ORIGINAL RESEARCH

Comparative evaluation of hydroxyapatite and nano-bioglass in two forms of conventional micro- and nano-particles in repairing bone defects (an animal study)

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ABSTRACT

Context: Many synthetic bone materials have been introduced for repairing bone defects.
Aim: The aim of this study is to comparatively evaluate the efficacy of nano-hydroxyapatite (HA) and nano-bioglass bone materials with their traditional micro counterparts in repairing bone defects.
Materials and Methods: In this prospective animal study, four healthy dogs were included. First to fourth premolars were extracted in each quadrant and five cavities in each quadrant were created using trephine. Sixteen cavities in each dog were filled by HA, nano-HA, bioglass, and nano-bioglass and four defects were left as the control group. All defects were covered by a nonrestorable membrane. Dogs were sacrificed after 15, 30, 45, and 60 days sequentially. All 20 samples were extracted by trephine #8 with a sufficient amount of surrounding bone. All specimens were investigated under an optical microscope and the percentage of total regenerated bone, lamellar, and woven bone were evaluated.
Statistical Analysis Used: Data analysis was carried out by SPSS Software ver. 15 and Mann–Whitney U-test (α =0.05).
Results: After 15 days, the bone formation percentage showed a significant difference between HA and nano-HA and between HA and bioglass ($P < 0.001$). The nano-HA group showed the highest rate of bone formation after 15 days. Nano-bioglass and bioglass and nano-HA and nano-bioglass groups represented a significant difference and nano-bioglass showed the highest rate of bone formation after 30 days ($P = 0.01$). After 45 days, the bone formation percentage showed a significant difference between nano-bioglass and bioglass and between nano-HA and nano-bioglass groups ($P = 0.01$).
Conclusions: Nano-HA and nano-bioglass biomaterials showed promising results when compared to conventional micro-particles in the repair of bone defects.

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Repairing bone defects is an important part of prosthetic and periodontal treatments. Single tooth replacement and different fix restorations require sufficient bone with acceptable quality and quantity.[1] On the other hand, the ultimate goal of periodontal treatments is the regeneration of lost bone and gingival tissues.[2] It has been shown that the use of bone graft significantly improves the outcome of periodontal treatments.[3]

In order to regenerate bone, an ideal bone graft substitute should be biocompatible, bio-restorable, and osteogenic.[4] Up to this point, researchers have proposed different methods

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for repairing bone defects. Autografts, xenografts, allografts, and alloplasts are the most common methods used in repairing bone defects; however, no conclusive results have been obtained about the superiority of one method over another.[5]

The replacement of autogenous bone by synthetic materials has been a dilemma for clinicians.[4] It has been shown that autogenous bone grafts are the gold standard for the treatment of segmental bone defect but surgical morbidity, bone sources limitation, and the need for the second surgery have hindered their frequent use.[6] Also, the use of bone allografts has been suggested but probable, immunological responses restrict its use.[7] To overcome these problems, synthetic biocompatible bone substitutions became the most widely used procedure. These materials consist of calcium, phosphate, ceramics, and organic materials and they can provide a stable scaffold for appropriate bone response and subsequent bone repair.[8] It has been declared that at the very least, a bone graft material should be osteoconductive to promote bone regeneration.[9] Synthetic materials fulfill the goal of repairing smaller defects; however, they are not suitable for correcting extended bone defects.[10]

Hydroxyapatite (HA) and bioglass are synthetic bone substitutes mostly used to repair bone defects. Their ability to accelerate bone repair have been proved in different studies.[11,12]

HA is the main component of the bone structure. Due to its biocompatibility and crystallographic structure, HA is used to produce a synthetic bone substitute.[13,14] In the best cases, these biomaterials should be biologically degraded and replaced by a new regenerated bone. In Thomazin and Nandi studies, the efficacy of HA in the healing of bone defects was assessed. It was concluded that this material shows an acceptable capability in repairing bone defects.[15,16] The main weakness of the earlier dense HA was that they could not be degraded and remodeled in the host. Therefore, disappointing results were seen when using porous block HA.[6,17] As a result, nano-HA was introduced as a favorable alternative to other bone materials. Compared to traditional micro-HA, this material is highly biocompatible and biodegradable and it can be rapidly substituted by the host.[18-20]

