# Characterization of Bovine Sponge Amnion (BSA) by a Novel Process for Dental Treatment

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**Abstract**

One of dental treatments in dentistry is tooth extraction. This treatment will cause injury after extraction and biomaterial is needed to accelerate wound healing. Bovine amniotic membrane is a biomaterial with collagen content that can accelerate wound healing. However, in the process of application, the membrane will tear easily and is difficult to apply.

The purpose of this study was to fabricate the bovine amniotic membrane into bovine sponge amnion (BSA) and characterize it. BSA was derived from bovine amniotic membrane with the addition of gelatin which then underwent freeze-drying process and lyophilization. BSA was then characterized using Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), and swelling test.

The result of characterization using FTIR indicated that amide A, amide B, amide I, amide II, and triple helix of collagen groups were shown in the FTIR graphs. The surface characterization using SEM showed a mean porosity size of 304.866 µm with a porosity proportion of 64%. The swelling analysis showed the proportion of swelling was 1320 times.

The BSA has an ideal pore size, functional group and swelling ability for biomaterial candidate in dentistry.

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## Introduction

Post extraction complications was ranked second for dental problems in Indonesia.[1] Complications that occur after tooth extraction will cause large wounds that have the potential to cause critically size bone defects which causes bone healing is not optimal.[2] The wound healing process is a complex process that involves bio-cellular and biochemical activities that run continuously.[3] The wound healing process will involve activation and synchronization of intracellular, intercellular, and extracellular mechanisms, including coagulation and inflammatory events, accumulation of fibrous tissue, collagen deposition, epithelialization, wound contraction, tissue granulation, and remodelling.[4] Wounds caused by extraction require materials that can accelerate wound healing.[5] One of the requirements for wound healing materials is that they can interact well with biological systems.[6]

Amniotic membrane is a biomaterial that has anti-inflammatory and antimicrobial properties that play a role in repairing and regenerating damaged tissue. Amniotic membrane has growth factors such as epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), keratinocyte growth factor receptor (KGFR), and hepatocyte growth factor receptor (HGFR).[7] The extracellular matrix of the amniotic membrane consists of collagen types I, II, III, IV,

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laminin, nidogen, fibronectin, and proteoglycans.[8] One source of the amniotic membrane is bovine. Some of the advantages of bovine amniotic membrane are the ease of legality, ethics, and that it is not affected by ritual beliefs as in the human amniotic membrane.[9] Bovine amniotic membrane can be produced in large quantities on farms, with good food quality so as to produce an amniotic membrane with good elasticity and thickness. The bovine amniotic membrane is rich in growth factors and collagen, which can accelerate the re-epithelialization process in wound healing.[10]

The limitation of the amniotic membrane is its low mechanical properties so that it is easily degraded.[11] This property also causes the amniotic membrane to tear easily upon application.[12] Ease of degradation of the amniotic membrane, will affect the integrity of the structure which is important when healing the extraction wounds with long-term treatment.[13] To solve this problem, modification of the amniotic membrane is required.[14] In dental treatment, the amniotic membrane can be combined with gelatin. Gelatin acts as a binder to form a structure and improve mechanical properties without losing the characteristics of the amniotic membrane.[15] Fabrication the amniotic membrane in the form of a sponge is carried out to provide ease of use. The amniotic membrane with freeze-drying treatment will form a porous structure that resembles a sponge.[16] The porous structure of the sponge helps the gas exchange and absorption of wound exudate. This structure also plays a role as a place for cell growth and vascularization as well as carrying medicaments to accelerate the wound healing process.[17,18]

Material characterization will provide information about the physical and chemical properties of the material. The physical and chemical properties of a material can be influenced by the size and components of the material.[19] The functional group, surface morphology, and swelling are important characteristics of a biomaterial to be used for biomedical applications. The bovine amniotic membrane contains collagen which plays a role in wound regeneration. For ease of application, it is fabricated into a bovine sponge amnion. Based on this, this study aimed to fabricate a bovine amniotic membrane in the form of a bovine sponge amnion (BSA) then analyze its characteristics using Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), and the percentage of swelling as candidates for dental treatment.[20]

## Materials and methods

### Fabrication of bovine amnion sponge specimen

This research is a laboratory experimental in vitro study Bovine amniotic membrane was obtained from the Installation Center for Biomaterials Bank Network Dr. Soetomo Surabaya. The fresh bovine amniotic membrane was cleaned in order to wash away the blood clots that sticked on the membrane and rinsed with saline solution containing antibiotics for four times.

