

**The Application Of Hydroxyapatite In Socket Preservation. A Review Of The Past And Current Advancement**

---

**ABSTRACT**

Alveolar ridge dimensional loss is a physiologic consequence of tooth extraction. Often, this event causes compromised feasibility of implant placement, prosthetic rehabilitation and esthetic outcome. As an attempt to minimize the shrinkage of the alveolar bone, socket preservation was introduced to intervene with the natural process by providing a scaffold with antibacterial and regenerative properties that aid in the healing process. For past decades, hydroxyapatites(HA) are one of the biomaterials used in socket preservation procedure and was thought to be biocompatible, long-term resorbable or non-resorbable and osteoconductive. Several improvements have been made to enhance the properties of hydroxyapatites that acted in providing a framework during the healing process to provide better outcomes. Here, we summarize the past, current advancements in the use of hydroxyapatite in socket preservation and its future direction.

*Key words: tooth extraction, socket preservation, hydroxyapatite, alloplast*

## INTRODUCTION

Maintenance and improvement of natural dentition to achieve optimum health, comfort, and function is an ultimate goal in periodontal therapy[1]. However, under certain circumstances based on the clinician's opinion on prognosis, dental extraction is preferable, indicating that the tooth cannot be successfully treated[2]. Following extraction, a local inflammatory response occurs, with a subsequent variable degree of alveolar ridge atrophy, characterized by a degree of bone resorption[3]–[5]. Depending on individual local and systemic factors, the alveolar ridge showed the mean reduction in the width of 3.87 mm and 1.21 mm clinically and radiographically[6]. This physiologic resorption may complicate implant placement, prosthetic rehabilitation and esthetic outcome[7]. Numerous studies have addressed socket preservation after tooth extraction as an effective treatment approach in compensating alveolar bone resorption, hence reduces the need for bone augmentation[8]–[11].

During the early stage of the healing process, the implantation of graft materials into the fresh extraction socket helps stabilize the coagulum and act as a scaffold that probably sustains in the socket until the formation of mineralized tissue is completed. It was reported that some graft materials have the potential to directly modulate the gene expression of osteoblastic cells, promoting increased production of the protein of the extracellular matrix[6]. Ideal bone grafts should enhance 1) osseointegration: provide direct attachment of the graft without fibrous tissue interference [12], [13] 2) osteoconduction: provide a framework for ingrowth of blood vessels, mesenchymal cells and osteoblasts 3) osteoinduction: recruitment of mesenchymal cells to differentiate into osteoblastic cells and 4) osteogenesis: provide osteoblast and stem cells for new bone formation[13]. They should have the capacity to be resorbed whilst being replaced by new bone and sustain mechanical force at the grafted site[14]. Various osseous graft materials are available, including autografts (harvested from the host), allografts (derived from a cadaver), xenografts (derived from other species) and alloplasts (synthetic materials obtained from natural and synthetic sources. Previously, autografts were considered as the 'gold standard' graft material[15], [16]. However, they are often associated with the risk of morbidity at the donor site, such as nerve damage, infection due to graft contamination, and loss of function[17]. Allografts have issues with tissue integration and revascularization, the potential of disease transmission, and often, patients refuse to have cadaver's bone implanted in their oral cavity [18], [19]. Xenografts have shown long term adverse effects, including the high risk of host immune response, displacement of graft materials, cystic formation and chronic inflammation[20]. These issues emphasize the interest in the exploration of new bone substitutes that mimic the properties of bone to overcome the weaknesses of currently available graft materials.

## Hydroxyapatite

Hydroxyapatite (HA) is one of the important classes of substitute materials belongs to the calcium phosphate ceramic group that has been extensively used in bone regeneration surgery. Bone is made up of 69 wt% mineral apatite, 22 wt% organic component (matrix proteins, lipid and osteogenic factors) and 9 wt% water. The mineral components of the bone were idealized as calcium hydroxyapatite, which contains irregularly shaped particles of various sizes ranges 30-45 nm length and width and an average of about 5nm thickness. HA with the general formula  $(Ca_{10}(PO_4)_6(OH)_2)$  contains a calcium phosphate ratio of 1.67, similar to the mineral components of the bone. HA is one of the most stable calcium phosphate-based materials, which is less soluble. Because it contains only calcium and phosphate, it does not cause any tissue inflammation, considered biocompatible[21]. HA properties depend on manufacturing methods, particle size, crystallinity, porosity, and specific surface area[22]. The commercially available HA includes HA cement, injectable form, porous and non-porous HA, nanocrystalline HA and implant coatings.

