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Characterization of Fresh Bovine Amnion Membrane Combined with Hydroxyapatite as Candidate Scaffold for Alveolar Bone Tissue Engineering

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ORIGINAL ARTICLE

Characterization of Fresh Bovine Amnion Membrane Combined with Hydroxyapatite as Candidate Scaffold for Alveolar Bone Tissue Engineering

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ABSTRACT

Objective: This research aims to analyze the characteristics of a BAM-HA biocomposite with ratios of 4:1 and 4:2. **Methods**: This research is an in vitro laboratory experiment that starts by grinding fresh BAM to produce amnion slurry and then, adding HA powder to the slurry and carrying out freeze-drying on the slurry. Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope-Energy dispersive X-ray (SEM-EDX) tests are used to analyze the characteristics of the BAM-HA biocomposite. **Results**: The FTIR test results showed that the BAM-HA biocomposite had amide functional groups I, II, III, A, B, OH, CO_3^{2-}, and PO_4^{3-}. SEM test results showed revealed different types of pores on the surface of the biocomposite with ratios of 4:1 and 4:2. The elements C, O, Na, Mg, Si, P, CL, Ca, and Nb were found in the BAM-HA biocomposite following testing by SEM-EDX. **Conclusion**: Based on the research results, this research has succeeded in combining BAM-HA. The ratio BAM-HA 4:2 has the potential as a scaffold for alveolar bone tissue.

Keywords: bovine amnion, characterization, hydroxyapatite, scaffold

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INTRODUCTION

Oral and maxillofacial surgery procedures often use alveolar bone engineering techniques to achieve effective clinical results.[1] Scaffolding is widely used in tissue engineering techniques. A scaffold formed as a three-dimensional structure can be implemented as a medium to support the growth of new tissues.[2] Scaffolds made from raw biomaterial are used to increase the growth of cells that continue the function of replaced tissue.[3] One biomaterial that can be used in making scaffolds is bovine amniotic membrane (BAM). BAM is a biomaterial located in the deepest part of the placenta. It surrounds the embryo with a thickness of 0.02 to 0.5 mm.[4] BAM is the same as the human amniotic membrane; the placenta covers the embryo. This membrane is a thin, strong, translucent, and avascular layer. BAM and the human amniotic membrane have similar physical and biological properties.[5] BAM contains carbonates (CO_3^{2-}), sodium (Na^+), magnesium (Mg^{2+}), iron (Fe^{2+}), fluoride (F^-), silicon (Si), and chloride (Cl^-).[6] BAM also has several materials for regeneration, such as growth factors, collagen, and antibacterial characteristics.[7,8] In addition, BAM has been shown to have the ability to accelerate the epithelialization process, having anti-inflammatory, antiangiogenic, and analgesic effects.[9] BAM is, therefore, useful as a biomaterial in regenerative medicine.[9]

While BAM offers significant potential for expediting bone regeneration, it also possesses inherent weaknesses. One notable limitation is its susceptibility to rapid degradation, leading to difficulties in maintaining the structural integrity needed for effective bone regeneration. This accelerated degradation is primarily attributed to the high collagen content within the membrane, which facilitates rapid absorption. Because of this degradation, bone healing is predominantly only effective in the marginal area of the bone being regenerated.[9,10] The limitation of bovine amnion membranes can be optimized by incorporating hydroxyapatite (HA) material. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the primary type of mineral that composes bones and teeth. HA is synthesized from cow bones, which have almost the same composition as human bones.[11] This material is biocompatible and osteoconductive.[10] In medical applications, HA is widely used in bone grafting, bone repair, and replacement of damaged bone.[12] However, biomaterial characterization tests are needed to prove the biological function of BAM and HA biocomposites as candidates for scaffold in regenerating alveolar bone.

This research draws on the results of previous research on the use of BAM and HA as tissue regeneration biocomposites. A previous study carried out by Munadziroh et al. found that BAM can be used for socket regeneration following tooth extraction by stimulating the proliferation and formation of new blood vessel fibroblasts in sockets.[7] Furthermore, HA has been used to accelerate the repair of bone damage by accelerating tissue vascularization without disrupting the process of remodeling bone.[12,13] However, previous research has been limited to the use of dry bovine amniotic membranes, so further research to combine HA with fresh amniotic membranes is needed.

Based on this background, this research aims to present a characterization of fresh BAM and HA biocomposite fabrication in the structure of a sponge. BAM-HA biocomposites are made in ratios of 4:1 and 4:2. The ratios of 4:1 and 4:2 were created in this research to improve on the properties of the materials used in previous research, which had ratios that could not be used to regenerate alveolar bone healing optimally compared to commercial xenograft products.[14] Therefore, the HA ratio was increased in this study, and a fresh amniotic membrane was used. Because HA is one of the most essential materials that forms alveolar bone. In addition, this research uses a fresh amniotic membrane because it has a greater growth factor when compared to dried BAM.[15] Characterization was carried out by functional group testing Fourier transform infrared (FTIR), morphological structure test via scanning electron microscope (SEM), and through element test energy-dispersive x-ray spectroscopy (EDX).

METHODS

The research was conducted at the Biomaterial Bank Tissue Center Installation Dr Soetomo Regional General Hospital (RSUD) Surabaya with ethics approval number 360/HRECC.FODM/VII/2021.

Fabrication of Biocomposite BAM-HA

Biocomposite fabrication begins with taking BAM from the deepest part of the cow's placenta. This collection is carried out without drying so that the membrane has a softer texture and higher immunogenicity.[16,17] The BAM should be cleaned until it is free from blood clots. In this study. the amniotic membrane was cleaned by washing it with a 0.05% saline solution four times over 10 minutes. The amniotic membrane was washed again using a distilled water solution until the saline solution was clear. Fresh cow amnion membranes were cut, and sodium chloride (NaCl) solution was added at a ratio of 1:1.[4] A blender was then used to produce amnion slurry.

The amnion slurry was extracted and combined with HA with ratios of 4:1 and 4:2. The amnion slurry and HA powder mixture was stirred until homogeneous and then put into a container with a diameter of 10 cm. The homogenized mixture was put in the freezer at a temperature of -80 ºC for 1x24 hours. The next step was freeze-drying for 2x24 hours with a temperature of -100 ºC until a sponge-shaped BAM and HA biocomposite was obtained.

Functional Group Test (FTIR)

Functional group testing (FTIR) begins with sample preparation. Fresh bovine amnion membrane-hydroxyapatite (BAM-HA) biocomposite samples were mixed with potassium bromide (KBr) in a ratio of 1:4. The mixture was ground until homogeneous and then put into a container with a diameter of 4 mm. Using an IR Prestidge-21 (Shimadzu, Japan), a homogeneous mixture of samples and KBr was measured using the diffuse reflectance Fourier transform infrared spectroscopy (DRSFTIR) method. The measurement range was 400–4000 cm^{-1}, the resolution was 2.0 cm-1, the sample was scanned as many as 20 times, and the value will appear as a transmittance percentage.

Scanning Electron Microscopy (SEM)

In SEM testing of morphology and structure, the BAM and HA biocomposite samples were cut to a size of 1 x 1 cm. Before testing, the samples were first coated with gold (fine coater JFC 1600). Samples were put in a chamber and then vacuumed with a 15kV SEI detector. The magnification of the tool was set at 100x. Then, the pore samples' surface morphology, size, and shape were analyzed using ImageJ.

Energy Dispersive X-ray Spectroscopy (EDX)

On the element test, the sample in the SEM sample chamber was analyzed, and the sample's material chemical elements was mapped using energy dispersive x-ray spectroscopy (EDX). The magnification was set at 100x and 300x with a distance of 10 mm. The outcomes of the chemical element analysis are presented visually in a figure with a color gradient.

RESULTS

This research has successfully combined fresh amnion membranes with HA into a biocomposite. The following are the results of biocomposites of fresh amnion membranes and HA with ratios 4:1 and 4:2.

FTIR analysis shows that all BAM-HA biocomposites contain amide I, II, III, A, B, and OH functional groups. Meanwhile, both samples can also be seen to contain PO_4^{3-} and CO_3^{2}.

Graph in Figure 1a. showed that the BAM-HA biocomposite with a ratio of 4:1 had an amide I absorption peak at 1642 cm^{-1}, amide II at 1548 cm^{-1}, amide III at 1241 cm^{-1}, amide A at 3571 cm^{-1}, amide B at 2962 cm^{-1}, OH^- at 3445 cm^{-1}, $PO4^{3-}$ at 1084 cm^{-1}, and CO_3^{2-} at a 1478 cm^{-1} wavelength.

On the other hand, as seen in Figure 1b, the BAM-HA biocomposite with a ratio of 4:2 has an absorption peak at 1634 cm^{-1} for amide I, at 1559 cm^{-1} for amide II, at 1263 cm^{-1} for amide III, at 3566 cm^1 for amide A, at 2966 cm^{-1} for amide B, at 3446 cm^{-1} for OH^- at 1025 cm^{-1} for $PO_4^{3-,}$, and at 1489 cm^{-1} for CO_3^{2-}.

SEM Analysis

Figure 2(a) is an image of the BAM-HA biocomposite ratio 4:1 surface using SEM at 100x magnification. Figure 2(b) is a surface image of the BAM-HA biocomposite ratio of 4:2 using SEM at 100x magnification. Calculation results using ImageJ show that the number of pores in BAM-HA biocomposites 4:1 and 4:2 is almost the same, with both having around 45 pores. However, the shape and size of the pore differ. BAM-HA, with a ratio of 4:1, has a relatively larger pore size with an average of 114.28 µm when compared to BAM-HA 4:2, which has a smaller pore size with an average of 69.10 µm. The surface morphology of BAM-HA 4:2 appears denser, with HA granules appearing to bond to the amnion membrane.

EDX Analysis

Both biocomposites of fresh bovine amnion membrane-hydroxyapatite ratio demonstrated the existence of C, O, Na, Mg, Si, P, Cl, Ca, and Nb elements. However, the element percentage values differed somewhat between the two biocomposites.

The results of the EDX test on the BAM-HA biocomposite with a ratio of 4:1 can be seen in Figure 3a. As shown in this figure, the fresh bovine amnion membrane-hydroxyapatite biocomposite with a ratio of 4:1 was shown to contain carbon (C) with a percentage of 69.16%, oxygen (O) 20.81%, sodium (Na) 3.82%, magnesium (Mg) 0.18 %, silicon (Si) 0.09%, phosphorus (P) 0.41%, chlorine (Cl) 3.99%, calcium (Ca) 1.33%, and niobium (Nb) 0.21%.

