Adjuvant therapies for the enhancement of microfracture technique in cartilage repair

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Abstract

The classic technique of microfracture does not promote hyaline cartilage restoration. Subchondral bone perforations lead to the formation of a clot containing pluripotent progenitor cells and finally the cartilage defect is filled by fibrocartilage tissue. Researchers have focused on enhancing the quality of the newly formed tissue in cartilage defects after microfracture arthroscopic surgery. Adjuvant treatments are categorized in four main groups: scaffolds, pharmaceutical agents, growth factors and combinations of the aforementioned. Several experimental studies utilize pharmaceutical or biological agents in combination with microfracture, to improve the quality of the regenerated cartilage. The mechanism of action of the agents used is either to exert a chondroprotective effect on the newly formed fibrocartilage tissue, or to induce the recruitment of mesenchymal stem cells towards chondrogenesis instead of osteogenesis during microfracture repair. Additionally, scaffolds have been used for both release of the biological agents and mechanical support of the newly formed blood clot. This review highlights current data regarding the combination of microfracture technique with adjuvant treatments in order to ameliorate the final outcome.

Introduction

Articular cartilage has low intrinsic reparative capacity.[1] Partial-thickness cartilage defects have a poor healing potential, whereas full thickness with penetration of the tidemark spontaneously may form a fibrocartilage tissue. Therefore, the penetration of tidemark and cartilage - subchondral bone interplay seems to play a significant role in cartilage reparative effort.[2,3] Researchers have focused on the development of marrow stimulating procedures such as subchondral drilling and microfracture techniques. All these procedures are easy, fast and inexpensive arthroscopic ways for treating small (up to 4 cm²), full thickness cartilage defects. However, the final result is the formation of a fibrocartilaginous tissue with inferior biomechanical properties when compared to hyaline cartilage.[4,5]

In marrow-stimulating procedures, perforation to the subchondral bone allows blood and marrow-derived cells to fill the defect and a blood clot is formed. The subsequent wound repair cascade comprises an acute inflammatory response and cell chemotaxis. It finally leads to the formation of a vascularized granulation tissue and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell-types.[6] In the first days following subchondral perforations, fibrinous arcades are formed across the surface of the defect. The framework they create serves to orient mesenchymal cell ingrowth along the long axes. Afterwards, undifferentiated mesenchymal cells progressively differentiate in fibroblasts, osteoblasts, articular chondroblasts, and chondrocytes. Finally, new bone forms into the deeper zones and fibrocartilage into the superficial zones of the newly formed tissue.[7-9]

The clinical effectiveness of treating chondral injuries with microfracture was outlined in a recently published case-control study.[10] The authors reported that after microfracture, all patient-reported outcomes (PROs), demonstrated clinically and statistically significant improvements at 5.7 years. However, fibrocartilage is mechanically weaker than hyaline cartilage and degenerates easily.[11,12] Achievement of hyaline cartilage formation without fibrous or ossified tissue in cartilage defects still remains challenging. Great scientific effort and much research have been conducted in order to find adjuvant treatments that would improve the quality of the microfracture repair tissue.

The aim of this review is to outline current data regarding the combination of microfracture technique with adjuvant treatments in order to improve the quality of cartilage repair tissue. The PubMed and Google Scholar databases were searched in June 2018 using the terms "cartilage repair", "microfracture" and "adjuvant therapies" and the following search limits: "any date", "English". Experimental studies were only included. Studies that used methods of bone marrow stimulation other than microfracture were excluded.

Overview of experimental studies

Studies aiming at adjuvant treatments to microfracture technique have three targets: (a) to optimize the environment of the clot created for the proliferation of the marrow derived MSCs. This is achieved through the use of membranes or scaffolds that cover the clot and therefore protect the cells contained within, (b) to utilize the effect of pharmaceutical agents that have chondroprotective properties, or can induce chondrogenesis (c) to biologically enhance the microfracture clot, by adding growth factors into it, in order to improve the quality and quantity of the regenerative tissue. Furthermore, the combination of more than one adjuvant treatments at one stage technique could offer an even better final outcome.

