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Review article

Autologous bone-marrow mesenchymal cell induced chondrogenesis (MCIC)

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ABSTRACT

Degenerative and traumatic articular cartilage defects are common, difficult to treat, and progressive lesions that cause significant morbidity in the general population. There have been multiple approaches to treat such lesions, including arthroscopic debridement, microfracture, multiple drilling, osteochondral transplantation and autologous chondrocyte implantation (ACI) that are currently being used in clinical practice. Autologous bone-marrow mesenchymal cell induced chondrogenesis (MCIC) is a single-staged arthroscopic procedure. This method combines a modified microfracture technique with the application of a bone marrow aspirate concentrate (BMAC), hyaluronic acid and fibrin gel to treat articular cartilage defects. We reviewed the current literatures and surgical techniques for mesenchymal cell induced chondrogenesis.

1. Introduction

Defects of articular cartilage in the knee joint are a common degenerative disease. Hyaline articular cartilage has cellular components, matrix composition, and molecular ultrastructure that optimally address the biomechanical demands of the joint surface. Several decades of attempts to heal the lesions of this vascular and aneural tissue has produced a logical, evolutionary progression of therapeutic options aimed at delivering a repair as close to natural hyaline cartilage as possible. Microfracture, evolved from the Pridie Drilling technique described over 50 years ago, still remains in widespread clinical use, relying on migration of chondrogenic cells in the subchondral bone marrow to the articular surface. However, this method results in fibrocartilage rather than hyaline cartilage regeneration, which is mechanically inferior and largely low in cells numbers, collagen Type II and hyaluronic acid.[1] Osteochondral autograft techniques, which replace articular cartilage defects with native hyaline cartilage, are limited by the size and number of the lesions and available donor sites. Significant donor site morbidity occurs even though the harvests are taken from non-weight bearing surfaces.[2] Autologous chondrocyte implantation (ACI) has shown some of the most successful results for regenerative healing of cartilage defect by delivering cells known to be chondrogenic after expansion in culture in association with a constraining or transporting membrane. However, ACI is a two-stage procedure, composed of an initial harvesting of normal cartilage, followed by a period of *in vitro* culture and subsequent transplantation in a second procedure. Moreover, the associated need for an arthrotomy increases surgical morbidity. The culture process is expensive and is associated with de-differentiation as the cultured cells progressively lose their chondrogenic potential during monolayer *in vitro* expansion.[3,4]

In recent years, several solutions have been introduced to overcome for these particular problems. Traditional microfracture relies on the potentially chondrogenic cells in subchondral bone marrow. Much of the cells that come through the microfracture holes are not chondrogenic, however, and the volume of the bone marrow emerging is very small. A technique that extracts the chondrogenic fraction of a larger volume of bone marrow aspirate logically could enhance the result of microfracture. Bone marrow aspirate concentrate (BMAC) uses density fractionation to yield a higher concentration of multipotent mesenchymal stem cells. It and also contains growth factors; this

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has been proven to be equal or superior to other current cartilage repair procedures.[5,6]

The molecular structural component applied to the treated lesion is also important. Pascarella et al. showed improved outcomes by using a bio-scaffold after microfracture procedure whether combined with BMAC or not.[7] Collagen based scaffolds appear to enhance the fixation of the graft.[8] Hyaluronic acid is a major constituent of articular cartilage and has been used as a scaffold with the ability to drive chondrotypic regeneration. Fibrin gel is a protein scaffold with the natural ability to change phase from liquid to gel in a controlled manner with the addition of thrombin.[9]

MCIC is a hybridization of these techniques that have the evidence of clinical success, delivering chondrogenic cells with a conductive biological matrix onto a surface prepared for regeneration. The single-staged, arthroscopic procedure involves injection of BMAC, hyaluronic acid, and fibrin gel after microfracture. MCIC is cost-effective, has low morbidity, and is a logical evolution of cartilage repair surgical techniques.

