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Clinical Applications of Autologous Cryoplatelet Gel for the Reconstruction of the Maxillary Sinus. A New Approach for the Treatment of Chronic Oro-sinusal Fistula

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Abstract. *The authors report their clinical experience regarding an original method of surgical repair of oro-sinusal communications. From September 1999 to December 2003, 13 patients (7 male and 6 female patients; mean age: 52 years, range: 24-68 years) underwent surgical repair of an oro-antral fistula by means of cryoplatelet gel: in three patients, it was mixed with bioglass granules; in two, it was mixed with Bioss™; in three, it was mixed with particulate bone extracted by means of a bone grafter from the oral cavity close to the operative site, with addition of demineralised bovine bone; in three, it was used together with porose hydroxyapatite, and in two patients the cryoplatelet gel was used only. No postoperative complication was reported; primary wound healing was achieved within seven to nine days. A bony orthopantoscintigraphy was performed a few months following the operative procedure, showing an active osteogenic process. In eight patients, a CT was performed after 8 to 12 months from the operation, showing a normal pneumatization with reconstruction of the floor of the maxillary sinus. Although preliminary, these findings seem to suggest that the use of bioengineered materials coupled with growth factors and osteoprogenitor cells may represent a valuable alternative to autologous bone transplantation for the reconstruction of the maxillary sinus.*

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The anatomic continuity between the maxillary sinus and polycuspidate teeth of the superior arcade, mostly the second premolar and the first molar teeth, is regarded as a predisposing factor for the onset of odontogenous maxillary sinusitis. The pathogenesis of the disease is actually related to the transmucosal spread of infection from these teeth, mostly in the elderly patient. An apical granuloma, a cystic granuloma or a radicular cyst may develop from a chronic periapical infection. Frequently, the bony wall interposed is very thin or completely lacking, so that the cystic sac directly communicates with the maxillary mucosa. These radicular cysts may also break through the antrum, mimicking primary tumors of the maxillary sinus.

The surgical treatment is pathogenesis-related, so that the involved tooth should be extracted coupled with the drainage or curettage of the maxillary sinus and/or removal of the cyst. This approach is frequently complicated by sinusal perforations of various size, depending on the extent of the chronic inflammatory reaction, with alveolar fistula and subsequent oro-sinusal fistulas. When these fistulas become rather large, their plastic reconstruction is difficult to achieve because the surrounding mucosa cannot be transposed.

Clinical experience has been gathered on the use of cryoplatelet gel to speed the tissue healing process as well as bony reconstruction (1-3). Actually, the early phase of bone reconstruction is characterized by the release of osteoinductive growth factors, such as platelet derived growth factor (PDGF), transforming growth factor-beta (TGF-β), insulin growth factor-1 (IGF-1) and IGF-2, which are derived from platelet α-granules (4-6).

Since 1999, an autologous fibrin-platelet glue has been used for the reconstruction of bone defects in maxillofacial surgery at the Division of Surgical Oncology of the National Cancer Research Institute of Genoa and the clinical results of this preliminary experience are reported.

Table I. *Clinical characteristics of patients.*

Age years	Type of fistula	Bony defect area cm^2	Biological model	Type of membrane	Capture time (months)
57	Post-extractive fistula	1	PG Bioss	No membrane	>12
66	Bilateral post-extractive fistula	1	PG+ particulate bone	GORE-RESOLUT	12
		2	PG+ particulate bone	GORE-RESOLUT	20
48	Post-extractive fistula	2	PG+hydroxiapatite	COLLAGEN (ossix) (6 mo)	>12
60	Post-extractive fistula	1.5	PG+ particulate bone bioss	COLLAGEN (3 mo)	12
24	Naso-palatine communication	2	PG+ hydroxyapatite	COLLAGEN (3 mo)	12
38	Alveolo-sinusal fistula after radicular cyst removal	1	PG+ hydroxyapatite	COLLAGEN (ossix) (6 mo)	12
62	Post-Caldwell-Luc fistula	0.5	PG+ particulate bone	Vicryl	n.e.
54	Post-extractive fistula	0.7	PG+Bioglass	COLLAGEN (3 mo)	>12
68	Post-extractive fistula	1	PG+Bioglass	COLLAGEN (3 mo)	> 12
53	Post-extractive fistula	0.5	PG	COLLAGEN (3 mo)	n.e.
27	Post-extractive fistula	1.5	PG+ Bioglass	COLLAGEN (3 mo)	n.e.
60	Post-Caldwell-Luc fistula	2	PG+particulate bone	No membrane	> 12
61	Palato-sinusal fistola after naso-palataline cyst removal	1.5	PG	No membrane	> 12