## HA in socket preservation - Historical context

Dennissen et al. (1979) reported the first trial in using HA as an alternative to overdenture and submerging vital root therapy in preserving the bulk of the alveolar ridge for better retention of prostheses. The study used HA in the form of solid, non-porous prepared by compression of calcium hydroxyapatite powder and sintered at 1100°C producing the desired shape dental root implants and placed in fresh extraction[23]. Based on the fact that HA can form chemical bonds with the surrounding bone, a few other studies also demonstrated the use of pre-shaped solid HA to fit closely

into the fresh extraction socket and revealed that this biomaterial is a promising root substitute[24], [25]. However, similar complications were encountered, including exposure of root implant, mucosal erosion, migration, wound dehiscence, and implant loss, all due to negligible resorption and weak bonding at the bone-implant interface[26], [27]. James et al. found the HA root implant remains as a confined solid particle with peripheral surface were surrounded by bone after two years of implantation[25]. Dennisen et al. (1989) showed that the HA root implant remains stable after eleven years of implantation with a direct attachment to the bone[28]. An animal study using dense ceramic HA in the form of granules showed HA remained in the socket after 12 months with a trace of fibrocartilages and bone jointly interfaced with HA particles[29].

The lack of data in the literature concerning the HA degradation making this material was initially believed to be non-resorbable, only applicable for the long-term preservative procedures such as augmentation of atrophic alveolar ridge and reconstruction of periodontal defects[30]–[32]. The intimate binding of HA to the bone with a lack of physiologic bone turnover raises an argument about the long term outcome of the implant placement at previously grafted sites[33]. Because of that, HA has been one of the most criticized biomaterials due to its limited ability to promote regeneration and unpredictable new bone formation. Furthermore, it does not provide any cellular elements required for osteogenesis, thus act as osteoconductive elements rather than osteoinductive [34].

The term osteoconductive refers to the ability to support tissue healing by promoting the osteogenesis on its surface and serving as a scaffold or template that guides new bone to be deposited along its surface[21], [35]. When implanted into the living tissues, HA forms interlocking between the bone with the surface irregularities and forming a neoformed layer that assures direct interaction between bone and biomaterials to prevent the interference of fibrous tissues[22]. Others described the existing calcium HA from natural bone bind to calcium phosphate biomaterial that further acts as a place holder to allow protein adsorption (from the biological environment) and further support the migration of bone cells, cytokines, and concentrates bone morphogenic proteins (BMP) that are required for production and mineralization of bony matrix[21].

## **The conception of nanotechnology has improved the material properties**

The utilization of nanotechnology in material production has changed material properties over the last two decades and provided a greater insight into the material interaction with host tissues. The paradigm shifted from a belief that HA is biocompatible, non-resorbable and osteoconductive to the idea that HA is rather more bioactive, bioresorbable, and osteoinductive material. Nanotechnology deals with designing and the production of matter at the dimension of approximately 1-100 nm enable material production with controlled particle morphology and porosity[36]. Nanostructured HA possess more similarity with the natural HA in terms of surface roughness, wettability, and larger surface area render more positive biological effects; improved protein adsorption, mesenchymal stem cells adhesion, differentiation and proliferation are enhanced and better osteoclastic response[37], [38].