2. Bone marrow aspirate concentrate (BMAC)

Mesenchymal Stem Cells (MSCs) are multipotent stromal cells that could differentiate into all cells of mesodermal origin, such as adipocytes, osteoblasts, chondrocytes, skeletal myocytes, and visceral stromal cells. The chondrogenic potential of MSCs has been investigated in recent years with studies reporting variable results.

MSC can be transformed into differentiated cells type by specific environmental stimuli.[10–12] Chondrogenic differentiation of bone marrow derived cells requires the use of medium added certain material including insulin, transferrin, selenium, and transforming growth factor-β (TGF-β).[13] Members of TGF-β have been shown to be major role in cartilage development. Recent studies suggest critical role of signaling by the TGF-β superfamily for chondrocyte-specific gene expression related to type II collagen expression.[14–16] MSCs are also known to secrete bioactive molecules that reduce T cell surveillance. It is also observed that other nucleated cells are able to restore the damaged cartilage[17–21] which mimics natural tissue regeneration.[22]

There are many sources of MSCs including bone-marrow, white adipose tissue, Wharton's jelly cells of the human umbilical cord and amniotic fluid. Among these, bone marrow is good source of mesenchymal stem cells (MSCs).[23] Reliable results have been reported in cartilage or bone healing using MSCs.[24–28] One of the advantages of using BMAC is that by avoiding a culture phase dedifferentiation into is reduced, in contrast with the *in vitro* culturing of chondrocytes in ACI.

However, there may be some concerns about MSCs in treating cartilage defects in the older patients. Stenderup et al. reported MSCs harvested from older donors had a decreased maximal life span and proliferative capacity compared to that from younger donors.[29]

3. Preparation of BMAC

After general or spinal anesthesia, which is a part of knee arthroscopic procedure, the patient's anterior superior iliac spine (ASIS) was marked, cleaned and draped. Bone marrow aspiration needle (SPASYTM, CYP Biotech, Seoul, Korea) and syringes preloaded with 5 mL anticoagulant citrate dextrose solution (Huons ACD Injection, Huons, Seongnam, Korea) were used to aspirate 35 mL of bone-marrow from the iliac crest. The bone marrow aspirate was fractionated using a dual centrifugation device (BMC kit™, nFinders, Seoul, Korea). The first cycle was for 6 min at 3500 rpm, followed by a second cycle for 5 min at 3300 rpm to obtain BMAC.

4. Hyaluronic acid (HA) and fibrin gel

Techniques that deliver cells to a chondral lesion face a number of technical challenges. One of these is the local retention of cells at the target lesion. MSCs delivered with a biomaterial scaffold have been shown effective in repairing chondral lesions,[30] but in some cases scaffold retention requires the use of suture or membrane causing trauma to adjacent tissue and adding another foreign structure. However a hyaluronic acid (HA) and fibrin mixture does not need any additional strategy for cell containment as the construct is naturally adhesive, readily fills the prepared defect, and quickly undergoes a stiffening transformation.

HA acts as a lubricant in the joint space and intraarticular injection is considered as a treatment for early osteoarthritis. By inhibiting matrix metalloproteinase synthesis, HA reduces breakage of cartilage matrix. Moreover, HA has anti-inflammatory effect which downregulates tumor necrotic factor-α (TNF-α).[31,32] Hyaluronic acid or hyaluronan is an anionic, non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissue. Hyaluronan is also an important component of articular cartilage in which it exists as a coating surrounding each chondrocyte.

Fibrin sealants mimic final course of coagulation cascade in the bleeding control, and are used as clinical material for bleeding control widely. Fibrin has high biocompatibility, biodegradability, but no toxicity. Recently fibrin gel has been interesting as a good material for use in cartilage reconstruction such as an injectable carrier for generating neo-cartilage.[33,34] Fibrinogen itself is inactive form, which converts to active form by thrombin in order to form soluble fibrin which becomes insoluble by factor VIII with cross-linking. However, Jang et al. reports fibrinogen and thrombin combination with HA as another component showed different levels of fibrin morphology, which appeared to form a compact, solid, wall-like layer.[35] They assumed HA at least locally influence the polymerization of fibrin.

