Tissue engineering applications in the management of bone loss

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Summary

Several conditions in Orthopaedics and Traumatology are characterized by a bone loss. Bone auto- or allo-grafting was considered sufficient to fulfill the defects decades ago; however, large bone defects were challenging for the Surgeons, particularly in case of necessity of structural and biological properties. Bioindustry proposed over the years synthetic biomaterials, as Demineralized Bone Matrix, bioactive surfaces for implant components, and recently recombinant Bone Morphogenetic Proteins. At the same time, the concept of the “biological chamber” and “diamond concept” allowed the scientific community to consider the need of a more complex interaction between scaffolds (matrix), cells (mesenchymal cells), and signaling (growth factors) in order to induce bone regeneration and also to fill small or large bone defects.

A brief overview is made on the processes of a physiologic bone metabolism (induction, conduction, osteogenesis), on the latest therapeutical procedures, based on the use of autologous growth factors and cells, and the recent prosthetic or synthetic scaffolds, and the common clinical conditions that may benefit of these modern approaches.

KEY WORDS: *tissue engineering; bone loss; bone graft.*

Treatment of large bone defects still represents a challenge in Orthopaedics and Traumatology. Many techniques and materials have been proposed over the last decades: however, more often we may find bone defects. Thus, we still are missing something in the prevention and/or in the treatment of these frequent clinical situations (1-3).

Bone is a self-healing tissue. However, alterations of local bone metabolism may occur in several situations, related to different causes. In Traumatology, we often assist to non unions or delayed consolidations of fractures: the reason regards mainly anatomical predisposition of specific sites of fracture, typically those with low blood supply (distal third of tibia, talus,

calcaneus in the lower limb; humeral diaphysis and forearm in the upper limb); also technical mistakes during fixation may cause delay or difficult healing. We may observe several orthopaedic pathologies in which a poor osteointegration of prosthetic components realizes (particularly after primary replacement in difficult cases, as Rheumatoid Arthritis and Haemophilic Arthropathy); in avascular necrosis (femoral head, semilunar); in revision arthroplasty, where host bone is already low-responding to biological stimuli due to slow but chronic loosening of the implant. Oncologic pathologies of the bone imply frequently the use of techniques of bone fulfillment, both for aggressive and non aggressive tumors. Furthermore, we may need a quicker bone healing after specific procedures, as osteotomy (proximal tibia, distal femur), in which a surgical controlled fracture is performed in order to correct a malalignment: healing normally occurs, but peculiar situations may need a faster callus formation or early recovery of weight-bearing, thus a strategy to reach this goal is requested.

During the last decades, many proposals were introduced to face these situations: acrylic cement, allografts, autografts, metallic elements, resorbable bioceramics (4-8). All of these options have showed advantages or pitfalls. Whatever the choice, in our opinion their outcomes may now be improved by modern approach to this surgery, by means of Tissue Engineering.

Tissue engineering is an interdisciplinary field that through the development of biological substitutes provides to restore bone defects and new tissue formation (1, 2). Even if it may regard all tissues, we will focus on the applications related to bone tissue, that represents the larger field of interest, and with better clinical results.

Tissue Engineering procedures consist in minimally manipulative operations regarding mesenchymal cells, growth factors, and scaffolds, in a way to develop a microenvironment with high osteogenic, osteoinductive, and osteoconductive properties. These modern and advanced techniques are considered “in line procedures”, and thus allowed by International Scientific Committee on Medical Products and Devices of European Community.

The goal of the Tissue Engineering in local bone metabolism is the interaction of these elements to produce new bone tissue. Bone formation passes through three complementary mechanisms called Osteoinduction, Osteoconduction, and Osteogenesis.

Osteoinduction is a process that supports the mitogenesis of mesenchymal cells, leading to the differentiation of osteoprogenitor cells to produce new bone by chemical interaction usually with mediators and growth factors.

Osteoconduction is the process that allows a matrix to support and facilitate adhesion and survival of undifferentiated cells, in a way that these may be duplicate.

Osteogenesis is the main pathway that induce effective tissue formation, and is possible if the other two processes are activated.

The guests of this complex activity are the mesenchymal cells, the scaffolds, and the growth factors.

