
Bone Marrow: A Validated Biological Driver for Bone Regeneration

Introduction

Cancellous iliac crest autograft has remained the gold standard for bone grafting due to its being osteoconductive, osteogenic and osteoinductive. There are numerous approaches being applied to reduce or eliminate the dependence on autologous bone graft in orthopedic procedures due to morbidity, time and cost concerns. The fundamental requirements are biological effectiveness (replicating autograft), ease of application and economic viability. For a replacement technology to meet the primary requirements, it should provide these three functions.

Autologous bone marrow aspirate (BMA) is a validated biological driver that can be used to provide osteogenic and osteoinductive properties to osteoconductive bone substitute materials. It is the purpose of this paper to review a portion of the extensive clinical and preclinical evidence of the biological and clinical value of bone marrow in bone grafting.

Over 175 references to bone marrow-based technologies have been produced/reported since Goujon in 1869. A significant number of these references involve the study of BMA with bone graft substitutes such as osteoconductive scaffold materials.

“Osteogenesis is a property found only in fresh autogenous bone and in bone marrow cells.”¹⁰¹

“Bone marrow contains BMP and is very osteoinductive.”¹⁰⁵

“Bone marrow is a source of osteogenic cells that may be under used in contemporary orthopaedic surgery.”¹⁰⁶

“Bone marrow alone may be used as an effective osteogenic graft and is probably under used in current clinical practice.”¹⁰⁸

“However, tissue engineering never can stray far from fundamental biologic principles, and one of these is that cells do all the work. No bone graft, no matrix, no growth factor, no cytokine can contribute to the generation of integration of new tissue, except through the influence it has on the behavior of cells.”¹¹⁰

“The use of cells for the purpose of orthopedic tissue engineering started more than 300 years ago. The osteogenic potential of transplanted bone marrow was later documented by Goujon in 1869, then by Macewen in 1881. Efforts of Albee and Phemister highlighted further the utility of bone transplantation for the healing of fractures and bone defects.”¹⁰⁸

“Osteogenic cells are cells capable of producing bone or differentiating into a bone-forming cell. These cells are found in periosteum, peritrabecular soft tissues, and bone marrow harvested by aspiration.”¹⁰⁸

“Fresh autologous bone marrow has the potential to induce osteogenesis in a variety of locations, including the spine. The principal advantages of using this approach

are that the harvesting/implantation of autologous bone marrow cells from the iliac crest is relatively inexpensive, simple and not subject to regulation by the Food and Drug Administration.”⁹⁶

“Bone marrow by itself has bone progenitor potential.”¹¹¹
“The use of bone marrow aspirates to augment osteoconductive scaffolds or in combination with osteoinductive growth factors may be an attractive treatment option.”⁹⁶

“Future biosynthetic bone implants may obviate the need for autologous bone grafts. The optimal graft will most likely be composed of osteoprogenitor cells and osteoinductive growth factors that are delivered on an osteoconductive, resorbable scaffold.”⁹⁶

Preclinical Validation

Early evidence of ectopic bone formation with the application of bone marrow was reported by Goujon in the nineteenth century.³⁸ A number of investigators over the past 130 years have demonstrated the osteogenic abilities of bone marrow. Researchers have observed that cell suspensions isolated from bone marrow can form bone and cartilage in diffusion chambers.^{39,40} Investigators have demonstrated that when access to local bone marrow is prevented, bone healing is minimal leading them to hypothesize that bone marrow precursor osteoblasts and mesenchymal stem cells (MSCs) synergistically act with BMPs to produce an effective healing response.²⁸ BMA injected into bone defects resulted in bone regeneration and fusion.^{25,41} Studies in animals have also demonstrated bone formation in posterolateral lumbar fusion (PLF) when appropriate volume and cellularity of BMA is used with a composite matrix (CM) of calcium phosphate plus collagen.^{80,81,82}

This bone marrow aspirate may contain all of the necessary components to stimulate bone regeneration.