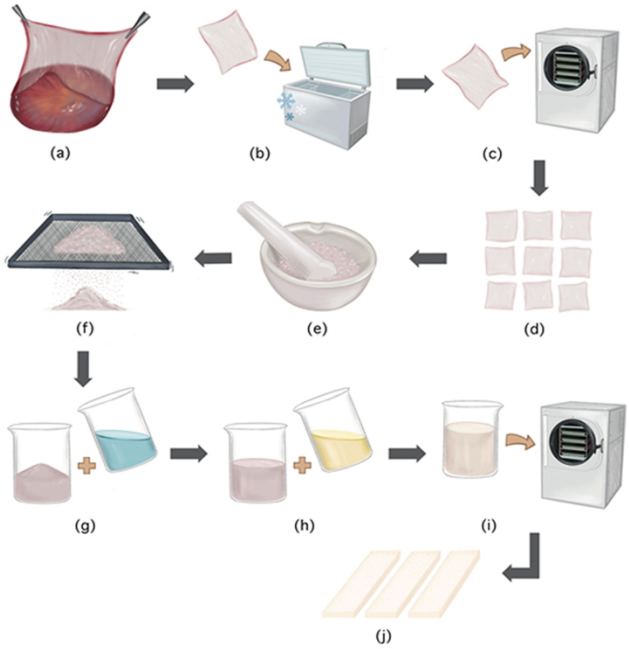


**Figure 1.** Bovine sponge amnion (BSA) fabrication scheme.

The inner amniotic membrane was separated from the chorion (Figure 1A) and put into deep freezer at -80$^{O}$C for 24 hours (Figure 1B). The membrane was then freeze-dried for eight hours (Figure 1C). After the freeze-drying process was carried out, the amniotic membrane would become a dry thin sheet. The next step was cutting the dry thin sheet of bovine amniotic membrane into small pieces (Figure 1D), then crushed with a mortar and pestle until it became smaller pieces of particles (Figure 1E). The particles were sieved with a sieving machine until it was 250 µm in size (Figure 1F). The bovine amniotic membrane was then added with water

($H_2O$) in a ratio of 1:1 to form the amniotic slurry (Figure 1G). The amniotic slurry was then mixed with bovine gelatin in 1:1 ratio and processed by freeze-drying method for 2×24 hours (Figure 1H and 1I). When the amniotic sponge was obtained, the sponge was cut into a size of 1.5×5 mm (Figure 1J).

**Fourier transform infrared spectroscopy (FTIR) analysis**

FTIR analysis was conducted at the Center for Isotopes and Radiation at the Nuclear Power Agency (PAIR BATAN). The sample of bovine sponge amnion was added with KBr powder *(Sigma Aldrich, Germany)* in a ratio of 1:50, crushed using a mortar and pestle. After it was mixed homogeneously, the sample was put into a sample compartment and then tested using an FTIR device for characterization. Functional group BSA analysis was using IR Prestige-21 *(Shimadzu, Japan).* The analysis in this study used the measurement of the percentage of transmittance with a resolution of 4.0 and at a wavelength of 4000 to 400 $cm^{-1}$.

