Biomimetic hydroxyapatite used in the treatment of periodontal intrabony pockets: clinical and radiological analysis

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Summary

Aim. **Hydroxyapatite (PA) has a chemical composition and physical structure very similar to natural bone and therefore it has been considered to be the ideal biomaterial able to ensure a biomimetic scaffold to use in bone tissue engineering. The aim of this study is to clinically test hydroxyapatite used as osteoconductive biomaterial in the treatment of periodontal bone defects.**
Clinical and radiological evaluations were conducted at 6, 12 and 18 months after the surgery.
Materials and methods. **Forty patients with 2- and 3-wall intrabony pockets were enrolled in this study. PPD, CAL, radiographic depth (RD) and angular defects were preoperatively measured. After surgery, patients were re-evaluated every 6 months for 18 months. Statistical analyses were also performed to investigate any differences between preoperative and postoperative measurements.**
Results. **Paired t-test samples conducted on the data obtained at baseline and 18 months after, showed significant ($p<0.01$) differences in each measurement performed. The role of preoperative RD was demonstrated to be a significant key factor ($p<0.01$). A relevant correlation between preoperative PPD and CAL gain was also found.**
Conclusions. **Within the limitations of this study, the absence of anatomical variables, except the morphology of the bone defect, emphasizes the importance of the proper surgical approach and the graft material used.**

Key words: biomimetic, hydroxyapatite, intrabony pockets, clinical, radiological.

Introduction

Bone substitutes represent one of the most widely used options for the regeneration of bone defects developed after a severe periodontal disease.
According to Ripamonti (1), correct bone regeneration is mainly based on 4 factors:

- osseo-inductive abilities
- scaffold able to support bone regeneration
- mesenchymal stem cells able to be simulated by these signals and able to differentiate towards the osteoblast phenotype
- appropriate timing of growth and proper environment. Grafting materials are commonly classified, based on the potential osteogenic activity, into: osteogenic, osteoinductive and osteoconductive materials (2).

Autogenous bone is the only graft material showing osteogenic, osteoinductive and osteoconductive abilities. However, the limited amount of bone obtainable from an intra-oral donor site, as well as the morbidity following surgery of the donor site and the fast resorption of the autogenous bone graft represents some of the most important limitations of this surgical procedure (3).
On the other hand, according to Berglundh & Lindhe (4), heterologous grafting materials possess only osteoconductive properties. Among these materials, the bovine bone matrix was the most investigated in periodontal-related literature. This class of grafting material showed a long resorption time, since bovine bone matrix particles were found even after more than 9 months (5).
Homologous bone graft holds intermediate features: studies on DBDFA reported that the freeze-drying process causes demineralization of the mineralized component, so freeing the BMPs able to regulate MSCs differentiation towards osteoblast phenotype (6).
Scientific progress has developed new synthetic biomaterials. Studies on grafting materials based on hydroxyapatite showed controversial outcomes: some Authors (7) demonstrated that the hydroxyapatite grafted in a bone defect induces new bone formation, without fibrotic tissue formation.

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On the contrary, other studies (8) demonstrated that hydroxyapatite works as an inert filling material, with no evidence of osteogenesis activity.
According to literature, the geometry of the grafting material could represent a differentiating key-factor able to influence the clinical outcomes. In fact, several studies demonstrated that the surface geometry is a critical factor for cell adhesion and differentiation (9, 10).
In the light of these chemical and physical features, biomaterials with a surface geometry similar to human bone represent the last generation of synthetic devices. These materials are supposed to produce molecular signalling able to modify the environment, so to facilitate a physiologic bone regeneration (11). Additionally, their stoichiometric instability potentially allows a fast and complete resorption (12).
Aim of this study was to clinically and radiographically evaluate the regenerative properties of synthetic hydroxyapatite in the surgical treatment of periodontal intrabony defects.

Materials and methods

This study was carried out with 40 patients affected by periodontal disease: the main inclusion criterion was that patients presented at least one periodontal intrabony defect. Patients were enrolled at the “Policlinico Universitario Mater Domini”, in the Department of Dental Diseases of the University “Magna Grecia” of Catanzaro. This study was approved by the University Ethical Committee (n.997 of 17/September/2010).
The research was performed following the principles of the Declaration of Helsinki on experimentation involving human subjects.

