

Evaluating the efficacy of eggshell-derived hydroxyapatite (EHA) and synthetic hydroxyapatite (SHA) in bone regeneration of maxillofacial defects-a randomized double-blind pilot comparative clinical-radiological study

Khushboo Changani^{a,*}, Anil Managutti^a, Shailesh Menat^a, Nirav Patel^a,
Vivekanand Kattimani^{a,b}, Darshal Panchal^a, E.K. Girija^c

^a Department of Oral and Maxillofacial Surgery, Narsinhbhai Patel Dental College and Hospital, Visnagar, Gujarat, India

^b NPDC-H-SPU, Visnagar, Gujarat, India

^c Biomaterials Lab, Department of Physics, Periyar University, Salem, Tamilnadu, India

ARTICLE INFO

Keywords:

Bone healing

Pathology

Apicectomy

Grafting

Osteoconductivity

ABSTRACT

Background: Defects of the maxillofacial skeleton lead to functional, aesthetic, social, and behavioral problems, that make the person isolate from the mainstream of society. So, bone regeneration is needed for the overall health. Since long time various materials have been used for bone regeneration with limitations. Recently, Eggshell derived hydroxyapatite [EHA] has been explored as a graft substitute. Hence EHA is evaluated and compared with SHA (synthetic hydroxyapatite) for its efficacy to accelerate bone regeneration in maxillofacial cystic defects.

Materials and methods: Total of 20 bony defects randomly allocated to SHA and EHA groups for grafting after removal of pathology (cyst and granuloma) having ≤ 2 cm in size. Signs and symptoms were evaluated post-surgically for 2 weeks. Bone regeneration was assessed by blinded observer for study duration and method of intervention postoperatively at 1st, 2nd, 4th, and 6th month and 1 year.

Results: The study found no statistical significant difference among clinical signs and symptoms between the groups. Whereas EHA showed better bone regeneration by the end of 6th month and 1 year compared to SHA with statistically significant difference in bone density [EHA group (136.04 ± 15.56) and SHA group (115.58 ± 16.26)]. Parameters like internal portion of surgical site, surgical site outline showed consistent results with density.

Conclusion: Both EHA and SHA graft materials are equally efficient in early bone regeneration. The EHA showed promising results and enhanced bone regeneration with osteoconductivity, which indicates the eggshell waste-bio mineral is worthwhile raw material for the production of HA and is Go Green procedure. EHA is economic, compared with SHA.

1. Introduction

Since ancient times graft materials have been used to promote the healing of large bony defects is well-known. Autogenous bone is the preferred augmentation material but the harvesting requires a second surgery, which is associated with donor site morbidity, prolonged hospitalization time, and high costs. Therefore, bone graft substitutes are used as alternatives to autogenous bone grafts.¹ Synthetic hydroxyapatite is the most widely used, due to its chemical composition, like human

bone. It is non-toxic, chemically stable, and causes less inflammation and antigenic reaction. The microstructure can be controlled to promote the formation of pores that can allow the migration of blood vessels and bone tissues into the material leading to osteoconductivity.² Hydroxyapatite (HA) is apatite calcium phosphate $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, has the highest level of bioactivity, and forms a quick bond with the bone. Hydroxyapatite is available for clinical use in porous/dense block or granular form. Recently, Hydroxyapatite (EHA) graft has been derived from agricultural waste “hen's eggshell” and is available in abundant

* Corresponding author.

E-mail addresses: drkhushichangani@gmail.com (K. Changani), drvivekanandsk@gmail.com (V. Kattimani).

<https://doi.org/10.1016/j.jobcr.2025.11.016>

Received 28 May 2025; Received in revised form 26 October 2025; Accepted 24 November 2025

Available online 30 December 2025

2212-4268/© 2025 The Authors. Published by Elsevier B.V. on behalf of Craniofacial Research Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

quantity. This has led to the curiosity to prepare the EHA using microwave-assisted technology.³ Hence the present study aimed to evaluate and compare the efficacy of EHA as a novel bone graft substitute with synthetic hydroxyapatite(SHA), clinically and radiographically for bone regeneration.^{1,2,17}

2. Materials and methods

The prospective double-blind randomized controlled clinical trial was conducted on 20 patients with maxillofacial pathology requiring surgical cyst removal according to a defined protocol in the Department of Oral & Maxillofacial Surgery. The protocol(****) was approved by the Institutional Ethics Committee.

