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A model of human bone regeneration: morphological, cellular and molecular aspects

ABSTRACT

Aim The postoperative healing of the cystic cavity was chosen as experimental model of bone regeneration. The morphological, cellular and molecular events characterizing the stages of regeneration, from the clot to the bone formation within the cystic cavity were investigated.

Materials and methods Samples of regenerating tissue taken 15 days, 1, 2 and 4 months after surgery were analyzed. A total of 8 samples were collected, 4 of which were from the same patient (longitudinal study) and 4 from different patients (cross-sectional study). The investigations performed were: SEM analysis of all samples; transmission electron microscopy of samples taken after 15 days and 1 month; primary cell cultures of samples taken after 15 days and 1 month; Western blotting of cell lysates to highlight the presence of proteins characteristic of osteoblasts, such as osteocalcin, BMP_2 and transglutaminase. Clinical and radiographic examinations completed the analyses.

Results Results confirm that a spontaneous bone regeneration occurs after enucleation of jaw cysts, even in patients with large bone defects. The bone healing process is slow, in particular bone maturation, which is still not completed after 4 months. Remodeling of the matrix begins after 15 days and is evident after 1 month, also demonstrated by the high expression level of transglutaminase in the preosteoblastic cells isolated from the samples. The first phase of bone healing, characterized by an osteoid matrix replacing the osteo-coagulum, can be considered completed after about 2 months.

Conclusion The basic conditions for spontaneous bone regeneration are: migration of undifferentiated mesenchymal cells from the overhanging periosteum and of osteoblasts from the surrounding endostium; fibrin matrix supporting the amplification and the differentiation of pluripotent mesenchymal cells; cooperation of platelets, macrophages and immunocompetent cells; significant neoangiogenesis ensuring new tissue vascularization.

Keywords Bone, stem cell, regeneration, clot, osteoblast, morphology.

INTRODUCTION

Bone disorders are among the clinical areas where there is a greater demand for replacement materials. According to the principles of modern tissue engineering, in order to replace a biological tissue, an artificial material must have not only adequate mechanical characteristics, but also be able to interact with cellular and/or extracellular components of the grafted area, providing or facilitating the complexity of biochemical events and cell-to-cell and cell-to-matrix relations (Cancedda et al., 2007). In this view, the understanding of biological mechanisms underlying the bone physiological repairing and regenerative processes, is of fundamental importance for the design and development of materials and procedures that promote the regeneration of this particular tissue.

The present study is centered on the healing of the cystic cavity, chosen as experimental model of bone regeneration. It represents nearly an ideal model to verify the biological processes occurring because spontaneous bone regeneration is obtained, even in patients with very large bone defects (Chiapasco et al., 2000; Ihan and Mijavec, 2008). For good healing it is important that the surgeon carefully preserves the

periosteum and bone walls as the source for osteogenesis and ensures the presence of blood in the entire volume of the cystic cavity following the removal of the cystic bag. Clinical observations highlighted the importance, for bone re-growth, of the presence of the blood clot resulting from the bleeding during surgery performed to enucleate the cyst. The bone regeneration is produced, in fact, because the presence of the clot (indicated as osteo-coagulum), without the help of either bone grafts or growth factors (Chiapasco et al., 2000). On the contrary, the use of autografts and allografts resulted in increased postoperative healing period, postoperative morbidity and complications (Marx et al., 1981, Horowitz and Bodner, 1989; Mitchell, 1992; Bodner, 1996; Bodner, 1998). Another model of bone regeneration studied in different animals and humans (Kuboki et al., 1988; Lin et al., 1994; Lekic et al., 2001; Cardaropoli et al., 2003, 2005; Kanayama et al., 2003; Sato and Takeda, 2007; Trombelli et al., 2008) is the healing of the extraction socket. Recently Trombelli et al. (2008) examined 27 biopsies in man, 10 representing early healing (2-4 weeks), 6 intermediate (6-8 weeks) and 11 a later phase of healing (12-24 weeks). They concluded that a great variability exists in man with respect to hard tissue formation in this model.

On the basis of the above concerns, the purpose of this study was to investigate and describe the morphological, cellular and molecular events characterizing the stages of spontaneous bone regeneration, from the clot to the newly formed hard tissue within the cystic cavity.

MATERIALS AND METHODS

Patients

This study included 5 patients with jaws cysts (4 inflammatory odontogenic cysts and 1 nasopalatine cyst) aged between 18 and 45 years, all males, to avoid the influence of menstrual cycle, pregnancy, lactation, menopause. All patients were not affected by systemic diseases and were not in therapy with drugs.

Surgical protocol

Cysts were removed with standardized techniques in the Department of Dental, Orthodontic and Surgical Sciences, Second University of Naples, Naples (Italy). All patients were treated with local anesthesia. The surgical protocol consisted in the elevation of a mucoperiosteal flap of adequate dimensions; access to the lesion was generally obtained using a round bur in a low speed hand-piece under irrigation with sterile saline solution. Particular care was taken to preserve the largest amount of bone to help subsequent bone regeneration and to provide adequate support to the soft tissue during the healing period. Whenever possible, the cysts were enucleated in one piece. The cyst cavity was vigorously curetted to remove all residual fragments and to reduce the risk of relapse. Primary closure was performed with non-resorbable sutures, that were removed after seven days postoperatively. All patients were prescribed systemic antibiotics and clorexidine digluconate 0.12% mouth rinses 3 times per day for two weeks. All cysts were examined microscopically after fixation in 4% formalin solution. The healing of the cystic cavity was controlled by radiographic exams, which allow to evaluate the stage of bone regeneration. For each case, radiographs were performed before the identification of the cysts, and after 1 month and 4 months from the surgical intervention; when necessary CT scanning analysis was also performed.

