Application of Mesenchymal Stem Cells for Repair and Regeneration of Cartilage and Bone

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Australian efforts to provide orthopaedic surgeons with living, load-bearing scaffolds suitable for current joint (knee and hip) replacement surgery, non-union fracture repair, and meniscal and growth plate cartilage regeneration are being lead by teams at the Institute for Medical and Veterinary Science and Women's and Children's Hospital in Adelaide; the Peter MacCallum and St Vincent's Medical Research Institutes in Melbourne; and the Mater Medical Research Institute and new Institute for Health and Biomedical Innovation at QUT, Brisbane. In each case multidisciplinary teams are attempting to develop autologous living tissue constructs, utilising mesenchymal stem cells (MSC), with the intention of effecting seamless repair and regeneration of skeletal trauma and defects. In this article we will briefly review current knowledge of the phenotypic properties of MSC and discuss the potential therapeutic applications of these cells as exemplified by their use in cartilage repair and tissue engineering based approaches to the treatment of skeletal defects.

Introduction

Current data clearly demonstrate that adult mammalian bone marrow contains at least two phenotypically and functionally discrete populations of stem cells. Hematopoietic stem cells (HSC) are the most extensively characterised population of tissue stem cells in vertebrate physiology and are responsible for maintaining lifelong production of blood cells. In contrast, the cellular characteristics and physiological role of the second marrow resident population of stem cells, most commonly referred to as bone marrow stromal cells or mesenchymal stem cells, are much less well understood.

Clonogenic Marrow Stromal Progenitors

Pioneering contributions to our understanding of marrow stromal precursor cells were made by Friedenstein, Owen and colleagues. Seeking to identify the cells in rodent bone marrow responsible for bone forma t ion in ectopic transplant experiments, Friedenstein and colleagues demonstrated the growth of colonies of cells morphologically resembling fibroblasts when single cell suspensions of bone marrow were explanted at appropriate cell densities in liquid cultures (1). The clonogenic stromal progenitor cells responsible for colony growth under these conditions, fibroblast colony-forming cells (CFU-F), were described as rapidly adherent, non-phagocytic clonogenic cells capable of extended proliferation *in vitro*. Although originally described in rodents, CFU-F have been detected in the bone marrow of essentially all species examined including cats, dogs, sheep, rabbits, non-human primates and humans.

The majority of information regarding the properties of marrow stromal cell progenitors is based on the analysis of cells derived from serial subcultivation of CFU-F-derived colonies, a population now most commonly referred to as mesenchymal stem cells (2,6). In contrast, relatively little is known about the precise phenotypic characteristics of the primary clonogenic stromal precursors in the bone marrow responsible for initiating stromal cell growth *in vitro*. The rarity of these cells, typically <0.01% of human bone marrow, is a major barrier to their prospective isolation. Until relatively recently, this problem was compounded by a shortage of specific antibodies to facilitate their isolation and enrichment. Monoclonal antibody STRO-1-positive cells from human bone marrow yield a 10-20-fold enrichment of CFU-F relative to their incidence in unseparated bone marrow. When combined with selection with an antibody to vascular cell adhesion molecule-1 (VCAM-1/CD106), a discrete population of STRO-1^{bright} VCAM-1^{+} cells with a CFU-F incidence of approximately 1 per 2 cells plated can be obtained (3,4). STRO-1^{bright} VCAM-1^{+} cells at the clonal level exhibit differentiation into cells with the characteristics of adipose, cartilage and bone cells *in vitro* and form human bone tissue following transplantation into immunodeficient SCID mice (4). Collectively, these data strongly suggest that primitive stromal precursors, including putative stromal stem cells with the capacity for differentiation into multiple mesenchymal lineages, are restricted to the STRO-1+ fraction in adult human bone marrow.

The differentiation capacity of cultured bone marrow derived stromal cells coupled with the apparent ease of *ex vivo* culture manipulation has not surprisingly engendered considerable interest in potential therapeutic applications of these cells in a range of clinical settings. Of the many potential targets for regenerative medicine and tissue engineering based upon the use of MSC, the most likely to meet with clinical success in the near future are cellular therapies for the repair of cartilage and skeletal defects.

MSC and Cartilage Repair

Cartilage is a specialised, avascular, aneural and alymphatic tissue with poor capacity to repair. This reparative inability is symptomatic of degenerative joint diseases, sports injuries, and premature growth arrest and deformity in growing children. Initial cell-based therapies were directed toward the use of cultured chondrocytes, however this treatment is restricted as it requires harvest of large numbers of chondrocytes from limited donor sites, and the effect of such removal on the donor cartilage tissue is unknown. As a consequence there is considerable interest in the use of MSC as an alternative for cartilage regeneration due to their ease of harvest and their rapid expansion in culture without loss of chondrogenic potential.

