The Fabrication and Characterization of Chitosan-Ethanol Extracted Aloe vera Scaffold for Alveolar Bone Healing Application

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Abstract

Aloe vera is an herbal plant that can stimulate osteoblast cell differentiation and accelerate alveolar bone healing. The combination of chitosan and ethanol extracted Aloe vera are potent to be suitable scaffold for bone tissue engineering. Biological activities of chitosan, however, depend on their molecular weight. The aim of this study was to synthesize and to evaluate the characteristics of chitosan-ethanol extracted Aloe vera scaffold (Ch-Av). It was divided two group: chitosan with low molecular weight-Aloe vera (ChL-Av) and chitosan with high molecular weight-Aloe vera (ChH-Av). Chitosan-Aloe vera with a ratio of 50:50 were made using freeze dry technique. Several characteristic tests were conducted, such as SEM examination, FTIR, XRD, XRF and compressive strength test. The average pore size and compressive strength of ChL-Av scaffolds were 124.85 µm and 2.21 ± 0.29 Mpa, while ChH-Av scaffolds were 94.71 µm and 0.06 ± 0.04 Mpa. Both of scaffolds had good pore interconnectivities and amorph structure. There were -OH group, $-NH_2$ group, and C = O group characteristic absorption bands in FTIR. ChL-Av scaffolds can potentially be used for alveolar bone healing application.

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Introduction

Aloe vera is one of plants growing in tropical or subtropical climates. Aloe vera is greatly grown in Batu, Malang, East Java, a region in Indonesia with a tropical climate. Aloe vera is categorized into the liliaceae family which has around 200 species. In Indonesia, Aloe vera with barbadansis type is generally found, known as Aloe vera linn or Aleo vera L.[1]

Aloe vera, moreover, is also called as a plant of immortality, or known as a magic plant since it has more than 75 active compounds playing an important role in the healing process, proteins (alloktin), amino acids, enzymes, alkaloids, flavonoids, saponins, collagen, vitamins, calcium, potassium, and polysaccharides including pectin, cellulose, hemisellulose, fructan, and mannan.[2-4] Aloe vera is also known to contain 20 of 22 kinds of non-essential amino acids and 7 of 9 kinds of essential amino acids.[5] Several previous researches even reported that Aloe vera extracted with ethanol has more powerful antibacterial and antioxidant activities as well as more protein and polysaccharides than that extracted with water.[6-8]

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Furthermore, alloktin bonds with mono or polysaccharide components can activate complement system, increasing coagulation process to prevent the loss of blood clot and improve scaffold function in bone healing process.[9-11] Alloktin can also stimulate progenitor osteoblasts from skeletal stem cells, increases osteoblast cell differentiation, stimulates signaling of Activator of Nuclear Kappa B Ligand (RANKL), increases Vascular Endothelial Growth Factor (VEGF), Bone Morphogenic Protein (BMP) and osteoprotegerin (OPG), as well as prevents osteoclastogenesis in order to accelerate bone remodeling process.[12,13]

Bone tissue engineering techniques actually have a major component of biomaterials as scaffolds, bone tissue-forming cells, and signals in the form of proteins, enzyme kinases, or growth factors.[14] In the innovation of bone tissue engineering, three-dimensional scaffolds can be absorbed by body, such as polymers chitosan which accelerate bone healing process since the scaffolds can affect cellular activities, including stimulating new cell growth and cell adhesion as well as supporting cell proliferation so that it accelerates bone healing process.[15,16] The molecular weight of chitosan powder can affect its biological activities.[17] The synthesized of scaffolds in combination with chitosan materials is widely carried out, including cross link chitosan-collagen-bovine hydroxyapatite.[18,19]

Bone graft material actually has certain requirements, such as non-toxic, biocompatible, osteogenic, osteoconductive, osteoinductive and good mechanical properties.[20] In producing scaffolds that can support bone tissue regeneration, single material scaffolds are less able for the requirements. A synergistic combination of two ingredients is needed to give optimal function.[15] The combination of chitosan and ethanol extracted Aloe vera will stimulate osteoconduction, osteoinduction, osteogenesis, mechanical strength, physical properties, including good pore size and interconnectivity. Therefore, the aim of this research was to synthesize and to evaluate the characteristics of chitosan -ethanol extracted Aloe vera scaffold.