Bioactivity is the ability of biomaterials to bond directly with a newly formed bone by allowing attachment and differentiation of cells directly on their surface and stimulate osteogenesis[39]. It is well known that the bone substitute needs to possess bioactive properties by forming a carbonate apatite layer on their surface in the living tissue and bond to the bone through this layer. The properties of HA can be improved to become bioactive by modifying the key parameters of material includes crystalline size, particle distribution and agglomeration[22]. Various methods have been used to manufacture nanocrystalline HA powder includes wet chemical precipitation, hydrothermal methods, plasma spraying, high-temperature solid-state reactions, sol-gel methods, microemulsion techniques, and microwave-assisted synthesis method[40] that produced HA with a greater surface area and significant bioactivity compared to larger sized crystal[41]. A study by Fathi et al. investigated the bioactivity and ionic dissolution rate of microcrystalline and nanocrystalline HA (average particle size 29 nm) found that bone-like apatite particle that was deposited on the superficial layer of nanocrystalline HA was greater than precipitation observed on the microcrystalline HA. The bioabsorbability rate of nanocrystalline HA was higher than that of conventional HA and almost identical to natural bone apatite. The study concluded that the bioactivity and bioresorbability of nanocrystalline HA crystals could be enhanced by controlling the crystalline size. The nanocrystalline HA can be more useful for treating alveolar bone deformity than conventional HA and could be more effective as a bone substitutes material to promote bone formation[42].

Constant resorption and complementary substitution of new bone tissue are desirable for long-term bone regeneration therapy. It is evident that resorption of graft material is mediated by the action of monocytes, macrophages and osteoclast through phagocytic activity. In vivo, HA graft is resorbed only when the particle size is suitable for phagocytosis. In-vitro, the osteoclast-like cell function was enhanced when cultured on nanophase-HA compared to larger than 100nm-sized-HA[43]. A histochemical and immunohistochemical study on human biopsies investigated the healing of nanocrystalline HA grafted in alveolar augmentation, and sinus lift procedures revealed the evidence of bone turn-over demonstrated by detection of TRAP-positive osteoclasts like cells on the graft surface and newly formed bone. An osteogenic area with bone deposition was also detected around the same graft. This postulates a possible integration of grafting materials into the physiologic bone remodelling process of the host[44]

Osteoinductivity is characterized by the ability of the material to induce new bone formation without the presence of osteogenic factors[21]. Generally, HA is osteoconductive; however, several synthetic HA without incorporating any osteogenic factors were reported to have osteoinductive properties whereby they were able to produce de novo bone formation when implanted at nonbony sites[45], [46]. The inductive event of some synthetic HA was associated with the composition, crystallinity, size, interconnecting macro and micro porosities and geometry<sup>38</sup>. Such features were believed to allow adsorption, entrapment and concentrating the circulating BMPs, osteogenic factors and/or osteoprogenitor cells, generating osteoinductive effects of synthetic HA[21]. Certain synthetic HA showed low osteoinductive effects in animal models because BMPs were superficially adsorbed, then rapidly released and exhausted before the osteogenic effects took place[48]. Such deficiency could be enhanced by an engineered combination of osteoprogenitor cells, bone growth factors (BMPs) and other bioactive factors to intensify the bone formation[21], [47]. The characteristics of HA can further be upgraded by adding porogen (e.g., naphthalene, silicon, hydrogen peroxide)[21], [49], creating a porous structure intending to intensify angiogenesis and provides a favourable environment for the migration of cells and tissue growth [50]. Due to that, it has a faster bone regeneration capacity demonstrated by a significant increase in osteophytes compared to non-porous HA[51].

Similar to the other calcium phosphate-based materials, HA is brittle and lacks elastic deformation properties. The low mechanical strength with lack of toughness, making its application in the load-bearing area restricted[52]. As the interconnecting porosity increases, the mechanical properties drastically decreases. Porous HA has a mechanical strength of 42 MPa, less than that of cortical bone (60-110 MPa)[53]. Low mechanical properties can contribute to material disintegration under cyclic stress and result in fibrous instead of bone tissue formation[54].

### **The usage of nano-hydroxyapatite in socket preservation**

Considering nano-HA has superior biological performance than conventional-HA, it has gained attention among clinicians to be in socket preservation procedures. Checchi et al. (2011) compared clinical-radiographic and histological outcomes of two nanostructured HA; biomimetic HA (HA enriched with magnesium ions) and nanocrystalline HA grafted in a fresh extraction socket – both showed evidence of resorption and osseointegration. The study concluded that there was no difference between both materials in the matter of new bone formation and total material resorption, in addition to the effectiveness in providing appropriate structural support during the healing period. The authors assumed that both materials could be used in the fresh extraction socket to limit the amount of alveolar ridge shrinkage[55]. Another study by Luigi et al. (2015) evaluated the effects of Mg-enriched nano-HA found that this material allowed complete tissue healing and demonstrated significant graft resorption after 12 months of the study period. The report suggested that due to this characteristic, Mg-enriched nano-HA is suitable to be grafted at postextraction sites and subsequent implant placement, reducing the possibility of failure in osseointegration due to the presence of residual graft materials[56]

