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Physical Modification of Bovine Amniotic Membrane for Dental Application

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Abstract

Collagen sponge are used as biomaterial in the field of tissue engineering to increase the life expectancy. Bovine amniotic membrane (BAM) contains collagen and growth factors that will accelerate wound healing after dental extraction procedure. The physical structure of the BAM in the form of a thin sheet will be difficult when applied to the extraction socket. The aims of this study were to modify the physical structure of BAM in the form of a thin sheet into a sponge after that analyze its characteristics by SEM and FTIR. Fresh BAM was washed and divided into 2 parts. The first part in the form of sheets is immediately freeze dried. The second part was cut into pieces then added PBS in a ratio of 1:1. BAM and PBS are then blended until they form a slurry and are sticky like jelly, then freeze drying. After fabrication BAM and modified BAM, then it characterized by FTIR and SEM. The result of fabrication BAM looks like a thin sheet, while the modified BAM looks like a sponge that has a porous structure by using SEM analysis. FTIR analysis showed the collagen content in the presence of functional groups amide A, Amide B, amide I, amide II, amide III in BAM and modified BAM. In this study, modification of BAM will form a porous physical structure like a sponge without losing its collagen as main component.

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Introduction

Collagen sponge is a biomaterial used for medical applications.¹ In dentistry this biomaterial can be used as a haemostatic sponge.² This material is applied into socket after extraction procedure to control bleeding.³ Besides being used as a haemostatic sponge, collagen sponge also has a role as a socket preservation biomaterial.⁴ Where the collagen sponge has a porous texture that will support the growth of osteoblast cells.⁵

Amniotic membrane is a biomaterial containing collagen and growth factors. The amniotic membrane is located in the deepest part of the placenta surrounding the embryo with 0.02 to 0.5 mm thickness. This membrane is a thin, tough, transparent, avascular composite layer.⁶ The amniotic membrane consists of three main layers: a single epithelial layer, a thick basement membrane, and avascular mesenchyme with the

main content of collagen.⁷ In the basement membrane, collagen types I, III, IV V, VI, XV were detected.⁸ The amniotic membrane contains growth factors EGF, TGF- α , KGF, HGF, bFGF, TGF- β 1, - β 2, - β 3, KGFR and HGFR.⁹ The content of the amniotic membrane plays a role in increasing immunomodulation and immunity; anti-microbial, reduce pain; reduce scar tissue and anti-inflammatory; tissue reparative activity with increased bone remodelling, osteogenesis and chondrogenesis; enhances fibrogenesis and angiogenesis; increase extracellular matrix deposition; the main source of mesenchymal stem cells.¹⁰ The bovine amniotic membrane (BAM) has similarities to the human amniotic membrane. BAM is proven, non-toxic, has a molecular weight of 70 kDa, is able to accelerate the formation of collagen which plays a role in the wound healing process.¹¹

BAM which has been fabrication in sheet form will have difficulty applying into the extraction socket after extraction.¹² Because of this, this study aims to make physical modifications of BAM so that it is easy to apply and has the potential to accelerate healing in extraction wounds. Physical modifications to the BAM will be analyzed with the characterization of

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Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR).

Materials and methods

Fabrication BAM

BAM comes from a female bovine from a livestock in Malang, Surabaya with normal births. The fabrication of BAM was carried out at the Biomaterial Center Dr. Soetomo Tissue Bank Surabaya, Indonesia. Fresh BAM were cleaned of blood clots and washed with 0.05% saline four times for 10 minutes. After that, it was washed again with distilled water until the saline solution was clean. Then the membrane is stretched and covered with gauze. After that, put it in the freezer with a temperature of -80°C for 1x24 hours. Then freeze drying for 2x24 hours at a temperature of -100°C . At the final stage, sterilization with 25kGy gamma rays.