PG: platelet gel; n.e.: not examined.

Patients and Methods

From September 1999 to December 2003, 13 patients (7 males and 6 female patients; mean age: 52 years, range: 24-68 years) underwent surgical repair of an oro-antral fistula by means of autologous fibrin-platelet glue. All patients were fully informed before giving their written consent to the procedure and the study protocol was approved by the Ethics Committee of the National Cancer Research Institute of Genoa.

No patient had preoperative infection at the operative site; an oral betalactamic antibiotic prophylaxis was started on the day before operation up to the seventh postoperative day. Patient characteristics are given in Table I along with repair details.

Technique for preparation of cryoplatelet gel. The day before operation, the patient was accepted at the Immunohematology Service where 450 mL of whole blood were collected. The blood was immediately centrifuged to obtain packed red blood cells (PRBC) and platelet-rich plasma (PRP). PRBC were reinfused to the patient. PRP was centrifuged to obtain platelet-poor plasma (PPP) and PC. PPP was immediately frozen at –80˚C in a mechanical refrigerator, the frozen plasma (FFP) was then kept at +4˚C for 18 h for spontaneous thawing. Cryodepleted plasma was removed and the residual cryoprecipitate dissolved in 30 mL of plasma. PC was kept at +22˚C under continuous agitation.

(i) Quality control

Platelet concentrate: Platelet count: $60x10^9$; residual leucocyte: $0.2x10^9$; volume: maximum 30 mL.

Cryoprecipitate: Factor VIII: 70 UI x100 mL; Fibrinogen: 140 mg/unit; Volume: maximum 30 mL.

(ii) Preparation

One platelet aliquot was mixed with one aliquot of cryoprecipitate in a sterile plastic Petri dish. For every 10 mL of PC-cryoprecipitate solution 1 mL of autologous thrombin and 1 mL of calcium gluconate were added then the contents of the Petri-dish were mixed in order to produce a gel-like material in 10-15 min. Cryoplatelet gel was kept at room temperature and used within 8 h.

Operative procedure. The procedure was always accomplished with local anaesthesia; the fistulous orifice was widely excised, including the tissue surrounding the fistulous tract as well as its endosinusal invagination, with exposure of the bony margins of the communication. The fistulous tract was cleared and micro-perforations were performed in order to ease the permeation of the fibrin glue; hence, the cryoplatelet gel was assembled with biogranules or autologous lamellar bone tissue that was collected from the hemimandible by means of a bone grafter (Figure 2). Bioss or Bioglass are biogranules of smaller size which most resemble natural bone and were used to repair small defects while larger fistulous tracts were usually repaired with hydroxyapatite (ENGIPORE, Finceramica, Faenza, Italy) or particulate bone.

The cryoplatelet gel was used to fill the fistulous tract, care being taken to completely fill the cavity as well as its margins. Finally, the operative site was covered by means of an absorbable collagen membrane or a non-absorbable Gore-Tex membrane, in order to avoid the colonization of the area of bony regeneration by epithelium or connective tissue, so that the slower migrating cells with osteogenic potential were allowed to repopulate the defect. This membrane was put under the periosteum both on the palatal and vestibular side, after adequate dissection. A buccinator myomucosal flap was used to close the surgical wound; the outcome was strictly related to the complete closure of the wound, in order to avoid the exposure of the biological graft.