**Scanning electron microscope (SEM) analysis**

SEM analysis was conducted in Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology (FMIPA ITB). BSA samples were prepared with a gold coating before testing. The prepared samples were then put into chamber and vacuumed. The analysis of sample surface, morphology, and components were characterized using SEM *(JEOL, JSM 6510-LA).* The pore size in this study was measured 30 times in different places with a 50x SEM magnification with Image-J software. The percentage of porosity was analyzed by using SEM images with a magnification of 100x with Origin Pro 2021 software.

**Swelling analysis**

Swelling analysis was conducted at DMTCORE FKG USAKTI. In the preliminary test, the swelling presentation on the sample was carried out for 1, 10, 15, 20 minutes, and 24 hours. However, the results of the swelling presentation were not significantly different. In this study, the 10-minute immersion showed the best results and used for swelling analysis calculation.

The initial weight (Ww) of the BSA, was weighed before immersion using an analytical balance *(Fujitsu FSAR-210, Japan)*. BSA samples were immersed in 5 mL phosphate buffer solution (PBS). After immersion, the samples were gently placed on filter paper with a diameter of 9 mm *(Whatman, US)* for three seconds to absorb excess water to measure the final weight (Wd). Swelling analysis was calculated using following equation:

$$Swelling = \frac{Ww - Wd}{Wd} \times 100\%$$

**Results**

**FTIR analysis**

As shown in Figure 2 that OH, Amide A, Amide B, Amide I, Amide II, Amide III, and triple helix of collagen functional group peaks can be observed in the graphs (OH = 3307.92 $cm^{-1}$; Amide Peak A = 3074.53 $cm^{-1}$; Amide peak B = 2929.87 $cm^{-1}$; Amide peak I = 1674.21 $cm^{-1}$; Amide Peak II = 1584.84 $cm^{-1}$; Amide Peak III = 1240.23 $cm^{-1}$; Triple helix of collagen: 1454.53 $cm^{-1}$).

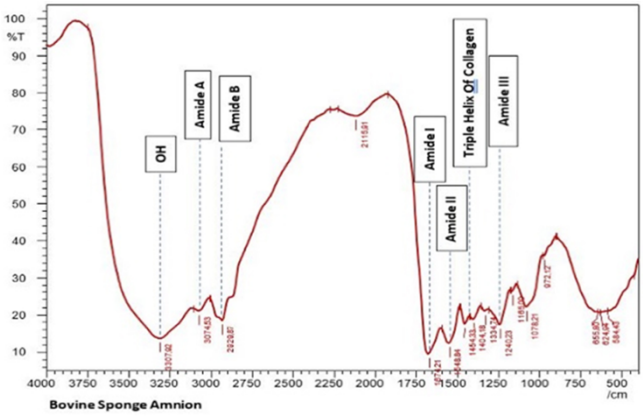


**Figure 2.** Analysis of bovine sponge amnion using FTIR.

The amide A absorption band occurred in the presence of an N-H strain bond from the amide group associated with a hydrogen bond and OH group. The absorption of the amide-I protein absorption band is associated with the C = O stretching mode. Amide-II protein absorption band is associated with the N – H bending mode and the C – N stretching mode. The amide band III is produced from a combination of the C – N stretching phase and the N – H plane bending, with some contribution from the C – C stretching and the C = O bending vibration.

## Discussion

In this study, a bovine amniotic membrane was fabricated with the addition of gelatin to form a bovine sponge amnion. The gelatin used was a ready-made bovine gelatin. The addition of gelatin in this study was carried out with the aim of bonding the biomaterial so that it became solid, producing a firm sponge that did not tear easily during biomedical applications. This study also aimed to analyze the interaction between collagen and gelatin at the time of manufacture. This material characterization can be analyze using FTIR, SEM, and swelling procedures.

Collagen is a protein substance in the form of fiber which is the main part of connective tissue needed for wound healing, scar tissue formation, and formation of bone matrices. Collagen is a key component in wound healing. Initially, type III collagen is more dominant, which then is replaced by type I collagen. After 20 days, the granulation tissue is replaced by collagen and bone begins to form at the base and margins of the extraction socket.[20] The bovine amniotic membrane has the main composition of collagen which plays a role in wound healing. This study was conducted to analyze what components were contained during the fabrication of BSA. The organic components contained in BSA are indicated by the peak of the functional group which can be analyzed using FTIR.