Samples

The study was conducted by taking samples

of regenerating tissue, after 15 days, 1 month, 2 months and 4 months from the surgery performed to enucleate the cyst. A total of eight tissue samples were collected, 4 of which were from the same patient (longitudinal study) and 4 from different patients (cross-sectional study). Four kind of investigations were performed:

1) scanning electron microscopy of all samples (SEM);
2) transmission electron microscopy (TEM) of samples taken 15 days and 1 month after surgery;
3) in vitro stimulation of primary cell cultures from samples (15 days and 1 month) with thyroxin or granulocyte-macrophage colony stimulating factor (GM-CSF);
4) western blot analysis of the primary cell lysates to detect osteocalcin, BMP 2 and transglutaminase.

The results obtained from these tests were completed by radiographs and clinical examinations.

All subjects signed the informed consent before participation and the local ethical committee approved the study protocol.

The techniques of sampling were different depending on when it was made, because the tissue consistency in the early stages of regeneration (15 days-1 month) was significantly lower compared to later stages (2-4 months). The first samples (osteo-coagula), were taken by the insertion of the surgical spoon to draw the desired amount of tissue. The samples after 2-4 months, requiring the access to mineralized tissue and the removal of a bone fragment, were taken with a core cutting machine in order to have a bone carrot. The samples obtained were washed with saline and transferred into cell culture medium or fixed for microscopic analysis.

Primary cell cultures of osteo-coagulum samples

Osteo-coagulum samples from patients were mechanically separated with a sterile pestel and cultured in lysine pre-coated Petri dishes in Dulbecco's modified Eagle's medium, DMEM, supplemented with 25% fetal calf serum, 2 mM glutamine, 100 IU/ml of penicillin, and 100 microg/ml of streptomycin. The cells were cultured as monolayers and the medium was changed every 2-3 days. To perform experiments the cells were treated, at exponential growth phase, with thyroxin (0,5 microg/ml) and 1.0 nM vit. D3 to induce osteoblast differentiation or with GM-CSF (5ng/mL) and 1.0 nM vit. D3 to induce osteoclast differentiation. After desired times the cells were mechanically harvested using a scraper in the presence of 5mM EDTA and 10 mM DTT, and used to perform further analysis.

Western blotting

Western blotting was performed according to the modified method of Towbin et al. (1979). The proteins were prepared in reduced forms using denaturating buffer containing 3% , beta-mercaptoethanol.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970) using and 12% polyacrylamide gel in a vertical slab gel apparatus (Bio-Rad Instrument) at a constant voltage of 150 volts. After the electrophoresis, proteins on the gel were electrophoretically blotted to the nitrocellulose membrane using Invitrogen apparatus. The membrane was incubated in 100 ml of rabbit appropriate antibody: anti-TGase, anti-osteocalcin, and anti-BMP 2 antibodies (Dako) at 1:2000 dilution in antibody diluent buffer overnight at 25ºC in a rocking motion. Subsequently the membrane was incubated with alkaline phosphatase-conjugated anti-rabbit antibody (diluted 1:2000) for two hours at room temperature. Finally the membrane was incubated with substrate nitroblue tetrazolium chloride (NBT) and BCIP (5-bromo-4-chloro-3-

indolyphosphate-p-toluidine salt) (Sigma), the reaction was stopped with stopping buffer containing 0.5 mM Na^2EDTA, and the membrane was air dried and photographed. The densitometric analysis was performed by using Image J, a public domain image tool processing by NIH, downloaded at http://rsbweb.nih.gov/ij/download.html.

Preparation of samples for TEM analysis

Samples for TEM, cut into 1-2 mm wide cubes (to facilitate the in depth penetration of the fixing and inclusing reagents) were fixed firstly with a solution of 2.5% glutaraldehyde in phosphate buffer, pH 7.2 - 7.4, and then with 1% v / v osmium tetroxide in phosphate or cacodilate buffer, pH 7.2-7.4. The fixed samples were then dehydrated with the alcohol scale (50%, 70%, 80%, 95%, absolute alcohol) and clarified in propylene oxide. The dehydrated samples were then included in epoxy resin (Epon 812), and cut into 400-500 Å slices using a ultramicrotome (Reicher, Ultracut E). The thin sections were then mounted on metal grids and observed at the transmission electron microscope (EM Zeis 109).