Besides HA, Bioglass (BG) is another bone substitute used for repairing bone defects. BG is a nonrestorable bio-ceramic, which has the ability of forming durable bonds with living tissues.[1] The capability of BG in promoting osseointegration and forming new bone in defects has been proved in different studies.[21,22] These capabilities may be explained by ion release rates (Na, Ca) from BG surfaces, which can promote the proliferation and differentiation of bone cells.[23] In recent years, researchers have declared that nano-BG has superiority over micro-BG in repairing bone defects.[24]

Because of the aforementioned capabilities of nanoparticles, these materials have changed the current trend in bone defect repairing. There are not enough animal studies with conclusive results regarding the efficacy of nanoparticles, and no study has investigated the effect of both nano-HA and nano-BG compared to micro-HA and BG. Therefore, the present study was designed to comparatively evaluate the efficacy of nano-HA and nano-BG with their traditional micro counterparts in repairing bone defects.

MATERIALS AND METHODS

This was a prospective animal study which was held in the Implant Research Center of Isfahan Dental School. This study was approved by the Animal Department of Torabinejad Dental Research Center and Local Ethical Committee of Isfahan University of Medical Sciences. During the research period, there was enough water and food available for all the animals and maintenance conditions were according to the standard protocols of Isfahan University of Medical Sciences.

Animal model

Inclusion criteria

If age and sex conditions were according to the inclusion criteria then the animals were undergone the health examination test and finally four healthy male dogs, aged 1.5–2 and weighing from 32 to 46 Kg, were included in this study. If each of the animals become ill or encountered uncontrollable complications such as fracture they were excluded from the research program.

Anesthesia induction

Under the aseptic condition, initially, atropin 0.1% of 0.04 mg/Kg (Alfasan, Woerden, Holland) was injected to the animals subcutaneously. Then dogs were anesthetized using acepromazine 1% of 0.02 ml/Kg (Alfasan, Woerden, Holland) and ketamine 10% of 10 mg/kg (Ketamine HCL, Alfasan, Woerden, Holland) and were then maintained under general anesthesia by 5% of halothane (Halothane, Bp, Nicholas Piramal India Limited, India) and N20. First to fourth premolars were extracted atraumatically in each quadrant with the preservation of surrounding bone. After the surgeries, ceftriaxone 20 mg/Kg (Jaber Ebne Hayyan Pharmaceutical Co., Tehran, Iran) was used to prevent from the infection and tramadol 5 mg/Kg (Caspian Tamin Pharmaceutical Co., Guilan, Iran) to control pain. After 3 months, parallel periapical radiographs were taken by XCP film holders (Rinn Co., USA) to evaluate the healing of tooth extraction sites.

Material placement

After appropriate bone healing, infiltration anesthesia comprising 3.6 ml of lidocaine (Darou Pakhsh Pharmaceutical, Mfg. Co., Tehran, Iran) was placed in the mucobuccal fold. After a linear incision on the crestal

ridge from molar to anterior segment, a full thickness mucoperiosteal flap was elevated by a mucoperiosteal elevator. Two defects with 5 mm of depth and 5 mm of diameter on the crestal ridge and three similar defects on the buccal surface of the ridge were surgically created using trephine #5. As a result, five defects in each quadrant and 20 defects in each dog were created. These 20 defects were randomly divided into 5 groups:

- HA group (Algipore, Dentsply Friadent, Mannheim, Germany)
- Nano-HA group (new Nano, Isfahan University of Technology, Isfahan, Iran)
- BG group (Novabone, Alachua, USA)
- Nano-BG group (new Nano, Isfahan University of Technology, Isfahan, Iran)
- Control group.

