

Mesenchymal stem cells in post-surgical cavities of large maxillary bone lesions

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Summary

Background. Recent studies have highlighted that MSCs are capable of regenerating large bone defects when used in combination with bone substitutes and increasing allograft osteointegration. We investigated the hypothesis that autologous MSCs may lead to increased bone regeneration and reduced healing time in post-surgical cavities of large maxillary bone lesions.

Methods. This study involved 10 patients (TEST GROUP) (6 males and 4 females). All patients had expansive mandibular lesions larger than 3 cm. From the surgical point of view, the 10 patients were treated with MSCs (withdrawal of the iliac crest bone marrow BMMSs) directly into the post-surgical cavity, without the addition of filler.

Results. Clinical and radiological data, in the postoperative, were compared to those of patients who did not receive any grafting of MSCs. The 7 patients with mandibular lesions showed a rapid and very good healing with an 85-90% ossification of the major defect at 12 months.

Conclusions. Through the use of stem cells a greater ossification of the residual cavity (85-90%) was observed at 12 months after surgical enucleation in contenitive defects.

KEY WORDS: bone regeneration; maxillary defects; maxillary bone cysts; MSCs; bone grafts.

Introduction

Enucleation and surgical debridement represent the most effective techniques to treat a massive benign expansive bone lesion of the maxilla.

Concerning the literature, the careful enucleation of small lesions plus a scrupulous surgical debridement (uncombined with biomaterials filling of the remaining cavity) is the unanimous procedure to ensure a satisfactory bone regeneration (1-6, 8-22).

In regard to post-surgical medium and large cavities (> 3 cm), defined by Horowitz's 1989 classification (1-7), there isn't any consensus on treatment procedure; conversely there're different alternative proposals to the only spontaneous regeneration.

The techniques about post-surgical enucleated large cavities include the use of biomaterials, autogenous bone and combinations of these materials. Many studies have shown, by periodical clinical-radiographic follow-ups, that residual large cavities can undergo to spontaneous regeneration in the totality of cases. Reviewing the literature, there's not evidence to fill cavity with autologous bone grafts or alloplastic materials, which could increase postoperative morbidity and would delay the healing time (8-22). After the surgery procedure, the fracture is the complication of large lesions due to the size and the excessive bone fragility. Whenever is possible, to reduce that risk, fixing plates are used intraoperatively and removed after healing.

After the enucleation of an expansive benign bone lesion, newly formed bone developed from primary clot organization (which spills from the cavity walls) and progressively fills the residual cavity. Usually an almost complete cavity ossification is radiographically visible since 6 to 12 months; however the time range is related to cyst initial size, number of intact residual walls and to patient's age [young patients heal faster (8, 9, 12, 22, 23)]. Concerning large cavities (more than 3 cm in diameter), literature indicates this data about the average percentage reduction (9, 12, 23):

- at 6 months: 12-15% reduction
- at 12 months: 43-49% reduction
- at 24 months: 83-91% reduction

In the last years, a tissue engineering procedure has been set up: as a graft material *autologous mesenchymal stem cells* (MSCs) are used in combination with an osteoconductive scaffold. MSCs are a population of bone marrow-derived (BMMSCs), or human adipose-derived stem cells (hADSCs), non-hematopoietic multipotent cells which can be expanded and *in vitro* differentiated into osteogenic phenotype (40, 41). Recent studies have highlighted that MSCs are capable of regenerating large bone defects when used in combination with bone substitutes (42, 43) and increasing allograft osteointegration (44). Another method that has been used to improve the outcome of bone grafting is based on autologous platelets. Indeed, they are a readily accessible, safe and cheap source of growth factors, such as *platelet-derived growth factor* (PDGF), *transforming growth factor beta* (TGF- β), *epidermal growth factor* (EGF), *vascular endothelial growth factor* (VEGF) and *insulin-like growth factor I* (IGF-I), which play a key role in tissue homeostasis (45, 46). Platelets are usually concentrated in a small volume of plasma called *platelet-rich plasma* (PRP), which can be an excellent addition to MSCs and freeze-dried bone grafts. In fact, MSCs express receptors for the growth factors contained in the PRP and *in vitro* studies have shown that the addition of PRP promotes MSC proliferation.

