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Collagen-chitosan- glycerol bio-composite as artificial tympanic membrane for ruptured inner ear organ

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Abstract. WHO data in 2012 shows that 5.3% of world population highly suffers from hearing loss and deafness. One of the deafness causes is rupture of tympanic membrane. Tympanic membrane damage which occurs often is perforated tympanic membrane, and it is also commonly known in medical term as tympanic membrane perforation. The causes, for instance, are high frequency of using earphones, traumatic accidents, noise, bacteria, viruses, and infectious microorganism. Tympanoplasty becomes the only treatment that can be widely accepted despite of deficiencies in postoperative complications. Therefore, this research aims to create artificial tympanic membrane made of natural materials such as type I collagen composited with chitosan and made of addition of glycerol to improve its mechanical strength and biodegradability. The method included the process of dissolving acetic acid in distilled water and mixation with chitosan. The solution is next added with glycerol and stirred to be homogeneous. After that, it was minted in petri dish and aerated before characterized. The sample characterization included tensile strength of which tensile test results showed that the value of the elasticity modulus tended to decrease with an increase in collagen concentration. The elasticity modulus values in a row for the variations of 7: 3, 8: 2, and 9: 1 were 35.10 MPa, 54,52 MPa, and 47,45 MPa respectively. The morphological test with 1000x, 2500x, and 5000x magnification showed their interaction in the formation of pores. Cytotoxicity results, moreover, showed that those samples were non-toxic and safe for the body due to the percentage of living cells. The sound absorption coefficient was between 1000 Hz - 2000 Hz which means that it could use as sound absorbing material. The antibacterial test results showed that all the sample variations were anti-bacterial due to the diameter of the clear zone. In conclusion, collagen and chitosan composite with addition of glycerol could be used for potential artificial tympanic for to its characterization

1. Introduction

WHO data on the number of hearing loss and deafness is shocking. In 2000, there were 250 million (4.2%) of the world population suffering from hearing loss, and approximately half (75-140 million) were in Southeast Asia where the prevalence of hearing loss and deafness was high at 4.6%, including Indonesia, which was steadily rising [1]. Tympanic membrane, which is an organ of the inner ear, carries forward impulse sound from the outer ear to the ossicles in the middle ear, then reinforces it before subsequently forwards it to the auditory nerve. The tympanic membrane damage that often occurs is perforated tympanic membrane which is also commonly known, in medical term, as perforation of the tympanic membrane. The causes are inter alia, trauma from an accident, high frequency of using earphones, noise, infectious microorganisms, viruses, and bacteria. The impact of deafness is profound which means that if someone cannot hear, then the individual is inhibited to talk and communicate.

Furthermore, the patient will have difficulty to study and become a retarded citizen meaning that he is a human with low quality and eventually a burden to the family, the community, and the country. Based on previous research, the use of polymer and natural polymers continues to be developed to find the best alloy in the manufacture of artificial tympanic membrane which meets the physical and mechanical properties. Natural material derived from collagen or its derivatives promises better properties in biocompatibility. Collagen is the most important structure in the inner lining of the tympanic membrane which helps to regenerate tissue including cells [2]. Additionally, Collagen in the manufacture of the membrane should be composited with other polymers such as chitosan to be potentially used as a *patch* in order to form the complex structure and interaction of aggregate matrix through covalent and ionic *cross-linking* [3]. Membrane is flexible to be a marker of mechanical quality improvement together with the addition of a *plasticizer* agent, i.e. glycerol, as amino acid-based *polyols* that bridges the polymerization and vegetable *cross-linking* bonds [4].

Therefore, in order to improve the quality of mechanical properties of the acoustic layer of biodegradable membrane and permanently restore hearing ability with sensitivity to power and velocity of sound in the material layer membrane system outside the tissue, functional group analysis test must be conducted via *Fourier Transform Infrared* (FTIR), tensile strength and elasticity modulus test, sound absorption coefficient test, and cytotoxicity assay test. Biomaterials are assumed to be made of chitosan-collagen composite with the addition of glycerol as potential artificial tympanic membrane in ruptured inner ear organ.