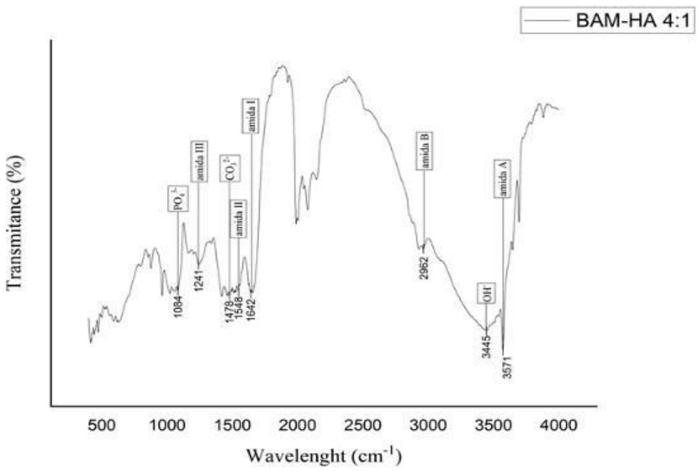

Figure 1a. Graph of FTIR analysis results of BAM-HA biocomposite with a ratio of 4:1

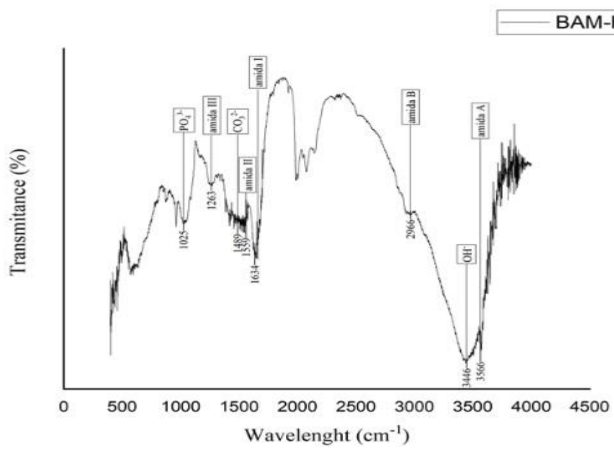

Figure 1b. Graph of FTIR analysis results of BAM-HA biocomposite with a ratio of 4:2

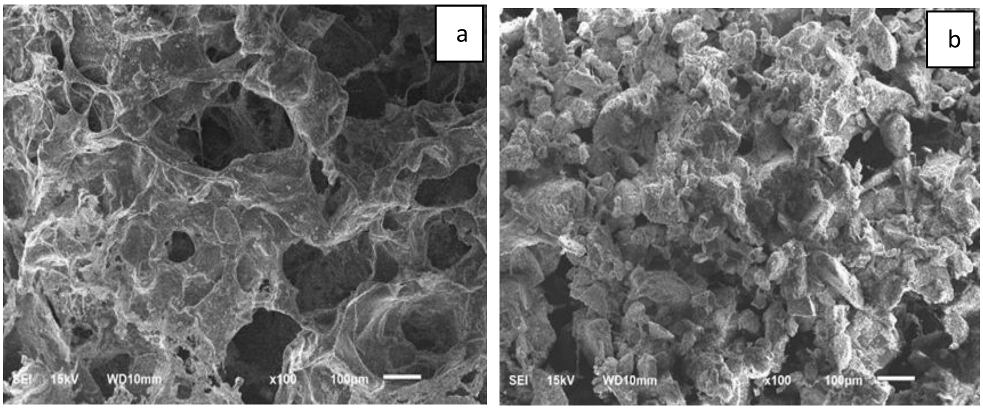

Figure 2. Analysis of surface and porosity using scanning electron microscopy (SEM) at 100x magnification (a) BAM-HA biocomposite ratio 4:1 (b) BAM-HA biocomposite ratio 4:2

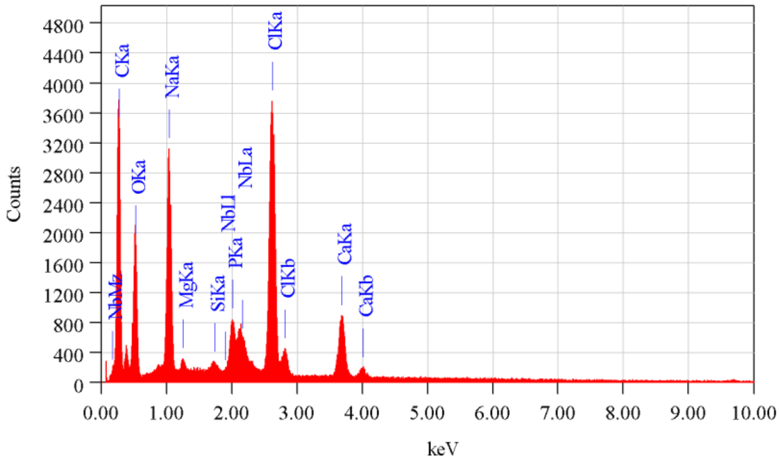

Figure 3a. Graph of EDX test results for BAM-HA biocomposite composition ratio 4:1

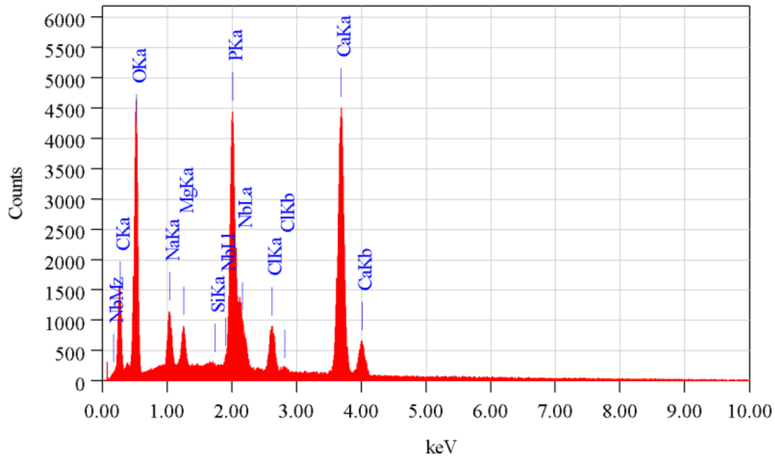

Figure 3b. Graph of EDX test results for BAM-HA biocomposite composition ratio 4:2

The results of the EDX BAM-HA test with a ratio of 4:2 are shown in Figure 3b. The graph shows the presence of the elements carbon (C) 31.29%, oxygen (O) 50.27%, sodium (Na) 3.82%, magnesium (Mg) 1.73%, silicon (Si) 0.96%, phosphorus (P) 5.20%, chlorine (Cl) 1.10%, calcium (Ca) 8.90%, and niobium (Nb) 0.54%.

BAM can be combined with hydroxyapatite (HA) biomaterial to maximize the performance of BAM in tissue regeneration. BAM has abundant growth factors and collagen with osteoinductive properties,[18,19] while HA has good osteoconductive properties as a bone substitute.[20] Biocomposite materials produced by combining collagen and HA can be used to optimize bone regeneration because they have a composition and structure similar to that of natural bone.[21] HA can be synthesized from inorganic components or natural resources, such as mammalian and marine bones, plants, and biogenic sources. In this study, HA from biological sources (natural hydroxyapatite) is extracted from the femurs and thigh bones of cows,[22] which is advantageous because cow bones morphologically and structurally resemble human bones.[23]

The application of BAM is prevalent in the field of tissue engineering, particularly in the medical domain. This is because BAM can help in tissue regeneration. In Indonesia, fresh BAM is abundant and can be obtained easily.[18] BAM contains regenerating ingredients, such as growth factors, collagen, and antibacterial properties. In this research, chemical characterization was carried out, including analysis of functional groups in the synthesized HA. The analysis used a Fourier transform infrared (FTIR) instrument to determine the functional groups of HA qualitatively based on the absorbance of infrared rays. The FTIR analysis showed all types of fresh BAM-HA biocomposites to have amide functional groups I, II, III, A, B, and OH; these functional groups show that fresh BAM and HA can bind to each other. The appearance of amides I, II, II, A, and B functional groups in the biocomposite showed that collagen was still present, not having been denatured yet. These characteristics are as expected for BAM-HA, which for HA is a major component of bone tissue and teeth. The presence of these characteristic functional groups in hydroxyapatite is consistent with the well-established chemical composition and biological significance of hydroxyapatite in tissue engineering. [22]

The presence of bonds, such as O-P-O (oxygen-phosphorus-oxygen), C-O (carbon-oxygen), and Ca-O (calcium-oxygen), indicates the presence of HA in the sample. FTIR test results show that collagen content is based on amide functional groups, which have bonds such as O-H (oxygen-hydrogen), N-H (nitrogen-hydrogen), C-H (carbon-hydrogen), and N-H (nitrogen-hydrogen). The BAM-HA biocomposite samples also showed the presence of PO_4^{3-} and CO_3^{2-}. The presence of phosphate and carbonate groups suggests that hydroxyapatite was well-bound or present in the BAM sample during fabrication. In addition, the amide and hydroxyapatite functional groups in the biocomposite indicate that the combination of fresh BAM-HA created in this study successfully bonds with each other. [10]

The formation and penetration of tissue in a biomaterial can be affected by the porosity, size, and pore structure of the scaffold used. Therefore, this research also carried out SEM analysis, a method of examining the surface of samples that provides information about their morphology.[24] Morphological characterization is essential for the biomaterials that can act as scaffolds. It helps in cell proliferation. The SEM analysis results revealed micro-sized pores on the sample surface. The size of pores plays a crucial role in fostering cell growth and the diffusion of nutrients, thereby promoting the attachment of cells and vascularization. In addition, pores can be used as a standard parameter for oxygen (O_2) transport and nutrients to an inner extracellular matrix scaffold.[22]

In this research, ImageJ was used to determine and measure pore area. ImageJ is a program designed to speed up the process of image analysis while also automatically calculating the areas of pores. The pore size of these two materials, both BAM-HA 4:1 and Bam-HA 4:2, they do the standards of a scaffold used for the regeneration of hard tissue and soft tissue. The optimal standard pore size for osteoconduction sockets ranges from 200–350 µm, while soft tissue regeneration ranges from 20–125 µm. [25]

The findings from the EDX examination of human alveolar bone indicate that calcium (Ca), phosphorus (P), and oxygen (O) are present in human bone, with these elements comprising the fundamental components of hydroxyapatite (HA). Additionally, the components iron (Fe), copper (Cu), zinc (Zn), bromium (Br), strontium (Sr), magnesium (Mg), lead (Pb), and sodium (Na) exist in limited quantities within the bones of humans.[26] The composition of the fresh BAM-HA biocomposite is almost the same as that of the previous study that used a combination of dried BAM and HA.[10] The EDX test results show that the biocomposite materials contain the elements Ca, P, and O, which are the essential components of bone structure. The composition of HA added at a ratio of 4:2 is greater, meaning that the Ca elements that appear in the EDX examination are higher than the ratio of 4:1. A higher HA content at a ratio of 4:2 BAM-HA made these samples have a higher potential to be used as a scaffold.[11]

More evidence-based research is needed to develop more effective methods to improve the ability of fresh bovine amnion as a base material for scaffold manufacture in regenerating alveolar bone. Furthermore, due to the limitations of this research, further research on cells, on experimental animals, and clinical trials need to be carried out to ensure that this material has potential as an alveolar bone scaffold.

