is extremely important to reconstruct a perfect surface that will withstand heavy loads. Unfortunately, articular cartilage lesions, with their inherent limited healing potential, remain a challenging problem for orthopedic surgeons. In the past few decades, surgeons often replaced the articular surface with expensive and sophisticated implants when articular lesions become full-blown osteoarthritis. However, recent studies have used new orthobiological techniques in cartilage lesions with increasing frequency and effectiveness as a way to regenerate tissue homeostasis and delay the progression of osteoarthritis. Growth factors and mesenchymal stem cells have been used successfully in many medical fields, such as maxillofacial, cosmetic, spine, orthopedic, and general wound healing applications.
CONSERVATIVE TREATMENT AND PREVENTIVE BIOLOGIC SOLUTION

Nonsurgical treatment of cartilage lesions, including diet, intra-articular injections, and rehabilitation were relegated to pain control and activity modifications. Recent studies on pulsed electromagnetic fields and platelet-rich plasma injections have shown that these methods have the capacity to help heal cartilage tissue and delay osteoarthritis.

A recent study by Focht et al\textsuperscript{19} analyzed 316 adults with obesity who underwent an arthritis, diet, and activity promotion trial. This 18-month single-blind, randomized controlled trial, compared the effects of exercise alone, dietary weight loss alone, a combination of exercise plus dietary weight loss, and a healthy lifestyle control intervention. The study concluded that exercise and dietary weight loss, compared with the healthy lifestyle control intervention, resulted in improved mobility-related self-efficacy and pain reduction.\textsuperscript{19}

In an animal study, Ciombor et al\textsuperscript{13} showed that pulsed electromagnetic fields preserved the morphology of articular cartilage and retarded the development of osteoarthritic lesions in Hartley guinea pigs compared with a control group. The study concluded that pulsed electromagnetic fields were disease modifying in this population. This supported a previous in vitro study of human chondrocytes that showed increased cell proliferation with exposure to pulsed electromagnetic fields.\textsuperscript{16} The study noted that electric and electromagnetic fields increased gene expression and synthesis of growth factors, and that this may amplify field effects through autocrine and paracrine signaling. Electric and electromagnetic fields may produce a sustained regulation of growth factors that enhance, but do not disorganize, endochondral bone formation.\textsuperscript{1}

Similarly, Massari et al\textsuperscript{35} summarized the results of the translational research of the Cartilage Repair and Electromagnetic Stimulation study group on the use of specific pulsed electromagnetic fields (I-ONE; IGEA, Carpi, Italy) (Figure 1) to control local joint inflammation and, ultimately, to have a chondroprotective effect on articular cartilage. The study showed that of patients who underwent chondral coblation at 3-year follow-up, the number of patients who completely recovered was higher in the group treated with I-ONE therapy compared with the control group. Clinical results show how I-ONE therapy is an effective chondroprotective treatment for patients immediately after arthroscopic surgery without any negative side effects and exerts a short-term effect in reducing functional recovery time.

However, platelet-rich plasma preparations have been used with effective results both in surgical and outpatient procedures in the treatment of musculoskeletal problems.\textsuperscript{29,52} Some studies suggested that platelet-rich plasma treatment prior to surgery prevented the necessity to undergo the surgical procedure.\textsuperscript{38} In addition, platelet-rich plasma combined with proper nutrition, including control of body mass index, exercise, and lifestyle, can act as a preventive agent in chronic and degenerative musculoskeletal disease.

SURGICAL TREATMENT

Traditional palliative or reparative treatment techniques have demonstrated variable results. Lavage and chondroplasty can provide symptomatic pain relief with no actual hyaline tissue formation. However, these techniques remove superficial cartilage layers that include collagen fibers responsible for the tensile strength, which creates a functionally inferior cartilage tissue.

