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Concentrated autologous bone marrow aspirate is not "stem cell" therapy in the repair of nonunions and bone defects

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A B S T R A C T

Autogenous bone grafting is the gold standard for replacing large bone defects. Due to limitations in the quantity and quality of harvested bone from the iliac crest, and the potential associated morbidity, the technique of cell grafting has been developed. Autogenous bone marrow aspirate is concentrated (so called BMAC) and delivered locally to the intended site with minimally invasive techniques. However, there are only about 1 in 30,000 Colony Forming Unit-Fibroblast (CFU-F) progenitor cells in unconcentrated iliac crest aspirate. Current techniques for cell concentration only increase these numbers by about 5-fold. Thus, BMAC is not equivalent to "stem cell therapy".

Introduction

There is an unmet clinical need for efficacious therapies for the treatment of fracture nonunions, residual bone defects due to trauma, infection, tumor, periprosthetic osteolysis, as well as spinal fusion and other conditions. Traditionally, the above clinical scenarios have been addressed by the use of autologous bone grafts, usually harvested from the iliac crest or another nearby source of cancellous bone, in metaphyseal bone or the spine. These grafts provide all the necessary ingredients for osteogenesis: a scaffold or framework on which to form new bone, growth factors and other substances for promoting bone formation, and cells that provide the machinery for production of bone. Autologous bone graft is still the gold standard for the repair of bone defects however, bone graft can be limited in quantity and quality, especially for larger "critical size" defects that have little chance of healing spontaneously. Furthermore, chronic diseases such as diabetes, chronic kidney or liver disease and many others, advanced age, medications such as non-steroidal anti-inflammatory drugs, corticosteroids and others can compromise the quality of bone graft and interfere with the processes of bone formation. In cases in which a large amount of autologous bone is needed, the incision and dissection for obtaining the bone graft may be associated with local pain, and potential complications including infection. Consequently, other methods to facilitate bone healing have been explored. Some of these strategies have included the use of allograft bone, different naturally occurring and artificial scaffolds, growth factors, other molecules etc. However, the above modalities are deemed osteoconductive (e.g., scaffold) and/or osteoinductive (e.g. growth factors) at best, and do not provide the final and most important element for osteogenesis, namely the cellular component. To this end, novel approaches have been designed to harvest cells, usually from the iliac crest, and add this key component to a scaffold to enhance bone formation [1].

The concept of bone marrow cell aspiration from the pelvis and percutaneous marrow injection to enhance bone healing in cases of nonunion of fractures has been attributed to Dr. John Connolly and colleagues in their preliminary series reported in 1989 [2]. However, prior to this report, Friedenstein et al. in Russia, as early as 1963, described the osteogenic capacity of transplanted cells from the bone marrow to form bone [3]. In fact, Friedenstein noted that observations of heterotopic bone formation by subcutaneous transplantation of bone marrow had been described even earlier, by Denis in 1958 [3]. The above observations spawned the concept of cell grafting, as opposed to bulk bone tissue grafting to heal nonunions and bone defects. Friedenstein cogently stated that the cells within the bone marrow aspirate were a heterogeneous mixture of "haemopoietic cells, reticular cells and endosteum elements". His work, together with that of Connolly and others foreshadowed new efforts to concentrate the marrow aspirate to provide a more manageable volume and efficacious product for subsequent local delivery (Fig. 1).

What constitutes the bone marrow?