Follow-up imaging procedures. The follow-up of the biological implant included a computerized tomography (CT) and a bone tomoscintigraphy in order to check the ossification process. Bone tomoscintigraphy required the administration of radiotracers characterized by a highly specific bony uptake, such as ^{99m}Tc bifosphonates (metilendiphosphonate – MDP or hidroxietilendiphosphfonate – HDP). ^{99m}Tc has an half-life of 6 h and a gamma-

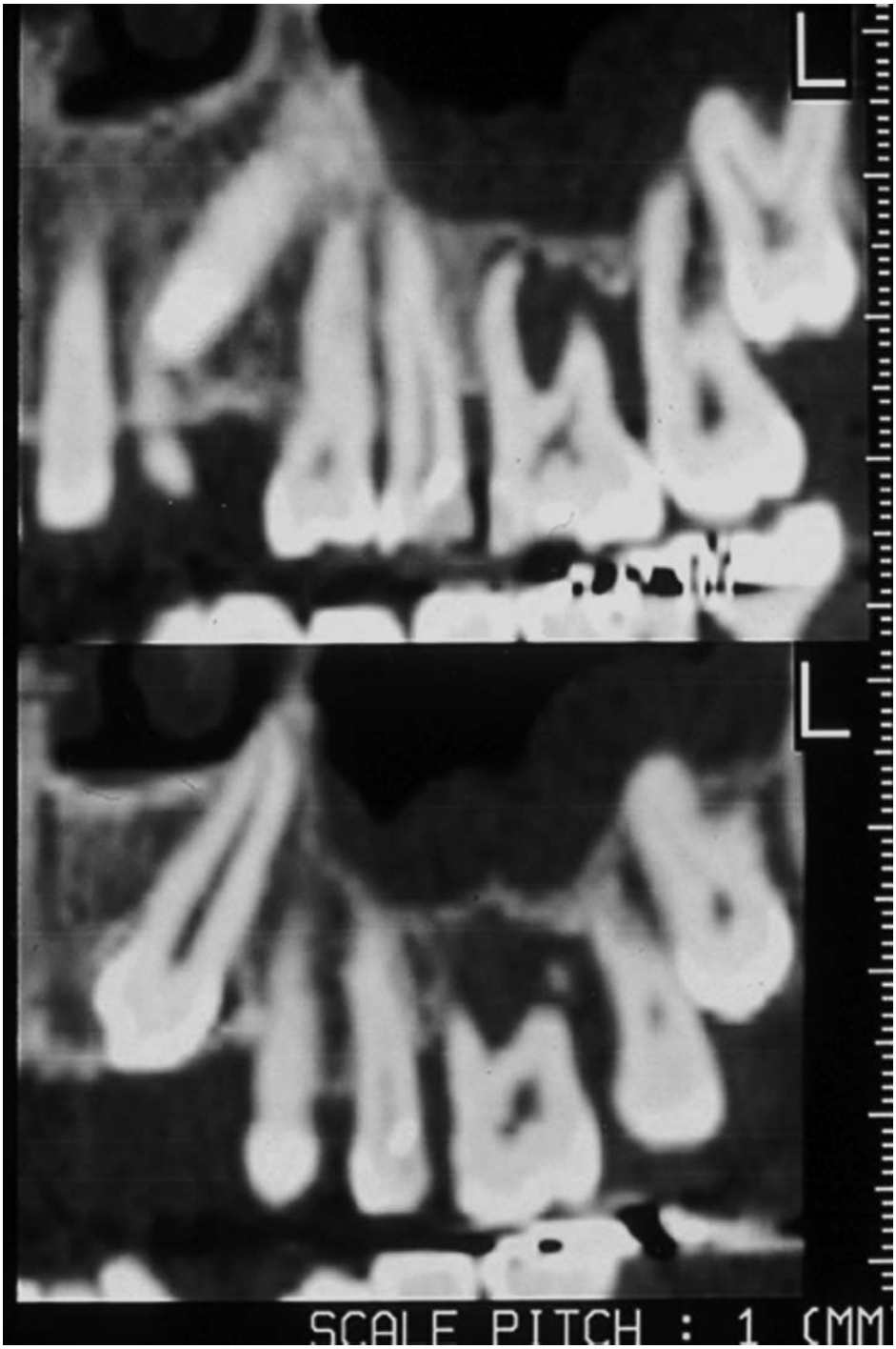

Figure 1. *Tomographic examination showing a case of chronic apical parodontopathy with communication within the floor of the maxillary sinus and inflammation of the sinusal cavity.*

emission of 140 KeV. At tissue level, the amount of radiotracer reaching the bone reconstruction area depends on the regional blood flow and bone metabolism. These processes justify the high sensitivity of this diagnostic procedure, which is far more accurate as compared to conventional X-ray radiography. Tomographic images (Single Photon Emission Tomography, SPET) are acquired using a "large-field of view" two head gamma-camera (Millenium, GE Wisconsin USA) equipped with high resolution collimators. Tomographic images were evaluated with a qualitative method (7-8). The study was completed with acquisition of planar images of the skull in anterior

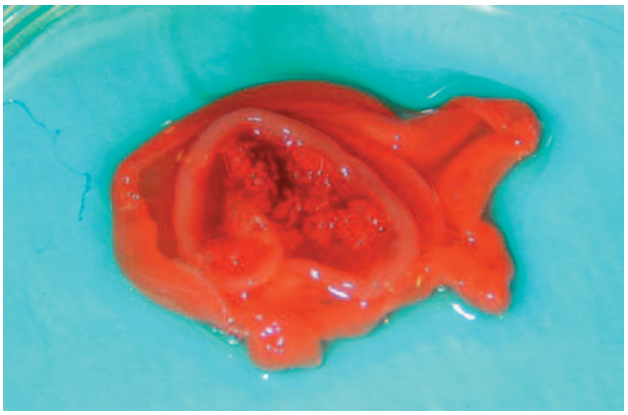

Figure 2. *Cryoplatelet gel in Petri-dish, mixed with autologous particulate bone tissue.*

and lateral views. The spatial resolution of the reconstructed tomograms and planar images was approximately 7 mm. Multiple regions of interest (ROIs) of the same size in the area corresponding to the osteointegrated implants and in the opposite regions (at the control sites) were generated using tomographic slices in order to assess the implant and peri-implant osteoblastic