FTIR analysis was carried out to ensure that the compound produced was gelatin by comparing the results of the sample spectrum and the gelatin standard. The structure of gelatin, like most proteins, has carbonyl, amine, and hydroxyl group. Gelatin gives rise to the typical IR absorption of amide A at wavenumbers 3600–2300 $cm^{-1}$, amide I at 1636–1661 $cm^{-1}$, amide II at 1560–1335 $cm^{-1}$, and amide III at 1300–1200 $cm^{-1}$ (Maryam *et al*. 2019).[21] This FITR image supports the findings of this study and shows that the gelatin and collagen in the bovine amniotic membrane bind well in the presence of the amide III functional group.[22] Collagen band characteristics shown in the graphs imply that collagen is retained at each decellularization process.[4]

Triple helix of collagen is type I collagen. In particular, among the various types of collagens, type I collagen is the most abundant extracellular matrix component and can be used as a scaffold, cell migration promotion, wound healing, and tissue regeneration. Resorbable collagen membranes have been used in an integrated manner in tissue regeneration and guided bone regeneration procedures due to its biocompatibility and ability to promote wound healing. The composition of collagen in the fabrication of BSA shows that this biomaterial has potential as a material used for dental applications.

### SEM analysis

The SEM analysis results showed irregular pore of BSA samples with magnification was 50x and 100x (Figure 3A and 3B). The pore is a combination of macro and micro pore sizes. The results of this study showed the smallest pore was 133.101 µm and the largest was 605.803 µm with an average pore of 304.866 µm. The porosity percentage in BSA showed 64% porosity (Figure 3C). Pore structure in biomaterial is needed and important in wound healing process where gas permeability, absorption of wound exudates, cell development, exchange of nutrients, and metabolites will occur inside the pores.[18] The microstructure, pore size, and porosity percentage of a biomaterial can be analyzed using SEM. The combination of macro and micro pores sizes in this study can be caused or influenced by the fabrication method. Freeze-drying method creates gelatin sponges with large and clearly visible pores.[23]

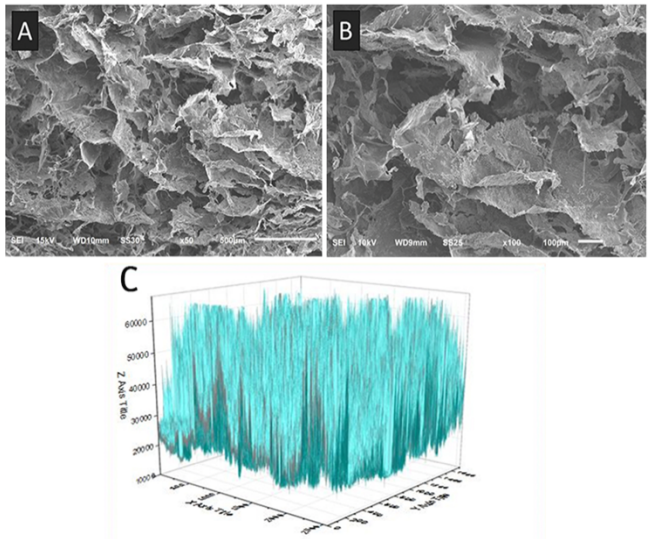


**Figure 3.** SEM overview of the morphology and microstructure of bovine sponge amnion. (A) 50x magnification, (B) 100x magnification; (C) Image of 3D color fill surface overview of bovine sponge amnion porosity percentage.

