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| Copyright Year | 2018 | |
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Abstract

Hyaline cartilage is an essential component for the form and function of articulating joints, such as the knee. With the annual incidence on the rise, there are between an estimated 30,000 and 100,000 chondral repair procedures that are performed yearly in the United States. Marrow stimulation is a commonly used technique for articular cartilage repair. Marrow stimulation involves the perforation of the subchondral bone plate, most commonly with an arthroscopic microfracture awl, for the release of marrow elements. The marrow elements fill the articular cartilage defect forming a fibrocartilage repair. Though arthroscopic microfracture is considered by some as the gold standard therapy for cartilage repair, short-term outcomes have been shown to be unreliable and unsustainable. Some experts now opine that marrow stimulation as it currently exists should be outright abandoned.

Recently, however, there has been a push for new innovations in the augmentation of the marrow stimulation techniques in order to attain more sustainable outcomes and decrease associated complications. The augmentation of microfracture via the addition of post-microfracture intra-articular platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), and adipose-derived stem cells (ASCs) is an exciting advancement in marrow stimulation. Also, the recent introduction of the nanofracture, "rebirth" of drilling, and biocartilage techniques offer promising technological advancement in the field of marrow stimulation. This chapter focuses on clinical indications, surgical technique, and the outcomes of marrow stimulation procedures and the augmentation of these procedures.

Keywords (separated by " - ")

Marrow stimulation - Cartilage restoration - Biologics - Microfracture - Platelet-rich plasma - Bone marrow aspirate concentrate - Adipose-derived mesenchymal stem cells - Subchondral drilling

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# Marrow Stimulation and Augmentation

# 16

Michael L. Redondo, Brian R. Waterman, Jack M. Bert, and Brian J. Cole

## Introduction

Hyaline cartilage is an essential component for the form and function of articulating joints, such as the knee. While the ideal management of chondral defects continues to be investigated, it is known that hyaline articular cartilage has limited capacity for healing due, in part, to the articular surface layer's lack of intrinsic blood supply, mitotic activity, and poor progenitor cell recruitment [1]. Therefore, the risk of symptoms (pain, effusion, decreased activity, loss of function) related to chondral defects and the likelihood of lesion progression to eventual osteoarthritis remains pervasive [2, 3]. With increases in the annual incidence reported at up to 5%, there are between 30,000 and 100,000 chondral repair procedures that are performed yearly in the United States [4, 5]. Currently, chondral lesions have been hypothesized to exist in approximately 12% of the population [6], most commonly, in the medial compartment of the knee with the second most common being the patellofemoral joint [7].

Intrinsic cartilage repair relies on chondrocyte activation and recruitment of mesenchymal stem cells (MSCs) and differentiation of surface chondroprogenitor cells [1]. However, an individual's response to chondral damage is patient-specific. Adult-aged patients have less potential for cartilage regeneration since fully differentiated chondrocytes have restricted mitotic activity and limited local progenitor cell recruitment [8]. Furthermore, cartilage tissue has limited ability to recruit MSCs at the articular surface for repair [1]. While the effect of chronological age on cartilage repair is inconsistent in existing clinical studies, several animal models that have suggested a negative correlation between age and chondrogenesis or MSC potential [9, 10]. Recent basic science models also support a trend toward suboptimal outcomes of cartilage repair procedures with advancing age [11]. In a study examining cartilage regeneration potential in a bovine model, there was a diminished collagen-forming capacity in adult chondrocytes, as well as less induction of MSCs. Likewise, fetal and juvenile model MSCs displayed greater comparative matrix and mechanical properties than that seen with adult model MSCs [10]. Therefore, due to the very low intrinsic regenerative healing of symptomatic full-thickness cartilage defects, particularly in the aging population, the progression of cartilage defects into osteoarthritis remains a concern.

Marrow stimulation was initially proposed as a treatment to recruit autogenous MSCs for full-thickness articular cartilage defects. After a

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J. Farr, A. H. Gomoll (eds.), *Cartilage Restoration*, https://doi.org/10.1007/978-3-319-77152-6_16

thorough debridement of overlying diseased or unstable cartilage flaps and the underlying calcified cartilage layer, all marrow stimulation techniques involve scoring or perforating the subchondral bone plate in order to release marrow elements into the base of the defect. MSCs can subsequently differentiate into fibrochondrocytes which facilitate formation and stabilization of a fibrocartilage clot. These "cartilage-like" fibrocartilage clots contain varying amounts of type I, II, and III collagen, which fills and ultimately remodels in the defect to replace native hyaline cartilage with fibrocartilage.

The idea of marrow stimulation was popularized in the late 1950s when Pridie described the technique of subchondral drilling, often termed Pridie drilling. Pridie drilling involves the open drilling of exposed subchondral bone with a Kirschner wire to stimulate bleeding and bone marrow recruitment [12]. Several techniques iterated on Pridie's technique. One of these iterations was spongialization, an aggressive approach in which the subchondral bone plate is completely removed exposing the cancellous bone or "spongiosa." [13]. Though Pridie drilling and its adaptations helped develop the concept of marrow stimulation techniques, they were quickly replaced by other less invasive procedures as arthroscopic techniques evolved. In the 1970's, Dr. Lanny Johnson popularized abrasion arthroplasty, an arthroscopic superficial abrasion performed to stimulate repair of osteoarthritic lesions [14]. As compared to the previous open drilling, this modified technique encouraged quicker postoperative rehabilitation and greater precision [14]. Abrasion arthroplasty was widely adopted as a viable method to treat osteoarthritis, until Bert and Rand reported that abrasion arthroplasty provided patients with no significant benefit over those treated with debridement only [15, 16]. Consequently, the technique was abandoned. Recently, however, a resurgence of abrasion arthroplasty investigations has occurred. Sansone et al. [17] recently displayed survivorship was 89.5% for patients younger than 50 years for small lesions (<4 $cm^2$) at mean follow-up of 20 years. Due to these recent investigations, reassessment of abrasion arthroplasty as a treatment of full-thickness cartilage defects may be warranted.

