



# The triad of osteobiology – Rehydrating calcium phosphate with bone marrow aspirate concentrate for the treatment of bone marrow lesions

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The topic of bone marrow lesions has been more widely discussed in recent years, especially in the knee literature. One such procedure to treat this pathology is back-filling the lesion with bone substitute materials, calcium phosphate (CaP), as just one example. This technique uses the principle of osteoconduction, CaP mimicking the composition of subchondral bone and creating a scaffold for bone cells to integrate onto and promote internal cancellous bone healing. After performing this procedure on the foot and ankle as the protocol describes, a technique was developed to increase healing potential by adding concentrated bone marrow aspirate (BMA) to the CaP. This addition in theory gives us increased osteobiology properties – osteogenesis, osteoconduction, and possibly osteoinduction through growth factors like transforming growth factor beta (TGF- $\beta$ ) and vascular endothelial growth factor (VEGF). Based off of other reported literature, this technique has theoretical benefit in the operating room to increase patient results. Here the method is discussed with a case example.

**Key words:** BioCUE<sup>TM</sup>, bone marrow lesion, bone marrow aspirate (BMA), calcium phosphate (CaP), osteogenesis, osteoconductive, osteoinductive, Subchondroplasty<sup>®</sup>

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**B**one marrow lesions (BML) first correlation to knee pain and osteoarthritis was cited by Felson et al in 2001 [1,2]. In a magnetic resonance imaging (MRI) study, 77.5% of participants with knee pain had evidence of BMLs, represented as an increased area of T2-weighted image signal intensity adjacent and deep to the subchondral bone plate. This represents a pathologic event of blood or fluid accumulation (synovial, water), resulting in an intraosseous increase in pressure [1]. Repetitive loading to the joint prevents healing, with the creation of bone infarcts, cartilage breakdown, and chronic pain.

One way to assist in healing is through filling these lesions with biologic materials to promote bone growth in order to restore the damaged subchondral architecture. Sometimes considered insufficiency fractures or non-healing/non-union fractures, surgeons have been searching for a technique to internally support these BMLs and stop the pathological process. This is the principle behind the Subchondroplasty<sup>®</sup> (Zimmer Knee Creations, Exton, PA) (SCP<sup>®</sup>) technique, targeted filling of the BML with a nanocrystal calcium phosphate (CaP) bone substitute material.

Osteoconductive materials such as CaP, calcium sulfate (CaS), or hydroxyapatite (HA) have been used to treat various osseous pathology. Each synthetic bone filling substitute has its own indication whether pure bone void filling versus structural support to a load-bearing bone is required. Regardless, the goal is

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to restore the natural osseous biology as effectively and efficiently as possible. However, many of these commercial products only deliver one of the key components of osteo-restoration: osteoconduction through acting as a scaffold for innate cells to respond to and remodel. Here we demonstrate a technique of enhancing the biology through adding osteogenic and possibly osteoinductive growth factor potential through incorporating bone marrow aspirate (BMA) concentrate (BMAC) [3-6]. Based on previous work, we suspect this combination leads to greater biological healing and restoration of the cancellous bone in the BML [5-8].

### **Design Rationale (Use of BMAC/CaP Rationale)**

The SCP<sup>®</sup> technique for repair of BMLs consists of filling the lesions with AccuFill<sup>®</sup> Bone Substitute Material (Zimmer Knee Creations, Exton, PA), a proprietary formulation of a highly-porous nanocrystalline CaP structure. This product acts as a point of internal fixation, providing support for osteoblast in-growth. The protocol requires its' rehydrated with 0.9% normal saline solution (NSS). Based on previously published literature, it was proposed in order to increase host response and healing potential, an autogenous BMAC flowable tissue could replace the NSS for rehydrating the CaP [5,7,8].

