Chapter

Cartilage Restoration and Allogeneic Chondrocyte Implantation: Innovative Technique

Anell Olivos-Meza, Mats Brittberg, Carlos Landa-Solis and Carlos Suárez-Ahedo

Abstract

Articular cartilage lesions are frequent in young people with deleterious results if not treated properly. Various restorative techniques have been developed with the aim to overcome the limitations and short-term results of cartilage repair procedures. Cell therapy and tissue engineering techniques as Autologous Chondrocyte Implantation (ACI) have proved to induce cartilaginous tissue in joint defects with considerable long-term durability, currently being the gold standard in the treatment of medium to large cartilage injuries. Although results are encouraging and overall, the patients are satisfied, this technique is not exempt of limitations. These include the technical complexity and the costs of the two surgical procedures, de-differentiation of chondrocytes during in-vitro expansion and the limited amount of cartilage from a small biopsy. Here, we describe the recent advances in chondrocytes-based therapies for cartilage restoration, with a focus on the latest development in the use of allogeneic chondrocytes as a cell source. In allogeneic chondrocyte implantation, cells are harvested from cadaveric articular cartilage, and implanted in a scaffold into the cartilage defect. The advantages of this procedure are that there is no need for double surgeries, reduced patient morbidity and the availability of a large chondrocyte depot.

Keywords: cartilage restoration, cartilage treatment, chondral lesions, chondrocyte implantation, allogeneic chondrocytes

1. Introduction

Injured cartilage and lack of intrinsic tissue healing capacity leave a relatively young and healthy population to the risk of development of degenerative osteoarthritis (OA). Currently, the standard surgical intervention for end-stage degenerative joint pathology is total joint replacement. Early surgical interventions for symptomatic focal cartilage injuries include reparative and restorative techniques. The restorative strategies include cell-based (with or without scaffolds) or whole-tissue transplantation techniques.

The short-term outcomes of reparative techniques prompted the development of Autologous Chondrocyte Implantation (ACI) by Mats Brittberg and Lars Peterson

in Sweden in 1987 [1, 2]. Chondrocyte implantation is a cell-based cartilage repair technique in which transplanted cells are used to allow de novo development of the articular hyaline cartilage. Over time, the original technique evolved with the aim of facilitating implantation, reducing complications and improving results. These modifications of the original ACI technique are popularly known as so-called "ACI generations" [3]. Since more than 30 years ago, there are now four generations described in the literature [4]. In the first and second generations the chondrocytes are injected under a membrane (living periosteal and collagen, respectively) while in the third generation the cells are seeded on a three-dimensional porous scaffold, then this construct is place in the cartilage damage area secured with a layer of fibrine glue. Unlike these three generations are performed in two steps, the fourth one can be done in one step using pieces of cartilage fragments or isolated chondrocytes, either autologous or allogeneic (**Table 1**).

The ACI procedure requires an in-vitro expansion of autologous chondrocytes harvested from a non-weight-bearing area of the articular joint and subsequent implantation into the defect after 4 to 8 weeks [2, 4]. Long-term case series with >10 years follow-up have demonstrated that ACI is an effective and durable treatment for large knee cartilage lesions being superior to other standard treatments in prospective randomized controlled clinical trials [5–7]. Compared to other reconstructive therapy options for cartilage defects, ACI shows the best quality of the induced repair tissue [8].

Although the implantation of mature cultured chondrocytes have shown good to excellent long-term results, there are still unresolved challenges associated with the maintenance of those cells in a stable state. The in vitro expansion of autologous chondrocytes is associated with de-differentiation [4]. Dedifferentiation refers to chondrocytes with a phenotype more reminiscent of fibroblasts with the consequent modification in the expressed proteins and the formation of fibrous like-tissue with inferior biochemical and biomechanical properties [9].

Allogenic transplantation of chondrocytes has been used with some success in animal models involving rabbits [10–12]. In those studies, chondrocytes did not show positive immunomodulatory properties [13]. However, chondrocytes express class I and, in some species, class II major histocompatibility complex (MHC). Successful allogeneic chondrocyte transplants in rabbits and hens could be due to an inbreeding among experimental animals, by the use of chondrocytes cultivated before grafting in artificial scaffolds and thus protected by matrix produced in vitro [14]. It subsequently seems that encasing the allogeneic chondrocytes in a matrix reduce the immunological activity. The advantages of the allogenic approach are a single surgery, high seeding densities with early or non-culture and decreased dedifferentiated cell use.