Based on the above grounds, in the present study we test the hypothesis that grafting of autologous BMMSCs and PRP may lead to increased bone regeneration and reduced healing time.

MSCs

A Mesenchymal Stem Cell (MSC) is a type of adult stem cell, immature and undifferentiated. It originates from the mesoderm, the germ of the blastocyst between ectoderm and endoderm. In recent years, MSCs have attracted a lot of attention thanks to their versatility. Their action is not limited to the trophic effect alone, given by the production of various types of cytokines and growth factors, but they are also able to migrate and differentiate into different types of mesenchymal tissues. In 2006 International Society for Cellular Therapy (ISCT) has proposed minimal criteria to define MSCs: a mesenchymal stem cell can be defined if it shows adhesion plastic in normal crop and if it has a fibroblast-like morphology (24-34). Some researchers also argue that MSC have the same functions of fibroblasts (35). The mesenchymal stem cells in culture express surface antigens CD73, CD90 and CD105, while not expressing CD11b, CD14, CD19, CD34, CD45, CD79a or HLA-DR (36). Mesenchymal stem cells have a role in tissue regeneration: they are divided cyclically and the newly formed cells replace those cells that show sign of aging via membrane receptors or damaged cells. They are mainly located in the bone marrow: here are on average 0.01% of the total bone marrow cells (35-37). Clinical trials with MSCs began in 1999 by Horowitz et al. demonstrating that "the bone marrow derived mesenchymal cells" increase the mineral content in the whole organism and the subsequent bone formation in children with osteogenesis imperfect (38). Since then, MSCs have been applied to patients suffering from osteitis and bone defects (39).

Materials and methods

This study was conducted between 2013 and 2015 and involved 10 patients (TEST GROUP) (6 males and 4 females) aged between 12 and 75 (average age 50).

Patients presented to our attention in the Department of Maxillofacial Surgery of the University of Florence. In the 80% of cases the lesion was occasionally detected, while in the remaining part (20%) there were painful symptoms of inflammatory origin which required a radiographic evaluation. To obtain a greater standardization, the surgical procedure was performed by the same surgical team using the same instruments. In the pre-surgical, the protocol provided for the collection of the patients' medical history and radiographic examinations (CT). All patients showed bone lesions: they were expansive, benign, voluminous maxillary or mandibular lesions with a diameter greater than 3 cm. All lesion were histologically assessed and the results were 3 keratocysts, 4 follicular cysts (Figure 1), 2 periapical inflammatory cysts and 1 ameloblastic fibro-odontoma (Figure 2). All patients were operated using the same anesthetic technique which involved general anesthesia via nasotracheal intubation. They all received pre-operative antibiotic coverage through the use of a broad-spectrum antibiotic (2 g of amoxicillin and clavulanic acid) administered at least 1 hour before operation and to be continued at the same dose twice a day for 6 days. Then, the oral hygiene protocol provided the use of mouthwash with chlorhexidine 0.12% three times a day for 15 days. From the surgical point of view, the 10 patients were treated with mesenchymal stem cells and PRP directly into the post-surgical cavity, without filler addition. Check-ups were carried out

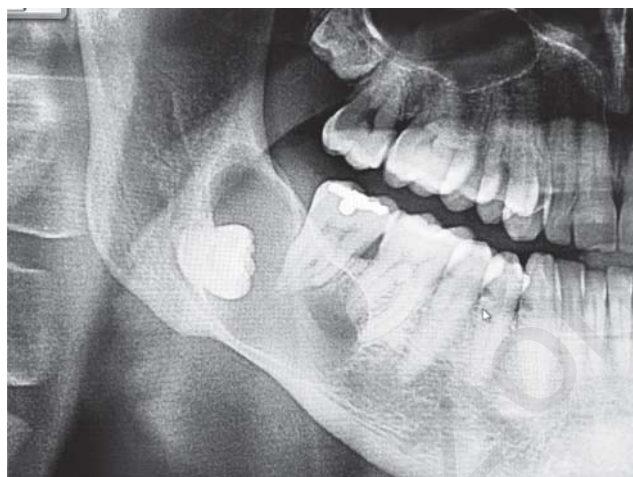


Figure 1 - An occasional radiological finding of follicular cysts in a patient of 29 years old with no clinical signs or symptoms.