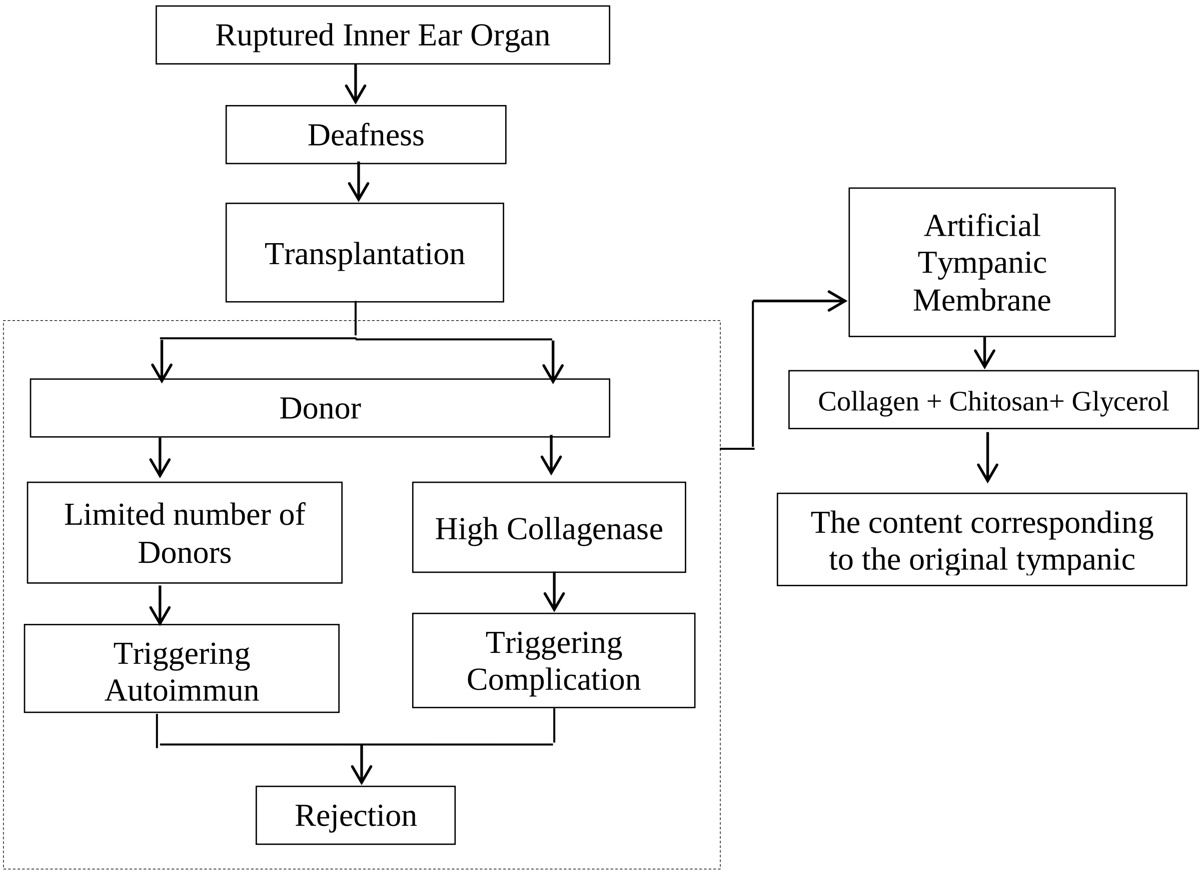

Figure 1. Scheme of research conceptual framework

2. Materials and method

2.1. Materials

Tools used in this research were digital Mettler Toledo scales, magnetic Yellow MAG HS 7 stirrer, Fourier Transform Infrared (FTIR) of Perkin Elmer, IMADA autograph, and Elisa reader. Meanwhile, materials used were collagen type I from red snapper produced by BATAN, chitosan, glycerol, distilled water, and acetic acid