Preparation of samples for SEM analysis

The samples for scanning electronic microscopy (SEM) were washed 3 times (5 min for each washing) in phosphate buffer at pH 7.2 - 7.4 and fixed in 2.5% glutaraldehyde in phosphate buffer at pH 7 , 2 - 7.4, maintaining the samples for 2-3 h at 4° C. After this time glutaraldehyde was replaced with 0.1 M cacodilate buffer, pH 7.3, taking care to fully cover the samples, which were left at room temperature for 15 min. The above procedure was repeated two more times. Subsequently samples are washed 3 times for 15 min with bidistilled water. At this point the fixed samples were dehydrated in alcohol, using the standard sequence of ethanol (30, 50, 70 and 95% v/v), leaving them in the alcoholic solution for 5 min. Later samples were washed with 3 changes of 100% ethanol for 15 min each. Before the metallization the samples were treated 2 times for 10 min with dimethylsilazane and were left to dry for 12 hours. Then, the dried samples were covered with a thin layer of gold/palladium by foil (Polaron 2000) and observed with a scanning electron microscope (Cambridge 100).

RESULTS

Radiological analysis

In figure 1 the radiographs of two subjects are shown, in particular the ortopan-tomographies regarding the bone

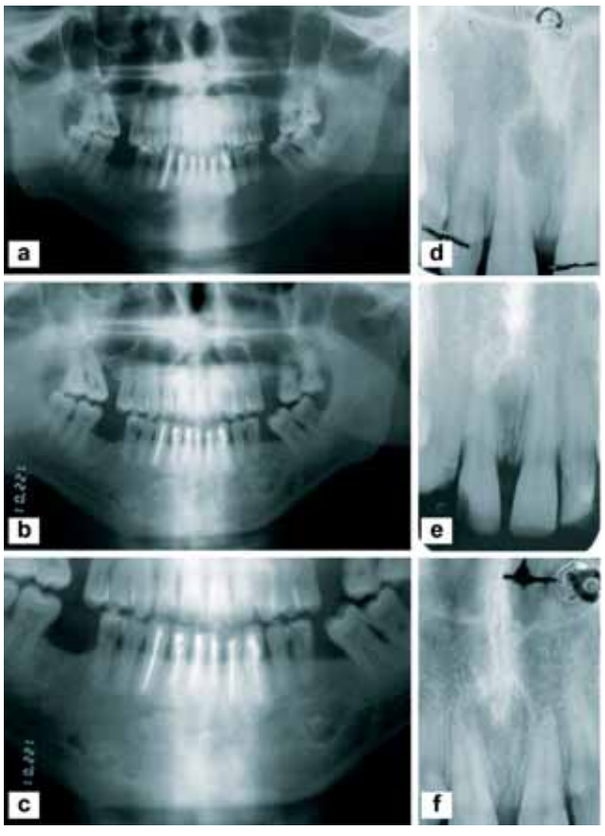

Fig. 1 Panoramic radiographs showing bone regeneration at 1 and 4 months (a, b) and a particular of the regenerated area at 4 months (c). Endoral views at 1 and 4 months after surgery of a small nose-palate cyst (d, f).

regeneration after 1 and 4 months (Fig. 1a, 1b), and a detail of the regenerated area (Fig. 1c) of the first patient, and the endoral views at 0, 1 and 4 months from the surgery (Fig. 1d-1f) of the second. The first case concerns the treatment of a large mandibular radicular cyst, stretching from teeth 45 to 37. The radiograph at 1 month shows that the bone regeneration is just appearing and the contours of the former cavity are still clearly visible. At 4 months it is evident that the regeneration is almost complete, although bone trabeculation is still unclear.
The second case concerns a small nose-palate cyst. The image at 1 month shows that the bone regeneration is in a fairly advanced stage, because some trabecular bone are visible. At 4 months it can be seen (Fig. 1c) that the regeneration is complete and even the newly formed bone appears thicker than the surrounding area.
Figure 2 shows the ortopantomographies of two cases before surgery (Fig. 2a, 2c) and in advanced regeneration (Fig. 2b, 2d) as well as the TC of another subject before surgery (Fig. 2e) and in advanced regeneration (Fig. 2f). The first case concerns the healing of a radicular cyst stretching from 43 to 34 teeth. The second case concerns a follicular cyst associated to tooth 48, which covers part of the ramus and the entire branch of the right mandible. The third case concerns a radicular cyst of the left jaw, which occupies much of the omolateral jaw sinus, deforming its anterior and medial wall, thus also affecting the left nasal fossa. It is noticeable that, in the complete regeneration, not only the bone is remodelled, but also the original shape of the anatomical structures affected by the disease is restored as well.