Inclusion criteria

From January 2008 to July 2009, 40 patients affected by at least one intrabony defect were selected consecutively. Defects showed the following characteristics: real 2- or 3-walls defects (radiographic intrabony component > 4mm), probing pocket depth (PPD) > 7mm, angular defect <30°.
Inclusion criteria were:
1. age >18
2. good oral hygiene
3. non smokers
4. no systemic disease
5. no previous periodontal surgery
6. no chronic assumption of NSAIDs
7. no allergy related to the used materials
8. no use of drugs such as nifepidine, steroids, allantoin, estrogens, cyclosporine, bisphosphonates
9. no pregnancy during the whole period of the study.

The selected patients underwent a non-surgical periodontal treatment. After one month, patients were re-evaluated and in all the reported cases the periodontal surgery was indicated to have a probing pocket depth (PPD) reduction, clinical attachment level (CAL) gain and defect filling. All patients were informed about the procedures and a signed informed consent form was obtained.


Figure 1. Preoperative probing.

Clinical data

The clinical data were evaluated, by means of a calibrated periodontal probe (Click-Probe®, Kerr, Bioggio, Switzerland), at the beginning of the study (baseline) and 6, 12 and 18 months after the surgery (Fig. 1).
In this study we evaluated the probing pocket depth (PPD), measured from the free gingival margin to the bottom of the pocket together with the Gingival Recession index (REC), measured from the Cement-Enamel Junction (CEJ) to the free gingival margin, and the clinical Attached Level (CAL), which is the sum of PPD+REC.

Surgical Procedure

Antibiotic therapy (Amoxicillin and Clavulanic Acid 2g a day for 6 days, starting the day before the surgery) and antiseptic therapy with Chlorhexidine 0.2% rinses


Figure 2. X-ray of the intrabony defects.

for 8 days were administered to patients. Before surgery, an X-ray was performed with the "parallel cone" technique (Fig. 2). After the local anesthesia (Mepivacain plus Adrenalin 1:100.000, Pierrel Italia), according to Cortellini et al. (13) an intrasulcular incision, was made with a blade (Beaver 64, Becton, Dickinson & Co, USA) between the mesial and distal tooth. The flap was raised at a split thickness. Granulation tissue was removed with ultrasonic tools and curettes. Defect morphology (2- or 3- walls) was characterised after flap elevation and the surgical debridement (Fig. 3).
Once the defect depth was measured, a topical therapy on the root surface was performed with tetracycline 0.5%. In addition, a biomaterial made by hydroxyapatite was customised to this bone defect (Engipore®, Finceramica, Faenza, Italy) (Fig. 4). We fill the bone defect with the biomaterial to be investigated, no membrane was used to cover this graft, while the flap was coronally repositioned (Poliglycolic Acid, diameter 4.0, Distrex Spa Italia) (Fig. 5). In all the reported cases, a periodontal dressing was placed to preserve the flap. After 8 days, periodontal dressing and sutures were removed and patients were carefully visited in order to evaluate the healing process.
Clinical and radiographic controls were performed 6, 12 and 18 months after surgery by an independent trained examiner (Figs. 6-9).

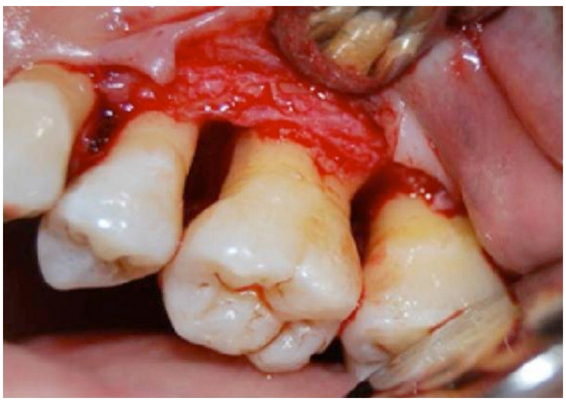

Figure 3. Assessment of defect after flap elevation and debridement.