2.2. Methods

2.2.1. Synthesis of Collagen and Chitosan Composite with Glycerol Addition.

First, 2.85 ml of 0.5 M acetic acid was dissolved in 97.2 ml of distilled water. Second, a composition of various solutions was made of a solution of collagen mixed with chitosan in acetic acid solution in order to be homogeneous. Next, the homogeneous solution resulted was added with 0.5 ml glycerol and stirred to be homogeneous. Finally, printing was performed on a petri dish and then aerated before characterized.

2.2.2. Fourier Transform Infrared (FTIR).

FTIR was used to determine the functional groups of the materials used as well as a new functional group obtained from the synthesis conducted on the sample. Next, samples were then added to taste KBr powder which was then compressed using a hydraulic clamp then placed on the specimen and irradiated with infrared wave number 4000-450 cm^{-1} [5].

2.2.3. Tensile Test and Elasticity Modulus Test.

Tensile test was used to determine the elastic properties of the materials when implanted and the mechanical response when the material interacted with the body's tissue samples formed into the shape of a dog bone with 63.5 mm in length and 10 mm and 5 mm in width on each sample variation in accordance with the American Society for Testing Materials (ASTM D 1822 L). Next, the samples were added with a 50 N load at the speed of 10 mm/min. The results obtained were the maximum voltage value (MPa) and the amount of strain and elongation [6]. The formula used to describe the correlation of stress with strain was:

$$E = \sigma / \varepsilon \quad (1)$$

2.2.4. Sound Absorption Coefficient Test.

In this method there are two techniques used to determine the normal sound absorption coefficient. They are standing wave tube method and two microphone tube method. The experiment was set up according to ASTM E1050/ISO 10534-2 test Method for Impedance and Absorption of acoustical Materials by Two Microphone Impedance Tube. This test method is similar to C384 test method which used an impedance tube with a sound source connected to one end, and the test sample was mounted to the other end. However, the measurements techniques for these two methods were different. ASTM E1050/ISO 10534-2 standard was used as a research screening tool, useful for manufactures and researchers in evaluating the absorption of materials. This method has been used in order to determine sound absorption coefficient of absorptive materials at normal incident that is 0 degree by using the reflector of a surface on material. The reflector of a surface on material can be used to determine the absorption coefficient of a surface. The absorption coefficient of a surface is a very useful parameter as it states the fraction of that energy that is absorbed when sound is incident to a surface. [7]

2.2.5. Cytotoxicity Test.

Cytotoxicity test aims to find the toxic effects of a material on cell cultures applied as biomaterial which has met the standards of medical applications [8] through MTT Assay test method. The samples were sterilized, incubated, and then added with MTT reagent in the 3-4 hour incubation before analyzed using *Elisa* reader. Data obtained from the ELISA reader, such as absorbance readings (OD) of each pitting, were then converted into a percentage of the cell.

3. Results and Discussion

The result of this research was artificial tympanic membrane in which the samples on all variations were made with a thickness of 30-90µm in accordance with the original of tympanic membrane (Ruah *et al.* 2000). Next, the artificial tympanic membrane generated was tested to conform to the parameters required to be applied through tensile test, morphology test, cytotoxicity test, and absorption coefficient test.