In recent years, the most popular iteration of marrow stimulation, microfracture (see drilling below), was popularized in the late 1990s by Steadman and is considered by some experts as the first-line gold standard treatment for isolated cartilage defects [18]. According to the large insurance database, approximately 78,000 microfracture procedures are performed annually in the United States. Though early clinical outcomes have been shown to be favorable, the highest level of evidence documenting the comparative effectiveness of microfracture is mostly derived from selected randomized control trials. Also, the mid- to long-term decline in benefit after primary microfracture has generated concerns about the sustainability of early clinical outcomes [19]. In a systematic review by Erggelet et al. [20], the status of microfracture as the gold standard for treatment of cartilage lesions is debated, stating that future comparative prospective trials are required to definitively acknowledge microfracture as a procedure of choice. Furthermore, some experts assert that microfracture does not predictably provide better outcomes than debridement alone, alters the microarchitecture of underlying bone, and should be outright abandoned [15].

Different drilling instrumentations impart distinct mechanical differences upon the subchondral bone. Mithoefer has opined (personal communication or ICRS annual meeting, September 25, 2016) that drilling with a 1 mm K-wire should be considered "second-generation microfracture" as a result of Eldracher's work confirming that drilling with a 1 mm drill bit avoids the formation of subchondral cysts and intralesional osteophytes [21, 22]. The use of a microfracture awl has been reported to result in more bone compaction. The dense fractured bone accumulations can block marrow space channels and inhibit MSC migration to the defect surface [23]. Subchondral drilling allowed more consistently patent channels for cell migration. Additionally, Chen et al. have demonstrated that drilling to greater depths (6 mm) allowed for greater fill of the cartilage defect with more hyaline character in the repair matrix [24].

The suspected predominant causal factors for variable to poor long-term clinical outcomes for microfracture include inadequate clot stability and the poor long-standing viability and durability of fibrocartilage regenerate. Fibrocartilage lacks the native type II collagen normally found in hyaline articular cartilage and offers a decreased capacity to tolerate the high stress and force with repetitive loading [25]. This decrease in longevity and durability would ultimately lead to poorer long-term outcomes seen with the microfracture technique [25]. Notably, the results following marrow stimulation are often attributed to poor-quality tissue formation. The senior author, however, believes that the results of marrow stimulation can in many cases mirror those of other cartilage repair procedures if traditionally recognized comorbidities are addressed at the time of treatment in addition to rigorous attention to technical details and postoperative rehabilitation. Thus, recently, there has been a push for new innovations in the augmentation of the microfracture techniques in order to attain more sustainable outcomes and decrease associated complications such as intralesional osteophytes, subchondral cysts, and weakness of the subchondral plate (see complications section below). The augmentation of microfracture via the addition of post-microfracture intra-articular platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), and adipose-derived stem cells (ASCs) is an exciting advancement in marrow stimulation. Also, the recent introduction of the nanofracture, "rebirth" of drilling, and biocartilage techniques offer promising technological advancement in the field of marrow stimulation. This chapter focuses on clinical indications, surgical technique, and the outcomes of marrow stimulation procedures and the augmentation of these procedures.

## Indications and Contraindications

Microfracture procedure is indicated in treatment of symptomatic grade III–IV articular cartilage lesions in younger patients (<40 years old). Microfracture is currently recommended for smaller (<2–3 $cm^2$) contained focal lesions about the trochlea, condylar surfaces. It should be avoided in the treatment of diffuse, large (>4 $cm^2$), or bipolar articular cartilage defects, and caution is warranted in patellar lesions in light of findings reported by Kreuz [26]. Similarly, the results of microfracture remain guarded when there are significant subchondral bone changes on MRI.

## Technique

### Preparation of the Lesion Site

The surgical procedure begins with the assessment and debridement of the full-thickness articular cartilage lesion. To debride the cartilage, sharpened ringed, angled, and/or straight arthroscopic curettes are used to remove any unstable cartilage overlying or encircling the chondral defect. It is critical to achieve a perimeter of healthy cartilage margins with vertical walls in order to optimize progenitor cell clot adherence and stabilization upon release from the underlying marrow channels, as well as to provide a discrete load-bearing transition zone. Finally, with care to avoid aggressive handling of the subchondral bone, the calcified cartilage layer at the base of the defect is removed using a curette to enhance nutrition diffusion and clot adherence at the base [27]. Any concomitant intra-articular disease should be addressed prior to microfracture or marrow stimulation.

### Microfracture and Drilling Marrow Stimulation

An arthroscopic awl is traditionally used to make multiple small perforations 2.5 mm in diameter and 2 mm deep in the exposed subchondral bone. The senior author now prefers drilling using a motorized shaver (i.e., PowerPick, Arthrex, Inc., Naples FL). The microperforation component of the procedure should commence only after all other procedures of the case are completed. The awl perforation or drilling process should begin

at the periphery and then progress toward the center of the defect. The author's preferred holes for drilling are 1.5 mm in diameter and approximately 6 mm deep, while nanofracture is 1.0 mm diameter and up to 9.0 mm deep. These are placed 3–4 mm apart allowing ample space to ensure that the holes do not become confluent during the perforation process (Fig. 16.1). Once microperforation is complete, arthroscopic fluid inflow is stopped to allow visualization of the egress of marrow elements from the marrow channels. If inadequate bleeding or fat droplets are evident, repeat drilling may be utilized for greater depth in order to enhance marrow access. Of note, microfracture of the patella is accompanied with distinctive technical challenges, involving a higher degree of difficulty with visualization and access of the lesions arthroscopically when compared with microfracture of the tibiofemoral joint. Also, microfracture of the patella requires counterpressure on the anterior aspect of the patella.

## Rehabilitation

Rehabilitation plays a crucial role in providing the optimal environment for chondrogenesis and the protection of the fibrocartilage clot matrix. Because of the high degree of inconsistency of chondral injuries, due to variability in location and size, the rehabilitation program may need to be altered to accommodate concomitant intra-articular pathology. The senior author has developed two basic protocols for microfracture postoperative rehabilitation based on location: tibiofemoral/femoral condyle (Table 16.1) or patellofemoral (Table 16.2).