CONCLUSION

Based on the research results, the characterization test for forming BAM-HA biocomposites with ratios of 4:1 and 4:2 has been successful. FTIR analysis showed that fresh bovine amnion membrane and hydroxyapatite bonded well. SEM-EDX test results show that the 4:2 fresh bovine amnion membrane hydroxyapatite biocomposite has small pores and higher HA content and, therefore, greater potential for use as a scaffold.

Thus, BAM-HA biocomposites can be an alternative material for tissue engineering, specifically in alveolar bone.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Esposito M, Grusovin MG, Felice P, Karatzopoulos G, Worthington HV, Coulthard P. The efficacy of horizontal and vertical bone augmentation procedures for dental implants - A Cochrane systematic review. Eur J Oral Implantol. 2009 ; 2(3):167-84.
2. Aufan MR, Daulay AH, Indriani D, Nuruddin A, Purwasasmita BS. Sintesis scaffold alginat-kitosan-karbonat apatit sebagai bone graft menggunakan metode freeze drying. J Biofisika. 2012; 8(1):16-24.
3. Hutama AS, Nugroho A. Optimasi Pembuatan Scaffold dengan Struktur Pori-Pori Beraturan Menggunakan Metode Response Surface Method. Jurnal Mater dan Proses Manufaktur. 2020; 4(1):1-11.
4. Octarina, Munadziroh E, Razak FA. Physical modification of bovine amniotic membrane for dental application. J Int Dent Med Res. 2021; 14(4):1425-8.
5. Octarina, Munadziroh E. Potential of bovine amniotic membrane and hydroxyapatite biocomposite materials for enhanced bone formation. Malays J Med Health Sci. 2021; 17:124-6.
6. Ratnayake JTB, Gould ML, Shavandi A, Mucalo M, Dias GJ. Development and characterization of a xenograft material from New Zealand sourced bovine cancellous bone. J Biomed Mater Res B Appl Biomater. 2017; 105(5):1054-62.
7. Faadhila TI, Valentina MN, Munadziroh E, Nirwana I, Soekartono H, Surboyo MDC. Bovine sponge amnion stimulates socket healing: A histological analysis. J Adv Pharm Technol Res. 2021; 12(1):99-103.
8. Oh D, Son D, Kim J, Kwon SY. Freeze-dried bovine amniotic membrane as a cell delivery scaffold in a porcine model of radiation-induced chronic wounds. Arch Plast Surg. 2021; 48(4):448-56.
9. Min S, Yoon JY, Park SY, Kwon HH, Suh DH. Clinical effect of bovine amniotic membrane and hydrocolloid on wound by laser treatment: Prospective comparative randomized clinical trial. Wound Repair Regen. 2014; 22(2):212-9.
10. Octarina, Munadziroh E, Razak FA, Surboyo MDC. Characterisation of bovine amniotic membrane with hydroxyapatite bio-composite. Coatings. 2022; 12(10):1403.
11. Filio P, Octarina, Komariah F. Characterization of fabricated bovine hydroxyapatite crystal as socket preservation material: An SEM-EDX and X-ray diffraction study. World J Dent. 2022; 13(S2):S175-81.
12. Budiatin AS, Gani MA, Samirah, Ardianto C, Raharjanti AM, Septiani I, et al. Bovine hydroxyapatite-based bone scaffold with gentamicin accelerates vascularization and remodeling of bone defect. Int J Biomater. 2021; 2021:5560891.
13. Lambert F, Léonard A, Drion P, Sourice S, Layrolle P, Rompen E. Influence of space-filling materials in subantral bone augmentation: blood clot vs. autogenous bone chips vs. bovine hydroxyapatite. Clin Oral Impl Res. 2011; 22;538-45.
14. Octarina, Munadziroh E, Razak FA, Handharyani E, Surboyo MDC. The role of bovine amniotic membrane and hydroxyapatite for the ridge preservation. Int J Biomater. 2024; 2024:4053527.
15. Ihsan P. The difference of epidermal growth factor concentration between fresh and freeze dried amniotic membranes. Jurnal Oftalmologi Indonesia. 2009; 7(2):62-6.
16. Park M, Kim S, Kim IS, Son D. Healing of a porcine burn wound dressed with human and bovine amniotic membranes. Wound Repair Regen. 2008; 16(4):520-8.
17. Gupta A, Kedige SD, Jain K. Amnion and chorion membranes: Potential stem cell reservoir with wide applications in periodontics. Int J Biomater. 2015; 2015:274082.
18. Indrawati DW, Munadziroh E, Sulisetyawati TIB, El Fadhlallah PM. Sponge amnion potential in post tooth extraction wound healing by interleukin-6 and bone morphogenetic protein-2 expression analysis: An animal study. Dent Res J (Isfahan). 2019; 16(5):283-8.
19. Madhan G, Singh M. Comparison of ability of Platelet-rich Fibrin vs CollaPlug in maintaining the buccal bone height of sockets following extractions in 20 patients. J Health Sci Res. 2017; 8(1):1-6.
20. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone grafts and substitutes in dentistry: A review of current trends

and developments. Molecules. 2021; 26(10):3007.
21. Zhang D, Wu X, Chen J, Lin K. The development of collagen based composite scaffolds for bone regeneration. Bioact Mater. 2017; 3(1):129-38.
22. Warastuti Y, Budianto E, Darmawan. Sintesis dan karakterisasi membran komposit hidroksiapatit tulang sapi-khitosan-poli(vinil Alkohol) untuk aplikasi biomaterial. Jurnal Sains Materi Indonesia. 2015; 16(2):83-90.
23. Noviyanti AR, Haryono H, Pandu R, Eddy DR. Cangkang telur ayam sebagai sumber kalsium dalam pembuatan hidroksiapatit untuk aplikasi graft tulang. Chimica et Natura Acta. 2017; 5(3):107-11.
24. Lopez Marquez A, Gareis IE, Dias FJ, Gerhard C, Lezcano MF. Methods to characterize electrospun scaffold morphology: A critical review. Polymers (Basel). 2022; 14(3):467.
25. López Angulo DE, do Amaral Sobral PJ. Characterization of gelatin/chitosan scaffold blended with aloe vera and snail mucus for biomedical purpose. Int J Biol Macromol. 2016 ; 92:645-53.
26. Stuckensen K, Lamo-Espinosa JM, Muiños-López E, Ripalda-Cemboráin P, López-Martínez T, Iglesias E, et al. Anisotropic cryostructured collagen scaffolds for efficient delivery of RhBMP-2 and enhanced bone regeneration. Materials (Basel). 2019; 12(19):3105.

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ABSTRACT

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INTRODUCTION

Oral and maxillofacial surgery procedures often use alveolar bone engineering techniques to achieve effective clinical results.[1] Scaffolding is widely used in tissue engineering techniques. A scaffold formed as a three-dimensional structure can be implemented as a medium to support the growth of new tissues.[2] Scaffolds made from raw biomaterial are used to increase the growth of cells that continue the function of replaced tissue.[3] One biomaterial that can be used in making scaffolds is bovine amniotic membrane (BAM). BAM is a biomaterial located in the deepest part of the placenta. It surrounds the embryo with a thickness of 0.02 to 0.5 mm.[4] BAM is the same as the human amniotic membrane; the placenta covers the embryo. This membrane is a thin, strong, translucent, and avascular layer. BAM and the human amniotic membrane have similar physical and biological properties.[5] BAM contains carbonates (CO_3^{2-}), sodium (Na^+), magnesium (Mg^{2+}), iron (Fe^{2+}), fluoride (F^-), silicon (Si), and chloride (Cl^-).[6] BAM also has several materials for regeneration, such as growth factors, collagen, and antibacterial characteristics.[7,8] In addition, BAM has been shown to have the ability to accelerate the epithelialization process, having anti-inflammatory, antiangiogenic, and analgesic effects.[9] BAM is, therefore, useful as a biomaterial in regenerative medicine.[9]

While BAM offers significant potential for expediting bone regeneration, it also possesses inherent weaknesses. One notable limitation is its susceptibility to rapid degradation, leading to difficulties in maintaining the structural integrity needed for effective bone regeneration. This accelerated degradation is primarily attributed to the high collagen content within the membrane, which facilitates rapid absorption. Because of this degradation, bone healing is predominantly only effective in the marginal area of the bone being regenerated.[9,10] The limitation of bovine amnion membranes can be optimized by incorporating hydroxyapatite (HA) material. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the primary type of mineral that composes bones and teeth. HA is synthesized from cow bones, which have almost the same composition as human bones.[11] This material is biocompatible and osteoconductive.[10] In medical applications, HA is widely used in bone grafting, bone repair, and replacement of damaged bone.[12] However, biomaterial characterization tests are needed to prove the biological function of BAM and HA biocomposites as candidates for scaffold in regenerating alveolar bone.

This research draws on the results of previous research on the use of BAM and HA as tissue regeneration biocomposites. A previous study carried out by Munadziroh et al. found that BAM can be used for socket regeneration following tooth extraction by stimulating the proliferation and formation of new blood vessel fibroblasts in sockets.[7] Furthermore, HA has been used to accelerate the repair of bone damage by accelerating tissue vascularization without disrupting the process of remodeling bone.[12,13] However, previous research has been limited to the use of dry bovine amniotic membranes, so further research to combine HA with fresh amniotic membranes is needed.