Generation	Chondrocytes	Membrane	Source	Steps
1st	In suspension	Living Periosteal	Autologous	2
2nd	In suspension	Collagen Flap	Autologous	2
3rd	Grown in scaffold	3D-Scaffold	Autologous	2
4th	Seeded in a scaffold	3D-Scaffold	Autologous / Allogeneic	1

Table 1.
Autologous chondrocyte implantation has evolved with the aim of facilitating cartilage treatment, reducing complications and improving results. The first three generations are performed in two steps with autologous chondrocytes while the fourth one can be done in one step using either autologous or allogeneic cells.

Although the use of allogeneic chondrocytes has been explored as an alternative technique of cartilage repair, the literature is limited regarding human practice [15]. Interesting are the pioneer results from Almqvist and Dhollander [16, 17]. They used in vitro expanded allogeneic chondrocytes seeded in alginate beads. No signs of clinical deterioration or adverse reactions to the alginate beads/allogenic chondrocyte implantation were observed after mean 6.3 years [17]. However, also with in vitro expansion of allogeneic chondrocytes, de-differentiation is a dis-advantage. The ideal is to use allogeneic chondrocytes without in vitro culture for cell expansion. Use of a cadaveric source of allogeneic chondrocytes offers a possibility to obtain a large number of chondrocytes ready to use fast and easy. We present here a model of how to make use of cadaveric chondrocytes.

2. Allogeneic chondrocyte implantation

In order to reduce costs with a multi-step procedure, a one-step technique called ALCI-Graft (Allogeneic Chondrocyte Implantation Graft) has been developed. In this technique chondrocytes are isolated from cartilage obtained from the knee of young cadaveric donors. The isolated cells are seeded in a membrane of hyaluronic acid sealed with a fibrin adhesive and left for a short period of time (4 days) in an incubator with culture media enriched with autologous serum. The ALCI-Graft is subsequently a one-stage cell-based repair therapy for isolated articular cartilage lesions.

This orthobiologic implant consists of 10 million of primary chondrocytes in 20 mm of hyaluronic acid membrane in Tisseel® tissue glue (Baxter B.V, Utrecht, the Netherlands) which will act as a cell carrier for implantation.

The number of allogeneic chondrocytes incorporated into a hyaluronic acid scaffold is determined by the area of the membrane using an injection of 10×10^6 cells in defects with a mean size of 2 cm^2. The calculated seeding density used is generally close to 5×10^6 cells/cm^2. It is important that the surgeon estimates the volume of the defect, in order to implant an amount of 50 million of chondrocytes for every 10 millimeters of cartilage lesion.

We have performed a pilot study with symptomatic patients that have full-thickness cartilage lesions in the hip, knee and ankle diagnosed by clinical examination and MRI. The ALCI-Graft surgery consists of an arthroscopy approach during which the cartilage defect is localized and debrided to create stable borders (**Figure 1**). Allogeneic cryopreserved chondrocytes are thawed 4 days before surgery and seeded in the hyaluronic acid membrane embedded in Tisseel® (**Figure 2**). Ten million of primary cadaveric chondrocytes per 20 millimeters of membrane are implanted into the cartilage defect.

In the 17 cases that we have treated with this technique we have not observed any early or late immunological rejection data, infection or foreign body response after surgery. Special attention was kept on the articular volume, local temperature, and possibility of detachment of the implant, the latter evaluated by MRI after 2 weeks of surgery.

Another great advantage of ALCI-Graft is that it is easy to obtain enough donor cartilage to treat large lesions and no limit of number of cells to carry out the surgical treatment as we can see in a bipolar lesion in trochlea and patella in the **Figure 3**. Furthermore, the graft requires only a short time to become ready for use in the operating room (< 1 week) (**Table 2**).