## Complications

As the body of knowledge in cartilage restoration grows, chondral damage has become increasingly characterized as a disease of the osteochondral unit rather than simply the articular surface. Marrow stimulation and microfracture has been suggested to have a significant impact on the architecture of the subchondral bone due to the penetration of the bone plate. These penetrating injuries to the subchondral bone have been suggested to trigger the activation of a secondary center of ossification leading to the eventual formation of intralesional osteophytes [28]. Intralesional osteophytes are bony advancements of the underlying subchondral bone that invade and disrupt de novo fibrocartilage regeneration and histological organization. Furthermore, this is not an uncommon occurrence. In a retrospective study examining microfracture by Cole et al. [29], 54% of patients had developed osteophytes at 6 months postoperatively, while approximately 70% of patients had developed osteophytes at 12 months. Perforation also has a known effect on the infrastructure of the subchondral bone plate. The penetrated subchondral bone plate displays reduced bone mineral density and thinner trabeculae of the subarticular spongiosa [30] .Thus, over exuberant subchondral drilling may induce changes



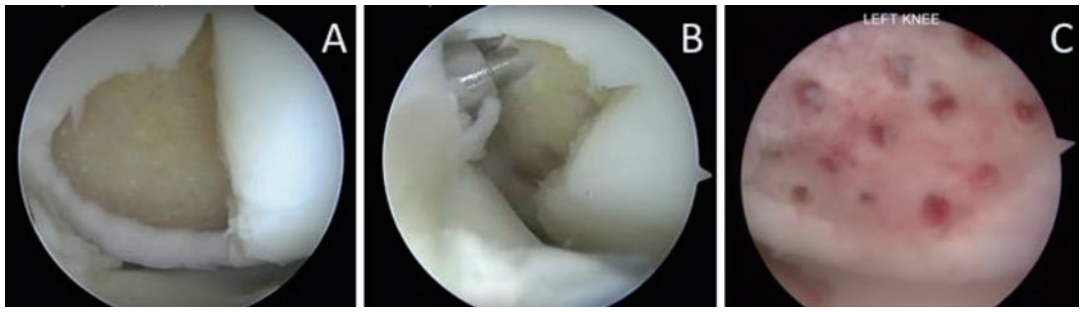


**Fig. 16.1** Arthroscopic images of the left knee joint of a (**a**) well-prepared chondral defect, (**b**) a standard microfracture drilling of the subchondral bone, and (**c**) fat and blood egress after the tourniquet is let down

**Table 16.1** Microfracture/BioCartilage of femoral condyle rehabilitation protocol

| Microfracture/BioCartilage of femoral condyle rehabilitation protocol | | | | |
|---|---|---|---|---|
| | Weight bearing | Brace | ROM | Exercises |
| Phase I: 0–6 weeks | Non-WB | 0–2 weeks: Locked in full extension at all times<br>Off for CPM and exercise only<br>Discontinue after 2 weeks | 0–6 weeks: Use CPM for 6 h/day, beginning at 0–40°; advance 5–10° daily as tolerated | 0–2 weeks: Quad sets, SLR, calf pumps, passive leg hangs to 90° at home<br>2–6 weeks: PROM/AAROM to tolerance, patella and tibiofibular joint mobs, quad, hamstring, and glut sets, SLR, side-lying hip and core |
| Phase II: 6–8 weeks | Advance 25% weekly until full | None | Full | Advance phase I exercises |
| PHASE III: 8–12 weeks | Full | None | Full | Gait training, begin closed chain activities: wall sits, shuttle, mini-squats, toe raises<br>Begin unilateral stance activities, balance training |
| Phase IV: 12 weeks–6 months | Full | None | Full | Advance phase III exercises; maximize core/glutes, pelvic stability work, eccentric hamstrings<br>May advance to elliptical, bike, pool as tolerated |
| Phase V: 6–12 months | Full | None | Full | Advance functional activity<br>Return to sport-specific activity and impact when cleared by MD after 8 months |

in the subchondral bone microarchitecture and intralesional osteophytes but also weaken the entire osteochondral unit [30].

Interestingly, bone cyst formation has also been reported in up to 33% of patients [19]. Also, a recent sheep model study by Beck et al. demonstrated that 42% and 92% of models had subchondral cyst formation at 13 and 26 weeks post-microfracture or augmented microfracture, respectively [31]. Experts hypothesize that subchondral bone cyst formation may be caused by an influx of synovial fluid in subarticular bone resulting in a localized increased synovial fluid pressure and cytokine-induced osteoclast-mediated bone resorption [30, 31]. Subchondral cysts are a cardinal feature of osteoarthritis and may represent a sign of progression of the cartilage defect. The senior author believes that these subchondral changes can minimized by drilling the lesion rather than using an awl, avoiding confluence of the drill holes, and avoiding postoperative loading of the newly prepared lesion for at least 6 weeks. Conceptually, if the patient loads the freshly prepared lesion, the bone responds similar to fracture repair including bone overgrowth and sclerotic changes.

## Clinical Outcomes

The reported outcomes of microfracture surgery have been widely variable. Many investigations have reported successful early short-term clinical outcomes (<24 months) for microfracture surgeries regardless of etiology of the chondral lesion [18, 32, 33]. However, the majority of existing studies are case series without control group comparison. In a seminal systematic review of 3122 patients, Mithoefer et al. [19] reported that microfracture had effectively improved knee function over the first 24 months, with 75–100% of microfracture patients indicating improved