2,5-9,13-15,19,25,29,33,41,42

“Autogenous marrow has the osteogenic capability to heal a bone defect. When live marrow was implanted, woven bone formed at 3 weeks, progressing to early lamellar bone at 6 weeks, with subsequent remodeling for as long as 12 weeks. Bone marrow, when placed in a fresh femoral defect and given in sufficient amounts, produced a rate of union comparable with that of autologous bone grafts. Mature lamellar bone formed by marrow was evaluated biomechanically; the results were statistically comparable with those of cancellous bone grafts at 12 weeks. Significant bone formation occurred when marrow was percutaneously injected in femoral non-unions. These results indicate that bone marrow can lead to structurally functional bone regeneration in an orthotopic location.”⁶⁴

“Bone formation in the marrow-injected non-unions was excellent. This demonstrated that it is not necessary to have freshly traumatized bone with release of inductive substances, since marrow itself contains sufficient osteogenesis for bone formation.”⁶⁴

Isolation and cultivation of MSCs to produce colony-forming units (CFUs) confirms their osteogenic potential in vitro and in vivo.^{36,37} Woven bone formation has been demonstrated at three weeks, and lamellar bone at six weeks and beyond.⁶⁴ Studies demonstrate that approximately one stem cell per 100,000 nucleated cells is present in BMA.^{58,59,7,57}

Clinical Validation

“Several clinical reports support the potential value of bone marrow as a graft material.”⁸⁶

Many investigators have demonstrated that bone marrow contains connective tissue progenitors, which can differentiate into osteoblast-like cells in vitro. Bone marrow delivered by injection or combined with other matrix materials significantly improves the bone healing response.

“Autologous bone marrow delivered by percutaneous injection or by direct transplant as a composite graft also has proven effective for osteogenic stimulation in a series of 100 skeletal healing problems, including delayed unions and non-unions of fractures, arthrodeses, and bone defects.”⁵⁸

“Marrow stimulated a callus formation sufficient to unite eight of the ten non-unions immobilized with casts and all ten of the fractures immobilized by intramedullary nails. Bone marrow injection was as effective as past open autologous grafting but with considerably fewer disadvantages. The technique provides a promptly renewable and reliable source of osteogenic stem cells with numerous advantages compared with standard open-grafting techniques.”⁶⁰

The effectiveness of BMA alone to generate bone fusion in non-unions has been observed clinically.^{65,59,66} A series of 20 tibial non-unions treated with autologous BMA as a substitute for operative grafting all healed following 150mL BMA injections.⁶⁰

A study of seven femoral non-unions treated with 150mL of BMA reported healing in five of the patients.⁶⁷ Two patients had infections of the non-unions and failed to fuse. Eight cancer patients with delayed and non-unions were studied.¹⁵ Five of the patients were receiving a course of chemotherapy, and one was undergoing radiation therapy. New bone formation was reported in seven patients and five fractures healed. Good results were reported on the use of BMA as a graft extender in experimental posterolateral spine fusion.⁶⁸ Insufficient autograft was augmented with BMA.

A study of four children with aneurysmal bone cysts treated with BMA injection reported healing in all of

the patients.⁶⁹ Ten children with simple bone cysts were treated with BMA injection.^{70,71} At two-year follow-up, all patients were healed. In a 31-month follow-up of eight children with simple bone cysts, seven showed gradual regression with a single injection of BMA.⁷² Investigators reported 10 of 12 children with simple bone cysts healed with a single injection of BMA.⁷³ The authors concluded that BMA injection contributed to a higher success rate and faster healing than conventional therapies. The use of BMA with sterilized, treated bovine bone (Kielbone) in 23 pediatric patients for non-unions, tumors and osteotomies produced good results.⁷⁵ Prophylactic injection of BMA into fresh fractures in 20 patients produced union in an average time of 2.5 months.^{76,77} The author concluded that the use of BMA contributed to acceleration of long bone healing. Additional investigators have recommended this technique for the use of BMA with the Ilizarov fixator when faced with delayed bone regeneration or delayed unions.^{59,78} Connolly, et al. reported successful treatment of 18 of 20 tibial non-unions treated closed or with intramedullary nails plus percutaneous injection of non heparinized bone marrow.¹⁰⁸

A study involving 11 patients with delayed union and non-union of fractures reports that after treatment with BMA injection, nine fractures united within an average of 10 weeks clinically and 17 weeks radiographically.⁷⁹ Clinicians reported 20 cases of non-union fractures treated with 15-20mL of BMA.⁶³ Seventeen of the fractures united in an average of 5 (3-7) months.