Autologous mesenchymal cells maybe easily harvested by several sites of the body, as iliac crest (anterior, posterior),

sternum, and proximal tibia, usually rich in bone marrow: the most frequent site, as also in our experience, is the iliac crest. Once harvested (usually an amount of 30 to 90 cc), the bone marrow is prepared in a dedicated cell separator, in order to isolate the multipotent cellular content (Colony Forming Units-CFUs) from other parts (serum, red cells). However, the number of cells obtained by this process is subjected to a high variability. Hernigou et al. examined the influence of the number of progenitor cells and the concentration property of the separator in autologous bone marrow injected percutaneously for the treatment of non unions (9). In their series, healing was obtained when the bone marrow injected contained more than 1500 progenitors/cm³, and an average total of 54,962 ± 17,431 progenitors. The concentration (634 ± 187 progenitors/cm³) and the total number (19,324 ± 6843) of progenitors injected into the non union site were both significantly lower ($p = 0.001$ and $p < 0.01$, respectively) when union was not obtained. These data showed a positive correlation between the volume of mineralized callus at four months and the number ($p = 0.04$) and concentration ($p = 0.01$) of mesenchymal stems cells in the graft. They concluded that there was a close relationship between clinical results and amount of mesenchymal cells; moreover, they suggested a reasonable cut-off of 1000 progenitors/cm³ to await a clinical effect on bone healing. Modern separators are generally standardized in a way that a sufficient amount of cells may be isolated and used.

Several types of scaffolds are longer available: the most frequently used are homologous bone chips or grafts (fresh frozen or lyophilized materials from cadavers), autologous bone plugs (typically harvested by iliac crest), or synthetic materials, as biodegradable polymers, recombinant collagen, resorbable cement, or Demineralized Bone Matrix products (DBM). Allografts are very cheap, available in wide quantity, but present only osteoconductive properties, a little risk of viral diseases transmission, and are generally partially resorbed few weeks after implantation by host tissues. Autografts are cost-free, very osteoconductive and inductive, but there is a very little available quantity, and donor site morbidity may be problematic for the patients (10). Resorbable cements are useful as osteoconductive substrates, but expensive, and may be only partially substituted over time by host bone. DBM presents tolerable costs, no risk of infections, good resorption without local reaction, and high osteoconductive (and somehow osteoinductive) properties.

Among modern scaffolds, we have to mention recently introduced osteoconductive synthetic materials belonging to several implants systems: sleeves, cones, wedges, and stems. They are metallic parts of a prosthesis useful to fill a defect by an osteoconductive stuff (Porous Tantalum, Trabecular Titanium, etc.) able to offer a 3D reticular frame in which osteoblasts and osteoclasts may find an ideal environment to duplicate and produce bone tissue. Even if expensive, they may be a helpful tool to be strongly considered in cases of revision surgery (7). Growth factors are substances able to act as signal between cells, in a way to promote or down-regulate biomolecular pathways. They are normally secreted both by circulating and local activated cells. Several families of growth factors are usually involved in the bone metabolism cascade: main growth factors belong however to *Transforming Growth Factors Beta* (TGF- β) family (11). To date, we are able to safely use two categories of growth factors: *Bone Morphogenetic Proteins* (BMPs) and *Platelet Growth Factors* (PRP). The formers are normally present in the bone and DBM, but they also be obtained by recombinant products, as highly concentrated commercial solutions of BMP-2 and BMP-7. They act as stimuli to differentiation of pre-osteoblasts in osteoblasts. The latter are generally obtained by concentration of autologous peripheral blood samples, harvested from the patients just before or during surgical

procedures. They are autologous, highly available in quantity, and with lower costs respect to BMPs.

All these three biological elements may be applied in bone loss sites as single factors or in variable combination. Our procedures provide generally the use of all the components to act as a single "biological composite": we had very good results in all cases, not depending on the type of diagnosis and basic procedure applied to treat the patients. In all cases we had complete integration, full healing, and no post-operative complications related to the use of this composite.

We also reached good success in cases of single use of one of the factors: typically, percutaneous injection of mesenchymal cells concentrate of limited non union sites (malleoli, forearm, humerus).

Orthopaedic surgery has several branches in which a bone defect may occur: severe joint destruction with large bone loss (Rheumatoid Arthritis, Haemophilic Arthropathy); ischemic bone pathology (avascular necrosis); tumoral conditions (cysts, pseudotumor, tumors); and revision arthroplasty, that is the most frequent scenario.