**Table 16.2** Microfracture of patella/trochlea rehabilitation protocol

| Microfracture of patella/trochlea rehabilitation protocol | | | | |
|---|---|---|---|---|
| | Weight bearing | Brace | ROM | Exercises |
| Phase I: 0–6 weeks | Full with brace | 0–1 week: Locked in full extension at all times<br>Off for CPM and exercise only<br>1–4 weeks: Unlocked and worn daytime only<br>Discontinue when quads can control SLR without extension lag | 0–6 weeks: Use CPM for 6 h/day, beginning 0–30° for 0–2 weeks<br>2–4 weeks: 0–60°<br>4–6 weeks: 0–90° | 0–2 weeks: Quad sets, SLR, calf pumps, passive leg hangs to 45° at home<br>2–6 weeks: PROM/AAROM to tolerance, patella and tibiofibular joint mobs, quad, hamstring, and glute sets, SLR, side-lying hip and core |
| Phase II: 6–8 weeks | Full | None | Full | Advance phase I exercises |
| Phase III: 8–12 weeks | Full | None | Full | Gait training, begin closed chain activities: wall sits, mini-squats, toe raises, stationary bike<br>Begin unilateral stance activities, balance training |
| Phase IV: 12 weeks–6 months | Full | None | Full | Advance phase III exercises; maximize core/glutes, pelvic stability work, eccentric hamstrings<br>May advance to elliptical, bike, pool as tolerated |
| Phase V: 6–12 months | Full | None | Full | Advance functional activity<br>Return to sport-specific activity and impact when cleared by MD after 8 months |

knee scores at short-term clinical follow-up. However, the long-term outcomes of microfracture were variable and suggested deterioration over time. After 2 years, 47–80% of microfracture patients reported functional decline from their original improvements, as also supported by Steinwachs et al. at longer-term follow-up. These authors also interestingly reported clinical decline at earlier time points (18 months postoperatively) among older patients and in patients with larger defects (>2.5 $cm^2$) [8].

Long-term outcomes in highly active and athletic patients have also exhibited suboptimal results. Steadman et al. initially reported favorable clinical outcomes in several subsets of professional athletes following microfracture, including professional alpine skiers [18, 34]. In this 2-year follow-up, Steadman reported that the median postoperative Tegner activity scale was 10, and there were significant improvements in mean postoperative Lysholm score and patient satisfaction score, with 95% of patients returning to competitive skiing [34]. In contrast, a prospective study of athletes by Gobbi et al. [33] demonstrated an improved Tegner activity scale at 2-year postoperatively, although 80% of the athletes in the study progressively declined in sport activity at the final follow-up. When examining return to sport (RTS) in National Football League athletes, Andrews et al. reported that players receiving microfracture were 4.4 times less likely to RTS than those treated with chondroplasty alone [35]. In two studies following National Basketball Association (NBA) athletes, there was a significant correlation observed between microfracture and decreased minutes per game, decreased player efficiency rating, or points per game [36, 37]. More importantly, 21% of the NBA players treated with microfracture did not return to professional competition in the NBA [36].

Other investigations have sought to evaluate long-term outcomes of athletes with microfracture

versus other cartilage repair techniques. Gudas et al. performed a randomized controlled trial in young active athletes under the age of 40 with mean follow-up of 37.1 months, and they revealed significant superiority of osteochondral autograft transplant (OAT) over microfracture for the repair of articular cartilage defects in the knee, and only 52% of microfracture athletes could return to sport at the preinjury level [38]. When investigating microfracture vs OAT at the 10-year follow-up, both groups retained significant clinical improvement in postoperative International Cartilage Repair Society (ICRS) scores compared to baseline, but results were significantly better in OAT patients than microfracture group [39]. Finally, a systematic review by Harris et al. suggested the overall rate of return to sport was worse after microfracture than seen with autologous chondrocyte implantation (ACI) or OAT, and the microfracture patients that were able to return to sport more frequently experienced diminished performance [40].

Mithoefer et al. [19] also described several preoperative factors and demographic factors associated with clinical outcomes after microfracture. Improved surgical results were identified in patients with defect size less than 4 $cm^2$. BMI was also inversely correlated to knee function postoperatively, and there were significantly worse outcomes described in patient population of BMI >30 $kg/m^2$. Moreover, higher Tegner activity scores of patients preoperatively were associated with improved clinical outcomes after microfracture. Age is likely the most commonly reported associated factor with microfracture outcomes. Overall, younger age has resulted in better clinical outcomes, with reported age thresholds varying between 30 and 40 years of age.

## Augmentation of Marrow Stimulation

Marrow stimulation augmentation techniques seek to improve upon the two current critical weaknesses in marrow stimulation derived repairs: the poor durability of the repaired clot and the lack of type II cartilage in the typical fibrocartilage repair.

## Hyaluronic Acid

Hyaluronic acid (HA) is a naturally occurring high molecular weight glycosaminoglycan present within articular cartilage and synovial fluid. HA provides the joint with viscoelastic properties, lubrication, and shock absorbancy, and it also contributes to the extracellular matrix. As osteoarthritis (OA) progresses, the concentration of high molecular weight HA decreases and shifts toward an increase in low molecular weight HA, causing a lessening of the viscoelastic properties usually provided to the joint. Historically, intra-articular HA injections have been used as palliative treatment for OA, via the process of chondroprotection [41]. HA in the joint has the ability to bind to cluster of differentiation 44 (CD44) and inhibit the expression of interleukin (IL)-1β, subsequently inhibiting the production of catabolic metalloproteinases. If allowed to activate, the catabolic metalloproteinases would then cause degradation and destruction of articular cartilage collagen and the joint surface. The HA-CD44 binding pathway also augments chondroprotection through decreased apoptosis of chondrocytes, allowing preserved synthesis of the cartilage extracellular matrix and slowed degeneration [41].

Currently, studies have suggested that HA viscosupplementation may enhance proliferation and differentiation of chondrocytes, and it may provide a framework for MSCs released from the bone marrow [42, 43]. Recently, basic science studies have reported varied outcomes in using HA augmentation in microfracture. Though several studies have reported significantly improved ICRS, gross appearance, and histology in rabbit models treated with combined microfracture and HA injection augmentation [42, 43], separate contrasting studies suggest that HA augmentation does not improve the quality of repair tissue [44].

