ONE-STAGE CELL THERAPIES – STATE OF THE ART TECHNIQUE

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INTRODUCING THE VILLAINS

Epidemiology of Cartilage Injuries

Cartilage injuries are potentially harmful lesions affecting around 60% of patients undergoing knee arthroscopy[1,2]. Full-thickness focal defects are more frequently found in athletes and may be present in up to 59% of them[3,4]. The most common locations are the patellar (36%) and medial femoral condyle (34%) surfaces and are often associated with a medial meniscus tear (42%) or anterior cruciate ligament injury (36%)[1]. While most isolated chondral injuries are asymptomatic, they may present with pain, locking or catching sensation, swelling and/or pseudoinstability[4].

Cartilage Healing Potential

Due to the highly specialized hyaline cartilage cells and tissue properties, its regeneration potential is low[5]. Additionally, the avascular nature of cartilage tissue and incapacity for clot formation hinders the main steps that occur in other tissues after an injury[6,7].

Cartilage tissue attempt at healing depends on defect size and depth[8]. Partial-thickness cartilage injuries do not violate the subchondral bone and do not repair spontaneously[5]. Cell adjacent to the defect margins undergoes cell death, and chondrocytes and migrating synovial cells fail to fill the defect after an injury[5,9,10].

On the other hand, the healing process after full-thickness injuries involves several cell types arising from the bone marrow after subchondral plate breaching[11]. In these cases, the resulting synthesized extracellular matrix after hematoma formation does not replicate the native morphology and mechanical characteristics of the native tissue but produces fibrocartilage[5]. This fibrocartilage tissue primarily consists of collagen I fibers with limited durability[12].

Furthermore, smaller lesions may dissipate weight-bearing forces across it, protecting the subchondral bone, but larger lesions may fail to do so. In those cases, the exposed subchondral bone will become abrasive to the opposite chondral surface, creating bipolar injuries and consequent subchondral edema[7,13]. When untreated, these defects may progress to knee osteoarthritis[5,14].

Several treatment approaches are available to address focal cartilage injuries of the knee. However, the standard treatment is yet to be defined. Non-surgical options include rehabilitation and physical therapy, and intra-articular injections[15]. On the other hand, surgical treatment options range from debridement and bone-marrow stimulation techniques to more complex procedures, including osteochondral autologous transplantation, osteochondral allografts, mosaicplasty, and cell-based therapies[16].

Costs of Autologous Chondrocyte Implantation (ACI)

Knee ACI, first performed in 1994, showed promising results in managing focal cartilage injuries[17]. Studies have addressed its cost-effectiveness with favorable results, with cost savings related to fewer work absences and disability[18]. This is especially relevant in the young and active population, in which regenerative techniques potentially allow better and sustained long-term outcomes compared to other techniques[19].

Everhart et al[18], in their systematic review, found that matrix-induced autologous chondrocyte implantation

Image: Illustration.

(MACI) had better cost-efficacy than its counterpart implementing a periosteal cover, with costs surpassing 50,000 USD per quality-adjusted life-year over ten years. However, this two-stage procedure is still expensive, costing approximately 16,226 EUR[20]. The need for a second procedure is a difficulty that translates into additional indirect costs from loss of productivity and qualitative deleterious effects from a time and monetary point of view[18].

Likewise, Its logistical complexity and the need for chondrocyte culture in highly specialized laboratories with processing costs exceeding 30,000 USD in the United States have limited its widespread implementation[21,22]. Since then, numerous modifications of this technique have been introduced, aiming for a single-stage definitive solution given its cost-saving potential.

CHOOSING THE WEAPONS

Chondrocytes: The Secluded Cell of Cartilage Tissue

Chondrocytes are mesenchymal cells specialized in extracellular matrix synthesis[5]. They represent only 2% of the articular cartilage volume and lead the cartilage homeostasis through secreting enzymes, growth factors, and inflammatory mediators[5,7]. Cartilage extracellular matrix is mainly composed of collagen II fibers, proteoglycans, and glycoproteins. The matrix interweaved architecture results in unique viscoelastic properties, providing a smooth and lubricated surface for low friction movement and load transmission[5].

Chondrocytes are the only cells capable of creating new hyaline cartilage. Thus, the quest for cartilage restoration has involved its implementation in several attempts. ACI has been demonstrated to be an effective treatment option in managing large, full-thickness symptomatic chondral lesions of the femoral condyles with early improvement and sustained at long-term follow-up[23,24].