Based on this background, this research aims to present a characterization of fresh BAM and HA biocomposite fabrication in the structure of a sponge. BAM-HA biocomposites are made in ratios of 4:1 and 4:2. The ratios of 4:1 and 4:2 were created in this research to improve on the properties of the materials used in previous research, which had ratios that could not be used to regenerate alveolar bone healing optimally compared to commercial xenograft products.[14] Therefore, the HA ratio was increased in this study, and a fresh amniotic membrane was used. Because HA is one of the most essential materials that forms alveolar bone. In addition, this research uses a fresh amniotic membrane because it has a greater growth factor when compared to dried BAM.[15] Characterization was carried out by functional group testing Fourier transform infrared (FTIR), morphological structure test via scanning electron microscope (SEM), and through element test energy-dispersive x-ray spectroscopy (EDX).

METHODS

The research was conducted at the Biomaterial Bank Tissue Center Installation Dr Soetomo Regional General Hospital (RSUD) Surabaya with ethics approval number 360/HRECC.FODM/VII/2021.

Fabrication of Biocomposite BAM-HA
Biocomposite fabrication begins with taking BAM from the deepest part of the cow's placenta. This collection is carried out without drying so that the membrane has a softer texture and higher immunogenicity.[16,17] The BAM should be cleaned until it is free from blood clots. In this study, the amniotic membrane was cleaned by washing it with a 0.05% saline solution four times over 10 minutes. The amniotic membrane was washed again using a distilled water solution until the saline solution was clear. Fresh cow amnion membranes were cut, and sodium chloride (NaCl) solution was added at a ratio of 1:1.[4] A blender was then used to produce amnion slurry.

The amnion slurry was extracted and combined with HA with ratios of 4:1 and 4:2. The amnion slurry and HA powder mixture was stirred until homogeneous and then put into a container with a diameter of 10 cm. The homogenized mixture was put in the freezer at a temperature of -80 ^oC for 1x24 hours. The next step was freeze-drying for 2x24 hours with a temperature of -100 ^oC until a sponge-shaped BAM and HA biocomposite was obtained.

Functional Group Test (FTIR)
Functional group testing (FTIR) begins with sample preparation. Fresh bovine amnion membrane-hydroxyapatite (BAM-HA) biocomposite samples were mixed with potassium bromide (KBr) in a ratio of 1:4. The mixture was ground until homogeneous and then put into a container with a diameter of 4 mm. Using an IR Prestidge-21 (Shimadzu, Japan), a homogeneous mixture of samples and KBr was measured using the diffuse reflectance Fourier transform infrared spectroscopy (DRSFTIR) method. The measurement range was 400–4000 cm^{-1}, the resolution was 2.0 cm-1, the sample was scanned as many as 20 times, and the value will appear as a transmittance percentage.

Scanning Electron Microscopy (SEM)
In SEM testing of morphology and structure, the BAM and HA biocomposite samples were cut to a size of 1 x 1 cm. Before testing, the samples were first coated with gold (fine coater JFC 1600). Samples were put in a chamber and then vacuumed with a 15kV SEI detector. The magnification of the tool was set at 100x. Then, the pore samples' surface morphology, size, and shape were analyzed using ImageJ.

Energy Dispersive X-ray Spectroscopy (EDX)
On the element test, the sample in the SEM sample chamber was analyzed, and the sample's material chemical elements was mapped using energy dispersive x-ray spectroscopy (EDX). The magnification was set at 100x and 300x with a distance of 10 mm. The outcomes of the chemical element analysis are presented visually in a figure with a color gradient.

RESULTS

This research has successfully combined fresh amnion membranes with HA into a biocomposite. The following are the results of biocomposites of fresh amnion membranes and HA with ratios 4:1 and 4:2.

FTIR analysis shows that all BAM-HA biocomposites contain amide I, II, III, A, B, and OH functional groups. Meanwhile, both samples can also be seen to contain PO_4^{3-} and CO_3^{2-}.

Graph in Figure 1a. showed that the BAM-HA biocomposite with a ratio of 4:1 had an amide I absorption peak at 1642 cm^{-1}, amide II at 1548 cm^{-1}, amide III at 1241 cm^{-1}, amide A at 3571 cm^{-1}, amide B at 2962 cm^{-1}, OH^{-} at 3445 cm^{-1}, $PO4^{3-}$ at 1084 cm^{-1}, and CO_3^{2-} at a 1478 cm^{-1} wavelength.

On the other hand, as seen in Figure 1b, the BAM-HA biocomposite with a ratio of 4:2 has an absorption peak at 1634 cm^{-1} for amide I, at 1559 cm^{-1} for amide II, at 1263 cm^{-1} for amide III, at 3566 cm^{1} for amide A, at 2966 cm^{-1} for amide B, at 3446 cm^{-1} for OH^{-} at 1025 cm^{-1} for PO_4^{3-}, and at 1489 cm^{-1} for CO_3^{2-}.

SEM Analysis

Figure 2(a) is an image of the BAM-HA biocomposite ratio 4:1 surface using SEM at 100x magnification. Figure 2(b) is a surface image of the BAM-HA biocomposite ratio of 4:2 using SEM at 100x magnification. Calculation results using ImageJ show that the number of pores in BAM-HA biocomposites 4:1 and 4:2 is almost the same, with both having around 45 pores. However, the shape and size of the pore differ. BAM-HA, with a ratio of 4:1, has a relatively larger pore size with an average of 114.28 μm when compared to BAM-HA 4:2, which has a smaller pore size with an average of 69.10 μm. The surface morphology of BAM-HA 4:2 appears denser, with HA granules appearing to bond to the amnion membrane.

EDX Analysis

Both biocomposites of fresh bovine amnion membrane-hydroxyapatite ratio demonstrated the existence of C, O, Na, Mg, Si, P, Cl, Ca, and Nb elements. However, the element percentage values differed somewhat between the two biocomposites.

The results of the EDX test on the BAM-HA biocomposite with a ratio of 4:1 can be seen in Figure 3a. As shown in this figure, the fresh bovine amnion membrane-hydroxyapatite biocomposite with a ratio of 4:1 was shown to contain carbon (C) with a percentage of 69.16%, oxygen (O) 20.81%, sodium (Na) 3.82%, magnesium (Mg) 0.18 %, silicon (Si) 0.09%, phosphorus (P) 0.41%, chlorine (Cl) 3.99%, calcium (Ca) 1.33%, and niobium (Nb) 0.21%.

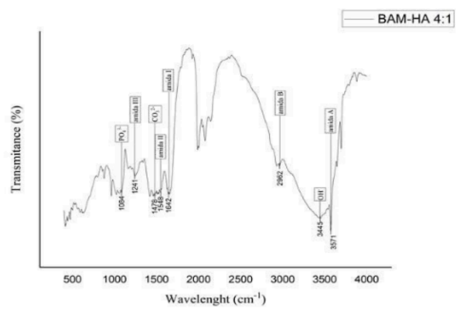

Figure 1a. Graph of FTIR analysis results of BAM-HA biocomposite with a ratio of 4:1

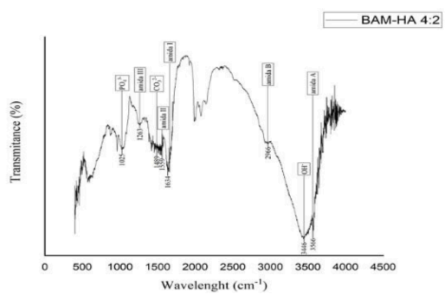

Figure 1b. Graph of FTIR analysis results of BAM-HA biocomposite with a ratio of 4:2

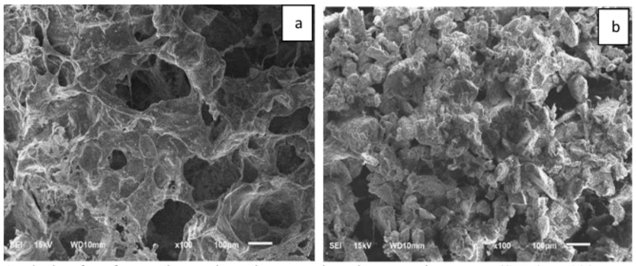

Figure 2. Analysis of surface and porosity using scanning electron microscopy (SEM) at 100x magnification (a) BAM-HA biocomposite ratio 4:1 (b) BAM-HA biocomposite ratio 4:2

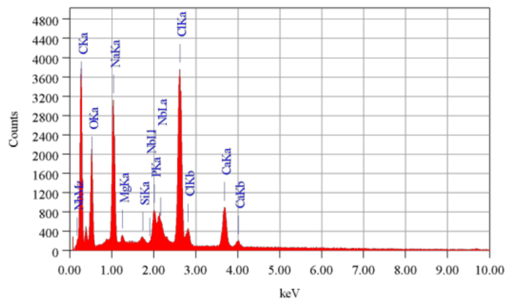

Figure 3a. Graph of EDX test results for BAM-HA biocomposite composition ratio 4:1

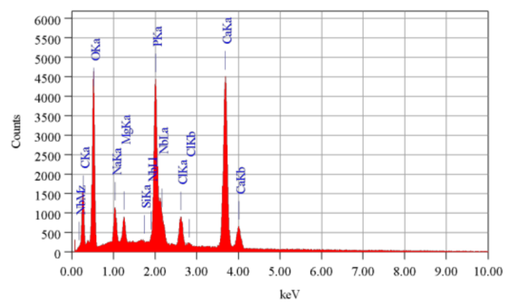

Figure 3b. Graph of EDX test results for BAM-HA biocomposite composition ratio 4:2

The results of the EDX BAM-HA test with a ratio of 4:2 are shown in Figure 3b. The graph shows the presence of the elements carbon (C) 31.29%, oxygen (O) 50.27%, sodium (Na) 3.82%, magnesium (Mg) 1.73%, silicon (Si) 0.96%, phosphorus (P) 5.20%, chlorine (Cl) 1.10%, calcium (Ca) 8.90%, and niobium (Nb) 0.54%.