Fibrin could promote retention of HA at the injected site, forming a polymer composite within a few minutes after being injected. The ratio of fibrinogen to thrombin has a critical influence on the handling time.[35] During the operation the liquid phase mixture can take a few minutes to fill a prepared chondral lesion, and may need to be manipulated after application to improve coverage and remove adhesions. A fibrinogen to thrombin ratio of 1:1 causes to consolidate too quickly making the post-application manipulation of the gel difficult. A fibrinogen to thrombin ratio of 4:1 shows the optimal consolidation time of 4–5 min.

5. Preparation of BMAC, fibrinogen and thrombin

For application to the chondral defect, two 1 mL syringes are connected to a Y-shaped mixing catheter. One syringe contains 0.8 mL of fibrinogen and 0.2 mL of HA. The second syringe contains 0.8 mL of BMAC and 0.2 mL of thrombin (Fig. 1).

6. Novel arthroscopic technique using CO_2 gas

For optimal visualization, arthroscopic procedures generally need to pump normal saline into the joint space under pressure. However injecting the biocellular matrix into the flowing fluid environment will cause immediate dissipation of the gel, particularly as the flow of the fluid is generally through the arthroscope cannula which would be directed at the lesion being treated. For this reason gel treatments of chondral lesions typically involve an arthrotomy.

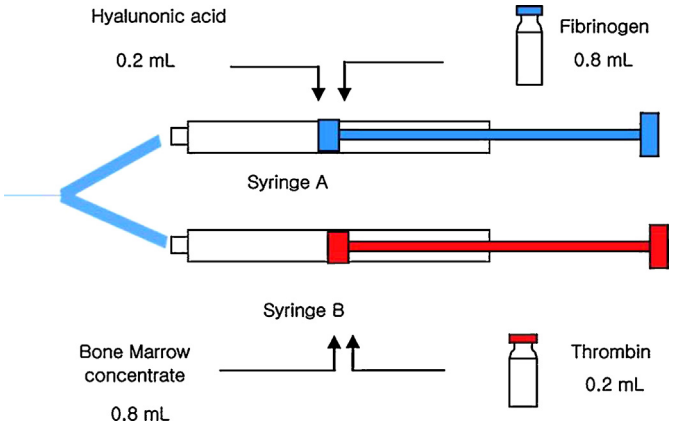

Fig. 1. Composition of MCIC.

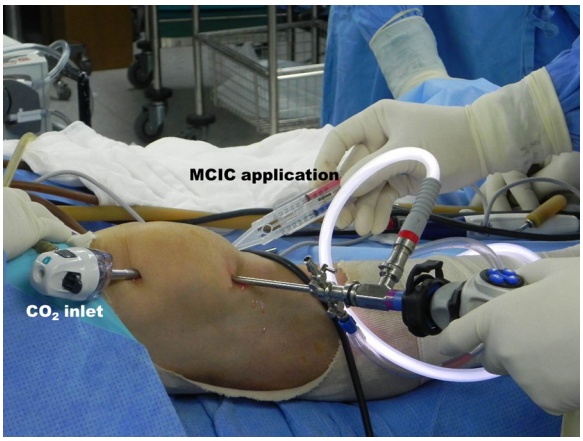

Fig. 2. Arthroscopy and CO_2 infusion setup and MCIC application.

Kim et al. introduced new arthroscopic technique to overcome this problems using CO_2 gas instead of normal saline during BMAC and gel matrix injection.[36] There are several advantages to using this method. An arthrotomy required in ACI is not necessary to implant the composite scaffold to the lesion. Areas that are traditionally difficult to approach such as the patella and the posterior femoral condyle are within easy access. Continuous CO_2 inflation insufflates the joint without irrigating the joint and has been safely used in laparoscopic surgery (Fig. 2). Furthermore, it appears that the continuous flow of CO_2 facilitates the placement of the mixture to the surface. The delivery of the composite liquid to the surface is followed by the consolidation as the action of thrombin on fibrinogen produces a fibrin clot that retains the HA and the cellular components of BMAC at the treated lesion.