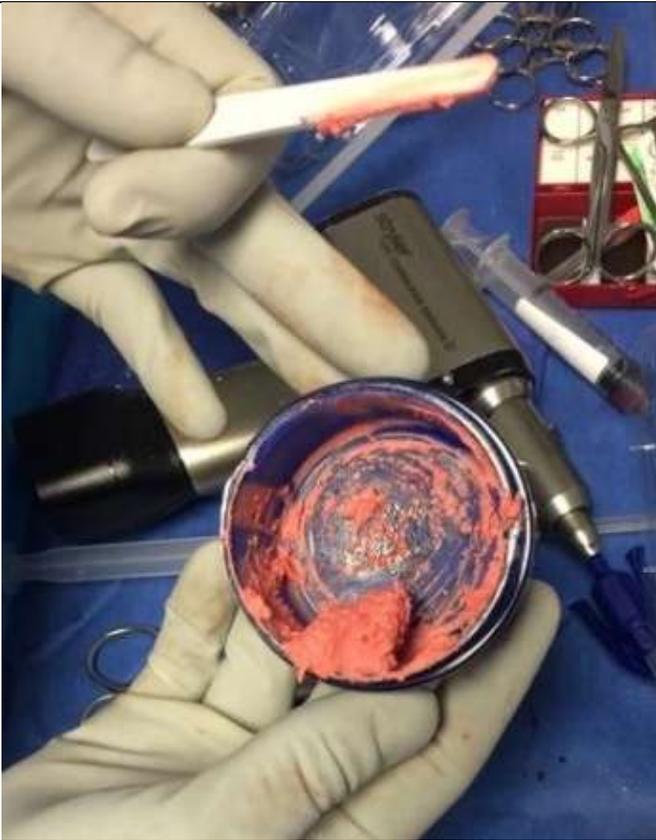
Bone marrow is aspirated to concentrate the cells contained within to augment and advance tissue repair, in this instance for bone regeneration. The target cells in bone marrow consist of the mesenchymal stem cells (MSCs), progenitor cells, hematopoietic stem cells (HSCs) and platelets [3,4,7]. The MSC differentiate into osteoprogenitor (OPG) cells, which further differentiate into osteoblasts (bone forming cells) with the assistant of growth factor signaling while the HSCs and platelets provide the important growth factors like transforming growth factor beta (TGF- $\beta$ ), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) [4]. The use of BMA with ceramic materials has been cited in the past as a marrow-impregnated graft. Reports have cited faster and more consistent defect healing compared to either product used individually [7]. Examples of use include bone defects (post-traumatic, iatrogenic), cysts, and tumors. It is felt a synergistic effect takes place, BMA

supplying the OPG cells which stay locally attached to the CaP scaffold for in-growth and mineralization to take place upon [9]. There have been reports of combining CaS and platelet-rich plasma (PRP) with good results [10]. It is this theory which we base our technique upon. By altering or "up-regulating" the healing/recovery potential, we feel this more biologically active local environment leads to quicker and more robust healing response. The introduction of BMAC adds osteogenic cells and growth factors to the osteoconductive CaP environment to create increased osteobiology for optimal healing potential. This composite provides an alternative to the 'gold standard' autograft with a much more minimalist approach in the harvesting technique [9].

### **Surgical Technique**

The basis of patient evaluation and work up for BML with SCP<sup>®</sup> intervention has been previously described by the senior author (J.R.M.) for foot and ankle pathology, although the indications are similar for other joints [2,11]. This should be reviewed to understand the theory behind and technique involved in performing SCP<sup>®</sup>. Once the decision has been made for treatment by SCP<sup>®</sup>, the described technique can be used to augment the normal process.

SCP<sup>®</sup> involves preparation of the injectable CaP bone substitute AccuFill<sup>®</sup> for placement into the BML through provided cannulated drills. These drills come in two sizes (11 and 15 gauge) and have two tip configurations in the larger size (end delivery and side-port delivery). The kit includes 5-cc of CaP powder that requires rehydration with 3.0-3.2-cc of the provided NSS. The two components are mixed until a putty that posses a toothpaste-like consistency is achieved. The mixing component of the procedure is surgeon preference, wanting to obtain the "right" consistency; too thin and it will not appropriately dry and harden while too thick and it will be difficult to inject and working time is decreased. It may take multiple procedures to get a feel for the product and therefore new surgeons to the procedure are cautioned to slowly rehydrate (drop-by-drop) the CaP once 3-cc of NSS is initially added. This material is then placed in syringes for injection, curing through an endothermic reaction in approximately ten minutes to form a nanocrystalline CaP structure [12].



**Figure 1** Intra-op demonstration of the AccuFill® and BMAC mixture. Normal color is white when hydrated with NSS.

This technique has been reported on by the senior author, with application to foot and ankle, as well as others for use in the knee [2,11,13].

With the new proposed technique, BMA is harvested from the surgeons' location of choice (distal tibia, proximal tibia, calcaneus, iliac crest). The senior author typically uses the distal tibia unless contraindicated. The BMA is harvested using the Biomet BioCUE™ (Zimmer-Biomet, Inc, Warsaw, IN) system. This process includes mixing 5-cc of the anticoagulant citrate dextrose solution formula-A (ACD-A) with each 25-cc of bone marrow aspirated. A trochar is tamped into the location of harvest, 30 cc syringe attached to the luer-lock, and constant back-pressure is applied to the syringe until 25-cc's of blood is aspirated (30-cc fluid total). The solution is spun down per protocol into a BMAC product.