3. Inclusion and exclusion criteria

Patients between 18 and 50 years of either gender with good general health were included in the study. Patients with possible compromised immune status or systemic disease that might affect the normal healing process (e.g. anemia, deficiency disorder, etc.) were excluded from the study.

4. Sample size and randomization

Randomization was performed using a computer-generated random table by an independent investigator. Allocation concealment was ensured using sealed opaque envelopes, and both patients and outcome assessors were blinded to the type of graft material used.

A total of twenty sites were randomly allocated into two groups of 10 each (Group EHA filled with EHA and Group SHA filled with SHA) using, a computer-generated random table. Synthetic hydroxyapatite is commercially available in market (G-bone synthetic granules, Surgiwear India). The sample size was calculated using G*power software with an 80 percent probability.

5. Preparation of eggshell hydroxyapatite

Hen's eggshells were washed thoroughly and calcined to prepare calcium oxide with 0.5 M di ammonium hydrogen phosphate solution mixed with calcium precursor in a ratio of Ca/p = 1.67. The mixed reactants were irradiated in a domestic microwave oven (BPL India, 245 GHz, 800 W). The product was then washed repeatedly with distilled water to remove unwanted ions and dried overnight in an oven at 100 °C to produce EHA.³ So prepared material has been procured from the bone regenerative biomaterial lab in a pre-sterile 1 cc vial using gamma irradiation. The prepared EHA material underwent sterilisation using **25 kGy gamma irradiation**, a validated and widely used technique for biomaterial sterilisation ensuring complete microbial inactivation without altering the physicochemical properties of hydroxyapatite.^{3,6} All material handling was performed under aseptic laminar airflow and sealed in sterile 1 cc vials to maintain infection control. Sterility confirmation was performed through microbial culture testing prior to surgical use. No intra- or postoperative infections were observed, confirming biosafety and the reliability of the sterilisation process.

6. Surgical procedure

Standard surgical protocols were followed under local anesthesia for the enucleation of maxillofacial pathologies “periapical cyst” (with apicoectomy wherever required). After thorough cleaning and hemostasis, the bone defects were filled with bone graft, and primary closure was done. The suture removal was done on the 7th postoperative day. All the patients were followed up during the first and second week post-surgically to evaluate the signs and symptoms of infection or any other complications related to the surgical procedure.

7. Clinical and radiographic criteria for assessment

Although cone-beam computed tomography (CBCT) provides superior densitometric analysis, ethical considerations (higher radiation exposure), availability, and cost factors led us to use standardized RVG with histogram analysis in Adobe Photoshop, a validated method in earlier studies.

The pain was assessed on the 2nd and 7th days post-operatively using the visual analog scale (VAS). Bone healing was assessed using the observer strategy and radiographic classification of healing adopted from Kattimani VS et al.^{4,5} The bone healing was assessed using Standard periapical RVG (radio-Visio gram). RVG was obtained at 1st week, 1st month and 2nd month, 4th month and 6th month and 1 year post-operatively using the following criteria:

- A) **Changes in surgical site outline** (1) Unchanged, (2) slightly changed, (3) Partly reduced, (4) Complete absence of margin ^{4,5}.
- B) **Internal portion of the surgical site (i.e. Bone formation characteristics);** (1) Changed, (2) Ground glass, (3) Spicular, and (4) Trabecular ^{4,5}.
- C) **Density of surgical site** at immediate postoperative 1st, 4th, 6th month and 1 year respectively in our study. ^{4,5}

The radiographs were examined by two oral and maxillofacial radiologists who were blinded for the duration of the surgery and the type of grafting. If any gross difference was found in the observation, a third observer interpreted the radiographs. Radiographic changes in all stages of bone healing in Group EHA and Group SHA are presented in (Fig. 2-a-e & Fig. 3-a-e) respectively. The criteria for bone density and scoring system were determined using a histogram in Adobe Photoshop software (7.0 version) based on mean and standard deviation values.

Statistical Analysis: Radiographic changes in surgical site outline, and internal portion of the surgical site after surgery were statistically compared using Mann- Whitney test and Wilcoxon matched-pairs test. The kappa (k) correlation was taken into consideration to assess the degree of observer agreement for radiologic assessment. The mean values and standard deviations of each parameter were calculated.

