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MESENCHYMAL STEM CELLS IN LONG BONE NON UNIONS

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The recent advances in fracture treatment and better surgical techniques led to better outcomes even in complex fractures. However, approximately 5%-10% of operated long bone fractures, are complicated by atrophic nonunion, in cases related to impaired osteogenic capacity (malnutrition, alcoholism, chronic tobacco use). Therefore, non union is the result of a cellular impairment of bone healing, so it is a good indication for cell-based therapies. The recent development of regenerative therapy and stem cell research, has led us to believe these therapies have great potential. In this review, we focus on current novel techniques of augmenting fracture healing based on cell therapy and tissue engineering and we will also discuss the current problems and future challenges.

Keywords: non union, stem cells, regenerative therapy

INTRODUCTION

Approximately 10% of long bone fractures result in impaired healing or nonunion. Non-union fractures form as the result of trauma or a metabolic disease in which injury to the bone becomes atrophic. This is a complication associated with reduced bone marrow (BM) progenitor cell numbers compounded by suppressed progenitor cell proliferation as a result of the disease itself[1]. Malunions result in severe impairment, and disability. The classical clinical methods for stimulating bone repair include autologous bone grafting, allograft demineralized bone matrix (DBM), and bone graft substitutes. However, these interventions are invasive and have high donor site morbidity. BM-derived mesenchymal stem cells (MSCs) represent an efficient therapeutic way to stimulate the repair of a long bone fracture with their osteogenic capability and potential to home to a site of injury2. When delivered in the presence of a long bone fracture, the circulating MSCs home to the fracture site[3] and integrate into the host marrow, bone, and cartilage[4]. Bruder and colleagues[5] first demonstrated that the implantation of a scaffold supplemented with BM MSCs supported osteogenesis over an empty scaffold, including the formation of a reparative callus that was absent in defects treated with scaffold alone. Perhaps more importantly, the administration of MSCs with DBM into a clinically relevant model of diabetes resulted in augmentation of fracture healing over those that did not receive MSCs[6,7].

Why should we use autologous bone marrow grafting in non-unions? Marrow samples, from non-union sites of the tibia showed that the number of progenitors after in vitro cloning of the marrow was very low as compared to the tibia of normal patients[8] This is often found in infections, previous trauma, tissue defects and scars, as well as a compromised vascularity frequently associated with non-unions[9.] Therefore, normal tissue repair may be limited by the decreased number of ofosteogenic cells. This suggests that normal tissue repair may be limited by the decreased population of progenitors in local tissues. The relationship between consolidation and activation of the bone marrow has been apparently first observed by Ilizarov[10] who demonstrated that a 1% loss of blood induced an accelerated consolidation of osteotomy in rabbits. In the same study he showed that, following the loss of blood, a hyper-active hematopoiesis in the iliac crest bone marrow was observed.

Treatment techniques

Bone marrow aspiration and cell harvesting

Bone marrow can be grafted from the anterior or posterior iliac crest. For normal weight patients a direct puncture of the needle is appropriate, but if the patient is obese a 2 mm incision should be made at the site of collection. Aspiration of bone marrow from the iliac crest is done by the use of an aspirating needle. A standard 10-cm^3 syringe should be used to obtain the bone marrow aspirate (BMA). The needle and syringe shoud undergo rinsing with heparin solution. The needle is turned 45° during successive aspirations to reorient the bevel, aspirating from the largest possible space. After one full turn, the needle is moved towards the surface and successive aspirations are begun, turning the needle 45° after each aspiration. The aspirated marrow is richer in stem cells when it is aspirated in small fractions, which reduces the degree of dilution by peripheral blood. All aspirates should be are filtered to separate cellular aggregates and fat.

Non union site injection of mesenchymal stem cells

The same trocar as that used to aspirate the marrow is placed in the non-union gap and around the bone ends under biplane fluoscopy. The trocar site should not be adjacent to tendons or major neurovascular structures. The marrow is injected slowly at a rate of about 20 mL/min with a 10 ml syringe. The volume of the bone marrow graft injected in the non-union should be 20 mL. In some cases, leakage of marrow may occur through the trephine site: in such a circumstance it is necessary to

change the position of the tip of the trocar. The perforation skin is closed with a circumferential suture to avoid leakage of marrow.

Post-procedural period

Weight bearing is not allowed during the first month after the bone marrow transplantation. This period should be respected to avoid the progression of tissue regeneration and tissue healing being stopped by mechanical failure. After one month, and only when callus is observed on X-rays, partial weight bearing is allowed.

The mechanism of healing in non-unions with MSCs

In the past decade, several clinical studies[11, 12-16] have demonstrated that transplantation of mesenchymal progenitors in aspirated bone marrow is able to provide bone healing in non-unions. Percutaneous autologous bone marrow cell grafting is an efficient and safe method of treatment of non-infected atrophic non-unions. It cannot be used when there are pre-existing angular deformities or prior shortening. Also the gap and the displacement of the fragments should be also limited.