Bone marrow stimulation techniques, such as subchondral plate drilling or microfracture, have been reported to stimulate production of hyaline-like tissue with variable properties and durability, compared with normal cartilage. However, many cases showed that these techniques tend to produce fibrocartilaginous tissue that will degenerate with time.\textsuperscript{23,32,53,54}

Kreuz et al,\textsuperscript{32} in a systematic review of 28 microfracture studies, noted that most authors have reported a decline in functional scores at medium term. However, at the last follow-up, patients still showed significant improvement from their preoperative scores.

Kon et al\textsuperscript{11} recently compared microfracture with second-generation autologous chondrocyte implantation and showed that at 5-year follow-up, sports activity of the microfracture group significantly decreased from the 2-year follow-up.

Microfracture is commonly used as first-line treatment because it is easy and does not require special instruments or implants. However, recent reports have shown that in a group of patients treated with autologous chondrocyte implantation, the worse results (failure and reoperation) oc-
The experience in patients previously treated with microfracture, compared with patients treated with autologous chondrocyte implantation as first-line surgery.\(^\text{32}\)

Osteochondral autologous transplantation and mosaicplasty can restore normal cartilage tissue, but the application is restricted to small defects and there are some concerns about donor-site morbidity.\(^\text{28}\)

First introduced by Peterson,\(^\text{10}\) autologous chondrocyte implantation has been proven capable of restoring hyaline cartilage tissue. Recent studies\(^\text{41,46,47}\) suggested the durability of this treatment, especially at long-term follow-up, primarily due to its ability to produce hyaline-like cartilage that is mechanically and functionally stable. Autologous chondrocyte implantation also allows integration with the adjacent articular surface. However, this method requires 2 surgical procedures and showed local morbidity for periosteal harvest.\(^\text{11}\)

The complexity of the Peterson periosteal technique and the possible complication of periosteal patch hypertrophy prompted surgeons to seek alternative techniques to enhance cell delivery and outcome.

At present, the most promising technique is tissue engineering, in which cells are combined with scaffolds to form a tissue; in general, the concept involves cultured autogenous chondrocytes integrated in biodegradable and biocompatible scaffolds. After the chondrocytes are cultivated and seeded on the scaffold, they must reacquire and maintain their chondrogenic phenotype to synthesize an extracellular matrix containing type II collagen, glycosaminoglycans, and proteoglycans, all of which are necessary to produce hyaline cartilage.

### SECOND-GENERATION AUTOLOGOUS CHONDROCYTE IMPLANTATION

The ideal scaffold should be biocompatible, biodegradable, not trigger any inflammatory response, and not cytotoxic. It should offer a temporary support to cells to promote replacement of a newly synthesized matrix and possibly induce proliferation of the transplanted cells. The matrix should also be permeable to nutrients and provide firm adhesion to the surrounding cartilage wound edges to promote integration. In addition, the scaffold must be reproducible and readily available, as well as versatile for repair and resurfacing.\(^\text{12,25,42,55}\)

After a systematic review of the available literature about different scaffolds in the market, which includes porcine collagen I and III (ie, membrane-seeded autologous chondrocyte implantation [Genzyme Europe BV, Naarden, The Netherlands]), three-dimensional bovine collagen (NeoCart; Histogenics, Waltham, Mass), and polyactic acid and polyglycolic acid fleece (Bioseed, Bangkok, Thailand), we concluded that a hyaluronan-based scaffold may be optimal for chondrocyte proliferation.\(^\text{30}\)