BAM can be combined with hydroxyapatite (HA) biomaterial to maximize the performance of BAM in tissue regeneration. BAM has abundant growth factors and collagen with osteoinductive properties,[18,19] while HA has good osteoconductive properties as a bone substitute.[20] Biocomposite materials produced by combining collagen and HA can be used to optimize bone regeneration because they have a composition and structure similar to that of natural bone.[21] HA can be synthesized from inorganic components or natural resources, such as mammalian and marine bones, plants, and biogenic sources. In this study, HA from biological sources (natural hydroxyapatite) is extracted from the femurs and thigh bones of cows,[22] which is advantageous because cow bones morphologically and structurally resemble human bones.[23]

The application of BAM is prevalent in the field of tissue engineering, particularly in the medical domain. This is because BAM can help in tissue regeneration. In Indonesia, fresh BAM is abundant and can be obtained easily.[18] BAM contains regenerating ingredients, such as growth factors, collagen, and antibacterial properties. In this research, chemical characterization was carried out, including analysis of functional groups in the synthesized HA. The analysis used a Fourier transform infrared (FTIR) instrument to determine the functional groups of HA qualitatively based on the absorbance of infrared rays. The FTIR analysis showed all types of fresh BAM-HA biocomposites to have amide functional groups I, II, III, A, B, and OH; these functional groups show that fresh BAM and HA can bind to each other. The appearance of amides I, II, II, A, and B functional groups in the biocomposite showed that collagen was still present, not having been denatured yet. These characteristics are as expected for BAM-HA, which for HA is a major component of bone tissue and teeth. The presence of these characteristic functional groups in hydroxyapatite is consistent with the well-established chemical composition and biological significance of hydroxyapatite in tissue engineering.[22]

The presence of bonds, such as O-P-O (oxygen-phosphorus-oxygen), C-O (carbon-oxygen), and Ca-O (calcium-oxygen), indicates the presence of HA in the sample. FTIR test results show that collagen content is based on amide functional groups, which have bonds such as O-H (oxygen-hydrogen), N-H (nitrogen-hydrogen), C-H (carbon-hydrogen), and N-H (nitrogen-hydrogen). The BAM-HA biocomposite samples also showed the presence of PO_4^{3-} and CO_3^{2-}. The presence of phosphate and carbonate groups suggests that hydroxyapatite was well-bound or present in the BAM sample during fabrication. In addition, the amide and hydroxyapatite functional groups in the biocomposite indicate that the combination of fresh BAM-HA created in this study successfully bonds with each other.[10]

The formation and penetration of tissue in a biomaterial can be affected by the porosity, size, and pore structure of the scaffold used. Therefore, this research also carried out SEM analysis, a method of examining the surface of samples that provides information about their morphology.[24] Morphological characterization is essential for the biomaterials that can act as scaffolds. It helps in cell proliferation. The SEM analysis results revealed micro-sized pores on the sample surface. The size of pores plays a crucial role in fostering cell growth and the diffusion of nutrients, thereby promoting the attachment of cells and vascularization. In addition, pores can be used as a standard parameter for oxygen (O_2) transport and nutrients to an inner extracellular matrix scaffold.[22]

In this research, ImageJ was used to determine and measure pore area. ImageJ is a program designed to speed up the process of image analysis while also automatically calculating the areas of pores. The pore size of these two materials, both BAM-HA 4:1 and Bam-HA 4:2, they do the standards of a scaffold used for the regeneration of hard tissue and soft tissue. The optimal standard pore size for osteoconduction sockets ranges from 200–350 µm, while soft tissue regeneration ranges from 20–125 µm.[25]

The findings from the EDX examination of human alveolar bone indicate that calcium (Ca), phosphorus (P), and oxygen (O) are present in human bone, with these elements comprising the fundamental components of hydroxyapatite (HA). Additionally, the components iron (Fe), copper (Cu), zinc (Zn), bromium (Br), strontium (Sr), magnesium (Mg), lead (Pb), and sodium (Na) exist in limited quantities within the bones of humans.[26] The composition of the fresh BAM-HA biocomposite is almost the same as that of the previous study that used a combination of dried BAM and HA.[10] The EDX test results show that the biocomposite materials contain the elements Ca, P, and O, which are the essential components of bone structure. The composition of HA added at a ratio of 4:2 is greater, meaning that the Ca elements that appear in the EDX examination are higher than the ratio of 4:1. A higher HA content at a ratio of 4:2 BAM-HA made these samples have a higher potential to be used as a scaffold.[11]

More evidence-based research is needed to develop more effective methods to improve the ability of fresh bovine amnion as a base material for scaffold manufacture in regenerating alveolar bone. Furthermore, due to the limitations of this research, further research on cells, on experimental animals, and clinical trials need to be carried out to ensure that this material has potential as an alveolar bone scaffold.

CONCLUSION

Based on the research results, the characterization test for forming BAM-HA biocomposites with ratios of 4:1 and 4:2 has been successful. FTIR analysis showed that fresh bovine amnion membrane and hydroxyapatite bonded well. SEM-EDX test results show that the 4:2 fresh bovine amnion membrane hydroxyapatite biocomposite has small pores and higher HA content and, therefore, greater potential for use as a scaffold.

Thus, BAM-HA biocomposites can be an alternative material for tissue engineering, specifically in alveolar bone.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Esposito M, Grusovin MG, Felice P, Karatzopoulos G, Worthington HV, Coulthard P. The efficacy of horizontal and vertical bone augmentation procedures for dental implants - A Cochrane systematic review. Eur J Oral Implantol. 2009 ; 2(3):167-84.
2. Aufan MR, Daulay AH, Indriani D, Nuruddin A, Purwasasmita BS. Sintesis scaffold alginat-kitosan-karbonat apatit sebagai bone graft menggunakan metode freeze drying. J Biofisika. 2012; 8(1):16-24.
3. Hutama AS, Nugroho A. Optimasi Pembuatan Scaffold dengan Struktur Pori-Pori Beraturan Menggunakan Metode Response Surface Method. Jurnal Mater dan Proses Manufaktur. 2020; 4(1):1-11.
4. Octarina, Munadziroh E, Razak FA. Physical modification of bovine amniotic membrane for dental application. J Int Dent Med Res. 2021; 14(4):1425-8.
5. Octarina, Munadziroh E. Potential of bovine amniotic membrane and hydroxyapatite biocomposite materials for enhanced bone formation. Malays J Med Health Sci. 2021; 17:124-6.
6. Ratnayake JTB, Gould ML, Shavandi A, Mucalo M, Dias GJ. Development and characterization of a xenograft material from New Zealand sourced bovine cancellous bone. J Biomed Mater Res B Appl Biomater. 2017; 105(5):1054-62.
7. Faadhila TI, Valentina MN, Munadziroh E, Nirwana I, Soekartono H, Surboyo MDC. Bovine sponge amnion stimulates socket healing: A histological analysis. J Adv Pharm Technol Res. 2021; 12(1):99-103.
8. Oh D, Son D, Kim J, Kwon SY. Freeze-dried bovine amniotic membrane as a cell delivery scaffold in a porcine model of radiation-induced chronic wounds. Arch Plast Surg. 2021; 48(4):448-56.
9. Min S, Yoon JY, Park SY, Kwon HH, Suh DH. Clinical effect of bovine amniotic membrane and hydrocolloid on wound by laser treatment: Prospective comparative randomized clinical trial. Wound Repair Regen. 2014; 22(2):212-9.
10. Octarina, Munadziroh E, Razak FA, Surboyo MDC. Characterisation of bovine amniotic membrane with hydroxyapatite bio-composite. Coatings. 2022; 12(10):1403.
11. Filio P, Octarina, Komariah F. Characterization of fabricated bovine hydroxyapatite crystal as socket preservation material: An SEM-EDX and X-ray diffraction study. World J Dent. 2022; 13(S2):S175-81.
12. Budiatin AS, Gani MA, Samirah, Ardianto C, Raharjanti AM, Septiani I, et al. Bovine hydroxyapatite-based bone scaffold with gentamicin accelerates vascularization and remodeling of bone defect. Int J Biomater. 2021; 2021:5560891.
13. Lambert F, Léonard A, Drion P, Sourice S, Layrolle P, Rompen E. Influence of space-filling materials in subantral bone augmentation: blood clot vs. autogenous bone chips vs. bovine hydroxyapatite. Clin Oral Impl Res. 2011; 22;538-45.
14. Octarina, Munadziroh E, Razak FA, Handharyani E, Surboyo MDC. The role of bovine amniotic membrane and hydroxyapatite for the ridge preservation. Int J Biomater. 2024; 2024:4053527.
15. Ihsan P. The difference of epidermal growth factor concentration between fresh and freeze dried amniotic membranes. Jurnal Oftalmologi Indonesia. 2009; 7(2):62-6.
16. Park M, Kim S, Kim IS, Son D. Healing of a porcine burn wound dressed with human and bovine amniotic membranes. Wound Repair Regen. 2008; 16(4):520-8.
17. Gupta A, Kedige SD, Jain K. Amnion and chorion membranes: Potential stem cell reservoir with wide applications in periodontics. Int J Biomater. 2015; 2015:274082.
18. Indrawati DW, Munadziroh E, Sulisetyawati TIB, El Fadhlallah PM. Sponge amnion potential in post tooth extraction wound healing by interleukin-6 and bone morphogenetic protein-2 expression analysis: An animal study. Dent Res J (Isfahan). 2019; 16(5):283-8.
19. Madhan G, Singh M. Comparison of ability of Platelet-rich Fibrin vs CollaPlug in maintaining the buccal bone height of sockets following extractions in 20 patients. J Health Sci Res. 2017; 8(1):1-6.
20. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone grafts and substitutes in dentistry: A review of current trends

and developments. Molecules. 2021; 26(10):3007.
21. Zhang D, Wu X, Chen J, Lin K. The development of collagen based composite scaffolds for bone regeneration. Bioact Mater. 2017; 3(1):129-38.
22. Warastuti Y, Budianto E, Darmawan. Sintesis dan karakterisasi membran komposit hidroksiapatit tulang sapi-khitosan-poli(vinil Alkohol) untuk aplikasi biomaterial. Jurnal Sains Materi Indonesia. 2015; 16(2):83-90.
23. Noviyanti AR, Haryono H, Pandu R, Eddy DR. Cangkang telur ayam sebagai sumber kalsium dalam pembuatan hidroksiapatit untuk aplikasi graft tulang. Chimica et Natura Acta. 2017; 5(3):107-11.
24. Lopez Marquez A, Gareis IE, Dias FJ, Gerhard C, Lezcano MF. Methods to characterize electrospun scaffold morphology: A critical review. Polymers (Basel). 2022; 14(3):467.
25. López Angulo DE, do Amaral Sobral PJ. Characterization of gelatin/chitosan scaffold blended with aloe vera and snail mucus for biomedical purpose. Int J Biol Macromol. 2016 ; 92:645-53.
26. Stuckensen K, Lamo-Espinosa JM, Muiños-López E, Ripalda-Cemboráin P, López-Martínez T, Iglesias E, et al. Anisotropic cryostructured collagen scaffolds for efficient delivery of RhBMP-2 and enhanced bone regeneration. Materials (Basel). 2019; 12(19):3105.