Clinically, there are a limited number of studies investigating HA augmentation outcomes, but

some promising evidence does exist, especially in regard to microfracture of talar cartilage defects. In a RCT including 57 patients (Doral et al.) [45], patients receiving microfracture for osteochondral talus lesions were then also randomly selected to receive intra-articular HA injections. Though both groups were found to have significantly higher postoperative American Orthopedic Foot and Ankle Society (AOFAS) scores when compared to preoperative scores, the increase in postoperative scores was also found to be significantly higher in the HA injection group when compared to a noninjection group at 2-year follow-up. Similarly, a RCT by Shang et al. also displayed a significant increase in AOFAS and Visual Analog Scale (VAS) for pain after talar microfracture augmented by HA vs microfracture alone at least 9 months of follow-up [46]. Although these studies show promising advances, further clinical evidence is required, especially in regard to microfracture in other large, weight-bearing joints and the impact of HA on long-term durability repairs.

## Platelet-Rich Plasma

Cellular growth factors have a critical effect on articular cartilage growth and homeostasis. Several of these critical growth factors are found and stored in the α-granules of platelets, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and many more [47, 48]. Platelet-rich plasma (PRP) is plasma containing supraphysiologic levels of platelets and autogenous growth factors derived from centrifuged peripheral venous blood. When activated with calcium chloride, targeted injections of PRP site of cartilage injury may act as a therapeutic modality in and augment cartilage repair techniques.

Recent in vitro and in vivo studies have demonstrated that PRP functions through modulation of several growth factors and cytokines, promoting differentiation, proliferation, signaling, and migration of chondrocytes and progenitor cells. Chondrocytes treated in vitro with PRP have shown increased proliferation and increased deposition of "hyaline-like" extracellular matrix type II collagen and glycosaminoglycans (GAGs) [49]. Subchondral bone progenitor cells have also been shown downstream effects from PRP. Kruger et al. [50] evaluated the migration capacity of human progenitor cells derived from subchondral bone in the presence of and without PRP and showed significantly greater migration of human subchondral progenitor cells on chemotaxis assays with exposure to PRP than untreated controls. Furthermore, histological analysis revealed that progenitor cells exposed to PRP displayed significantly improved immunohistochemical staining for proteoglycans and increased concentration of type II collagen, suggesting that PRP significantly increased cartilage matrix formation when compared to the control progenitor cells. Finally, PRP injections have been reported to be protective against further cartilage degradation via inhibition of nuclear factor-κB (NF-κB), an important transcription factor required for expression of many inflammatory mediators, such as cytokines IL-1β, tumor necrosis factor-α (TNF- α), and interleukin-6 [48, 49]. Modulation of the NF-κB allows evasion of this dangerous and destructive pro-inflammatory pathway.

Clinical outcomes of PRP injection augmentation of microfracture (Fig. 16.2), however, have conveyed mixed results. In a prospective cohort study comparing knee microfracture with PRP augmentation and classic microfracture alone, the authors found no statistically significant difference between the two groups in IKDC subjective scale, VAS, or SF-36 at any of the follow-up timeframes (3, 6, 12, and 24 months) [51]. Similarly, in a level II randomized clinical study, Manunta et al. failed to show a statistically significant difference in International Knee Documentation Committee (IKDC) or VAS at any outcome timeframe between PRP-enhanced microfracture and microfracture of the knee alone [52]. By contrast, several studies have shown more promising results in PRP injections with microfracture in talus osteochondral defects [53–55]. In particular, a level II evidence study by Guney et al. revealed that

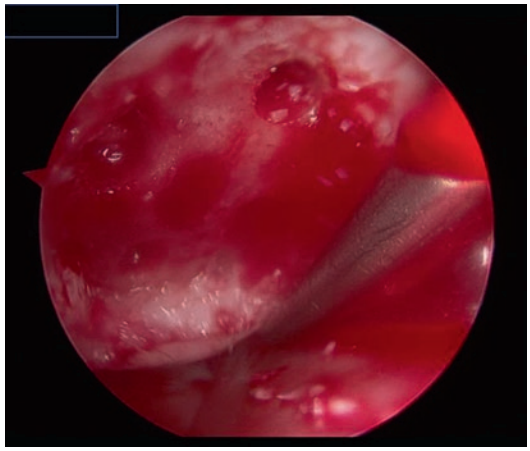

**Fig. 16.2** PRP super clot generated on top of a microfractured cartilage defect

patients who underwent talus microfracture with PRP injection did significantly better on AOFAS scoring system, Foot and Ankle Ability Measure (FAAM), and VAS for pain at an average of 16.2 months of follow-up [54].

Contrasting outcomes of PRP-augmented marrow stimulation may be due to the varying amounts of specific factors in the PRP. Dragoo et al. [56] have reported that the choice of commercial PRP system causes a variance in factor concentration, and not all of the factors included in PRP are chondrogenic. PRP with high concentrations of white blood cells (leukocyte-rich) or red blood cells resulted in promotion of pro-inflammatory markers and significant synovial cell death, resulting in the destruction of cartilage extracellular matrix. Though further studies are needed to elucidate the impact of leukocyte-rich vs leukocyte-poor PRP, Dragoo postulates that removal of undesired factors, such as leukocytes, can impact local inflammation and enhance chondrocyte recovery [56, 57]. The deletion process would, however, require additional FDA approval and regulatory guidelines due to "manipulation" of the PRP.

## Bone Marrow Aspiration Concentrate Injections

Mesenchymal stem cells (MSCs) are multipotent stromal cells that could differentiate into all cells of mesodermal origin, including chondrocytes. As the interest in MSC use in cartilage restoration increases, bone marrow aspiration (BMA) has emerged as a preferred technique for the acquisition of MSCs. The harvest site for BMA is typically the iliac crest (Fig. 16.3) due to its greater MSC concentration when compared to femoral or tibial aspirates [58]. In a typical BMA specimen, stem cells account for only 0.001 to 0.01% of nucleated cells in bone marrow [59]. Aspirate samples require concentration, usually through density-gradient centrifugation, in order to produce higher concentrations of MSCs. However, new innovations in harvesting methods via a novel needle system have been able to produce high MSCs numbers as well [60]. Bone marrow aspiration concentrate (BMAC) is then used for targeted injection of MSCs into joint of interest either as an isolated treatment or an augmentation to surgical treatment, such as marrow stimulation.

