Journal of Dental Sciences (2015) 10, 115—122

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

REVIEW ARTICLE

Fresh frozen bone in oral and maxillofacial surgery

Luigi Fabrizio Rodella [a*], Marco Angelo Cocchi [a], Rita Rezzani [b], Pasquale Procacci [c], Lena Hirtler [d], Pierfrancesco Nocini [e], Massimo Albanese [c]

[a] *Section of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, University of Brescia, Italy*
[b] *Resident Chief, Section of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, University of Brescia, Italy*
[c] *Section of Dentistry and Maxillofacial Surgery, Department of Surgery, University of Verona, Italy*
[d] *Centre for Anatomy and Cell Biology, Department for Systematic Anatomy, University of Wien, Austria*
[e] *Resident Chief, Department of Surgery, Section of Dentistry and Maxillofacial Surgery, University of Verona, Italy*

Received 4 June 2014; Final revision received 5 August 2014
Available online 31 January 2015

KEYWORDS
fresh frozen bone;
maxillofacial surgery;
oral surgery

Abstract The aim of the current study was to review the use of fresh frozen bone (FFB) in oral and maxillofacial surgery. We performed a review of the articles published in the literature between 1976 and May 2014 analyzing three medical databases (PubMed, Cochrane Library, and Embase) and using specific search terms. Literature analysis on FFB applications in oral and maxillofacial surgery revealed 47 articles between 1976 and May 2014. There are 46 clinical articles and one review. Clinical articles are represented by 22 case reports and case series and 24 retrospective studies. Classifying the scientific production by year of publication, it is evident that especially during the last 6 years there was an increase of FFB graft use in oral and maxillofacial approaches. The literature analysis on FFB's use shows that its application in oral and maxillofacial surgery began slowly in 1992 with Perrott and since 2006 it had a real development. The recent significant increase emphasizes the importance of FFB for bone regeneration in oral and maxillofacial surgery. This review found consistent evidence of FFB's use increase in oral and maxillofacial surgery suggesting a valid instrument for bone regeneration. To date, risks connected to the infections' transmission and to the immunogenic

* Corresponding author. Section of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, Viale Europa, 11, 25123, Brescia, Italy.
E-mail address: luigi.rodella@unibs.it (L.F. Rodella).

http://dx.doi.org/10.1016/j.jds.2014.08.002

potential are extremely low and could be considered practically absent. So, this is an important alternative in the preimplant reconstructive surgery.

Introduction

Bone reconstruction techniques are extensively used in oral and maxillofacial surgery.[1–5] The most important applications include the maintenance of postextraction alveolar volume, maxillary sinus elevation, restoration of the maxilla and mandible ridge, treatment of odontogenic cysts, and orthognatic surgery.

With the exclusion of the osteogenetic distraction techniques and grafts of vascularized flaps, all the other bone reconstruction procedures involve the use of bone or bone substitute materials.[6–8] Osteogenetic graft material directly stimulates osteoblasts inducing the production of bone tissue, osteoinductive material induces differentiation of mesenchymal cells into chondroblasts and/or osteoblasts, and finally, osteoconductive material facilitates the proliferation, cell migration, and apposition of new bone tissue on its surface or, if it has adequate porous structure, in its interior.[9–11]

The bone grafts can be classified into two major groups: bone blocks and particulate bone. Bone blocks can be subdivided as follows: cortical, cancellous, and cortical-cancellous.[12] In addition, on the basis of their structure, even if with some differences in their mechanical characteristics, all these type of bone can be adequately modeled and adapted to the defects.

The integration of bone grafts is a sequential process involving inflammation, neovascularization, osteogenesis, and bone remodeling in which graft stabilization and vascularization play a pivotal role.[13]

Bone grafts can additionally be classified into heterologous bone if it is transferred from one species to another; autologous bone if it is transferred on the same patient; and bone allografts or homologous bone if transferred between members of the same species.

Autologous bone graft material has always been considered as the gold standard because it showed osteogenic osteoconductive and osteoinductive properties.[14] In addition, no immunological reactions are expected.[15] Nevertheless, it presents some disadvantages, including increased operating time that must include the time of bone harvesting, and an increase in morbidity and postoperative risks in case of extraoral sampling. The autograft is widely used both as particulate and blocks, alone or in combination with osteoconductive materials. Those grafts are implanted either with or without membranes for the guided regeneration or together with preparations intended to improve the regeneration such as platelet-derived growth factors.[16]

Heterologous bone is mainly represented by deproteinized bovine bone and deantigenated equine bone.[17,18] Contrary to autologous bone it is available in unlimited quantities, but it is not osteogenic and osteoconductive.[19] In addition, it is associated with high costs and the possibility of pathogen transmission.[20]

Homologous bone is obtained from cadavers or from patients undergoing hip replacement surgery with removal of the head of the femur. femoral head. It has osteoconductive properties and it is potentially osteoinductive because its matrix contains growth factors such as bone morphogenetic protein (BMP) or vascular endothelial growth factor.[21–23] However, freezing causes almost total loss of cell viability; therefore, it has no osteogenic properties. Nevertheless, some authors showed a residual cell viability.[24]

Homologous fresh frozen bone (FFB) has already been widely used in orthopedic surgery and neurosurgery; recently, its advantages in oral and maxillofacial surgery could be shown.[25–27]

The sterility and the antigenicity represent two critical points. Regarding the sterility, irradiation increases the degree of safety. Regarding immunogenicity, no complications related to histocompatibility have been reported.[28] This could be due to the loss of viable cells by freezing.