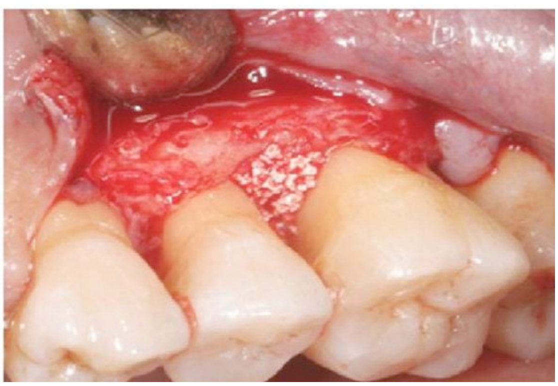

Figure 4. Defect filled with the biomaterial.

Figure 5. Flap sutured and coronally repositioned.

Figure 6. Clinical control at 6 months from baseline.

Radiographic analysis

Standard X-ray analyses were performed by using a custom-made resin bite with the "parallel cone" technique, at the baseline and 6, 12 and 18 months after surgical therapy. The radiographic depth of the intrabony defect (RD) was evaluated as a vertical dimension between the projection of the coronal bone crest onto the root surface and the most apical bone level where the periodontal ligament was considered to have a normal width.
A computerised measuring technique was applied to digital periapical radiographs. The evaluation of the

Figure 7. Intraoral X-rays at 18 months from baseline.

Figure 8. Clinical control at 18 months from baseline.

Figure 9. Control probing at 18 months from baseline.

defect sizes were assessed in mm and performed using an image analysis software with RVG equipment (CDR Dicom 4.5, Schick Technologies, Long Island City, NY).

Statistical analysis

Paired sample t-test was carried out to evaluate any significant differences between RD, PPD, REC and CAL values before surgery and the same variables 18 months after surgery.
A general linear model was achieved in order to evaluate the role of variables preoperative RD, angular and number of bone walls on the realisation of the dependent variable (Bone Gain, calculated as RD at 18-month follow-up- preoperative RD). In particular, we measured direct and indirect effects between the variables included in the model. Pearson's correlation coefficient was calculated to determine the relationship between CAL Gain (calculated as CAL at 18-month follow-up - preoperative CAL) and PPD values.

Results

Forty patients were observed (24 females, 16 males), aged from 18 to 65 years old (mean 42.2 ± 4.97 years old).
Intraoperative evaluations assessed that 17 cases showed 2-wall defects and 23 cases showed 3-wall defects.
No patient reported intra-operative or post-operative pain.
No patient dropped out during the study.

Clinical evaluation

As reported in Table 1, clinical results at 18 months showed a complete filling of the defect, with a mean PPD of 3 mm, and a mean CAL of 3.3 mm.
Paired sample t-tests carried out on PPD, REC and CAL before surgery and after 18 months showed strongly ($p<0.01$) significant differences for each measurements performed between pre- and post (18 months) operative steps.
The Pearson coefficient shows a relevant correlation (0.797) between preoperative PPD and CAL gain.

Radiographic evaluation

The radiographic evaluation was performed by analysing both the radio-opacity on digital periapical radi-

Table 1. Clinical and radiographic mean values (mm values) at the time of surgery, 6, 12 and 18 months from baseline.

	Pre-operative	6-months follow-up	12-months follow-up	18-months follow-up
PPD	10.52 SD: 1.36	4.57 SD: 0.65	5.30 SD: 0.67	2.98 SD: 0.50
CAL	11.77 SD: 1.37	4.84 SD: 0.65	5.59 SD: 067	3.32 SD: 0.54
REC	1.26 SD: 0.75	0.288 SD: 0.44	0.34 SD: 0.43	0.35 SD: 0.49
Rx Depth	8.95 SD: 1.46	2.41 SD: 0.60	2.35 SD: 0.44	1.10 SD: 1.02

