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ORIGINAL RESEARCH

Lindia Ayu Hafshah, Azhari Azhari, Farina Pramanik

Differences in the Assessment of Dental Implant Osseointegration with Changes in Orthopantomography Exposure Settings on the Rabbit Tibia

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:6] [Pages No:S119 - S124]

Keywords: Bone density, Dental implant, Fractal dimension, Orthopantomography, Osseointegration

DOI: 10.5005/jp-journals-10015-2134 | Open Access | How to cite |

Abstract

Aim: This study investigates the differences in assessing dental implant osseointegration with changes in orthopantomography (OPG) exposure setting in the rabbit tibia. **Materials and methods:** This research design is quasi-experimental. The sample of this research is 18 panoramic radiographs of rabbit tibia bone that had been installed with a dental implant for 28 days with different exposure settings and were divided into two groups of settings based on exposure time (14 and 16 seconds). Data were obtained by measuring bone density and fractal dimension using ImageJ 2.3.0 software. Data were analyzed using the Kruskal–Wallis test, independent *t*-test, one-way analysis of variance (ANOVA) test, and *post hoc* test at *p*-value < 0.05. Using Statistical Package for the Social Sciences (SPSS) 21.0 software. **Results:** The results of the *p*-value analysis showed an average image quality of 5.33 (*p*-value > 0.05), the largest bone density value was 0.1827, and the largest fractal dimension value was 0.7990 (*p*-value < 0.05). **Conclusion:** There is a difference in bone density and fractal dimension in the 14 and 16 seconds exposure setting variation groups. **Clinical significance:** Differences in assessing dental implant osseointegration in different OPG exposure setting groups can obtain the best exposure setting to evaluate dental implant osseointegration.

ORIGINAL RESEARCH

Fabrizio A Wiess-Laurencio, Gabriela López-Rodriguez, Stefany Caballero-García

Evaluation of Solvent Efficacy of the Myrciaria Dubia (Camu-camu) Essential Oil in Root Canal Re-treatment Procedures: An In Vitro Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:4] [Pages No:S125 - S128]

Keywords: Eucalyptus oil, Gutta-percha solvent, Myrciaria dubia, Orange peel oil, Xylene

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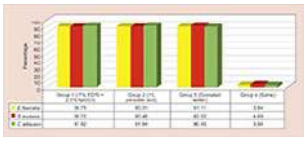
Abstract

Aim: To evaluate the solvent efficacy of an experimental substance based on the *Myrciaria dubia* (camu-camu) essential oil in root canal re-treatment procedures. **Materials and methods:** Sixty polylactic acid tubes (PLA) were used and divided into five groups (distilled water, *M. dubia* oil (camu-camu), xylene, orange peel, and eucalyptus oil). In each group, 12 tubes were filled with a temporary restorative material (Coltosol®). The upper and the middle part were filled with gutta-percha. A total of 0.1 mL solvent was added depending on the study group and left for 5 minutes. Once the solvent was placed, the samples were taken to the Instron 3382 machine. The force used to penetrate the spreader into the 5 mm depth was recorded in Newton (N). The Kruskal–Wallis test and Dunn's *post hoc* test were used for multiple comparisons ($p < 0.05$). Statistical analysis was performed using Stata® v.15.0 package. **Results:** We found significant statistical differences when comparing all solvents ($p = 0.001$), obtaining 14.02 N for the experimental substance. The results of the superficial dissolution depth and the force used to penetrate the spreader to 5 mm revealed that the *M. dubia* oil (camu-camu) was the solvent that significantly softened the gutta-percha the most ($p < 0.05$). These values were followed by xylene and orange peel oil. We also found that the solvent with the lowest efficacy was eucalyptus oil. **Conclusion:** The *M. dubia* (camu-camu) essential oil had more softening power than other solutions in the study. **Clinical significance:** The efficacy of the *M. dubia* (camu-camu) essential oil is relevant as it is a nonharmful solvent that would not harm the periapical tissue and would reduce the time of endodontic re-treatments procedures, which is beneficial for patients.



ORIGINAL RESEARCH

Kiran Kumar Aheibam, Sagolsem Chandarani, Elizabeth Moirangthem, Albert Ashem, Deepak Ningombam Singh, Khwairakpam Chaoton Singh

Antimicrobial Efficacy of Endodontic Irrigants after Reciprocating System of Instrumentation in Infected Root Canals with Complex Curvature of Mandibular Molars: An Ex Vivo Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:6] [Pages No:S129 - S134]

Keywords: Enterococcus faecalis, Ethylenediaminetetraacetic acid, Ozonated water, Peracetic acid, Reciprocating instrumentation, Sodium hypochlorite

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Abstract

Aim: The aim of the *ex vivo* study was to assess the efficacy of 17% ethylenediaminetetraacetic acid (EDTA) with 2.5% sodium hypochlorite (NaOCl), 1% peracetic acid (PAA), and ozonated water in mesiobuccal (MB) canals with curved mandibular molars infected with *Candida albicans*, *Streptococcus mutans*, and *Enterococcus faecalis* and to compare with normal saline irrigation. **Materials and methods:** A total of 240 mandibular molars were selected. The WaveOne Gold (WOG) primary reciprocating system was used to prepare the MB canals. The selected teeth were allocated randomly into 12 groups, 20 in each group. Based on the microbiological strains, irrigants were employed following the instrumentation of the tooth. Microbiological samples were collected prior to instrumentation and also following the irrigation after instrumentation. The load of the microbes was measured using the colony-forming units (CFU/mL). The paired *t*-test and one-way analysis of variance (ANOVA) were used to evaluate the data, followed by Tukey's *post hoc* test. **Results:** The tested irrigants demonstrated a significant decrease ($p < 0.001$) in CFU/mL following the irrigation after instrumentation compared to the saline group. However, PAA, ozonated water, and EDTA with NaOCl showed statistically insignificant differences from each other in the mean reduction of microbial loads. The positive control (17% EDTA with 2.5% NaOCl) had a mean reduction of 90.75%; test groups PAA and ozonated water produced a reduction of 90.31 and 91.11%, respectively, against *E. faecalis*; while the saline group showed the least reduction in microbial counts (3.84%). **Conclusion:** According to the findings of this study, chemomechanical preparations incorporating irrigants such as PAA and ozonated water could be as beneficial as EDTA and NaOCl in reducing microbial content in root canal therapy. **Clinical significance:** Microbial lysis results from the oxidative action of PAA and ozonated water on cell membranes. These irrigants have better applicability and rather encouraging results, and they could be an important therapeutic tool for endodontists.

ORIGINAL RESEARCH

Akshata Jagadish Airsang, Adarsha Mandya Shankaregowda, Naganath Meena, Upasana Lingaiah, Vijayalakshmi Lakshminarasimhaiah, Shreya Harti

Comparative Assessment of Complete Pulpotomy in Mature Permanent Teeth with Carious Exposure Using Calcium Silicate Cement: A Randomized Clinical Trial

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:9] [Pages No:S135 - S143]

Keywords: Calcium silicate cements, Complete pulpotomy, Mature permanent teeth, Symptomatic irreversible pulpitis

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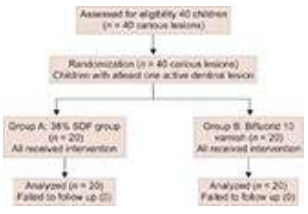
Abstract

Aim: To assess the clinical and radiographic outcome of complete pulpotomy procedure with NeoMTA and Biodentine in mature permanent vital teeth with carious exposure after 6 months and 1 year. **Materials and methods:** A total of 60 mature permanent mandibular molar teeth with a clinical diagnosis of irreversible pulpitis (IP) were included in the study. After the teeth were anesthetized, a complete pulpotomy procedure was performed under an aseptic environment. The cases were randomly divided into two groups [group I = NeoMTA (*n* = 30) and group II = Biodentine (*n* = 30)]. The teeth were temporized using type VII pink glass ionomer cement (GIC) for 2 weeks, following which a resin-modified GI (RMGI) was placed as a liner over the pulpotomy agent, and direct composite resin restorations were done. The cases were reevaluated postoperatively, clinically, and radiographically after 6 months and 1 year. **Results:** Following pulpotomy, complete pain relief was reported in 92.45% of the cases. External root resorptions were observed at the end of 6 months in two cases (group I: *n* = 1 and group II: *n* = 1). Radiographic evidence of periapical pathosis was present in three cases in group II at 6 months intervals. Radiographic evidence of periapical pathosis was present in three new cases at the end of a 1-year interval (group I: *n* = 2 and group II: *n* = 1). The success rate at 1 year for group I was 85.71%, and for group II was 84%. **Conclusion:** NeoMTA and Biodentine have favorable and comparable clinical and radiographic outcomes as therapeutic materials for complete pulpotomy procedures in mature permanent teeth with carious exposure. NeoMTA is a promising therapeutic agent for complete pulpotomy procedures in mature permanent teeth with carious exposure. **Clinical significance:** Considering the outcome of the study, complete pulpotomy procedures with calcium silicate-based cement can be considered a valid treatment alternative to non-surgical endodontic therapy (NSET) for mature permanent vital teeth with carious exposure.



ORIGINAL RESEARCH

Nagarathna Chikkanarasaiah, N Mounashree, Navin Hadadi Krishnamurthy, Umapathy Thimmegowda, B Amrutha

Effectiveness of Various Fluoride Varnishes in Arresting Cavitated Dentinal Lesion in Preschool Children: A Randomized Clinical Trial

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S144 - S148]

Keywords: Caries arrest, Cavitated dentinal lesion, Early childhood caries, Fluoride varnish, Preschool children, Silver diamine fluoride

DOI: 10.5005/jp-journals-10015-2156 | Open Access | How to cite |

Abstract

Aim: The aim of this randomized clinical trial was to compare the effectiveness of various fluoride varnishes in arresting cavitated dentinal lesions among preschool children. **Materials and methods:** Children aged 3–5 years old with at least one active cavitated dentinal carious lesion were recruited and randomly assigned into two groups as follows. Children in group I received 38% silver diamine fluoride (SDF) (FAgamin, Tedequim SRL, Argentina) application and children in group II received bifluorid 10 varnish (VOCO Bifluorid 10) application on carious lesion respectively. Lesion activity was assessed by visual-tactile examination using the World Health Organization (WHO) consumer price index (CPI) probe. Baseline and follow-up examinations were done by different examiners to collect the data. **Result:** Baseline and 1-week follow-up data was collected and subjected to statistical analysis using the Chi-squared test. The caries arrest rate in group I (100%) was highly significant. **Conclusion:** Silver diamine fluoride (SDF) was effective in arresting caries in preschool children and facilitating the early restoration of the cavitated lesion and was also helpful in instilling positive behavior in preschool children toward dental treatment. **Clinical significance:** Caries control methods used here are simple, noninvasive, highly efficient, cost-effective, well accepted, and can be used in all children. The results were satisfactory as cavitated lesions were restored in a short time in achieving good oral health.

ORIGINAL RESEARCH

AlWaleed Abushanan, Abdulfatah Alazmah, Uthman S Uthman, Adel S Alqarni, Abdulhamid Al Ghwainem, Narendra Varma Penumatsa

Assessment of the Efficacy of Different Storage Media for Maintaining an Avulsed Tooth: An In Vitro Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S149 - S153]

Keywords: Avulsion, Cell viability, Periodontal ligament, Storage media

DOI: 10.5005/jp-journals-10015-2149 | Open Access | How to cite |

Abstract

Aim: The aim of the current research was to assess the effectiveness of four different storage media for maintaining an avulsed tooth. **Materials and methods:** A total of 80 premolars implicated for extraction as part of orthodontic treatment were chosen for this research. The extraction of premolars was performed with minimal likely trauma and the least amount of injury to the periodontal ligament (PDL) membrane. Following removal, the premolars were caught from the crown area employing forceps as well as 3 mm of the PDL was removed from the crown region with the aid of a curette to facilitate the elimination of injured cells. The specimens were then allocated at random to one of the four investigational cohorts as group I—Hank's balanced salt solution (HBSS) (positive control), group II—Ringer's lactate, group III—*Aloe vera,* and group IV—egg albumin, each comprising 20 specimens in every group. The specimens in the investigational cohorts were subjected to storage to permit drying for 30 minutes and then immersed for 60 minutes in the individual storage medium. Every tube was subsequently subjected to centrifugation for 4 minutes at 1000 rpm, following which the supernatant was eliminated with disinfected micropipettes. The cells resulting from the supernatant were subjected to coloring with 0.5% trypan blue for determining viability. The quantity of viable PDL cells was calculated beneath a light microscope with a hemocytometer at 20× magnifying power. **Results:** The highest mean viable periodontal cell was exhibited by HBSS at 38.48 ± 2.32, pursued by the use of *Aloe vera* at 30.36 ± 1.86, then the egg albumin group at 24.58 ± 2.38, and finally the Ringer's lactate group at 21.12 ± 3.06. The dissimilarity noted amid the groups was greatly significant, with a *p*-value < 0.001. **Conclusion:** Among the confines of the limitations of this research, it may be inferred that HBSS exhibited the highest efficacy as a storage medium for an avulsed tooth than the other three investigational groups. As an alternative, *Aloe vera* may be utilized in regions where HBSS may not be accessible. **Clinical significance:** Trauma to the dentoalveolar segment is a frequent occurrence, with avulsion accounting for a large amount of harm. The therapy of preference for postavulsion is instant reimplantation. Nevertheless, in instances where this may not be likely, the prognosis of reimplanted teeth can be enhanced by the choice of a suitable storage medium. Avoidance of ankylosis plus replacement resorption is influenced by the ability of storage media to preserve cell viability to a greater extent vs the time tenure outside the alveolus.

ORIGINAL RESEARCH

Abhinandan Patel, G Girish, G Nikhila, R Akarsh, Preethi Bhat, Ahamed Irfan Khazilane

Efficiency of 2 mm Titanium Lambda Plate for Open Reduction and Internal Fixation of Subcondylar Fractures: A Prospective Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:7] [Pages No:S154 - S160]

Keywords: Lambda plate, Mouth opening, Occlusal bite forces, Occlusion, Plate adaptability, Subcondylar fractures

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Abstract

Aim: To evaluate the clinical efficiency of a 2 mm lambda plate for open reduction and internal fixation (ORIF) of condylar fractures in terms of surgical time, plate adaptability, mouth opening, restoration of occlusion, and ramus height. **Materials and methods:** A prospective study was conducted, including 16 patients presenting with subcondylar fractures. Under general anesthesia, a surgical approach was employed, and fracture fixation was accomplished using the titanium (Ti) lambda plate of 2 mm thickness and having seven holes. Patients were postoperatively (post-op) followed up clinically and radiologically at 1 week and 1 and 3 months. **Results:** Plate adaptability was good. The mean surgical time was 110.5 minutes. All patients showed a statistically significant increase in mouth opening following surgery. Around 93.8% (15 patients) had occlusion, which was deranged preoperatively (pre-op). This improved significantly in the post-op period. Pre-op, seven patients (43.75%) had a deviation on the opening of the mouth, and after surgery, it significantly decreased. The variation in mean bite force at central incisor (CI), right premolar, left premolar, and right and left molar between various time intervals was statistically significant $p < 0.001$. **Conclusion:** We conclude that the use of a 2 mm seven-hole lambda plate for fractures in the subcondylar region presents a good option to achieve stable osteosynthesis with less surgical time and complications. **Clinical significance:** There is limited data regarding surgical management of subcondylar fractures, owing to the challenge in accessing the region and the complexity of reduction of the displaced fragments. Fixation techniques also add to the issue. Exploration of surgical management of subcondylar fractures and data on *in vivo* evaluation of lambda plate design will enhance the prospects and knowledge for the development and implementation of the treatment protocol.