8. Results and observations

A total of 20 subjects included 10 in each group. Group EHA consist of 7 females and 3 males whereas Group SHA consists of 8 females and 2 males. The study showed no statistical significant difference in pain scores (Mann Whitney U test) between the groups pre-operatively on the 2nd and 7th day [Table 1]. The study showed no statistical significant difference in surgical site margin changes between the groups at 1st month, 6th month and 1 year whereas statistical significant difference was present at 2nd and 4th months [Table 2]. The internal portion of the site was changed at the end of 1st month in both groups. Statistically, a significant difference was present between the groups at 2nd month. At the end of 4th month, one subject had a spicular apperance and 9 subjects had a trabecular apperance in the Group-EHA whereas one subject had ground glass apperance, 5 had a spicular appearance and 4 subjects

Table 1
Comparison of pain in both groups.

Time	Groups	Median	P-value
Pre-Operative	EHA*	2	0.908 NS***
	SHA**	3	
2 Days	EHA	3	0.907 NS
	SHA	4	
7 Days	EHA	1	0.967 NS
	SHA	1.5	

*EHA- Eggshell-derived hydroxyapatite.
**SHA- Synthetic hydroxyapatite.
***NS- not significant.

Table 2
Comparison of surgical site margin between two groups.

Time	Groups	Median	P- value
1 Month	EHA*	1	1.000 NS***
	SHA**	1	
2 Month	EHA	3	0.000 S****
	SHA	1	
4 Month	EHA	4	0.021 S
	SHA	3	
6 Month	EHA	4	0.317 NS
	SHA	4	
1 Year	EHA	4	0.317 NS
	SHA	4	

*EHA- Eggshell-derived hydroxyapatite.

**SHA- Synthetic hydroxyapatite.

***NS- not significant.

****S - significant.

had a trabecular site in Group-SHA. Statistically, a significant difference was present among both groups at 4th month. [Graph –1]. At the end of the 6th month and 1 year density was higher in the Group- EHA (136.04 ± 15.56) than Group- SHA (115.58 ± 16.26) with a statistical significant difference but no difference has been observed at 1st, 2nd, and 4th month.[Graph –2] (see Fig. 3).

9. Discussion

In recent years, the use of synthetic or processed bone graft substitutes gained popularity over traditional grafting methods. These materials are available either as porous or dense granules of various sizes, shape and are analogous to the natural mineral found in human bone in many ways. They have osteoconductive properties, are biocompatible, easily sterilized for clinical use.¹The regeneration of bone following pathological destruction has an important bearing on success. Inadequate or inconsistent bone healing is caused by the ingrowth of connective tissue into the bone space, preventing osteogenesis. The bone regeneration following periapical surgery can be facilitated by placing a bone graft into the periapical defects. The ideal bone replacement material should be clinically and biologically inert, non-carcinogenic, easily maneuverable to suit the osseous defect, and



Fig. 2a. Group EHA- 1st month postoperative radiograph showing slightly change in surgical outline and changes in internal portion of site.

should be dimensionally stable, and serve as a scaffold for new bone formation.⁶ The sterilisation and infection-control aspects of EHA are crucial for its safe clinical use. Gamma irradiation effectively eliminates microbial contaminants while preserving the crystal morphology and osteoconductive potential of hydroxyapatite. The absence of post-operative infections in this study indicates that appropriate sterilisation and aseptic handling ensured patient safety. Similar observations have been reported in previous evaluations of sterilized eggshell-derived hydroxyapatite.^{3,6} Synthetic hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is often stoichiometric with a specific atomic Ca/P molar ratio of 1.67 and is similar in the chemical and crystallographic structure of bone.⁶ Recently, eggshell formulations have been tried as a regenerative bone graft material. The eggshell powder has been examined in rats for bone healing as osteoconductive bone-filling material with variable benefits of