In addition to MSCs, BMAC has also been found to have a valuable platelet component that contains high levels of growth factors and cytokines, such as VEGF, PDGF, TGF-β, and bone morphogenic protein 2 and 7 (BMP-2, BMP-7) [61]. These bioactive factors are essential components of BMAC and allow increased anabolic, signaling, and anti-inflammatory activity [42, 61, 62]. Members of the TGF-β superfamily have specifically been suggested to play a major role in cartilage development [62, 63]. Several studies displayed TGF-β's critical role for increased gene expression related to chondrocyte type II collagen expression [62, 63]. Recently, BMP-7 has also been shown to be useful for the stimulation of chondrocyte proliferation, differentiation, and metabolism in animal models, making its inclusion attractive for cartilage regeneration therapy [42, 64].

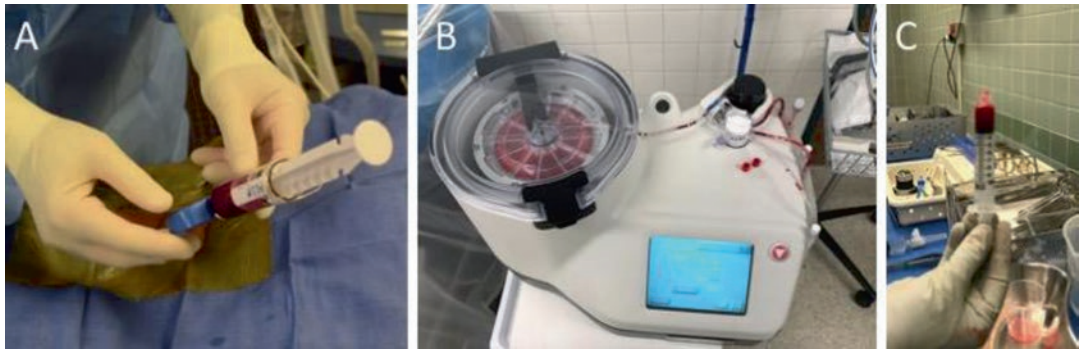


**Fig. 16.3** An intraoperative image of (**a**) BMA harvest from the iliac crest. The BMA is prepared by (**b**) centrifugation to concentrate the mesenchymal stem cells into BMAC. The BMAC is placed in (**c**) small syringes to ME injected at the site of marrow stimulation

Currently, the evidence for the significance of BMAC enhancement of marrow stimulation in several animal models is promising [12]. Fortier et al. treated 12 young adult horses with full-thickness chondral defects of the trochlear ridge with microfracture alone or BMAC-enhanced microfracture [65]. Arthroscopically, BMAC and thrombin were injected into the microfracture-treated defects. After 8 months, radiological and histological evaluations discovered a significant increase in defect filling, improved repair integration into the surrounding cartilage, and a significantly increased type II collagen and glycosaminoglycan repair composition. Similarly, in goat models, Saw et al. [66] reported on the cartilage defects treated with either subchondral drilling, drilling with intra-articular HA injection, or drilling with intra-articular injection of both HA and BMAC. At 6 months postoperatively, comparable findings were found between the subchondral drilling alone and HA arms, yet the HA and BMAC combination group displayed significantly improved proteoglycan content and repair integration.

Although a paucity of evidence exists in regard to the clinical outcomes of BMAC-augmented marrow stimulation for articular cartilage repair, there are some studies that have reported optimistic results. De Girolamo et al. examined pain or adverse events in chondral lesions repaired with microfracture in combination with implantation of a type I/III porcine collagen matrix and application of BMAC [67]. Clinically, no pain or adverse events were seen in patients at 6-month follow-up; however, these clinical outcomes were not compared to a negative control. In a cohort study by Gobbi et al. [68], full-thickness cartilage defects of the knee were treated with microfracture or a HA-based scaffold plus BMAC (HA-BMAC). The cartilage defect was prepared in the same fashion between the two groups prior to introduction of the HA scaffold and BMAC. At 2-year follow-up, the HA-BMAC group demonstrated a normal or nearly normal IKDC objective score in 100% of repairs, while the microfracture group obtained normal IKDC in only 64%. Moreover, HA-BMAC treated patients maintained a significantly improved knee function at 5 years and IKDC objective scores when compared with microfracture patient group. The improvement in long-term clinical outcomes suggests that BMAC may play a role in increased defect repair durability when compared to marrow stimulation alone. BMAC-enhanced microfracture has also been investigated in cartilage defects of the ankle. Hannon et al. [69] compared microfracture alone with BMAC-enhanced microfracture of talar defects in 34 patients, with improvements in the FAOS pain score and the short-form 12 general health questionnaire physical component summary (SF-12 PCS) in both groups postoperatively. The magnetic resonance observation of cartilage repair tissue (MOCART) score in the BMAC-enhanced microfracture group was significantly higher than that in microfracture alone

group, signifying better quality of tissue repair. Presently, the current evidence for BMAC used in conjunction with marrow stimulation is promising, yet it still requires high levels of evidence investigations to qualify as a standard of care.

## Adipose-Derived Mesenchymal Stem Cells

Adipose tissue contains MSCs referred to as adipose-derived mesenchymal stem cells (ASC) [70]. ASCs have been found to have endodermal, mesodermal, and ectodermal proliferative potential, making them useful aids in cartilage restorative marrow stimulation procedures. ASCs stimulated by various bioactive factors, especially the TGF-β superfamily, have been shown to induce their differentiation and proliferation into a chondrocytic phenotype [71–73], and several in vitro studies have demonstrated ASCs to have a potent capacity to fill animal model osteochondral defects [74, 75]. ASCs are obtained via local harvest, typically via liposuction, in the abdominal region. Many orthopedic surgeons lack experience with liposuction techniques required for ASC harvest. Recently, however, Dragoo et al. [76] have developed an entirely arthroscopic method of harvesting ASCs from the infrapatellar fat pad. This technique functions to remove barriers associated with other liposuction techniques. The ease of access, low harvest site morbidity, and comparatively higher stem cell concentrations make ASCs an attractive source of MSCs [70, 71].