Among several other proteins, hyaluronan is a naturally occurring and highly conserved glycosaminoglycan widely distributed in the body. It has proven to be an ideal molecule for tissue engineering strategies in cartilage repair because of its impressive multifunctional activity through its structural and biological role. The three-dimensional nonwoven scaffold, HYAFF (Fidia Farmaceutici, Padova, Italy), supports the in vitro growth of highly viable chondrocytes and promotes the expression of the original chondrogenic phenotype.\(^\text{12}\) Chondrocytes that were previously expanded on plastic and seeded into the scaffold produce a characteristic extracellular matrix rich in proteoglycans and express typical markers of hyaline cartilage, such as collagen II and aggrecan.\(^\text{25}\) When implanted in full-thickness defects of the femoral condyle in rabbits, chondrocytes regenerated a cartilage-like tissue.\(^\text{26,55}\)
The main indications for second-generation cartilage transplantation are symptomatic focal, full-thickness cartilage lesions (ie, International Cartilage Repair Society grades III to IV) in the absence of significant arthritis in physiologically young patients (between ages 15 and 50). Additional factors to consider include the patient’s motivation and willingness to comply with the postimplantation rehabilitation regimen. Defect sizes (range, 2-12 cm²) have been shown to be favorable to regeneration. Osteochondritis dissecans is not a contraindication for cartilage transplantation as long as the bone loss is not >8 mm. Adverse events of second-generation autologous chondrocyte implantation are apparently lower than first generation, as reported by Mandelbaum. Several reports from controlled trials in patients undergoing surgery with the use of these hyaluronic acid scaffolds have been presented. However, the largest collection of data using the hyaluronic acid scaffold in clinical practice is represented by a multicenter observational study conducted in Italian Orthopedic Centers since

Autologous chondrocyte implantation can be performed through the conventional arthrotomy approach. However, recent advances in scaffold technology has enabled surgeons to perform this technique arthroscopically (Figures 2 and 3). Some technical limitations prevail, including treatment of patellar lesions and posterior portions of femoral condyles or the tibial plateau. These limits are common to all arthroscopic techniques and could partly be resolved with the development of new arthroscopic tools.

In a prospective, nonrandomized study on patellofemoral lesions treated with second-generation autologous chondrocyte implantation, we analyzed a group of patients at the 5-year follow-up, using International Knee Documentation Committee (IKDC) subjective and objective scores, the EuroQoL pain scale, and Tegner scores. The authors noted significant improvement from preoperative scores to the final follow-up. Objective preoperative data improved from 8 of 34 (23.5%) patients classified as IKDC A or B scores to 31 of 34 (91.2%) classified IKDC A or B scores at the 5-year follow-up. Mean subjective scores improved from 46.09 points preoperatively to 70.39 points 5 years postoperatively, and Tegner scores improved from 2.56 to 4.68. EuroQol visual analog scale scores improved from 54.81 to 78.24.

In another study, we compared second-generation autologous chondrocyte implantation with microfracture and found a higher improvement in the IKDC objective and subjective scores in the group treated with second-generation autologous chondrocyte implantation at the 5-year follow-up. Analyzing the resumption of sports activity obtained with the Tegner score, we observed similar level in both groups at the 2-year follow-up, which remained stable at the 5-year follow-up in the second-generation autologous chondrocyte implantation group, whereas return to sport activities worsened in the microfracture group.

Future Implications

Third-Generation Autologous Chondrocyte Implantation

Promising results have been shown with autologous chondrocyte implantation. Autologous chondrocyte implantation is a technology that involves the implantation of an expanded chondrocyte population derived from a cartilage biopsy. These expansions result in the loss of phenotypic traits, also called dedifferentiation. This produces chondrocytes with a decreased capacity to regenerate hyaline cartilage cells.

Characterized chondrocyte implantation is a new generation of autologous chondrocyte implantation procedures that uses ChondroCelect (TiGenix NV, Haasrode, Belgium). ChondroCelect was developed to limit the loss of phenotype and is an expanded population of chondrocyte with a proven ability to produce stable cartilage in vivo (Figure 4). The highly controlled and consistent manufacturing process is based on the expression of a marker profile to characterize this cell population.

A recent prospective, randomized controlled trial that compared characterized chondrocyte implantation ver-
sus microfracture as treatment for a single symptomatic cartilage defect of the femoral condyle showed that characterized chondrocyte implantation produced a superior type of tissue regenerate. The primary aims of the trial were to demonstrate superiority of characterized chondrocyte implantation over microfracture in overall quality of structural regeneration of the articular tissue at 12 months posttreatment using histomorphometry and the overall histology assessment score. In addition, the study aimed to demonstrate that clinical outcomes at 12 to 18 months posttreatment were comparable in both treatment groups. For the first time, it was proved that joint surface repair and regeneration using cell technology produced a higher quality regenerate than did intrinsic repair. Recently, the authors released their 36-month results, which showed that the ChondroCelect group continues improvement, according to Knee Injury and Osteoarthritis Outcome Scores; these results suggest characterized chondrocyte implantation may lead to an improved long-term clinical outcome (Saris DB, oral communication, May 2008).