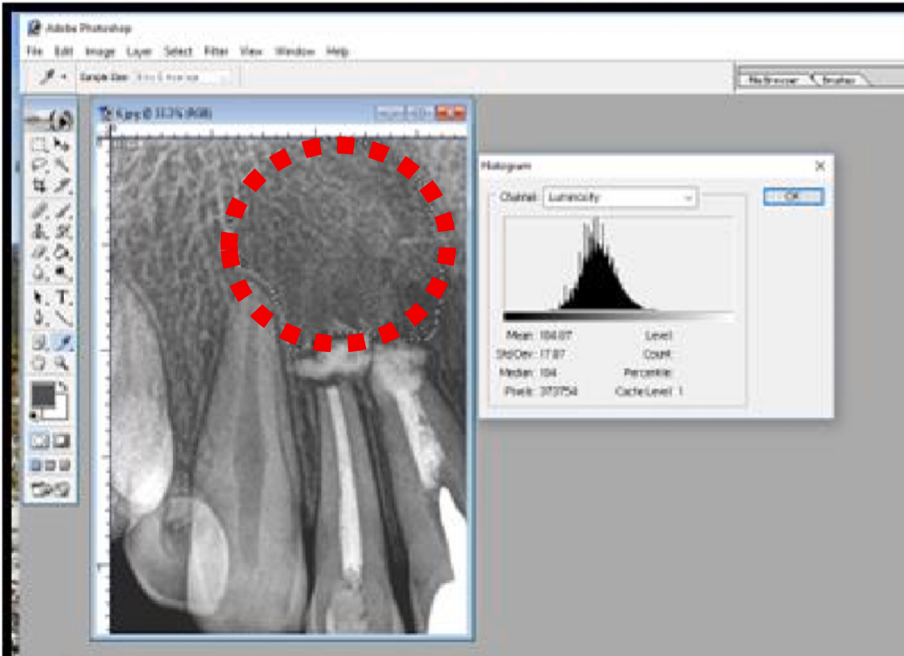


Fig. 1. Density of surgical site [Add arrows/labels to indicate healing changes].

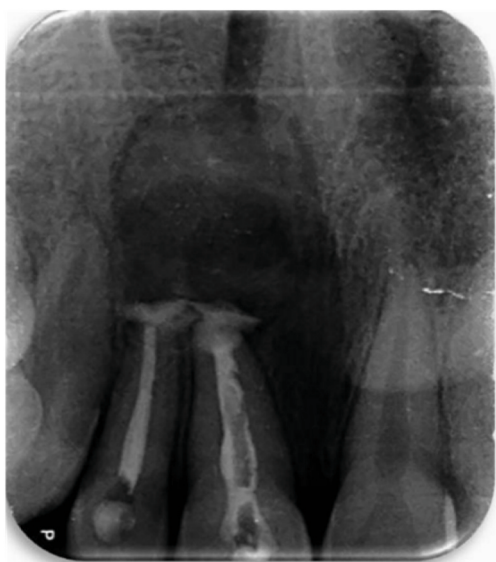


Fig. 2b. Group EHA-2nd month postoperative radiograph showing slightly change in surgical outline and ground glass appearance in internal portion of site.



Fig. 2d. Group EHA-6th month postoperative radiograph showing entirely absent surgical outline and trabecular appearance in internal portion of site.



Fig. 2c. Group EHA-4th month postoperative radiograph showing partial change in surgical outline and spicular appearance in internal portion of site.



Fig. 2e. Group EHA - 1Year postoperative radiograph showing entirely absent surgical outline and trabecular appearance in internal portion of site.

regeneration.⁷ Clinical and experimental studies have shown several positive effects of eggshell powder and calcium carbonate derived from eggshells demonstrating biocompatible and osteoconductive properties.⁷ Histomorphometric evaluation showed that the EHA has excellent new bone formation ability.⁸ In our study both the groups had no statistical significant difference in pain scores indicating they are biocompatible during follow up periods. Bone regeneration was assessed using standard radiography which is the most common imaging method to assess bone healing because it is widely available, economical, and relatively safe.^{9,10} In our study, we used Histogram mean value using Adobe Photoshop software (version 7.0) for the assessment of bone healing. The results of our study showed statistical significant increase in mean bone density from 1st week to 1st month and 1st month to 2nd month in both groups. However, the intergroup comparison at 1st, 2nd, and 4th months showed no statistical difference except at the 6th month and 1 year indicating bone graft material is radiodense and subsequent early bone regenerative change remains similar at 1st, 2nd and 4th