An ALCI-Graft consists of chondrocytes isolated from articular cartilage (knee and/or patella) from young donors (<20 years), seeded on a three-dimensional

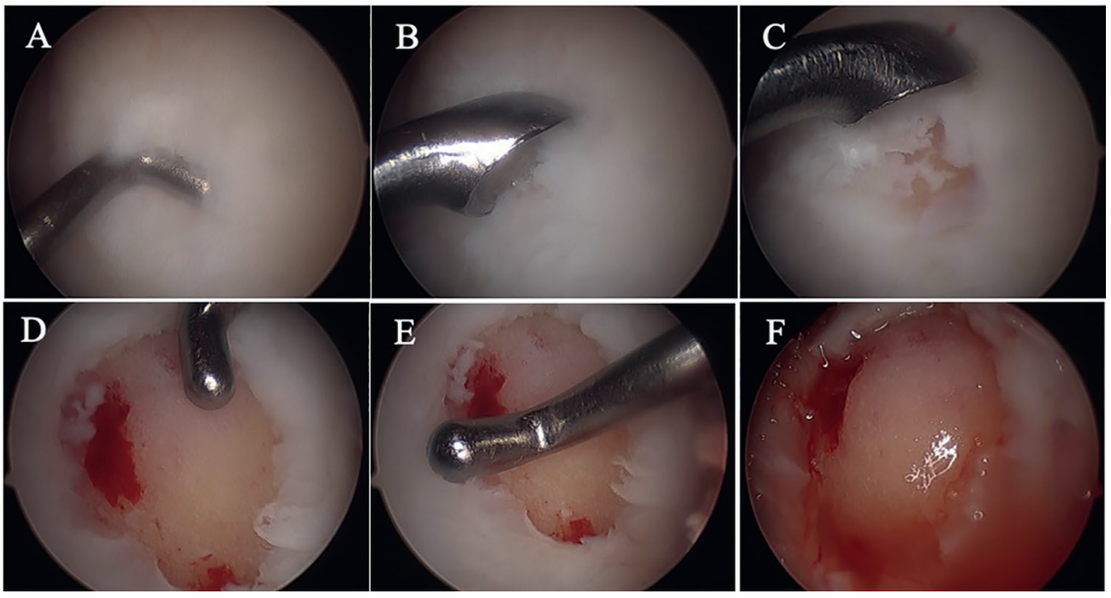

Figure 1.
Cartilage lesion in medial femoral condyle (A). The lesion is stabilized by debridement around the edges and down to the subchondral bone using a curette. Any delaminated cartilage is removed from the defect (B and C). Vertical walls are established to maximize the probability of complete cartilage fill (D and E). The calcified layer of cartilage is removed before implantation (F).

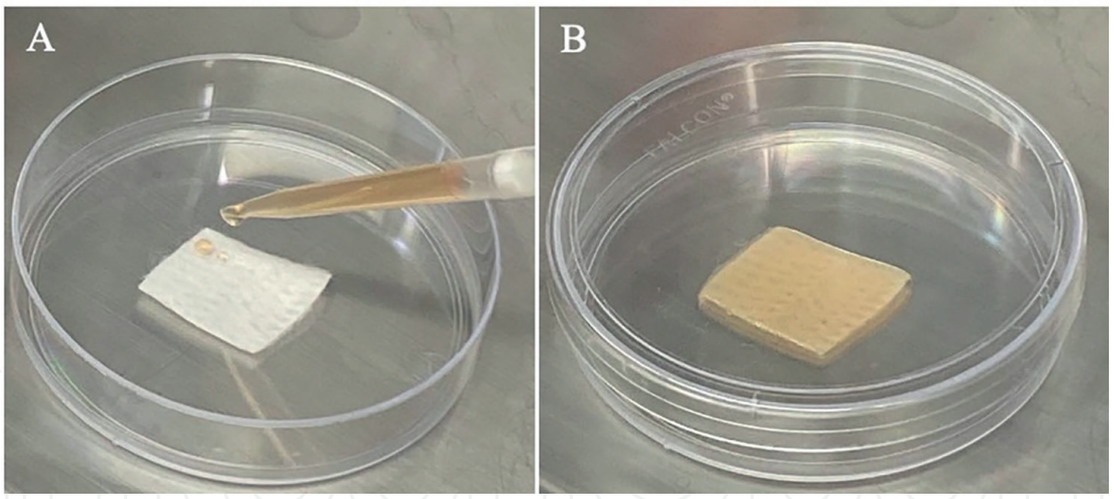

Figure 2.
Allogeneic chondrocytes seeding. The cells diluted in culture medium enriched with autologous patient serum are seeded in the hyaluronic acid membrane and then covered by fibrin glue.