activity. Count density ratios (counts/pixel) obtained from each ROI were used for a quantitative/relative assessment of the tracer uptake, reflecting the bony metabolic status. A slow decrease with time in tracer uptake was considered a good prognostic factor (Figures 3-4).

Results

The operative procedure was always well-tolerated, with mild postoperative pain that was well controlled with non-steroideal anti-inflammatory drugs in the first postoperative day. No infection or postoperative bleeding occurred; in the early postoperative period, no symptom of oro-sinusal communication was reported. Primary wound healing was always achieved within seven to nine days.

As regards the intraoperative preparation of the cryoplatelet gel, a good clot was always obtained; as soon as calcium gluconate and thrombin were added to the PRP, platelets were activated and released their granules with the corresponding growth factors. At the same time, thrombin and calcium induced the clotting process that converted fibrinogen into fibrin with the production of an easily manipulated gel. Fibrinogen concentration in the cryoprecipitate was close to 1000 mg/dl, while platelet concentration within the cryoprecipitate was $15x10^6$ /mm^3.

A bony orthopantoscintigraphy was performed a few months following the operative procedure, showing an active osteogenic process. A computerized tomography was performed in eight patients after 8 to 12 months from the operation, and a normal pneumatization with reconstruction of the floor of the maxillary sinus was observed (Figure 5). In one patient, the flap underwent necrosis but no re-operation was required because, after its clearing 14 days from the operation, a well vascularized tissue developed, completely filling the bony defect and, after 20 days, complete epithelization occurred.