ORIGINAL RESEARCH

S Ashwini, Kavitha Prasad, B K Sujini

Assessment of Porphyromonas gingivalis and Filifactor alocis Levels in Gestational Diabetes Mellitus Patients with Periodontitis Post Nonsurgical Periodontal Therapy

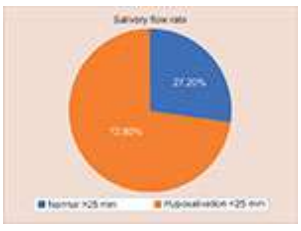
[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:9] [Pages No:S161 - S169]

Keywords: Filifactor alocis, Gestational diabetes mellitus, Nonsurgical periodontal therapy, Periodontitis, Porphyromonas gingivalis

DOI: 10.5005/jp-journals-10015-2154 | Open Access | How to cite |

Abstract

Aim: To evaluate the effect of nonsurgical periodontal therapy (NSPT) on microbial alterations (*Porphyromonas gingivalis* and *Filifactor alocis*), in pregnant periodontitis study participants with and without gestational diabetes mellitus (GDM). **Materials and methods:** The present study has been designed as a prospective and interventional trial. A total of 120 pregnant women were selected according to predetermined inclusion and exclusion criteria. Group I included pregnant study participants with GDM and periodontitis, who underwent oral hygiene instructions (OHI) throughout the study. Group II had pregnant study participants with GDM and periodontitis, who underwent scaling and root planning (SRP), followed by OHI. Group III had pregnant study participants with periodontitis maintained on OHI, and group IV had pregnant study participants with periodontitis who underwent SRP, followed by OHI. After the patients were categorized into respective groups, subgingival plaque samples were collected. All the patients were given OHI, and patients in groups II and IV underwent SRP. Clinical parameters were reevaluated 3 months after NSPT, before parturition. Subgingival plaque samples were also collected at the follow-up appointment. The study data was analyzed using Statistical Package for Social Sciences (SPSS) software V.22, IBM, for Windows. **Results:** An improvement in the microbial load was observed in all patients. However, better improvement was observed in group II, which included patients with GDM and periodontitis, who were provided with NSPT and OHI. **Conclusion:** Nonsurgical periodontal therapy (NSPT) in pregnant periodontitis study participants with and without GDM demonstrated a reduction in *P. gingivalis* and *F. alocis* loads. The results are in accordance with the hypothesis of the current study, suggesting a positive impact of NSPT on microbial load and its effect on periodontal status. **Clinical Significance:** The detrimental effect of microorganisms on host periodontal tissues lead to periodontitis. After NSPT, there was a reduction in the microbiological load with an improvement in the clinical periodontal parameters, thereby improving the oral health of the study participants.



ORIGINAL RESEARCH

Deepa Sara John, Nina Shenoy

Assessment of Oral Manifestations, Periodontal Status, and Vitamin D Levels in Postmenopausal Women: A Cross-sectional Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S170 - S174]

Keywords: Estrogen, Oral manifestations, Postmenopausal women, Vitamin D

DOI: 10.5005/jp-journals-10015-2158 | Open Access | How to cite |

Abstract

Aim: The aim of this study was to assess oral manifestations, periodontal status, and vitamin D levels in postmenopausal women. **Materials and methods:** Seventy systemically healthy postmenopausal women were included in this cross-sectional study. To determine the periodontal status, clinical parameters such as clinical attachment level (CAL), probing depth (PD), plaque index (PI), and bleeding on probing (BOP) were used. Oral manifestations were recorded using a standard questionnaire. The unstimulated salivary flow rate was recorded using the modified Schirmer test strips. The collected salivary samples were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits to estimate the salivary levels of vitamin D. Results were then subjected to statistical analysis. Statistical significance was defined as $p < 0.05$. Spearman's correlation was used to correlate vitamin D levels and the periodontal parameters. **Results:** More than 50% of the participants reported xerostomia and burning mouth syndrome, with 72.08% reporting hyposalivation. A total of 78% had insufficient salivary vitamin D levels. More than 50% of the sites with BOP and mean CAL of 2.89 ± 0.79 was detected. A statistically significant strong negative correlation was observed between salivary vitamin D levels and the periodontal parameters ($p < 0.001$). **Conclusion:** The majority of postmenopausal women exhibited oral discomfort in the form of xerostomia, burning mouth, taste alterations, and hyposalivation. There was a marked insufficiency in vitamin D levels. A significant negative correlation was demonstrated between vitamin D levels and periodontal status, owing to the increased risk of development of periodontitis in the subjects. **Clinical significance:** The majority of women in the postmenopausal period experience oral symptoms related to estrogen deficiency. However, due to a lack of sufficient awareness, these issues have not been adequately addressed. Vitamin D deficiency is also commonly encountered at this stage, which complicates systemic as well as periodontal health.

ORIGINAL RESEARCH

Pascal Filio, Octarina, Komariah

Characterization of Fabricated Bovine Hydroxyapatite Crystal as Socket Preservation Material: An SEM-EDX and X-ray Diffraction Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:7] [Pages No:S175 - S181]

Keywords: Biomaterial, Bovine hydroxyapatite, Socket preservation

DOI: 10.5005/jp-journals-10015-2155 | Open Access | How to cite |

Abstract

Aim: This research aimed to fabricate and characterize bovine hydroxyapatite (BHA) crystal by using scanning electron microscope-energy dispersive X-ray (SEM-EDX) and X-ray diffraction (XRD) test for the evaluation of its potential as socket preservation material. **Materials and methods:** Bovine hydroxyapatite was fabricated from bovine cancellous bone that was washed and cut into small pieces. The bone was placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000°C for 1 hour and dried. The bone was then grounded into a powder and sieved through 150 µm sieves. Powdered BHA was then analyzed with SEM-EDX to analyze the structure and content of the HA element, and an XRD test was used to analyze the HA crystal. **Results:** From the SEM test, BHA had a crystalline particle with hexagonal crystal with an average size of 197 µm. The EDX test indicated BHA has the elements of carbon (C) (50.71%), oxygen (O) (34.59%), sodium (Na) (0.35 %), magnesium (Mg) (0.35%), phosphorus (P) (4.29%), calcium (Ca) (8.97%), and niobium (Nb) (0.74%). The XRD test showed that BHA contains hydroxyapatite (HA) crystal with 66% crystallinity degree. **Conclusion:** The BHA was successfully fabricated through the process of calcination. Based on the characterization and analysis using SEM-EDX and XRD tests of BHA, it had the potential as socket preservation material to preserve alveolar bone after tooth extraction. **Clinical significance:** The addition of BHA after tooth extraction has the potential to maintain alveolar bone quality and prevent dimension loss. This biomaterial helps and supports prosthesis treatment, such as implants, which need good alveolar bone quantity and quality.

ORIGINAL RESEARCH

Radha Vellayappan, Sheeja S Varghese

Effectiveness of Low-intensity Pulsed Ultrasound as an Adjunct to Periodontal Regenerative Therapy: A Randomized Controlled Clinical Trial

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:7] [Pages No:S182 - S188]

Keywords: Alkaline phosphatase, Gingival crevicular fluid, Intrabony defect, Low-intensity pulsed ultrasound, Periodontal regeneration, Periodontitis

DOI: 10.5005/jp-journals-10015-2160 | Open Access | How to cite |

Abstract

Aim: This study was performed to evaluate the effectiveness of low-intensity pulsed ultrasound (LIPUS) as an adjunct to periodontal regenerative therapy [open flap debridement (OFD) with or without bone graft (BG)] in intrabony defects of chronic periodontitis patients. **Materials and methods:** A total of 40 angular periodontal defect sites were included in this study by recruiting 18 systemically healthy volunteers. Sample sites were randomly allotted to four groups: group I, OFD; group II, OFD + LIPUS; group III, OFD + BG; and group IV, OFD + BG + LIPUS. The clinical parameters such as plaque index (PI), gingival sulcus bleeding index (SBI), probing depth (PD), clinical attachment loss, radiographic depth of the defect site, and alkaline phosphatase (ALP) level in gingival crevicular fluid (GCF) were analyzed. **Results:** When compared between the groups, there was no significant difference in clinical and radiographic parameters at 3 and 6 months of postevaluation, whereas ALP level showed a significant increase at 6 weeks in group II and group IV when compared to other groups. **Conclusion:** It can be concluded that LIPUS did not improve periodontal regeneration in terms of clinical and radiographic parameters when used as an adjunct to periodontal regenerative therapy. But it has shown the potential to increase the ALP level in GCF. **Clinical significance:** There is no added clinical benefit in the short-term usage of LIPUS in periodontal therapy.

ORIGINAL RESEARCH

Pratik B Kariya, Sweta Singh

Correlation between Nutritional Status and Dental Caries in 3–18-year-old Indian School-going Children: A Cross-sectional Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S189 - S193]

Keywords: Body mass index, Dental caries, Nutritional status

DOI: 10.5005/jp-journals-10015-2136 | Open Access | How to cite |

Abstract

Aim: This study aimed to examine the prevalence of dental caries and its correlation with nutritional status in 3–18-year olds. **Materials and methods:** The cross-sectional study was conducted among 3–18-year olds from public schools in a rural district in India. A total of 829 subjects from 29 schools participated in the study. The body mass index (BMI) was used to assess the nutritional status of participants. The anthropometric measurements for BMI were recorded within the school premises. The World Health Organization (WHO) Child Growth Standards Reference for BMI was used to categorize the participants into obese/overweight, normal, or underweight for age. Dental caries was assessed using decayed, missing, and filled primary teeth (DMFT) and decayed extracted filled teeth (deft) index. A parent-administered questionnaire was used to obtain data on oral hygiene practice, parental education, dental visits, and sugar exposure. **Results:** The prevalence of dental caries was found to be 48.8%. A total of 54.6% of students were malnourished, and 47.7 % were underweight for their age. The nutritional status was found to be inversely related to dental caries. The children with higher BMI (obese and overweight) were likely to have less caries experience. **Conclusion:** Nutritional status was found to be inversely related to dental caries. Children with lesser BMI were at higher risk of having dental caries and *vice versa*. **Clinical significance:** Dental caries and nutritional status have common risk factors. Diet is the major risk factor, common to both conditions. Diet is a modifiable risk factor. Therefore, strategies can be developed and targeted at the prevention of both dental caries, and malnutrition in the community. Healthy dietary habits and practices can be promoted for the control of dental caries and malnutrition.

ORIGINAL RESEARCH

Sumit Singla, Satyavan Gangaram Damle, Abhishek Dhindsa, Shalini Garg, Ashish Loomba, Pragati Poddar

Comparative Efficacy of Bioceramics Apexification in Periradicular Healing and Root-end Calcific Tissue Repair in Immature Traumatized Permanent Anterior Teeth

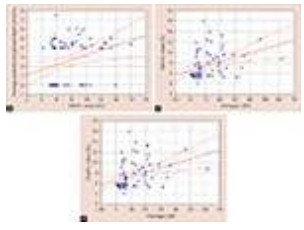
[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:9] [Pages No:S194 - S202]

Keywords: Apexification, Biodentine, Mineral trioxide aggregate

DOI: 10.5005/jp-journals-10015-2153 | Open Access | How to cite |

Abstract

Aim: To compare the efficacy of bioceramics [Biodentine (BD) and mineral trioxide aggregate (MTA)] apexification in periradicular healing and root-end calcific tissue repair in immature traumatized permanent anterior teeth as compared to calcium hydroxide [$Ca(OH)_2$] apexification. **Materials and method:** A total of 33 (N) traumatized nonvital immature permanent teeth in 21 children between the age-group of 8–16 years, fulfilling the inclusion criteria, were selected. They were randomized into three equal groups (n = 11) each for treatment by apexification. In the study groups, MTA and BD were compacted as apical plug barriers. The conventional method of apexification by placing $Ca(OH)_2$ paste in the root canal space was used in the control group. The recall was done at 1, 3, 6, and 9 months clinically and at 3, 6, and 9 months radiographically to check for healing by improved periapical index (PAI) scores and induction of calcific tissue repair and/or continued root formation apical to the placed materials. Statistical assessment was done using Statistical Package for the Social Sciences (SPSS) version 15.0 analytical software. **Results:** Success was 100% at the final follow-up (p < 0.001) according to clinical parameters. The resolution of the periradicular radiolucency took minimum time in the BD group, followed by the MTA group. Calcific repair and/or increase in apical length was observed in 100% of teeth in both groups at 6 months compared to only 40% of teeth in control group $Ca(OH)_2$ with resultant radiopaque hard tissue barrier apical to placed material plug. **Conclusion:** BD, followed by MTA, has superior properties for inducing periradicular healing and calcific tissue repair than $Ca(OH)_2$ for apexification. For conclusive inferences regarding better efficacy, a larger sample size with a prolonged follow-up period needs to be taken on. **Clinical significance:** To preserve the young permanent teeth from trauma, apexification is the treatment of choice. $Ca(OH)_2$ has been used as a gold standard for apexification procedures. However, newer materials such as MTA and BD have superior properties, are less time-consuming, have better handling properties and faster results compared to $Ca(OH)_2$ in terms of periradicular healing and induction of root-end calcific repair by the formation of hard tissue.



ORIGINAL RESEARCH

Sunila B Sangappa, Sahana Alwar Mandayam Krishnian, Srinath M Kenkere, Ravindra Shivamurthy

Assessment of Salivary Lactate Dehydrogenase as a Noninvasive Biomarker for Chronic Periodontitis and Tooth Loss in Type II Diabetics

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:10] [Pages No:S203 - S212]

Keywords: Case-control study, Chronic periodontitis, Diabetes mellitus, Salivary biomarker, Salivary lactate dehydrogenase, Tooth loss

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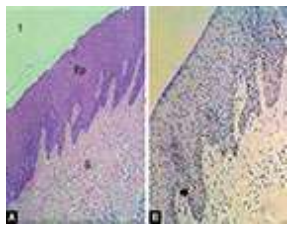
Abstract

Aim: This study aims at evaluating the association between salivary lactate dehydrogenase (SLDH) levels among type II diabetes mellitus (T2DM) subjects with chronic periodontitis (CP) and tooth loss to check the diagnostic value of SLDH as a noninvasive biomarker. **Materials and methods:** Seventy-six subjects aged from 30 to 70 years with at least 15 remaining teeth were selected for the study. Hemoglobin A1c (HbA1c) levels confirmed diabetic status while CP was assessed based on periodontal parameters with a full-mouth periodontal examination, following which the subjects were classified into four groups. Group I: controls—systemically healthy individuals (HbA1c levels ≤6.4%), without CP; group II: non-T2DM with CP—systemically healthy individuals (HbA1c ≤6.4%) with CP; group III: T2DM (HbA1c ≥6.5%) with CP; group IV: T2DM (HbA1c ≥6.5%) with CP and tooth loss due to periodontitis. Unstimulated whole saliva in fasting was collected from subjects and quantitatively assessed using a colorimetric lactate dehydrogenase assay kit. Analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used for statistical analysis. Statistical significance was set at 5% level ($p < 0.05$). Karl Pearson's correlation coefficient compared the relationship between different variables. **Results:** ANOVA of the four groups' mean scores yielded significant variation in SLDH levels ($F = 11.2889$, $p < 0.05$). The relationship between variables of HbA1c levels with SLDH activity levels (mU/mL) and average periodontal pocket depth (APPD) was statistically significant and a moderate correlation was found with Karl Pearson's correlation coefficient. **Conclusion:** Study outcomes indicate a high prevalence of CP in T2DM. The severity of periodontitis and tooth loss significantly altered LDH enzyme activity and exhibited elevated LDH levels indicating a valuable noninvasive salivary biomarker in the detection of periodontitis. **Clinical significance:** In view of saliva as a mainstay noninvasive screening method of periodontal disease, SLDH can assume a significant role in detecting elevated glycemic levels and distinguishing the severity of periodontitis. Routine use in general practice and dental offices can ensure early detection and monitoring.



ORIGINAL RESEARCH

Sapna Chandran, Rekha K Pillai, Bindhu P Ramakrishnan, Priya Thomas

Significance of SOX2 in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: An Immunohistochemical Study

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:7] [Pages No:S213 - S219]

Keywords: Biomarker, Cancer stem cells, Immunohistochemistry, Oral epithelial dysplasia, Oral squamous cell carcinoma, SOX2

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Abstract

Aim: The present study aimed to determine the role of SRY (sex-determining region Y)—box 2 (SOX2) as a potential marker of stemness and pluripotency and to compare its association with the normal oral mucosa (NOM), different grades of oral epithelial dysplasia (OED), and oral squamous cell carcinoma (OSCC). **Materials and methods:** A total of 70 study samples, including 30 cases of OED (10 each of mild, moderate, and severe dysplasia), 30 cases of OSCC [10 each of well-, moderate-, and poorly differentiated (WD, MD, and PD)], and 10 NOM samples were immunohistochemically stained to evaluate SOX2 association. Quantitative analysis was based on the percentage of positive cells with a scoring of 0–5 (0%, 1–10%, 11–30%, 31–50%, 51–80%, and >80%), and qualitative evaluation was based on the intensity of staining grading from 0–3 (negative, weak, moderate, and strong). Differences in staining intensity were evaluated using the Kruskal–Wallis test. Statistical comparison of positive cells among three groups was performed using one-way analysis of variance and a pairwise comparison within groups using Tukey analysis. **Results:** SOX2 expression was more prominent in OSCC than OED and weakest in normal mucosa. A significant increase in SOX2 expression was observed from mild to severe dysplasia, whereas the expression decreased from WDOSCC to PDOSCC. **Conclusion:** In conformity with the present study, SOX2 expression is seen to be upregulated in higher grades of OED and early OSCC. Thus, highlighting its role in identifying the potential lesions at an early stage. **Clinical significance:** Identification of cancer stem cells (CSCs) using biomarkers may help in the early detection of tumor development. SOX2 showing evidence of expressing these cells can be utilized as a definitive biomarker.