Materials and Methods

Materials used in this research were chitosan powder purchased from Sigma chemical, St. Louis, USA. The degree of deacetylation was more than 75%, chitosan powder with a high molecular weight of 310,000-375,000 Da (Product number: 419419, Lot number: MKBH5816V) and chitosan powder with a low molecular weight of 50,000-190,000 Da (Product number = 448869, Lot number = MKBH7256V). Aloe vera plants were taken from a plantation in Batu, Malang, East Java, Indonesia.

Preparation of ethanol extracted Aloe vera

Extraction of Aloe vera was performed using maceration method. Aloe vera plants were cleaned, then their spines were removed, and their gel was taken. The inner skin of those Aloe vera plants were cut into small pieces. The smooth gel taken was dried using freeze dry technique and then dissolved in 70% ethanol with a ratio of 1: 4, it was stirred for 30 minutes with a magnetic stirrer, and it was allowed for 48 hours. The maceration result was filtered 3 times with a buctner funnel coated with filter paper and Erlenmeyer. The filtered filtrate was evaporated with a vacuum rotary evaporator. This research used ethanol extracted Aloe vera at a concentration of 50%. Thus, to make 50 mL of ethanol extracted Aloe vera at the concentration of 50%, 25mL of Aloe vera extract was dissolved with 25mL of 3.5% Na-CMC.

Scaffold synthesis

1% chitosan gel (w/v) was made of one gram of chitosan powder diluted in 100 ml of 2% acetic acid. It was then added with 1.25% NaOH solution to get neutral pH. It was stirred until the gel was completely formed. Chitosan powder used had high and low molecular weights. Scaffolds made of the combination of chitosan and ethanol extracted Aloe vera was prepared by mixing 50% ethanol extracted Aloe vera and 1% chitosan gel with a ratio of 50:50. The mixture of chitosan and ethanol extracted Aloe vera was put into scaffold molds, and then frozen at -80^{0} C for 1x24 hours. Freeze drying technique was carried out for 3x24 hours. Scaffolds made of the combination of chitosan powder with low molecular weight and ethanol extracted Aloe vera were called ChL-Av. Meanwhile, scaffolds made of the combination of chitosan powder with high molecular weight and ethanol extracted Aloe vera were known as ChH-Av.

Scaffold pore size and pore interconnectivity (SEM) examination

Scaffolds made of the combination of chitosan and ethanol extracted Aloe vera and coated with sputter coater were vacuumed for 30 minutes. After the vacuum was performed with plasma coating using Au and Pb, the scaffolds were examined with Scanning Electron Microscope (SEM) machine (JCM-5700, JEOL, Tokyo, Japan) at a magnification of 250x. The data then were collected.

FTIR (Fourier Transform Infrared Spectroscopy) Examination

FTIR was conducted using FTIR Thermo Scientific examination tool, Nicolet, and iS10 with a wavelength of 400-4000 cm^{-1}. Reading then was performed using OMNIC program.

X-ray Diffraction (XRD) and X-ray Fluorosence (XRF) Examination

The density of samples was observed using X-ray diffraction examination. This X-ray diffraction examination was performed using a Xray Diffractometer (XRD JEOL JDX-3530) with an angle range of 2ϴ = 10-80^0 as well as X-ray fluoresence tool (PANalytical on version 4.0 of the software for MiniPal spectrometers). XRD diffractogram data were obtained in the form of numbers (x, y), in which x is the angle of 2ϴ, while y is the intensity presented in the form of a diffractogram image. The XRF absorbance data then was processed by comparing the data of the standard elements emerged at spectra peaks, and then converted into the percentage of elements (%) contained.

Compressive strength examination

Compressive strength examination was carried out with Universal Testing Machine (Shimadzu AGS-X). The samples were pressed at 5 mm / minute with a capacity of 1000 N.The reading was performed using TRAPEZIUMX software program. The compressive strength examination was conducted on six samples of every ChL-Av and ChH-Av scaffolds.

Results

Scaffold synthesis

ChLAv and ChH-Av scaffolds can be seen in Figure 1. On the ChL-Av scaffolds, their surface was smoother, denser, and less cavity than ChH-Av scaffolds.