Haemophilic Arthropathy is the most common complication of Haemophilia, related to recurrent bleedings in specific target joints. Severe destruction of the joints usually lead to large subchondral defects: in case of primary replacement, it is helpful to provide the use of complex and modular implants to fill any bone loss areas. However, use of engineered composites may be useful.

In revision surgery we often need to manage bone loss. When an implant fails, either aseptic or septic, a loosening of the components realizes with secondary bone loss. It is of paramount importance to fulfill this deficiency in a way to recreate a solid substrate in which a revision component may be stable. Historically made with allografts or acrylic cements (2, 12), it was possible over the time to use Autografts (particularly in knees and hips), resorbable matrix as DBM, allografts, and, as before mentioned, modular prosthetic parts. To date, a different combination of allografts mixed with biological autologous products and sleeves/wedges/stems make this kind of surgery easier with the great advantage to adapt an implant to a patient, rather than a patient's joint to the prosthesis, as usually until last decade (Figure 1). Of paramount importance remains the fact that this surgery has to be performed by experienced Surgeons in high-volume Centers.

Other field of interest is the avascular necrosis, particularly of the femoral head. Historic core decompression procedures were considered for long time the gold standard, but able to produce a good biological stimulus with no mechanical solution for the involved bone (13): no weight-bearing strategies were proposed for months until bone formation on the decompressed sites. Today, several proposals have been made, and recently Civinini et al. reported interesting results with a brand new procedure, consisting in core decompression, allograft mixed with concentrate autologous mesenchymal cells and injection of a resorbable bioceramic (14). Outcomes were satisfactory related to no recurrence or progression of the necrosis through the femoral head, no complications, complete resorption of the grafts, progressively substituted by host bone, and faster weight-bearing recovery (Figure 2).

Traumatic disorders are showing a tremendous increase during last years, with subsequent rising of complications: as mentioned, we are assisting more frequently to delays of fracture healing or non unions. Usually, management of these complex issues consisted in hardware removal and new fixation by more secure and stable devices, or local conservative and physical methods that however might give positive outcomes only after long intervals (15). Tissue Engineering may help Surgeons in different ways.

Either for delays or non unions of long bones, we may use direct

Figure 1 A-D- **A)** Forty-seven years-old male patient affected by severe Haemophilia with a previous implant failed after 4 years (aseptic loosening of acetabular cup with severe bone loss); **B)** Revision surgery with modular components in Trabecular Metal, allografts engineered with autologous mesenchymal cells concentrate; **C)** Good outcome after 1 month with expected resorption of the graft. **D)** Radiologic evaluation after 6 months with complete integration.

Figure 2 A-F - **A,B)** Fifty-five years-old male patient affected by Steinberg 2b Avascular Necrosis of the femoral head (x-ray and MRI evaluation); **C,D)** Intraoperative pictures with bone marrow harvest from iliac crest, mixed with allograft; **E,F)** X-rays at one and three months postoperatively, with progressive resorption of the graft and healing of the necrosis.

Figure 3 A-D - **A,B)** Twenty-five years-old patient with an isolated fracture of the radius treated by cast; **C)** Since non union occurred, percutaneous injection of autologous mesenchymal cells concentrate under fluoroscopy was performed directly on the non union site; **D)** Healing after just 36 days postoperatively.

injection of mesenchymal cells concentrate (by open or percutaneous approach) in a specific sites, by amploscope assistance (16). Surprising results have been reported with these technique, that however, may be indicated only for limited areas of anomalous healing, in presence of stable fixation or in combination with immobilization (i. e. by cast) (Figure 3).

Other approach is a mini-open surgery with debridement of a non union site, and grafting with a biological composite (bone chips or DMB + mesenchymal cells +/- PRP). After an interval generally shorter than other techniques, we may observe in most cases a quick healing, with possibility of early functional recovery and weight-bearing.

Finally, engineered composite may find application in osteotomies, particularly in High Tibial Osteotomy: usually healing occurs in an interval of two to four months, with suggested weight-bearing not before the third month. By plating with new generation devices and filling the osteotomy site with an engineered composite, we may allow early weight-bearing (6 to 8 weeks after surgery) and observing full healing after two months.

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