Scaffolds and membranes

In a sheep microfracture experimental model, full thickness articular cartilage defects were generated in the medial

femoral condyle of the left stifle joint. Eight animals were divided in two groups: Four were treated with microfracture only and served as controls. In the rest of them, cell-free, freeze-dried implants made of a poly-glycolic acid (PGA) scaffold (Alpha Research Deutschland GmbH, Berlin, Germany) and hyaluronan were immersed in autologous serum and used for covering the microfractured cartilage defects. Animals were sacrificed 6 months after surgery. The results according to histological scoring showed that the repair tissue formed when the defects were covered with the cell-free implant, compared to controls, was significantly better.[13]

In another experimental model, microfracture defects were created in the medial femoral condyle of fourteen sheep, and either were left untreated (n =6), or were treated with chitosan-glycerol phosphate/blood implant (BioSyntech, Laval, Quebec, Canada) (n = 8). Animals were euthanized 6 months after surgery. The quality of repair was assessed with histology, histomorphometry, and biochemistry at six months postoperatively. Repair tissue from defects that had been treated with chitosan-glycerol phosphate/blood, contained more cells and more collagen compared with the control group.[14]

Pharmaceutical agents

Combination of microfracture technique with intra-articular injection of Hyaluronic acid

Full-thickness cartilage defects were experimentally created in the medial femoral condyle in 36 female New Zealand white rabbits weighing between 3.5 and 4.5 kg and were treated with microfracture. Their exact age was not stated. Six rabbits were randomly assigned to receive 3 weekly injections of hyaluronic acid (5 mg/0.50 mL) (group A), 5 weekly injections (5 mg/0.50 mL) (group B), or control injections of normal saline (0.50 mL of normal saline) (group C) for a total period of either three or six months. Repair tissue was assessed both grossly, using a modified component of the International Cartilage Repair Society (ICRS) Cartilage Repair Assessment scoring scale and histologically, using the modified O'Driscoll histological cartilage scoring system. Supplementing the microfracture technique with 3 weekly injections of intra-articular hyaluronic acid had a positive effect on the repair tissue that formed within the chondral defect at the early follow-up. This improvement was not found for the 3-injection group at 6 months

or for the 5-injection group.[15]

In another experimental model, full-thickness cartilage defects were created in the medial femoral condyle of thirty New Zealand rabbits. Three groups were formed: control, microfracture (MF), and microfracture with hyaluronic acid (MFHA). One week post surgery, 1 mL of saline was injected into the knees of the control and MF groups, whereas 1 mL (15 mg/mL) hyaluronic acid was injected into the knees of the MFHA group 3 times weekly. Animals were sacrificed 6 months after surgery. According to the ICRS and Wakitani scales, no significant difference was observed between the MF and MFHA groups.[16]

Combination of microfracture technique with intra-articular injection of the small molecule compound Kartogenin

Full-thickness cartilage defects were generated on the right patellar groove of 24 female skeletally mature New Zealand White rabbits. Two groups were formed: (group A) microfracture plus weekly intra-articular injection of 0.3 mL of 10 μM KGN and (group B) microfracture plus dimethyl sulfoxide. Kartogenin is known to direct human bone marrow-derived mesenchymal stem cells (BD-MSCs) into chondrocytes by mediating the CBFβ-RUNX1 signaling pathway.[17] The outcome was assessed macroscopically, by using the International Cartilage Repair Society (ICRS) evaluation system, and histologically, by using the modified O'Driscoll scoring system. At 12 weeks, group A showed statistically significantly higher ICRS scores and modified O'Driscoll scores compared with group B, indicating that intra-articular injection of KGN enhances the quality of fibrocartilage tissue.[18]

Combination of microfracture technique with intermittent recombinant parathyroid hormone

In a rabbit microfracture model of cartilage regeneration, (a 6- mm full-thickness defect was made on the weight-bearing surface of the trochlea) twelve adolescent animals were divided into three equal groups: microfracture alone, microfracture + 10 μg/kg human recombinant (1–34) PTH for 7 days and microfracture + 10 μg/kg recombinant (1–34) PTH for 28 days. PTH was administered subcutaneously on a daily basis starting on the day of surgery. The animals were sacrificed at 12 weeks. Histologic and gross analysis showed that treatment with either 1 or 4 weeks of intermittent parathyroid hormone inhibited cartilage formation.[19]

