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Review article

Cell therapy for bone repair

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ABSTRACT

When natural bone repair mechanisms fail, autologous bone grafting is the current standard of care. The osteogenic cells and bone matrix in the graft provide the osteo-inductive and osteo-conductive properties required for successful bone repair. Bone marrow (BM) mesenchymal stem cells (MSCs) can differentiate into osteogenic cells. MSC-based cell therapy holds promise for promoting bone repair. The amount of MSCs available from iliac-crest aspirates is too small to be clinically useful, and either concentration or culture must therefore be used to expand the MSC population. MSCs can be administered alone via percutaneous injection or implanted during open surgery with a biomaterial, usually biphasic hydroxyapatite/β-calcium-triphosphate granules. Encouraging preliminary results have been obtained in patients with delayed healing of long bone fractures or avascular necrosis of the femoral head. Bone tissue engineering involves in vitro MSC culturing on biomaterials to obtain colonisation of the biomaterial and differentiation of the cells. The biomaterial-cell construct is then implanted into the zone to be treated. Few published data are available on bone tissue engineering. Much work remains to be done before determining whether this method is suitable for the routine filling of bone tissue defects. Increasing cell survival and promoting implant vascularisation are major challenges. Improved expertise with culturing techniques, together with the incorporation of regulatory requirements, will open the way to high-quality clinical trials investigating the usefulness of cell therapy as a method for achieving bone repair. Cell therapy avoids the drawbacks of autologous bone grafting, preserving the bone stock and diminishing treatment invasiveness.

Physiological bone repair results in the production of normal bone. Unfavourable local conditions (e.g., inadequate blood supply, soft tissue injury, or mechanical instability) and/or extensive bone tissue loss may result in failure of physiological bone repair with delayed healing, nonunion, or a persistent bone defect. In these situations, autologous bone grafting is the current standard of care. The osteogenic cells and bone matrix in the graft provide the osteo-inductive and osteo-conductive properties required for new bone formation. However, drawbacks of autologous bone grafting include donor-site morbidity [1], limited availability of autologous bone, and loss of bone stock. Attention has therefore turned to other options, such as allogeneic bone grafts and bone substitutes, which supply an osteo-conductive matrix. Cytokines, most notably bone morphogenetic proteins (BMPs) can be added to produce osteo-inductive effects. Physical methods (e.g., electromagnetic fields and ultrasounds) remain to be evaluated.

Cell therapy holds promise as an alternative to autologous bone grafting for promoting bone repair. Bone progenitor cells are supplied to the injury site, either alone or in combination with a mineral or protein matrix and/or cytokines. In bone tissue engineering, the cells are cultured, alone or on a biomaterial, before implantation. Cell therapy spares the bone stock and diminishes treatment invasiveness.

This conference reviews the current use of cell therapy for bone repair in humans, chiefly at long bone sites, to achieve either fracture healing or bone defect filling. Cell therapy for disorders of bone metabolism (osteoporosis), osteogenesis imperfecta, and gene therapy will not be discussed.

1. Physiology of bone repair

Bone tissue is capable of self-repair, which results in the production of new bone exhibiting all the characteristics of normal bone. Fracture healing or bone defect filling by an autologous cancellous bone graft results from interactions among osteogenic cells,

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cytokines, an osteo-conductive matrix, and a mechanically stable environment with a good blood supply, according to the 'diamond concept' [2].

In rare cases, the cortices undergo primary healing after perfect fracture reduction and stabilisation. Usually, however, fracture healing involves intra-membranous and enchondral ossification. This complex dynamic process requires the precise orchestration of various events during four overlapping stages [3] having distinctive histological characteristics: an inflammatory response, formation of a cartilaginous soft callus, formation of a bone hard callus, and bone union with remodelling. This process involves a sequence of anabolic and catabolic events, some of which are non-specific (production then remodelling of the cartilaginous callus) and others specific (formation of the bone callus, which is then remodelled into normal bone). Thus, bone resorption plays a crucial role, and the resorption and formation processes are not separate or independent in time and space. The final result of the bone repair process is the production by the cells of a collagen matrix, whose ossification restores the normal mechanical properties of the bone. These histologically defined stages of bone repair require a number of cellular events (migration, proliferation, and differentiation), whose coordination is ensured by cytokines and growth factors.

Inflammation plays a role of paramount importance at the beginning of the bone repair process. The injury triggers the release of pro-inflammatory cytokines (interleukins IL-1β and IL-6, TNFα), whose chemotactic effects attract inflammatory cells and stimulate angiogenesis at the fracture site. Cell types that are more specific to the bone repair process are involved subsequently. Although the molecular mechanisms that regulate cell proliferation and differentiation have been partly elucidated, no biological markers of use for the clinical monitoring of bone healing have been identified to date.