The powders of the above materials were mixed with distilled water according to the manufacturers' instructions for each material, and they were then placed separately in different defects. Therefore, 16 defects in each dog were filled randomly by HA, nano-HA, BG, and nano-BG, and four defects were left as the control group. Then, all the defects were covered by a nonresorbable membrane (PTFE Whatman, Kent, UK).

The anesthesia induction steps and postoperative care were all as above.

Follow-up

The dogs were sacrificed at 4 time intervals (15, 30, 45, and 60 days, each dog at each time point). 15 days after the surgery, a lethal injection of 40 ml of pentobarbital sodium at 100 mg/ml in 290 g/1000 ml of spilritus fortis, 100 mg/kg was given to one of the dogs. All the 20 samples were extracted using trephine #8 with the sufficient amount of surrounding bone. These procedures were also applied on other dogs at 30, 45, and 60-day periods.

Specimens' analysis

Extracted specimens were kept in glutaraldehyde solution for 6 h. Longitudinal ground sections were then prepared using microtome (Accutom-50, Stuers, Copenhagen, Denmark). The samples were then stained by hematoxylin and eosin stain and mounted on the histological lams. All stained specimens were investigated under an optical microscope (×40) (Zless, Germany) and the percentage of different types of regenerated bones (lamellar and woven) was recorded. The cross sections of specimens were surveyed by Adobe Photoshop 7.0 (Adobe Systems, San José, CA, USA). and the amount of the regenerated bone was re-evaluated to confirm data.

Statistics

Data analysis was carried out by SPSS software ver. 15 (SPSS Inc., Chicago, IL, USA) and Mann–Whitney test (α =0.05).

RESULTS

In the present study, four healthy dogs were selected. First to fourth premolars were extracted from each dog in each quadrant and finally five bony defects were created in each quadrant. Twenty prepared cavities in each dog were randomly divided into five groups. Sixteen cavities were filled by HA, nano-HA, BG, and nano-BG and four cavities were left unfilled as the control group.

Mann–Whitney test showed that the difference between nano-HA and nano-BG groups with the control group was significant in all the time intervals regarding the amount of bone formation ($P < 0.01$). Also, this difference was significant for BG at 15 and 30 days and for HA at 15, 30, and 60 days, compared to the control group.

The total amount of regenerated bone showed a significant difference between HA and nano-HA and between HA and BG after 15 days ($P < 0.001$). However, this difference was not significant between BG and nano-BG and between nano-HA and nano-BG ($P > 0.05$). It should be emphasized that the amount of regenerated lamellar and woven bone showed a significant difference between HA and nano-HA, and this rate was higher in the nano-HA group. Nano-HA group showed the highest rate of bone formation after 15 days.

After 30 days, it can be declared that nano-BG and BG and nano-HA and nano-BG groups revealed a significant difference in the amount of bone regeneration ($P = 0.01$), and that nano-BG showed the highest rate of bone regeneration. Also, the amount of lamellar and woven bones showed a significant difference as they were higher in nano-BG and nano-HA when compared to BG and nano-BG, respectively.

After 45-day period, the bone formation percentage revealed a significant difference between nano-BG and BG and nano-HA and nano-BG groups ($P = 0.01$) as nano-HA showed the highest rate of bone regeneration after 45 days. This difference was not significant between HA and nano-HA ($P = 0.8$) and HA and BG groups ($P = 0.22$).

After 60 days, the difference between HA and nano-HA, nano-HA and nano-BG, and HA and BG groups was significant regarding the amount of regenerated bone ($P < 0.01$). Although the difference of total formed bone after 60 days was significant between HA and nano-HA, there was no significant difference in the types of the regenerated bone (woven and lamellar, P value for woven bone = 0.42, P value for lamellar bone = 0.19). Also, nano-BG showed the highest rate of bone formation after 60 days compared to the other bone materials.