Growth factors and biological factors

Combination of microfracture technique with use of adipose tissue mesenchymal stem cells (AD-MSCs)

Thirty mature male rabbits were randomly divided into three groups and osteochondral defects were created in the medial condyle of all right knees. The control group (group A) received no treatment. In group B, microfractures were performed. In group C, microfractures were supplemented by application of 3×10^6 ADSCs to the defect area. At eight weeks post-operation, the animals were sacrificed. Cartilage repair was evaluated histopathologically according to the International Cartilage Repair Society (ICRS) scale. There was no statistically significant difference between the group which was treated with microfractures only and the group which received additional AD-MSCs treatment.[20]

Combination of microfracture technique with autologous conditioned plasma application

In another study 30 mature sheep underwent chondral defect creation on the medial femoral condyle and were treated with microfractures. Animals were divided into two groups: in group A, 5 weekly injections of autologous conditioned plasma (ACP) were performed, whereas group B did not receive further treatment. ACP was produced by harvesting 60 mL of blood from each animal before the induction of anesthesia. It was centrifuged at 2400 rpm for 3 min. The precipitate was separated and supernatant was centrifuged at 3000 rpm for 12 min. The final product was 6–8 mL of liquid PRP, which was used for intra-articular injection. Animals were sacrificed 6 months after surgery. The microfracture plus autologous conditioned plasma injections, showed significantly better macroscopical, histological, and biomechanical results than the control group.[21]

Combination of microfracture technique with insulin like growth factor 1 (IGF- 1)

Twenty-four New Zealand white rabbits aged, 4-6 months; weighing, 2.5-3.5 kg underwent generation of cartilage defects in the bilateral femoral condyles and were randomly divided into 4 groups: micro-fracture and recombinant human IGF-1 (rhIGF-1), micro-fracture only, rhIGF-1 without microfracture, and blank control group. Groups A and C received intra-articular injections of rhIGF-1, 0.1 mL rhIGF-1 (0.01 microg/microL) , three times per week for

four weeks postoperatively, while 0.1 mL saline was injected in groups B and D at the same time points. Histological score was evaluated according to Wakitani's criteria. The histological scores of group A (microfracture supplemented by recombinant human IGF-1), were significantly better than those of the other three groups at 4, 12, and 24 weeks.[22]

Combination of microfracture technique with intra-articular injection of recombinant human fibroblast growth factor-18

Experimentally generated cartilage defects in the medial femoral condyle of 80 skeletally mature female Welsh Mountain Sheep (mean age 3.9 years) were treated with microfracture alone, or microfracture plus intra-articular injection of 100ng/mL rhFGF□18, three times per week, for one or two weeks. The authors did not mention the volume of the injection. Animals were humanely sacrificed at 13 or 26 weeks postoperatively. The outcome was assessed macroscopically, by using the International Cartilage Repair Society (ICRS) evaluation system, and histologically, by using the modified O'Driscoll scoring system. Results showed that the use of intra-articular rhFGF-18 combined with microfracture in sheep improved quality and quantity of repair tissue in the defect at 6 months, compared to microfracture alone.[23]

Combination of adjuvant treatments

Combination of microfracture technique with autologous conditioned plasma application and periosteum as scaffold

Chondral defects were created in the right medial femoral condyles of New Zealand rabbits. Twenty-one mature (18 weeks old) rabbits, with a mean weight of 2450 g (1950–2900 g) were used in this study. Three groups were formed: Group A was treated with microfractures only. Group B was treated with microfractures plus intraarticular injections of autologous conditioned plasma. Group C received microfractures, the defect was covered by periosteum, and then, ACP was applied subperiosteally and intraarticularly. Approximately 2 mL of thrombocyte-rich plasma (ACP) were injected in Groups B and C, which were produced from 9 mL of venous blood that were centrifuged at 1500 rpm for 5 min (Hettich Rotofix 32A). Animals were euthanized 12 weeks after surgery. The histological sections that were

finally produced were assessed using the International Cartilage Repair Society (ICRS) visual histological scale. Intra-articular administration of ACP following microfracture, demonstrated a beneficial effect in cartilage healing. Histological scores in Group B and Group C were better compared to Group A.[24]