membrane, composed of one of the main chondrogenic-promoting substances (hyaluronic acid, HYALOFAST®, Anika Therapeutics, Inc), in high densities (10×10^6) with autologous serum and sealed with a fibrin adhesive. The structural nature of the hyaluronic acid membrane allowed us to obtain an implant with the desirable flexibility and strength to be manipulated during arthroscopy (**Figure 4**). Likewise, during the development of the present invention and with the described methods, we found that more than 80% of the chondrocytes obtained from cadaver donor cartilage, up to 48 hours after death, maintain their viability and preserve the capacity to form cartilage in in-vitro cultures.

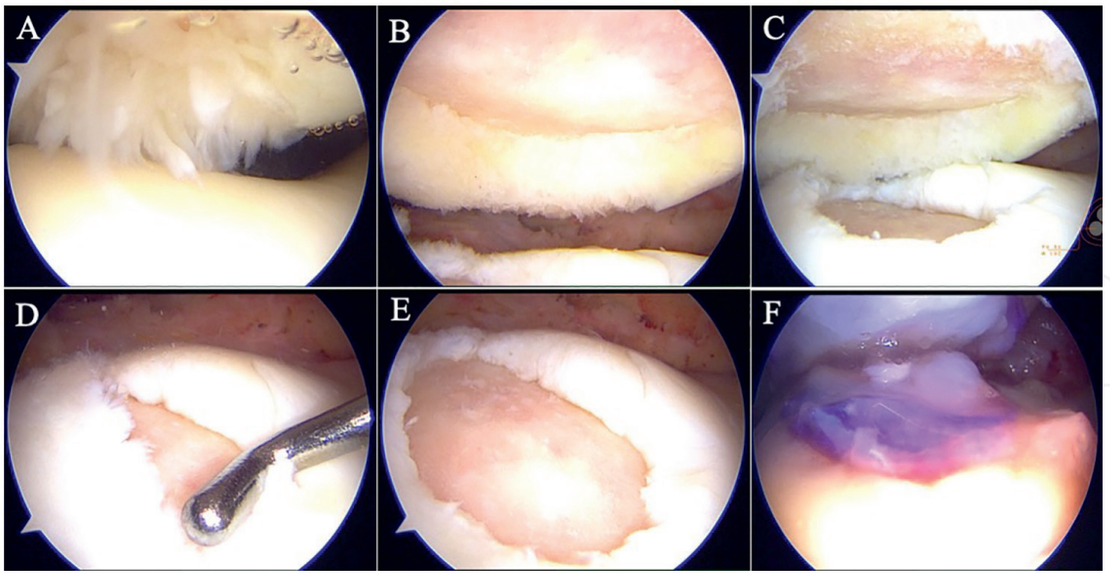

Figure 3.
Treatment of big size bipolar cartilage lesion in the patella (A) and trochlea (D) with ALCI-graft technique. The chondral defects are cleaned with a curette to remove the pathologic cartilage from within the defect (B and E). Once the cartilage lesions have stable borders (C) a final measurement is performed to cut the graft to the appropriate sizes, then the pump water is closed and the construct introduced into the joint and place in the bottom of the lesion until completely covered, finally fibrin glue is applied on the surface to fix the implant (F).

Characteristic	ALCI	ACI
Source of cells	Allogeneic	Autologous
Number of surgeries	One	Two
Cell culturing	No	Yes
Days to prepare the graft	4	42–56
Limit of lesion size	No limit	4 cm^2
Recommended age of patient	No limit	<50 years

Table 2.
Comparative characteristics of allogeneic versus autologous chondrocyte implantation. ALCI-graft does not require cell culture or biopsy of the patient, so it becomes a one-step technique with which it is possible to treat large lesions (> 4 cm^2).

Once a cadaveric donor is selected, the cartilage is harvested in sterile conditions and then sent to a panel of safety tests to rule out the presence of infectious diseases that can be transmitted through the donated tissue. The panel includes antibodies against hepatitis B (Core and Surface), hepatitis C, HTLV, syphilis, HIV and detection of nucleic acids (NAT test) against hepatitis B, C and HIV (Viromed/Labcorp laboratory, in Minnesota, United States). Once the biopsies or the tissue samples show negative serology, they are considered suitable for use in the formation of the implant.