The use of homologous FFB represents a promising alternative to autologous bone for bone reconstruction in oral and maxillofacial surgery. The purpose of this study was to review the scientific literature in order to define the state-of-the-art use of FFB in surgery.

Materials and methods

Three medical databases were used to analyze the articles published in the literature until May 2014: PubMed, Cochrane Library, and Embase. The keywords and medical subject headings used were: "*fresh frozen bone, FFB, deep frozen allogenic, maxillofacial, oral, human*".

Publications were divided by year of publication and type of article; the articles were subdivided by:

- clinical trials: these studies included case reports, case series, and retrospective studies;
- review.

For each clinical trial were considered the following parameters:

- patient number;
- bone type used;
- donor site;
- presence of irradiated bone;
- surgical procedure site: upper maxilla, lower maxilla;
- surgical procedure type: preprosthetic surgery with implant rehabilitation;

- maxillary sinus elevation, removal of cysts/benign neoplasms;
- histological and/or molecular analysis;
- follow-up.

Results

We found 47 articles in the time period between 1976 and May 2014.

There are 46 clinical articles and one review.

Clinical articles are represented by 22 case reports/case series and 24 retrospective studies.

In Table 1 the number of patients included in each respective article is reported.[29–73]

Thirty-nine articles regard preprosthetic surgery with implant rehabilitation; five articles concern maxillary sinus elevation (three articles cover both the preprosthetic surgery for implant rehabilitation that the maxillary sinus elevation), four articles concern removal of cysts/tumors, and one regarding correction of deformities.[50,70,73]

Twenty-nine articles are about the maxilla, 18 on the mandible, and 12 about both districts (Fig. 1).

Classifying the articles by year of publication we showed an increase of FFB use in clinical practice (Fig. 2). This increase was found mainly during the past 6 years. Most of those publications regard preprosthetic surgery (Fig. 3).

Concerning the type of grafted bone, the main donor sites are the iliac crest, and the head of the femur and tibia with a preference for the iliac crest. However, in several studies the type of bone used was not reported. In most of the articles, the bone grafts were nonirradiated. Irradiated bone was used only in one study and in 24 articles there are no data about the tissue irradiation.[59] Histology of the bone graft was analyzed in 24 studies describing the type of regenerating bone.

With regard to surgical technique and the type of surgery, most of the studies concerned the preprosthetic surgery and the maxillary sinus elevation. The surgical technique used was highly variable, comparing the different articles as well as within the same study. Both increases in vertical and horizontal volume carried out with techniques of bone apposition and interpositional grafts were described. The use of morcellized bone was also reported.

Thirty-four studies described the use of bone blocks: three inlay, 17 onlay, and two veneer. In 25 studies implant surgery was carried out and in 15 of these studies an implant failure was reported.

The follow-up of the patients in the different studies was highly variable, even in the same study, with a minimum of 1 month and a maximum of 134 months.[30,55] Also, the time between bone regeneration and the implant surgery was highly variable, ranging from a minimum of 4 months to a maximum of 11 months.[35,36,62] In two studies the implant surgery was made in the same session of the bone regeneration.[41,50]

Discussion

Bone regeneration is the objective of several surgical fields. Although there are different materials of natural and synthetic origin, some preparations also containing bone growth factors such as BMP2Rh, the use of both autologous and homologous bone grafts is an important tool in

Table 1 Number of patients for clinical article.

Retrospective studies	Patients	Case report and case series	Patients
1) Acocella et al, 2011a[29]	16	1) Albanese et al, 2011[52]	1
2) Acocella et al, 2011b[30]	15	2) Albanese et al, 2012[53]	1
3) Bertossi et al, 2013[31]	20	3) Barone et al, 2009[54]	13
4) Carinci et al, 2009°[32]	22	4) Boniello et al, 2013[55]	20
5) Carinci et al, 2009b[33]	21	5) Borgonovo et al, 2010[56]	1
6) Carinci et al, 2010[34]	69	6) Borgonovo et al, 2013[57]	2
7) Chen et al, 2008[35]	4	7) Buffoli et al, 2012[58]	10
8) Contar et al, 2009[36]	15	8) Chiapasco et al, 2013a[59]	20
9) Contar et al, 2011[37]	18	9) Chiapasco et al, 2013b[60]	15
10) D'Aloja et al, 2011[38]	14	10) D'Aloja et al, 2008[61]	2
11) Franco et al, 2009a[39]	16	11) Deluiz et al, 2013[62]	24
12) Franco et al, 2009b[40]	36	12) Ferraz et al, 2013[63]	1
13) Franco et al, 2009c[41]	81	13) Lumetti et al, 2012[64]	24
14) Grecchi et al, 2009[42]	11	14) Pimentel et al, 2012[65]	6
15) Hiatt et al, 1978[43]	Not reported	15) Rochanawutanon et al, 2002[66]	4
16) Nocini et al, 2011[44]	7	16) Spin-Neto et al, 2012[67]	33
17) Perrott et al, 1992[45]	10	17) Spin-Neto et al, 2013a[68]	26
18) Rigo et al, 2011[46]	17	18) Spin-Neto et al, 2013b[69]	34
19) Rodella et al, 2010[23]	6	19) Tetè et al, 2013[70]	20
20) Stacchi et al, 2008[47]	10	20) Viscioni et al, 2010a[71]	21
21) Viscioni et al, 2009a[48]	58	21) Vos et al, 2009[72]	1
22) Viscioni et al, 2009b[49]	41	22) Xavier et al, 2014[73]	15
23) Viscioni et al, 2010b[50]	17		
24) Viscioni et al, 2011[51]	12		