It should be emphasized that the mean amount of total regenerated bone reached to its highest rate after 30 days by the application of nano-BG.

Tables 1-4 show the mean percentage of all the types of regenerated tissues in the four groups of biomaterials in 15, 30, 45, and 60 days of evaluation.

DISCUSSION

In the present study, the use of nanoparticles showed promising results compared to conventional micro-particles in repairing bone defects. In Götz *et al.* study in 2008, it was concluded that nano-porous HA materials show osteoconductive capacity and that they can be integrated to the host bone.[25] Nandi *et al.* in 2009 declared that BG blocks can enhance the bone regeneration in bone defects.[26]

In another study, Huber *et al.*[27] compared the therapeutic effects of nano-HA pastes (Ostim) with solid HA ceramics (Cerabone) in the treatment of bone defects in rabbits. A uniform and rapid bone growth was seen following the use of Ostim. In Schwarz study in 2006,[28] different cases with intrabony defects around dental implants were investigated. Following the application of nanocrystalline HA and bovine-derived xenograft in combination with a collagen membrane (BDX + BG), all the bone defects were repaired and the pocket depth was reduced.[28] In Fathi and Doostmohammadi study, the superiority of nano-BG materials over traditional micro-particle in the treatment of bone defects was shown.[29] All aforementioned studies show that the use of nano-HA and nano-BG materials can promote the healing process of bone defects. In the first stage of bone healing, the bone matrix proteins can be attracted by nano-bone granules and a vascular rich protein matrix is formed. The osteogenesis then takes place on this matrix and the final bone is regenerated. Since nanoparticles provide a larger surface area, the rate of attracted proteins would be increased. This can explain why the use of nanomaterials can enhance the bone regeneration capacity compared to micro-particle. Also, the large surface area of nanoparticles causes them to act as biological materials[30] and therefore, the mechanical reliability and osseoconductivity of nanoparticles can be improved.[31]

Table 1: The percentage of regenerated tissues in nano-HA in different times

Material and time of evaluation	Bone	Connective tissue	Lamellar bone	Woven bone
Nano-HA 15 days	0.35±48.18	0.35±51.81	0.54±30.43	1.05±18.37
Nano-HA 30 days	2.65±49.40	2.65±50.59	1.40±29.53	3.22±19.87
Nano-HA 45 days	2.64±44.5625	2.64±55.43	2.02±27.93	1.07±16.68
Nano-HA 60 days	1.78±38.65	2.92±60.71	1.60±22.43	1.49±15.84

HA=Hydroxyapatite

Table 2: The percentage of regenerated tissues in nano-BG in different times

Material and time of evaluation	Bone	Connective tissue	Lamellar bone	Woven bone
Nano-BG 15 days	6.52±43.46	6.37±56.65	6.27±26.84	1.07±16.53
Nano-BG 30 days	1.13±51.68	1.17±48.37	1.16±28.53	2.12±23.09
Nano-BG 45 days	3.82±39.43	3.82±60.56	3.31±23.43	2.79±16.00
Nano-BG 60 days	7.99±49.28	7.99±50.71	2.60±29.62	5.93±19.65

BG=Bioglass

Table 3: The percentage of regenerated tissues in HA in different times

Material and time of evaluation	Bone	Connective tissue	Lamellar bone	Woven bone
HA 15 days	43.21±2.88	56.78±2.88	28.09±3.04	15.25±1.57
HA 30 days	48.00±1.83	52.00±1.83	29.28±1.25	18.78±0.83
HA 45 days	44.18±3.80	55.81±3.80	26.00±4.20	18.25±1.06
HA 60 days	36.84±1.48	62.84±1.58	21.56±1.50	15.28±1.85