Nanocrystalline HA in socket preservation procedures resulted in similar, less alveolar ridge dimensional alteration following tooth extraction compared with xenografts. A study by Gholami et al. (2011) concluded that no conclusive evidence of the superiority of one specific material was obtained from the histologic and histomorphometric standpoint. The only difference was in respect to the source of the material; one material is animal derivative, and another is synthetic[57]. In contrast with the finding by Rodrigo et al. (2019), when nanostructured HA was substituted with carbonate ions, a greater amount of new bone formation and higher biodegradation was demonstrated compared to bovine xenograft (Bio-Oss). The physicochemical structure of carbonated HA produces greater wettability, thermal stability and uniform morphology that have biological performance and bonding

capacity between graft materials and bone. However, their findings should be interpreted with caution due to lack of comparison in regards to alveolar ridge dimensional alteration with and without biomaterial filling[58]. Other than that, efforts have been made to produce more ideal economic graft substitutes by utilizing eggshell-derived nano-HA, which showed desirable bone regeneration potential in the grafted than that in the ungrafted sites[59].

## CONCLUSION

Since more than 3 decades, numerous studies have investigated the performance of different types of HA in socket preservation. Nanostructured HA can be considered as a promising alternative material for regenerating new bone in socket preservation procedures. The improved material properties such as osteoinduction are biodegradable, and, more importantly, it mimics extracellular bony matrix to allow undifferentiated mesenchymal cell infiltration and proliferation to produce new bone. However, most of the studies mentioned only involved a small number of subjects using different types of nano-HA with the lack of important parameters needed for comparison in alveolar ridge dimensional changes. When metal/ions are substituted, the biological behaviour of the materials is altered. This warrants a larger sample with well-design studies for each material modification to provide a deeper understanding of bone regeneration patterns.

## REFERENCES

- [1] AAP, "Comprehensive Periodontal Therapy: A Statement by the American Academy of Periodontology," *J. Periodontol.*, vol. 82, no. 7, pp. 943–949, Jul. 2011, doi: 10.1902/jop.2011.117001.
- [2] K. RT, "Strategic extraction: a paradigm shift that is changing our profession," *J. Periodontol.*, vol. 79, no. 6, pp. 971–977, Jun. 2008, doi: 10.1902/JOP.2008.070551.
- [3] M. H. Amler, "The time sequence of tissue regeneration in human extraction wounds," *Oral Surgery, Oral Med. Oral Pathol.*, vol. 27, no. 3, pp. 309–318, 1969, doi: 10.1016/0030-4220(69)90357-0.
- [4] J. L. Saffar, J. J. Lasfargues, and M. Cherruau, "Alveolar bone and the alveolar process: The socket that is never stable," *Periodontol. 2000*, vol. 13, no. 1, pp. 76–90, 1997, doi: 10.1111/j.1600-0757.1997.tb00096.x.
- [5] M. G. Araújo, C. O. Silva, M. Misawa, and F. Sukekava, "Alveolar socket healing: What can we learn?," *Periodontol. 2000*, vol. 68, no. 1, pp. 122–134, 2015, doi: 10.1111/prd.12082.
- [6] R. Farina and L. Trombelli, "Wound healing of extraction sockets," *Endod. Top.*, vol. 25, no. 1, pp. 16–43, Sep. 2011, doi: 10.1111/ETP.12016.
- [7] C. V, E. O, R. M, S. K, N. LP, and B. D, "Ridge alterations post-extraction in the esthetic zone: a 3D analysis with CBCT," *J. Dent. Res.*, vol. 92, no. 12 Suppl, Dec. 2013, doi: 10.1177/0022034513506713.
- [8] S. García-González, A. Galve-Huertas, S. A. H. Centenero, S. Mareque-Bueno, M. Satorres-Nieto, and F. Hernández-Alfaro, "Volumetric changes in alveolar ridge preservation with a compromised buccal wall: A systematic review and meta-analysis," *Med. Oral Patol. Oral y Cir. Bucal*, vol. 25, no. 5, pp. e565–e575, 2020, doi: 10.4317/MEDORAL.23451.
- [9] E. Couso-Queiruga, S. Stuhr, M. Tattan, L. Chambrone, and G. Avila-Ortiz, "Post-extraction dimensional changes: A systematic review and meta-analysis," *Journal of Clinical Periodontology*, vol. 48, no. 1. Blackwell Munksgaard, pp. 126–144, Jan. 01, 2021, doi: 10.1111/jcpe.13390.
- [10] M. N, T.-E. A, D. N, and M. N, "Hard and soft tissue changes following alveolar ridge