Discussion

Until recently, the surgical approach to oro-sinusal fistula included the surgical debridement on its borders with marginal resection and endosinusal invagination. The reconstruction of the operative field was accomplished by means of a buccinator myomucosal flap and/or free lingual flaps (9-10). Martin-Granizo (11) suggested filling the fistulous tract with adipose tissue collected from the cheek in order to ease the proliferation of the original connective tissue, although it depends on the amount of adipose tissue that can be harvested from the patient. However, all these techniques try to obtain the closure of the fistula without recreating the bony tissue of the alveolar process.

In order to reconstruct the bone defect, autologous bone transplantation was performed. Haas *et al.* (12) reported that five patients with oro-antral fistulas of different pathogenesis were treated with autogenous monocortical bone block harvested from the chin: press-fit closure for bony repair of the basal maxilla was accomplished in three patients, the other two patients required additional internal graft fixation. Watzak *et al.* (13) treated 21 patients with oro-antral fistulas using monocortical bone blocks harvested from the retromolar or interforaminal regions of the mandible. Press-fit closure for repair of the bony sinus floor was sufficient in 17 patients. Four of them needed additional internal fixation. Three patients developed wound dehiscence at the grafted sites with secondary healing. The use of autologous cancellous bone is associated with postoperative morbidity due to longer operative time for tissue harvesting, higher bleeding, greater risk of infection and postoperative pain, and the amount of bone may not be enough with progressive re-absorption along time.

On these grounds, a technique of tissue bioengineering with autologous growth factors and biomaterials was developed. The role of growth factors in bone regeneration is well established: thirty years ago, Marshall Urist (14) described the role of bone morphogenetic protein (BMP) in the osteogenetic process. In 1998, Marx *et al.* (15) proposed a model of bone regeneration that was observed in cancellous cellular marrow grafts.

More recently, the use of autologous cryoplatelet gel in order to speed the tissue healing process, as well as to ease bony reconstruction, has gained special interest because it can be well fitted for filling tissue defects, especially in oro-maxillofacial surgery (3, 16-17). Autologous cryoplatelet gel is rich in platelets, which in