When ready, the BMAC is placed back onto the surgical table. The AccuFill® kit is opened and slowly the BMAC is mixed with the CaP powder (Figure 1).



**Figure 2** Mixing AccuFill® with NSS and BMAC results in small crystal precipitate formation which can clog the injection syringe.

Reconstitution should be performed slowly with gradual addition of the liquid BMAC until the desired consistency is achieved. This amount will be more variable from case to case compared to the normal process which we typically find uses 3.2-cc of NSS. Differences in BMA/BMAC from patient to patient (cell concentration and density) as well as the overall increased viscosity of BMAC over NSS typically results in more marrow liquid volume than NSS to be utilized. We have seen ranges from 3.5 – 4.2cc of BMAC per standard 5cc of CaP. Once the desired consistency is achieved, the material is then prepared and injected into the BML per normal protocol. The surgeon should appreciate the blushing effect as the material interdigitates within the cancellous bone and BML. Side table analysis shows a similar 10 minute hardening time once components are combined to give an estimate of handling and waiting time before cannulated drills are removed from the bone.

Some keys to the technique include:

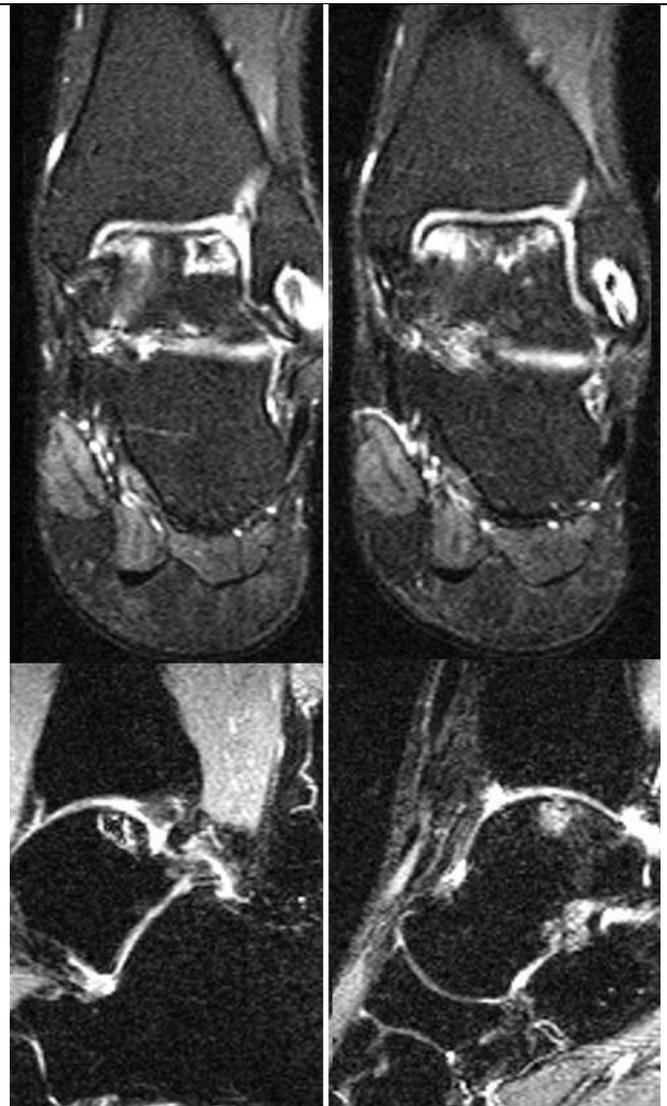
1. Do not mix NSS and BMAC into the CaP. This results in formation of small precipitated crystals that will clog the syringe during injection. (Figure 2) Use either NSS or BMAC.
2. One filled syringe of BMAC/CaP could be saved and placed in a basin of warm NSS to mimic the curing process (addition of heat) to approximate the time to hardening for determining cannulated drill removal.

### Case Report

A 26-year-old female presented to the office with complaint of left ankle pain. She had been in a motor vehicle accident two years prior but had not sustained any fractures on her emergency department evaluation despite pain and bruising to the ankle. She underwent a period of protected weight-bearing, physical therapy, and discharge to resume normal activities. Due to continued discomfort, she underwent left ankle arthroscopy and lateral ankle ligament stabilization one year later. Still with continued daily pain on weight-bearing to the ankle one year after the surgical intervention, she ended up seeing the senior author (J.R.M.) for evaluation.