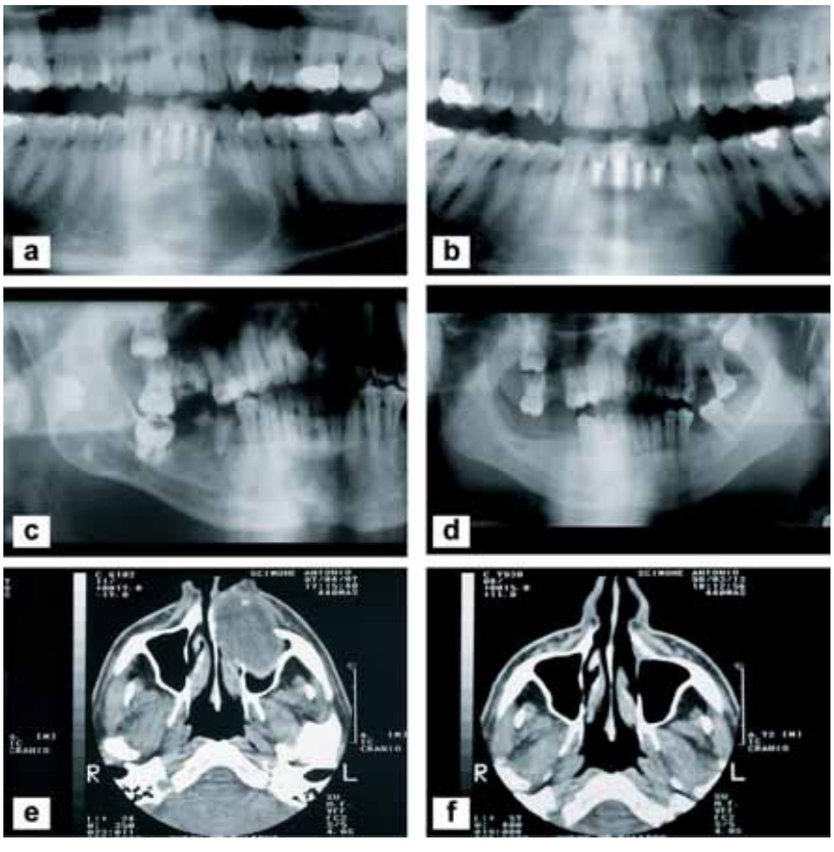

Fig. 2 Panoramic radiographs of two cases before surgery of the radicular and follicular cyst respectively (a, c) and during the advanced regeneration (b, d) and the TC of another case before surgery of the radicular cyst (e) and during the advanced regeneration (f).

In vitro stimulation of osteo-coagulum-derived cells and protein expression

To shed light into the early events characterizing the osteo-coagulum remodeling, the cell populations within the sample tissues were isolated and stimulated to differentiate into osteoblasts or osteoclasts.

Figure 3 shows the optical microscopic images of the cultured primary cells in the absence and after the stimulus with factors that can induce differentiation into osteoblasts or osteoclasts. It is known that cells isolated from tissue samples have, in the absence of specific stimuli, a fibroblastoid appearance, characterized by the tapered shape (Fig. 3a). The addition of thyroxin (0.5 mg/mL) and vit. D3 (1 nM/mL) to the medium induces the maturation of cells into osteoblasts, as evident from figure 3c and 3d, in which cells have a hexagonal shape and are more "spreading" compared to fibroblastoides. However, the stimulation with factors that induce differentiation into osteoclasts (5ng/mL, GM-CSF and 1nM/mL vit. D3) was not able to induce formation of cells with osteoclastic characteristics, clearly demonstrating the absence of progenitors of this type of cells (Fig. 3b).

Cell lysates have also been analyzed from a biochemical point of view, by Western blotting (Fig. 4), to highlight the presence of

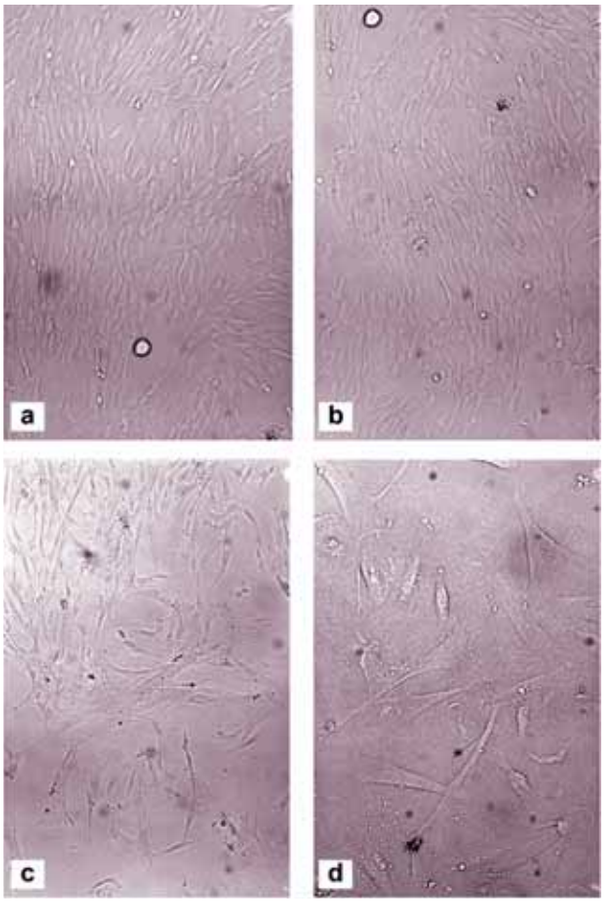

Fig. 3 Optical microscopic images of the cells obtained from the osteo-coagulum in the absence (a) and after the stimulus with GM-CSF + vit. D3 (b), or thyroxin + vit. D3 (c, d); Magnification 400x(a-c), 600x (d).