Moseley et al[23], in a multicenter observational study comprising 72 patients, reported that 75% of them improved from their baseline scores at 1 to 5-year follow-up, and 87% maintained their improvement to the last follow-up (mean 9.2 years), with an early failure rate in 17% of patients (mean 2.5 years). Similarly, Peterson et al[24] have reported similar outcomes in 224 patients with follow-up as long as 20 years.

While first-generation ACI has demonstrated satisfactory outcomes, there is still a gap for improvement in clinical outcomes, failure rates, and costs. Current practices aim to harvest chondrocytes from non-weight-bearing cartilage zones and implement fast isolation protocols, avoiding cell culture and two-stage procedures[25]. Moreover, it has been suggested that implementing chondrocytes from the injury rim or even arthritic cartilage seems not to alter the quality of newly synthesized cartilage, which may help to avoid donor-site morbidity[22,25].

Hyaline cartilage is harvested using a shaver or curettes from the medial margin of the medial femoral condyle, medial margin of the trochlea, or the lesion rim area to obtain approximately 0.3 g[25]. This tissue is recycled using enzymatic reactions to obtain chondrons (chondrocytes with their pericellular matrix) within an hour, enabling one-stage procedures. Cells are washed and counted to meet the density and ratio according to the defect[17,25].

Bone-Marrow-Derived Mesenchymal Stem Cells (BM-MSC): The Most Popular Stem Cell

Mesenchymal stem cells (MSC) are an adult lineage of multipotent cells with the potential to differentiate to the bone, cartilage, and other connective tissues by local signaling and genetic potential at embryonic stage[26,27]. However, according to the current understanding all MSC are pericytes, embeded in the capillaries, and