Evidence for clinical outcomes of ASC-enhanced marrow stimulation (Fig. 16.4) is limited, but its potential is encouraging. In a level III evidence study, Kim et al. reported on clinical outcomes on ASC-enhanced microfracture procedures compared to microfracture alone in varus ankle osteoarthritis patients. At 12-month follow-up, significant improvements in VAS and AOFAS scores, as well as better ICRS grades, were achieved after marrow stimulation enhanced with ASC injection, when compared with after marrow stimulation alone [77]. Additionally, in a prospective cohort study on osteochondral talus lesions, 50 ankles were treated with either marrow stimulation with concomitant injection of stromal vascular fraction containing ASC or marrow stimulation alone. The clinical outcomes, including the VAS, AOFAS, and Tegner scores, improved significantly in the ASC group when compared with the marrow stimulation exclusive group [78]. Interestingly, these authors also reported that patient age (≥46.1 years) and large lesion size (≥151.2 $mm^2$) were significantly associated with poor outcomes in conventional marrow stimulation, but not in the ASC group. This suggests that ASC augmentation may be a viable method to overcome these known barriers of conventional marrow stimulation [78]. Currently, there are few randomized prospective studies that have examined ASC use in marrow stimulation, but in a recent prospective randomized comparative trial by Koh et al. [79], patients with full-thickness femoral condyle cartilage defects were randomly selected to receive ASCs with fibrin glue with concomitant microfracture treatment or conventional microfracture alone. At a mean clinical follow-up period of 27.4 months, the mean KOOS pain and symptom subscores were significantly more improved in the ASC group than with conventional microfracture technique alone. However, there was no significant difference in activity, sports, or quality-of-life subscores achieved by the addition of ASC to microfracture. Further randomized control trials and investigation into long-term clinical outcomes are required, but the addition of concomitant intra-articular ASCs to marrow stimulation techniques remains a promising therapeutic option for symptomatic chondral lesions.

# Advancements in Marrow Stimulation Technique

## Nanofracture, PowerPick, and Drilling

Nanofracture represents an innovation of the initial microfracture technique where a device or small-diameter wire are preferentially used for drilling [80]. The 1 mm diameter needle allows deeper drilling of the subchondral bone (up to

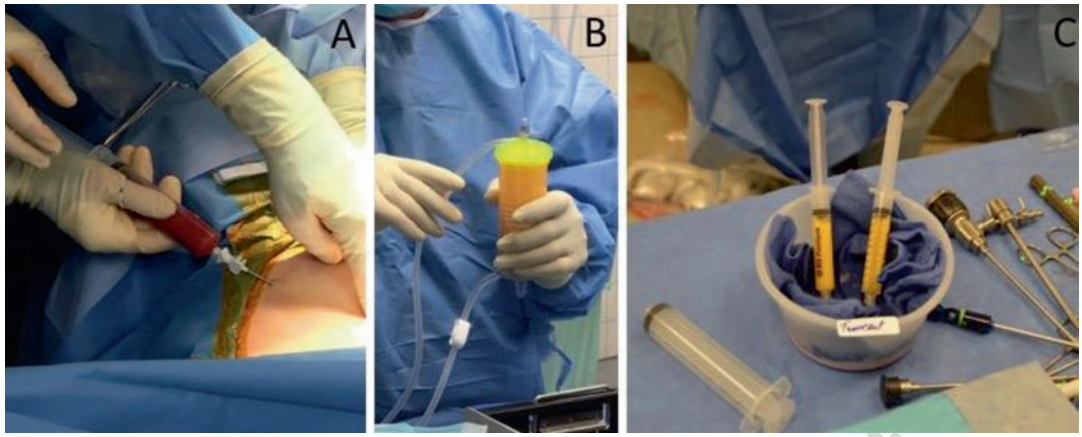


**Fig. 16.4** (**a**) Fat containing adipose-derived stem cells is harvested from the abdomen via insertion of a thin-harvesting cannula. (**b**) The fat sample is then processed with a LIPOGEMS® device to isolate the lipoaspirate. (**c**) The resulting lipoaspirate is placed into several small syringes to be injected at the site of marrow stimulation AU6

9 mm), a more consistent uniform cylindrical shape of the entire perforation, and more accurate drill depth [80]. Optimal subchondral bone perforation is an area of interest for many experts. Chen et al. have reported that a drill depth of at least 6 mm, a depth standard microfracture awls do not achieve, is required for the proper release of MSC [24]. These authors also demonstrated that increased drill depth was correlated with an increased percentage of type II collagen found in the fibrocartilage repair. The nanofracture technique also aligns itself with the recent increase emphasis on preservation of the subchondral bone architecture following penetration. In a basic science study comparing microfracture to nanofracture in ovine models, Zedde et al. demonstrated that nanofractured subchondral bone displayed better preservation of trabecular structures when compared with microfracture and that bone remodeling after nanofracture resulted in a trabecular structure remarkably similar to that of native subchondral bone (Figs. 16.5 and 16.6) [81]. There is currently a paucity in peer-reviewed literature comparing nanofracture to other cartilage repair procedures, but Tahta et al. [82] did demonstrate that the use of nanofracture achieved an improvement in PROs of talus cartilage defect repairs equal to scaffold-augmented microfracture technique. Despite these optimistic findings, more clinical trials are currently required to elucidate the further use of nanofracture as a viable improvement over microfracture.