The bone marrow contains cells of the hematopoietic and mesenchymal cell lineages at many different stages of proliferation, differentiation and maturation. This includes red and white blood cells, megakaryocytes and platelets, and cells in the stroma comprised of endothelial cells, adipocytes, fibroblasts, osteoblasts and osteoclasts. Of the non-stromal cells, 50% are white blood cells (WBC) i.e., monocyte/macrophages, polymorphonuclear leukocytes, mast cells etc. and

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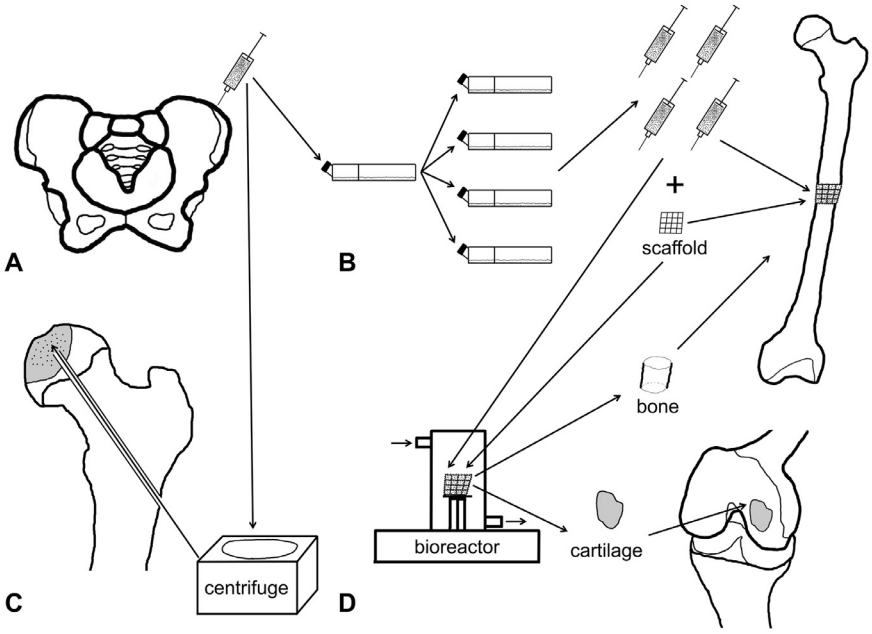

Figure 1. Options to process bone marrow aspirate in orthopaedic surgery. Bone marrow aspirate is usually harvested from the iliac crest (A). It contains an extremely low number of precursors of the bone forming cells, the Colony Forming Unit-Fibroblast (CFU-F). By cell culturing techniques, CFU-F cells can be isolated and expanded by 1×10^5 or more (B). The most common method of concentrating nucleated cell portion in the bone marrow aspirate is centrifugation (C). The number of CFU-F cells stays extremely low and is therefore not "stem cell" therapy. Concentrated bone marrow aspirate is clinically used to support the treatment of osteonecrosis of the hip. Applying further in vitro steps, like a bioreactor, isolated and expanded CFU-F cells can be differentiated towards the target tissue such as bone or cartilage (D).

their precursors, 25% are in the erythropoietic lineage, and the balance, about 25%, are in the lymphocyte lineage. The precursors of the bone forming cells, the Colony Forming Unit-Fibroblast (CFU-F) cells are extremely rare. Hernigou et al., one of the strongest advocates of cell therapy for the treatment of nonunions, osteonecrosis and other bone deficiencies, found an average of 612 ± 134 CFU-Fs /ml (range 60-6120 cell/ml) during careful aspiration of the anterior iliac crest in 60 patients 18-80 (mean of 40) years of age [4]. Only 1 in 30,000 nucleated cells harvested from the anterior iliac crest proved to be a CFU-F. This number would amount to approximately 600 CFU-F cells in 1 cc of bone marrow aspirate. Muschler aspirates only 2cc at one time per location when harvesting bone marrow from the iliac crest, so as not to dilute the aspirate with red blood cells. Using this technique, their group found that 2 cc contained approximately 2400 alkaline phosphatase positive CFUs, in 32 patients 14-77 (mean of 41) years of age [5]. Similar to the findings of Hernigou et al, Muschler et al. reported that 1 in 35,000 nucleated cells was an alkaline phosphatase positive CFU [5]. Furthermore, Muschler et al. observed that the numbers of nucleated cells decreased with increasing age; however, whereas the number of CFU-F cells decreased with age in women, this phenomenon was not found with aging in men [6].