Figure 2 - Subject 9 years old: radiological appearance of ameloblastic fibro-odontoma; the 4.6 tooth is pushed by benign tumor at the base of the jaw.

according to our protocol that included new X-rays and clinical visits at 3, 6, 12 and 24 months after surgery.

All possible postoperative complications were considered, paying particular attention to the absence of infection, pain or neurological disorders. Bone regeneration was evaluated by X-ray examinations observing the size variations of the operated area. For the feedback and the calculation of residual areas we used the dedicated digital software provided by the radiology center. All this data were compared with 10 patients treated without MSCs (control groups).

Surgical procedure with stem cells:

- withdrawal of the iliac crest bone marrow and stem cell preparation
- enucleation of the bone lesion
- the lesion is fixed in formalin and sent to the Department of Histology and Pathology for histological analysis
- application of mesenchymal stem cells
- suture.

Preparation of stem cells, of platelet-rich plasma (PRP) and autologous thrombin serum (ATS)

Our protocol involves the use of Regenkit Extracell Glue (RegenLab SA Losanne, Swiss) disposable kits, dedicated to the preparation of concentrated autologous stem cells from blood samples of iliac crest bone marrow and PRP platelet concentrate complete with

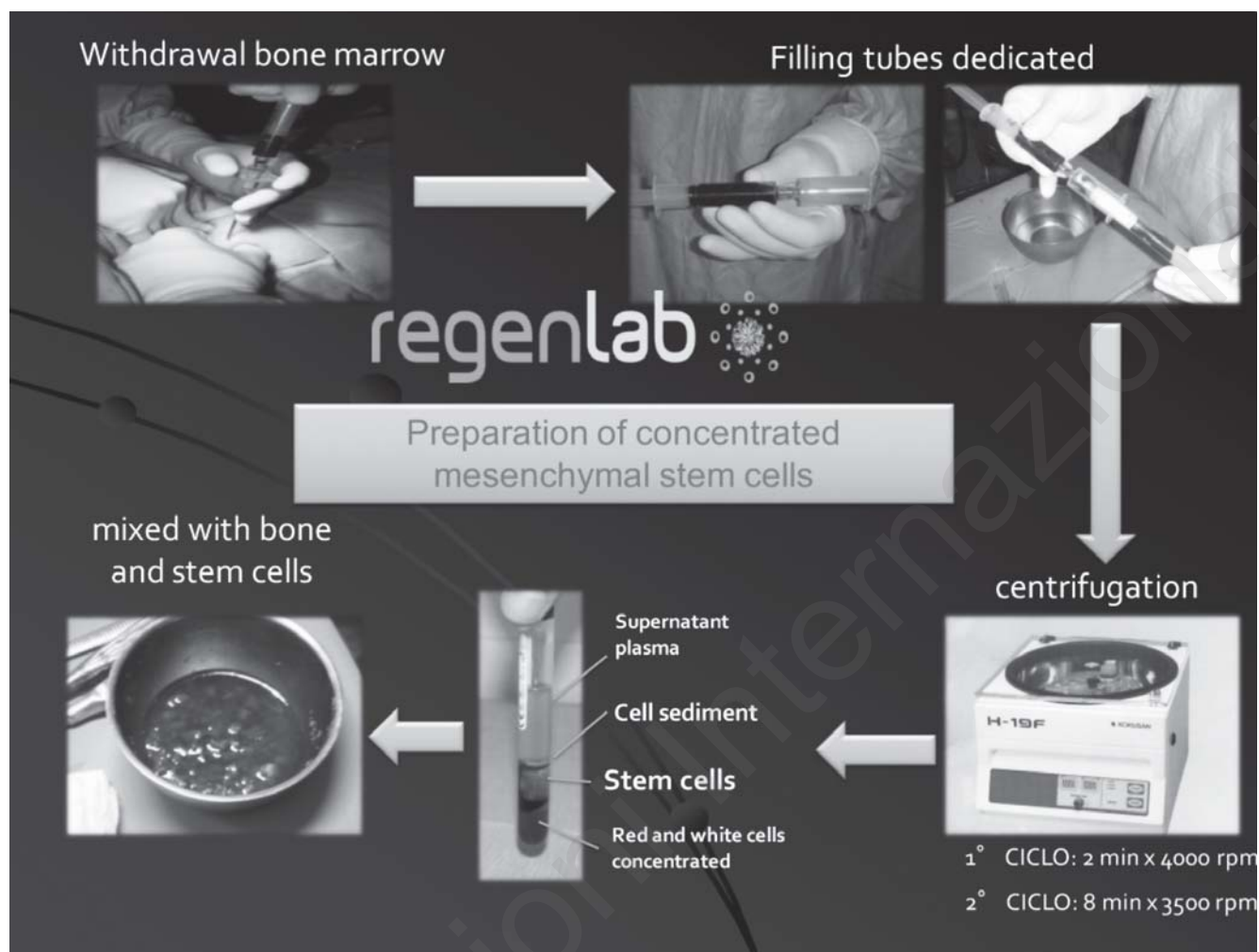


Figure 3 - Step 1a – The Regenlab procedure: withdrawal of blood marrow (iliac crest) Step 2a – transfer tube Step 3a – centrifugation Step 4a – collection and concentration of stem cells.

autologous thrombin serum ATS. This protocol refers to the Brandi-Innocenti study in which it is shown that the number of MSC's per ml was 8 times higher than the control group (47). The procedure to obtain the concentrate of stem cells has been already described in our previously published item (48). It consists of four steps plus the one of mixing and application (Figure 3).