ographs and the defect depth in mm measured from the bone crest to the bottom of the defect, during the surgery and after 6, 12 and 18 months.
At baseline, the angular defect was 23.05° on average (SD: 1.64). At the following follow-ups, these data resulted in 0. In fact, after 6 months, the biomaterial showed a radio-opacity very similar to the surrounding bone. At 12 months, the radio-opacity was even more similar to surrounding bone, while at 18 months it was indistinguishable from the native bone tissue and was well integrated. On the pre-surgery digital periapical radiographs, the depth of the defect, evaluated from the bone crest to the bottom of the defect, was on average 8.9 mm. This value decreased to an average of 2.4 mm, 2.3 mm and 1.1 mm. On the digital periapical radiographs performed 6, 12 and 18 months after the surgery, there were several ($p<0.01$) significant differences shown.
Our linear model was built to determine how the variables preoperative RD, angular and number of walls were crucial in contributing to the achievement of bone gain, and it showed interesting results (R Squared: 0.84). The single effect of preoperative RD was strongly significant ($p<0.01$), while the other single effects and all the cumulative effects played no statistically significant role (Tab. 2).

Discussion

Bone regeneration mechanisms depend on mechanical factors and physical factors such as humidity, mineral content, density, porosity, collagen fiber orientation and interfacial bonding between constituents (14).
In the field of periodontal tissue regeneration, the use of synthetic materials, mostly hydroxyapatites, is growing every day because of their mechanical properties very similar to human bone characteristics. So these "Miming bone materials" were studied for their ability to trigger the Guided Tissues Regeneration (GTR) mechanisms (15-17).
The present study was aimed to evaluate the capability of new bio-mimetic hydroxyapatite biomaterial to treat the complex periodontal intrabony defects. Hydroxyapatite (PA) has a composition and structure very similar to natural bone tissue and therefore it has been considered to be the ideal biomaterial to use in bone tissue engineering, also in the light of its osteoconductivity and a likely osteoinductivity.
PA has already been proven to show good biocompatibility with many human cells and tissues, probably thanks to its similarity to collagen (18).
Nano-structured hydroxyapatite presents chemical and morphologic properties similar to natural bone. PA porosity was demonstrated to reach 90% of the entire volume, with macro-pores ranging between 200 and 500 µm, and pores of interconnection ranging between 80 and 200 µm. Additionally, its Ca/P (Calcium/Phosphate) ratio is almost to the same as the natural bone Ca/P ratio. These features allow the material to present a geometric configuration similar to natural bone and, according to Ripamonti et al. (12), allow this material to adsorb the bioactive proteins and the grow factors concentrated in the clot. It allows the progressive releasing of these factors, able to induce migration, adhesion and proliferation of cells inside the pore network and to promote a faster angiogenesis and a more effective osteo-genesis inside these pores (19-21). Theoretical data was confirmed by clinical outcome (22). In fact, according to several studies (23-26), new generation hydroxya-

Table 2. Analysis of variance, tests of between-subjects effects - dependent variable: bone gain.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	299.306	29	4.338	2.281	0.007
Intercept	2635.376	1	2635.376	1386.027	0.000
Rx depth preop	136.729	12	11.394	5.992	0.000
Angle preop	4.745	11	0.431	0.227	0.994
Number of walls preop	1.692	1	1.692	0.890	0.353
Rx depth preop * Angle preop	9.649	23	0.420	0.221	1.000
Rx depth preop * Number of walls preop	2.276	6	0.379	0.199	0.974
Angle preop * Number of walls preop	4.548	5	0.910	0.478	0.789
Rx depth preop * Angle preop * Number of walls preop	3.278	5	0.656	0.345	0.881
Error	57.042	10	1.901389		
Total	6510.750	40			
Corrected Total	356.348	39			

R Squared = 0.840 (Adjusted R Squared = 0.472)
Df: degrees of freedom
F: statistical result of F Test

patites demonstrated high osteo-conductive properties when used in sinus-lift procedure and in vertical ridge-augmentation of atrophic posterior mandible (27). Moreover, the osseointegration period was confirmed to range between 9 and 18 months. On the other hand, bovine bone matrix showed, in the same conditions, a longer resorption time, even with controversial histomorphometrical outcomes (28).