Figure 1. Ch-Av Scaffolds. (A) ChL-Av Scaffolds. (B) ChH-Av Scaffolds.

Pore size and pore interconnectivity (SEM)

Figure 2 shows the SEM results of Ch-Av scaffolds. The largest pore size in ChL-Av scaffolds was 139.9 µm, the smallest one was 110.5 µm and the average pore size was 124.85 µm. The largest pore size in ChH-Av scaffolds was 99.13 µm, the smallest one was 85.09 µm and, the average pore size was 94.71 µm. Open interconnectivity was also seen in both ChL-AV and ChH-Av scaffolds.

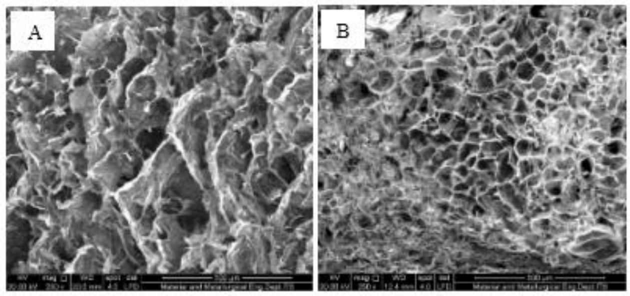

Figure 2. SEM results (A) ChL-Av scaffolds and (B) ChH-Av scaffolds.

FTIR (Fourier Transform Infrared Spectroscopy)

The analysis results of FTIR tests on scaffolds made of the combination of chitosan and ethanol extracted Aloe vera were shown in Figure 3. ChL-Av scaffolds reached their peaks at 1027.63 cm^{-1}, 1053.84 cm^{-1}, 1245.52 cm^{-1}, 1321.93 cm^{-1}, 1408.83 cm^{-1}, 1558.04 cm^{-1}, 1568.24 cm^{-1}, 1732.79 cm^{-1}, 2138.65 cm^{-1}, 2921.82 cm^{-1}, and 3271.81 cm^{-1}. Meanwhile, ChH-Av scaffolds reached their peaks at 922.57 cm^{-1}, 1030.92 cm^{-1}, 1054.06 cm^{-1}, 1247.94 cm^{-1}, 1322.81 cm^{-1}, 1404.33 cm^{-1}, 1558.00 cm^{-1}, 1574.07 cm^{-1}, 1731.27 cm^{-1}, 2134.29 cm^{-1}, 2933.77 cm^{-1}, and 3280.02 cm^{-1}.

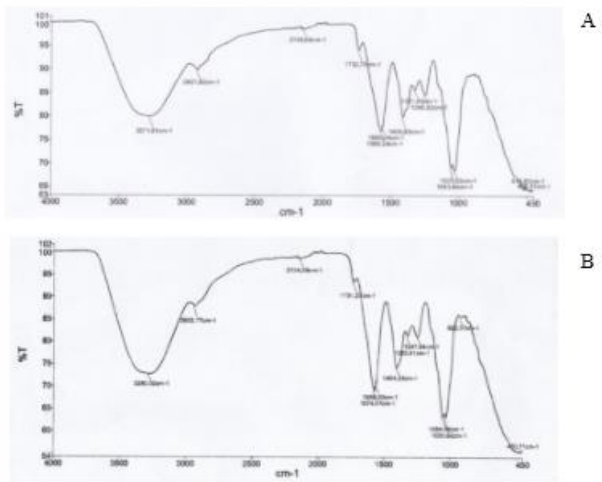

Figure 3. FTIR result (A) ChL-Av scaffolds and (B) ChH-Av scaffolds.

The Ch-Av bond spectrum then indicates the interaction between the two components, namely typical peaks of C-N stretching at 1027.63 cm^{-1} (ChL-Av) and 1030.92 cm^{-1} (ChH-Av), C = O stretching at 1732.79 cm^{-1} (ChL-Av) and 1731.27 cm^{-1} (ChH-Av), O-H stretching at 2921.82 cm^{-1} (ChL-Av) and 2933.77 cm^{-1} (ChH-Av), and N-H stretching at 3271.81 cm^{-1} (ChL-Av) and 3280.02 cm^{-1} (ChH-Av).