Image-J is a software for analyzing digital images that has been widely used in medicine and biology.[24] In this study, the magnification was at 50x SEM image to calculate pore size. These results are in line with the study results of Murphy *et al*. (2010) that the ideal mean size of the pores for cell proliferation and migration in bone tissue formation is 325 µm. Minimum pore size for bone growth is in the optimal range of 100–135 µm. The pore size exceeding 300 µm will play a role in the process of vascularization.[25] Combined pores larger than 300 µm lead to direct osteogenesis while pores smaller than 300 µm can promote osteochondral ossification. As a scaffold, the pores on sponges have an oval structure with an ideal diameter ranging from 100 to 500 µm.[26]

To allow cell growth to occur, the scaffold must have a porous structure with a porosity value of 80-90% to support regeneration. The greater the percentage of scaffold porosity, the faster the cell proliferation and differentiation.[27] In this study, the percentage porosity analysis was less than 80%. This can be affected by the freeze temperature and the freeze-drying method used.[28] This porosity percentage value can be influenced by the location where SEM images were taken. The definition of the size of the porosity is limited to the pixel size of the SEM images. Image pixel size affects the smallest measurable scale value and causes large deviations in the mean of porosity percentage. Biomaterials used for biomedicine generally have soft pores that formed a three-dimensional network.[29] Analysis with the use of Micro-computed X-ray tomography (Micro-CT) can improve the accuracy of the calculation of the percentage of porosity in biomaterials with porous structures. This analysis can also provide three-dimensional characterization of complex multi-porous microarchitectures.[30] After all, SEM results in this study indicate that this material has a combination of macro and micro size pore structures that can act as a material for wound healing in dental treatment.

| | Before Immersion (gr) | After Immersion (gr) | Swelling percentage (%) |
|---|---|---|---|
| Specimen 1 | 0.0019 | 0. 0270 | 1321 |
| Specimen 2 | 0.0017 | 0.0223 | 1211 |
| Specimen 3 | 0.0017 | 0.0260 | 1429 |
| Mean ± SD | | | 1320 ± 108 |

**Table 1.** The percentage of swelling after immersion in PBS solution.

The swelling ability plays an important role in tissue healing process, where the transfer of nutrients and body fluids into the biomaterial will be maximized if a biomaterial has good swelling capability.[31] In this study, when the sample was put into the PBS solution, the sample immediately expanded and after ten minutes, the mean swelling percentage reached 1320%. This result is in line with that conducted by Angulo and Sobral (2016) who used a incorporation of gelatin to fabricate scaffolds for tissue engineering. Gelatin has the ability to increase the swelling ability of the biomaterial.[32] BSA contains collagen which causes this biomaterial to have high hydrophilicity properties. Hydrophilicity is the ability of collagen to bind water through hydrogen bonds, so that collagen is more easily dissolved in water. If the hydrogen bonds meet water ($H_2O$), then there is an intermolecular attraction at the H atom which is attached to the very electronegative atoms (N, O, and F).[33] A study by Cheng *et al*. (2017) shows that collagen sponges show a higher water absorption rate this can be caused with the presence of a porous collagen sponge structure and water retention of the collagen fibers.[34]

Research conducted by Guclu et al (2017) increases the swelling capacity of a biomaterial that can occur up to 1200% to 1900% as a candidate biomaterial for bone healing.[35-37] This shows that swelling is also an important feature for developing suitable tissue engineering constructs for bone defect regeneration.[27] The study by Indrawati *et al*. (2019) shows that BSA increase the bone morphogenetic protein-2 (BMP-2), as a marker of osteogenesis from the first day to the seventh day after the application in post-extraction of rat teeth.[12] This swelling presentation has been able to make BSA as a material for wound healing not only of soft tissue but also of hard tissue.

## Conclusions

Based on the results of this study, it shows the potential characteristics of BSA as a biomaterial that can improve wound healing in dental treatment. Further studies are recommended for in vivo animal studies to analyze the effect of biomaterials on wound healing and regeneration after tooth extraction.

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### Declaration of Interest

The authors declare that there are no conflicts of interest.

### Ethical policy and institutional review board statement

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Airlangga University, Surabaya (No. 293/KKEPK. FKG/XII/2016).