At the end of this study, the reported outcomes showed a strong bone regeneration, with a bone regeneration mean value of 7.85±1.9 mm. These data report a bone regeneration slightly higher than the outcome reported by Trombelli et al. (6) which compared different grafting materials, and the outcome reported by Sculean et al. (28) which used bovine bone matrix with or without membrane. It might be explained because of the bio-mimetic properties of the hydroxyapatites associated to the surgical technique used and to proper soft tissue management. Split thickness flap used has ensured an adequate blood supply to the soft tissues during the surgical treatment and the possibility to replace the gingival flap more coronally.

The surgical technique used exploited the reparative and regenerative properties of underlying bone tissue, given that periosteum is rich in totipotential staminal cells. Moreover, this flap technique allowed for complete flap stability, which is a key condition to obtain good healing of the tissues (29).

Trombelli et al. (6) and Cortellini & Tonetti (29), according to a Literature systematic review, stressed the importance of the surgical approach. In fact, it was shown that a minimally invasive technique with or without regenerative materials resulted in significant clinical and radiographic improvements. In this light, our results from the present study showed that, by using the nano-structured hydroxyapatite as filling biomaterial, a membrane might not be a critical factor for bone regeneration, even in non-containing bone defects.

The data from published controlled clinical studies do not seem to clearly indicate improved clinical outcomes related to probing pocket depth (PPD) reduction, clinical attachment level (CAL) gain and defect fill when the combination of grafting materials and a covering membrane is compared with the membrane alone or with the grafting materials alone (30-33). It was shown that treatment of intrabony defects with a complicated, non-containing morphology by using membranes and grafting materials showed better clinical outcomes if compared with the use of the membranes alone (30, 33). Such better clinical outcomes are confirmed by more suggestive histological pictures describing a good bone repair, after the surgical technique combining grafting materials and membranes in non-containing periodontal defects. Otherwise, in containing bone defects (i.e. fenestration defects, three-wall intrabony defects or Class II furcation defects) no additional advantage by a combination of grafting materials and barrier membranes compared with grafting materials alone or barrier membranes alone was reported (23).

However, the lack of a control group suggests the need to perform further clinical trials.

In our clinical study no statistical differences regarding the probing depth reduction values, the clinical attachment level (CAL) gain and the defect fill were observed, both in contained (3 wall) and non-contained (2 wall) periodontal defects. Even if such clinical outcomes are not investigated by histology assay, it can be argued that the periodontal defects can be successfully regenerated only by means of the tested biomaterial without any use of membrane.

Statistical analyses found no statistically differences in tree-wall intrabony pockets compared to two-wall defects. These data are in agreement with Cortellini & Tonetti (29), which described the same healing pattern in three- and two-wall intrabony pockets.

Furthermore, after having analyzed the results, we found that angular defects seemed not to influence the final outcomes of the study; we have to consider, however, that the inclusion criteria required a preoperative angular defect <30°.

On the other hand, the length of the defect appeared to be another key factor: in fact, the better results were demonstrated in the deeper defects.

Results of the reported study seem to disagree with Needleman et al. (34), which stated that a better healing can be obtained in the three-wall defects.

At the end of our study, the marginal soft tissue level was found to be healed more coronally than we expected. REC reduction after surgery might be explained by the overfilling of the defect, to maintain an adequate space, and by the possibility to move and suture coronally the flap, to obtain a perfect soft tissue closure and the stability of the coagulum for a proper healing process. This data seems to be in contrast with the Literature (35) which suggests to place the soft tissues at the same level or slightly apically after the surgery.

Conclusions

In the presented study, the absence of anatomical determinants, with the exception of the length of the defect, might suggest the importance of surgical approach and of the grafting material used as well. With an accurate patients selection and the proper surgical technique, the here investigated biomaterial gives an important aid in the treatment of periodontal intrabony defects, leading to a normalisation of the clinical and radiological parameters. These assumptions were supported by histological evidence, as previously demonstrated by Figliuzzi et al. (36).