Chondrogenic Potential of MSC

Numerous studies have demonstrated the chondrogenic capacity of MSC *in vitro*. Chondrocytes isolated from cartilage tissue will dedifferentiate if cultured in monolayer, so differentiation of MSC to chondrocytes requires a three-dimensional arrangement such as that in pellet culture or high-density micromass. MSC harvested from bone marrow aspirate have been stimulated to develop towards a chondrogenic lineage by addition of growth factors TGF-β1 (5), TGF-β3 (6), or FGF-2 (7). Supplementation of cultures containing TGF-β3 with BMP-6 or IGF-1 was shown to enhance synthesis of chondrogenic markers (8).

When attempting to engineer cartilage tissue, the selection of a suitable biomaterial scaffold is of major importance. Many biomaterials have already been shown to support *in vitro* chondrogenesis. In order to support and encourage chondrogenesis and synthesis of the cartilage extracellular matrix components, the scaffold is required to be biocompatible, resorptive, provide mechanical stability and maintain the precursor cells evenly within the transplant. Both naturally occurring and synthetically manufactured scaffolds have potential as biomaterials. Successful *in vivo* chondrogenesis has also been described with the subcutaneous transplantation of bone marrow MSC into immunocompromised mice with synthetic polymers porous [polyvinyl formal resin and polylactide-caprolactone (9)], a hyaluronic acid coat (10), and a gelatin/hyaluronan composite sponge (11).

Repair of Joint Cartilage with MSC

Osteoarthritis is a degenerative joint disease of the articular cartilage surface of joints. Although predominantly an age-related disease affecting the middle-aged and elderly, osteoarthritis can be triggered prematurely by sports injuries and obesity. Injuries that penetrate into the subchondral bone of articular cartilage are repaired poorly with fibrocartilage, which has biochemical and biomechanical inferiority to hyaline cartilage. Defects that do not penetrate into subchondral bone are not repaired at all. Extensive research in the transplantation of cultured chondrocytes to repair articular cartilage defects has been undertaken, but this approach is somewhat restricted due to accessibility and yield of chondrocytes from donor tissues. In addition, differentiated cells isolated from specialised adult tissues often have reduced proliferative capacity. Transplantation of MSC within a type-1 collagen gel to a large full thickness (subchondral) articular cartilage defect led to chondrogenesis and repair of the damaged subchondral bone region. However, some thinning of the articular cartilage was observed over time (12). Large full thickness articular cartilage defects necessitate the regeneration of both the articular cartilage and subchondral bone.

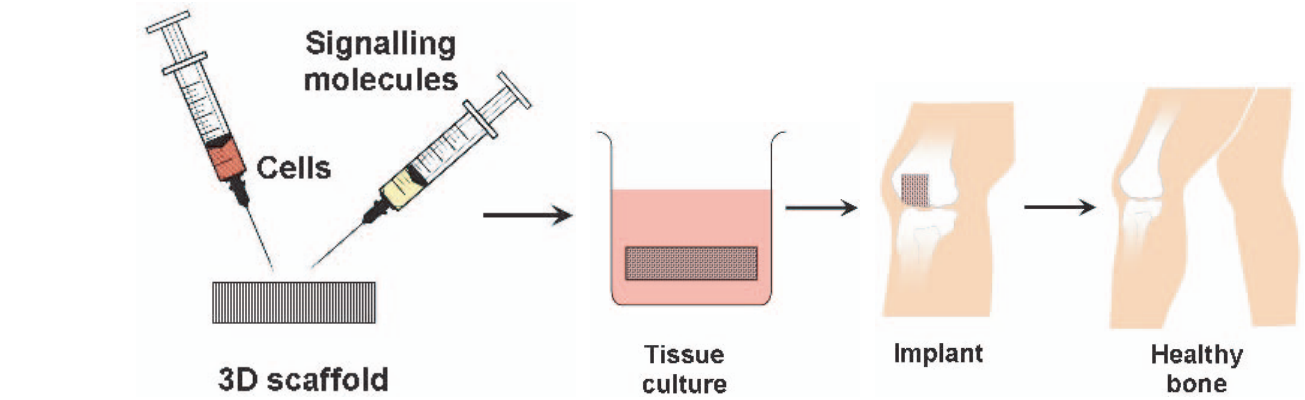

Fig. 1. Mesenchymal Stem Cell Repair and Regeneration.