- preservation: a systematic review," *Clin. Oral Implants Res.*, vol. 28, no. 8, pp. 982–1004, Aug. 2017, doi: 10.1111/CLR.12911.
- [11] G. Avila-Ortiz, L. Chambrone, and F. Vignoletti, "Effect of alveolar ridge preservation interventions following tooth extraction: A systematic review and meta-analysis," *J. Clin. Periodontol.*, vol. 46, no. S21, pp. 195–223, Jun. 2019, doi: 10.1111/JCPE.13057.
- [12] C. PD, H. D, G. S, and M. J, "Bone healing and bone substitutes," *Facial Plast. Surg.*, vol. 18, no. 1, pp. 13–26, 2002, doi: 10.1055/S-2002-19823.
- [13] T. J. Cypher and J. P. Grossman, "Biological principles of bone graft healing," *J. Foot Ankle Surg.*, vol. 35, no. 5, pp. 413–417, Sep. 1996, doi: 10.1016/S1067-2516(96)80061-5.
- [14] W. Gotz and S. Papageorgiou, "Molecular, Cellular and Pharmaceutical Aspects of Synthetic Hydroxyapatite Bone Substitutes for Oral and Maxillofacial Grafting," *Curr. Pharm. Biotechnol.*, vol. 18, no. 1, pp. 95–106, Dec. 2016, doi: 10.2174/1389201017666161202103218.
- [15] J. V. D. S. Canellas *et al.*, "Histomorphometric evaluation of different grafting materials used for alveolar ridge preservation: a systematic review and network meta-analysis," *International Journal of Oral and Maxillofacial Surgery*, vol. 49, no. 6. Churchill Livingstone, pp. 797–810, Jun. 01, 2020, doi: 10.1016/j.ijom.2019.10.007.
- [16] A. H. Dewi and I. D. Ana, "The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation , sinus augmentation , and periodontal bone defect : A systematic review," *Heliyon*, no. May, p. e00884, 2018, doi: 10.1016/j.heliyon.2018.e00884.
- [17] C. M. Misch, "Complications of autogenous bone grafting," in *Dental Implant Complications: Etiology, Prevention, and Treatment: Second Edition*, Wiley Blackwell, 2015, pp. 332–361.
- [18] H. Opris *et al.*, "Clinical applications of avian eggshell-derived hydroxyapatite," *Bosnian Journal of Basic Medical Sciences*, vol. 20, no. 4. Association of Basic Medical Sciences of FBiH, pp. 430–437, 2020, doi: 10.17305/bjbms.2020.4888.
- [19] D. Baranes and G. M. Kurtzman, "Biphasic Calcium Sulfate as an Alternative Grafting Material in Various Dental Applications," *J. Oral Implantol.*, vol. 45, no. 3, pp. 247–255, Jun. 2019, doi: 10.1563/AAID-JOI-D-18-00306.
- [20] A. E. Rodriguez and H. Nowzari, "The long-term risks and complications of bovine-derived xenografts: A case series," *J. Indian Soc. Periodontol.*, vol. 23, no. 5, pp. 487–492, Sep. 2019, doi: 10.4103/jisp.jisp\_656\_18.
- [21] L. RZ, "Calcium phosphate-based osteoinductive materials," *Chem. Rev.*, vol. 108, no. 11, pp. 4742–4753, Nov. 2008, doi: 10.1021/CR800427G.
- [22] C. Rey, "Calcium Phosphates for Medical Applications," *Calcium Phosphates Biol. Ind. Syst.*, pp. 217–251, 1998, doi: 10.1007/978-1-4615-5517-9\_10.
- [23] D. HW and de G. K, "Immediate dental root implants from synthetic dense calcium hydroxylapatite," *J. Prosthet. Dent.*, vol. 42, no. 5, pp. 551–556, 1979, doi: 10.1016/0022-3913(79)90253-1.
- [24] P. Kangyonkit, V. J. Matukas, and D. J. Castleberry, "Clinical evaluation of Durapatite submerged-root implants for alveolar bone preservation," *Int. J. Oral Maxillofac. Surg.*, vol. 15, no. 1, pp. 62–71, Feb. 1986, doi: 10.1016/S0300-9785(86)80012-6.
- [25] Q. JH, K. JN, H. RG, and S. CM, "Preservation of the alveolar ridge with hydroxylapatite tooth root substitutes," *J. Am. Dent. Assoc.*, vol. 110, no. 2, pp. 189–193, 1985, doi: 10.14219/JADA.ARCHIVE.1985.0276.
- [26] B. DH, "Particles versus solid forms of hydroxyapatite as a treatment modality to preserve residual alveolar ridges," *J. Prosthet. Dent.*, vol. 56, no. 3, pp. 322–326, 1986, doi: 10.1016/0022-3913(86)90013-2.
- [27] C. AN, R. E, S. R, T. G, and D. JB, "Hydroxylapatite (H/A) particulate versus cones as post-extraction implants in humans. Parts I & II," *J. Biomed. Mater. Res.*, vol. 22, no. 12, pp. 1165–