ORIGINAL RESEARCH

Shweta Bali, Akshay Bhargava, Shobhit Arora, Priyanka Aggarwal, Aruna Nautiyal, Deepali Singhal

Oxygen-releasing Gel vs 0.2% Chlorhexidine Gel as an Adjuvant to Scaling and Root Planing: A Randomized Controlled Trial

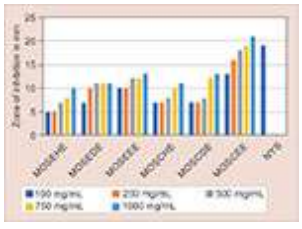
[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S220 - S224]

Keywords: Chlorhexidine, Chronic periodontitis, Local drug delivery, Nonsurgical periodontal treatment, Oxygen-releasing gel

DOI: 10.5005/jp-journals-10015-2137 | Open Access | How to cite |

Abstract

Aim: To compare and evaluate oxygen-releasing gel vs 0.2% chlorhexidine (CHX) gel as an adjuvant to scaling and root planning (SRP). **Materials and methods:** A total of 11 patients (22 sites) with chronic periodontitis were randomly assigned into test and control groups by the flip of a coin method. Pre-op clinical parameters recorded were plaque index (PI), gingival index (GI), probing pocket depth (PPD), and wound healing index (WHI). Post-completion of SRP, test group (n = 11) received subgingival delivery of oxygen-releasing gel and control group (n = 11) received 0.2% CHX gel. A periodontal pack and oral hygiene instructions (OHI) were given. Post-op clinical parameters were measured at 1 and 3 months. **Results:** The intergroup comparison of test sites (oxygen-releasing gel) with the control sites (0.2% CHX gel) showed a statistically higher significant reduction in test group when compared to control group in periodontal parameters such as PPD (p < 0.0001), clinical attachment level (CAL) (p < 0.0001), WHI (p, 0.0001). However, no statistically significant difference was found in PI and GI between both groups. **Conclusion:** Oxygen-releasing gel, when used as a local drug delivery, showed better results when compared to the current gold standard, that is, 0.2% CHX gel in the nonsurgical management of moderately deep pockets in chronic periodontitis. **Clinical significance:** Nonsurgical periodontal therapy lays the foundation for most periodontal procedures. It also stands a chance of higher acceptability to the patients. Hence, whenever the clinical situation is conducive, oxygen-releasing gel serves to be an excellent local drug delivery alternative.



ORIGINAL RESEARCH

Tanvi Tekwani, Sunila Bukanakere Sangappa, SubbaRao V Madhunapantula, Tejashree Anantha Balraj Urs, Krishna Karthik

Evaluation of Antifungal Activity of Moringa oleifera Seeds on Oral Candida Isolated from Type 2 Diabetic and Nondiabetic Complete Denture Wearers

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:6] [Pages No:S225 - S230]

Keywords: Antifungal agents, Antifungal resistance, Candida, Ethanolic extract, Moringa oleifera seed coat, Oral candidiasis, Type 2 diabetes

DOI: 10.5005/jp-journals-10015-2157 | Open Access | How to cite |

Abstract

Aim: To evaluate the antifungal potential of *Moringa oleifera* seed extracts on *Candida* isolated from oral cavities of diabetic (case) and nondiabetic (control) complete denture wearers. **Materials and methods:** Serial exhaustive extraction of *M. oleifera* seed coat and seed endosperm was done using hexane, dichloromethane, and ethanol. The salivary samples collected from the oral cavities of edentulous complete denture wearers were evaluated for susceptibility with *M. oleifera* seed coat and endosperm extracts on 60 salivary samples diabetic [case group, 30 individuals hemoglobin A1C (HbA1c) >6.4%] and nondiabetic (control group, 30 individuals HbA1c <6.4%). The potent extract's mean percent inhibition was compared using an independent *t*-test. The mean zone of inhibition was compared using an independent *t*-test between diabetic (case group) and nondiabetic (control group) groups using Statistical Package for Social Sciences (SPSS) 24 software, and a *p*-value of <0.05 was statistically significant. The potent *M. oleifera* extracts were tested for their *in vitro* anti-inflammatory potential. **Results:** *M. oleifera* seed coat ethanolic extract of 1000 mg/mL concentration showed a zone of inhibition of 20 mm against positive control nystatin (NYS) with a zone of 19 mm is the most potent antifungal agent. The susceptibility of *Candida* isolated from the saliva of nondiabetics (control) was higher when compared to that isolated from diabetics (case group). The anti-inflammatory potential of the potent extract revealed a dose-dependent % inhibition. **Conclusion:** The current study provides a potential herbal alternative in the form of *M. oleifera* seed coat extract against oral *Candida*. The study also explains the low toxicity of the *M. oleifera* ethanolic extract on human erythrocytes, making it a safer alternative to NYS. **Clinical significance:** Oral candidiasis due to the overgrowth of *Candida* in denture wearers is common. Herbal alternative to conventional antifungal agents against oral *Candida* that are effective and safe is successfully discussed in this study.

CASE REPORT

Vinay Dutta, Shubhangi Wate, Arun Khalikar, Sattyam Wankhede, Suryakanth Deogade, Archit Kapadia, Pradyumna Doibale

Fabrication of a Two-piece Magnet-retained Orbital Prosthesis in a Post-COVID-19 Mucormycosis Case: A Simplified Evidence-based Approach

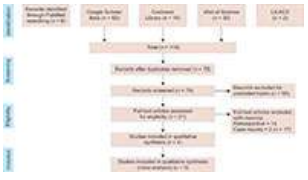
[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:5] [Pages No:S231 - S235]

Keywords: Artificial eye, Post-coronavirus disease 2019 mucormycosis, Rhino-orbital cerebral mucormycosis, Silicon facial prosthesis

DOI: 10.5005/jp-journals-10015-2147 | Open Access | How to cite |

Abstract

Aim: To bring into light an evidence-based approach to fabricate an orbital prosthesis in a post-coronavirus disease 2019 (COVID-19) mucormycosis-affected individual, which is simple, efficient, and economically advantageous. **Background:** Rehabilitation of facial defects becomes a demanding task as it is noticed distinctly and might be a socially awkward situation for the individual. Especially, a missing eye/contents of the orbit have a negative impact on the patients, which necessitates immediate rehabilitation. Various treatment modalities are at hand to rehabilitate such patients, of which implant-supported prosthesis has a finer retentive outcome. However, it cannot be administered among all patients due to economic or other unfavorable surgical limitations. **Case description:** This case report projects a 54-year-old male; a case of exenterated orbit secondary to post-COVID-19 mucormycosis surgery. The reconstruction of this patient was planned by a two-piece magnet-retained prosthesis with a silicon outer prosthesis. A heat-processed acrylic conformer was fabricated for the defected part, and a silicon superstructure to reconstruct the missing anatomy was done. Retention was achieved using natural undercuts, magnet, and adhesive. **Conclusion:** A systematic and meticulous approach is warranted based on the tissue makeup, material handling, and retentive aids as per the patient's expectations and affordability for maxillofacial rehabilitation. An evidence-based, multidisciplinary approach with the skill of the clinician is essential in providing effective and optimum rehabilitation. The treatment strategy adopted in this case is simple and economical and can be adapted for mass rehabilitation of orbital defects at an accelerated pace. **Clinical significance:** Much research is necessary in the digital domain of maxillofacial prosthodontics such that it is financially affordable, accessible, and an approach that reduces chairside time. Also, retentive aids which have a more predictable outcome, like implants, should be considered so as to improve the quality of life of the patient.



REVIEW ARTICLE

Sowmithra Devi, Aravind Kumar Subramaniyan

Treatment Efficacy and Patient Acceptance of Removable Mandibular Retractor Appliance and Bone-anchored Maxillary Protraction in Growing Skeletal Class III Patients: A Systematic Review

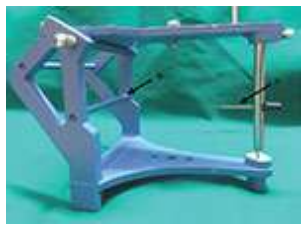
[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:6] [Pages No:S236 - S241]

Keywords: Class III functional appliance, Growing skeletal class III patients, Removable mandibular appliance

DOI: 10.5005/jp-journals-10015-2152 | Open Access | How to cite |

Abstract

Background: There are various treatment modalities in correcting the developing class III, of which the efficacy of removable mandibular retractor (RMR) in class III correction has not been discussed much in the literature. Available literature has compared various fixed and removable appliances for class III correction, which did not take RMR into inclusion. Hence, this systematic review focuses on the efficacy of the RMR appliance in bringing about favorable skeletal, dentoalveolar, and soft tissue changes in growing skeletal class III patients and patient acceptance of the appliance. **Objective:** To assess the skeletal, dentoalveolar, and soft tissue effects of RMR appliance and acceptability of the appliance in growing skeletal class III patients. **Search methods:** A systematic review of articles was performed using different electronic databases (PubMed, Cochrane Library, MEDLINE, Embase, Google Scholar, LILACS, and Web of Science) along with a manual search of orthodontic journals from 2011 to the year 2021. **Selection criteria:** Search items consisted of “removable appliances” and “skeletal class III.” The selection criteria were set to include only randomized controlled trials (RCTs). Four RCTs were included in the systematic review that evaluated dental, skeletal, and soft tissue effects and patient acceptance of RMR appliances. **Data collection and analysis:** Data regarding patient acceptance, skeletal, dentoalveolar, and soft tissue outcomes were extracted to collect study characteristics. Two separate authors independently extracted the data. Review manager (RevMan) version 5.1, Denmark, was the software used to analyze the data. After evaluating the risk of bias, standardized mean differences and 95% confidence intervals were calculated. **Results:** Of the four RCTs included, two RCTs evaluated the dentoalveolar, skeletal, and soft tissue changes with bone-anchored mandibular protraction (BAMP) and RMR appliance, and two RCTs evaluated the patient compliance and acceptability of the appliance. The results of the study by Majanni and Hajeer in 2016 revealed that skeletal and soft tissue changes were more pronounced with BAMP and dental changes were more pronounced with RMR appliance. However, a study by Saleh et al. concluded that hard and soft tissue changes were pronounced with RMR appliances when compared with no treatment controls. Two RCTs that evaluated patient compliance revealed that compared to RMR, bone-anchored intermaxillary traction (BAIMT) produced more pain, pressure, and tension of soft tissues that gradually reduced over time, and speech difficulty and social avoidance were noted in patients wearing RMR, which decreased gradually in time. **Conclusion:** Studies reviewed provide insufficient evidence to form a conclusion regarding the effects of the use of MR appliances. The available evidence suggests that the use of MR produces predominant dental changes and causes speech difficulty and social avoidance for the patients. More trials are needed to produce stable results on the use of RMR appliances.



VR Arun Kumar, A Kirubakaran, S Anand, VC Karthik, Sonia Abraham, S Nithyapriya

Simplified Technique for Mounting the Cast in the Mean Value Articulator Using a Newly Designed Mounting Device: An Innovative Clinical Technique

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:4] [Pages No:S242 - S245]

Keywords: Articulation, Custom mounting device, Laboratory mounting, Mounting cast

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Abstract

Aim: To demonstrate the procedure for mounting the maxillary cast in the mean value articulator using a newly designed mounting device. **Background:** Fabrication of dental prostheses, especially by indirect technique, requires the use of an articulator. Apart from reducing the chair side time it has numerous advantages. However, accurate mounting of the cast in the articulator is mandatory to avoid fabrication errors. The techniques currently in use are either difficult to master or require costly equipment. This article describes a new technique for mounting the cast in a mean value articulator using a custom-made mounting device. **Technique:** The newly designed mounting device consists of a U-plate, an anterior rod, and two posterior rods. The mounting device is fixed to the articulator so that the anterior rod is inserted into the hole in the incisal pin and the posterior rods rest on the posterior reference bar. This places the U-plate at the level of the reference plane of the articulator. The maxillary cast with occlusal rim is placed over the U-plate and mounted using type II gypsum. The mandibular cast is later mounted in the articulator using the interocclusal record. **Conclusion:** As the wax mount is not necessary for this technique; the newly fabricated mounting device reduces the chances of error during the mounting of the maxillary cast that could occur during the conventional procedure. **Clinical significance:** Routine procedures commonly used were based on arbitrary methods (wax mounting) with a high chance of error, while other devices were expensive and selective in purpose. Therefore, the need for more accurate and precise methods are required instead.

OBITUARY

Dr Beena Rani Goel

Dr Senia leaves a void in the field of Endodontics

[Year:2022] [Month:Supplementary Issue 2] [Volume:13] [Number:S2] [Pages:1] [Pages No:S246 - S246]

DOI: 10.5005/WJOUD-13-S2-246 | Open Access | How to cite |

ORIGINAL RESEARCH

Characterization of Fabricated Bovine Hydroxyapatite Crystal as Socket Preservation Material: An SEM-EDX and X-ray Diffraction Study

Pascal Filio[1], Octarina[2], Komariah[3]

Received on: 22 February 2022; *Accepted on:* 02 May 2022; *Published on:* 31 December 2022

Abstract

Aim: This research aimed to fabricate and characterize bovine hydroxyapatite (BHA) crystal by using scanning electron microscope-energy dispersive X-ray (SEM-EDX) and X-ray diffraction (XRD) test for the evaluation of its potential as socket preservation material.

Materials and methods: Bovine hydroxyapatite was fabricated from bovine cancellous bone that was washed and cut into small pieces. The bone was placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000°C for 1 hour and dried. The bone was then grounded into a powder and sieved through 150 µm sieves. Powdered BHA was then analyzed with SEM-EDX to analyze the structure and content of the HA element, and an XRD test was used to analyze the HA crystal.

Results: From the SEM test, BHA had a crystalline particle with hexagonal crystal with an average size of 197 µm. The EDX test indicated BHA has the elements of carbon (C) (50.71%), oxygen (O) (34.59%), sodium (Na) (0.35 %), magnesium (Mg) (0.35%), phosphorus (P) (4.29%), calcium (Ca) (8.97%), and niobium (Nb) (0.74%). The XRD test showed that BHA contains hydroxyapatite (HA) crystal with 66% crystallinity degree.

Conclusion: The BHA was successfully fabricated through the process of calcination. Based on the characterization and analysis using SEM-EDX and XRD tests of BHA, it had the potential as socket preservation material to preserve alveolar bone after tooth extraction.

Clinical significance: The addition of BHA after tooth extraction has the potential to maintain alveolar bone quality and prevent dimension loss. This biomaterial helps and supports prosthesis treatment, such as implants, which need good alveolar bone quantity and quality.

Keywords: Biomaterial, Bovine hydroxyapatite, Socket preservation.

World Journal of Dentistry (2022): 10.5005/jp-journals-10015-2155

Introduction

Tooth extraction is the second most frequently performed dental procedure in Indonesia, covering 7.9% of all dental procedures on research done by the Ministry of Health in 2018.[1] After tooth extraction, the dimensions of the alveolar bone will decrease by 30% due to resorption.[2,3] Socket preservation was carried out to maintain the dimensions of the postextraction alveolar bone socket. Socket preservation material in the form of bone graft could come from natural or synthetic materials.[4] Natural socket preservation materials are easier to obtain and do not require complex or expensive processing to be fabricated.[5] Natural grafts can be in the form of autografts from the patient's body, allografts from the same species, and xenografts.

Human bone consists of 70% inorganic components, most of which are HA crystals and 30% organic components, most of which are type 1 collagen.[5] Alveolar bone consists of 67% inorganic material and 33% organic material.[6] HA [calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$] is the main inorganic compound that composes various hard tissues such as bones, teeth, dentin, and others. Currently, the demand for HA as a bone graft is very high. Due to the limited supply of HA autograft and allograft, HA from xenograft was developed.[7] Xenograft is a graft taken from a different species from the recipient, such as an animal.[8] There are two categories of HA xenografts that are often used, namely synthetic and natural. One of the natural sources of this material is bovine bone.[9] In the field of dentistry, several commercial products use HA as a bone graft.[5] The price of commercial HA is quite expensive when compared to the fabrication price of HA from bovine bone. One method of fabricating HA from bovine is the calcination method. The calcination method with thermal decomposition is easy to do and can produce HA in the form of nanorods.[10]

Bovine hydroxyapatite fabricated from bovine bone has shown potential as socket preservation material; still, it needs to be tested both elementally and structurally to prove its biological function. SEM is a tool that provides very accurate microscopic images of material. SEM could analyze organic and inorganic material on a nanometer to micrometer scale. EDX test could be done while doing SEM analysis. EDX test measures the X-ray pattern

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Source of support: Nil

Conflict of interest: None

emitted by the material to determine the chemical composition and quantity.[11] XRD is a test for analyzing crystal structure, size, component, parameter, and degree of crystallinity by using an X-ray beam.[12] XRD works by measuring diffracted X-ray angles emitted from the material to the detector.[13] Therefore, the present study aimed to fabricate HA derived from bovine bone and see the characterization of this material with an SEM-EDX and XRD to analyze the potential of this material as a substitute for alveolar bone for socket preservation.