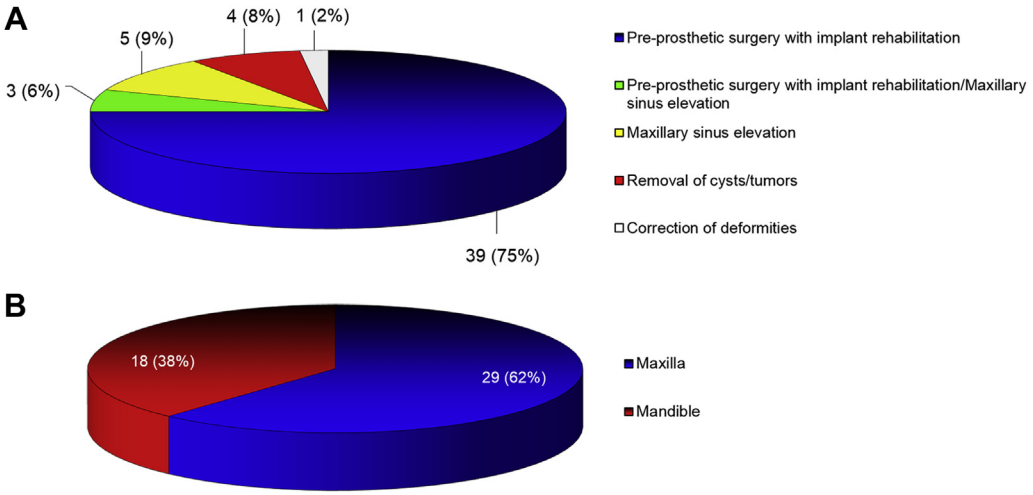

Figure 1 The different applications of FFB (A) and surgical applications in the maxilla and mandible (B).

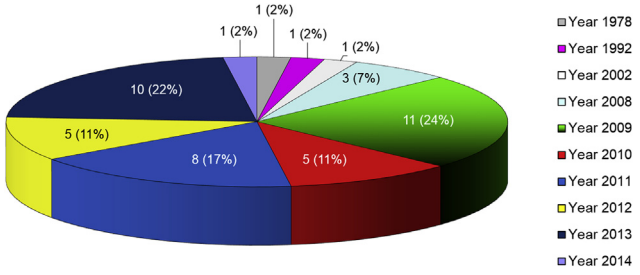

Figure 2 The scientific production regarding FFB per year.

reconstructive maxillofacial surgery.[8,74–77] Although autogenous bone still represents the gold standard in bone reconstruction, the use of FFB could represent a valid alternative for several reasons.[78,79] If the graft site is extraoral, general anesthesia is often recommended, which then increases both the risks for the patient and the costs for the surgery. In addition, some recent studies also showed that the FFB homologous bone grafts undergo a slower remodeling compared to autologous bone grafts.[80] This evidence could be particularly useful in preimplant reconstructive surgery, because a slower remodeling could allow a more stable maintenance of bone volumes also in the absence of load, as can be found in the months that follow bone graft insertion before the insertion of the implant and its functional load. The use of FFB as an inlay graft in sinus elevation or as onlay graft in the maxillary bone must be carefully evaluated, because, even if valid results have been reported in literature, there are still no long-term evaluations of implant survival.

However, some critical points exist. In addition to a low number of donations, there are some biological aspects, such as the possible transmission of infections and its potential immunogenicity. Concerns about the transmission of

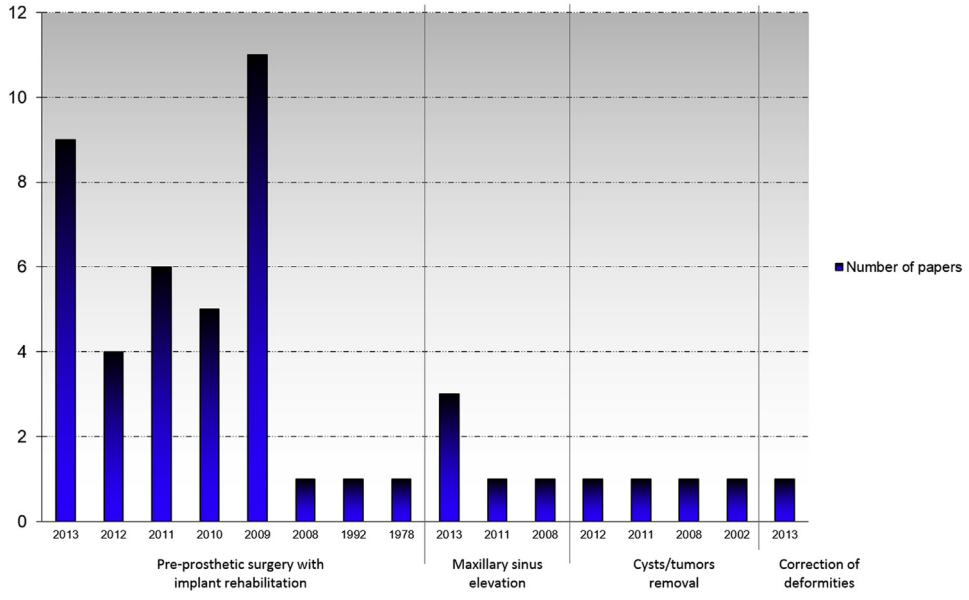

Figure 3 Distribution of different clinical applications by year.