3. Implant formation

Cryopreserved chondrocytes stored in a tissue banking are thawed and seeded 4 days before patient is scheduled for chondrocyte implantation. Every vial of stored

Figure 4.
ALCI-graft in the operating room. The construct is transported in a sterile culture box to the operating room, where it is appropriate to the size and shape of the lesion to be treated.

cells contains $10x10^6$ primary chondrocytes that are seeded in every 20 mm parts of hyaluronic membrane. The seeded cells are then covered by tissue glue and left in the incubator for 15 minutes before to be started in the media culture (Dulbecco's Modified Eagle Medium F12 GIBCO, Grand Island, NY with 20% autologous serum) and finally left in the incubator at 37°C, 5% CO2 and 5% humidity. The autologous serum is obtained from the patient to whom the construct is to be implanted, thus avoiding the use of fetal bovine serum which may cause secondary reactions in the patient.

4. Arthroscopic implantation

The ALCI-Graft is useful for the treatment of cartilage lesions in all kinds of articular joints. The construct is provided as a kit comprising a culture box transported under sterile conditions in a portable incubator that maintain standard parameters (37°C, 5% CO2 and 5% humidity). It is recommended that prior to the use of the implant, the treating physician should evaluate the injury during surgery, identifying the location, size, and shape.

In order to have a good integration of the graft to surrounding native cartilage, it is recommended that, before the implantation of the construct, an adequate debridement of the lesion should be performed (**Figure 3B** and **E**). All damaged tissue should be removed creating stable vertical walls of healthy cartilage. It is important that after debridement the lesion is measured again, as it is very certain that the size of the lesion is now larger than before removal of the injured and unstable edges. If the surgery is open, the measurement can be made with a surgical ruler; if the procedure is arthroscopic, the hook probe, or a flexible ruler, is used to determine the exact size of the lesion in the proximal-distal and medio-lateral planes (**Figure 1D** and **E**). Likewise, it is highly recommended that the lesion be given a square or rectangular shape, to facilitate measuring and matching the size and shape of the graft. It is equally important not to damage the subchondral bone.

Once the final lesion measurements are obtained, after debridement of the lesion, it is recommended to trim the graft with an excess of at least 2 mm at each edge. It is preferable to have a larger construct, which can be compressed and adapted to the lesion, than to leave a fair or smaller construct, with the consequent risk that one or more of its walls will fail to integrate with the edges of the healthy cartilage.

The graft is fixated at the bottom of the cartilage lesion with fibrin glue. When the construct is placed into the lesion, fluid entry into the arthroscopy is closed to prevent loss of chondrocytes. A cannula is placed in the portal of best access to the lesion, and through this portal the graft is introduced into the joint (**Figure 5A**), placed and spread over the entire area (**Figure 5B**) until the lesion is completely covered and ensuring existing contact of the graft with all edges of the adjacent native cartilage (**Figure 5B**). Finally, fibrin glue is applied to the edges and surface of the graft to ensure fixation to the lesion, (**Figure 5C** and **D**). In the case of the knee, it is recommended to keep it in extension for at least a couple of days to avoid friction and loss of the graft, when a lesion is treated in hip or ankle, we also recommend a couple of days of immobilization.