Materials and Methods

Universitas Airlangga, Faculty of Dental Medicine, Dr Soetomo Surabaya approved this research with ethical clearance No: 360/HRECC.FODM/VII/2021. This research fabricated HA at the Tissue Bank of Rumah Sakit Umum Daerah (RSUD), Dr Soetomo Surabaya. Bovine bones used in this study were derived from female cow bones from farms in Malang, East Java, Indonesia. At first, the bovine bones were cleaned and washed from any blood clots. Then, the part of the cancerous bone that contains a lot of sponges was cut into small pieces as needed (Fig. 1A). Washing was carried out in detail using hydrogen peroxide (H_2O_2) (Fig. 1B). The bone was cleaned with an ultrasonic cleaner at a temperature of 60°C so that the fat would melt (Fig. 1C). Ultrasonic cleaning is repeated until the bone is completely clean of fat and the color becomes white. Washing was carried out again with distilled water to remove H_2O_2 until it was clean (Fig. 1D). The bones were then dried at room temperature to minimize moisture content (Fig. 1E). Next, the powder was heated in a furnace with a temperature of 1000°C for 1 hour (Fig. 1F). After the heating process, the bone was rinsed with distilled water 3–4 times to remove the toxic properties (Fig. 1G). Then dried again in an oven at a temperature of 60°–100°C until dry (Fig. 1H). After drying completely, the bone was ground with a bone miller until particles were formed (Fig. 1I). The fine and coarse particles were separated by a sieving machine with a size of 150 µm (Fig. 1J).

Specimen Characterization

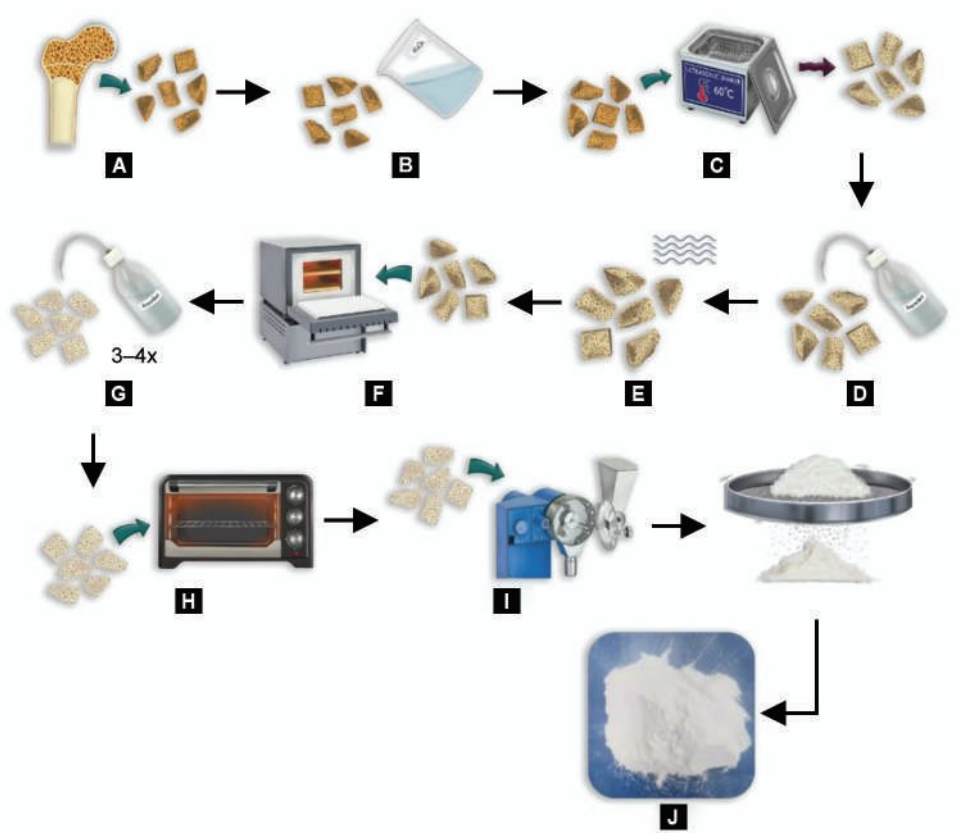
After the fabrication of HA powder from bovine bone, then it was characterized by SEM-EDX and XRD.

Scanning Electron Microscopy-Electron Dispersive X-ray Test

Scanning Electron Microscope test was carried out at Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Teknologi Bandung (JEOL, JSM 6510-LA). A total of 0.1 gm of BHA material was used in this analysis. The sample was then coated with gold (fine coater JFC-1600) before testing. The prepared sample was inserted in the chamber and vacuumed in high vacuum mode with a 15 kV secondary electron imaging detector. Magnification was set to 10,000 and 20,000×, after which the focus X stigma and Y stigma were adjusted to refine the image. Then the surface morphology of the pores, size of the pores, and the shape of the pores were analyzed.

Electron Dispersive X-ray Test

The samples in the SEM chamber were analyzed and mapped the chemical elements of the material using EDX spectroscopy (JEOL, JSM 6510-LA) at 10 µm magnification settings with 10 mm spacing. The results of the chemical element analysis are displayed in the form of graphs and color gradation maps.



Figs 1A to J: Process of fabrication HA from bovine bone: (A) Cutting of cancellous bovine bone; (B) Washing with H_2O_2; (C) Fat removal; (D) Washing with H_2O_2; (E) Drying at room temperature; (F) Burning in the oven; (G) Washing with distilled water; (H) Bone drying; (I) Bone grinding;

X-ray Diffraction Test

X-ray diffraction test was carried out at the National Research and Innovation Agency (BRIN) for Physics Serpong. A total of 1 gm of BHA powder was used in this study. The Bruker D8 XRD machine (Germany) with cobalt cathode is used with a scan range of 10–90° with a speed of 0.1 seconds/step.

Results

In this study, the calcination method with thermal decomposition was used to extract HA from bovine bone to remove organic material. The bovine bone powder is heated in a furnace to a temperature of 1000°C for 1 hour until the organic matter evaporates.

Scanning Electron Microscopy-Electron Dispersive Test Results

Figure 2 is an SEM image of BHA at (A) 10,000× and (B) 20,000× magnification. In this study, Image J software was used on SEM images with a magnification of 20,000× to measure the size of HA crystals. The picture shows an irregular hexagonal crystal structure with an average size of 1.97 m, with the smallest size of 0.144 µm and the largest size of 4.494 µm.

Figures 3 and 4 are the results of BHA analysis with EDX. These results indicate the elements contained in BHA. In the picture, BHA shows the presence of the elements C (50.71%), O (34.59%), Na (0.35%), Mg (0.35%), P (4.29%), Ca (8.97%), and Nb (0.74%).

X-ray Diffraction Test Results

The analysis of HA peaks and measurements of the degree of crystallinity were carried out using the Origin 2019b program. In the results of the HA analysis, there is a sharp peak at 30.2°, 33.8°, 37.1°, 37.6°, 38.4°, 39.8°, 46.6°, 54.8°, 56.5°, 58.2°, 59.4°, 60.4°, 61.3°, and 62.7°2θ (Fig. 5). The measurement of crystallinity degree was carried out with $\frac{\text{crystalline area}}{\text{amorph area + crystalline area}} \times 100\%$. The degree of crystallinity in this HA sample is 66% which is HA crystal with a moderate degree of crystallinity. The X'Pert Highscore program was used to calculate the crystal size by Scherrer calculation. The crystal size of HA in the HA sample was 1087 nm.

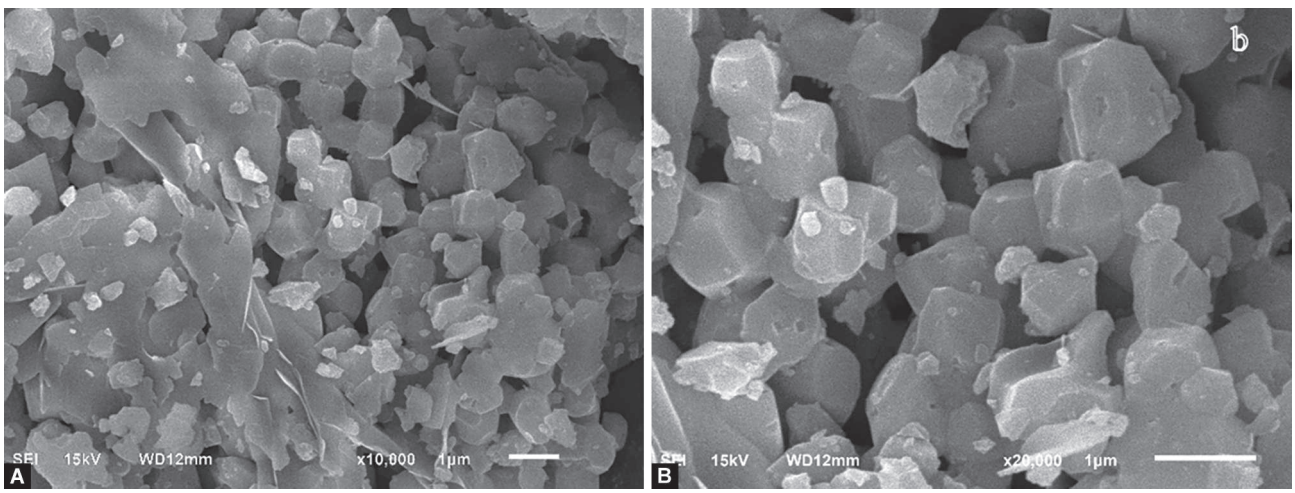
From the test above, BHA contains HA crystals that has an average crystal size of 1.97 µm containing elements such as C, O, Na, Mg, P, Ca, and Nb with 66% degree of crystallinity. The result of the test shows favors that BHA has the shape, elements, and crystal structure of a bone formation that has potential as a socket preservation material.

Discussion

Tooth extraction will cause alveolar bone resorption, which could lead to dimension loss. Using graft material HA after extraction could help maintain alveolar bone height. Commercial natural HA is very expensive and hard to get for postextraction use. This study is aimed to create a cheaper and more readily available HA from bovine bone and analyze it using SEM-EDX and XRD. SEM-EDX test is to show the microscopic size and chemical component of HA crystal. XRD is used to characterize HA crystals.

Alveolar bone resorption can be divided into two stages. The first stage of this process is bone resorption which causes a decrease in bone dimensions. The second stage in this process is the remodeling of the buccal bone and woven bone, which will lead to more reduction in the dimensions of the alveolar bone. Alveolar bone disuse atrophy, decreases blood supply, and local inflammation also plays a role in bone resorption.[14] Alveolar bone resorption complicates even the failure of placement of restorations such as implants. Implant placement requires ideal alveolar bone volume to achieve an adequate implant-to-bone ratio.[15] Bone quality is also an important factor in the placement of implant restorations.[16] Socket preservation material could come in many forms, such as HA powder from many sources. HA could come from allograft, xenograft, and alloplast. Allograft materials come from the same species but from a different individual. Allograft HA could come from frozen bone, freeze-dried bone, demineralized freeze-dried bone, and deproteinized bone. Xenograft material comes from a natural origin but from different species from the recipient. These include animal bones and coral. Alloplast is a graft that comes from a synthetic origin. Alloplast graft is made from calcium phosphates, glass ceramics, and polymers.[17,18]

Bovine bone has a morphology and structure that is similar to human bone. HA from bovine bone also has the same chemical composition as human bone.[19] Bovine bone also has the main mineral of human bone, namely HA.[20] HA extracted from bovine bone by thermal decomposition has good biocompatibility so that it can decompose naturally, can be absorbed naturally, and can be integrated with the bone. BHA is also more available, not limited to religious beliefs, and more economic.[5] HA also has mechanical



Figs 2A and B: Scanning electron microscope images (A) BHA at 10,000x magnification and (B) BHA at 20,000x magnification

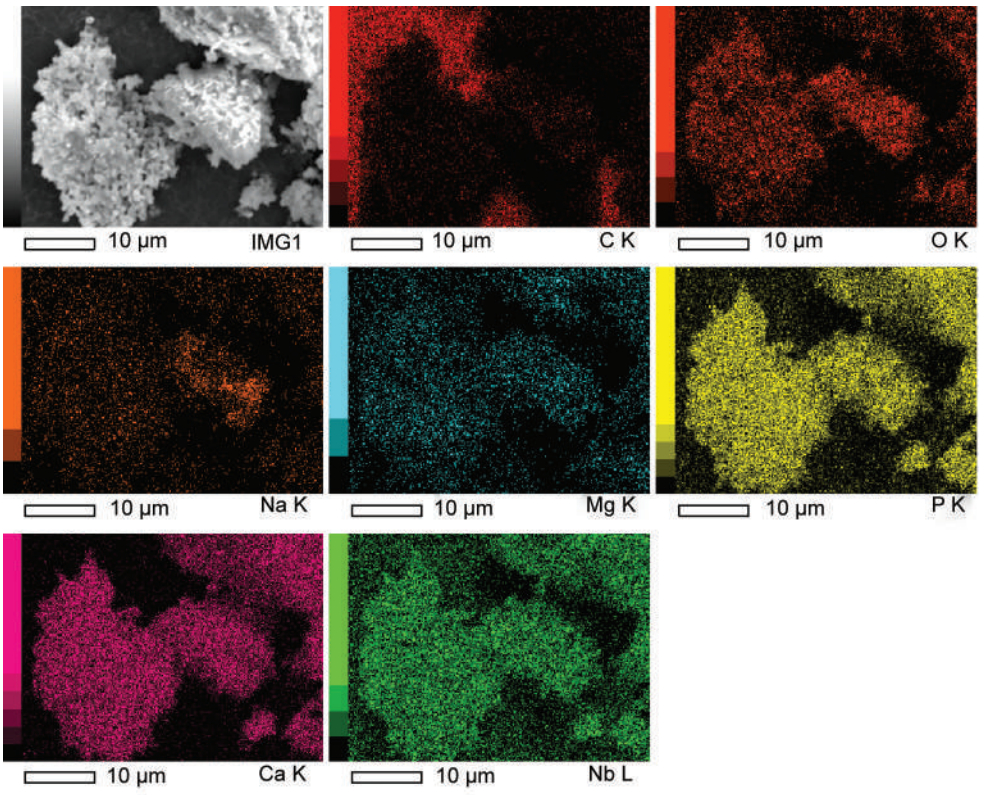


Fig. 3: Energy dispersive X-ray images of C, O, Na, Mg, P, Ca, and Nb element mapping from BHA

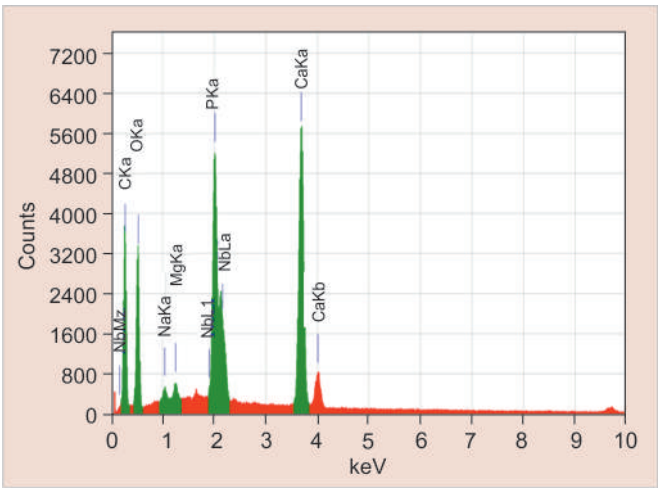


Fig. 4: Energy dispersive X-ray graph test results from BHA

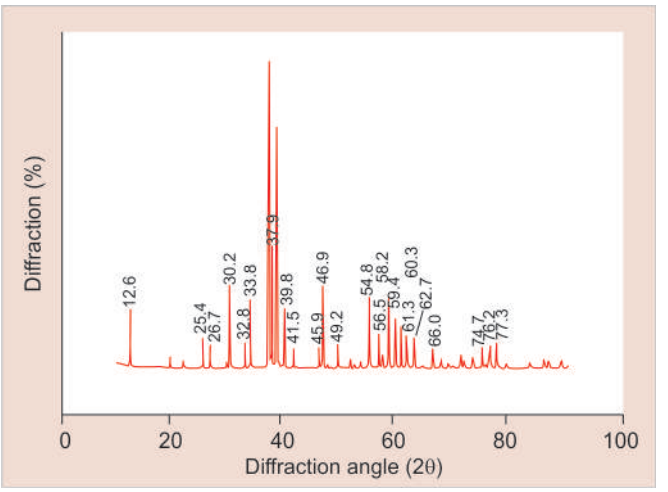


Fig. 5: X-ray diffraction graph from BHA

properties that are almost the same as human bone. This material has an important role in socket preservation.[21,22]

Beef consumption in Indonesia is quite high. In 2020, 1,276,473 beef cattle were slaughtered and produced around 168,267 tons of waste beef bones.[23–25] So, bovine bone waste produced in Indonesia is quite a lot, but the processing is not optimal, which makes bovine bones have a low selling value. To increase the selling value of bovine bones, processing can be done, one of which is extracting HA from the bones.