### Swelling analysis

In this study, the 10-minute immersion showed the results of the swelling percentage of 1321%, 1211%, and 1429% which are shown in Table 1.

### References

1. Riskesdas. Laporan Riskesdas 2018. Badan Penelitian dan Pengembangan Kesehatan. Kementrian Kesehatan Repubilik Indonesia. 2018:181-222
2. Walsh DP, Raftery RM, Chen G, Heise A, O'Brien FJ, Cryan SA. Rapid healing of a critical-sized bone defect using a collagen-hydroxyapatite scaffold to facilitate low dose, combinatorial growth factor delivery. J Tissue Eng Regen Med. 2019;13:1843–53.
3. Eming S, Paul M, Tomic-Camic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci Transl Med. 2014;6:1–36.
4. Ballesteros ACV, Puello HRS, Lopez-Garcia JA, Bernal-Ballen A, Mosquera DLN, Forero DMM, et al. Bovine decellularized amniotic membrane: Extracellular matrix as scaffold for mammalian skin. Polymers (Basel). 2020;12.
5. Pei Y, Ye D, Zhao Q, Wang X, Zhang C, Huang W, et al. Effectively promoting wound healing with cellulose/gelatin sponges constructed directly from a cellulose solution. J Mater Chem B. 2015;3:7518–28.
6. O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Mater Today. 2011;14:88–95.
7. Koizumi N, Inatomi T, Sotozono C, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. Curr Eye Res. 2000;20:173–7.
8. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian M, et al. Properties Of The Amniontic Membrane For Potential Use In Tisseu Enginering. Eur Cells Mater. 2008:88-99
9. Putra NHD, Matulatan F, Wibowo MD, Danardono E. Effects of dried bovine amniotic membrane as prosthetics of abdominal fascial defect closure observed by the expression of platelet-derived growth factor in Rattus norvegicus wistar strain. Syst Rev Pharm. 2020;11:987–91.
10. Yang Z, Cui Q, An R, Wang J, Song X, Shen Y, et al. Comparison of microbiomes in ulcerative and normal mucosa of recurrent aphthous stomatitis (RAS)-affected patients. BMC Oral Health. 2020;20:1–8.
11. Cai M, Zhang J, Guan L, Zhao M. Novel implantable composite biomaterial by fibrin glue and amniotic membrane for ocular surface reconstruction. J Mater Sci Mater Med. 2015;26.
12. 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;16:283–8.
13. Dimitriou R, Mataliotakis GI, Calori GM, Giannoudis P V. The role of barrier membranes for guided bone regeneration and restoration of large bone defects: Current experimental and clinical evidence. BMC Med. 2012;10:1-24
14. Dadkhah Tehrani F, Firouzeh A, Shabani I, Shabani A. A Review on Modifications of Amniotic Membrane for Biomedical Applications. Front Bioeng Biotechnol. 2021;8:1–25.
15. Zhang Q, Qian C, Xiao W, Zhu H, Guo J, Ge Z, et al. Development of a visible light, cross-linked GelMA hydrogel containing decellularized human amniotic particles as a soft tissue replacement for oral mucosa repair. RSC Adv. 2019;9:18344–52.
16. Kang M, Choi S, Cho Lee AR. Effect of freeze dried bovine amniotic membrane extract on full thickness wound healing. Arch Pharm Res. 2013;36:472–8.
17. Cheng H, Li C, Jiang Y, Wang B, Wang F, Mao Z, et al. Facile preparation of polly sacharide-based sponges and its potential application in wound dressing. J Mater Chem B. 2013;1–8.
18. Long H, Ma K, Xiao Z, Ren X. Preparation and characteristics of gelatin sponges crosslinked by microbial transglutaminase. PeerJ. 2017;1–18.