The MSC repair/regeneration concept involves seeding highly porous biodegradable scaffolds in the shape of the desired bone with cells and protein growth factors/signalling molecules. This construct is then cultured *ex vivo* before being transplanted into the defect site to induce and direct the growth of new bone. The goal is for the cells to attach to the scaffold, multiply, differentiate, organise and integrate seamlessly into normal, healthy bone as the scaffold degrades.

Loss or damage of meniscal cartilage is frequently associated with sports injury and removal rapidly leads to progression of arthritis. Following induction of osteoarthritis through meniscectomy in a caprine (goat) model, the intra-articular injection of MSC with dilute hyaluronan (a cartilage extracellular matrix component) resulted in retention of cells within the joint and formation of neo-meniscus (13).

Mesenchymal Stem Cells to Treat Growth Plate Injury

Longitudinal bone growth occurs at either end of the long bones at sites known as the growth plate. Responsible for elongation of the long bones, the growth plate is a highly organised cartilage tissue. Trauma to the growth plate leads to incorrect repair and formation of a bone bridge across the cartilage and can often result in permanent limb deformity. Current clinical treatment includes external frame bone lengthening and as yet there is no biological cell-based therapy to correct this type of injury. Applications of MSC within agarose (14), chitin (15), and gelatin (16) scaffolds have demonstrated prevention of bone bridge and regeneration of growth plate cartilage in a rabbit model, but success in larger animals awaits demonstration.

MSC in the Repair of Critical Skeletal Defects

Numerous preclinical studies in animal models convincingly demonstrate the feasibility of localized transplantation of MSC grafts as a cellular therapy for the reconstruction of critical size bone defects in the skull and appendicular skeleton (17, 18) . As previously noted for cartilage repair, the choice of biomaterial scaffold is of major importance in promoting the osteogenic potential of MSC following transplantation. *De novo* bone formation has been reported following reimplantation of MSC with scaffolds such as demineralised bone matrix, tricalcium phosphate/hydroxyapatite (TCP/HA), polycaprolactone (PCL), polyglycolides/polylactides (PLA/PGA), hydrogels of collagen, hyaluronan, fibrin, or even cells alone (19-21).

While data are not yet available for humans, these preclinical *in vivo* animal studies nevertheless provide an all important proof-in-principle suggesting the likely clinical efficacy of MSC in the treatment of skeletal defects in humans. Thus autologous MSCs (harvested from a patient's own marrow thereby obviating immune-mediated rejection) might be expanded *ex vivo* and returned to the patient with an appropriate scaffold, as a 'living prosthesis' with the potential to integrate with existing tissue(s). One application of this approach might be the provision to orthopaedic surgeons of a living, loadbearing 'polyfilla' to be used in current arthroplasty practices to fill the space between metallic joint implants and native bone. Surgeons currently use 'bone cement' (usually polymethylmethacrylate) for this purpose. Bone cement is brittle and is frequently a source of inflammation-induced osteolysis.

Autologous, cellular 'polyfilla' will circumvent these problems, as it is patient-derived and will stimulate the regrowth of native bone to replace the synthetic implant with autogenous regenerated bone. In addition to minimising motion at the interface between the metal implant and native bone, it will facilitate a tight bond (osseointegration) between native bone and the metal implant. In the longer term it will be preferable to replace the metallic components of the implant with a resorbing scaffold that will guide autogenous regeneration resulting in native bone. This application will be enhanced by delivering MSC in pre-formed scaffolds with different resorption characteristics in order to facilitate targeted and controlled release of biological response modifiers such as growth factors, co-factors (protease activators) and pharmaceuticals to further promote bone regeneration. Several groups around the world are developing multi-phase nanostructured composite scaffolds to deliver growth factors in association with extracellular matrix elements. As one phase of the scaffold is resorbed, growth factor complexes are temporally released; the second scaffold phase provides continued mechanical support, other growth factors and is a template guide for the regenerating tissue. Preliminary *in vitro* results indicate that these structures can support MSC survival and growth. Ongoing pre-clinical research will rapidly translate these promising laboratory results into clinical outcomes, available to surgeons throughout the world.

Fig. 2. Mesenchymal Stem Cells Growing on Synthetic Scaffold.

Patient-derived (human) mesenchymal stem cells adhere to a synthetic bone material (fluorine-substituted apatite) and spread over the scaffold surface. This image was captured 24 hours after placing and culturing the cells within the construct. The cells display extensive contacts with the scaffold and exhibit smooth and ' hairy ' (microvilli) morphologies. [The unusual clump of material in the centre is residual debris from materials manufacture] © QUT

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