Bovine bones contain 70% HA which is similar to HA in human bones. The content of HA in bovine bones can make it a good candidate for socket preservation material.[24] HA from bovine bone has long been developed so that it has an affordable price and better quality. Bovine bone is considered as organic waste. Bovine bones can be recycled and reused to increase their resale value. One way to increase the market value of bovine bone is to make it a source of HA for graft biomaterials in dental practice.[21] This research tried to process bovine cancellous bone and fabricate biomaterials that are useful as bone replacement materials or to maintain bone tissue after tooth extraction.

In this study, HA biomaterial derived from bovine bone was made by calcination process with thermal decomposition. Ramirez-Gutierrez et al. successfully created pure HA without any organic compound from the bovine bone using calcination through thermal degradation at 900°C.[26] The bovine bone powder in this research is heated at 1000°C for 1 hour.[27] This was done to remove other organic matter that will evaporate at a temperature of 350°C.[28] HA also has stability up to a temperature of 1200°C.[29] The

advantage of the calcination method is that it can remove all organic components and all disease genomes. This provides safety when used as a graft material.[27]

The bone used in this study was taken from the cancellous bone. Cancellous bone is a porous bone that is easily powdered and calcined. The porosity of the graft material is required for bone regeneration. The size and amount of porosity of the graft material can affect the osteoconductive properties, biodegradation, connection, migration, adhesion, proliferation, and differentiation of cells.[30,31] The higher the porosity of the graft material, the easier it is for growth factors to penetrate the material and increase vascularity and angiogenesis.[30]

Scanning electron microscope is a type of electron microscope that images the surface of a sample by scanning it with an electron beam. The electrons that interact with the atoms that make up the sample produce a signal that contains information about the sample from the surface topography, morphology, and composition of a material. SEM can detect and analyze surface fractures, provide microstructural information, view surface contamination, perform qualitative chemical analysis, and identification of crystalline structures.[32]

The results of the SEM test show that the HA crystal structure is hexagonal, which is in accordance with the research by Ruksudjarit et al., which examined HA from bovine extracted using a thermal calcination process.[33] The results indicate an average HA crystal size of 1.97 µm. The crystal size of these HA sample crystals is within the micro size (2–3 m). Micro-sized crystals can help stem cell differentiation and matrix synthesis, stimulate cell proliferation, chondrogenic gene expression, and matrix production.[34] HA with micro size can also increase the osteogenic and cementogenic ability because it can help the attachment, spread, and proliferation of cells.[35] The process of making HA by thermal decomposition produces HA in the form of rods.[10]

Energy dispersive X-ray is one of the most frequently used tests to analyze materials. This test is very easy to do because this test kit is an addition to the SEM machine and can be done in a matter of minutes.[36] X-rays have a specific energy response for each element. This can happen because the higher the atomic number, the stronger the emission of energy from the X-rays.[37] In this study, EDX was used to determine the elemental content in HA powder.

The EDX test results from human alveolar bone showed that human bone contains elements of calcium, phosphorus, and oxygen which are the main constituents of HA. There are also trace elements of iron, copper, zinc, bromium, strontium, Mg, lead, and Na which are found in human bones in small amounts. The results of the EDX test of BHA indicate that BHA contains elements of Ca, P, and O which are the major components. Also found, elements of Mg and Na as minor components. This is in accordance with the research of Maidaniuc et al. also found elements of Ca, P, O, Mg, and Na in BHA.[38] The similarity of the elemental content of alveolar bone and BHA makes this material can be used as a bone replacement material.

In this study, HA was produced with a Ca/P ratio of 2.09. According to research by Jaber et al., the lower the thermal temperature used, the higher the Ca/P ratio.[39] The Ca/P ratio in human bone is 1.67, if the Ca/P ratio of an HA is below 1.5, it produces a biphasic polarized HA material, namely β-tricalcium phosphate. The higher the Ca/P ratio, the purer the resulting HA. The presence of Na content in BHA can increase biocompatibility and osteointegration.[40]

X-ray diffraction analysis is an analysis that uses the degree of XRD to see the characteristics of a crystal.[41] XRD analysis can also be used to identify the structure, crystal size, elements, lattice parameters, and degree of crystallization through the diffraction peaks that appear.[12] Therefore, XRD is a technique that can analyze the crystalline phase of various materials such as HA.[41]

After analyzing the XRD peaks on HA which was carried out with the X'Pert highscore program, HA bovine according to the mineral reference HA $Ca_{10}(PO_4)_6(OH)_2$ from The International Centre for Diffraction Data, Powder Diffraction File™.[42] Calculation of the degree of crystallinity showed that the resulting HA had a moderate degree of crystallinity, which was 66% which indicated natural HA.[41] HA with a moderate degree could increase the proliferation of osteogenic cells and can release particles from HA crystals in the surrounding tissue.[44,45] BHA has a moderate degree of crystallinity and micro size that can help bone healing because HA is not easily absorbed.[43,46]

Hydroxyapatite in powder form is easier and more useful for dentistry. Also provides a repeatable quality in clinical use.[47] HA powder can be used for a variety of applications. HA powder can be applied as bone filler in the repair of bone defects, fixation of implants, and bone grafts.[48] BHA is a bioactive material that has osseointegration, osteoinduction, and osteogenesis properties.[49] The bioactive properties of BHA can stimulate osteoblast cells to form osteoid, which will accelerate osteocyte formation.[22] However, HA has limitations, namely its slow resorption so it cannot be absorbed properly. The osteoconductive nature of HA makes it difficult for resorption. The remaining unresorbed HA can have a negative effect on the mechanical properties of the regenerated bone.[5] Currently, there's no literature about contraindications for BHA in clinical usage.[50]

This study on BHA is currently limited to SEM-EDX and XRD characterization tests. In the future, it's necessary to run other supportive tests, such as cytotoxicity tests and analyze them to observe BHA biocompatibility as socket preservation material. After the cytotoxicity test, clinical tests on BHA will be done on the animal subject and then on human subjects.

Conclusion

Hydroxyapatite can be produced naturally from bovine bone. The results of the characterization analysis using SEM-EDX and XRD showed that this material has a crystalline structure and elements that can replace human alveolar bone. Therefore, this material has the potential to replace the lost bone structure or as socket preservation after tooth extraction.

Acknowledgments

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ORIGINAL RESEARCH

Characterization of Fabricated Bovine Hydroxyapatite Crystal as Socket Preservation Material: An SEM-EDX and X-ray Diffraction Study

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Abstract

Aim: This research aimed to fabricate and characterize bovine hydroxyapatite (BHA) crystal by using scanning electron microscope-energy dispersive X-ray (SEM-EDX) and X-ray diffraction (XRD) test for the evaluation of its potential as socket preservation material.

Materials and methods: Bovine hydroxyapatite was fabricated from bovine cancellous bone that was washed and cut into small pieces. The bone was placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000°C for 1 hour and dried. The bone was then grounded into a powder and sieved through 150 µm sieves. Powdered BHA was then analyzed with SEM-EDX to analyze the structure and content of the HA element, and an XRD test was used to analyze the HA crystal.

Results: From the SEM test, BHA had a crystalline particle with hexagonal crystal with an average size of 197 µm. The EDX test indicated BHA has the elements of carbon (C) (50.71%), oxygen (O) (34.59%), sodium (Na) (0.35 %), magnesium (Mg) (0.35%), phosphorus (P) (4.29%), calcium (Ca) (8.97%), and niobium (Nb) (0.74%). The XRD test showed that BHA contains hydroxyapatite (HA) crystal with 66% crystallinity degree.

Conclusion: The BHA was successfully fabricated through the process of calcination. Based on the characterization and analysis using SEM-EDX and XRD tests of BHA, it had the potential as socket preservation material to preserve alveolar bone after tooth extraction.

Clinical significance: The addition of BHA after tooth extraction has the potential to maintain alveolar bone quality and prevent dimension loss. This biomaterial helps and supports prosthesis treatment, such as implants, which need good alveolar bone quantity and quality.

Keywords: Biomaterial, Bovine hydroxyapatite, Socket preservation.

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Introduction

Tooth extraction is the second most frequently performed dental procedure in Indonesia, covering 7.9% of all dental procedures on research done by the Ministry of Health in 2018.[1] After tooth extraction, the dimensions of the alveolar bone will decrease by 30% due to resorption.[2,3] Socket preservation was carried out to maintain the dimensions of the postextraction alveolar bone socket. Socket preservation material in the form of bone graft could come from natural or synthetic materials.[4] Natural socket preservation materials are easier to obtain and do not require complex or expensive processing to be fabricated.[5] Natural grafts can be in the form of autografts from the patient's body, allografts from the same species, and xenografts.

Human bone consists of 70% inorganic components, most of which are HA crystals and 30% organic components, most of which are type 1 collagen.[5] Alveolar bone consists of 67% inorganic material and 33% organic material.[6] HA [calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$] is the main inorganic compound that composes various hard tissues such as bones, teeth, dentin, and others. Currently, the demand for HA as a bone graft is very high. Due to the limited supply of HA autograft and allograft, HA from xenograft was developed.[7] Xenograft is a graft taken from a different species from the recipient, such as an animal.[8] There are two categories of HA xenografts that are often used, namely synthetic and natural. One of the natural sources of this material is bovine bone.[9] In the field of dentistry, several commercial products use HA as a bone graft.[5] The price of commercial HA is quite expensive when compared to the fabrication price of HA from bovine bone. One method of fabricating HA from bovine is the calcination method. The calcination method with thermal decomposition is easy to do and can produce HA in the form of nanorods.[10]

Bovine hydroxyapatite fabricated from bovine bone has shown potential as socket preservation material; still, it needs to be tested both elementally and structurally to prove its biological function. SEM is a tool that provides very accurate microscopic images of material. SEM could analyze organic and inorganic material on a nanometer to micrometer scale. EDX test could be done while doing SEM analysis. EDX test measures the X-ray pattern

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emitted by the material to determine the chemical composition and quantity.[11] XRD is a test for analyzing crystal structure, size, component parameter, and degree of crystallinity by using an X-ray beam.[12] XRD works by measuring diffracted X-ray angles emitted from the material to the detector.[13] Therefore, the present study aimed to fabricate HA derived from bovine bone and see the characterization of this material with an SEM-EDX and XRD to analyze the potential of this material as a substitute for alveolar bone for socket preservation.

Materials and Methods

Universitas Airlangga, Faculty of Dental Medicine, Dr Soetomo Surabaya approved this research with ethical clearance No: 360/HRECC.FODM/VII/2021. This research fabricated HA at the Tissue Bank of Rumah Sakit Umum Daerah (RSUD), Dr Soetomo Surabaya. Bovine bones used in this study were derived from female cow bones from farms in Malang, East Java, Indonesia. At first, the bovine bones were cleaned and washed from any blood clots. Then, the part of the cancerous bone that contains a lot of sponges was cut into small pieces as needed (Fig. 1A). Washing was carried out in detail using hydrogen peroxide (H_2O_2) (Fig. 1B). The bone was cleaned with an ultrasonic cleaner at a temperature of 60°C so that the fat would melt (Fig. 1C). Ultrasonic cleaning is repeated until the bone is completely clean of fat and the color becomes white. Washing was carried out again with distilled water to remove H_2O_2 until it was clean (Fig. 1D). The bones were then dried at room temperature to minimize moisture content (Fig. 1E). Next, the powder was heated in a furnace with a temperature of 1000°C for 1 hour (Fig. 1F). After the heating process, the bone was rinsed with distilled water 3–4 times to remove the toxic properties (Fig. 1G). Then dried again in an oven at a temperature of 50°–100°C until dry (Fig. 1H). After drying completely, the bone was ground with a bone miller until particles were formed (Fig. 1I). The fine and coarse particles were separated by a sieving machine with a size of 150 µm (Fig. 1J).

Specimen Characterization

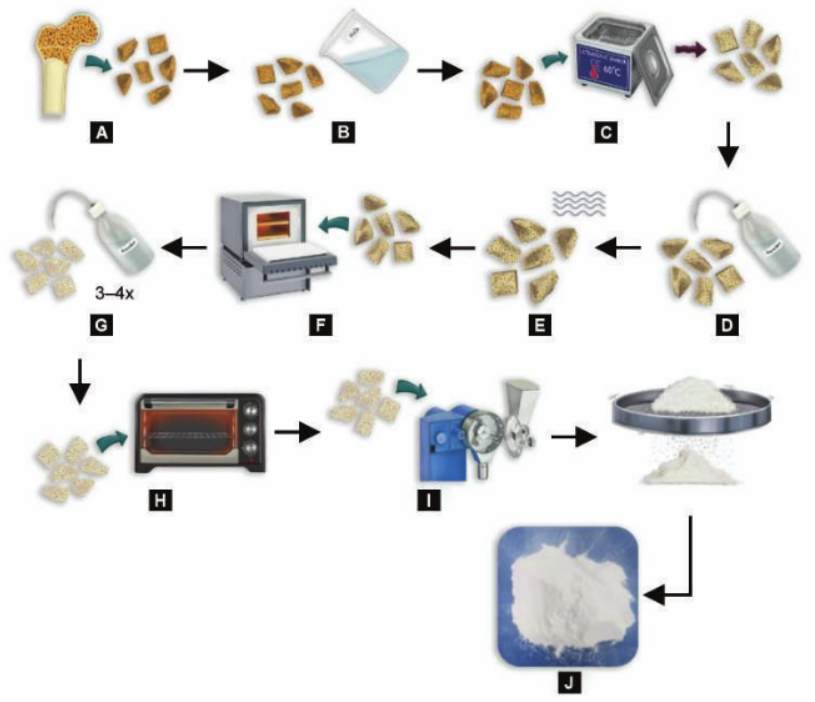
After the fabrication of HA powder from bovine bone, then it was characterized by SEM-EDX and XRD.

Scanning Electron Microscopy-Electron Dispersive X-ray Test

Scanning Electron Microscope test was carried out at Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Teknologi Bandung (JEOL, JSM 6510-LA). A total of 0.1 gm of BHA material was used in this analysis. The sample was then coated with gold (fine coater JFC-1600) before testing. The prepared sample was inserted in the chamber and vacuumed in high vacuum mode with a 15 kV secondary electron imaging detector. Magnification was set to 10,000 and 20,000×, after which the focus X stigma and Y stigma were adjusted to refine the image. Then the surface morphology of the pores, size of the pores, and the shape of the pores were analyzed.

Electron Dispersive X-ray Test

The samples in the SEM chamber were analyzed and mapped the chemical elements of the material using EDX spectroscopy (JEOL, JSM 6510-LA) at 10 µm magnification settings with 10 mm spacing. The results of the chemical element analysis are displayed in the form of graphs and color gradation maps.



Figs 1A to J: Process of fabrication HA from bovine bone: (A) Cutting of cancellous bovine bone; (B) Washing with H_2O_2; (C) Fat removal; (D) Washing with H_2O_2; (E) Drying at room temperature; (F) Burning in the oven; (G) Washing with distilled water; (H) Bone drying; (I) Bone grinding;

X-ray Diffraction Test

X-ray diffraction test was carried out at the National Research and Innovation Agency (BRIN) for Physics Serpong. A total of 1 gm of BHA powder was used in this study. The Bruker D8 XRD machine (Germany) with cobalt cathode is used with a scan range of 10–90° with a speed of 0.1 seconds/step.

Results

In this study, the calcination method with thermal decomposition was used to extract HA from bovine bone to remove organic material. The bovine bone powder is heated in a furnace to a temperature of 1000°C for 1 hour until the organic matter evaporates.