At the present time, it is difficult to know the exact mechanism that allows the transformation of fibrous tissue into callus. Finally, bone-marrow derived mononuclear cells are also able to stimulate formation of new blood vessels[17.] Besides the generation of new capillaries, the growing endothelia enhance mobilization and growth of mesenchymal progenitors through angiopoietin1–Tie2 pathway, which generate pericytes and vascular mural cells required for new vessel growth and stabilization18. A broad capacity of differentiation of pervascular mesenchymal cells has been shown[19, 20].

Designing composites of cells and matrices

All successful bone healing requires the presence of a sufficient number of osteoblastic progenitor cells. This is important because the number of local osteoblastic progenitors is limited. Thus, implanting an osteo-conductive material alone or even an osteoconductive material with some osteoinductive growth factors may not be adequate to accomplish a reliable and optimal bone-healing response. Instances in osteoblastic cells may be defficient include sites of large bone defects, sites containing extensive scar tissue from previous surgery or trauma, sites of previous infection or radiation, sites in which local bone may be diseased, or sites with compromised vascularity. Other systemic conditions, such as diabetes or metabolic bone disease, systemic glucocorticoids, or chemotherapy, may also limit the number or function of progenitor cells.

Designing cell-matrix composites opens the opportunity to design or refine matrix materials to function specifically as delivery systems for transplanted osteoblastic progenitors. Potential matrix substrates include processed allograft bone, matrices formed from purified collagen or hyaluronic acid, synthetic polymers, calcium phosphate ceramics, and bioglasses. Improvements or design of new materials require specific chemical surface modifications,

like the addition of surface coatings to optimize biologic performance, such as adhesion molecules, or addition of defined growth factors linked to the matrix surface.

The autologous approach for isolation and osteogenic differentiation of MSCs is highly demanding in terms of logistics, production and safety of culture conditions leading to a costly therapeutic procedure. The association of biomaterials and osteoprogenitor cells raises technical challenges and regulatory issues (devices with medicinal drugs) for the implementation of clinical trials.

How much is enough for callus formation?

Histologic observations and methods of quantitative histomorphometry pioneered by Parfitt et al[21] and Frost[22] have provided understanding of the functional and dynamic parameters associated with bone formation and remodeling at the tissue level. In adult bone remodeling, these processes of bone formation take place in the context of the basic multicellular unit (BMU) described by Frost[22]. Osteoblasts begin secreting matrix within a day, and matrix synthesis increases over several days to a maximum rate of approximately 1.5 μm per day over an area of approximately 150 μm2 per osteoblast, resulting in synthesis of approximately 225 μm3 per day per osteoblast. The total matrix synthesis per osteoblast is approximately 6000–9000 μm3, or 3–5 times its cell volume. Therefore it can be estimated that the volume of bone matrix made by one osteoblast is approximately 5000 μm3. During the process of bone formation, some osteoblasts become embedded in the newly synthesized matrix as osteocytes. These osteocytes reside within cavities known as lacunae and interconnect with one another through multiple processes extending through an interconnected plexus of channels called canaliculi. Some osteoblasts also undergo apoptosis. The mean lifespan of an osteoblast is slightly less than the 50 days needed to complete the wave of bone formation, probably about 40 days. The osteocyte density[23] is reported to be greater in cancellous bone (0.000047 osteocytes/μm3) than in cortical bone (0.000026 osteocytes/μm3). Estimates of osteocyte density as low as 0.000013 osteocytes/μm3 have been reported in the human iliac crest. As a first approximation, based on a mean bone volume of 11 to 25% in cancellous bone, one can estimate the number of osteocytes in one cubic centimeter of cancellous bone to be in the range of 5 to 10 million. It is estimated that the volume of bone matrix made by one osteoblast is approximately 5000 μm3. There are approximately 20 million osteoblasts or osteocytes per ml of new bone. Since there are approximately 2500 progenitors per ml of prepared marrow graft, each must have divided a minimum of 12 or 14 times to obtain 1 ml of new bone, assuming that all the bone marrow graft retained the ability to make bone (2500×214 = 20 million osteoblasts). According to the high number of cells necessary to produce a callus, the highest number of MSCs in the bone marrow graft increases the rate of union[24].

CONCLUSIONS

Improving knowledge of the biology of osteogenic cells and the ability to manipulate these cells presents clinicians with the opportunity to harness their capacity of regeneration and repair of skeletal tissues. The ultimate goal of the orthopedic tissue engineer is to augment the body's repair mechanisms to stimulate the repair or regeneration of viable remodeling bone tissue. The proliferation and differentiation of these cells can be influenced by selected bioactive molecules. The capacity to design matrices that act as osteoconductive scaffolds,

customized surface chemistries, creates further opportunities to optimize the delivery of highly selected osteogenic cells. Realization of the full potential of engineered matrix materials and cell-matrix composites will provide new solutions for skeletal reconstruction. The challenge for the upcoming decade of orthopedic research is therefore one of translation: to render, from the outstanding recent advancements in progenitor cell identification and our improved understanding of the therapeutic mechanism of action, clinically relevant therapies.

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