HA=Hydroxyapatite

Table 4: The percentage of regenerated tissues in BG in different times

Material and time of evaluation	Bone	Connective tissue	Lamellar bone	Woven bone
BG 15 days	40.43±2.24	59.56±2.24	25.12±4.20	15.31±2.12
BG 30 days	47.40±2.71	52.59±2.71	27.84±1.85	19.50±0.93
BG 45 days	42.56±1.48	57.43±1.48	25.15±0.97	17.37±1.71
BG 60 days	44.75±7.30	55.87±7.52	26.75±6.40	17.37±2.91

BG=Bioglass

In the present study, there was a significant difference in the amount of bone formation after 15, 30, and 60-day periods between HA and the control groups. This result can be explained by osseoconductivity and biocompatibility of HA bone material, and it is in line with the results of Welch *et al.*,[32] Den Boer *et al.*,[33] and Kruse *et al.*[34] studies.

In Zamet *et al.*,[35] and Nandi *et al.*[26] studies, the positive effects of BG on bone formation were shown. The bioactivity of BG is initiated exactly after it was mixed with saline or blood,[21] and this breaks down the silicon oxide bonds. As a result, silicic acid can be aggregated on the surface of the particles and it forms a negatively charged gel. As time elapses, calcium hydroxide is being formed on this surface to form a new apatite layer which initiates the bioactivity of BG.[1] These can be a good reason why the formation of new bone revealed a significant difference in BG after 15 and 30 days compared to control group. Also, it may be assumed that this bioactivity cannot play a significant role after 30 days since the difference between control and BG groups was not significant after that time.

Moreover, the bone formation percentage in the present study showed a remarkable difference at 30 and 45 days between BG and nano-BG groups and this rate was higher for nano-BG and BG after 30 and 45 days, respectively. It can be assumed that this difference goes back to the size of the particles as the particle size can play a determining role in breaking down the silicone oxide bond and forming calcium hydroxide.

It was shown that there was a significant difference between nano-HA and nano-BG materials after 30, 45, and 60 days in the amount of bone formation. This amount was higher in

the nano-BG group after 30 and 60 days. Since bone-related genes are just affected by BG and the mechanism of HA bone material is just confined to matrix formation, it seems rational that nano-BG particles showed superiority over nano-HA particles.

Nano-HA particles are highly biodegradable and biocompatible compared to conventional micro-particles.[19] Also, nano-particles accelerate the substitution of biomaterials by vital bones[18] and they can induce the osteogenic differentiation of stem cells.[36] All of the mentioned capabilities can explain the significant difference seen between HA and Nano-HA in repairing bone defects.

The effect of nano-particles is highly dependent on their concentration. It was shown that particle overload can impede the osteogenic differentiation of mesenchymal stem cells.[36] So, it is recommended to investigate the effect of the concentration of nanoparticles on the bone regeneration capacity in future studies. Owing to the complexity of human tooth growth and development, the regeneration of a whole tooth structure, including enamel, dentin/pulp complex, and periodontal tissues, as a functional entity in humans is not possible given the available regenerative biotechnologies. The end goal of tissue engineering is to develop the products capable of healing diseased or lost tissues and organs; thus, representing a departure from conventional biomedical research, whose primary focus is an understanding of mechanisms.[37]