- A.
- Step 1a – withdrawal of blood marrow (iliac crest)
 - Step 2a – transfer tube
 - Step 3a – centrifugation
 - Step 4a – collection and concentration of stem cells

- B.
- Step 1b – collection of peripheral whole blood
 - Step 2b – centrifugation
 - Step 3b – preparation of serum thrombin
 - Step 4b – collection of concentrates
 - Step 5 – mixing and application

In sterile field the PRP was placed in a provided container. Then, the stem cell concentrate was added together with the autologous thrombin serum to activate coagulation. All this was placed inside the residual bone cavity after enucleation and surgical debridement. After that the flap was sutured.

Results

Only one patient didn't come to control visits. The 7 patients with mandibular lesions showed a rapid and very good healing with an 85-90% ossification of the major defect at 12 months (Figures 4, 5). The 2 patients with maxillary bone lesions showed good tissue healing but a large loss of vestibular bone. Withdrawals of bone were performed, eight months after surgery, for a histological evaluation. Histological examination showed a complete new bone formation (Figure 6). No complications were observed.

Discussion and conclusion

Radiographic controls of test group showed a faster bone healing than the control group (patients without use of MSCs) (Figure 7) and the one described in the literature concerning spontaneous bone healing after enucleation. For large cysts the literature reports a time value of 24 months to obtain an ossification of about 85-90% of the residual cavity. By the use of MCs, it was observed an ossification of 85-90% of the residual bone cavity at only 12 months from the surgery, halving, in fact, the time.