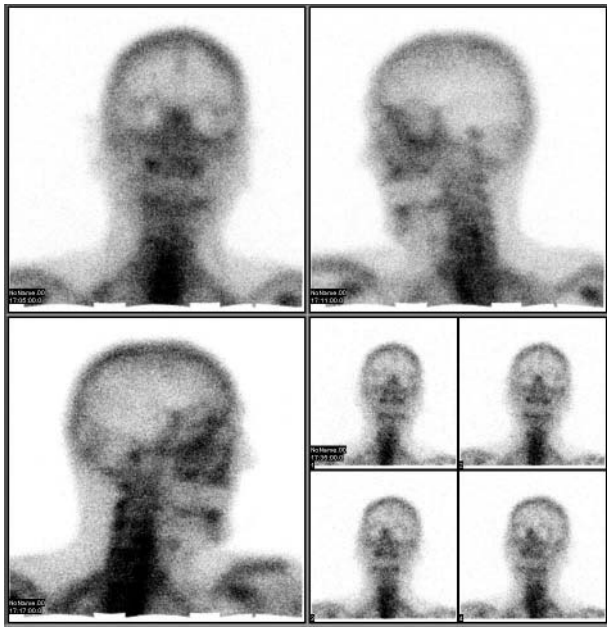

Figure 3. *Orthopantomographic hypercaptation after three months.*

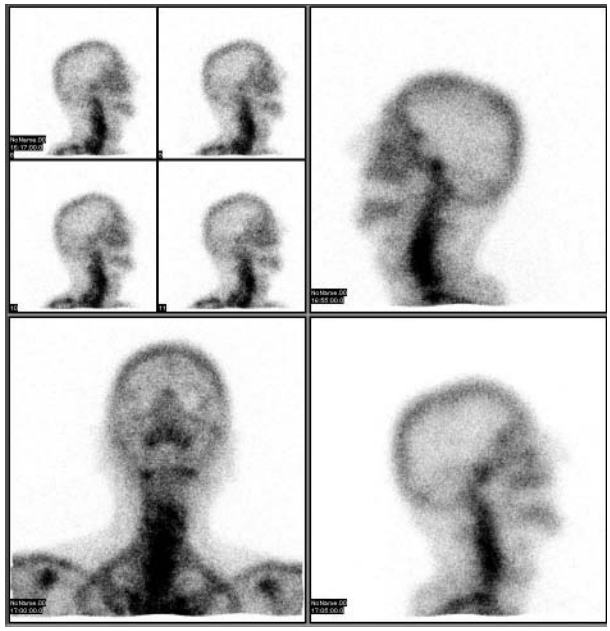

Figure 4. *Orthopantomographic: no captation ten months after surgery.*

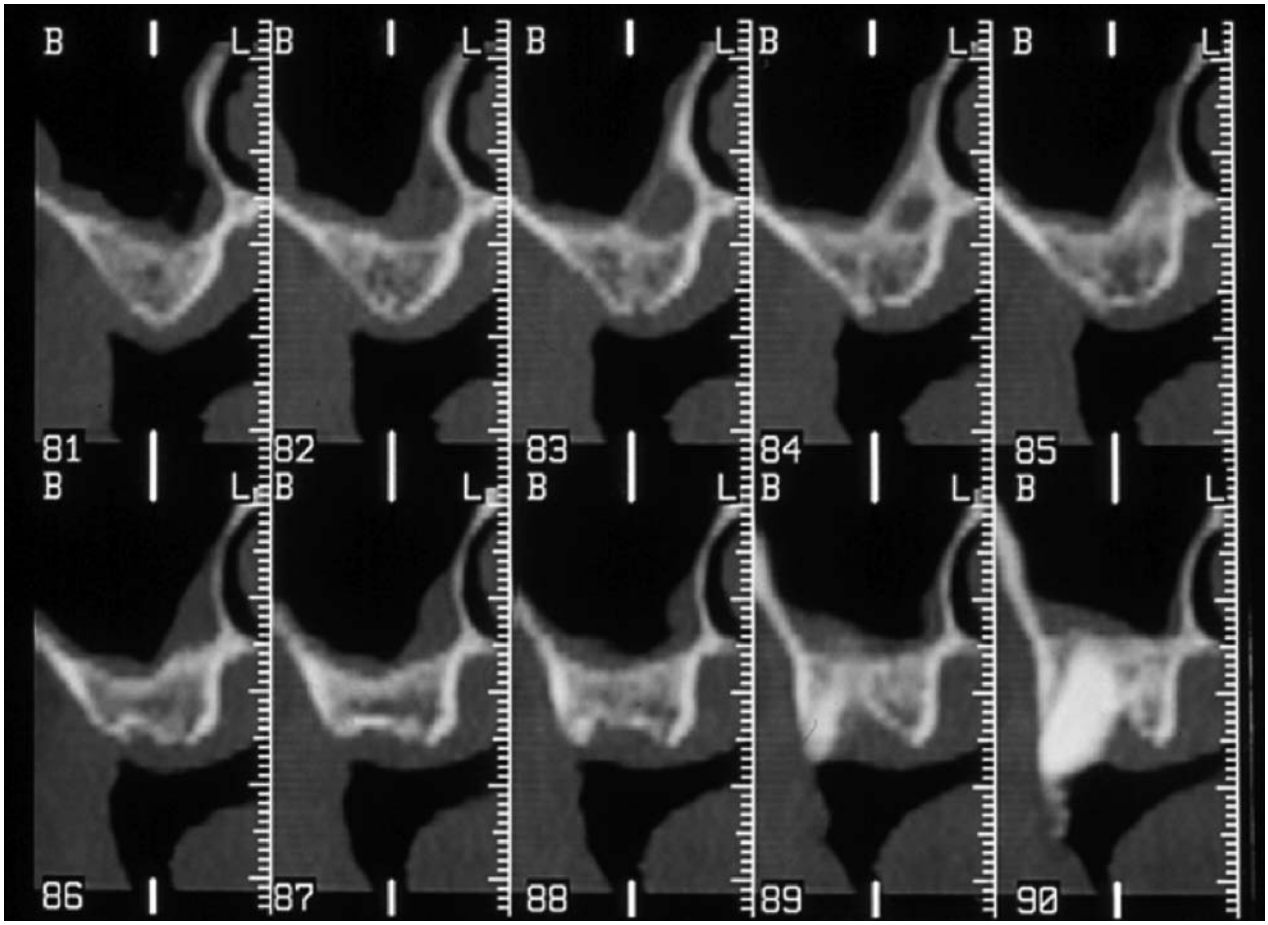

Figure 5. *Tomographic examination after one year.*

turn release growth factors, such as PDGF, TGF-β1 and TGF-β2, EGF, and IGF-1 and IGF-2, which play an important role for cell differentiation, wound healing, and stimulation of both proliferation and differentiation of osteoblasts. Platelet-derived growth factors are among the first growth factors that can be detected within a wound and promote connective tissue healing, including bone regeneration. The most relevant activities of PDGF include mitosis, angiogenesis and macrophage activities (18). Quarto *et al.* (19) reported three cases of large bone defect repair (right tibia, right ulna, right humerus) with the use of autologous marrow stromal cells grown on scaffolds of macroporous hydroxyapatite. In all patients, radiographs and tomographic scans revealed abundant callus formation along the implants and good integration at the interfaces with the host bones within the second postoperative month.

Robiony *et al.* (20) proposed a new method for restoring severe atrophic mandible using platelet-rich plasma during alveolar ridge distraction osteogenesis, indicating an adequate mandibular restoration and the possibility of implant placement just 60 days after the surgery. Warnke *et al.* (21) reported the results of a technique of heterotopic bone induction to obtain a mandibular replacement inside the latissimus dorsi muscle. The authors used a titanium mesh cage filled with bone mineral blocks and infiltrated with recombinant human BMP-7 and bone marrow mixture. Seven weeks after surgery, the patient underwent transplantation of the mandibular replacement as a free bone-muscle flap.