Combination of microfracture technique, with administration of recombinant SRY-type high mobility group box-9 and collagen membrane

In mature female New Zealand white rabbits, osteochondral defects at the right knee trochlea were experimentally produced. The defects were either (a) left untreated, (b) treated with microfractures, or (c) treated with microfractures and covered with a Sox-9 bound collagen membrane.(n=4-11) A commercial bilayer collagen membrane (Bio-Gide, Geistlich Pharma AG, Switzerland) was utilized to serve as a carrier for scSox9. The diameter of the membrane was 4 mm and it was soaked in 25 μL of 100 μg/mL of scSOX9 solution for one hour. Rabbits were euthanized 8 weeks after surgery. By local application of cell penetrating Sox-9, it was shown that the quality of microfracture induced cartilage repair was superior. Histological analysis revealed that the newly formed tissue produced by microfracture with SOX9 had features of hyaline cartilage.[25]

Combination of microfracture technique with Bone Morphogenetic Protein-7 and collagen scaffold

Forty fifteen-week-old male New Zealand white rabbits weighing 2.5–3 kg were used in this study. They were divided into five groups and osteochondral defects were created in the patellar groove. Eight animals were then assigned to (a) no further treatment (control), (b) microfracture only, (c) 10 mg of recombinant human BMP7 (Stryker Corporation, Hopkinton, MA) dissolved in 10 mL of 5 mM hydrochloric acid that was painted onto the bone at the base of the chondral lesion and allowed to adsorb for 5 minutes, (d) 10 μg of BMP-7 dissolved in 10 μL of 5 mM hydrochloric acid was adsorbed onto a 2 mm diameter by 3 mm tall cylinder of a type I collagen sponge (Helistat Sponge, Integra LifeSciences, Plainsboro, NJ) for 15 min. This sponge was then press-fit into the distal microfracture hole, and (e) 2 mm diameter by 3 mm tall cylinder of type I collagen sponge soaked in 10 μL of 5 mM hydrochloric acid was press-fit into the distal microfracture hole. The animals were sacrificed at 24 weeks . The quality of the repair tissue was determined using the International Cartilage

Repair Society Visual Histological Assessment Scale. Compared to either single treatment, the combination of microfracture and BMP -7 in a collagen sponge increased both quality and quantity of repair tissue.[26]

Combination of microfracture technique with Bone Morphogenetic Protein-4 and scaffold

Full- thickness cartilage defects were created in the trochlear groove of 72 mature rabbits weighing 2.5–3.0 kg (4–6 months) and then microfracture was applied. MSCs were derived from bone marrowaspirates of 4-week rabbit taken from the distal femur. Each aspirate was combined with 25 mL of MSCs. Transfection of MSCs was performed. The medium was removed and the cells were incubated with bone morphogenetic protein-4 recombinant adenovirus (Ad-BMP4). Afterwards; adenovirus-BMP-4 was placed in a biomaterial scaffold of perforated decalcified cortical bone matrix (DCBM) and set to the microfracture site. Four groups were assigned: (n=18) Ad-BMP4/perforated DCBM composite (group I), perforated DCBM alone without Ad-BMP4 (group II), DCBM without perforated (group III) and microfracture alone (group IV). Animals were sacrificed 6, 12 and 24 weeks post operation. The addition of adenovirus-BMP-4 resulted in a more effective repair, leading to regeneration of hyaline articular cartilage at 6 weeks and to complete repair of articular cartilage and subchondral bone by 12 weeks, compared to the DCBM-alone group, the DCBM-perforated group, and the microfracture-alone group.[27]

Combination of microfracture technique with recombinant human fibroblast growth factor-18 and a collagen membrane

Chondral defects were created in the medial femoral condyle of 40 skeletally mature female Welsh Mountain Sheep (mean age 3.9 years). They were treated with microfracture only, microfracture and intra-articular injection of rhFGF-18 30 μg, or microfracture and rhFGF-18 applied on a membrane. The membrane used was an 8 mm diameter bilayer collagen membrane (Chondrogide, Geistlich, Manchester, UK) at concentrations between 0.064 and 32 μg. Animals were humanely sacrificed at 13 or 26 weeks post□operatively. The administration of rhFGF-18 on a collagen membrane significantly enhanced the healing of the defect in comparison to the other groups.[28]

The aforementioned studies are summarized in Table 1.