## Biocartilage

BioCartilage (Arthrex Inc., Naples, FL) is a novel technique that combines a dehydrated allograft cartilage extracellular matrix (ECM) scaffold and

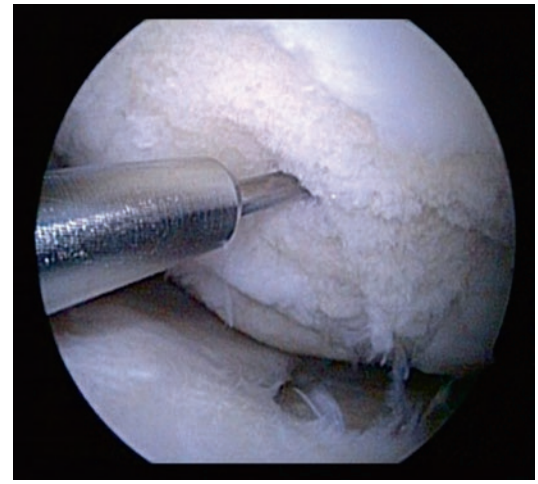

**Fig. 16.5** Tip of nanofracture© needle after insertion into an ICRS grade IV cartilage defect. (Reprinted from Benthien and Behrens [80]. With permission from Springer Berlin Heidelberg)

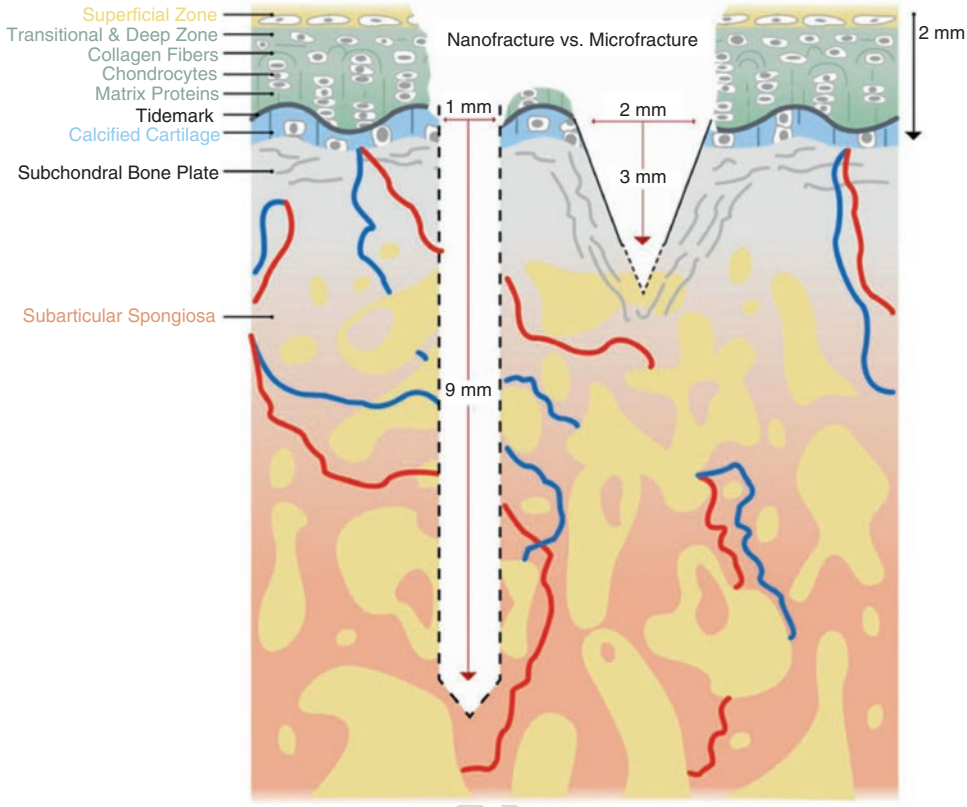


**Fig. 16.6** Demonstrating the difference between the deeper nanofracture© (left) which reaches the subchondral bone plate more regularly, in a consistent cylindrical shape and at a more defined depth than microfracture (right). (Reprinted from Benthien and Behrens [80]. With permission from Springer Berlin Heidelberg)

the addition of PRP (Fig. 16.7) [83]. The ECM is composed of type II collagen, proteoglycans, and growth factors, which are native components of articular cartilage [83]. Little peer-reviewed studies examining BioCartilage outcomes are available; however, a study by Fortier et al. [84] reported that BioCartilage-treated knee lesions had significantly higher ICRS repair scores when compared with microfracture alone at 2, 6, and 13 months postoperatively via repeat arthroscopy

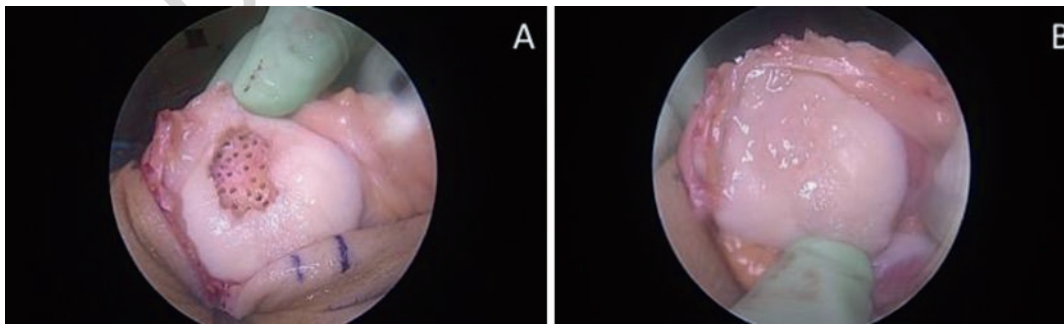


**Fig. 16.7** An intraoperative photograph of (**a**) a microfractured patellar cartilage defect and (**b**) the defect following repair with BioCartilage

in equine models. Additionally, histology revealed BioCartilage-repaired defects had significantly better deposition of hyaline-like type II collagen than the control defects, which is optimal for repair [84].

## Conclusion

Marrow stimulation remains a popular treatment for isolated cartilage lesions with positive short-term patient-reported outcomes. However, due to the paucity of prospective comparative trials, poor long-term outcomes, and the potential worsening of the underlying bone microarchitecture, the indications for marrow stimulation remain controversial. The addition of intra-articular PRP, BMAC, and ASCs as well as new technical advances may assist in overcoming marrow stimulation weaknesses. In summary, additional prospective comparative trials are required before marrow stimulation can be considered the treatment of choice for isolated cartilage lesions in large weight-bearing joints.

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