19. Khalid P, Suman VB, Shameema S, Vinodini NA, Shela RP, Chattergee PK. Analytical methods for nanomaterial characterization. Int J Nano Dimens Anal methods Nanomater Charact. 2014;5:309–20.
20. Octarina, Munadziroh E, Razak FA. Physical Modification of Bovine Amniotic Membrane for Dental Application. Journal of International Dental and Medical Research. 2021;14(4):1425-8
21. Steiner GG, Francis W, Burrell R, Kallet MP, Steiner DM, Macias R. The healing socket and socket regeneration. Compend Contin Educ Dent. 2008;29:1-14
22. Maryam S, Effendi N, Kasmah K. Produksi dan Karakterisasi Gelatin dari Limbah Tulang Ayam dengan Menggunakan Spektrofotometer Ftir (Fourier Transform Infra Red). Maj Farm. 2019;15:96.
23. Muyonga JH, Cole CGB, Duodu KG. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus). Food Chem. 2004;86:325–32.
24. Kabiri M, Emami SH, Rafinia M, Tahriri M. Preparation and characterization of absorbable hemostat crosslinked gelatin sponges for surgical applications. Curr Appl Phys. 2011;11:457–61.
25. uarez-Arnedo A, Figueroa FT, Clavijo C, Arbeláez P, Cruz JC, Muñoz-Camargo C. An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays Alejandra. bioRxiv. 2020;1–21.
26. Murphy CM, Haugh MG, Brien FJO. Biomaterials The effect of mean pore size on cell attachment , proliferation and migration in collagen – glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials. 2010;31:461–6.
27. León-Mancilla BH, Araiza-Téllez MA, Flores-Flores JO, Piña-Barba MC. Physico-chemical characterization of collagen scaffolds for tissue engineering. J Appl Res Technol. 2016;14:77–85.
28. Tohamy KM, Mabrouk M, Soliman IE, Beherei HH, Aboelnasr MA. Novel alginate/hydroxyethyl cellulose/hydroxyapatite composite scaffold for bone regeneration: In vitro cell viability and proliferation of human mesenchymal stem cells. Int J Biol Macromol. 2018;112:448–60.
29. Oikonomopoulou VP, Krokida MK, Karathanos VT. The influence of freeze drying conditions on microstructural changes of food products. Procedia Food Sci. 2011;1:647–54.
30. Ahumada M, Jacques E, Calderon C, Martínez-Gómez F. Porosity in biomaterials: A key factor in the development of applied materials in biomedicine. Handb Ecomater. 2019;5:3503–22.
31. Nair M, Shepherd JH, Best SM, Cameron RE. MicroCT analysis of connectivity in porous structures: Optimizing data acquisition and analytical methods in the context of tissue engineering. J R Soc Interface. 2020;17:1-16
32. Maji K, Dasgupta S, Pramanik K, Bissoyi A. Preparation and Evaluation of Gelatin-Chitosan-Nanobioglass 3D Porous Scaffold for Bone Tissue Engineering. Int J Biomater. 2016;2016:1-14
33. 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.
34. Peng L, Xiang RC, Jia WW, Dong XX, Wang GE. Preparation and evaluation of porous chitosan/collagen scaffolds for periodontal tissue engineering. J Bioact Compat Polym. 2006;21:207–20.
35. Cheng X, Shao Z, Li C, Yu L, Raja MA, Liu C. Isolation, characterization and evaluation of collagen from jellyfish Rhopilema esculentum kishinouye for use in hemostatic applications. PLoS One. 2017;12:1–21.
36. Guclu ZA, Hurt AP, Ohia L, Coleman NJ. Composite Sponges for in Situ Alveolar Bone Regeneration Following Tooth Extraction. Acta Phys Pol A. 2017;131:580–3.
37. Munadziroh E, Soekartono RH, Rafli RBV, Yuliati A, Kartikasari N. Effect of rSLPI Amnion Membrane Application on Incision Wound of Rattus Norvegicus in Collagen and VEGF Expression. Journal of International Dental and Medical Research. 2017;10(2):239-43.