CONCLUSION

Based on the results of the present study, it can be concluded that nano-HA and nano-bioglass biomaterials show promising results in repairing bone defects compared to conventional micro-particles. Since nanoparticles regenerated certain amount of bone faster than micro-particle, it seems rational that the use of nanoparticles reduces the healing time prior to prosthetic and periodontal treatments.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gosain AK; Plastic Surgery Educational Foundation DATA Committee. Bioactive glass for bone replacement in craniomaxillofacial reconstruction. Plast Reconstr Surg 2004;114:590-3.
2. Chitsazi MT, Shirmohammadi A, Faramarzie M, Pourabbas R, Rostamzadeh An. A clinical comparison of nano-crystalline hydroxyapatite (Ostim) and autogenous bone graft in the treatment of periodontal intrabony defects. Med Oral Patol Oral Cir Bucal 2011;16:e448-53.
3. Mellonig JT, Bowers GM, Cotton WR. Comparison of bone graft materials. Part II. New bone formation with autografts and allografts: A histological evaluation. J Periodontol 1981;52:297-302.
4. Kweon H, Lee KG, Chae CH, Balázsi C, Min SK, Kim JY, *et al.* Development of nano-hydroxyapatite graft with silk fibroin scaffold as a new bone substitute. J Oral Maxillofac Surg 2011;69:1578-86.
5. Gosain AK, Persing JA. Biomaterials in the face: Benefits and risks. J Craniofac Surg 1999;10:404-14.
6. Cottrell DA, Wolford LM. Long-term evaluation of the use of coralline hydroxyapatite in orthognathic surgery. J Oral Maxillofac Surg 1998;56:935-41.
7. Lane JM, Glaser AR, Forster RA Gazdag D. Alternatives to autogenous bone graft: Efficacy and indications. J Am Acad Orthop Surg 1995;3:1-8.
8. Sculean A, Barbé G, Chiantella GC, Arweiler NB, Berakdar M, Brecx M. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. J Periodontol 2002;73:401-8.
9. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. J Am Acad Orthop Surg 2005;13:77-86.
10. Henkel KO, Gerber T, Lenz S, Gundlach KK, Bienengräber V. Macroscopical, histological, and morphometric studies of porous bone-replacement materials in minipigs 8 months after implantation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:606-13.
11. Sculean A, Pietruska M, Schwarz F, Willershausen B, Arweiler NB, Auschill TM. Healing of human intrabony defects following regenerative periodontal therapy with an enamel matrix protein derivative alone or combined with a bioactive glass. A controlled clinical study. J Clin Periodontol 2005;32:111-7.
12. Oberg S, Kahnberg KE. Combined use of hydroxy-apatite and Tisseel in experimental bone defects in the rabbit. Swed Dent J 1993;17:147-53.
13. Williams RL, Brown SA, Merritt K. Electrochemical studies on the influence of proteins on the corrosion of implant alloys. Biomaterials 1988;9:181-6.
14. Hing KA, Revell PA, Smith N, Buckland T. Effect of silicon level on rate, quality and progression of bone healing within silicate-substituted porous hydroxyapatite scaffolds. Biomaterials 2006;27:5014-26.
15. Nandi SK, Kundu B, Ghosh SK, De DK, Basu D. Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goat. J Vet Sci 2008;9:183-91.
16. Soccol AT, Bettega S, Noronha L, Sass S, Soccol VT, Scholz MR, *et al.* Defect repair in rat mandible with hydroxyapatite cement compared to small intestine submucosa. Braz J Otorhinolaryngol 2006;72:195-9.
17. Pohunková H, Adam M. Reactivity and the fate of some composite bioimplants based on collagen in connective tissue. Biomaterials 1995;16:67-71.
18. Strietzel FP, Reichart PA, Graf HL. Lateral alveolar ridge augmentation using a synthetic nano-crystalline hydroxyapatite bone substitution material (Ostim): Preliminary clinical and histological results. Clin Oral Implants Res 2007;18:743-51.
19. Webster TJ, Siegel RW, Bizios R. Osteoblast adhesion on nanophase ceramics. Biomaterials 1999;20:1221-7.
20. Kim HW, Kim HE, Salih V. Stimulation of osteoblast responses to biomimetic nanocomposites of gelatin-hydroxyapatite for tissue engineering scaffolds. Biomaterials 2005;26:5221-30.
21. Blaydon S, Amato MM, Neuhaus R, Shore JW. The orbito-facial uses of Nova Bone C/M, a bioactive glass synthetic bone graft particulate for craniofacial and maxillofacial surgery. Dallas, Texas: Presented at the American Society of Oculoplastic Plastic Reconstructive Surgery Scientific Symposium, October 20, 2000.
22. Zhong JP, LaTorre GP, Hench LL. The kinetics of bioactive ceramics: Part VII. Binding of collagen to hydroxyapatite and bioactive glass. In: Anderson H, Yli-Urpo A, editors. Proceedings of the 7th International Symposium on Ceramics in Medicine: Bioceramics. Vol. 7. Oxford: Butterworth Heinemann; 1994. p. 61-6.