Autologous cryoplatelet gel has found different clinical applications at the Division of Surgical Oncology of the National Cancer Research Institute of Genoa, with satisfactory results. Since 2000, we have been successfully using this technique after oncological surgical procedures of the maxillofacial region, mandibular reconstruction, surgical repair of alveolar defects, and associated oro-antral nasal fistulas. This latter application seems to show different advantages, such as: use of autologous tissue, relatively easy surgical procedure, possibility of performing the operation with local anaesthesia, no need for wide flap reconstruction with low postoperative complications and postoperative discomfort, as well as low cost. Moreover, this technique allows close oro-antral communications not only with soft tissue reconstruction but also with bony reconstruction of the floor of the maxillary sinus.

Regarding bone tomoscintigraphy assessment, significantly lower levels of tracer uptake immediately after grafting and during graft healing represented a lack of bone turnover due to decreased revascularization. Our findings indicated that the time required to completely stabilize the biological process of reconstruction of the oro-sinusal communications ranged from 12 to 18 months, and at that time the neo-formed bone was quite similar to pre-existent bone. However, clinical follow-up is still limited for us to be able to draw final conclusions.

An experimental randomized study is currently ongoing for better definition of the response of the biological model with regard to the time of stabilization of the bony regeneration as well as the possibility of using titanium implants within bone neoformation. Notwithstanding these preliminary findings, the use of bioengineered materials coupled with growth factors and osteoprogenitor cells seems to represent a valuable alternative to autologous bone transplantation and, hopefully, the future of bone reconstructive surgery.

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