Fabrication Modified BAM

The modification of BAM was carried out at the Biomaterial Center Dr. Soetomo Tissue Bank Surabaya, Indonesia. Fresh BAM were cleaned of blood clots and washed with 0.05% saline four times for 10 minutes. After that, it was washed again with distilled water until the saline solution was clean. BAM was then cut into pieces with scissors as much as possible about with a size of approximately 3x5 cm. Then, added with 0.9% NaCl in a ratio of 1:1. After that, it was mashed using a blender and rotated for 30 seconds to 1 minute periodically until it produced amniotic slurry and have consistency like a jelly. Then put into a mould with a diameter of 10 cm and freezer with a temperature of -80°C for 1x24 hours. After that, freeze drying for 2x24 hours at a temperature of -100°C . At the final stage, sterilization with 25kGy gamma rays.

Scanning Electron Microscope (SEM) Characterization

SEM analysis was conducted at the Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology (FMIPA ITB). BAM was prepared with a size of 0.5 cm x 0.5 cm. The sample was then coated with gold (fine coater JFC 1600) prior to testing. SEM analysis was used (JEOL, JSM 6510-LA, Japan). The prepared sample was then put into the chamber and vacuumed in high vacuum mode with a 15 kV SEI detector. Magnification settings are 50x and 500x, then focus and stigma X and Y stigma are adjusted to refine the image. The

morphological description of the porous surface, the size of the pore and the shape of the resulting pore are then analyzed.

Fourier Transform Infrared (FTIR) Characterization

The FTIR analysis was carried out at the Isotope and Radiation Central of the Nuclear Energy Agency (PAIR BATAN). BAM sample preparation with a maximum size of 0.5 x 0.5 mm. The prepared sample was then added with potassium bromide (Sigma Aldrich, Germany) in a ratio of 1:50. The mixture was ground using mortar and paste until homogeneous. After being homogeneous, a pan with a diameter of 4 mm was inserted for testing on the FTIR tool. Functional group analysis using IR Prestige-21 (Shimadzu, Japan). Transmittance percentage measurement method, 4.0 resolution, measuring distance 400 to 4000 cm^{-1} .

Results

Fabrication of BAM and Modified BAM

In this study, fabrication of BAM and modified BAM was carried out. The fabrication of BAM appeared to be a thin sheet like a paper (Figure 1a). While the modified BAM looks like a porous sponge (Figure 1b).

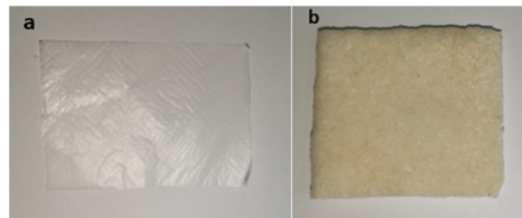


Figure 1. Fabrication (a) Bovine amniotic membrane, (b) Modified bovine amniotic membrane.

SEM Analysis

In this research, an analysis of the surface structure and morphology of the material was carried out using SEM. SEM images on BAM at 50x and 500x magnification (figures 2a and 2b) show a flat surface. Meanwhile, the modified BAM showed macro and micro pore structures with magnifications of 50x and 500x (Figures 3a and 3b).

FTIR analysis

Figure 3 shows the results of functional group of BAM and modified BAM with FTIR analysis. The

BAM (figure 3a) shows the peak of amide A 3091 cm^{-1} , amide B band 2958 cm^{-1} , amide I band 1680 cm^{-1} , amide II band 1570 cm^{-1} , amide III band 1244 cm^{-1} . While in the modified BAM (figure 3b) shows peak of amide A 3076 cm^{-1} , amide B 2960 cm^{-1} , amide I 1670 cm^{-1} , amide II 1547 cm^{-1} , amide III 1245 cm^{-1} .

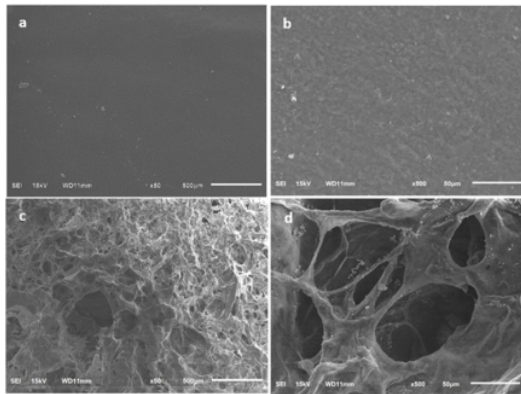


Figure 2. SEM overview (a) 50x magnification BAM, (b) 500x magnification BAM, (c) 50x magnification modified BAM, (d) 500x magnification modified BAM.