month. But density has increased by 6th month significantly hence the EHA group showed (136.04 ± 15.56) better density than the SHA group (115.58 ± 16.26). The similar results have been observed in published literature.^{1,6} Kattimani et al. study compared the density (in eight patients) of the grafted site with surrounding normal bone with time intervals of 1st, 2nd, and 3rd month,¹ whereas in the present study, EHA and SHA grafted sites were compared but not with normal bone and the time interval were also different that is 1st, 2nd, 4th and 6th month and 1 year. Another study compared EHA and SHA using similar parameters with intervals of 1st, 2nd, 3rd, and 6th month showed better bone regeneration in the EHA group compared to SHA whereas in our study similar results have been observed. The study concluded that both EHA and SHA graft materials are equally efficient in early bone regeneration.⁶ Indicating EHA is a worthwhile, economic graft material compared to SHA produced using a go-green procedure.⁶ Some of the



Fig. 2f. Group EHA-preoperative radiograph showing defect.

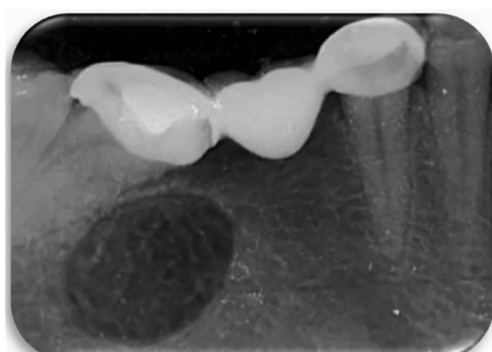


Fig. 3a. Group SHA-1st month postoperative radiograph showing slightly change in surgical outline and change in internal portion of site.

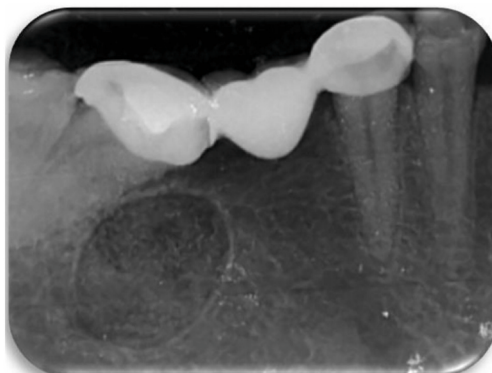


Fig. 3b. Group SHA-2nd month postoperative radiograph showing slightly change in surgical outline and change in internal portion of site.

studies by Jin Woo Park⁷ and Uraz⁸ used hydrothermally treated eggshells and calcium carbonate in animal models showing osteoconductivity and biocompatibility indicating eggshell biomineral is worthwhile raw material for the production of hydroxyapatite. The In vitro study in the animal model by Yudha Mathan Sakti showed equal efficiency between the Eggshell Waste derived-Hydroxyapatite graft and



Fig. 3c. Group SHA-4th month postoperative radiograph showing partial change in surgical outline and ground glass appearance in internal portion of site.



Fig. 3d. Group SHA-6th month postoperative radiograph showing partially reduced surgical outline and spicular appearance in internal portion of site.

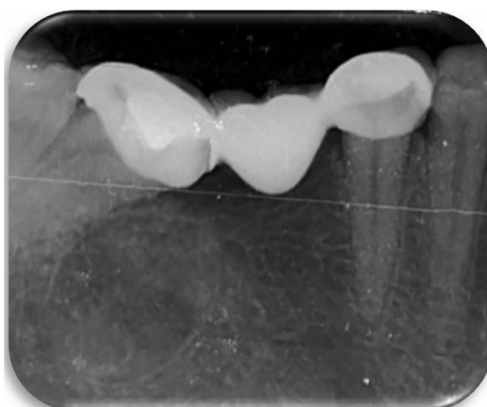


Fig. 3e. Group SHA- 1 Year postoperative radiograph showing partially reduced surgical outline and spicular appearance in internal portion of site.