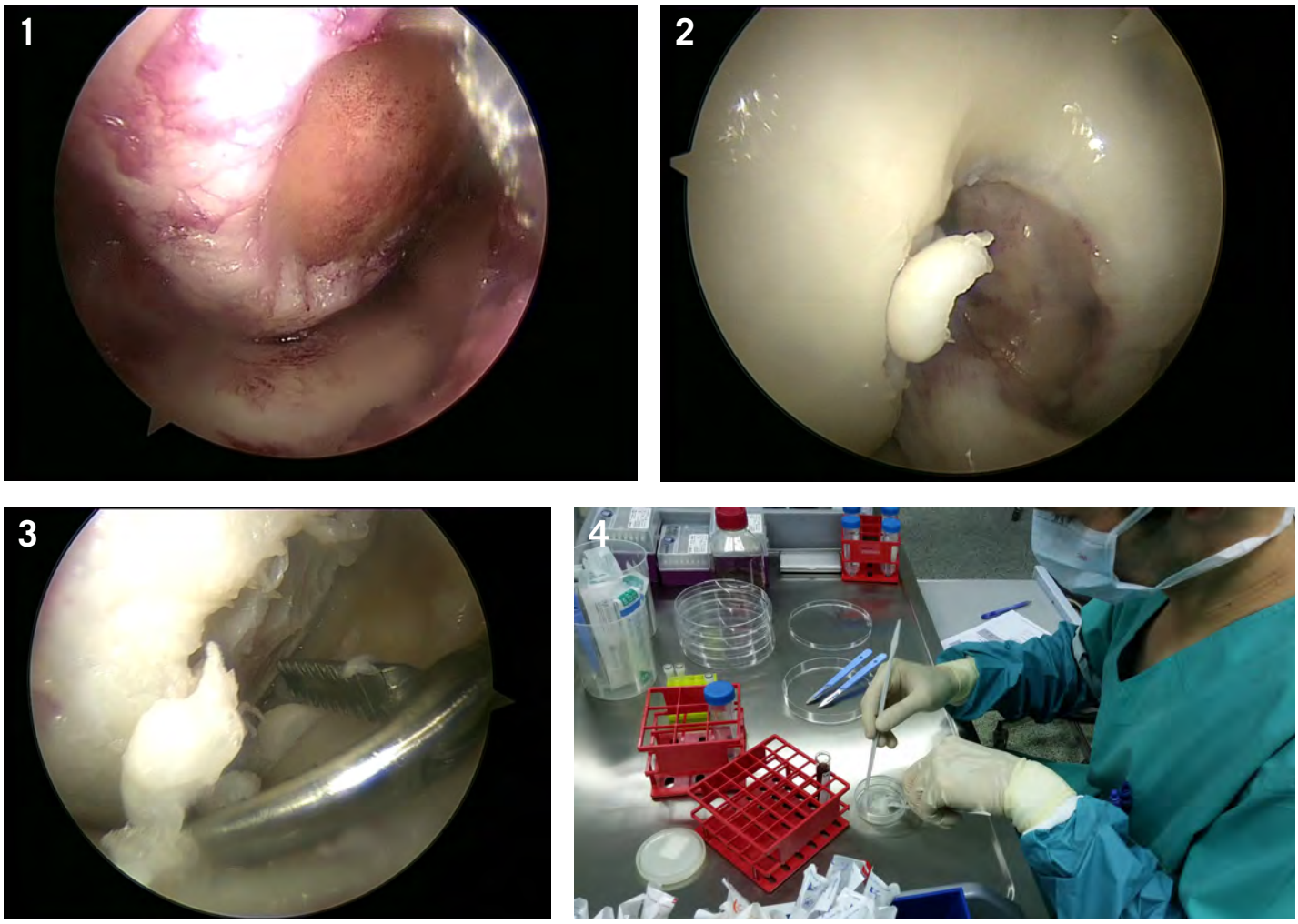


Figure 1: Cartilage defect of MFC after debridement and creation of stable shoulders.

Figure 2: Harvesting of healthy piece of cartilage from non-weight bearing area (notch).

Figure 3: Harvesting cartilage pieces from the defect.

Figure 4: Cartilage pieces minced and enzymatically digested to chondrons in the In-Theater portable lab.

do not differentiate to other cells type, but, when activated, secret growth factors that have influence on surrounding cell types[28].

Pericytes are stimulated by soluble growth factors and chemokines to become activated MSC, which respond to the microenvironment by secreting trophic (mitogenic, angiogenic, anti-apoptotic or scar reduction), immunomodulatory or antimicrobial factors[28].

They are currently the most widely used stem cells[29]. According to the International Society for Cellular Therapy criteria[30], a MSC must be (a) plastic adherent, (b) express CD105, CD73, and CD90, and not CD45, CD34, CD14, or CD11b, CD79 alpha or CD19, and HLA-DR surface molecules, and (c) differentiate into osteoblasts, adipocytes, and chondroblasts in vitro.

These cells are typically harvested in the iliac crest by aspiration, although the number of collected cells is minimal[31,32]. In the bone marrow of skeletally mature patients, the number of MSC ranges from 1:50000 to 1:100000, a few hundred per milliliter of marrow aspirate[31]. Furthermore, the implementation of allogeneic MSC has shown not to activate an adverse immune response while promoting chondrogenic potential of the surrounding chondrocytes, presenting as a safe option to be implemented.

Theoretically, the chondrogenic and trophic potential of MSC and homing are the most critical mechanisms in which these cells participate in the restoration of cartilage[27,33-35]. The first one, in which the cells differentiate to cartilage cells restoring the lost function and morphology; and the second, secreting several bioactive factors to promote repair environment[31]. The latter being the most accepted after de Windt et al[17] revealed that tracking these cells showed a temporary behavior, enhancing joint homeostasis before disappearing.

In a case series by Gobbi et al[21], successful comparable long-term outcomes in IKDC, KOOS, and Tegner activity scale were obtained when implementing BM-MSC in a hyaluronan-based scaffold for the treatment of full-thickness cartilage injuries ≥ 1 cm². The implementation of BM-MSC in a hyaluronan-based scaffold is an emerging therapeutic option among one-stage cartilage restorative procedures.

Synergistic Effect of Combined Chondrocyte and Mesenchymal Stem Cells

It has been suggested that a combination of chondrocytes and BM-MSC may increase the chondrogenic potential of the firsts[36,37]. Although MSC have shown no differentiation into chondrocytes in these

circumstances in recent investigations[17], paracrine trophic and immunomodulatory effects contribute to the regeneration of the lesion[25]. It seems that MSC fade over time but secreting site-specific factors that promote tissue regeneration[17]. Complementing chondrocytes with MSC ensures a higher cell density in the defect and stimulates further hyaline matrix synthesis[25,38-41].