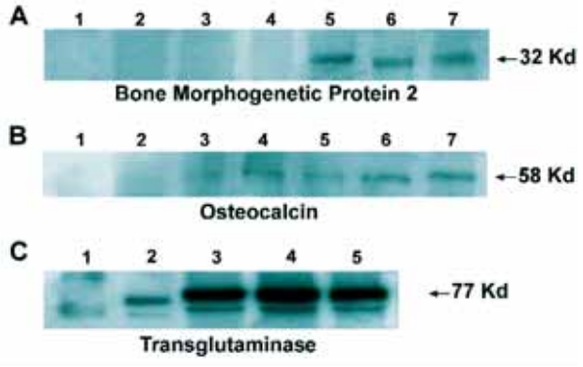

Fig. 4 Western blotting of bone morphogenetic protein 2 (BMP 2), osteocalcin (OC), and transglutaminase (Tgase) performed on protein extract of the primary cell cultures stimulated with vit. D3, GM-CSF , or thyroxin as specified below.

A: lane 1: not stimulated cells; lanes 2-4: GMCSF / vit.D3 stimulated cells; lanes 5-7: thyroxin / vit. D3 stimulated cells.

B: lane 1: molecular weight standard; lane 2: not stimulated cells; lanes 3-4: 2 and 4 days treatment of cells with GM-CSF / vit.D3; lane 5: 4 days thyroxin stimulated cells; lanes 6-7: 2 and 4 days thyroxin / vit. D3 stimulated cells.

C: time dependent immuno-expression of TGase in vit.D3 / thyroxin stimulated cells. Lane 1: molecular weight standard; lane 2: not stimulated cells; lane 3: 2 days stimulated cells; lane 4: 4 days stimulated cells; lane 5: 7 days stimulated cells.

specific osteoblasts proteins, like osteocalcin and BMP 2, a potent local factor with a pro-osteogenic action (Pagani et al., 2005; Samee et al., 2008; Kato et al., 2009). The results obtained with specific antibodies indicated the presence of osteocalcin and BMP-2, demonstrating unequivocally the differentiation in osteoblasts of the stimulated cells. Thyroxin (Fig. 4a, Lanes 5-7) but not GM-CSF (Fig. 4a, Lanes 2-4) induces BPM-2 expression in vit. D3 treated cells. Thyroxin and vit. D3 positively regulate osteocalcin immuno expression (Fig. 4b, Lanes 6-7), whereas GM-CSF inhibits (ten fold, meaured by densitometric analysis) the vit. D3 osteocalcin-induced expression (Fig. 4b, Lanes 3-4). The positive effects of thyroxin (Fig. 4b, Lanes 5) and vit. D3 appear additive and not synergic.

The expression of transglutaminase (Tgase), a key regulator of remodeling and mineralization of the extracellular matrix and of osteoblast differentiation (Aeschlimann et al., 1996, 2000; Nurminskaya and Kaartinen, 2006; Al-Jallad et al., 2006) was also analyzed in the early events of clot remodeling. As observable in figure 4c Lane 2, transglutaminase is expressed in not stimulated cells and a great increase of expression is obtained after stimulation with thyroxin and vit. D3, with a maximum of expression after four days of stimulation (Fig. 4c Lane 3-5).

Ultrastructural morphological analysis of the samples taken at different times.

- SEM and TEM analysis of the samples at 15 days from surgery: SEM analysis highlights the presence of cells immersed in a loose matrix of fibrin (Fig. 5a, 5b). Most of these have a round shape, although rare cells with fibroblastic aspect are also evident. TEM analysis reveals typical elements of a granulation tissue with white blood

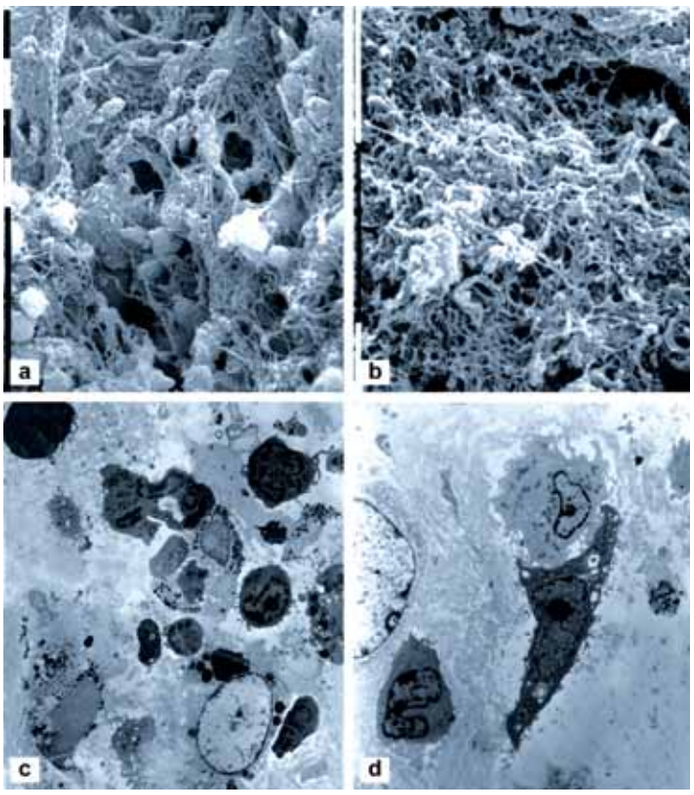

Fig. 5 SEM and TEM analysis of the osteo-coagulum after 15 days from surgery. The presence of cells immersed in a loose matrix of fibrin were highlighted at SEM (a, b). The TEM analysis reveals typical elements of a granulation tissue with white blood cells and osteoblasts. Neo-angiogenesis is absent. Magnification: a,1100 x; b, 4000x; c,4600x; d, 2800x.

cells and preosteoblasts (Fig. 5c, 5d). Neo-angiogenesis is absent.