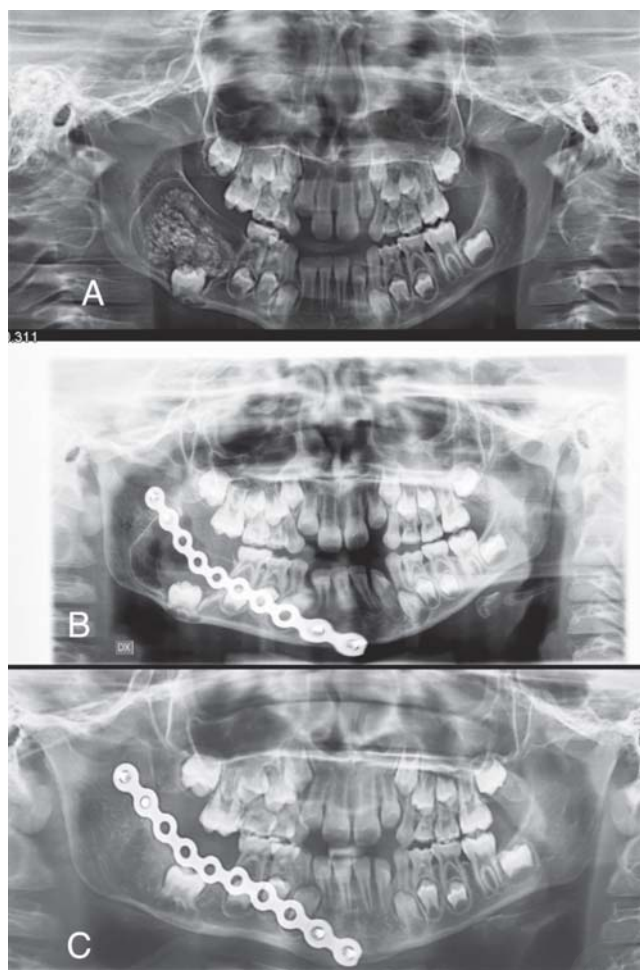


Figure 4 A-C - Same case as in Figure 2; A) ameloblastic fibro odontoma; B) radiographic check after surgery in C) radiographic check at 6 months after surgery; note the growth of the tooth 4.6.

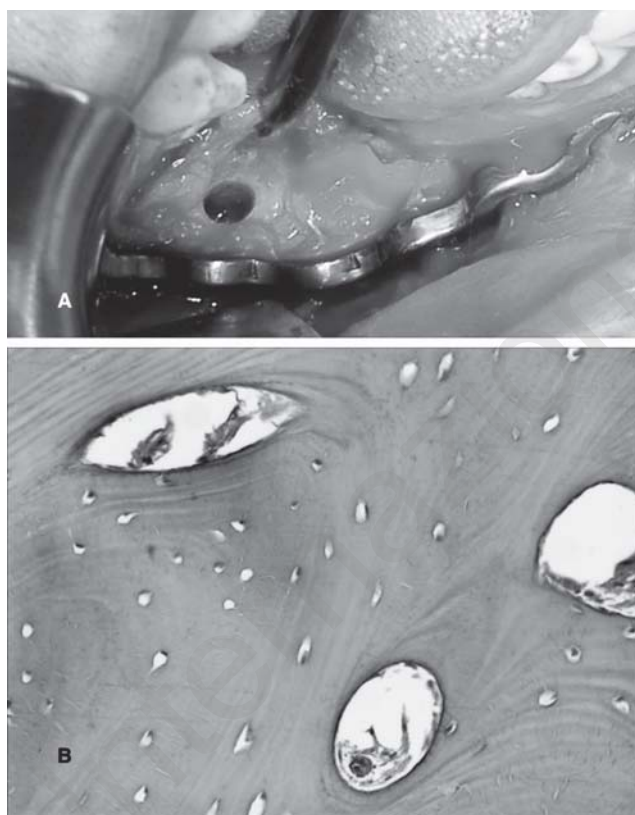


Figure 6 A, B - Same case as in Figure 2; A) ameloblastic fibro-odontoma: 8 months after surgery a huge new bone regeneration; B) histological features, hematoxylin-eosin 400 x, compact lamellar cortical bone, with alive osteocytes.