infections with frozen bone allografts include one case of hepatitis B virus (HBV) in 1954, seven cases of hepatitis C virus (HCV) transmission, and nine cases of HIV transmission reported between 1985 and currently.[81–83] Therefore, even if we cannot exclude the possibility to contract infections, it is important to highlight that these cases have been documented mainly in the early years of FFB-use and currently, thanks to sophisticated screening methods, risks are reasonably smaller. Moreover, recent methods such as irradiation improve graft safety. Nevertheless, the correct limit of gamma irradiation exposure to inactivate viral transmission together to maintain a good BMP expression and osteoblast differentiation needs to be defined. In addition, rigorous background checks on the donor must be deeply performed.[8,74] Comparing FB to freeze-dried bone allograft (FDB) some authors showed that lyophilized bone, stored at room temperature for 15 days or more, did not transmit HIV, HCV, or HBV.[81] Freezing preserves the infectivity of enveloped viruses and presumably by keeping their lipid membranes intact for years, whereas the process of drying and storing at room temperature results in the collapse of the lipid membrane.[81]

With regard to the immunogenic potential, freezing allows killing of the immunogenic potential by eliminating almost completely vital bone cell component.[28,84,85] Nevertheless, even if in some experimental studies some authors did not show any detectable antibody response after healing of long-term frozen bone allograft[80,81]; other authors showed an evoked detectable humoral and cell-mediated immunity with frozen bone, comparing it to FDB (which failed to sensitize the recipients).[86] Using FDB, in fact, Lingaraj et al[87] did not report any noticeable immunological activation in patients subjected to periapical surgery and FDB graft; also, studies from Mellonig[88] and Quattlebaum et al[89] showed a reduction both of humoral and cell-mediated type of antigenicity up to a not observed incidence of donor specific antibodies and so reporting clinical success in oral and maxillofacial surgeries. Mechanisms concerning the interactions between the host immune system and grafting are not fully understood, but recent studies on animal models show that from the immunological point of view the homologous frozen bone has behavior comparable to autologous fresh bone not inducing any significant response in the recipient.[84,85]

The literature analysis regarding the use of FFB shows that its application in oral and maxillofacial surgery began in 1992 with Perrott[45]; it had a real development since 2006 and a significant increase only in the past six years.[26] In preprosthetic surgery with implant rehabilitation, studies should target increases in horizontal and vertical volume. Also, should the degree of rise be assessed, this would also be of significant importance in maxillary sinus elevation. In fact, there are no randomized controlled trials that would be needed to properly validate the procedure, making it independent of the personal preferences of individual surgeons. The stratification of articles by type proves that most of the articles are case reports and case series or retrospective studies regarding oral preprosthetic surgery. Reports on maxillary and mandibular rehabilitation and the orthognathic surgery currently are numerically limited. These results suggest some considerations. In contrast to orthopedic bone, in reconstruction surgery or reconstructive maxillofacial surgery, the risk for the patient, although both improving the quality of life of the patient, is considerably higher than in oral preprosthetic surgery. For those, alternative techniques should be considered.

The FFB has different characteristics as it originates from different donor sites. In oral and maxillofacial applications the iliac crest and the femur's head is mainly used. The femur's head consists of cancellous bone coated with a thin layer of compact bone that is not maintained during the bone segment shaping; the iliac crest has both cortical and cancellous parts. This different structural organization, together with different remodeling of cortical bone compared to cancellous bone, could influence graft integration.[90] However, because of the small sample sizes, further studies are definitely needed to assess how, where, and if there should be specific guidelines regarding the right choice of harvesting the bone from a specific donor site over another. In addition, bones of the splanchnocranium originate through membranous ossification from neural crest cells[91–93]; they exhibit some special characteristics both concerning innervation and the periosteum as well as repair mechanisms.[94–96] This diversity, along with more details on the biology of bone tissue and the graft integration mechanisms, should also be investigated further.[97] This is not the only difference with orthopedics, in which FFB is widely used. Some authors show a certain degree of bone graft resorption.[98] This resorption, which might depend on the bone type used (cortical, cancellous, corticocancellous, morcellized), may be of little significance in orthopedics but it can assume great importance in oral and maxillofacial surgery where bone thickness is extremely limited. In conclusion, although the overall advantages of the use of FFB have already been outlined in the literature, a better characterization of its use in oral and maxillofacial surgery is necessary. Therefore, it is essential to plan larger studies to transfer the results and current discussions into the evidence-based medicine of the future by providing the necessary guidelines and protocols.

Conflicts of interest

The authors have no potential conflicts of interest.

Acknowledgments

We thank Dr. Gaia Favero and Dr. Barbara Buffoli for helpful suggestions.