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Jurnal : Journal of Dentistry Indonesia

Penulis : Octarina[1*], Elly Munadziroh[2]

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INTRODUCTION

Oral and maxillofacial surgery procedures are often used alveolar bone engineering techniques to get some effective clinical results.[1] Scaffold widely used in tissue engineering techniques (tissue engineering). A scaffold formed as a three-dimensional structure implemented as a medium to support the growth of new tissues.[2] Scaffold made from biomaterial raw as a bone tissue restoration material to increase the growth of cells that will continue the function of the replaced tissue.[3] One of the biomaterials that can be used in making scaffolds is bovine amniotic membrane (BAM). BAM is a biomaterial which it's location is in the deepest part of the placenta. It surrounds the embryo with a thickness of 0.02 to 0.5 mm.[4] BAM is similar to human amniotic membrane based on its physical and biological properties. BAM contains carbonates (CO_3^{2-}), sodium (Na^+), magnesium (Mg^{2+}), iron (Fe^{2+}), flouride (F^-), silicon (Si), and chloride (Cl^-).[5] BAM also has several contents for regeneration, such as growth factors, collagen, and antibacterial characteristic.[6,7] In addition, BAM has been shown to have the ability to accelerate the epithelialization process, having anti-inflammatory, antiangiogenic, and analgesic effects.[8] Therefore, BAM is useful as an biomaterial in regenerative medicine.[9]

The function of bovine amnion membranes in regeneration can be optimized by incorporating hydroxyapatite (HA) material. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the primary type of mineral that compose bones and teeth. HA formed from cow bones that has almost the same composition with human bones. This material is biocompatible and osteoconductive.[9] In medical applications, HA is widely used in bone grafting, bone repair, and replacement of damaged bone.[2] However, it requires biomaterials characterization tests to prove the biological function of BAM and HA biocomposites as candidates for scaffold in regenerating alveolar bone. Characterization tests are also carried out to determine physical and chemical character based on the influence of size and composition.[11]

This research refers to several results of previous research regarding BAM and HA as tissue regeneration biocomposites. A previous study done by Munadziroh et al. (2021) found that BAM can be used for socket regeneration after extracting tooth by stimulating the proliferation and formation of new blood vessel fibroblasts.[6] Meanwhile, HA has also been researched and developed to accelerate the repair of bone damage by accelerating tissue vascularization without disrupting the process of remodeling bone.[10,11] However, further research is still needed because previous research was limited to the use of dry cow amniotic membranes.

Based on this background, this research aims to present a characterization of the BAM and HA biocomposite fabrication method, which will produce a preparation in the structure of a sponge. BAM-HA biocomposites are made in ratios of 4:1 and 4:2 according to material standards that can be a substitute for alveolar bone. Characterization was carried out by functional group testing Fourier Transform Infrared (FTIR), morphological structure test via Scanning Electron Microscope (SEM), and through element test Energy-Dispersive X-ray Spectroscopy (EDX).

METHODS

The laboratory experimental research in vitro was conducted. The research was conducted at the Biomaterial Bank Tissue Center Installation Dr Soetomo Regional General Hospital (RSUD) Surabaya with Number of Ethics 360/HRECC.FODM/VII/2021.

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Biocomposite fabrication begins with taking BAM from the deepest part of the cow's placenta. This collection is carried out without drying so that the membrane has a softer texture and higher immunogenicity.[12,13] The BAM should be cleaned until it is free from blood clots**.** The amniotic membrane was washed using the saline solution with a concentration of 0.05% four times within 10 minutes. Hereafter, the amniotic membrane was washed again using a distilled water solution until the saline solution is clear. Fresh cow amnion membranes were cut, and sodium chloride (NaCl) solution was added with a ratio of 1:1.[4]

The amnion membrane was cut and added with NaCl, then ground using a blender to produce amnion slurry. Amnion pulp was taken and added with HA powder twice in a ratio of 4:1 and 4:2. The amnion slurry and HA powder mixture was stirred until homogeneous and then put into a container with a diameter of 10 cm. The homogenized mixture is put in the freezer at a temperature of -80^{0}C for 1x24 hours. If it was done, then the next step was freeze-drying for 2x24 hours with the temperature of 100^{0}C until a sponge-shaped BAM and HA biocomposite was obtained.

Functional Group Test (FTIR)

Functional group testing (FTIR) begins with sample preparation. Fresh bovine amnion membrane-hydroxyapatite (BAM-HA) biocomposite samples were mixed up with potassium bromide (KBr) in a ratio of 1:4. The mixture was grinded until homogeneous and put into a container with a diameter of 4 mm. Using an IR Prestidge-21 (Shimadzu, Japan), a homogeneous mixture of samples and KBr was measured using the Diffuse Reflectance – Fourier Transform Infrared Spectroscopy (DRS-

FTIR) method. The measurement range is 400 – 4000 cm^{-1}, resolution 2.0 cm^{-1}, scanned as many as 20, and the percentage measurement results transmittance.

Morphological Structure Test (SEM)

In structure-morphology (SEM) testing, BAM and HA biocomposite samples were cut to a size of 1 x 1 cm. Before testing, the samples were first coated with gold (fine coater JFC 1600). Samples are put in a chamber and then vacuumed with a 15kV SEI detector. The magnification of the tool is set at 50x and 500x. Then, the pores surface morphology, size and shape will be analyzed using Image-J.

Element Test (EDX)

On the elemental test (EDX), the sample found on chamber SEM was analyzed and performed mapping material chemical elements using Energy Dispersive X-ray Spectroscopy (EDX). The magnification was set as 100 µm and 300 µm with a distance of 10 mm. The outcomes of the chemical element analysis are presented visually on a map with a colour gradient.

RESULTS

FTIR analysis

FTIR analysis shows that all BAM-HA biocomposites have amide I, II, III, A, B and OH functional groups. Meanwhile, both samples also show the the existence of PO_4^{3-} and CO_3^{2}.

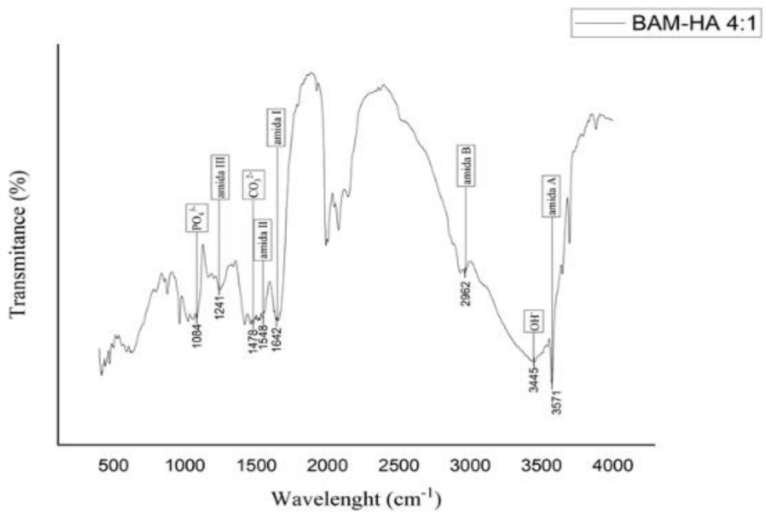

Figure 1a. Graph of FTIR analysis results of BAM-HA biocomposite with a ratio of 4:1

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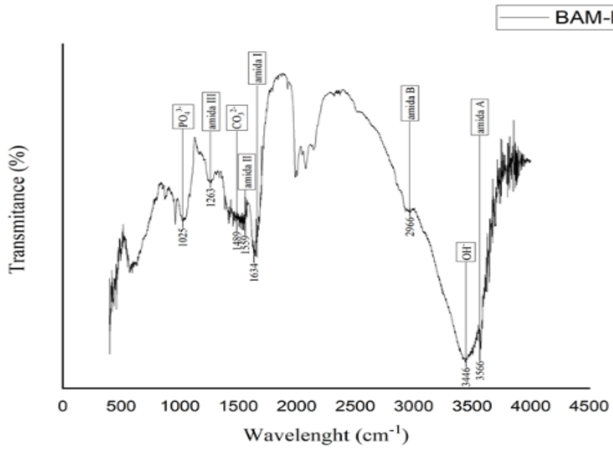

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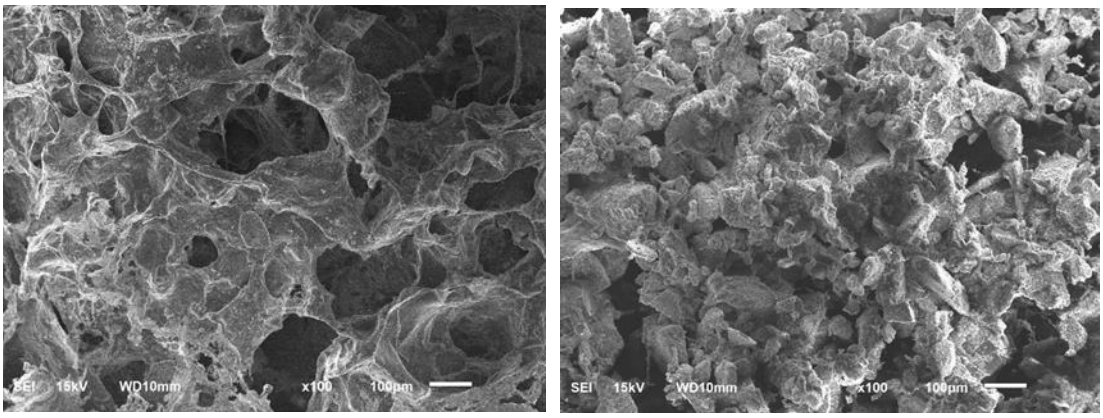

(a) (b)

Figure 2. Analysis of surface and porosity using Scanning Electron Microscopy (SEM) at 100x magnification (a) BAM-HA biocomposite ratio 4:1 (b) BAM-HA biocomposite ratio 4:2

Figure 2(a) is an image of the BAM-HA biocomposite ratio 4:1 surface using SEM at 100x magnification. Figure 2(b) is a surface image of the bovine amnion membrane-hydroxyapatite biocomposite ratio of 4:2 using SEM at 100x magnification.

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Both biocomposites of fresh bovine amnion membrane-hydroxyapatite ratio demonstrated the existence of C, O, Na, Mg, Si, P, Cl, Ca, and Nb elements. However, the element percentage values between the two biocomposites are pretty different.