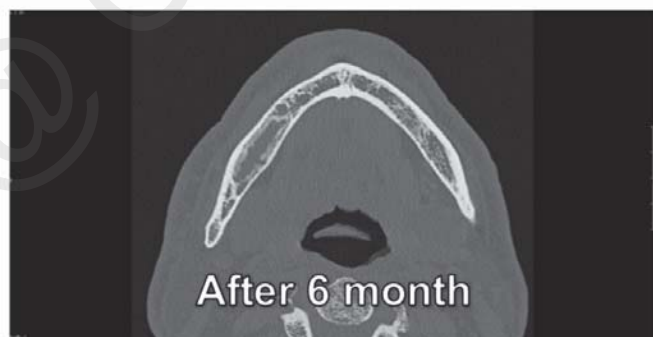


Figure 5 - Cheratocyst in a 31 y.o. man: radiological check after the surgical therapy at 3, 6 and 12 months. Note the good bone healing.

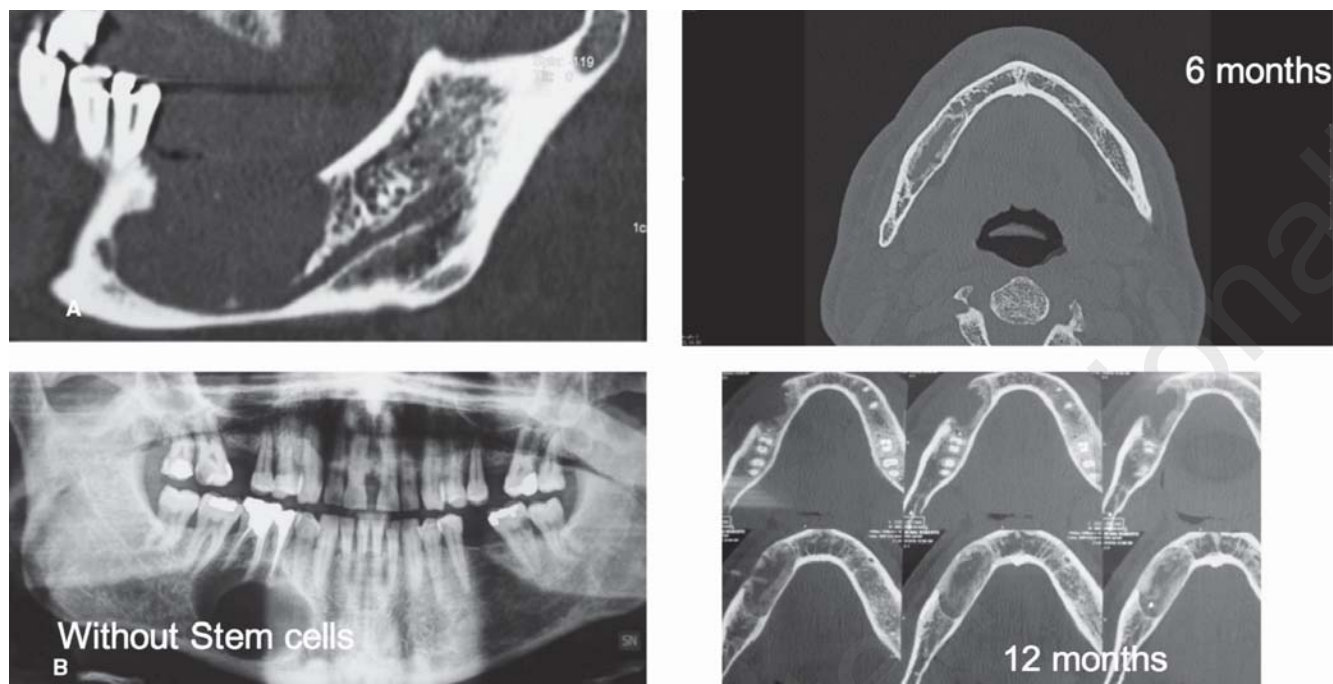


Figure 7 A, B - A) keratocysts after surgery with the use of MSCs, and X-ray control to 6 months; the good bone healing and faster when compared with a surgical cavity (B dentigerous cyst) in which are not used MSCs.