Physical exam noted pain on palpation of the medial and lateral malleoli, the anterior joint line, and deep palpation to the ankle gutters. There was also a decrease in ankle dorsiflexion. Suspecting a possible osteochondral lesion or BML, an MRI was ordered. The results demonstrated a low T1 / high T2 signal in the lateral and central-medial aspect of the talar dome, indicative of a BML. (Figure 3) Conservative and surgical options were presented to the patient and she opted for SCP<sup>®</sup> of the talar dome with BMAC.

On the day of surgery, the above described surgical technique was implemented. In this instance, the 5-cc of CaP powder was rehydrated with 4-cc of BMAC to achieve the desired consistency. Using fluoroscopic C-arm guidance, the BML was targeted and 5-cc of BMAC/CaP was used. The cannulated drills were left in place for 10 minutes and then removed. The joint was evaluated arthroscopically and no extravasation of the material was seen intra-articularly.



**Figure 3** MRI T2 coronal (top) and sagittal (bottom) images of the left ankle. Images demonstrate the BML to the lateral (left, top-bottom) and central-medial (right, top-bottom) talus.

Arthroscopy portal incision were closed with nylon, a soft dressing was applied, and the patient was placed in a CAM boot to be weightbearing as tolerated in the boot until follow-up in two weeks.

At her first follow-up two weeks after the surgery, the patient stated only mild pain and the need to take opioid medication for relief over the first two days post-operation. Sutures were removed, and the patient was to continue CAM weight bearing with implementation of physical therapy in two weeks with transition into a sneaker as tolerated. At 6 weeks post-operation, the patient stated feeling greater than 90% improved from the pre-operative setting with VAS

scale decrease from a consistent 9/10 pain to occasional 1-2/10 discomfort to the surgical ankle. She has remained at this level through continued follow-up.

## Discussion

Osteoarthritis (OA) is a multifactorial disease process that involves all of the structures around the joint: articular cartilage, subchondral bone plate, bone marrow, synovial fluid, and the surrounding soft tissue structures [14]. Through the work of Radin the relationship between bone and cartilage was established, revealing damage to the subchondral bone correlated to knee OA pain and joint destruction [15]. BMLs are a result of subchondral damage, often a response to continued stress, similar to that of a stress fracture and are sometimes referred to as insufficiency fractures or non-healing (non-union) fractures [2]. The bone responds through stimulating a repair process, creating a focal area of sclerotic bone - the core of the developing BML. On MRI these areas are visualized as decreased signal uptake on T1 (sclerotic core of the BML) and increased signal uptake on T2 weighted-images (blood, synovial fluid, or water content) [2]. Continued weightbearing results in forces being transmitted through this core to the weaker cancellous periphery, resulting in further breakdown, creating the insufficiency fracture. This bone histologically shows decreased mineralization, increased vascularity, and a fibrotic quality [14]. Furthermore, this now soft focal area does not transmit joint loads normally, leading to a process that causes cartilage attrition [2]. This imbalance and cyclic process of damage over repair, lesion evolution, cartilage damage, and bone or joint pain has been well described by Sharkey et al (2012) [2]. Ultimately, the patient has to make a decision between palliative care or joint replacement. However, the SCP<sup>®</sup> technique has been introduced as a minimally invasive procedure that solves the problem of BML-related pain while preserving joint motion.

The SCP<sup>®</sup> procedure with our BMA addition offers another conservative surgical approach for the treatment of BML not just in the ankle as demonstrated here, but other appropriate joints or regions of BMLs seen on MRI. Historically the senior author diagnoses this condition based on patient

subjectively complaining of chronic deep ankle pain, specifically with pain they either wake up with or have whether weightbearing or not. The subsequent ordered MRI oft correlates with marrow edema to that area of discomfort. Often the lesions persist many months after the inciting event and represent an insufficiency fracture of the cancellous bone. This has been seen on serial MRI studies greater than three months apart at our practice. This procedure is often offered as a “no-bridges burned” solution to put off more complex surgery such as ligament reconstructions, ankle fusions, or ankle replacements. Knee results for treatment of advanced arthritis with concomitant BMLs have demonstrated that the SCP<sup>®</sup> procedure reduced VAS pain scores by greater than 4 points and delayed the need for total knee arthroplasty (TKA) in 70% of patients by at least two years [13]. We look to achieve the same results in the ankle when we offer the procedure to appropriate patients.