Key Issues – Source, Technique, Delivery, etc.

Harvest Locations

While the iliac crest has traditionally been considered the best source for BMA, investigators have demonstrated the equivalence of cellularity of BMA harvested from the vertebral bodies.^{65,66} Recent tests and studies demonstrate that the cellularity of vertebral body marrow is at least as high as crest marrow and as osteoinductive.^{84,85} Bone marrow can be aspirated fairly easily from the iliac crest or vertebral body with minimal morbidity.^{5-10,13,15,17,27,33,35,41,42} “Of all of the available sources of osteoblastic cells for bone grafting, however, the only one that does not require an open surgical procedure or the added time, cost and risk of growing cells in vitro is bone marrow harvested by aspiration. Bone marrow aspiration from the pelvis has little risk or morbidity.”¹⁰⁸ “The posterior iliac crest has been shown to be an area extremely rich in red active marrow and it therefore provides a source of cells with osteoinductive and osteogenic potential.⁴¹ The advantage of red active marrow aspirate is that it can be quickly and easily obtained from the posterior iliac crest with negligible morbidity. It may contain all of the components necessary to promote bone growth without the need for

processing of the material.”⁴¹ “The posterior iliac crest is rich in RAM (Red Active Marrow) and provides a readily available source of osteoinductive and osteogenic cells. The concentration of these cells in the presence of routine cancellous bone grafting appears to enhance osseous consolidations in conditions where bone healing is known to be delayed or difficult. Aspiration of RAM and its use with cancellous bone graft is a safe, simple and effective way to improve the rate of bone union.”⁴¹

Technique - Pelvis

“Aspiration of bone marrow is a simple, safe and effective means of harvesting connective tissue progenitors in significant numbers. Bone marrow-derived cells also provide a rich growth factor environment that may enhance the bone healing response. Methods for harvesting bone marrow by aspiration have been defined and can be taught and learned. If done properly, bone marrow aspiration presents little risk of injury or morbidity at the site of aspiration. One author has performed more than 600 bone marrow aspiration procedures in the past ten years. No significant complications have been reported.”¹⁰⁷

Ideally, the first 2-3mL of marrow aspirated from any needle site provides the best opportunity to capture the osteoprogenitor cells in their highest concentration. The dilution of marrow-derived cells by peripheral (venous) blood occurs with higher aspiration volumes at the needle site. Therefore, after the first 2-3mL aspiration, the needle is moved deeper for additional aspiration of 2-3mL of marrow and then redirected to another site. This is repeated in a “fanning” method to provide for the most concentrated cells.⁵⁷

Early studies were undertaken to determine the most appropriate volume of BMA to be injected into defects to produce bone regeneration. Volumes of up to 150mL injected into tibial non-unions have been reported^{59,5,60,61} as well as volumes of 50mL¹⁵, 30mL⁶² and 15-20mL injections of BMA.⁶³ Muschler, et al. determined in an adult human study that 4mL aspirated at each site before progressing to additional sites is the largest volume for BMA injections.⁵⁷ Their presumption is that they would capture 85% of aspiratable stem cells from the site with this volume of BMA containing approximately 70 million marrow cells, of which 700 would be undifferentiated stem cells. Volume increases beyond this 4mL would effectively dilute the osteoprogenitor cell population with peripheral (venous) blood. The study demonstrated that four one-mL aspirates would yield approximately two times the number of stem cells as one 4mL aspiration.^{58,59}

Technique - Vertebral Bodies via Pedicles

“A viable population of autograft progenitors resides within that portion of the vertebral body routinely entered for pedicle screw placement. This alternative procurement source will further reduce the time and morbidity associated with iliac crest harvest.”⁸⁴

Optimization of Delivery

While the value of bone marrow as a biological driver is clearly established, its clinical efficacy is optimized when used with an ideal osteoconductive scaffold. It is critical that the scaffold used in conjunction with bone marrow have a high degree of porosity to carry a large volume of marrow, thus maximizing the number of stem cells delivered as well as maximizing the quantity of signaling molecules.