Hydroxyapatite control graft radiologically.¹¹ Other studies support the use of EHA as a bone graft material in the third molar¹² and extracted site.^{13,14} These are the most common bone defects encountered clinically for testing of any new material for enhancing bone regeneration. Hence in the present study irrespective of the size of the lesion (≤ 2 cm) has been considered for examining the efficacy of EHA in comparison to SHA. Cystic defects in the periapical area sometimes heal with scarring that may require intervention. Considering the size of the lesion and exposed part of the root within the cystic defects warrants the

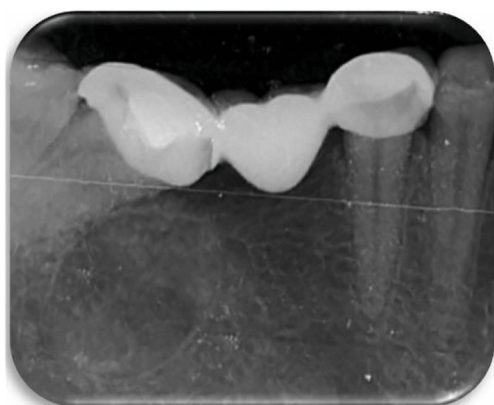


Fig. 3f. Group SHA-preoperative radiograph showing defect.

grafting. ^{15, 6, 18, 19, 20}

10. Limitation

Additionally, the study combined both maxillary and mandibular lesions, which differ in blood supply and healing potential, and lesion sizes showed variability. These factors may have influenced the outcomes. Future studies with larger, multicenter cohorts and advanced imaging (e.g., micro-CBCT) are recommended.

The smaller sample size and shorter duration of the follow-up warrants a larger sample size and longer duration multicentre studies. In the present study, histomorphometric analysis has not been performed keeping ethical considerations in mind according to Sammartino et al.¹⁶ bone biopsy in this region should not be done because of the bioethics principles since a second surgical procedure on the graft area is unnecessary.

11. Conclusion

Within the limitations of this pilot randomized controlled study, both EHA and SHA were found to be effective in promoting early bone regeneration. EHA showed relatively higher density values and promising osteoconductive properties. While EHA may offer an economical and sustainable option, further large-scale studies are required before definitive conclusions can be drawn.

Patient consent form

The details of the study have been given to me in writing & explained to me in my own language in detail. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in this study is voluntary. I agree not to restrict any use of data or results that arise from this study provided such a use is only for scientific purpose. I have been given an information sheet giving details of the study. I fully consent to participate in the above study.

Ethical clearance

Issued BY INSTITUTE OF NARSINHBHAI PATEL DENTAL COLLEGE AND HOSPITAL COMMITTEE.

Funding source

The study was partly funded for the production of EHA under the WMT/576/TDT program by DST, Govt. of India.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. KHUSHBOO CHANGANI reports equipment, drugs, or supplies was provided by Narsinhbhai Patel Dental College & Hospital. DR. KHUSHBOO CHANGANI reports a relationship with Narsinhbhai Patel Dental College & Hospital that includes: If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Authors thanks -Dr. Kattimani VS. Department of Oral and Maxillo-facial Surgery, Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India, and Dr.E K Girija for the material support Under DST, GOI, funded Waste Management Technology Project.