Future clinical studies using combinations of characterized chondrocyte implantation technology and hyaluronic acid could analyze whether the combination of viable cells and scaffold can lead to a more stable and long-lasting regenerated cartilage.

**Mesenchymal Stem Cells Implantation: Toward 1-Step Surgery**

Second-generation and third-generation autologous chondrocyte implantation represents a modern and viable technique for cartilage full-thickness chondral lesion repair. However, these are 2-step procedures that include an arthroscopic biopsy, cell cultivation, and subsequent implantation. Aside from the risks associated with harvest-site morbidity, 2 surgical procedures, and the total cost of the operation, scaffold and chondrocytes cultivation is still high.

Future directions in cartilage repair consider the possibility of 1-step surgery, including the use of stem cells and growth factors. The use of autologous mesenchymal stem cells and growth factors represent an improvement on the currently available techniques, which avoids the first surgery for cartilage biopsy and chondrocyte cultivation.

Many authors have recognized that nucleated cells found in bone marrow are a useful source of cells for restoration of damaged tissue. After mesenchymal stem cells are cultured in the appropriate microenvironment, they can differentiate from chondrocytes and form cartilage. The onset of chondrogenesis requires a chemically defined serum-free medium supplemented with dexamethasone, ascorbic acid, and growth factors, such as transforming growth factor-beta. Along with appropriate scaffolds, these cells can be used to regenerate cartilage in a variety of applications. In addition, the combination of mesenchymal stem cells and platelet-rich plasma make it possible to improve the healing response of cartilage lesions in a 1-step procedure. Some animal and laboratory studies have shown the chondrogenic potential of mesenchymal stem cells, but only few clinical human studies have been published that show these results.

Wakitani et al used autologous cultures of expanded bone marrow for repair of cartilage defects in osteoarthritisic knees. The study examined 24 knees in 24 patients with knee osteoarthritis who underwent a high tibial osteotomy. The patients were divided into a cell-transplanted group and cell-free group. After 16 months of follow-up, the study concluded that mesenchymal stem cells were capable of regenerating a repair tissue for large chondral defects.

**Conservative Biological Approach to Osteoarthritis: Platelet-Rich Plasma**

Recently the idea of a “biological solution for biological problems” has lead to the development of less invasive procedures and accelerated treatments that usually reduce morbidity while enhancing functional recovery. Platelet-rich plasma, which was first introduced by Ferrari et al in 1987 in open heart surgery, is an interesting therapy. Later, this therapy increased in popularity because of its versatility, biocompatibility, and low costs, which has stimulated its therapeutic use in many medical fields. Scientific research and technology have provided new insight to understand the biological potential of platelets in the

**THE JOURNAL OF KNEE SURGERY**
wound-healing process.\textsuperscript{3,52} It is well known that platelets have many functions beyond simple hemostasis. Platelets contain many important bioactive proteins and growth factors, such as platelet-derived growth factor, insulin-like growth factor, transforming growth factor, epidermal growth factor, fibroblast growth factor, and vascular endothelial growth factor. These factors, if secreted, regulate key processes involved in tissue repair, including cell proliferation, chemotaxis, migration, cellular differentiation, and extracellular matrix synthesis.\textsuperscript{6,39}