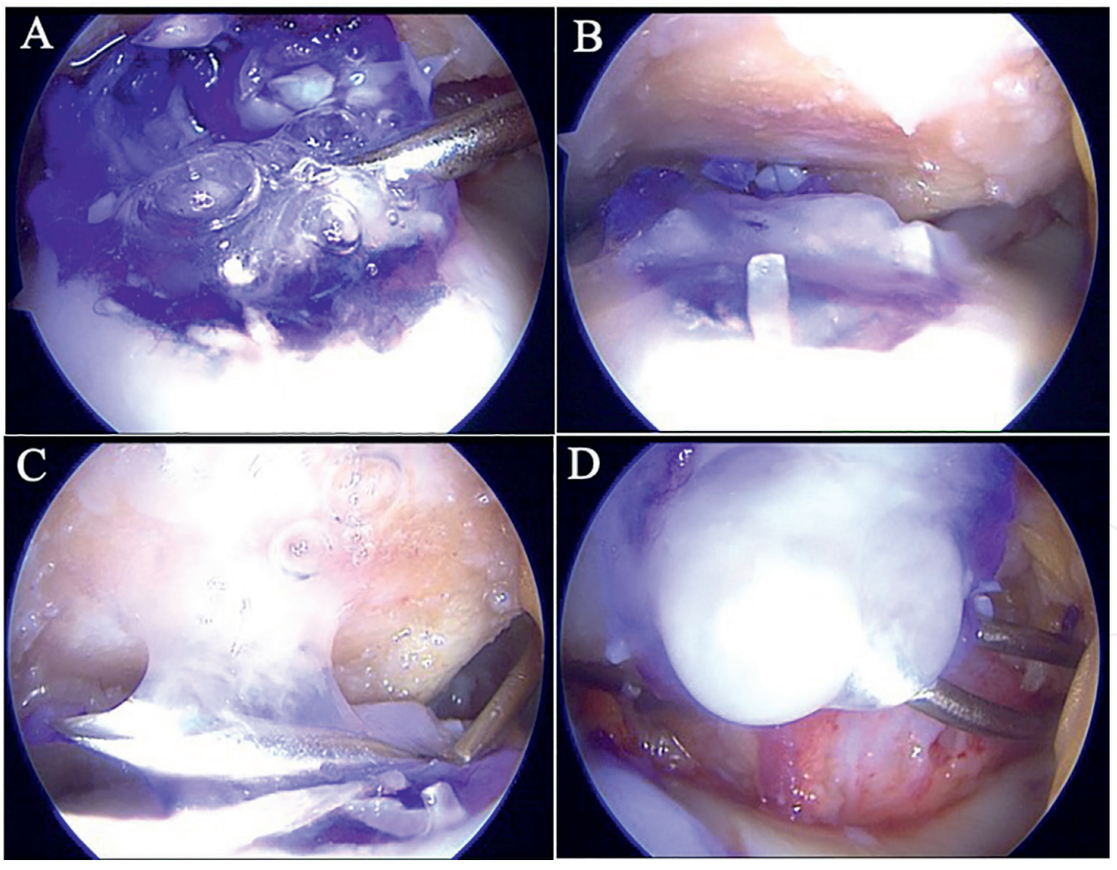

Figure 5.
Allogeneic chondrocyte implantation in a bipolar lesion in the patella and trochlea. A) At the time the construct is placed into the lesion, water entry into the arthroscopy is closed to prevent loss of chondrocytes. B) the graft is introduced into the joint, placed and spread over the entire area until the lesion is completely covered and ensuring contact of the graft with all edges of the adjacent native cartilage. Fibrin glue is applied to the edges and surface of the graft to ensure fixation to the lesion (C and D).

5. Rehabilitation protocol

Post-operatively the rehabilitation starts directly the same day with cryotherapy, after 2 days of immobilization in a brace locked in extension, continuous passive motion is started with gradually movement that increases over the time depending of the location of the graft and its stability during arthroscopy motion. Weight bearing is avoided during 4 weeks and if the repaired lesion is in a loading zone, then partial discharge is permitted at week 5 and 6. Progressive open-chain strengthening is started after the first iso-kinetic evaluation at 3 months after surgery. Patients are allowed to return to sports activities after 12 months and when isokinetic evaluation reported 90% of strength of the contralateral limb.

6. Post-operative follow-up

Different evaluation system can be used for post-operative evaluation. Visual analog scale (VAS) for pain, the IKDC knee scoring, Lysholm knee score, and Tegner activity scale are all used to assess the clinical evolution. With MRI, T2-mapping evaluation is performed after surgery to evaluate the graft integration and maturity compared to a native cartilage adjacent zone.

7. Conclusions

Since the first ACI-autologous chondrocyte implantation was performed in humans in 1987, several variants of ACI have been developed. All those generations of ACI are based on autologous cells. To use allogeneic cells, the availability to treat patients faster, with a smaller number of operations and with more stable repair possibility with differentiated cells is possible. Implantation of allogeneic chondrocytes in focal full cartilage lesions in hip, knee and ankle has been shown to be a safe procedure that can be performed arthroscopically and improve pain, function and quality of life of the patients in studies up to 3 years post-surgery. Use of a cadaver allogeneic chondrocytes gives benefits in terms of a possible one step technique, minor cell culturing, larger treatment sizes and short period of graft preparation compared to traditional Gold ACI Standard technique.