Scanning Electron Microscopy-Electron Dispersive Test Results

Figure 2 is an SEM image of BHA at (A) 10,000× and (B) 20,000× magnification. In this study, Image J software was used on SEM images with a magnification of 20,000× to measure the size of HA crystals. The picture shows an irregular hexagonal crystal structure with an average size of 1.97 m, with the smallest size of 0.144 µm and the largest size of 4.494 µm.

Figures 3 and 4 are the results of BHA analysis with EDX. These results indicate the elements contained in BHA. In the picture, BHA shows the presence of the elements C (50.71%), O (34.59%), Na (0.35%), Mg (0.35%), P (4.29%), Ca (8.97%), and Nb (0.74%).

X-ray Diffraction Test Results

The analysis of HA peaks and measurements of the degree of crystallinity were carried out using the Origin 2019b program. In the results of the HA analysis, there is a sharp peak at 30.2°, 33.8°, 37.1°, 37.6°, 38.4°, 39.8°, 46.6°, 54.8°, 56.5°, 58.2°, 59.4°, 60.4°, 61.3°, and 62.7°2θ (Fig. 5). The measurement of crystallinity degree was carried out with $\frac{\text{crystalline area}}{\text{amorph area} + \text{crystalline area}} \times 100\%$. The degree of crystallinity in this HA sample is 66% which is HA crystal with a moderate degree of crystallinity. The X'Pert Highscore program was used to calculate the crystal size by Scherrer calculation. The crystal size of HA in the HA sample was 1087 nm.

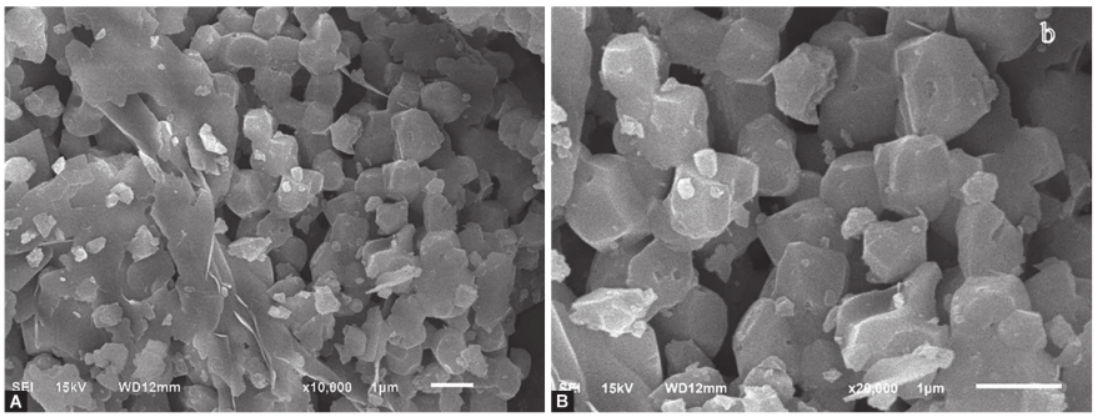
From the test above, BHA contains HA crystals that has an average crystal size of 1.97 µm containing elements such as C, O, Na, Mg, P, Ca, and Nb with 66% degree of crystallinity. The result of the test shows favors that BHA has the shape, elements, and crystal structure of a bone formation that has potential as a socket preservation material.

Discussion

Tooth extraction will cause alveolar bone resorption, which could lead to dimension loss. Using graft material HA after extraction could help maintain alveolar bone height. Commercial natural HA is very expensive and hard to get for postextraction use. This study is aimed to create a cheaper and more readily available HA from bovine bone and analyze it using SEM-EDX and XRD. SEM-EDX test is to show the microscopic size and chemical component of HA crystal. XRD is used to characterize HA crystals.

Alveolar bone resorption can be divided into two stages. The first stage of this process is bone resorption which causes a decrease in bone dimensions. The second stage in this process is the remodeling of the buccal bone and woven bone, which will lead to more reduction in the dimensions of the alveolar bone. Alveolar bone disuse atrophy, decreases blood supply, and local inflammation also plays a role in bone resorption.[14] Alveolar bone resorption complicates even the failure of placement of restorations such as implants. Implant placement requires ideal alveolar bone volume to achieve an adequate implant-to-bone ratio.[15] Bone quality is also an important factor in the placement of implant restorations.[16] Socket preservation material could come in many forms, such as HA powder from many sources. HA could come from allograft, xenograft, and alloplast. Allograft materials come from the same species but from a different individual. Allograft HA could come from frozen bone, freeze-dried bone, demineralized freeze-dried bone, and deproteinized bone. Xenograft material comes from a natural origin but from different species from the recipient. These include animal bones and coral. Alloplast is a graft that comes from a synthetic origin. Alloplast graft is made from calcium phosphates, glass ceramics, and polymers.[17,18]

Bovine bone has a morphology and structure that is similar to human bone. HA from bovine bone also has the same chemical composition as human bone.[19] Bovine bone also has the main mineral of human bone, namely HA.[20] HA extracted from bovine bone by thermal decomposition has good biocompatibility so that it can decompose naturally, can be absorbed naturally, and can be integrated with the bone. BHA is also more available, not limited to religious beliefs, and more economic.[5] HA also has mechanical



Figs 2A and B: Scanning electron microscope images (A) BHA at 10,000x magnification and (B) BHA at 20,000x magnification

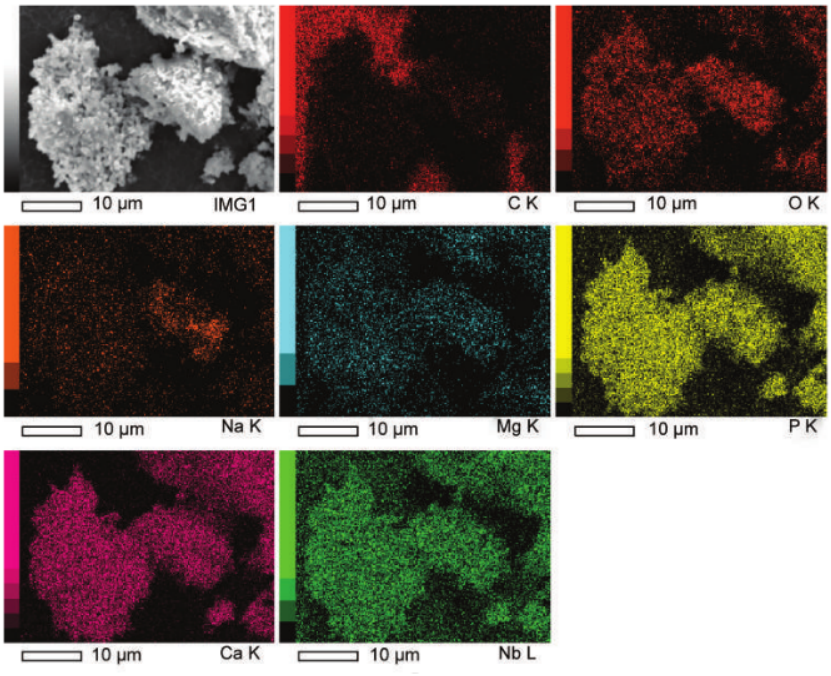


Fig. 3: Energy dispersive X-ray images of C, O, Na, Mg, P, Ca, and Nb element mapping from BHA

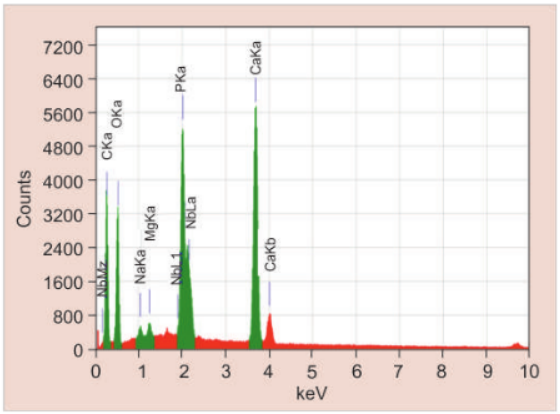


Fig. 4: Energy dispersive X-ray graph test results from BHA

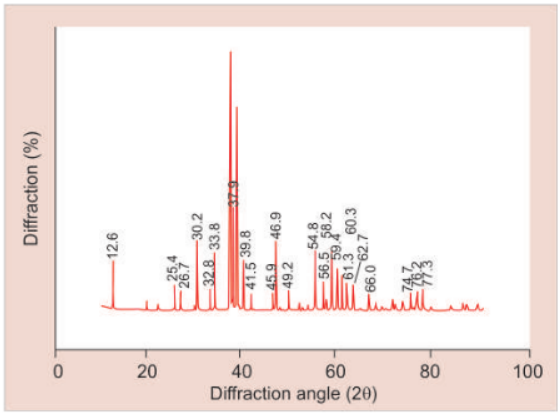


Fig. 5: X-ray diffraction graph from BHA

properties that are almost the same as human bone. This material has an important role in socket preservation.[21,22]

Beef consumption in Indonesia is quite high. In 2020, 1,276,473 beef cattle were slaughtered and produced around 168,267 tons of waste beef bones.[23–25] So, bovine bone waste produced in Indonesia is quite a lot, but the processing is not optimal, which makes bovine bones have a low selling value. To increase the selling value of bovine bones, processing can be done, one of which is extracting HA from the bones.

Bovine bones contain 70% HA which is similar to HA in human bones. The content of HA in bovine bones can make it a good candidate for socket preservation material.[24] HA from bovine bone has long been developed so that it has an affordable price and better quality. Bovine bone is considered as organic waste. Bovine bones can be recycled and reused to increase their resale value. One way to increase the market value of bovine bone is to make it a source of HA for graft biomaterials in dental practice.[21] This research tried to process bovine cancellous bone and fabricate biomaterials that are useful as bone replacement materials or to maintain bone tissue after tooth extraction.

In this study, HA biomaterial derived from bovine bone was made by calcination process with thermal decomposition. Ramirez-Gutierrez et al. successfully created pure HA without any organic compound from the bovine bone using calcination through thermal degradation at 900°C.[26] The bovine bone powder in this research is heated at 1000°C for 1 hour.[27] This was done to remove other organic matter that will evaporate at a temperature of 350°C.[28] HA also has stability up to a temperature of 1200°C.[29] The

advantage of the calcination method is that it can remove all organic components and all disease genomes. This provides safety when used as a graft material.[27]

The bone used in this study was taken from the cancellous bone. Cancellous bone is a porous bone that is easily powdered and calcined. The porosity of the graft material is required for bone regeneration. The size and amount of porosity of the graft material can affect the osteoconductive properties, biodegradation, connection, migration, adhesion, proliferation, and differentiation of cells.[30,31] The higher the porosity of the graft material, the easier it is for growth factors to penetrate the material and increase vascularity and angiogenesis.[30]

Scanning electron microscope is a type of electron microscope that images the surface of a sample by scanning it with an electron beam. The electrons that interact with the atoms that make up the sample produce a signal that contains information about the sample from the surface topography, morphology, and composition of a material. SEM can detect and analyze surface fractures, provide microstructural information, view surface contamination, perform qualitative chemical analysis, and identification of crystalline structures.[32]

The results of the SEM test show that the HA crystal structure is hexagonal, which is in accordance with the research by Ruksudjarit et al., which examined HA from bovine extracted using a thermal calcination process.[33] The results indicate an average HA crystal size of 1.97 µm. The crystal size of these HA sample crystals is within the micro size (2–3 m). Micro-sized crystals can help stem cell differentiation and matrix synthesis, stimulate cell proliferation, chondrogenic gene expression, and matrix production.[34] HA with micro size can also increase the osteogenic and cementogenic ability because it can help the attachment, spread, and proliferation of cells.[35] The process of making HA by thermal decomposition produces HA in the form of rods.[10]

Energy dispersive X-ray is one of the most frequently used tests to analyze materials. This test is very easy to do because this test kit is an addition to the SEM machine and can be done in a matter of minutes.[36] X-rays have a specific energy response for each element. This can happen because the higher the atomic number, the stronger the emission of energy from the X-rays.[37] In this study, EDX was used to determine the elemental content in HA powder.

The EDX test results from human alveolar bone showed that human bone contains elements of calcium, phosphorus, and oxygen which are the main constituents of HA. There are also trace elements of iron, copper, zinc, bromium, strontium, Mg, lead, and Na which are found in human bones in small amounts. The results of the EDX test of BHA indicate that BHA contains elements of Ca, P, and O which are the major components. Also found, elements of Mg and Na as minor components. This is in accordance with the research of Maidaniuc et al. also found elements of Ca, P, O, Mg, and Na in BHA.[38] The similarity of the elemental content of alveolar bone and BHA makes this material can be used as a bone replacement material.

In this study, HA was produced with a Ca/P ratio of 2.09. According to research by Jaber et al., the lower the thermal temperature used, the higher the Ca/P ratio.[39] The Ca/P ratio in human bone is 1.67, if the Ca/P ratio of an HA is below 1.5, it produces a biphasic polarized HA material, namely β-tricalcium phosphate. The higher the Ca/P ratio, the purer the resulting HA. The presence of Na content in BHA can increase biocompatibility and osteointegration.[40]

X-ray diffraction analysis is an analysis that uses the degree of XRD to see the characteristics of a crystal.[41] XRD analysis can also be used to identify the structure, crystal size, elements, lattice parameters, and degree of crystallization through the diffraction peaks that appear.[12] Therefore, XRD is a technique that can analyze the crystalline phase of various materials such as HA.[41]

After analyzing the XRD peaks on HA which was carried out with the X'Pert highscore program, HA bovine according to the mineral reference HA $Ca_{10}(PO_4)_6(OH)_2$ from The International Centre for Diffraction Data, Powder Diffraction File™.[42] Calculation of the degree of crystallinity showed that the resulting HA had a moderate degree of crystallinity, which was 66% which indicated natural HA.[41] HA with a moderate degree could increase the proliferation of osteogenic cells and can release particles from HA crystals in the surrounding tissue.[44,45] BHA has a moderate degree of crystallinity and micro size that can help bone healing because HA is not easily absorbed.[43,46]

Hydroxyapatite in powder form is easier and more useful for dentistry. Also provides a repeatable quality in clinical use.[47] HA powder can be used for a variety of applications. HA powder can be applied as bone filler in the repair of bone defects, fixation of implants, and bone grafts.[48] BHA is a bioactive material that has osseointegration, osteoinduction, and osteogenesis properties.[49] The bioactive properties of BHA can stimulate osteoblast cells to form osteoid, which will accelerate osteocyte formation.[22] However, HA has limitations, namely its slow resorption so it cannot be absorbed properly. The osteoconductive nature of HA makes it difficult for resorption. The remaining unresorbed HA can have a negative effect on the mechanical properties of the regenerated bone.[5] Currently, there's no literature about contraindications for BHA in clinical usage.[50]

This study on BHA is currently limited to SEM-EDX and XRD characterization tests. In the future, it's necessary to run other supportive tests, such as cytotoxicity tests and analyze them to observe BHA biocompatibility as socket preservation material. After the cytotoxicity test, clinical tests on BHA will be done on the animal subject and then on human subjects.

Conclusion

Hydroxyapatite can be produced naturally from bovine bone. The results of the characterization analysis using SEM-EDX and XRD showed that this material has a crystalline structure and elements that can replace human alveolar bone. Therefore, this material has the potential to replace the lost bone structure or as socket preservation after tooth extraction.

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Reply to the reviewers' comments

Dear Editor of World Journal of Dentistry,

Thank you for your kindness in providing invaluable input to our manuscript. We also thank you for the opportunity to improve the writing of our manuscript. We really hope that our manuscript will be accepted.