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References

1. Ripamonti U. Soluble, insoluble and geometric signals sculpt the architecture of mineralized tissues. Journal of Cellular and Molecular Medicine. 2004;8:169-180.
2. Lambert F, Léonard A, Drion P, Sourice S, Layrolle P, Rompen E. Influence of space-filling materials in subantral bone augmentation: blood clot vs. autogenous bone chips vs. bovine hydroxyapatite. Clinical Oral Implants Research. 2011;5:538-454.
3. Becker W, Becker B, Bergstrom C, Polizzi G. Autogenous bone grafting of bone defects adjacent to implants placed into immediate extraction sockets in patients: a prospective study. International Journal of Oral and Maxillofacial Implants. 1994;94:389-396.
4. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. Clin Oral Implants Res. 1997;8:117-124.
5. Sculean A, Chiantella GC, Windisch P, Arweiler NB, Brecx M, Gera I. Healing of intra-bony defects following treatment with a composite bovine-derived xenograft (Bio-Oss Collagen) in combination with a collagen membrane (Bio-Gide PERIO). Journal of Clinical Periodontology. 2005;7:720-724.
6. Trombelli L, Heitz-Mayfield LJ, Needleman I, Moles D, Scabbia A. A systematic review of graft materials and biological agents for periodontal intraosseous defects. Journal of Clinical Periodontology. 2002;29:117-135.
7. Götz W, Gerber T, Lossdorfer S, Henkel KO, Heinemann F. Immunohistochemical characterization of nanocristalline hydroxyapatite silica gel (Nanobone) osteogenesis: a study on biopsies from human jaws. Clinical Oral Implants Research. 2008;19:1016-1026.
8. Nery EB, Legeros RZ, Lynch KL, Lee K. Tissue response to biphasic calcium phosphate ceramic with different ratios of HA/B-TCP in periodontal osseous defects. Journal of Periodontology. 1992;63:729-735.
9. Ripamonti U, Ferretti C, Heliotis M. Soluble and insoluble signals and the induction of bone formation: molecular therapeutics recapitulating development. Journal of Anatomy. 2006;209:447-68.
10. Ripamonti U, Richter PW, Thomas ME. Self-inducing shape memory geometric cues embedded within smart hydroxyapatite-based biomimetic matrices. Plastic and Reconstructive Surgery. 2007;120:1796-807.
11. Ripamonti U, Richter PW, Nilen RW, Renton L. The induction of bone formation by smart biphasic hydroxyapatite tricalcium phosphate biomimetic matrices in the non-human primate Papio ursinus. Journal of Cellular and Molecular Medicine. 2008;12:2609-21.
12. Ripamonti U, Crooks J, Khoali L, Roden L. The induction of bone formation by coral-derived calcium carbonate/hydroxyapatite constructs. Biomaterials. 2009;30:1428-39.
13. Cortellini P, Pini Prato G, Tonetti MS. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. International Journal of Periodontics & Restorative Dentistry. 1999;19:589-59.
14. Du C, Cui FZ, Zhang W, Feng QL, Zhu XD, de Groot K. Formation of calcium phosphate collagen composites trough mineralization of collage matrix. J Biomed Mater Res. 2000; 50(4):518-27.
15. Götz W, Reichert C, Canullo L, Jäger A, Heinemann F. Coupling of osteogenesis and angiogenesis in bone substitute healing – A brief overview. Journal of Anatomy. 2011;10: 12-14.
16. Neel EAA, Chrzanowski W, Salih VM, Hae-Won K, Knowles JC. Review. Tissue engineering in dentistry journal of dentistry. 2014;42:915-928.
17. Fricain JC, Schlaubitz S, Le Visage C, Arnault I, Derkaoui SM, Siadous R, Catros S, Lalande C, Bareille R, Renard M, Fabre T, Cornet S, Durand M, Léonard A,Sahraoui N, Letourneur D. A nano-hydroxyapatite-pullulan/dextran polysaccharide composite macroporous material for bone tissue engineering. J Biomaterials. 2013 Apr;34(12):2947-59.
18. Springer ING, Fleiner B, Jepsen S, Ali Y. Culture of cells gained from temporomandibular joint cartilagine on non-absorbible scaffolds. Biomaterials. 2001;22:2569-77.
19. Wang H, Li Y, Zuo Y, Li J, Ma S, Cheng L. Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering. Biomaterials. 2007;28:3338-3348.
20. Shiels SM, Solomon KD, Pilia M, Appleford MR, Ong JL. BMP-2 tethered hydroxyapatite for bone tissue regeneration: coating chemistry and osteoblast attachment. J Biomed Mater Res A. 2012 Nov;100(11):3117-23.
21. Jun SH, Lee EJ, Jang TS, Kim HE, Jang JH, Koh YH. Bone morphogenic protein-2 (BMP-2) loaded hybrid coating on porous hydroxyapatite scaffolds for bone tissue engineering. J Mater Sci Mater Med. 2013 Mar;24(3):773-82.
22. Lee EU†, Kim DJ†, Lim HC, Lee JS, Jung UW, Choi SH. Comparative evaluation of biphasic calcium phosphate and biphasic calcium phosphate collagen composite on osteoconductive potency in rabbit calvarial defect. Biomaterials Research. 2015;19:1.
23. Mangano C, Scarano A, Perrotti V, Iezzi G, Piattelli A. Maxillary sinus augmentation with a porous synthetic hydroxyapatite and bovine-derived hydroxyapatite: a comparative clinical and histologic study. International Journal of Oral and Maxillofacial Implants. 2007;22.980-6.
24. Neiva RF, Tsao YP, Eber R, Shotwell J, Billy E, Wang HL. Effects of a putty-formhydroxyapatite matrix combined with the synthetic cell-binding peptide P-15 on alveolar ridge preservation. Journal of Periodontology. 2008;79:291-299.
25. Canullo L, Dellavia C. Sinus lift using a nano-crystalline hydroxyapatite silica gel in severely resorbed maxillae: histological preliminary study. Clinical Implant Dentistry and Related Research. 2009;11:7-13.
26. Rebaudi A, Maltono AA, Pretto M, Benedicenti S. Sinus grafting with magnesium-enriched bioceramic granules and autogenous bone: a microcomputed tomographic evaluation of 11 patients. International Journal of Periodontics and Restorative Dentistry. 2010;30:53-61.
27. Figliuzzi M, Mangano FG, Fortunato L, De Fazio R, Macchi A, Iezzi G, Piattelli A, Mangano C. Vertical ridge augmentation of the atrophic posterior mandible with custom-made, computer-aided design/computer-aided manufacturing porous hydroxyapatite scaffolds. J Craniofac Surg. 2013 May;24(3):856-9.
28. Sculean A, Chiantella GC, Windisch P, Arweiler NB, Brecx M, Gera I. Healing of intra-bony defects following treatment with a composite bovine-derived xenograft (Bio-Oss Collagen) in combination with a collagen membrane (Bio-Gide PERIO). Journal of Clinical Periodontology. 2005;7:720-724.
29. Cortellini P, Tonetti MS. Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials: a randomized-con-