References

1. Resnick CM, Kaban LB, Troulis MJ. Minimally invasive orthognathic surgery. *Facial Plast Surg* 2009;25:49–62.
2. Brown JS, Shaw RJ. Reconstruction of the maxilla and midface: introducing a new classification. *Lancet Oncol* 2010;11:1001–8.
3. Chiapasco M, Zaniboni M. Failures in jaw reconstructive surgery with autogenous onlay bone grafts for pre-implant purposes: incidence, prevention and management of complications. *Oral Maxillofac Surg Clin North Am* 2011;23:1–15.

4. Pirgousis P, Eberle N, Fernandes R. Reoperative mandibular reconstruction. *Oral Maxillofac Surg Clin North Am* 2011;23:153—60.
5. Tasoulis G, Yao SG, Fine JB. The maxillary sinus: challenges and treatments for implant placement. *Comp Cont Educ Dent* 2011;32:10—4.
6. Sacco AG, Chepeha DB. Current status of transport-disc-distraction osteogenesis for mandibular reconstruction. *Lancet Oncol* 2007;8:323—30.
7. Chiapasco M, Romeo E, Coggiola A, Brusati R. Long-term outcome of dental implants placed in revascularized fibula free flaps used for the reconstruction of maxillo-mandibular defects due to extreme atrophy. *Clin Oral Implants Res* 2011;22:83—91.
8. Rodella LF, Favero G, Labanca M. Biomaterials in maxillofacial surgery: membranes and grafts. *Int J Biomed Sci* 2011;7:81—8.
9. Simion M, Fontana F. Autogenous and xenogeneic bone grafts for the bone regeneration. A literature review. *Minerva Stomatol* 2004;53:191—206.
10. Oltramari PV, de Lima Navarro R, Henriques JF, et al. Orthodontic movement in bone defects filled with xenogenic graft: an experimental study in minipigs. *Am J Orthod Dentofacial Orthop* 2007;131. 302e10—7.
11. Shahgoli S, Levine MH. Introduction and overview of bone grafting. *N Y State Dent J* 2011;77:30—2.
12. Dasmah A, Thor A, Ekestubbe A, Sennerby L, Rasmusson L. Particulate vs. block bone grafts: three-dimensional changes in graft volume after reconstruction of the atrophic maxilla, a 2-year radiographic follow-up. *J Craniomaxillofac Surg* 2011;40:654—9.
13. Vacanti CA, Bonassar LJ. An overview of tissue engineered bone. *Clin Orthop Relat Res* 1999;367:S375—81.
14. Sittitavornwong S, Gutta R. Bone graft harvesting from regional sites. *Oral Maxillofac Surg Clin North Am* 2010;22:317—30.
15. Hoexter DL. Bone regeneration graft materials. *J Oral Implantol* 2002;28:290—4.
16. Kang YH, Jeon SH, Park JY, et al. Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. *Tissue Eng Part A* 2011;17:349—59.
17. Stievano D, Di Stefano A, Ludovichetti M, et al. Maxillary sinus lift through heterologous bone grafts and simultaneous acid-etched implants placement. Five year follow-up. *Minerva Chir* 2008;63:79—91.
18. Santini S, Barbera P, Modena M, Schiavon R, Bonato M. Equine-derived bone substitutes in orthopedics and traumatology: authors' experience. *Minerva Chir* 2011;66:63—72.
19. Aichelmann-Reidy ME, Yukna RA. Bone replacement grafts. The bone substitutes. *Dent Clin North Am* 1998;42:491—503.
20. Pappalardo S, Puzzo S, Carlino V, Cappello V. Bone substitutes in oral surgery. *Minerva Stomatol* 2007;56:541—57.
21. Lalani Z, Wong M, Brey EM, Mikos AG, Duke PJ. Spatial and temporal localization of transforming growth factor-beta1, bone morphogenetic protein-2, and platelet-derived growth factor-A in healing tooth extraction sockets in a rabbit model. *J Oral Maxillofac Surg* 2003;61:1061—72.
22. Lalani Z, Wong M, Brey EM, et al. Spatial and temporal localization of FGF-2 and VEGF in healing tooth extraction sockets in rabbit model. *J Oral Maxillofac Surg* 2005;63:1500—8.
23. Rodella LF, Favero G, Boninsegna R, Borgonovo A, Rezzani R, Santoro F. TGF-beta1 and VEGF after fresh frozen bone allograft insertion in oral-maxillo-facial surgery. *Histol Histopathol* 2010;25:463—71.