Synthetic forms of CaP have been created to repair and augment natural cancellous bone pathology. AccuFill<sup>®</sup>, the product used in this technique, is a proprietary nanocrystalline CaP powder formulation, when mixed with NSS creates a flowable putty that can be injected into lesions percutaneously. Once it hardens, activated endothermically by body temperature (37°C), it is slowly converted to and replaced by normal bone over subsequent months. Its structure has a 65% total porosity and 1-300µm pore size, allowing for a high surface area to facilitate bony in growth. The 10MPa of compressive strength, achieved after 10 minutes of set time, is comparable to normal cancellous bony and permits immediate weightbearing post-operation.

In trying to achieve greater osteo-biology, we postulated that the addition of BMAC to the AccuFill<sup>®</sup> would give us the properties of osteoconduction, osteogenesis, and inductive growth factors. With the scaffold present, BMACs ability to add MSCs, OPG cells, HSCs, and platelets (degranulating growth factors VEGF & TGF-β) to this technique would in theory make sense to achieve a more superior local environment to healing [3,4,6,7]. While osteoinductive qualities to BMAC may be debated due to the lack of bone morphogenic protein (BMP), the protein most closely and traditionally linked to osteoinduction, growth factors like VEGF

and TGF- $\beta$  have been linked to inductive activity and play a role in regulating osteogenesis [6,16,17]. Further, TGF- $\beta$  superfamily proteins have been shown to aid in articular (hyaline) cartilage restoration [17].

Similar theories have been attempted in combining osteobiologic materials. Mixing CaS with PRP has been shown to demonstrate bone regeneration qualities in humans [10]. Torres et al (2015) reported on a BMAC/HA combination in vitro [18]. Desired results of the combination versus controls included: (1) scanning electron microscopy visualization of marrow cells adherent to the scaffold surface in a scattered pattern days after preparation; (2) higher mean cell viability/proliferation as time increased; (4) greater cell growth rate; and (5) greater osteoblastic gene expression (ex. Col 1, ALP, BMP-2). Combinations of PRP, BMAC, and CaP granules (CPG) have also been studied in animal models [8]. Kadiyala et al (1997) demonstrated abundant bone tissue formation after 8 weeks in rats treated with a MSC/HA-triCaP graft [19]. To the authors' knowledge, no one has reported on the combination of BMA concentrate as the material to re-constitute a nanocrystalline CaP-type product.

The goal of our report is to describe an additional technique for an established procedure that may increase patient results and decreased surgical complications. As osteobiology is combined through synthetic (CaP, CaS, HA) and minimally invasive extraction technique (aspirating bone marrow, venipuncture for PRP harvest), the surgeon can create materials comparable to autogenous bone graft with a wide array of clinical applications [8,9]. The procedure can be adapted for the management of delayed or non-unions, tumors, cysts, or revision joint replacement. It can be implemented when greater osteobiology is demanded (autogenous bone graft, iliac crest source) but donor site morbidity is not desired. It can also be utilized in instance where the host is impaired (diabetes, chronic steroid, osteoporosis, post-menopausal, elderly, tobacco use) and the necessity of bone growth supplementing techniques is desired. We have used this technique in treating BMLs to the tibial (malleoli), talus, calcaneus, cuboid, cuneiforms, 2<sup>nd</sup> metatarsal base, and 1<sup>st</sup> metatarsal head with good results.

We realize this technique is not an exact science and poses many questions. Differences in BMA cell concentration and density from patient to patient would lead to differences in product characteristics. This consists of varied preparation and handling standards (time to cure; volume of BMAC to hydrate), altered curing rates in-vivo, and potentially change either scaffold structure or physiological properties (pore size, compressibility) of the AccuFill<sup>®</sup>. Another point of debate is the osteoinductivity and addition of growth factors by BMA/BMAC [3-5,16,17,19]. It has been studied with limited publications or clarity. This process requires much more testing for it to become an exact science and understand what is exactly happening biochemically. However, the mixing of biologics is common in medical use and this technique should not be avoided because of the unknown. We ultimately feel that this technique provides an alternate or adjuvant to the currently available treatments for bone regeneration therapy.

#### Authors Note

The senior author has been performing foot and ankle SCP<sup>®</sup> for the past 2 years. In the time from the first time this technique was attempted until publication, he has seen no difference in patient complications or adverse events. Additionally, as stated above, where as the normal technique used 3.2cc of NSS to create a reproducible consistency in the putty, he has used variable amounts of BMAC to achieve the same consistency.

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