References

- Kattimani VS, Chakravarthi PS, Kanumuru NR, et al. Eggshell derived hydroxyapatite as bone graft substitute in the healing of maxillary cystic bone defects: a preliminary report. *J Int Oral Health*. 2014;6(3):15. PMID: 25083027; PMCID: PMC4109239.
- Shankar R, Singh D, Shaikh S, Singh G, Yadav A, Jain R. Bone regeneration in osseous defects using hydroxyapatite graft and the extent of ossification in osseous defects treated without grafts: a comparative evaluation. *J Maxillofac Oral Surg*. 2011;10(2):123–126. <https://doi.org/10.1007/s12663-011-0189-x>.
- Muthu D, Kumar GS, Kattimani VS, Girija EK. Optimization of conversion of eggshell biowaste into hydroxyapatite using microwave reactor. *Ceram Int*. 2020;46(16): 25024–25034. <https://doi.org/10.1016/j.ceramint.2020.06.288>.
- Kattimani VS, Bajantani NV, Sriram SK, Sriram RR, Rao VK, Desai PD. Observer strategy and radiographic classification of healing after grafting of cystic defects in maxilla: a radiological appraisal. *J Contemp Dent Pract*. 2013;14(2):227–232. <https://doi.org/10.5005/jp-journals-10024-1304>.
- Kattimani VS, Chakravarthi SP, Neelima Devi KN, Sridhar MS, Prasad LK. Comparative evaluation of bovine derived hydroxyapatite and synthetic hydroxyapatite graft in bone regeneration of human maxillary cystic defects: a clinico-radiological study. *Indian J Dent Res*. 2014;25(5):594–601. <https://doi.org/10.4103/0970-9290.147100>.
- Kattimani V, Lingamaneni KP, Chakravarthi PS, Kumar TS, Siddharthan A. Eggshell-derived hydroxyapatite: a new era in bone regeneration. *J Craniofac Surg*. 2016;27(1):112–117. <https://doi.org/10.1097/SCS.0000000000002288>.
- Park JW, Bae SR, Suh JY, et al. Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: a pilot study. *J Biomed Mater Res*. 2008;87(1): 203–214. <https://doi.org/10.1002/jbm.a.31768>.
- Uraz A, Gultekin SE, Senguen B, et al. Histologic and histomorphometric assessment of eggshell-derived bone graft substitutes on bone healing in rats. *J Clin Exp Dent*. 2013;5(1):e23–e29. <https://doi.org/10.4317/jced.50968>.
- Geraets WG, Lindh C, Verheij H. Sparseness of the trabecular pattern on dental radiographs: visual assessment compared with semi-automated measurements. *Br J Radiol*. 2012;85(1016):e455–e460. <https://doi.org/10.1259/bjr/32962542>.
- Lindh C, Petersson A, Rohlin M. Assessment of the trabecular pattern before endosseous implant treatment: diagnostic outcome of periapical radiography in the mandible. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1996;82(3):335–343. [https://doi.org/10.1016/s1079-2104\(96\)80363-5](https://doi.org/10.1016/s1079-2104(96)80363-5).
- Sakti YM, Magetsari R. Structural evaluation and animal implantation of porous eggshell waste-derived hydroxyapatite graft as bone substitution. *J Med Sci*. 2013;45(4):176–186. <https://doi.org/10.19106/JMedScie004504201304>.
- Kattimani V, Lingamaneni KP, Yalamanchili S, Mupparapu M. Use of eggshell-derived nano-hydroxyapatite as novel bone graft substitute: a randomized controlled clinical study. *J Biomater Appl*. 2019;34(4):597–614. <https://doi.org/10.1177/0885328219863311>.
- Kattimani VS, Lingamaneni KP, Kreedapathi GE, Kattappagari KK. Socket preservation using eggshell-derived nanohydroxyapatite with platelet-rich fibrin: a new technique. *J Korean Assoc Oral Maxillofac Surg*. 2019;45(6):332–342. <https://doi.org/10.5125/jkaoms.2019.45.6.332>.
- Nainoor N, Shobha ES, Prashanth NT, et al. Clinical and radiographic assessment of eggshell-derived nanohydroxyapatite vs DFDBA in socket preservation: a randomized controlled clinical trial. *J Maxillofac Oral Surg*. 2024. <https://doi.org/10.1007/s12663-024-02187-4>.
- Wang J, Yao QY, Zhu HY. Efficacy of bone grafts in jaw cystic lesions: a systematic review. *World J Clin Cases*. 2022;10(9):2801–2810. <https://doi.org/10.12998/wjcc.v10.i9.2801>.
- Sammartino G, Tia M, Gentile E, Marenzi G, Claudio PP. Platelet-rich plasma and resorbable membrane for prevention of periodontal defects after deeply impacted lower third molar extraction. *J Oral Maxillofac Surg*. 2009;67(11):2369–2373. <https://doi.org/10.1016/j.joms.2009.04.093>.

17. Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Amanatullah DF. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: a review. *Biomater Res*. 2017;21:9. <https://doi.org/10.1186/s40824-017-0095-5>.
18. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. *Eur Spine J*. 2001;10(Suppl 2):S96–S101. <https://doi.org/10.1007/s005860100282>.
19. Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: which is the ideal biomaterial? *J Clin Periodontol*. 2019;46(Suppl 21):92–102. <https://doi.org/10.1111/jcpe.13058>.
20. Shi B, Zhou Y, Wang Y, Ma Q, Liu Y. Bone substitute materials for oral and maxillofacial bone defect repair: a review. *J Oral Sci Res*. 2021;37(2):123–135. <https://doi.org/10.2334/josnusd.21-0123>.