23. Price N, Bendall SP, Frondoza C, Jinnah RH, Hungerford DS. Human osteoblast-like cells (MG63) proliferate on a bioactive glass surface. J Biomed Mater Res 1997;37:394-400.
24. Fathi M, Doostmohammadi A. Bioactive glass nanopowder and bioglass coating for biocompatibility improvement of metallic implant. J Mater Process Technol 2009;209:1385-91.
25. Götz W, Gerber T, Michel B, Lossdörfer S, Henkel KO, Heinemann F. Immunohistochemical characterization of nanocrystalline hydroxyapatite silica gel (NanoBone(r)) osteogenesis: A study on biopsies from human jaws. Clin Oral Implants Res 2008;19:1016-26.
26. Nandi SK, Kundu B, Datta S, De DK, Basu D. The repair of segmental bone defects with porous bioglass: An experimental study in goat. Res Vet Sci 2009;86:162-73.
27. Huber FX, McArthur N, Hillmeier J, Kock HJ, Baier M, Diwo M, *et al.* Void filling of tibia compression fracture zones using a novel resorbable nanocrystalline hydroxyapatite paste in combination with a hydroxyapatite ceramic core: First clinical results. Arch Orthop Trauma Surg 2006;126:533-40.
28. Schwarz F, Bieling K, Latz T, Nuesry E, Becker J. Healing of intrabony peri-implantitis defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide). A case series. J Clin Periodontol 2006;33:491-9.
29. Fathi M, Mortazavi V, Doostmohammadi A. Bioactive glass nano powder for the treatment of oral bone defects. J Dent Tehran Univ Med Sci 2007;4:115-22.
30. Quinones C, Lovelace T. Utilization of a bioactive synthetic particulate for periodontal therapy and bone augmentation techniques. Pract Periodont Aesthet Dent 1997;9:1-7.
31. Tadjoedin ES, de Lange GL, Lyaruu DM, Kuiper L, Burger EH. High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. Clin Oral Implants Res 2002;13:428-36.
32. Welch RD, Berry BH, Crawford K, Zhang H, Zobitz M, Bronson D, *et al.* Subchondral defects in caprine femora augmented with *in situ* setting hydroxyapatite cement, polymethylmethacrylate, or autogenous bone graft: Biomechanical and histomorphological analysis after two-years. J Orthop Res 2002;20:464-72.
33. den Boer FC, Wippermann BW, Blokhuis TJ, Patka P, Bakker FC, Haarman HJ. Healing of segmental bone defects with granular porous hydroxyapatite augmented with recombinant human osteogenic protein-1 or autologous bone marrow. J Orthop Res 2003;21:521-8.
34. Kruse A, Jung RE, Nicholls F, Zwahlen RA, Hämmerle CH, Weber FE. Bone regeneration in the presence of a synthetic hydroxyapatite/silica oxide-based and a xenogenic hydroxyapatite-based bone substitute material. Clin Oral Implants Res 2011;22:506-11.
35. Zamet JS, Darbar UR, Griffiths GS, Bulman JS, Brägger U, Bürgin W, *et al.* Particulate bioglass as a grafting material in the treatment of periodontal intrabony defects. J Clin Periodontol 1997;24:410-8.
36. Tautzenberger A, Kovtun A, Ignatius A. Nanoparticles and their potential for application in bone. Int J Nanomedicine 2012;7:4545-57.
37. Saini R, Saini S, Sharma S. Therapeutics of stem cells in periodontal regeneration. J Nat Sci Biol Med 2011;2:38-42.