Scaffolds and Carriers

The use of scaffolds has also been widely studied during the last decades. They show advantages such as the uniform distribution of the seeded cells, provide a temporary platform for the new to be synthesized extracellular matrix which components may be implemented for such role[42-44].

Hyaluronan-based scaffolds and fibrin glue are among the most popular options, but new biomaterial are being continuously developed and studied for cartilage restoration[29,45-47]. To date, hyaluronan-based scaffolds have shown to be superior to other types, as they "recrerate" or mimic embroynic environment in limb buds development.

THE AVENGER

Indications

One-stage cartilage restoration with chondrocytes and MSC is the preferred technique for focal cartilage lesions on the femoral condyles or trochlear, ICRS II or III, > 1 cm², in adult patients with stable and well-aligned knees and meniscal loss < 50%[22,25].

One-Stage Restoration with Chondrocytes and Bone Marrow-Derived Mesenchymal Stem Cells: Surgical Technique[22,25]

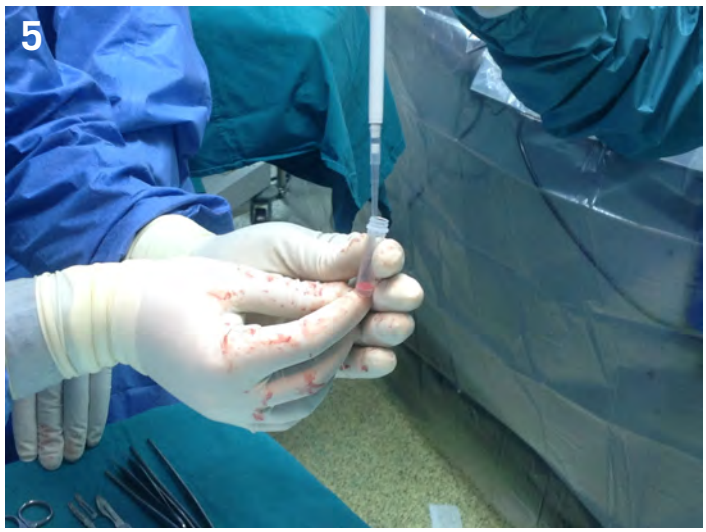
Surgery can be performed via arthroscopy or a mini-arthrotomy approach. Cartilage defects are

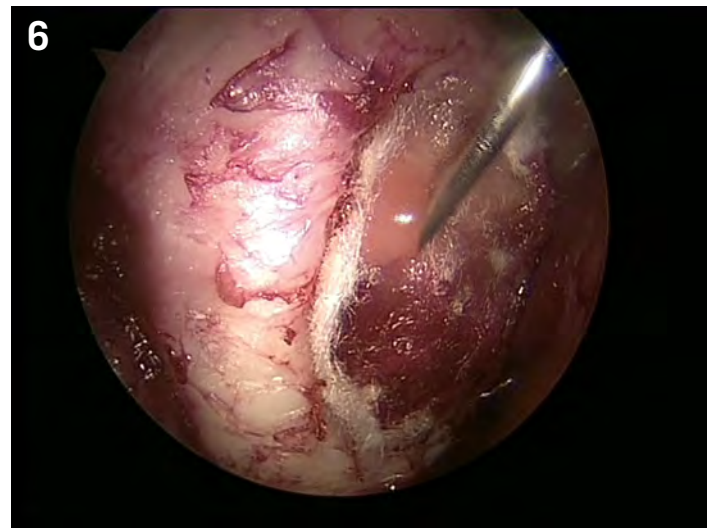
debrided with curettes, removing the calcified layer and creating vertical and stable margins (Figure 1).

Cartilage pieces and BM-MSC are harvested afterward (Figures 2 and 3). Autologous chondrons (after enzymatic digestion of the minced cartilage) and MSCs are combined in a 1:9 ratio (standard) or 2:8 ratio (high yield) (Figures 4 and 5), depending on the number of isolated chondrons[17].

The lesion is measured, and a scaffold is prepared to meet the shape and thickness of the defect when implemented. In the next step the scaffold is implanted in the defect seeded with the cell mixture and further stabilized with the use of fibrin glue (Figures 6 and 7). Seeding after the fixation of the scaffold results in less cellular death resulting from manipulation[36]. The implantation of the cell mixture is also feasible directly in the fibrin glue without a scaffold.