Discussion and Conclusions

Microfracture technique is a simple, fast and low-cost procedure targeted in inducing the intrinsic healing potential of cartilage by utilizing and stimulating the subchondral bone marrow.

The effort to optimize the outcomes of microfracture can be pursued by either enhancing the microfracture procedure itself, or by developing adjuvant treatments. Regarding the first strategy the usage of a cannulated awl may offer certain advantages, as it can yield more patent marrow channels at the adjacent subchondral bone of the microfracture hole and result in greater mobilization of the reparable cells to the defect, according to the findings of a recent study.[29]

The exact mechanism through which the released pluripotent mesenchymal progenitor cells react into the microfracture clot is complex. Understanding the interplay between pathways taking part into the cartilage defect healing, would offer valuable information for improving the properties of the newly formed fibrocartilage tissue.

Regarding the effectiveness of the adjuvant treatments studied, some of them did not achieve statistically significant superiority than microfracture alone.[16,20] On the contrary, pharmaceutical agents like Kartogenin and most of the growth or biological factors demonstrated encouraging results.[18,21,22,23] Additionally, the use of scaffolds seems to further enhance the efficacy of biologics.[25,27,28] Each adjuvant treatment seems to apply cumulatively to the healing potential of microfracture, while several treatments do not necessarily share common pathways.

In order to achieve optimal results and reach the intended purpose for a pharmaceutical or biological agent, the selection of a suitable delivery mechanism is extremely important. Moreover, a proper delivery strategy can affect the dosage and ultimately determine whether an effective pharmacokinetic profile is achieved. Techniques for the delivery of soluble reagents targeting cartilage defect treatment have evolved over the years. Simple intra-articular injections into the defect have been overshadowed by more focused delivery systems, like bulk phase scaffolds or membranes.[30]

Table 1. Summary of previous-published studies.

Author, year [ref.]	Animals per group; time of sacrifice	Animal: defect	Adjuvant treatment	Results compared to mf only
Erggelet et al. 2009[13]	4; 6 months	Sheep: medial femoral condyle	PGA scaffold superior histological scores	Statistically significant
Hoemann et al. 2005[14]	8; 6 months	Sheep: medial femoral condyle	Chitosan-glycerol phosphate membrane	Statistically significant superior histological scores
Strauss et al. 2009[15]	6; 3/6 months	N.Z.W. Rabbits: medial femoral condyle	Intrarticular hyaluronic acid (5 mg/0.50 mL)	Statistically significant higher ICRS and modified O'Driscoll scores
Gunes et al. 2012[16]	10; 6 months	N.Z.W. Rabbits: medial femoral condyle	Intrarticular hyaluronic acid (1 mL ,15 mg/mL)	No significant difference
Xu et al. 2015[18]	12; 4 and 12 weeks	N.Z.W. Rabbits: patellar groove	Kartogenin intrarticular inj. (0.3 mL of 10 M KGN)	Statistically significant higher ICRS and modified O'Driscoll scores
Feeley et al. 2010[19]	4; 3 months	N.Z.W. Rabbits: patellar groove	10 g/kg recombinant (1–34) PTH subcutaneously	Inhibited cartilage formation
Ceylan et al. 2016[20]	10; 8 weeks	N.Z.W. Rabbits: medial femoral condyle	3×10(6) ADSCs to the defect area	No statistical significance
Milano et al. 2012[21]	15; 6 months	Sheep: medial femoral condyle	2mL of (ACP)	Statistically significant superior histological scores
Zhang et al. 2014[22]	6; 4, 12, 24 weeks	N.Z.W. Rabbits: medial femoral condyle	0.1 mL rhIGF-1 (0.01 microg/microL)	Statistically significant superior histological scores
Power et al. 2014[23]	16; 13/26 weeks	Sheep: medial femoral condyle	Intra-articular injection of 100ng/mL rhFGF 18	Statistically significant superior histological scores
Karakaplan et al. 2015[24]	7; 12 weeks	N.Z.W. Rabbits: medial femoral condyle	2 mL ACP subperiostaly and inrarticularly	Statistically significant superior histological scores
Zhang et al. 2017[25]	4-11; 8 weeks	N.Z.W. Rabbits: patellar groove	Bilayer membrane soaked in 25 L of 100 g/mL of scSOX9 solution	Statistically significant superior histological scores
Kuo et al. 2006[26]	8; 24 weeks	N.Z.W. Rabbits: patellar groove	10 g of BMP-7 on a type I collagen sponge	Statistically significant higher ICRS scores
Zhang et al. 2008[27]	18; 6, 12, 24 weeks	N.Z.W. Rabbits: patellar groove	Adenovirus-BMP-4 in a biomaterial scaffold of perforated decalcified cortical bone matrix (DCBM)	Statistically significant superior histological scores
Howard et al. 2015[28]	5; 13/26 weeks	Sheep: medial femoral condyle	rhFGF-18 applied on a bilayer collagen membrane (Chondrogide, Geistlich, Manchester, UK) at concentrations between 0.064 and 32 g	Statistically significant higher modified O'Driscoll scores