trolled trial in intra-bony defects. Journal of Clinical Periodontology. 2011;38,365-73.
30. Blumenthal N, Steinberg J. The use of collagen membrane barriers in conjunction with combined demineralized bone-collagen gel implants in human intrabony defects. Journal of Periodontology. 1990;61:319-327.
31. Chen CC, Wand HL, Smith F, Glickman GM, Shyr Y, O'Neal RB. Evaluation of a collagen membrane with and without bone grafts in treating periodontal intrabony defects. Journal of Periodontology. 1995;66:838-847
32. Trejo PM, Weltman R, Caffesse R. Treatment of intraosseous defects with bioabsorbable barriers alone or in combination with decalcified freeze-dried bone allograft: a randomized controlled clinical trial. Journal of Periodontology. 2000;71:1852-1861.
33. Paolantonio M. Combined regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A controlled clinical study. Journal of Periodontology. 2002;73:158-166.
34. Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal intrabony defects. Cochrane Database System Review. 2006;19: CD001724.
35. Zucchelli G, De Sanctis M. A novel approach to minimizing gingival recession in the treatment of vertical bony defect. Journal of Periodontology. 2008;79:567-574.
36. Figliuzzi M, De Fazio R, Tiano R, De Franceschi S, Pacifico D, Mangano F, Fortunato L. Histological evaluation of a biomimetic material in bone regeneration after one year from graft. Ann Stomatol (Roma). 2014 Jul 20;5(3):103-7.