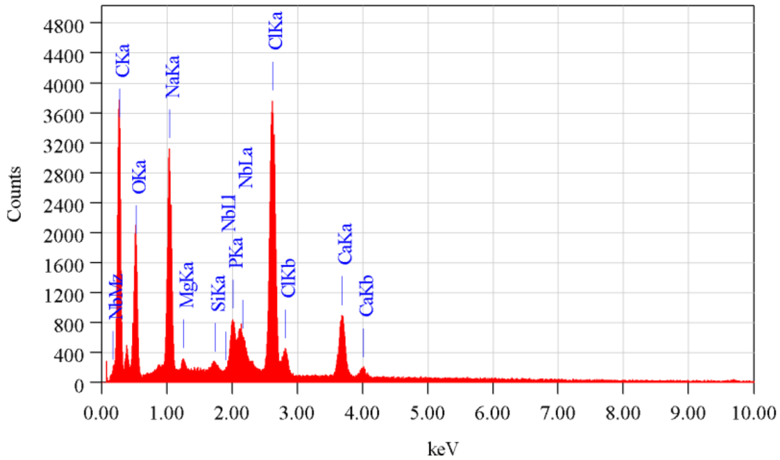
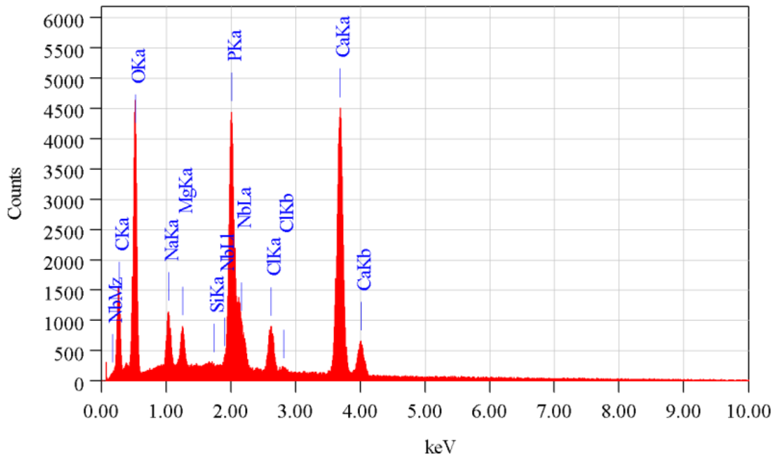


Figure 3a. Graph of EDX test results for BAM-HA biocomposite composition ratio 4:1

The EDX test on the BAM-HA biocomposite with a ratio of 4:1 shows the results as depicted in figure 3a. Based on the graph, the fresh bovine amnion membrane-hydroxyapatite biocomposite with a ratio of 4:1 shows elements including Carbon (C) with a percentage of 69.16%, Oxygen (O) 20.81%, Sodium (Na) 3.82%, Magnesium (Mg) 0.18 %, Silicon (Si) 0.09%, Phosphorus (P) 0.41 %, Chlorine (Cl) 3.99%, Calcium (Ca) 1.33%, Niobium (Nb) 0.21%.



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Meanwhile, the EDX BAM-HA test with a ratio of 4:2 shows results as shown in the figure 3b. The graph shows the presence of the elements Carbon (C) 31.29%, Oxygen (O) 50.27%, Sodium (Na) 3.82%, Magnesium (Mg) 1.73%, Silicon (Si) 0.96%, Phosphorus (P) 5.20%, Chlorine (Cl) 1.10%, Calcium (Ca) 8.90%, Niobium (Nb) 0.54%.

DISCUSSION

BAM can be combined with hydroxyapatite (HA) biomaterial to maximize the performance of BAM in tissue regeneration. BAM has abundant growth factors and collagen with osteoinductive properties[15], while HA has good osteoconductive properties as a bone substitute.[16] Biocomposite materials produced by combining collagen and HA using several techniques can be an option for optimizing bone regeneration because they have a composition and structure similar to natural bone.[17] HA can be synthesized from inorganic components or natural resources, such as mammalian and marine bones, plants and biogenic sources. In this case, HA from biological sources (natural hydroxyapatite) is extracted from cow bone parts of the femur or thighs.[18] This is an advantage because cow bones morphologically and structurally resemble human bones.[19]

In line with previous studies, the application of bovine amnion membranes (BAM) is prevalent in the field of tissue engineering, particularly within the medical domain. This use is carried out because BAM can help tissue regeneration. In Indonesia, BAM is so abundant that it can be obtained easily.[14] BAM contains regenerating ingredients such as growth factors, collagen, and antibacterial properties. In this research, chemical characterization has been carried out, including analysis of functional groups on the synthesized HA. The analysis uses a Fourier Transform Infrared (FTIR) instrument to determine the functional groups of hydroxyapatite qualitatively based on the absorbance of infrared rays. The analysis is characterized by a shift in wavelength in the wave number area of 4000-400 cm-^{1}, an increase or decrease in intensity, and the disappearance and emergence of new spectra.[18]

Based on the results of FTIR analysis, all types of BAM-HA biocomposites have amide functional groups I, II, III, A, B, and OH. The existence of amide chemical compounds is characterized by the presence of the functional group RnE(O) x NR'2, where R and R' can be hydrogen (H) or organic groups. BAM-HA biocomposite with a ratio of 4:1 has an Amide I absorption peak of cm^{-1}, Amida II 1,548 cm^{-1}, Amida III 1.241 cm^{-1}, Amida A 3,571 cm^{-1}, Amida B 2962 cm^{-1}, OH^- group 3,445 cm^{-1}, PO_4^{3-} at 1.084 cm^{-1}, CO_3^{2-} at a wavelength of 1,478 cm^{-1}. Meanwhile, the BAM-HA biocomposite with a ratio of 4:2 has an Amide I absorption peak of 1634 cm^{-1}, Amida II 1559 cm^{-1}, Amida III 1263 cm^{-1}, Amida A 3566 cm^{-1}, 2966 cm^{-1}, OH group$^-$ 3446 cm^{-1}, PO_4^{3-} 1025 cm^{-1} and CO_3^{2-} clusters at a wavelength of 1489 cm^{-1}. These absorption peaks show characteristic peaks of protein macromolecules in the bovine bone matrix.[18]

The presence of bonds such as O-P-O (Oxygen-Phosphorus-Oxygen), C-O (Carbon-Oxygen), and Ca-O (Calcium-Oxygen) indicates the presence of HA in the sample. FTIR test results show that collagen content is based on amide functional groups which have bonds such as O-H (Oxygen-Hydrogen), N-H (Nitrogen-Hydrogen), C-H (Carbon-Hydrogen) and N-H (Nitrogen-Hydrogen). The

BAM-HA biocomposite samples also showed the presence of the PO_4^{3-} and CO_3^{2-}. The presence of phosphate and carbonate groups indicates that hydroxyapatite is well-bound or present in the BAM sample during fabrication. In addition, the amide and hydroxyapatite functional groups in the biocomposite indicate that combination BAM-HA is feasible.

The formation and penetration of tissue in a biomaterial can be affected by the porosity, size, and pore structure of the scaffold. Therefore, this research also carried out SEM analysis, a method of examining the surface of samples, which provides information about their morphology.[20] Morphological characterization is essential for the primary purpose of the scaffold. It helps in cell proliferation. The SEM analysis results show micro-sized pores on the sample surface. The size of pores plays a crucial role in fostering cell growth and the diffusion of nutrients, thereby promoting the attachment of cells and vascularization. In addition, pores can be used as a standard parameter for oxygen (O_2) transport and nutrients to the inner extracellular matrix scaffold.[18]

The optimal standard pore size for osteoconduction sockets ranges from 200-350 µm, while soft tissue regeneration ranges from 20-125 µm.[21] To determine and measure the pore area, Image-J is used. Imgae-J is a program designed to speed up the process of image analysis while also automatically gauging the area of pores area. The pore size is in the range of 40-80 µm for the BAM-HA 4:1 biocomposite. Meanwhile, no pores were found in the BAM-HA biocomposite with a ratio of 4:2.

The findings from the EDX examination of human alveolar bone indicate that Calcium (Ca), Phosphorus (P), and Oxygen (O) are present in human bone, comprising the fundamental components of hydroxyapatite (HA). Additionally, there are components iron (Fe), copper (Cu), zinc (Zn), Bromium (Br), strontium (Sr), magnesium (Mg), lead (Pb), and sodium (Na) which exist in limited quantities within the bones of humans. The composition of this material is almost the same as that contained in the BAM-HA biocomposite.[22] The EDX BHA test results show that BHA contains the elements Ca, P, and O, which are the essential components. The elements Mg and Na were also found as minor components. The EDX results of the BAM-HA biocomposite show that the fiber surface contains hydroxyapatite, with calcium and phosphorus peaks, but at a lower level than the carbon peak.

More evidence-based research is needed to develop more effective methods to improve the ability of fresh bovine amnion as a base material for scaffold manufacture in regenerating alveolar bone. Furthermore, due to the limitations of this research, as a recommendation it is also necessary to carry out further research on cells, experimental animals and clinical trials to ensure that this material really has potential as an alveolar bone scaffold.

CONCLUSION

Based on the research results, the characterization test for forming BAM-HA biocomposites with a ratio of 4:1 and 4:2 has been successful. FTIR analysis showed that fresh bovine amnion membrane and hydroxyapatite bonded well. Meanwhile, SEM test results show that the fresh bovine amnion membrane-hydroxyapatite 4:1 biocomposite can regenerate soft tissue but is not osteoconductive. This result is better than the 4:2 fresh bovine amnion membrane-hydroxyapatite biocomposite, with no pores. The EDX results also showed that the fresh bovine amnion membrane-hydroxyapatite biocomposite contained several elements in joint with scaffold bones. Thus, BAM-HA biocomposites can be an alternative as a material for tissue engineering, especially in alveolar bone.