Systemic administration was utilized in just one of the experimental models studied. That was the case of subcutaneously injected recombinant (1-34) PTH.[19] Notably, the findings of the study concerning the final outcome were opposite from the desired, as systemic administration of PTH led to induction of bone formation. On the other hand, the same findings indicate that systemic administration as a delivery method could prove rather effective.

Cartilage formation is the desirable outcome in osteochondral lesions, but there are cases like fracture nonunion when it is quite unwelcome. An important observation made in fracture nonunion is the presence of cartilage between the bone ends, associated with the formation of fibrous tissue and minimal bone regeneration.[31-33] In endochondral ossification, cartilage formation is an important intermediate step to new bone formation. In several models of fracture nonunion, cartilage formation is not followed by efficient endochondral ossification, while fibrous tissue forms instead. The transition from cartilage to bone is a process which is regulated by locally produced growth factors.[34,35] In a study by Kwong et al. it was shown that imbalance in the expression of BMPs and BMP inhibitors within cartilaginous areas of developing non-unions may account for their reduced bone formation ability.[36] These findings imply that, if microfracture regenerative procedure was considered as a special case of fracture repair, several pathways could be targeted in an attempt to promote the recruitment of progenitor cells towards the chondroblast instead of osteoblast lineage during endochondral ossification. Both fracture healing and endochondral bone formation are directly regulated by BMPs,[37,38] fibroblast growth factor 2 (FGF-2).[39] Wnt proteins and Wnt signaling antagonists.[40,41] Several of these morphogenetic processes participate in interactive feedback loops, including the interplay between BMPs and Wnt signaling proteins.[42,43]

Based on the previous observations one could describe the main properties of an "ideal" adjuvant therapy, in terms of a) way of administration and b) mechanism of action. Systemic administration of the proper reagent ensures that, through blood flow, it is transported inside the forming clot itself and therefore exerts its action effectively. On the other hand, application of the agent through intra-articular injection would form an interface with the surface of the clot only. Incorporating the utilization of a scaffold additionally to the pharmaceutical agent or growth factor further enhances cartilage regeneration. Regarding the desired signal transduced by the agent to the cells inside the forming clot, induction of chondrogenesis should be its target. It should prevent progenitor mesenchymal cells from differentiating towards the osteoblast lineage and instead, divert them to committing themselves to the chondroblast series.

Conclusively, several studies have focused on adjuvant treatments to improve the quality of the microfracture repair tissue. Their results indicate that it may be further enhanced. Targeting the recruitment of progenitor cells accumulated in the microfracture clot, towards the chondroblast lineage might be an effective strategy.

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