- 1180, 1988, doi: 10.1002/JBM.820221208.
- [28] D. HW, K. W, V. AA, and van den H. A, "Eleven-year study of hydroxyapatite implants," *J. Prosthet. Dent.*, vol. 61, no. 6, pp. 706–712, 1989, doi: 10.1016/S0022-3913(89)80047-2.
- [29] S. AD, S. RG, R. SS, and D. HP, "Evaluation of implanted durapatite particles in fresh extraction sockets to maintain the alveolar ridge in beagle dogs," *J. Prosthet. Dent.*, vol. 57, no. 3, pp. 331–337, 1987, doi: 10.1016/0022-3913(87)90308-8.
- [30] F. SJ, K. L, S. IW, and S. SS, "Human clinical and histologic responses to Durapatite implants in intraosseous lesions. Case reports," *J. Periodontol.*, vol. 53, no. 12, pp. 719–725, Dec. 1982, doi: 10.1902/JOP.1982.53.12.719.
- [31] B. R and B. OR, "Effect of hydroxylapatite, tricalcium phosphate, and collagen on the healing of defects in the rat mandible," *J. Oral Maxillofac. Surg.*, vol. 46, no. 7, pp. 589–594, 1988, doi: 10.1016/0278-2391(88)90150-4.
- [32] F. Ye *et al.*, "A long-term evaluation of osteoinductive HA/ $\beta$ -TCP ceramics in vivo: 4.5 Years study in pigs," *J. Mater. Sci. Mater. Med.*, vol. 18, no. 11, pp. 2173–2178, Nov. 2007, doi: 10.1007/S10856-007-3215-2.
- [33] M. C, P. A, P. V, and I. G, "Dense hydroxyapatite inserted into postextraction sockets: a histologic and histomorphometric 20-year case report," *J. Periodontol.*, vol. 79, no. 5, pp. 929–933, May 2008, doi: 10.1902/JOP.2008.070245.
- [34] H. F. Nasr, M. E. Aichelmann-Reidy, and R. A. Yukna, "Bone and bone substitutes," *Periodontol. 2000*, vol. 19, no. 1, pp. 74–86, 1999, doi: 10.1111/j.1600-0757.1999.tb00148.x.
- [35] G. W and P. SN, "Molecular, Cellular and Pharmaceutical Aspects of Synthetic Hydroxyapatite Bone Substitutes for Oral and Maxillofacial Grafting," *Curr. Pharm. Biotechnol.*, vol. 18, no. 1, 2017, doi: 10.2174/1389201017666161202103218.
- [36] H. Zhou and J. Lee, "Nanoscale hydroxyapatite particles for bone tissue engineering," *Acta Biomater.*, vol. 7, no. 7, pp. 2769–2781, Jul. 2011, doi: 10.1016/J.ACTBIO.2011.03.019.
- [37] F. K, T. PA, and T. N, "Recent advances in research applications of nanophase hydroxyapatite," *Chemphyschem*, vol. 13, no. 10, pp. 2495–2506, Jul. 2012, doi: 10.1002/CPHC.201200080.
- [38] A. Pilloni *et al.*, "Analysis of human alveolar osteoblast behavior on a nano-hydroxyapatite substrate: an in vitro study," *BMC Oral Heal. 2014 141*, vol. 14, no. 1, pp. 1–7, Mar. 2014, doi: 10.1186/1472-6831-14-22.
- [39] R. Z. Legeros, "Properties of Osteoconductive Biomaterials: Calcium Phosphates List of Abbreviations Used BMP bone morphogenetic protein ECM extracellular matrix MSC mesenchymal stem cell," *Clin. Orthop. Relat. Res. Number*, vol. 395, pp. 81–98, 2002.
- [40] M. N. Hassan, M. M. Mahmoud, A. A. El-Fattah, and S. Kandil, "Microwave-assisted preparation of Nano-hydroxyapatite for bone substitutes," *Ceram. Int.*, vol. 42, no. 3, pp. 3725–3744, 2016, doi: 10.1016/j.ceramint.2015.11.044.
- [41] W. TJ, E. C, D. RH, S. RW, and B. R, "Enhanced osteoclast-like cell functions on nanophase ceramics," *Biomaterials*, vol. 22, no. 11, pp. 1327–1333, Jun. 2001, doi: 10.1016/S0142-9612(00)00285-4.
- [42] M. H. Fathi, V. Mortazavi, and S. I. R. Esfahani, "Bioactivity Evaluation of Synthetic Nanocrystalline Hydroxyapatite," *Dent. Res. J. (Isfahan).*, vol. 5, no. 2, Mar. 2009, Accessed: Aug. 22, 2021. [Online]. Available: <http://drj.mui.ac.ir/index.php/drj/article/view/55/30>.
- [43] W. TJ, E. C, D. RH, S. RW, and B. R, "Enhanced osteoclast-like cell functions on nanophase ceramics," *Biomaterials*, vol. 22, no. 11, pp. 1327–1333, Jun. 2001, doi: 10.1016/S0142-9612(00)00285-4.
- [44] G. W, G. T, M. B, L. S, H. KO, and H. F, "Immunohistochemical characterization of nanocrystalline hydroxyapatite silica gel (NanoBone(r)) osteogenesis: a study on biopsies from

