

## CELL THERAPY AND BONE REPAIR

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**INTRODUCTION:** Bone damage, due to pathology or trauma, is a common occurrence, requires costly medical and/or surgical intervention and involves several human resources as well as a great deal of suffering in the patients. Present techniques are only partially successful, due to the length of treatment and shortage of donor bone tissue. In many cases grafts of new tissue are required to achieve functional recovery. Bone grafts or synthetic materials are needed to replace missing bone and enhance new bone formation. Grafts are either of autologous source (from the patient itself; autograft) or from other donors (allograft). In the first case, the autograft procedures require a second operation to harvest enough bone, increases risk factors, hinders the patient's recovery and substantially raises costs. The allografts are instead associated with the risk of blood-borne diseases and are limited to the countries in which fresh frozen grafts are stored and correctly donor-receiver matched; moreover, many grafts are produced from cadavers by heat and dehydration procedures which are expensive, time-consuming and strictly law-regulated.

At present, the use of autologous bone graft is considered the best option. Regenerative Medicine and Tissue Engineering are new research areas that investigate how to repair and regenerate organs and tissues using the natural signaling pathways and components of the organism (stem cells, growth factors, etc.) Cell biology gave several growth factors a role in the control of the proliferation and differentiation capacity of specific cell types. In addition, today's *in vitro* cell culture protocols allow the expansion of specifically selected cell populations. Cells harvested from the patient, including adult stem cells, are expanded in culture and associated with resorbable biomaterials both of synthetic and extractive origin. Therefore, damaged tissues that would normally not be repairable with the available traditional techniques could be in this way reconstituted. The employment of biomaterials as both vehicles and inductors of the "*ex vivo*" expanded cells, has and will always promote an increase in the research and development of a new generation of transplantable biomaterials. Of great relevance is the fact that these engineered tissues could be obtained and used with no quantity limitations.

**METHODS: Cell cultures** Stromal cells were obtained from iliac crest marrow aspirates from healthy donors. Donor age ranged between 3 and 50 years and donors were all white Caucasians. Informed consent was obtained from all donors and all procedures were approved by institutional ethical committee. Mononuclear cells (MNC) were counted,

plated at  $2-5 \times 10^6$  MNC/100mm dish in Coon's modified Ham's F-12 medium supplemented with 10% Fetal Calf Serum (FCS). Some plates were cultured in the presence of 1ng/ml human recombinant FGF-2. The medium was changed after 3 days and then twice a week. When dishes reached confluence, BMSC were detached with 0.05% trypsin/0.01% EDTA and replated. To evaluate CFU-f frequency (CFU-f assay), 100µl of the original marrow suspension were plated in 100 mm dishes. The medium was changed after 3 days and then twice a week. After 2 weeks of primary culture, cells were washed, fixed with 3.7% formaldehyde in PBS, and stained with 1% methylene blue in borate buffer. Colonies were counted.

**Tissue engineering of bone: preclinical and clinical studies.** Bone formation can be assessed in small animals by implanting BMSCs combined with mineralized tridimensional scaffolds subcutaneously in immunodeficient mice

To treat full-thickness gaps of tibial diaphysis in adult sheep, autologous cells isolated from bone marrow and expanded *in vitro*, were loaded onto highly porous ceramic cylinders (100% hydroxyapatite; 70- 80% porosity; pore size distribution: < 10 µm ~ 3% vol.; 10- 150 µm ~ 11% vol.; > 150 µm ~ 86% vol) and implanted in critical-sized segmental defects in the tibia of the animals. External fixation was used to stabilize the grafts. For the human patient, the study protocol was approved by the ethical committee of the orthopaedic centers involved. Patients had non neoplastic pathologies, and were selected for this treatment after failure of an alternative surgical therapy, and/or to avoid important donor site morbidity, an expected long recovery time and likely future complications. The patients were informed of the nature of the treatment and gave their written consent.

**RESULTS:** Cultured Bone Marrow Stromal Cells (BMSC) can be regarded as a mesenchymal progenitor/precursor cell population derived from adult stem cells. They can differentiate into different lineages: osteoblasts, chondrocytes, adipocytes and myocytes. BMSC stimulation with particular growth factors (i.e. FGF2) induces a critical modification in the proliferation and differentiation ability of these cells, which remain in a osteo-chondrogenic stem/progenitor status. BMSC undergo limited mitotic divisions and do not express telomerase activity. We have also observed a sequential loss of lineage potential in tripotent cloned cell populations, suggesting a model of predetermined BMSC differentiation. Thus BMSC do not display full features of stem cells and should be regarded as early

mesenchymal progenitors. A search for specific markers expressed by BMSC at the early progenitor stage is in progress.

When implanted in immunodeficient mice, BMSCs combined with mineralized tridimensional scaffolds form a primary bone tissue highly vascularized. We have used this animal model to test efficacy of cells expanded according to different protocols and different biomaterial scaffolds of different nature and with variable degree of reabsorbability.

Autologous BMSC/bioceramic composites were used also to treat full-thickness gaps of tibial diaphysis in adult sheep. Gross morphology, x rays, histology, microradiography and SEM studies showed complete integration of ceramic with bone and good functional recovery. We have also reported the existence of complementary integration and disintegration mechanisms within HA ceramic (HAC) implants used to replace the critical-sized segmental defects

The healing process involves four main steps: 1) bone formation on the outer surface of the implant; 2) bone formation in the inner cylinder canal; 3) formation of fissures and cracks in the implant body; 4) bone formation in the bioceramics pores. By radiography and by tomography, bone formation was far more prominent over the external surface and within the inner canal of the implants. This might be due to a higher density of loaded cells and/or to a better survival of cells within the outermost portions of the HA bioceramics. Alternatively the implanted cells could stimulate, via a paracrine loop, resident osteoprogenitor cells, located within the skeletal tissues at the resection ends.

Given the results obtained in the animal models, similar composites were implanted at the lesion sites of six patients. External fixation was used. Some of the patients have been followed for more than 3-4 years. A full functional recovery of the treated limb occurred within 6 to 7 months from surgery. An initial integration at the bone/implant interface was already evident one month after surgery. Bone formation progressed steadily during the following months. A full functional recovery of the treated limb occurred within 6 to 7 months after surgery.

The pattern of the bone healing process in the patients was similar to the one described in the large animal model.

**DISCUSSION & CONCLUSIONS:** A tissue engineering approach has been followed in the orthopedics field. This approach via the composite biomaterial and cell technology, and the characterization of cell growth conditions, implantation protocols and surgical procedures is allowing significant progresses for bone repair.

Regardless of the low number of patients so far treated, we consider the results obtained very promising and we propose the use of culture-expanded osteoprogenitor cells in conjunction with HA bioceramics as a real and

significant improvement in the repair of critical size long bone defects.

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