Finally, the knee is tested for passive range of motion, checking the implant stability.





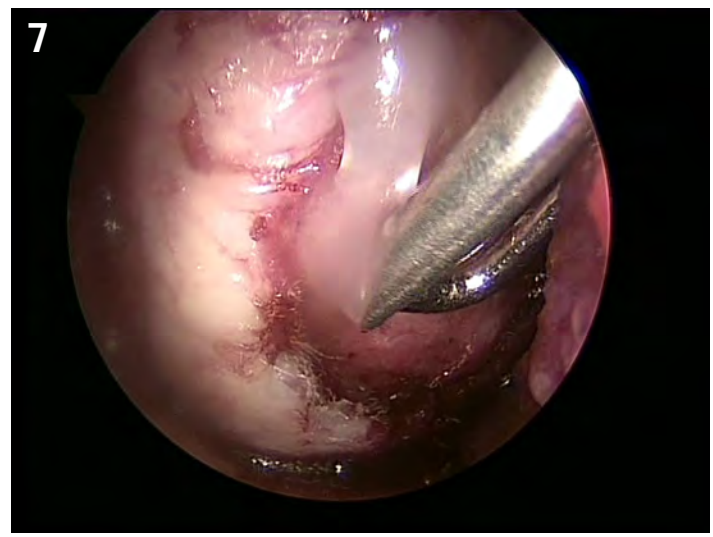


Figure 5: Dilution of Chondrons and MSCs provided for final implantation.

Figure 6: Properly sized scaffold implanted and seeded with the dilution.

Figure 7: Fibrin glue used for final stabilization of the implant.

One-stage cartilage restoration using chondrocytes and MSC has proved to be a safe and reproducible technique, improving clinical outcomes and tissue quality of its predecessor two-stage ACI at two-year follow-up[17,25].

Outcomes: The Promise of a Definite Solution

One-stage cartilage restoration using chondrocytes and MSC has proved to be a safe and reproducible technique, improving clinical outcomes and tissue quality of its predecessor two-stage ACI at two-year follow-up[17,25].

Similarly, de Windt et al[17] implemented a combination of recycled chondrons from the lesion rim and cryopreserved allogeneic BM-MSC suspended in fibrin glue in 35 patients with full-thickness cartilage injuries with a mean size of 3.2 cm^2 ± 0.7, in a first-in-man clinical trial. Patient-reported clinical outcomes KOOS and VAS significantly improved from baseline scores up at 18 months after surgery, with the most considerable improvement at 3-month follow-up. Moreover, biochemical MRI, second-look arthroscopies, and histologic evaluation revealed a similar or higher quality in the new cartilage than in that obtained after ACI at 12 months. Hyaline-like cartilage was confirmed in almost 95% of the patients.

At a 5-year follow-up, the same patient cohort maintained the clinical benefits along with the follow-up, with fluctuations around the second year, probably related to the return to sporting activities. No serious adverse effects were recorded, and five patients required reintervention[22].

Similarly, in a prospective multicenter study using a combination of primary chondrocytes and bone marrow mononucleated cells in a hyaluronan-based scaffold, Slynarski et al[25] reported successful lesion filling in all 40 patients with ICRS II and III chondral lesions ≤ 2.6 cm^2 at 3-month follow up and in all patients that completed the 2-year follow-up (20% loss to follow-up). Significant improvement in KOOS and IKDC patient-reported outcomes were achieved throughout the study with confirmed hyaline-like cartilage in 22 of 40 patients post-operative biopsies.

Similar complications have been reported in one-stage procedures compared to those observed in ACI and microfractures[25]. Arthralgia, joint effusion, and reoperation were the most common among them[22,25].

Future investigations should evaluate the differences in outcomes when higher cellularity is seeded in the chondral defect or differences in the chondrogenic potential of chondrocytes harvested from different local donor sites.

CONCLUSION

One-stage cartilage restoration using a combination of chondrocytes and BM-MSC is a safe and reproducible surgical procedure with satisfactory short- and mid-term clinical outcomes. Similar or better new synthesized cartilage should be expected in the defect compared to ACI with superior cost-effectiveness. Further research may consolidate one-stage cell-based cartilage restoration procedures as the standard of treatment for focal cartilage injuries.

References

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