X-ray Diffraction (XRD) and X-ray Fluorescence (XRF)

Figure 4 illustrates the results of x-ray diffraction tests on ChL-Av and ChH-Av scaffolds using peak search analysis of diffraction patterns. ChL-Av and ChH-Av scaffolds were known to have amorphous or non-crystalline particles. Based on the absorbance data of the XRF examination, the contents of the elements emerged at the peak spectra indicates that scaffolds made of the combination of chitosan and ethanol extracted Aloe vera contains some mineral components K (12.7%), Ca (15%), Na (57%), Mg (9.2%), and Zr (6.1%).

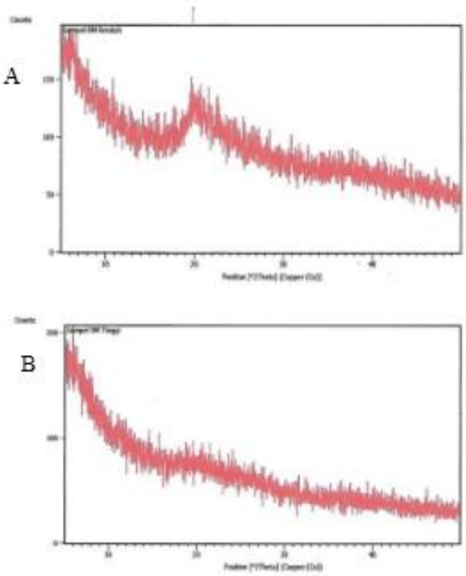

Figure 4. XRD Patterns (A) ChL-Av scaffolds and (B) ChH-Av scaffolds.

Compressive strength

Table 1 and Figure 5 shown that the average of the compressive strength in ChL-Av scaffolds was 2.21 ± 0.29 Mpa, while in ChH-Av scaffolds was 0.06 ± 0.04 Mpa. The data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. T test value p=0.00 (P≤0.05). There was a significant difference between ChL-Av and ChH-Av scaffolds.

Sample no	Compressive strength (Mpa) ChL-Av scaffolds	Compressive strength (Mpa) ChH-Av scaffolds
1	2.60	0.09
2	1.98	0.12
3	2.49	0.05
4	2.18	0.03
5	2.17	0.02
6	1.82	0.03
Mean ±SD	2.21 ± 0.29	0.06 ± 0.04

Table 1. The average of compressive strength.

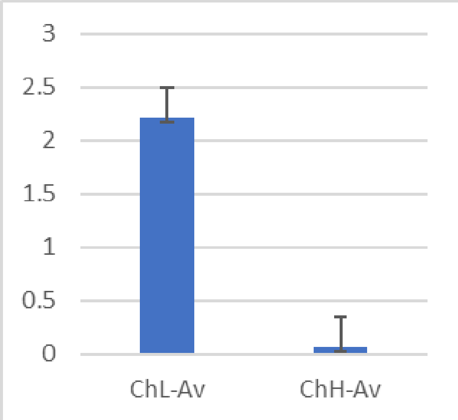

Figure 5. Grafic of Compressive strength.

Discussion

In medical tissue engineering applications, a combination of biomaterials has been developed and used as scaffolds. For instance, Aloe vera has been used as a natural herbal ingredient combined to make scaffolds. Scaffolds made of a combination of chitosan and ethanol extracted Aloe vera can provide a synergistic effect on bone healing process. Chitosan is osteoconductive, the nature of matrix bone graft that can support the attachment of bone-forming cells. Aloe vera, on the other hand, is osteoinductive and osteogenic that can stimulate differentiation of osteoprogenitor cells into osteoblast cells, then triggering new bone formation and bone regeneration.[21] Chitosan can also improve the stability of polysaccharide components in Aloe vera, in which chitosan can also maintain its biological activity. Therefore, the combination of chitosan and Aloe vera is assumed to have degradation, hardness, moisture, and satisfactory mechanical properties.[2] In some previous researches on the manufacture of chitosan scaffolds combined with other ingredients, such as hydroxyapatite, gelatin, and collagen reveals ideal scaffolds for bone tissue enginering.[19]