Best Regards,
Authors

Reviewer Number	Comments of the reviewer	Reply by the author(s)	Changes done on page number and line number
Reviewer 1	1.Title of manuscript need revision	Title have been changed to: "Characterization of Fabricated Bovine Hydroxyapatite Crystal as Socket Preservation Material: A SEM-EDX and X-Ray Diffraction study"	Page no.-1 Line number 1

Reviewer 1	2. Graphs: fig 3 and 5 can be represented as graph 1 and 2 instead. Kindly revise	Corrections have been done accordingly	Page no.-18&19 Paragraph Line number 464&468
Reviewer 1	3. Kindly revise the running title as Characterization of fabricated bovine hydroxyapatite	Corrections have been done accordingly	Page no.- Paragraph Line number
Reviewer 1	4. Kindly note that all the abbreviations have to be mentioned in full form once prior to their usage in the manuscript	Corrections have been done accordingly	Page no.- Paragraph Line number
Reviewer 1	5. It is mentioned that- The bone is then placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000 ºC for 1 hour, and dried..Kindly narrate abstract methodology in past tense	We already revised it to: ”The bone placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000 ºC for 1 hour, and dried”	Page no.-1 Paragraph 1 Line number 9
Reviewer 1	6. It is mentioned that- extraction has the potential maintain alveolar bone quality and prevent dimension loss… kindly revise	We already revised it to: “The addition of BHA after tooth extraction has the potential to maintain alveolar bone quality and prevent dimension loss”	Page no.-1 Paragraph 1 Line number 24

Reviewer 1	7. Kindly structure the last paragraph of the introduction	Corrections have been done accordingly	Page no.-2 Paragraph 3 Line number 53-65
Reviewer 1	8. Methodology needs to be narrated in past tense. kindly verify and revise: This research has been granted ethical approval from the Faculty of Dentistry, Airlangga University	We already revised it to: "Airlangga University Faculty of dentistry Surabaya approved this research with ethical clearance No: 360/HRECC.FODM/VII/2021"	Page no.-3 Paragraph 1 Line number 67
Reviewer 1	9. Methodology needs to be narrated in past tense. kindly verify and revise: Fabrication of HA is carried out at the Tissue Bank of RSUD Dr. Soetomo Surabaya	We already revised it to: "This research fabricated HA at the Tissue Bank of RSUD Dr. Soetomo Surabaya"	Page no.-3 Paragraph 1 Line number 68
Reviewer 1	10. Methodology needs to be narrated in past tense. kindly verify and revise: The bone is then placed inside an ultrasonic cleaner with a temperature of 60°C so that	We already revised it to: "The bone was cleaned with an ultrasonic cleaner at a temperature of 60°C so that the fat would melt (Figure 1c)."	Page no.-3 Paragraph 1 Line number 73

Reviewer 1	11. Methodology needs to be narrated in past tense. kindly verify and revise: This process is then carried out until it is completely clean of fat and the color is white.	We already revised it to: “Ultrasonic cleaning is repeated until the bone is completely clean of fat and the color becomes white.”	Page no.-3 Paragraph 1 Line number 75
	12. Methodology needs to be narrated in past tense. kindly verify and revise: The prepared sample is then put into the chamber	We already revised it to: “Prepared sample inserted in the chamber and vacuumed in high vacuum mode with a 15 kV SEI detector”	Page no.-4 Paragraph 1 Line number 91

Characterization of Fabricated Bovine Hydroxyapatite for Socket Preservation

ABSTRACT

Aim: This research aimed to fabricate and characterize bovine hydroxyapatite (BHA). Hydroxyapatite (HA) crystal of BHA characterized using Scanning Electron Microscopy-Electron Dispersive X-Ray (SEM-EDX) and X-Ray Diffraction (XRD) test for its potential as socket preservation material.

Materials and Method: BHA was fabricated from bovine cancellous bone that was washed and cut into small pieces. The bone is placed in an ultrasonic cleaner to remove the fat, heated in the oven at 1000 ºC for 1 hour, and dried. The bone was then grounded into a powder and sieved through 150 µm sieves. Powdered BHA was then analyzed with SEM-EDX and XRD tests. SEM-EDX test was used to analyze the structure and content of HA element within BHA and the XRD test was used to analyze HA crystal within BHA.

Results: From the SEM test, BHA had a crystalline particle with hexagonal crystal with an average size of 197 µm. The EDX test indicated BHA has the elements of C (50.71%), O (34.59%), Na (0.35 %), Mg (0.35%), P (4.29%), Ca (8.97%), Nb (0.74%). The XRD test showed that BHA contains Hydroxyapatite (HA) crystal with 66% crystallinity degree.

Conclusion: The BHA was successfully fabricated through the process of calcination. Based on the characterization and analysis using SEM-EDX and XRD tests of BHA, it had the potential as socket preservation material to preserve alveolar bone after tooth extraction.

Clinical significance: The addition of BHA after a tooth extraction is expected to play a role in maintaining alveolar bone quality and preventing dimension loss.

Keywords: Bovine hydroxyapatite, biomaterial, socket preservation.

INTRODUCTION

Tooth extraction is the second most frequently performed dental procedure in Indonesia, covering 7.9% of all dental procedures.[1] After tooth extraction, the dimensions of the alveolar bone will decrease by 30% due to resorption.[2,3] Socket preservation was carried out to maintain the dimensions of the post-extraction alveolar bone socket. Socket preservation material in the form of bone graft could come from nature or synthetic materials.[4] Natural socket preservation materials are easier to obtain and do not require complex or expensive processing to be fabricated.[5] Natural grafts can be in the form of autografts from the patient's body, allografts from the same species, and xenografts.

Human bone is composed of inorganic components as much as 70% HA crystals and organic components as much as 30% type 1 collagen and other organic molecules.[5] Alveolar bone consists of 67% inorganic material and 33% organic material.[6] HA ($Ca_{10}(PO_4)_6(OH)_2$) is the main inorganic compound that composes various hard tissues such as bones, teeth, dentin, and others. Currently, the demand for HA as a bone graft is very high. Due to the limited supply of HA autograft and allograft, HA from xenograft was developed.[7] Xenograft is a graft taken from a different species from the recipient.[8] There are two categories of HA xenografts that are often used, namely synthetic and natural. One of the natural sources of this material is bovine bone.[9] In the field of dentistry, several commercial products use HA as a bone graft.[5] The price of commercial HA is quite expensive when compared to the fabrication price of HA from bovine bone. One method of fabricating HA from bovine is the calcination method. The calcination method with thermal decomposition is easy to do and can produce HA in the form of nanorods.[10]

The purpose of this study was to fabricate hydroxyapatite derived from bovine bone and observe the characterization of this material with a Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), and X-Ray Diffraction (XRD) to analyze the potential of this material as a substitute for alveolar bone for socket preservation. SEM is a tool that provides very accurate microscopic images of material. SEM could analyze organic and inorganic material on a nanometer to micrometer scale. EDX test could be done while doing SEM analysis. EDX test measures the x-ray pattern emitted by the material to determine the chemical composition and quantity.[11] XRD is a test for analyzing crystal structure, size, component, parameter, and degree of crystallinity by using an x-

ray beam.[12] XRD works by measuring diffracted x-ray angles emitted from the material to the detector.[13]

MATERIALS AND METHOD

This research has been granted ethical approval from the Faculty of Dentistry, Airlangga University, Surabaya with No: 360/HRECC.FODM/VII/2021. Fabrication of HA is carried out at the Tissue Bank of RSUD Dr. Soetomo Surabaya. Bovine bones used in this study were derived from female cow bones from farms in Malang, East Java. At first, the bovine bones are cleaned and washed from any blood clots. Then, the part of the cancellous bone that contains a lot of sponges was cut into small pieces as needed (Figure 1a). Washing was carried out in detail using H_2O_2 (Figure 1b). The bone is then placed inside an ultrasonic cleaner with a temperature of 60°C so that the fat melts (Figure 1c). This process is carried out until it is completely clean of fat and the color is white. Washing was carried out again with distilled water to remove H_2O_2 until it was clean (Figure 1d). The bones were then dried at room temperature to minimize moisture content (figure 1e). Next, the bone was heated in the furnace at a temperature of 1000 °C for 1 hour (Figure 1f). After the heating process, the bone was rinsed with distilled water 3 to 4 times to remove the toxic properties (Figure 1g). Then dried again in an oven at a temperature of 60°C-100°C until dry (figure 1h). After dried completely, the bone was ground with a bone miller until particles were formed (Figure 1i). The fine and coarse particles were separated by a sieving machine with a size of 150 µm (Figure 1j).

Specimen Characterization

After the fabrication of hydroxyapatite powder from bovine bone, then it was characterized by Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) and X-Ray diffraction (XRD).

SEM-EDX test. SEM testing was carried out at FMIPA ITB Bandung (JEOL, JSM 6510-LA). A total of 0.1 g of BHA material was used in this analysis. The sample was then coated with gold (fine coater JFC 1600) before testing. The prepared sample is then put into the chamber. The sample was then vacuumed in high vacuum mode with a 15 kV SEI detector. Magnification was set to 10000x and 20000x, after which the focus X stigma and Y stigma were adjusted to refine the image. Then the surface morphology of the pores, size of the pores, and the shape of the pores were analyzed.

EDX test. The samples in the SEM chamber were analyzed and mapped the chemical elements of the material using Energy-dispersive X-ray spectroscopy (EDX) (JEOL, JSM 6510-LA). 10 µm magnification settings at 10 mm spacing. The results of the chemical element analysis are displayed in the form of graphs and color gradation maps

XRD Test. XRD testing was carried out at the National Research and Innovation Agency (BRIN) for Physics Serpong. A total of 1 g of BHA powder was used in this study. The Bruker D8 XRD machine (Germany) with cobalt cathode is used with a scan range of 10-90^{O} with a speed of 0.1 seconds per step**.**

RESULTS

In this study, the calcination method with thermal decomposition was used to extract hydroxyapatite from bovine bone to remove organic material. The bovine bone powder is heated in a furnace to a temperature of 1000 °C for 1 hour until the organic matter evaporates.

SEM-EDX test results

Figure 2 is an SEM image of BHA at 10000x(a) and 20000x(b) magnification. In this study Image J software was used on SEM images with a magnification of 20000x to

measure the size of hydroxyapatite crystals. The picture shows an irregular hexagonal crystal structure with an average size of 1.97 m with the smallest size of 0.144 µm and the largest size of 4.494 µm.

Figures 3 and 4 are the results of BHA analysis with EDX. These results indicate the elements contained in BHA. In the picture, BHA shows the presence of the elements Carbon (50.71%), Oxygen (34.59%), Sodium (0.35%), Magnesium (0.35%), Phosphorus (4.29%), Calcium (8.97%), Niobium (0.74%).

XRD test results

The analysis of HA peaks and measurements of the degree of crystallinity were carried out using the Origin 2019b program. In the results of the HA analysis, there is a sharp peak at 30.2°; 33.8°; 37.1°; 37.6°; 38.4°; 39.8°; 46.6°; 54.8°; 56.5°; 58.2°; 59.4°; 60.4°; 61.3°; and 62.7° 2θ. The measurement of crystallinity degree was carried out with $\frac{\text{crystalline area}}{\text{amorph area + crystalline area}} \times 100\%$. The degree of crystallinity in this HA sample is 66% which is HA crystal with a moderate degree of crystallinity. The X'Pert Highscore program was used to calculate the crystal size by Scherrer's calculations. The crystal size of HA in the HA sample was 1087 nm.

DISCUSSION

Tooth extraction will cause alveolar bone resorption which could lead to dimension loss.[14] Using graft material HA after extraction could help maintain alveolar bone height. Commercial natural HA is very expensive and hard to get for post-extraction use. This study is aimed to create a cheaper and more readily available HA from bovine bone and analyze it using SEM-EDX and XRD. SEM-EDX test is to show the microscopic size and chemical component of HA crystal. XRD is used to characterize HA crystal.

Alveolar bone resorption can be divided into 2 stages. The first stage of this process is bone resorption which causes a decrease in bone dimensions. The second stage in this process is the remodeling of the buccal bone and woven bone which will lead to more reduction in the dimensions of the alveolar bone. Alveolar bone disuse atrophy decreased blood supply, and local inflammation also plays a role in bone resorption.[15] Alveolar bone resorption complicates even the failure of placement of restorations such as implants. Implant placement requires ideal alveolar bone volume to achieve an adequate implant-to-bone ratio.[16] Bone quality is also an important factor in the placement of implant restorations.[17] Socket preservation material could come in many forms such as HA powder from many sources. HA could come from allograft, xenograft, and alloplast. Allograft materials come from the same species but from a different individual. Allograft HA could come from frozen bone, freeze-dried bone, demineralized freeze-dried bone, and deproteinized bone. Xenograft material comes from a natural origin but from a different species from the recipient. These include animal bone and coral. Alloplast is a graft that comes from a synthetic origin. Alloplast graft is made from calcium phosphates, glass ceramics, and polymers.[18,19]

Bovine bone has a morphology and structure that is similar to human bone. HA from bovine bone also has the same chemical composition as human bone.[20] Bovine bone also has the main mineral of human bone, namely HA.[21] HA extracted from bovine bone by thermal decomposition has good biocompatibility so that it can decompose naturally, can be absorbed naturally, and can be integrated with the bone. BHA is also more available, not limited to religious beliefs, and more economic.[5] HA also has mechanical properties that are almost the same as human bone. This material has an important role in socket preservation.[22,23]

Beef consumption in Indonesia is quite high. In 2020, 1,276,473 beef cattle have been slaughtered.[24] If beef bones cover 25 percent of the beef carcass weight and Indonesia produces 504,802 tons of beef, the beef bones produced are 168,267 tons.[25,26] So bovine bone waste produced in Indonesia is quite a lot, but the processing is not optimal which makes bovine bones have a low selling value. To increase the selling value of bovine bones, processing can be done, one of which is extracting Hydroxyapatite (HA) from the bones.

Bovine bones contain 70% Hydroxyapatite which is similar to Hydroxyapatite in human bones. The content of HA in bovine bones can make it a good candidate for socket preservation material.[26] HA from bovine bone has long been developed so that it has an affordable price and better quality. Bovine bone is considered as an organic waste. Bovine bones can be recycled and reused to increase their resale value. One way to increase the market value of bovine bone is to make it a source of HA for graft biomaterials in dental practice.[22] This research tried to process bovine cancellous bone and fabricate biomaterials that are useful as bone replacement materials or to maintain bone tissue after tooth extraction.

In this study hydroxyapatite biomaterial derived from bovine, bone was made by calcination process with thermal decomposition. Ramirez-Gutierrez.C et.al (2016) successfully created pure HA without any organic compound from the bovine bone using calcination through thermal degradation at 900 ◦C.[27] The bovine bone powder in this research is heated at 1000 ◦C for 1 hour.[28] This was done to remove other organic matter that will evaporate at a temperature of 350 ºC.[29] HA also has stability up to a temperature of 1200 ºC.[30] The advantage of the calcination method is that it can remove all organic components and all disease genomes. This provides safety when used as a graft material.[28]

The bone used in this study was taken from the cancellous bone. Cancellous bone is a porous bone that is easily powdered and calcined. The porosity of the graft material is required for bone regeneration. The size and amount of porosity of the graft material can affect the osteoconductive properties, biodegradation, connection, migration, adhesion, proliferation, and differentiation of cells.[31,32] The higher the porosity of the graft

material, the easier it is for growth factors to penetrate the material and increase vascularity and angiogenesis.[31]

SEM is a type of electron microscope that images the surface of a sample by scanning it with an electron beam. The electrons that interact with the atoms that make up the sample produce a signal that contains information about the sample from the surface topography, morphology, and composition of a material. SEM can detect and analyze surface fractures, provide microstructural information, view surface contamination, qualitative chemical analysis, and identification of crystalline structures.[33]

The results of the SEM test show that the HA crystal structure is hexagonal, which is in accordance with the research by Ruksudrajit.A.et al 2008 which examined HA from bovine extracted using a thermal calcination process.[34] The results indicate an average HA crystal size of 1.97µm. The crystal size of these HA sample crystals is within the micro size (2-3 m). Micro-sized crystals can help stem cell differentiation and matrix synthesis, stimulate cell proliferation, chondrogenic gene expression, and matrix production.[35]. HA with micro size can also increase the osteogenic and cementogenic ability because it can help the attachment, spread, and proliferation of cells.[36] The process of making HA by thermal decomposition produces HA in the form of rods.[10]

EDX is one of the most frequently used tests to analyze materials. This test is very easy to do because this test kit is an addition to the SEM machine and can be done in a matter of minutes.[37] X-rays have a specific energy response for each element. This can happen because the higher the atomic number the stronger the emission of energy from the X-rays.[38] In this study, EDX was used to determine the elemental content in HA powder.

The EDX test results from human alveolar bone showed that human bone contains elements of calcium, phosphorus, and oxygen which are the main constituents of HA. There are also trace elements of iron, copper, zinc, Bromium, strontium, magnesium lead, and sodium which are found in human bones in small amounts. The results of the EDX test of BHA indicate that BHA contains elements of calcium, phosphorus, and oxygen which are the major components. Also found elements of magnesium and sodium as

minor components. This is in accordance with the research of Maidaniuc.A et al 2018 also found elements of calcium phosphorus, oxygen, magnesium, and sodium in BHA.[39] The similarity of the elemental content of alveolar bone and BHA makes this material can be used as a bone replacement material.