- human jaws," *Clin. Oral Implants Res.*, vol. 19, no. 10, pp. 1016–1026, Oct. 2008, doi: 10.1111/J.1600-0501.2008.01569.X.
- [45] R. M. Gruber *et al.*, "Mandibular reconstruction using a calcium phosphate/polyethylene glycol hydrogel carrier with BMP-2," *J. Clin. Periodontol.*, vol. 41, no. 8, pp. 820–826, Aug. 2014, doi: 10.1111/JCPE.12264.
- [46] P. Y. Yun, Y. K. Kim, K. I. Jeong, J. C. Park, and Y. J. Choi, "Influence of bone morphogenetic protein and proportion of hydroxyapatite on new bone formation in biphasic calcium phosphate graft: Two pilot studies in animal bony defect model," *J. Cranio-Maxillofacial Surg.*, vol. 42, no. 8, pp. 1909–1917, Dec. 2014, doi: 10.1016/J.JCMS.2014.07.011.
- [47] S. Sprio *et al.*, "Surface Phenomena Enhancing the Antibacterial and Osteogenic Ability of Nanocrystalline Hydroxyapatite, Activated by Multiple-Ion Doping," *ACS Biomater. Sci. Eng.*, vol. 5, no. 11, pp. 5947–5959, Nov. 2019, doi: 10.1021/ACSBMATERIALS.9B00893.
- [48] J. Tazaki *et al.*, "BMP-2 release and dose-response studies in hydroxyapatite and  $\beta$ -tricalcium phosphate," *Biomed. Mater. Eng.*, vol. 19, no. 2–3, pp. 141–146, 2009, doi: 10.3233/BME-2009-0573.
- [49] M. Virginia, A. D. Laksono, W. P. Kusuma Asih, Sulistijono, and D. T. Agustiningtyas, "Study on Biocompatibility of Chitosan/Hydroxyapatite Doped Silicon Composite as Material for Alveolar Socket Preservation," in *Journal of Physics: Conference Series*, Jan. 2021, vol. 1726, no. 1, doi: 10.1088/1742-6596/1726/1/012007.
- [50] I. R. Bordea *et al.*, "Nano-hydroxyapatite use in dentistry: a systematic review," *Drug Metab. Rev.*, vol. 52, no. 2, pp. 319–332, Apr. 2020, doi: 10.1080/03602532.2020.1758713.
- [51] S. J. Jang, S. E. Kim, T. S. Han, J. S. Son, S. S. Kang, and S. H. Choi, "Bone regeneration of hydroxyapatite with granular form or porous scaffold in canine alveolar sockets," *In Vivo (Brooklyn)*, vol. 31, no. 3, pp. 335–341, May 2017, doi: 10.21873/INVIVO.11064.