24. Simpson D, Kakarala G, Hampson K, Steele N, Ashton B. Viable cells survive in fresh frozen human bone allografts. *Acta Orthop* 2007;78:26—30.
25. Vanaclocha V, Bazan A, Saiz-Sapena N, Paloma V, Idoate M. Use of frozen cranial vault bone allografts in the repair of extensive cranial bone defects. *Acta Neurochir* 1997;139:653—60.
26. D'Aloja E, Santi E, Franchini M. Utilizzo di osso omologo fresh frozen in chirurgia. *Ital Oral Surg* 2006;5:19—26.
27. Grover V, Kapoor A, Malhotra R, Sachdeva S. Bone allografts: a review of safety and efficacy. *Indian J Dent Res* 2011;22:496.
28. Costain DJ, Crawford RW. Fresh-frozen vs. irradiated allograft bone in orthopaedic reconstructive surgery. *Injury* 2009;40:1260—4.
29. Acocella A, Bertolai R, Ellis 3rd E, Nissan J, Sacco R. Maxillary alveolar ridge reconstruction with monocortical fresh-frozen bone blocks - a clinical, histological and histomorphometric study. *J Craniomaxillofacial Surg* 2011a;40:525—33.
30. Acocella A, Bertolai R, Nissan J, Sacco R. Clinical, histological and histomorphometrical study of maxillary sinus augmentation using cortico-cancellous fresh frozen bone chips. *J Craniomaxillofacial Surg* 2011b;39:192—9.
31. Bertossi D, Albanese M, Nocini PF, D'Agostino A, Trevisiol L, Procacci P. Sliding genioplasty using fresh-frozen bone allografts. *JAMA Facial Plast Surg* 2013;15:51—7.
32. Carinci F, Brunelli G, Franco M, et al. A retrospective study on 287 implants installed in resorbed maxillae grafted with fresh frozen allogenous bone. *Clin Implant Dent Relat Res* 2010;12:91—8.
33. Carinci F, Brunelli G, Zollino I, et al. Mandibles grafted with fresh-frozen bone: an evaluation of implant outcome. *Implant Dent* 2009b;18:86—95.
34. Carinci F, Guidi R, Franco M, et al. Implants inserted in fresh-frozen bone: a retrospective analysis of 88 implants loaded 4 months after insertion. *Quintessence Int* 2009a;40:413—9.
35. Chen SY, Liu ZH, Lu Y, Hu YJ. Application of autologous bone fresh-frozen and re implantation in treatment of craniomaxillofacial bone tumors: report of 4 cases. *Shanghai Kou Qiang Yi Xue* 2008;17:545—7.
36. Contar CM, Sarot JR, Bordini Jr J, Galvão GH, Nicolau GV, Machado MA. Maxillary ridge augmentation with fresh-frozen bone allografts. *J Oral Maxillofac Surg* 2009;67:1280—5.
37. Contar CM, Sarot JR, da Costa MB, et al. Fresh-frozen bone allografts in maxillary ridge augmentation: histologic analysis. *J Oral Implantol* 2011;37:223—31.
38. D'Aloja C, D'Aloja E, Santi E, Franchini M. The use of fresh-frozen bone in oral surgery: a clinical study of 14 consecutive cases. *Blood Transfus* 2011;9:41—5.
39. Franco M, Rigo L, Viscione A, et al. CaPO4 blasted implants inserted into iliac crest homologue frozen grafts. *J Oral Implantol* 2009a;35:176—80.
40. Franco M, Viscioni A, Rigo L, et al. Clinical outcome of narrow diameter implants inserted into allografts. *J Appl Oral Sci* 2009b;17:301—6.
41. Franco M, Viscioni A, Rigo L, Guidi R, Brunelli G, Carinci F. Iliac crest fresh frozen homografts used in pre-prosthetic surgery: a retrospective study. *Cell Tissue Bank* 2009c;10:227—33.
42. Grecchi F, Zollino I, Parafioriti A, Mineo G, Pricolo A, Carinci F. One-step oral rehabilitation by means of implants' insertion, Le Fort I, grafts, and immediate loading. *J Craniofac Surg* 2009;20:2205—10.
43. Hiatt WH, Schallhorn RG, Aaronian AJ. The induction of new bone and cementum formation. IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and nongraft periodontal regenerative procedures. *J Periodontol* 1978;49:495—512.
44. Nocini PF, Bertossi D, Albanese M, D'Agostino A, Chilosi M, Procacci P. Severe maxillary atrophy treatment with Le Fort I, allografts, and implant-supported prosthetic rehabilitation. *J Craniofac Surg* 2011;22:2247—54.