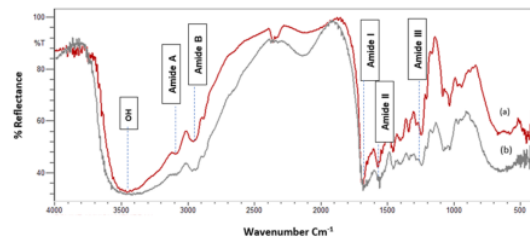


Figure 3. FTIR spectrum of (a) BAM and (b) modified BAM.

Discussion

Bovine amniotic membranes are used in tissue engineering because of their potential like the human amniotic membrane. The advantage of the bovine amniotic membrane (BAM) is the ease of legality, ethics and is not influenced by ritual beliefs as in the human amniotic membrane.¹³ BAM can be produced in large quantities on livestock, with good nutrition so as to produce amniotic membranes with good elasticity and thickness. BAM has growth factors like the human amniotic membrane. This growth factor

will accelerate the re-epithelialization process so that wound healing will be faster.^{14,15} Collagen can accelerate wound healing and serve as a template, initiating and disseminating mineralization independent of matrix vesicles during bone tissue formation.¹⁶

In this research, a modified BAM was made using the technical principles and procedures of sol and gel. This method is a synthesis method that applies two important phases namely sol and gel phase. The fabrication step procedure is as follows: fabrication of the sol phase, gelation of the sol and removal of the liquid phase.¹⁷ PBS was used in this study as an organic solvent, to facilitate the refining of BAM with a blender. During this procedure, the gelation of the sol occurs, which is characterized by the presence of a sticky, jelly-like amniotic slurry. To remove the liquid phase at this stage, the super critical freeze-drying method (aerogel) was used. This freeze-drying method will sublimate the water content without removing components or changing the structure of the biomaterial. This process will cause a pore structure in BAM.¹⁸

SEM is a useful tool in tissue engineering studies that provides good detail of changes in cell morphology and cell/scaffold interactions.¹⁹ The SEM description of BAM is the same as the research conducted. BAM looks like a sheet without a porous structure.²⁰ The porous structure of the modified BAM will act as a medium for gas exchange, absorption of wound exudate. This pore also acts as a site for cell growth, vascularization and carrier medicaments so that bone regeneration will be faster.^{21,22,23}

The two spectra (Figures 3a and 3b) show that the BAM and modified BAM contains collagen which is indicated by the presence of amide A, amide B, amide I, amide II, and amide III spectra. The amide A bands associated with the N-H stretching of BAM and modified BAM are present at wave numbers 3091 cm^{-1} and 3076 cm^{-1} , while the amide B bands are at wave numbers 2958 cm^{-1} and 2960 cm^{-1} , respectively. The amide I band, of hydrogen bonding between the N-H and C-O (Gly) strains was confirmed at absorption peaks at 1680 cm^{-1} and 1670 cm^{-1} for BAM and modified BAM, respectively. N-H in bending and stretching vibrations of C-N as amide II were confirmed at wave numbers 1570 cm^{-1} and 1547 cm^{-1} for BAM and modified BAM. Amide III was found at wave numbers 1244 cm^{-1} and 1245 cm^{-1} .²⁴

Conclusions

Physical modification of BAM will create a porous spongy structure without removing the main component of collagen from BAM which plays a role in wound healing. The porous structure of the sponge will be a potential medium for cell growth to accelerate healing.

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Declaration of Interest

The authors report no conflict of interest.

Ethical policy and institutional review board statement

Ethical clearance for this experimental study laboratory was granted by Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission No: 360/HRECC.FODM/VII/2021.

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