2. Cell types involved in bone repair

The bone repair process mobilises many cell types. Despite having no direct role in bone formation, the cell types involved in the inflammatory and angiogenic responses are indispensable to the development of the bone formation mechanisms. They release cytokines and growth factors (PDGF, BMPs, VEGF, and interleukins) that attract and activate the mesenchymal stem cells (MSCs) directly involved in bone repair.

MSCs are precursors of osteoprogenitor cells. They play a key role in cell therapy for bone repair, as they are the best characterised multipotent cells and can now be produced reliably for clinical purposes. The osteoclast lineage makes a major contribution to bone remodelling but is not currently used for clinical applications.

Friedenstein et al. [4] were the first to demonstrate new bone formation from cultured bone marrow (BM) cells. The BM cells proliferated in vitro, generating colonies of fibroblast-like cells, or 'colony-forming unit fibroblasts' (CFU-Fs). MSCs are defined as multipotent non-haematopoietic cells capable of differentiating into functional cell types found in various mesenchymatous tissues (bone, cartilage, muscle, tendon, adipose tissue, and haematopoietic stroma) [5]. The self-renewal capacity of MSCs ensures that they maintain their multipotency throughout their life span.

MSCs can be identified in vitro based on their ability to adhere to plastic culture dishes and to generate CFU-Fs after several days of culture in standard medium containing foetal calf serum. Then, depending on the available induction influences, MSCs can differentiate into bone tissue cells (osteoblasts), cartilage cells (chondrocytes), and adipose cells (adipocytes) [5]. The in vitro MSC phenotype is characterised by absence of expression of membrane molecules specific of haematopoietic cells (CD45, CD14, and CD34), contrasting with the presence of other molecules (CD73, CD44, CD105, CD90, and CD146). No marker strictly specific of MSCs is available [6], a fact that complicates the reliable identification of MSCs and their extraction from the pool of nucleate BM cells.

MSCs were first identified in BM [5] then in adipose tissue [7], cord blood, the placenta [8], the periosteum [9], and other tissues. MSCs from these different sources share similar phenotypic characteristics but differ regarding their differentiation and proliferation properties. It should be noted that these MSCs are identified only after culturing. Native MSCs (naturally found in tissues), in contrast, are poorly characterised and difficult to identify. Native MSCs have been identified in blood vessel walls [10].

The source of the MSCs present at sites of bone repair, particularly after a fracture, is difficult to determine. In animal models, these cells come from the periosteum, BM, and neighbouring soft tissues. The most obvious source of MSCs is the BM, in which MSCs contribute 0.001% to 0.01% of all mononuclear cells in healthy adults, with a decrease over the life span [11,12]. One millilitre of BM contains only $18 \pm 7 \times 10^6$ mononuclear cells including 612 ± 134 MSCs [13]. BM concentration and culturing techniques are therefore valuable to expand the MSC population available for clinical use.

Methods are now available for expanding MSCs in compliance with current regulatory requirements for use in clinical applications [14]. Current culture media contain no animal products and are based on human platelet lysates designed for optimal safety. Within 2–3 weeks, a 30-mL sample of iliac-crest BM generates several million MSCs, depending on the available culture surface area. During culturing, MSC differentiation to cartilage, adipose, or bone cells can be induced. Acquiring a high level of expertise with MSC production and differentiation to osteoprogenitor cells is crucial to successful bone repair. Differentiation of cultured MSCs to the osteoblastic lineage (osteo-induction) can be obtained by adding BMP (BMP2 or BMP4) or dexamethasone.

Differentiating MSCs release growth factors and cytokines that contribute to regulate the bone repair process. The fluctuations over time in the production of growth factors and cytokines are poorly known, a fact that limits our ability to obtain precise therapeutic effects by using these molecules.

An interesting characteristic of MSCs pertains to the immune system: MSCs are not immunogenic, because they express little or no major histocompatibility complex Class II molecules and induce no T-cell proliferation. On the contrary, MSCs have immunosuppressive properties related to their ability to inhibit T-cell proliferation and NK-cell lysis under allogeneic conditions [15,16]. These properties may enable allogeneic MSC transplantation without immunosuppressive therapy of the recipient and suggest a role for universal MSC banks for regenerative medicine.

Another major advantage of MSCs is a high level of resilience with preservation of bone repair capabilities even after several hours of transport.

3. Cell therapy approaches to bone repair

According to the diamond concept [2], MSCs play a crucial role in bone repair. Cell therapy can serve as an alternative to autologous bone grafting. A large number of osteoprogenitor cells are implanted at the injury site, either alone or combined with a matrix. BM MSCs are currently the most appropriate cells for inducing bone repair, as they have a strong osteogenic potential and are easily obtained by culturing iliac-crest aspirates.

Several MSC-based cell therapy modalities have been developed, i.e., with and without cell culturing and with or without a matrix.

The mononuclear cell fraction of the BM, which contains the MSCs, can be used directly by percutaneous injection of