45. Perrott DH, Smith RA, Kaban LB. The use of fresh frozen allogeneic bone for maxillary and mandibular reconstruction. *Int J Oral Maxillofac Surg* 1992;21:260—5.
46. Rigo L, Viscioni A, Franco M, et al. Overdentures on implants placed in bone augmented with fresh frozen bone. *Minerva Stomatol* 2011;60:5—14.
47. Stacchi C, Orsini G, Di Iorio D, Breschi L, Di Lenarda R. Clinical, histologic, and histomorphometric analyses of regenerated bone in maxillary sinus augmentation using fresh frozen human bone allografts. *J Periodontol* 2008;79:1789—96.
48. Viscioni A, Franco M, Rigo L, Guidi R, Brunelli G, Carinci F. Implants inserted into homografts bearing fixed restorations. *Int J Prosthodont* 2009a;22:148—54.
49. Viscioni A, Franco M, Rigo L, Guidi R, Spinelli G, Carinci F. Retrospective study of standard-diameter implants inserted into allografts. *J Oral Maxillofac Surg* 2009b;67:387—93.
50. Viscioni A, Rigo L, Franco M, et al. Reconstruction of severely atrophic jaws using homografts and simultaneous implant placement: a retrospective study. *J Oral Implantol* 2010b;36:131—9.
51. Viscioni A, Franco M, Paolin A, et al. Effectiveness of fresh frozen and cryopreserved homologue iliac crest grafts used in sinus lifting: a comparative study. *Cell Tissue Bank* 2011;12:263—71.
52. Albanese M, Procacci P, Sancassani G, Nocini PF. Fresh-frozen human bone graft to repair defect after mandibular giant follicular cyst removal: a case report. *Cell Tissue Bank* 2011;13:305—13.
53. Albanese M, Nocini PF, Fior A, et al. Mandibular reconstruction using fresh frozen bone allograft after conservative enucleation of a mandibular odontogenic myxoma. *J Craniofac Surg* 2012;23:831—5.
54. Barone A, Varanini P, Orlando B, Tonelli P, Covani U. Deep-frozen allogeneic onlay bone grafts for reconstruction of atrophic maxillary alveolar ridges: a preliminary study. *J Oral Maxillofac Surg* 2009;67:1300—6.
55. Boniello R, Gasparini G, D'Amato G, et al. Reconstruction of severe atrophic jaws with Fresh Frized Bone Allografts: clinical histologic and histomorphometric evaluation. *Eur Rev Med Pharmacol Sci* 2013;17:1411—8.
56. Borgonovo AE, Tommasi F, Panigalli A, Bianchi AC, Boninsegna R, Santoro F. Use of fresh frozen bone graft in rehabilitation of maxillar atrophy. *Minerva Stomatol* 2012;61:141—54.
57. Borgonovo AE, Marchetti A, Vavassori V, Censi R, Boninsegna R, Re D. Treatment of the atrophic upper jaw: rehabilitation of two complex cases. *Case Rep Dent* 2013:1—7. http://dx.doi.org/10.1563/AAID-JOI-D-13-00102. ID154795.
58. Buffoli B, Boninsegna R, Rezzani R, Poli PP, Santoro F, Rodella LF. Histomorphometrical evaluation of fresh frozen bone (FFB) allografts for alveolar bone reconstruction: preliminary cases comparing femoral head versus iliac crest grafts. *Clin Implant Dent Relat Res* 2013;15:791—8.
59. Chiapasco M, Giammattei M, Carmagnola D, Autelitano L, Rabbiosi D, Dellavia C. Iliac crest fresh-frozen allografts and autografts in maxillary and mandibular reconstruction: a histologic and histomorphometric evaluation. *Minerva Stomatol* 2013a;62:3—16.
60. Chiapasco M, Di Martino G, Anello T, Zaniboni M, Romeo E. Fresh frozen versus autogenous iliac bone for the rehabilitation of the extremely atrophic maxilla with onlay grafts and endosseous implants: preliminary results of a prospective comparative study. *Clin Implant Dent Relat Res* 2013. http://dx.doi.org/10.1111/cid.12191 [Epub 2013].
61. D'Aloja E, Santi E, Aprili G, Franchini M. Fresh frozen homologous bone in oral surgery: case reports. *Cell Tissue Bank* 2008;9:41—6.
62. Deluiz D, Oliveira LS, Pires FR, Tinoco EM. Time-dependent changes in fresh-frozen bone block grafts: tomographic, histologic, and histomorphometric findings. *Clin Implant Dent Relat Res*. http://dx.doi.org/10.1111/cid.12108 [Epub 2013].
63. Ferraz EP, Rosa AL, de Oliveira PT, et al. Clinical, histological and cellular evaluation of vertico-lateral maxillary reconstruction associating alveolar osteogenic distraction and fresh-frozen bone allograft. *J Oral Implantol*. http://dx.doi.org/10.1563/AAID-JOI-D-13-00102 [Epub 2013].
64. Lumetti S, Consolo U, Galli C, et al. Fresh-frozen bone blocks for horizontal ridge augmentation in the upper maxilla: 6-month outcomes of a randomized controlled trial. *Clin Implant Dent Relat Res* 2014;16:116—23.
65. Pimentel AC, Napimoga MH, Manzi MR, Sendyk WR. Reconstruction of the edentulous mandible with fresh frozen bone grafts and implants: a 4-year report of a prospective clinical study. *Cell Tissue Bank* 2012;15:1—6.
66. Rochanawutanon S, Suddhasthira T, Pairuchvej V, Vajaradul Y. Long term follow-up of reconstruction with allogeneic mandibular bone crib packed with autogenous particulate cancellous bone marrow. *Cell Tissue Bank* 2002;3:183—97.
67. Spin-Neto R, Stavropoulos A, de Freitas RM, Pereira LA, Carlos IZ, Marcantonio Jr E. Immunological aspects of fresh-frozen allogeneic bone grafting for lateral ridge augmentation. *Clin Oral Implants Res* 2012;24:963—8.
68. Spin-Neto R, Stavropoulos A, Dias Pereira LA, Marcantonio Jr E, Wenzel A. Fate of autologous and fresh-frozen allogeneic block bone grafts used for ridge augmentation. A CBCT-based analysis. *Clin Oral Implants Res* 2013a;24:167—73.
69. Spin-Neto R, Stavropoulos A, Coletti FL, Faeda RS, Pereira LA, Marcantonio Jr E. Graft incorporation and implant osseointegration following the use of autologous and fresh-frozen allogeneic block bone grafts for lateral ridge augmentation. *Clin Oral Implants Res* 2014;25:226—33.
70. Tetè S, Zizzari VL, D'Aloja E, et al. Histological evaluation of fresh frozen bone integration at different experimental times. *J Craniofac Surg* 2013b;24:836—40.
71. Viscioni A, Dalla Rosa J, Paolin A, Franco M. Fresh-frozen bone: case series of a new grafting material for sinus lift and immediate implants. *J Ir Dent Assoc* 2010a;56:186—91.
72. Vos MD, Raghoebar GM, van der Wal JE, Kalk WW, Vissink A. Autogenous femoral head as grafting material for mandibular augmentation. *Int J Oral Maxillofac Surg* 2009;38:1320—3.
73. Xavier SP, Dias RR, Sehn FP, Kahn A, Chaushu L, Chaushu G. Maxillary sinus grafting with autograft vs. fresh frozen allograft: a split-mouth histomorphometric study. *Clin Oral Impl Res* 2014:1—6. http://dx.doi.org/10.1111/clr.12404 [Epub 2014].
74. Kao ST, Scott DD. A review of bone substitutes. *Oral Maxillofac Surg Clin North Am* 2007;19:513—21.
75. McKay WF, Peckham SM, Badura JM. A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). *Int Orthop* 2007;31:729—34.
76. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: current challenges in BMP delivery. *Biotechnol Lett* 2009a;31:1817—24.
77. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: delivery systems for BMPs in orthopaedic and craniofacial tissue engineering. *Biotechnol Lett* 2009b;31:1825—35.
78. Tatum SA, Kellman RM. Cranial bone grafting in maxillofacial trauma and reconstruction. *Facial Plast Surg* 1998;14:117—29.
79. Nadal E, Sabàs M, Dogliotti P, Esposito R. Secondary alveolar bone grafting: our experience with olecranon bone graft. *J Craniofac Surg* 2010;21:371—4.
80. Spin-Neto R, Landazuri Del Barrio RA, Pereira LA, Marcantonio RA, Marcantonio E, Marcantonio Jr E. Clinical