- SEM analysis of the samples after 30 days from surgery: SEM analysis at 30 days from surgery shows a tissue organization significantly different from the previous sample. In particular, there is a compact surface of the tissue, with the presence of vascular elements, indicative of an intense neo-angiogenesis (Fig. 6 a, b). It is possible to observe a remarkable deposition of collagen fibers arranged in a very orderly way and the presence of vessels that permeate the fibers themselves (Fig. 6c, 6d).

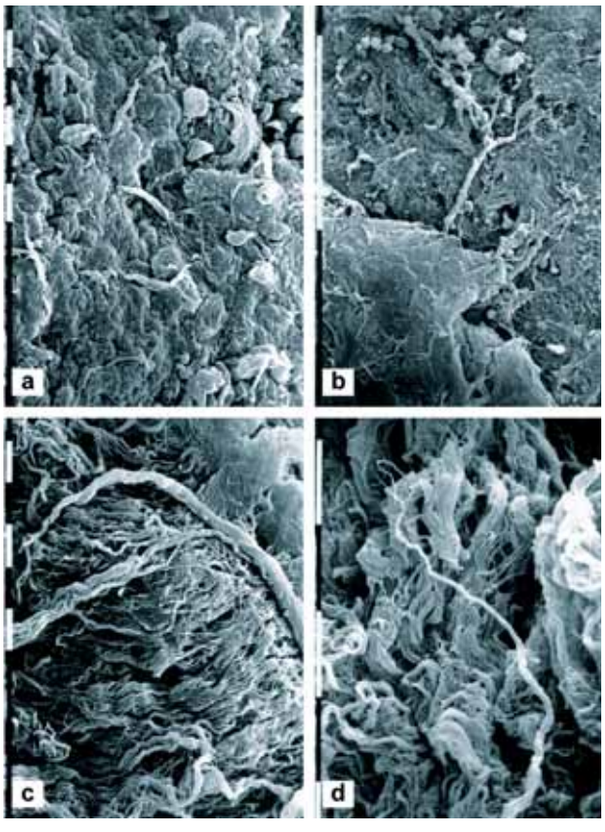

Fig. 6 The SEM analysis of the osteo-coagulum after 30 days from surgery. Compact surface of the osteo-coagulum, with the presence of vascular elements, a sign of intense neo-angiogenesis (a, b). Remarkable deposition of collagen fibers arranged in a very orderly way and presence of vessels that permeate the fibers themselves (c, d). Magnification: a,1100 x; b, 500x; c,1100x; d, 2200x.

- TEM analysis of samples obtained after 30 days from surgery: TEM shows the presence of collagen deposits (Fig. 7a, 7b) and fibroblastoid cells with active synthesis and secretion (Fig. 8a). Ossification nuclei at an initial stage are also observable (Fig. 8c).
- SEM analysis of the sample after 60 days from surgery: SEM analysis of the samples after two months from surgical treatment shows an increased organization of collagen fibers and in particular their "packaging" (Fig. 9a). It is interesting to note the presence of precipitates in the context of collagen fibers (Fig. 9b).
- SEM analysis of the samples after 120 days from surgery: SEM analysis after 120 days from surgery shows an osteogenic maturation of the sample (Fig. 10a, b). Remodeling and the presence of vessels are still evident; areas of incomplete transformation of bone is observable in some districts of the sample.

DISCUSSION

In the present study the postoperative healing of the cystic cavity was chosen as experimental model of bone regeneration. The morphological, cellular and molecular

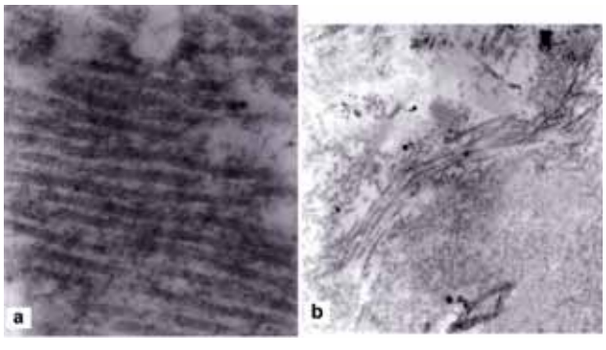

Fig. 7 TEM analysis of samples obtained after 30 days from surgery. Presence, in the osteo-coagulum context, of collagen deposits (a, b). Magnification: a,60000 x; b, 22000x.

events characterizing the stages of regeneration, from the clot to the bone formation within the cystic cavity were investigated. Samples of regenerating tissue after 15 days (osteo-coagulum), 1, 2 and 4 months from the surgery procedure were analyzed.