- [52] D. SV, "Nanosized and nanocrystalline calcium orthophosphates," *Acta Biomater.*, vol. 6, no. 3, pp. 715–734, Mar. 2010, doi: 10.1016/J.ACTBIO.2009.10.031.
- [53] V. S. Kattimani, S. Kondaka, and K. P. Lingamaneni, "Hydroxyapatite—Past, Present, and Future in Bone Regeneration:," <https://doi.org/10.4137/BTRI.S36138>, vol. 7, p. BTRI.S36138, Sep. 2016, doi: 10.4137/BTRI.S36138.
- [54] B. A *et al.*, "Effect of micro- and macroporosity of bone substitutes on their mechanical properties and cellular response," *J. Mater. Sci. Mater. Med.*, vol. 14, no. 12, pp. 1089–1097, Dec. 2003, doi: 10.1023/B:JMSM.0000004006.90399.B4.
- [55] C. V, S. L, M. M, F. P, and C. L, "Clinical-radiographic and histological evaluation of two hydroxyapatites in human extraction sockets: a pilot study," *Int. J. Oral Maxillofac. Surg.*, vol. 40, no. 5, pp. 526–532, May 2011, doi: 10.1016/J.IJOM.2010.12.005.
- [56] C. L, W. M. G, T. M, C. E, M. F, and D. C, "Histological and Histomorphometrical Evaluation of Postextractive Sites Grafted with Mg-Enriched Nano-Hydroxyapatite: A Randomized Controlled Trial Comparing 4 Versus 12 Months of Healing," *Clin. Implant Dent. Relat. Res.*, vol. 18, no. 5, pp. 973–983, Oct. 2016, doi: 10.1111/CID.12381.
- [57] G. GA, N. B, M. F, G. W, and N. S, "Clinical, histologic and histomorphometric evaluation of socket preservation using a synthetic nanocrystalline hydroxyapatite in comparison with a bovine xenograft: a randomized clinical trial," *Clin. Oral Implants Res.*, vol. 23, no. 10, pp. 1198–1204, Oct. 2012, doi: 10.1111/J.1600-0501.2011.02288.X.
- [58] R. RFB *et al.*, "Randomized Controlled Clinical Trial of Nanostructured Carbonated Hydroxyapatite for Alveolar Bone Repair," *Mater. (Basel, Switzerland)*, vol. 12, no. 22, Nov. 2019, doi: 10.3390/MA12223645.
- [59] K. V, L. KP, Y. S, and M. M, "Use of eggshell-derived nano-hydroxyapatite as novel bone graft substitute-A randomized controlled clinical study," *J. Biomater. Appl.*, vol. 34, no. 4, pp. 597–614, Oct. 2019, doi: 10.1177/0885328219863311.

UNDER PEER REVIEW