In this study, HA was produced with a Ca/P ratio of 2.09. According to research by Jaber.H et al 20193, the lower the thermal temperature used, the higher the Ca/P ratio.[40] The Ca/P ratio in human bone is 1.67, if the Ca/P ratio of an HA is below 1.5, it produces a biphasic HAp material, namely β -TCP. The higher the Ca/P ratio, the purer the resulting HA. The presence of Na content in BHA can increase biocompatibility and osteointegration.[41]

XRD analysis is an analysis that uses the degree of X-ray diffraction to see the characteristics of a crystal.[42] XRD analysis can also be used to identify the structure, crystal size, elements, lattice parameters, and degree of crystallization through the diffraction peaks that appear.[12] Therefore XRD is a technique that can analyze the crystalline phase of various materials such as HA.[42]

After analyzing the x-ray diffraction peaks on HA which was carried out with the X'Pert Highscore program, HA bovine according to the mineral reference HA $Ca_{10}(PO_4)_6(OH)_2$ from *The International Centre for Diffraction Data (ICDD®) Powder Diffraction File™ (PDF®)*.[43] Calculation of the degree of crystallinity showed that the resulting HA had a moderate degree of crystallinity, which was 66% which indicated natural HA.[44] HA with a moderate degree can increase the proliferation of osteogenic cells and can release particles from HA crystals in the surrounding tissue.[45,46] BHA has a moderate degree of crystallinity and micro size that can help bone healing because HA is not easily absorbed.[44,47]

HA in powder form is easier and more useful for dentistry. Also provides a repeatable quality in clinical use.[48] HA powder can be used for a variety of applications. HA powder can be applied as bone filler in the repair of bone defects, fixation of implants, and bone grafts.[49] BHA is a bioactive material that has osseointegration, osteoinduction, and osteogenesis properties.[50] The bioactive properties of BHA can stimulate osteoblast cells to form osteoid which will accelerate osteocyte formation.[23] However, HA has

limitations, namely its slow resorption so it cannot be absorbed properly. The osteoconductive nature of HA makes it difficult for resorption. The remaining unresorbed HA can have a negative effect on the mechanical properties of the regenerated bone.[5] Currently there's no literature about contraindication for BHA in clinical usage[51]

This study on BHA is currently limited to SEM-EDX and XRD characterization tests. In the future, it's necessary to run other supportive tests such as cytotoxicity tests and analyze them to observe BHA biocompatibility as socket preservation material. After the cytotoxicity test, clinical tests on BHA will be done on the animal subject and then on human subjects

CONCLUSION

Hydroxyapatite can be produced naturally from bovine bone. The results of the characterization analysis using SEM-EDX and XRD showed that this material has a crystalline structure and elements that can replace human alveolar bone. Therefore, this material has the potential to replace the lost bone structure or as socket preservation after tooth extraction.

CONFLICT OF INTEREST

No conflict of interest

ACKNOWLEDGMENT

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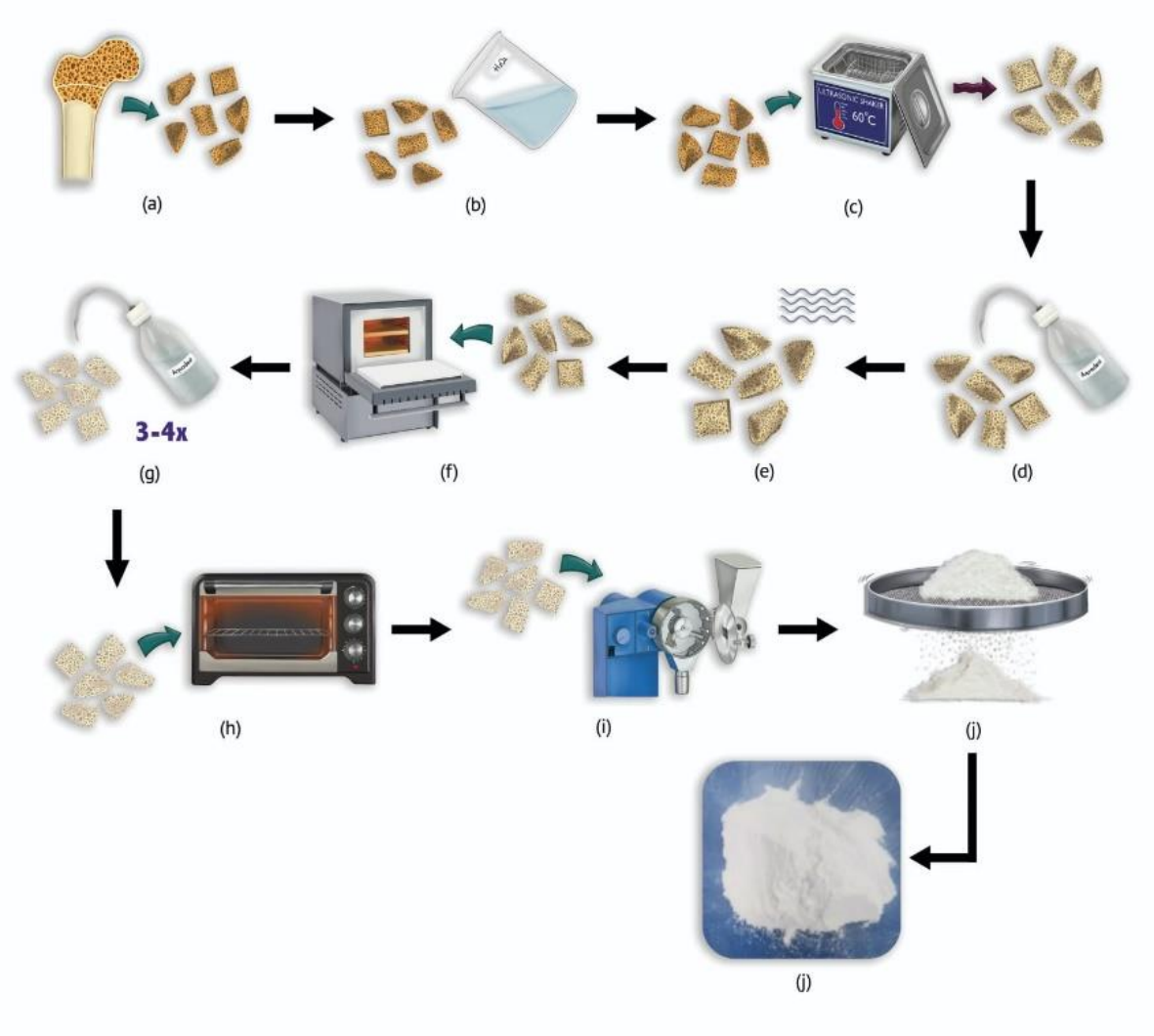

Figure 1: Process of fabrication HA from bovine bone (a) cutting of cancellous bovine bone, (b) washing with H_2O_2, (c) fat removal, (d) washing with H_2O_2 (e) drying at room

temperature (f) burning in the oven, (g) washing with distilled water, (h) bone drying, (i) bone grinding (j) hydroxyapatite powder

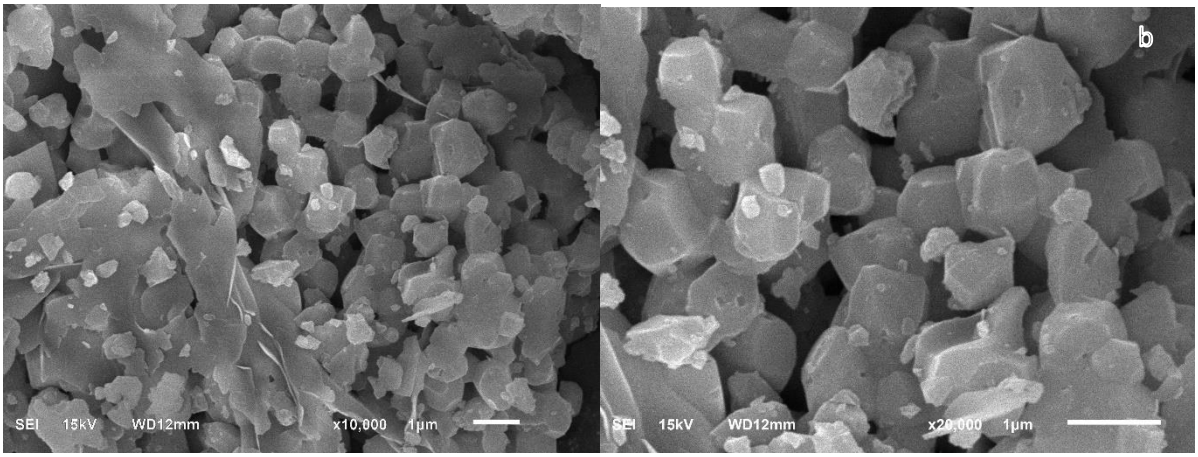

Figure 2: SEM images (a) BHA at 10000x magnification and (b) BHA at 20000x magnification

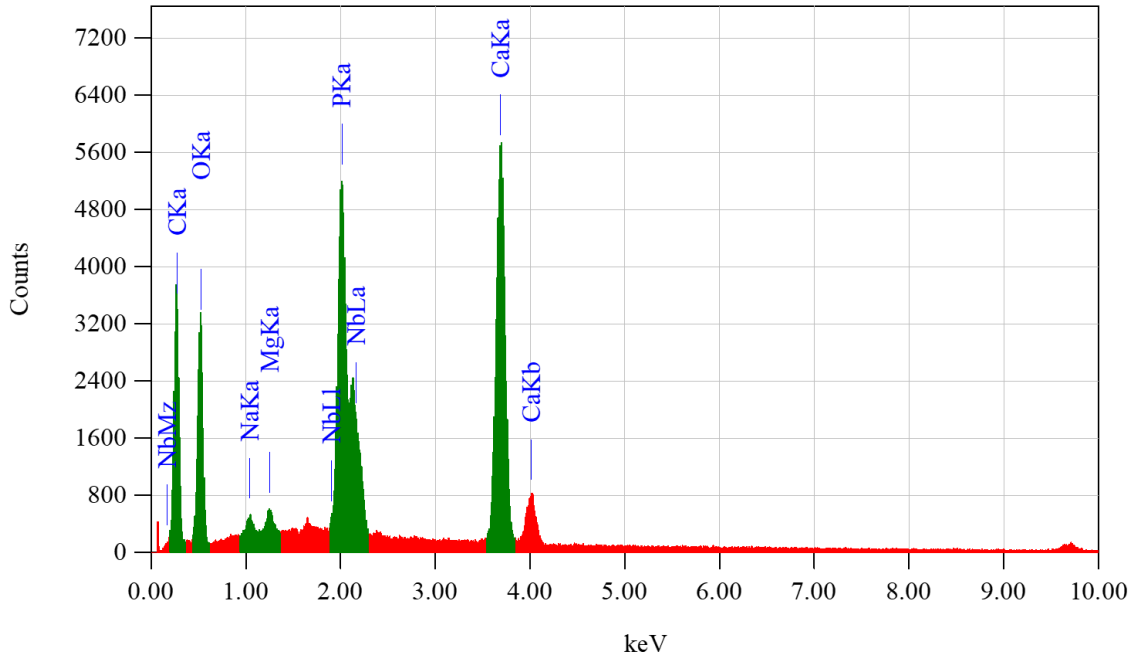

Figure 3: EDX graph test results from BHA

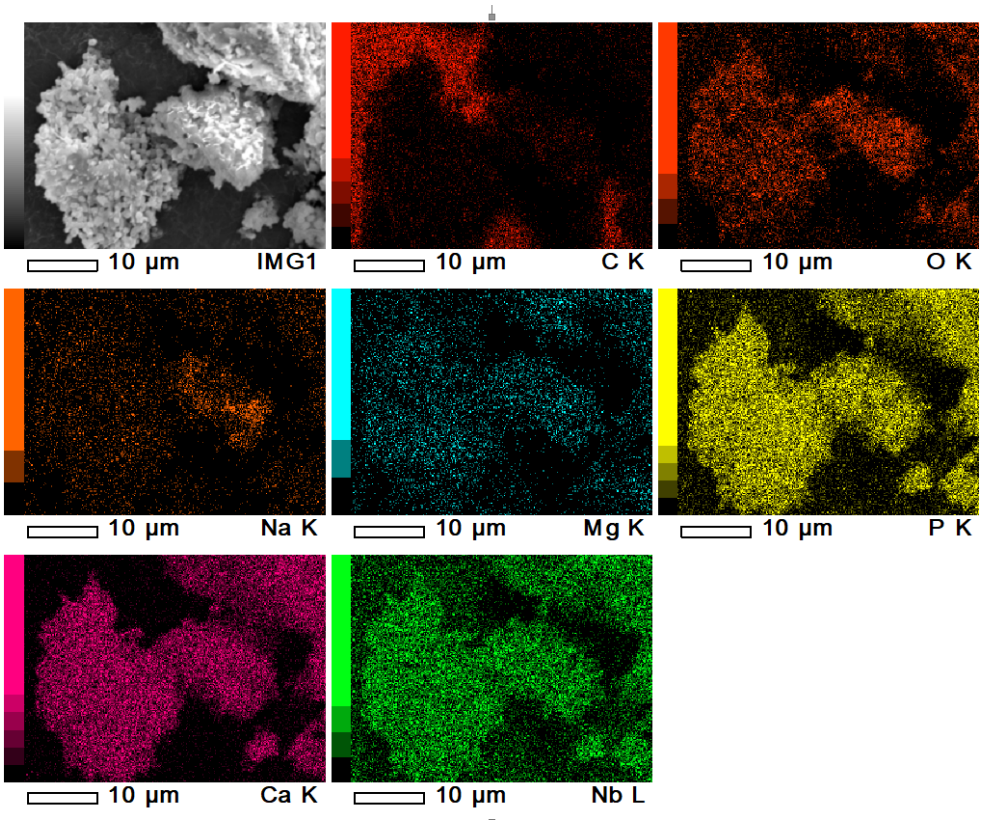

Figure 4: EDX images of element mapping from BHA

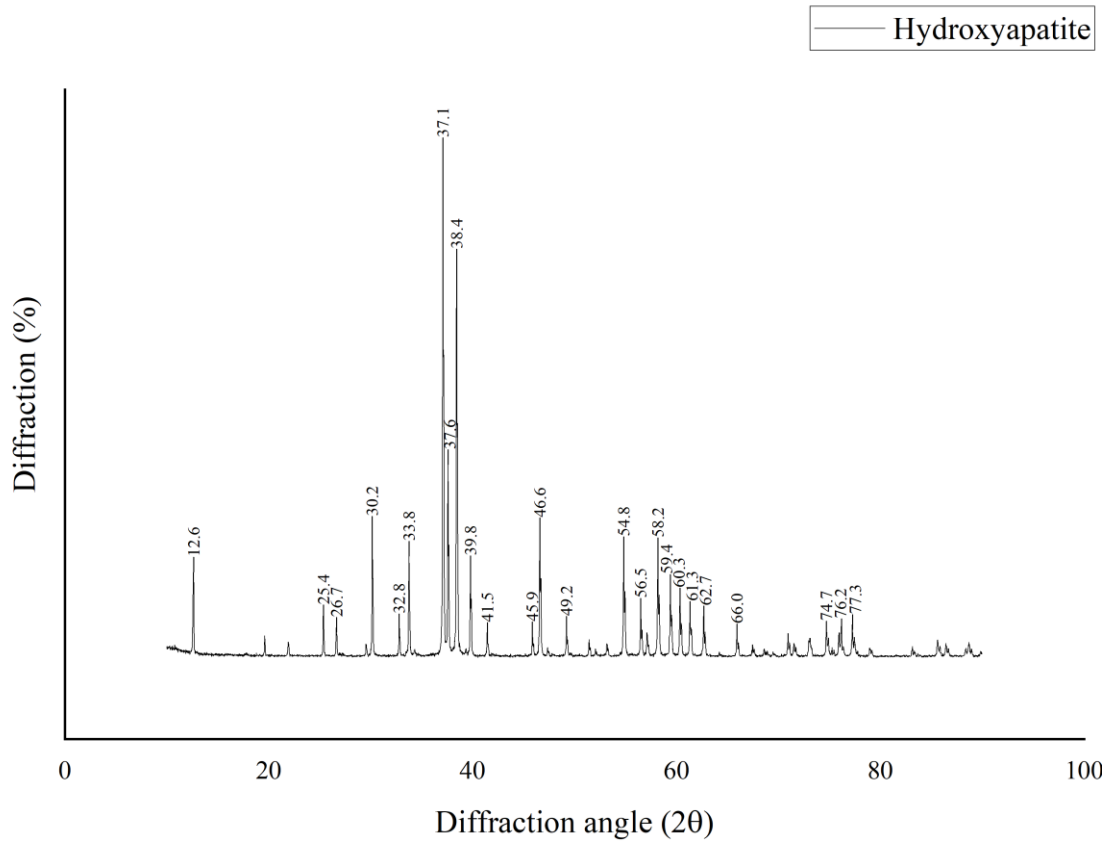

Figure 5: XRD graph from BHA