7. Application of BMAC, HA, fibrin-gel mixture

The saline is drained from the knee joint after microfracture and CO_2 gas was introduced at pressure of 20 mmHg and rate of 20 l/min using the cannula at the supero-lateral portal which is already used as an outflow during microfracture procedures. Any residual saline is aspirated from the knee using both a syringe and an angled suction tube under low pressure in order to avoid bleeding. The microfracture site was dried using cotton buds. A 20-gauge spinal needle is inserted into the joint *via* any suitable portal and is connected to the double syringe containing BMAC, thrombin and HA, fibrinogen. Under arthroscopic guidance, the mixture is gently applied over the lesion. Due to the pressure effect of the flowing CO_2 and the adhesiveness of the gel, the graft adheres to the lesion even against gravity (Fig. 3). The graft consolidates in 5 min. The knee is then moved several times through its range of motion in order to anatomically sculpt the graft and test its stability.

8. Osteochondral lesions: regeneration *versus* replacement

Total knee arthroplasty (TKA) is undoubtedly the most effective definitive treatment for advanced osteoarthritis. It is one of the most successful surgical procedures, and 80–90% of patients have pain relief with improved knee motion and function.[37] Despite these successful results, potential complications after TKA remain a significant concern.[38–40] Infection, deep vein thrombosis, pulmonary embolism are significant, potentially life changing events; further morbidity may be caused by surgical-procedure related problems in alignment and soft-tissue balancing. These complications require other treatments ranging from medication to re-operation.

The pain after surgery is another issue.[41] In spite of many apparently successful results of TKA in joint registries, where success is measured in the time to revision of an implant, many patients suffer from continued pain after TKA. Even though TKA longevity has been extended with modern implant materials and surgical techniques, the number of revision surgery is projected to continue increasing with the increase in life span of the general population.

The irreversibility of arthroplasty, the difficulty in predicting outcomes, the increased demands of the younger patient with

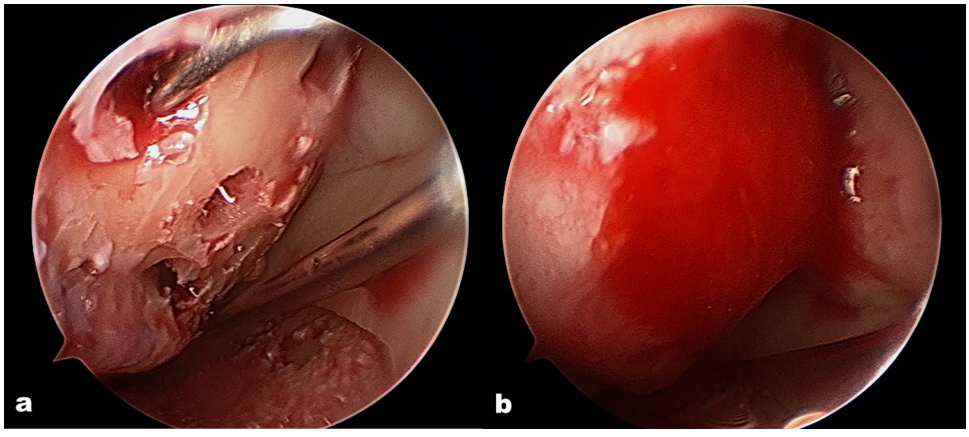

Fig. 3. (a) Drilling or microfracture and dry up. (b) After MCIC application.

osteoarthritis, and the prospect of eventual revision surgery have impacted on the surgeons' and patients' decisions in the treatments of this debilitating condition. Joint preserving techniques can offer an interim alternative that carries the potential for symptoms relief without significant surgical insult and morbidity. Biological treatments using stem cells, cultured cells, and bone marrow aspirate concentrate are becoming a popular alternative, promoting the paradigm shift from replacement toward regeneration as the primary treatment.

9. Conclusion

This novel technique uses microfracture, BMAC, HA, and fibrin gel to treat articular cartilage defects. We hope that this technique will add to the ever-increasing arsenal against artificial joint replacements.

Conflicts of interest

The authors have none to declare.

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