The rationale for topical use of platelet-enriched preparations is to stimulate the natural healing cascade and tissue regeneration by a supraphysiological release of platelet-derived factors directly in the site of treatment. Several systems are available to prepare the platelet-rich plasma and the platelet gel. Through these systems, a 2- to 6-fold enrichment of platelet concentration is obtained to achieve comparable growth factor enrichment (Figures 5 and 6). The amount of growth factor available to the tissue healing depends on the growth factors actually stored in platelets and the kinetics of the adsorption of the platelet gel.\textsuperscript{17} In addition, evidence is mounting to show that the crucial factors in the effectiveness of this treatment are the number of platelets used (ie, \( \geq 1.2 \times 10^9/\text{mL} \) in platelet-rich plasma) and the way in which they are processed, as the growth factor content of the gel is highly sensitive to these 2 variables.\textsuperscript{8,9,36,37}

Recent studies\textsuperscript{2,15,29,40} have documented the effectiveness of growth factors in chondrogenesis and in preventing degeneration of the joints. In particular, Kon\textsuperscript{29} studied 30 patients with symptomatic degenerative disease of the knee joints treated with 3 platelet-rich plasma intra-articular injections weekly. The 6-month follow-up showed positive effects on function and symptoms with an improvement of 85\% in scores evaluated for patients with median age <60 years, whereas in patients >60 years, the improvement shown was only 30\%. Nakagawa et al\textsuperscript{40} demonstrated the efficacy of autologous platelet-rich plasma in stimulating the proliferation and collagen synthesis of human chondrocytes, suggesting the use of this method in the treatment of cartilage defects. Anitua et al\textsuperscript{2} showed that an intra-articular injection of platelet-rich plasma could induce an increase in production of hyaluronic acid structure and promote angiogenesis and cell proliferation. Cugat et al\textsuperscript{15} used platelet-rich growth factors to treat chondral defect in athletes and obtained good results. According to their experiences with other connective tissue repair, they showed that platelet-rich growth factors in physiological concentration are effective for the recovery of connective tissue. In addition, local treatment is safe and does not alter the systemic concentrations of these proteins. They also had good results using platelet-rich growth factors in articular cartilage lesion treatment and presented the following positive results: platelet-rich growth factors can increase total glycosaminoglycans collagen II synthesis and can decrease degradation. In addition, platelet-rich growth factors induce chondrogenesis of mesenchymal stem cells and promote chondrocyte proliferation, differentiation, and adhesion.\textsuperscript{7}
In an experimental study done on animals, Wu et al. showed the effectiveness of intra-articular injections of platelet-rich plasma with chondrocytes grown in vivo that resulted in the formation of new cartilage tissue. Finally, as observed by Nishimoto et al., we believe that simultaneous concentration of platelets and bone marrow cells, acting as a sources of growth factors and “working cells,” could play important roles in future regenerative medicine.

**REHABILITATION PROGRAM AFTER CARTILAGE TRANSPLANTATION**

The importance of rehabilitation after cartilage transplantation cannot be overemphasized. These protocols are an important part of the success of cartilage regeneration studies in Italy. A standardized postoperative functional rehabilitation protocol is adopted based on current knowledge of the biology of graft healing and on functional criteria and therapy goal progression. Patients will progress through 4 rehabilitation phases:

- Phase 1: Protection of the transplant and the recovery of normal gait.
- Phase 2: Recovery of a correct run.
- Phase 3: Recovery of sports-specific skills.
- Phase 4: Maintenance of the physical fitness attained during rehabilitation and prevention of the risk of reinjury.

Progression between phases is decided according to the achievement of specific criteria (Table), with particular care to avoid swelling and pain in the joint.

**CONCLUSION**

A biological approach to cartilage lesions is a new challenge. A number of viable options have been made available over the years to address problems concerning cartilage damage, and each technique has its advantages and disadvantages. Numerous studies are currently in progress to clarify some of the questions that still remain unanswered regarding the long-term durability of these procedures and the possible modifications that can still be done to achieve better results.

Biotechnology is progressing at a rapid pace, exploring new horizons and allowing the introduction of numerous products for clinical application. However, carefully conducted randomized, prospective studies for each of these innovations should be conducted to validate the safety and efficacy of cartilage regeneration.

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