Acknowledgements

We thank to the Novoinjertos Tissue Bank of Mexico and Medica Sur Hospital of Mexico for the collaboration of this project.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.107292

Author details

Anell Olivos-Meza[1*], Mats Brittberg[2], Carlos Landa-Solis[3] and Carlos Suárez-Ahedo[1]

1 Orthopedic Department, Hospital Médica Sur, Mexico City, Mexico

2 Cartilage Research Unit, University of Gothenburg, Region Halland Orthopaedics, The Varberg Hospital, Varberg, Sweden

3 Tissue Engineering Unit, Instituto Nacional de Rehabilitación, México City, Mexico

*Address all correspondence to: aolivos_meza@hotmail.com

References

[1] Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. The New England Journal of Medicine. 1994;**331**:889-895

[2] Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA. Repair and tissue engineering techniques for articular cartilage. Nature Reviews Rheumatology. 2015;**11**(1):21-34

[3] Bohacek I, Bojanic I, Gajovic S, Josipovic M, Jelic M. Articular cartilage repair techniques exploiting intrinsic healing capacity – Which one is the best. Periodicum Biologorum. 2015;**117**(1):125-133

[4] Brittberg M. Clinical articular cartilage repair—An up-to-date review. Annals of Joint Journal. 2018;**3**:1-9

[5] Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: A long-term follow-up. The American Journal of Sports Medicine. 2010;**38**:1117-1124

[6] Minas T, Von Keudell A, Bryant T, Gomoll AH. The John Insall award: A minimum 10-year outcome study of autologous chondrocyte implantation. Clinical Orthopaedics and Related Research. 2014;**472**:41-51

[7] Behery OA, Harris JD, Karnes JM, Siston RA, Flanigan DC. Factors influencing the outcome of autologous chondrocyte implantation: A systematic review. The Journal of Knee Surgery. 2013;**26**:203-211

[8] Vavken P, Samartzis D. Effectiveness of autologous chondrocyte implantation in cartilage repair of the knee: A systematic review of controlled trials. Osteoarthritis and Cartilage. 2010;**18**(6):857-863

[9] Medvedeva EV, Grebenik EA, Gornostaeva SN, Telpuhov VI, Lychagin AV. Repair of damaged articular cartilage: Current approaches and future directions. International Journal of Molecular Sciences. 2018;**19**(8):2366

[10] Boopalan PR, Sathishkumar S, Kumar S, Chittaranjan S. Rabbit articular cartilage defects treated by allogenic chondrocyte transplantation. International Orthopaedics. 2006;**30**:357-361

[11] Schreiber RE, Ilten-Kirby BM, Dunkelman NS, Symons KT, Rekettye LM, Willoughby J, et al. Repair of osteochondral defects with allogeneic tissue engineered cartilage implants. Clinical Orthopaedics and Related Research. 1999;**367S**:S382-S395

[12] Wakitani S, Goto T, Young RG, Mansour JM, Goldberg VM, Caplan AI, et al. Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. Tissue Engineering. 1998;**4**:429-444

[13] Davies RL, Kuiper N. Regenerative medicine: A review of the evolution of autologous chondrocyte implantation (ACI) therapy. Bioengineering. 2019;**6**(22):2-16

[14] Moskalewski S, Hyc A, Osiecka-Iwan A. Immune response by host after allogeneic chondrocyte transplant to the cartilage. Microscopy Research and Technique. 2002;**58**(1):3-13

[15] Lohan P, Treacy O, Lynch K, Barry F, Murphy M, Griffin MD, et al. Culture

expanded primary chondrocytes have potent immunomodulatory properties and do not induce an allogeneic immune response. Osteoarthritis and Cartilage. 2016;**24**:521-533

[16] Almqvist KF, Dhollander AA, Verdonk PC, Forsyth R, Verdonk R, Verbruggen G. Treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. The American Journal of Sports Medicine. 2009;**37**(10):1920-1929

[17] Dhollander AA, Verdonk PC, Lambrecht S, Verdonk R, Elewaut D, Verbruggen G, et al. Midterm results of the treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. The American Journal of Sports Medicine. 2012;**40**(1):75-82