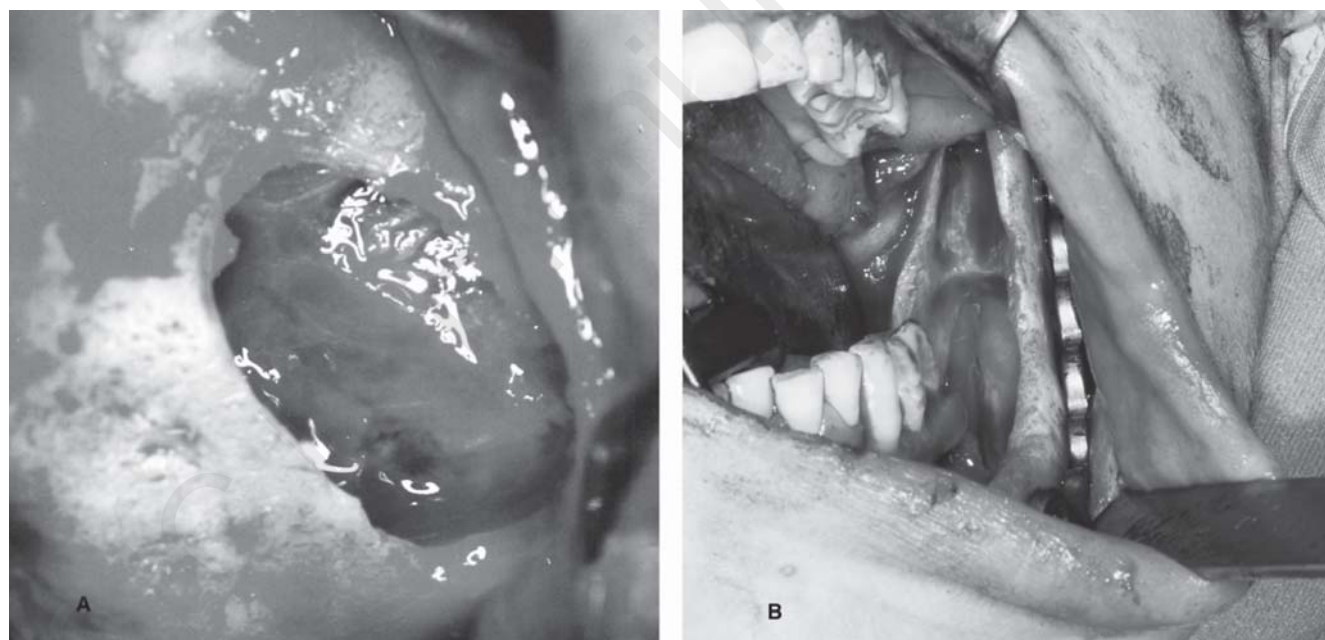


Figure 8 A, B - A) bone cavity after sinus lifts procedure; the vascularization is given only by raised upward mucosa and by outside palatine mucosa; B) a post surgical cavity where it is evident a large blood supply coming from bony walls.

Comparing the results of this study with those of our previously published work, concerning the use of the MSCs in sinus lift procedure (48), we have been able to see different times of osteogenesis. In fact, the bone healing times between the double sinus lift and the ones obtained without the use of stem cells are fully comparable. Instead, the use of stem cells in the post-surgical cavity seems to speed up significantly the bone healing times. This seems to be related to the vascular environment of the post surgical cavity because different from the cavities after sinus lift, owing to a lack of vascularity (Figure

8). A well-vascularized post surgical cavity appears to be a more favorable environment for the activity of MSCs in order to exploit their full potential. The excellent results obtained seem to be due to the receiving site, which is ideal for the clot stability and vascularization. In fact, very good results have been achieved in cases where the bone defect to be regenerated was contentive (residual bone cavities with three walls), whereas in patients treated with absence of vestibular wall support, there were no striking results from the bone regeneration point of view.

Since the aim of MSCs using is reaching a faster “restitutio ad integrum”, the result considerably reduces the incidence of post-operative complications and an easier operative outcome. Further studies will be needed to confirm our results and the encouraging evidences encountered so far.

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