“Scaffolds must possess an open-pore, fully-interconnected geometry in a highly porous structure with large surface-to-area volume ratios that will allow cell in-growth and an accurate cell distribution throughout the porous structure, and will facilitate the neovascularization of the construct from the surrounding tissue. Furthermore, the scaffolds should also exhibit adequate microporosity in order to allow capillary in-growth. Porosity and interconnectivity are also important for an accurate diffusion of nutrients and gases and for the removal of metabolic waste resulting from the activity of the cells that had meanwhile grown into the scaffold.”¹⁰³ It has been shown that scaffolds without such properties only facilitate cell growth on the exterior of the scaffold, resulting in a cellular shell only protruding into the first 250 microns.¹²⁰

The ceramics HA and β -tricalcium phosphate have been studied in bone healing and found to possess a substantial capacity for delivering osteoinductive factors and osteogenic cells.^{24, 100}

“The newer ultraporous version of β -TCP, Vitoss® Bone-Graft Substitute, may be well-suited for use with BMA in a composite graft.”¹⁰⁴

Conclusions

Bone marrow is a validated biological driver for bone formation. Decades of research and clinical application have confirmed the safety, efficacy, performance and economy of this natural, potent biological driver described in over 175 publications. Clinical application of bone marrow is safe and effective. Significant preclinical and clinical data exists demonstrating the efficacy of BMA. BMA mixed with the optimum osteoconductive scaffold performs equivalent to autograft in controlled studies.

Common Criticisms or Objections: Bone marrow as a biological driver for orthopedic applications.

Lack of studies and evidence of efficacy.

As briefly introduced in this paper, there is a preponderance of published evidence on the efficacy of bone marrow as a biological driver for bone formation. Iliac crest autogenous bone graft is the gold standard due to the osteogenic cells and osteoinductive signals contained in its bone marrow component.

There may not be enough stem cells to be useful. Variability of number and concentration of stem cells (patient age, gender, location, technique).

The optimal number of cells or progenitors required in a graft site is not known.¹⁰⁷ However, autologous bone marrow has been proven effective in a range of ages and indications (see Clinical Validation).

Bone marrow is not as powerful as BMP.

Bone marrow has been shown to be equivalent to autograft when collected appropriately and combined with an ideal matrix. To date, the commercially available BMP formulations have been shown to be equivalent to, and not better than, autograft. In addition to having the potential of similar efficacy, bone marrow has a much more attractive safety profile and economics as compared to BMP. Remember, “However, tissue engineering never can stray far from fundamental biologic principles, and one of these is that cells do all the work. No bone graft, no matrix, no growth factor, no cytokine can contribute to the generation of integration of new tissue, except through the influence it has on the behavior of cells.”¹¹⁰

Aspiration is too cumbersome.

Methods for harvesting bone marrow by aspiration have been defined and can be taught and learned.^{24,107} On the average hemi-pelvis, there are at least 75 sites where you could aspirate. The technique for aspiration is simple. Advance the needle through the cortex, remove the obturator, attach a syringe and aspirate.¹¹⁴

Morbidity of aspiration.

If done properly, bone marrow aspiration presents little risk of injury or morbidity at the site of aspiration.¹⁰⁷ “We have done over 600 donors so far with this kind of technique in either anterior or posterior crests. I have had two women tell me they had a bruise where aspirated ... but the bruises were gone by the time I saw them at two weeks.”¹¹⁴ The advantage of red active marrow aspirate is that it can be quickly and easily obtained from the posterior iliac crest with negligible morbidity.^{5-10,13-15-,17,27,33,35,41}

Delivery of marrow has not been optimized.

An optimized osteoconductive scaffold must possess a fully interconnected geometry in a highly-porous structure with large surface-to-area volume ratios that allow cell in-growth and efficient cell distribution throughout the porous structure. Scaffolds should also exhibit adequate microporosity to promote capillary in-growth. Porosity and interconnectivity are critically important for efficient diffusion of nutrients and gases, and for the removal of metabolic waste resulting from the activity of bone regeneration throughout the scaffold.¹⁰³ BMA delivery can be further optimized if the scaffold chemistry, structure and porosity combine to provide a superior “soak and hold” environment within which BMA-driven bone regeneration opportunities are maximized.

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