In this research, Ch-Av scaffolds were made with a ratio of 50:50 using freeze drying method. Chitosan powder which has low molecular weight will have a lower particle size so that it can dissolve easily in acetic acid to produce a gel. When this gel was combined with ethanol extracted Aloe vera gel, it will be more homogeneous and have better consistency. By using the freeze-drying technique, scaffolds produced will have smoother and denser surfaces. The levels of water and shrinkage during the freeze drying process can also affect the pore size of the scaffolds. Based on the results of SEM, ChLAv and ChH-Av scaffolds were known to have porous structures with various pore sizes and open interconnectivity. The average pore size of ChH-Av scaffolds was 94.71 μm, while that of ChL-Av scaffolds was 124.85 μm. The minimum pore size recommended for scaffolds is 100 μm, which can provide a suitable environment for osteoblast and mesemnchymal stem cell proliferations, cell attachment and migration, as well as nutrient diffusion. The open interconnectivity can also increase tissue vascularization and oxygenation.[22,23]

Moreover, based on the results of FTIR in this research, ChLAv and ChH-Av scaffolds were known to have specific interactions between chitosan components and ethanol extracted Aloe vera. Both interact because of the formation of hydrogen bonds. The -OH groups and $-NH_2$ groups in chitosan can form hydrogen bonds with -OH groups and $-NH_2$ groups in Aloe vera. In this research, this was shown from the characteristic absorption broads and 'the strong intensity bands at 3271.81 cm^{-1} (ChL-Av) and 3280.02 cm^{-1} (ChH-Av). Hydrogen bonds are also formed between -C = O groups and $-NH_2$ groups in Aloe vera with OH groups and $-NH_2$ groups in chitosan at 2921.82 cm^{-1} (ChL-Av) and 2933.77 cm^{-1} (ChH-Av).[24,25] The mixing ratio between chitosan and Aloe vera is proportional, which can be seen from the characteristic bands of spectra results.[2] Ionic bonds between chitosan and Aloe vera are also possible, cationic polysaccharida of chitosan and anionic -COOH groups of Aloe vera, leading to a complex polyanionic and polycationic interaction. However, this complex ionic interaction cannot be demonstrated by FTIR results.[25] In this research, the FTIR results of ChL-Av and ChH-Av scaffolds had almost the same spectrum profile, but with different intensity. ChH-Av has a lower intensity than ChL-Av scaffold since it has a longer molecular chain absorbing more vibration energy.[26]

Furthermore, the x-ray diffraction pattern of ChL-Av scaffolds showed that there were low crystalline peak patterns, only at one peak at around 19.50, while there was none found in ChH-Av scaffolds. This means that both ChL-Av and ChH-Av scaffolds have amorphous or non-crystalline particles. The X-ray diffraction results even showed amorphous structure for all Aloe vera samples using both fresh and freeze dries processed techniques.[27,28] Based on XRF test, Ch-Av scaffolds contain some minerals: K, Ca, Mg, and Na. Those mineral components are very important in bone healing process.[29]

The average of compressive strengths between ChL-Av and ChH-Av scaffolds was statistically significantly different. The average of compressive strength on ChL-Av scaffolds was 2.21 ± 0.29 MPa, while on the ChH-Av scaffolds was 0.06 ± 0.04 MPa. The compressive strength of mandibular cancellous bone is 0.5 - 50 MPa.[30] ChL-Av scaffolds are made of chitosan powder which has a lower molecular weight and a smaller particle size so that the scaffolds formed will be more solid and have greater mechanical strength. Compressive strength characteristics are needed by scaffolds to have sufficient strength to resist the hydrostatic pressure of the tissue fluid and maintain the space needed for cell growth and matrix formation.[24,31]

Conclusion

The scaffold combination low molecular weight of chitosan and ethanol extracted Aloe vera (ChL-Av scaffold) can potentially be used as scaffolds for alveolar bone healing process.

Declaration of interest

The authors hereby declare that we have no pecuniary or other personal interest, direct or indirect, in any matter that raises or may raise a conflict with our research. We also gratefully acknowledge the support and generosity of RISTEKDIKTI for the 2016 BUDI DN Scholarship financially supporting this research.

Conflict of Interest

Authors report that there is no conflict of interest and this article is not funded by any research grant.

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