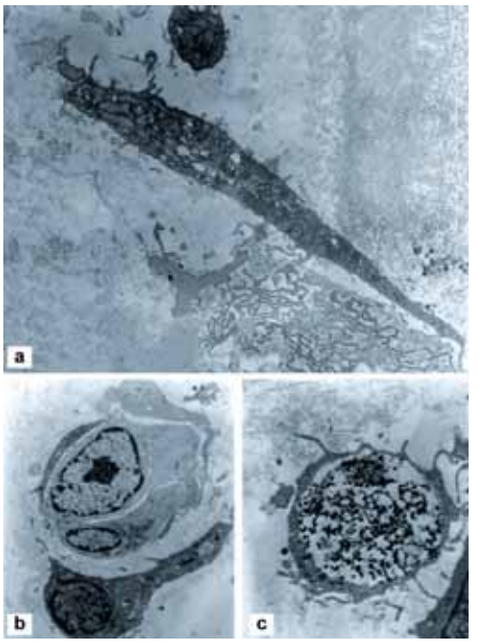

Fig. 8 TEM analysis of samples obtained after 30 days from surgery. Fibroblastoid cells with active synthesis and secretion (a); signals of neo-angiogenesis (b); ossification nuclei at an initial stage (c). Magnification: a,10000 x; b, 6000x; c,10000x.

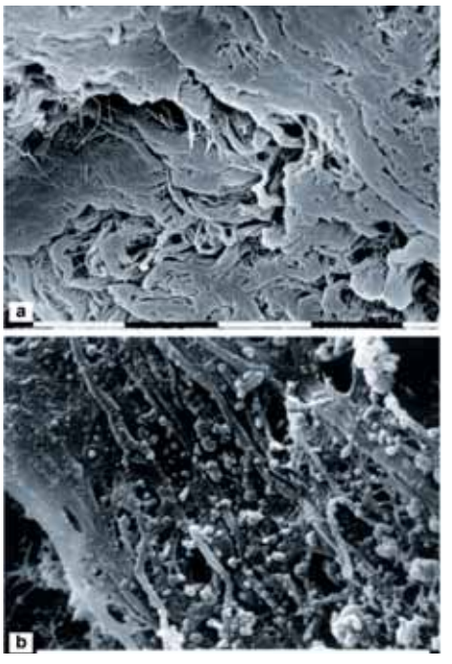

Fig. 9 SEM analysis of the osteo-coagulum after 60 days from surgery. "Packaging" of collagen fibers (a). Presence of precipitates in the context of collagen fibers (b). This sample emphasizes the passage from the first to the second phase of the bone regeneration. Magnification: a,2200 x; b, 9100x;.

From the ortopantomographies and TC scanning analyses of the cases reported it is evident that there is a complete regeneration of bone affected by the osteolytic disease, both for small (1 cm) and large (10-12 cm) cysts, although in different times. This observation is particularly significant and it confirms that the bone regeneration inside the cystic cavities occurs spontaneously, only owing to the remodeling of the clot (osteo-coagulum remodeling) resulting from bleeding during surgery, without the help neither of bone grafts nor of growth factors, whatever the cyst size (Chiapasco et al., 2000; Ihan and Mijavec, 2008).

The in vitro stimulation of osteo-coagulum-derived cells and protein expression demonstrated that thyroxin and vit. D3

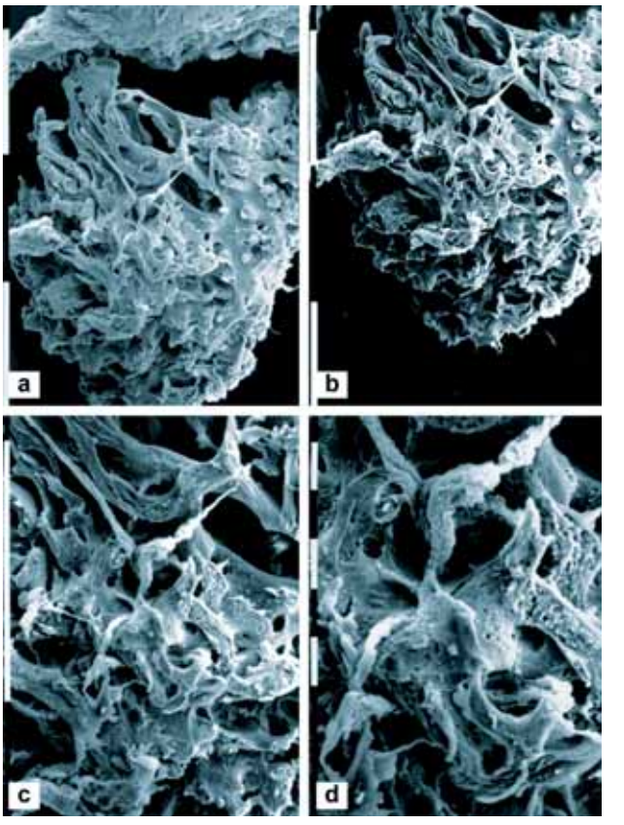

Fig. 10 SEM of the osteo-coagulum after 120 days from surgery. Maturation in the osteogenic sense of the osteo-coagulum (a, b). Magnification: a,350 x; b, 330x; c,660x; d, 1300x.