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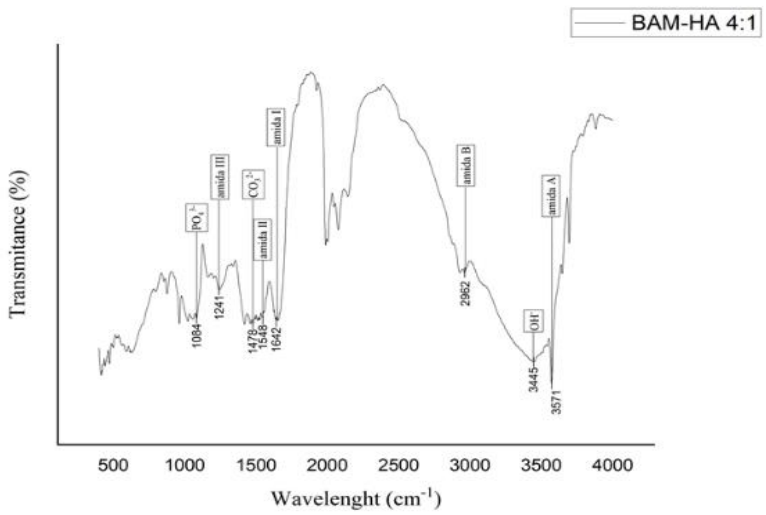

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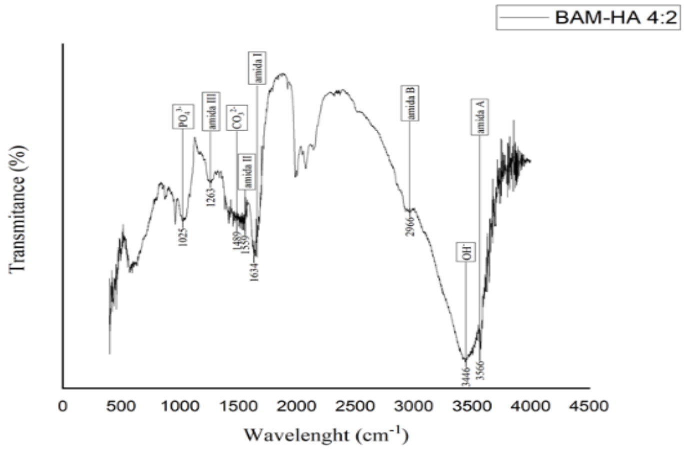

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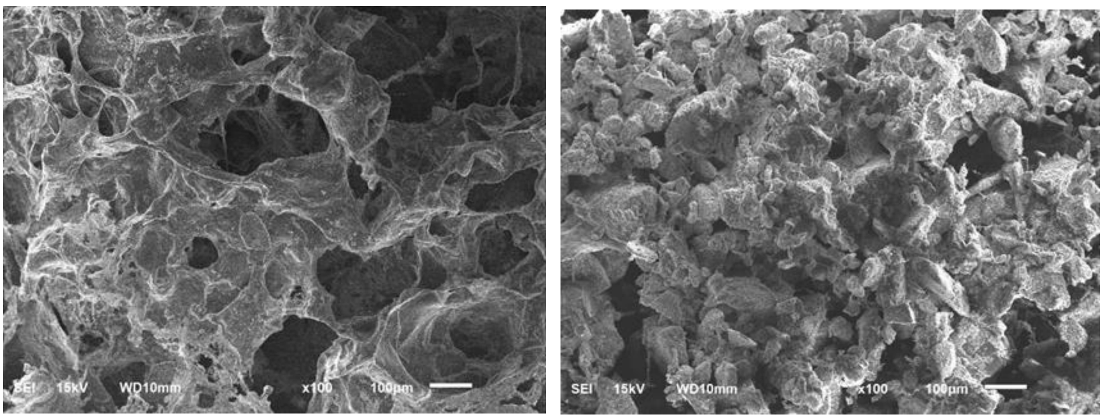

(a) (b)

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Figure 2(a) is an image of the BAM-HA biocomposite ratio 4:1 surface using SEM at 100x magnification. Figure 2(b) is a surface image of the bovine amnion membrane-hydroxyapatite biocomposite ratio of 4:2 using SEM at 100x magnification.

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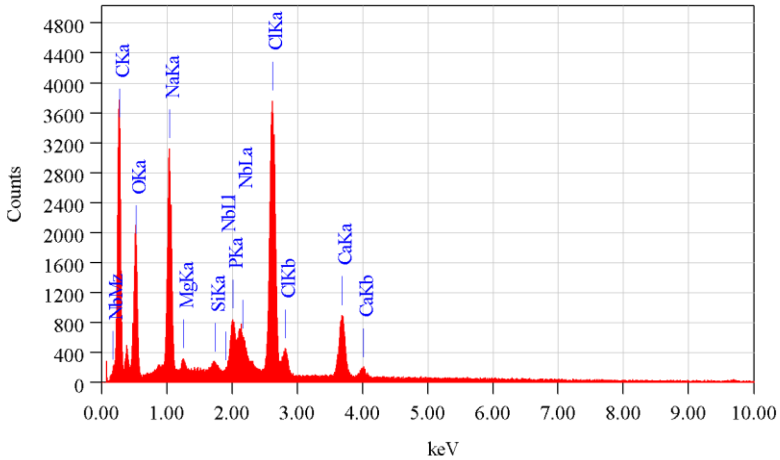

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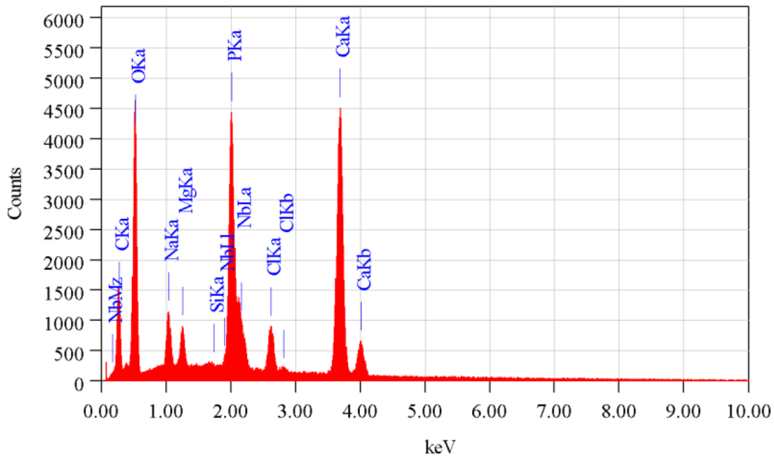

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The presence of bonds such as O-P-O (Oxygen-Phosphorus-Oxygen), C-O (Carbon-Oxygen), and Ca-O (Calcium-Oxygen) indicates the presence of HA in the sample. FTIR test results ~~show~~ that collagen content is based on amide functional groups which have bonds such as O-H (Oxygen-Hydrogen), N-H (Nitrogen-Hydrogen), C-H (Carbon-Hydrogen) and N-H (Nitrogen-Hydrogen). The

BAM-HA biocomposite samples also showed the presence of the PO_4^{3-} and CO_3^{2-}. The presence of phosphate and carbonate groups indicates that hydroxyapatite is well-bound or present in the BAM sample during fabrication. In addition, the amide and hydroxyapatite functional groups in the biocomposite indicate that combination BAM-HA is feasible.

The formation and penetration of tissue in a biomaterial can be affected by the porosity, size, and pore structure of the scaffold. Therefore, this research also carried out SEM analysis, a method of examining the surface of samples, which provides information about their morphology.[20] Morphological characterization is essential for the primary purpose of the scaffold. It helps in cell proliferation. The SEM analysis results show micro-sized pores on the sample surface. The size of pores plays a crucial role in fostering cell growth and the diffusion of nutrients, thereby promoting the attachment of cells and vascularization. In addition, pores can be used as a standard parameter for oxygen (O_2) transport and nutrients to the inner extracellular matrix scaffold.[18]

The optimal standard pore size for osteoconduction sockets ranges from 200-350 µm, while soft tissue regeneration ranges from 20-125 µm.[21] To determine and measure the pore area, Image-J is used. Imgae-J is a program designed to speed up the process of image analysis while also automatically gauging the area of pores area. The pore size is in the range of 40-80 µm for the BAM-HA 4:1 biocomposite. Meanwhile, no pores were found in the BAM-HA biocomposite with a ratio of 4:2.

The findings from the EDX examination of human alveolar bone indicate that Calcium (Ca), Phosphorus (P), and Oxygen (O) are present in human bone, comprising the fundamental components of hydroxyapatite (HA). Additionally, there are components iron (Fe), copper (Cu), zinc (Zn), Bromium (Br), strontium (Sr), magnesium (Mg), lead (Pb), and sodium (Na) which exist in limited quantities within the bones of humans. The composition of this material is almost the same as that contained in the BAM-HA biocomposite.[22] The EDX BHA test results show that BHA contains the elements Ca, P, and O, which are the essential components. The elements Mg and Na were also found as minor components. The EDX results of the BAM-HA biocomposite show that the fiber surface contains hydroxyapatite, with calcium and phosphorus peaks, but at a lower level than the carbon peak.

More evidence-based research is needed to develop more effective methods to improve the ability of fresh bovine amnion as a base material for scaffold manufacture in regenerating alveolar bone. Furthermore, due to the limitations of this research, as a recommendation it is also necessary to carry out further research on cells, experimental animals and clinical trials to ensure that this material really has potential as an alveolar bone scaffold.

CONCLUSION

Based on the research results, the characterization test for forming BAM-HA biocomposites with a ratio of 4:1 and 4:2 has been successful. FTIR analysis showed that fresh bovine amnion membrane and hydroxyapatite bonded well. Meanwhile, SEM test results ~~show~~ that the fresh bovine amnion membrane-hydroxyapatite 4:1 biocomposite can regenerate soft tissue but is not osteoconductive. This result is better than the 4:2 fresh bovine amnion membrane-hydroxyapatite biocomposite, with no pores. The EDX results also showed that the fresh bovine amnion membrane-hydroxyapatite biocomposite contained several elements in joint with scaffold bones. Thus, BAM-HA biocomposites can be an alternative as a material for tissue engineering, especially in alveolar bone.

3.Bukti konfirmasi revisi final dan Accepted

6 Oktober 2024

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Octarina <octarina@trisakti.ac.id>

Minor Revisions

11 messages

Dr. Yuniardini Septorini Septorini Wimardhani <editor-jdi-1722-3145072@dcuischolarhub.bepress.com>
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Cc: The Authors <authors-jdi-1722@dcuischolarhub.bepress.com>, The Editors <editors-jdi-1722@dcuischolarhub.bepress.com>

Sun, Oct 6, 2024 at 2:16 PM

Dear Octarina Octarina and Elly Munadziroh

"Characterization Of Combined Fresh Bovine Amnion Membrane With Hydroxyapatite As Candidate Scaffold Alveolar Bone" has been accepted into Journal of Dentistry Indonesia.

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1 message

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To: Octarina Octarina <octarina@trisakti.ac.id>, Elly Munadziroh <elly-m@fkg.unair.ac.id>
Cc: The Editors <editors-jdi-1722@dcuischolarhub.bepress.com>

Dear Octarina Octarina and Elly Munadziroh,

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