If cell grafting is to be efficacious, the number of CFU-F cells must be increased. Although this can be accomplished with cell culturing techniques, including isolating, expanding and harvesting of selected cell types, this strategy may not be desired for many reasons. First, the aspiration and delivery portions would entail 2 separate interventions which would be unacceptable to most patients who are looking for "one-stop shopping" i.e., harvesting and placement of the cell graft at a single point of care. Second, two interventions are generally more costly than one single procedure. Third, the cell culturing procedure, storage and delivery would need to be accomplished in a strict environment with good laboratory practices, ensuring confidentiality and logistical precision at every step. Fourth, in the USA, the FDA does not permit more than minimal manipulation of cells and tissues; cell culture and isolation of specific subtypes of cells is contrary to this doctrine. Thus, novel strategies for point-of-care isolation and delivery of cells needed to be developed.

Autologous Bone Marrow Aspirate Concentrate (BMAC)

The most common method of concentrating the nucleated cell portion in the bone marrow aspirate is centrifugation with/without differential layering using Ficoll-Paque. These methods have been reported to concentrate nucleated cells up to 5-fold or more in some cases [7-9]. However, different patient characteristics, devices, methodologies and anticoagulants may affect the final end product [9]. Other methods of cell concentration are available such as Fluorescence-Activated Cell Sorting (FACS), selective retention, magnetic separation and others, however these techniques are generally not used to any major degree in the clinical setting at this time.

The composition of bone marrow aspirate that has been concentrated (BMAC) by different devices has been examined in several studies. In one study, the concentration of different cells increased by 4.30-4.91 for CFU-F, 4.71-6.95 for CD34+ cells (precursors for hematopoietic and endothelial cells), and 4.49-5.99 for WBC [9] However in the same study, the percentage yield for 3 different devices varied from 25.8% to 82.4% for CFU-F, 36.6%-81.1% for CD34+ cells and 29.7% -77.3% for WBC. The 10 donors in this study were young, ranging in age from 18-35. In another study examining the composition of BMAC via flow cytometry in patients aged 28-59, CD90+105+31−45− cells accounted for 0.03% and CD34+ cells accounted for approximately 1.9% of the total cells in BMAC [10].

What cells are actually found in BMAC? At the Orthopaedic Research Society in 2020, Professor Quanjun Cui reported on bone marrow aspirate from 3 male and 1 female donors aged 25-45 years old. His group centrifuged and concentrated the nucleated cells, and assessed the concentrate using FACS analysis and mass cytometry. Interestingly, the BMAC was composed of CD11b+ macrophages (approximately 70% of cells) and T cells (approximately 15% of cells). The BMAC contained only 2-5 CFUs/10^6 cells.

Discussion

The injection of BMAC for the treatment of bone defects, delayed or nonunion of fractures is often marketed as "stem cell therapy".

Indeed, the use of BMAC as “stem cell therapy” has been offered for the treatment of other diseases in the musculoskeletal system and in other organ systems. However, it should be emphasized that BMAC is not “stem cell therapy”, but is a heterogenous agglomeration of numerous cell types, most of which are in the hematopoietic lineage and not the mesenchymal cell lineage. Indeed, mesenchymal progenitors comprise a very small percentage of the cells in BMAC. These facts should help clarify the potential or proposed indications for the use of BMAC in the treatment of different clinical conditions. Furthermore, this information emphasizes that proper use of specific biological terms is necessary for full transparency in scientific reports, to government agencies and the public.

Several other points merit mentioning. First, as commonly used, the term “stem cell therapy” is imprecise and should be differentiated from the term “expanded stem cell therapy” in which a single type of stem cell is obtained and expanded. Second, even when considering expanded mesenchymal stem cell therapy to treat bone defects, the required number of cells to obtain bone healing is unknown. Finally, in BMAC and expanded stem cell therapy, autologous cells provide both cells for potential engraftment and paracrine effects. Although both mechanisms are feasible, especially in the use of BMAC, the paracrine effects of these cells seem to be the most favored explanation.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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