similarities and histological diversity comparing fresh frozen onlay bone blocks allografts and autografts in human maxillary reconstruction. *Clin Implant Dent Relat Res* 2011;15:490—7.
81. Salvucci JT. Bone tissue, lyophilized and stored at room temperature for 15 days or more, is not capable of transmitting HIV, HCV or HBV. *Cell Tissue Bank* 2011;12:99—104.
82. Shutkin NM. Homologous-serum hepatitis following the use of refrigerated bone-bank bone. *J Bone Joint Surg Am* 1954;36-A:160—2.
83. Simonds RJ, Holmberg SD, Hurwitz RL, et al. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. *N Engl J Med* 1992;326:726—32.
84. Reikerås O, Sigurdsen UW, Shegarfi H. Impact of freezing on immunology and incorporation of bone allograft. *J Orthop Res* 2010;28:1215—9.
85. Reikerås O, Reinholt FP, Zinöcker S, Shegarfi H, Rolstad B. Healing of long term frozen orthotopic bone allografts is not affected by MHC differences between donor and recipient. *Clin Orthop Relat Res* 2011;469:1479—86.
86. Friedlaender GE, Strong DM, Sell KW. Studies on the antigenicity of bone. I. Freeze-dried and deep-frozen bone allografts in rabbits. *J Bone Joint Surg Am* 1976;58:854—8.
87. Lingaraj JB, Kotrashetti SM, Gupta N. Healing assessment of osseous defects of periapical lesions with use of freeze dried bone allograft. *J Maxillofac Oral Surg* 2009;8:362—5.
88. Mellonig JT. Freeze-dried bone allografts in periodontal reconstructive surgery. *Dent Clin North Am* 1991;35:505—20.
89. Quattlebaum JB, Mellonig JT, Hensel NF. Antigenicity of freeze-dried cortical bone allograft in human periodontal osseous defects. *J Periodontol* 1988;59:394—7.
90. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord* 2010;11:219—27.
91. Trainor PA. Specification and patterning of neural crest cells during craniofacial development. *Brain Behav Evol* 2005;66:266—80.
92. Gitton Y, Heude E, Vieux-Rochas M, et al. Evolving maps in craniofacial development. *Semin Cell Dev Biol* 2010;21:301—8.
93. Li S, Quarto N, Longaker MT. Activation of FGF signaling mediates proliferative and osteogenic differences between neural crest derived frontal and mesoderm parietal derived bone. *PLoS One* 2010;5:e14033.
94. Bataille C, Mauprivez C, Haÿ E, et al. Different sympathetic pathways control the metabolism of distinct bone envelopes. *Bone* 2012;50:1162—72.
95. Wiren KM, Hashimoto JG, Semirale AA, Zhang XW. Bone vs. fat: embryonic origin of progenitors determines response to androgen in adipocytes and osteoblasts. *Bone* 2011;49:662—72.
96. Horton WA, Degnin CR. FGFs in endochondral skeletal development. *Trends Endocrinol Metab* 2009;20:341—8.
97. Oppenheimer AJ, Mesa J, Buchman SR. Current and emerging basic science concepts in bone biology: implications in craniofacial surgery. *J Craniofac Surg* 2012;23:30—6.
98. Macedo LG, Mazzucchelli-Cosmo LA, Macedo NL, Monteiro AS, Sendyk WR. Fresh-frozen human bone allograft in vertical ridge augmentation: clinical and tomographic evaluation of bone formation and resorption. *Cell Tissue Bank* 2011;13:577—86.