positively regulate osteocalcin immuno-expression (Fig. 4B, Lanes 6-7), whereas GM-CSF inhibits (ten fold, meaured by densitometric analysis) the vit. D3 osteocalcin-induced expression (Fig. 4B, Lanes 3-4). The positive effects of thyroxin (Fig. 4B, Lanes 5) and vit. D3 appear additive and not synergic. The observed inhibitory effect of GMCSF on vit. D.3 osteocalcin-induced is in accord with Evans et al. (1989).
As regard the expression of transglutaminase, the reported data appear particularly intriguing, because this enzyme is able to remodel the extracellular matrix, creating isopeptidic covalent bonds between the various proteins in the matrix itself and to induce polymerization of pericellular skeletal matrix calcium-binding proteins that promote nucleation and/or growth of calcium-containing crystals (Aeschlimann et al., 1996). Kaartinen et al. (1997, 1999) demonstrated that osteocalcin is permanently linked by transglutaminase to the bone proteic matrix. Although the results reported on cell cultures have been obtained in vitro, it is likely that a similar biological phenomenon also occurs in vivo.
The ultrastructural morphological analysis shows different stage of evolution at different times.
At 15 days the presence of immunocompetent cells in the early stages of the repair process, as well as the intimate contacts established between leucocytes cells and preosteoblasts, support the view that the cytokines produced by these cells induce osteoblastic maturation of undifferentiated mesenchymal cells (Fig. 5d) (Meng et al., 2008; Horowitz and Lorenzo, 2007).
At 30 days it is possible to note neo-angiogenesis (Fig. 8b), important for the development of the new tissue. Neo-angiogenesis plays a pivotal role for the surviving of the regenerating tissue in the maintenance of homeostasis and providing the communicative network to neighboring tissues to respond to requirements as needed (Kanczler and Oreffo 2008). Recently Mödder and Kossla (2008) hypothesized another important role of the developing blood vessels as a source of progenitor bone cells.
At 60 days there is the passage from the first to the second phase of bone regeneration. The first phase is characterized by the generation of an osteoid matrix replacing the osteo-coagulum. This matrix is compact, confused, not mineralized and rich in vessels. The second phase arises with the osteoclastic action that digesting the osteoid matrix promotes the secretion of several growth factors (BMPs) present in the matrix inducing osteoblasts recruitment, proliferation, and differantion. In this way begins the production of an ordered matrix that starts to mineralize up to become mature bone.
Finally, data indicate that after 4 months bone maturation is still not completed.
In the experimental model of bone cyst we have highlighted that, in the case of cavities resulting from cyst enucleation, the situation for the osteogenesis is almost ideal if the presence of the clot is guaranteed across the entire length of the cavity and the integrity of the overlying periosteum. However, these conditions, which are stringent for a good osteogenesis, do not occur when you want to get an appositional bone growth, because in this case it is necessary to implant a material, which defines the area to regenerate and allows the biological processes above described.
The autologous bone, although currently considered the best grafting material, limits the completion of the biological processes necessary for an effective osteogenesis, especially because of the difficult cell migration and revascularization across the length of the graft.
Currently, there is no graft material that provides a 100% controlled bone

regeneration. The understanding of the involved biological phenomena is stimulating the development of techniques that are more and more distant from the simple "filling" made with several different materials. The aim of these techniques is the creation of an environment that supports the biological processes.
The results obtained indicate that the ideal material for bone regeneration, beyond its physical and mechanical characteristics, must be especially able to facilitate the migration of progenitor cells from the peripheral tissue, the adhesion to the material, the amplification, the differentiation and to promote the deposition of the extracellular matrix. This must occur through the presence of specific biochemical signals, normally present in the matrix of the normal bone tissue. The material must also possess a high degree of porosity to allow the passage of newly formed vessels in order to ensure the vascularization of the regenerating tissue. Moreover it must not interfere with the processes of the osteo-coagulum reorganization, that is madatory for all the models of regeneration. Obtaining a material of this kind, able to ensure the normal carrying out of the physiological processes of regeneration, would be undoubtedly a major achievement.

CONCLUSION

The results obtained confirm that a spontaneous bone regeneration occurs after enucleation of jaw cysts even of large dimensions.
Remodeling of the matrix begins after 15 days and is evident after 1 month, also demonstrated by the high expression level of transglutaminase in the preosteoblastic cells isolated from the samples. The first phase of bone healing, characterized by an osteoid matrix replacing the osteo-coagulum, can be considered completed after about 2 months.
The present findings support the view that the basic conditions for a spontaneous bone regeneration are:

1) the migration of undifferentiated mesenchymal cells from the overhanging periosteum and of osteoblasts from the surrounding endostium;
2) the presence of a fibrin matrix, that is supporting the amplification and the differentiation of pluripotent mesenchymal cells;
3) the cooperation of platelets, macrophages and immunocompetent cells;
4) a significant neoangiogenesis which guarantees the vascularization of the new tissue.

All of these conditions, which emerge from the analysis of the osteogenesis process of the bone cystic cavity, allow to make a more general strategy which defines the conditions necessary to effectively activate the osteogenetic process when required by a specific pathology or surgical need.
Finally, it is our knowledge that a higher number of cases – possibly on one homogeneous type of cyst - has to be analyzed in order to confirm the reported data.

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