3.1. Fourier Transform Infra-Red (FTIR),

It was found that cross-links occur in group C = O stretch which is an ester formation at 1647,43 $cm^{-1.}$

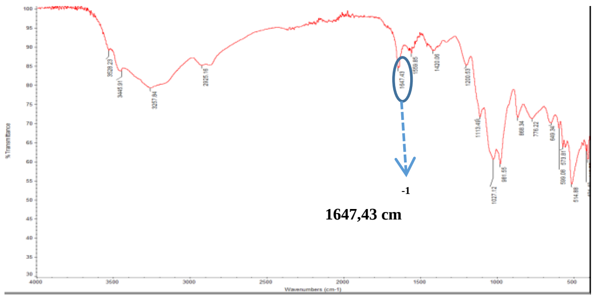

Figure 2. Fourier Transform Infrared (FTIR) Result

3.2. Tensile Strength Test and Elasticity Modulus Test

Tensile strength test was then performed to determine the elasticity of the materials. The test results showed the elasticity modulus values tended to decline with increased concentrations of collagen.

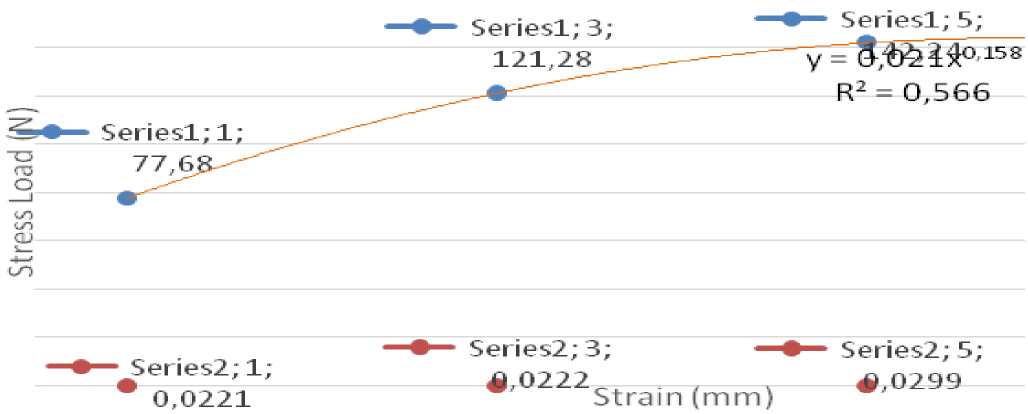

Figure 2. Composite Tensile Test Results

The values of elasticity modulus in a row for the variations of 7: 3, 8: 2, and 9: 1 were 35.10 MPa, 54.52 MPa, and 47.45 MPa respectively. In general, the values of the modulus elasticity decreased as the collagen concentration increased because collagen could conduct brittleness properties in the composite. In accordance with a literature [9], the modulus of elasticity of the human tympanic membrane of 20-60 MPa indicated that the entire sample variations were still in accordance with the functional standard range of human tympanic membrane.

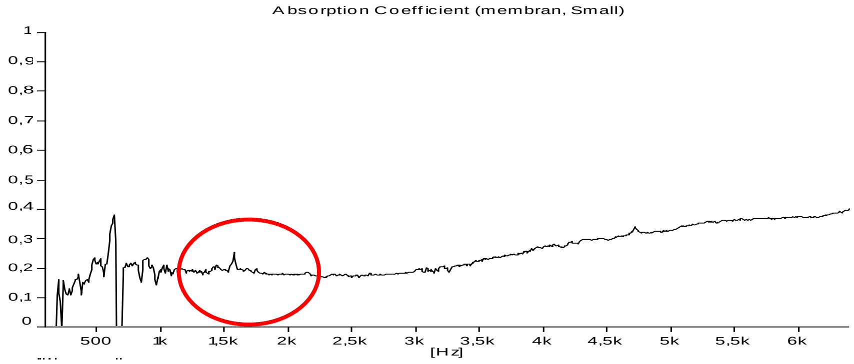

Figure 3. Membrane Absorption Coefficient Spectrum Tympanic Membrane

3.3. Sound Absorption Coefficient Test
The frequency range of human hearing is between 20- 20000 Hz [10]. According to when the coefficient is large (> 0.2), the material is referred to as sound absorbent material, otherwise if <0.2 is called reflective material. In addition to the results graph of FFT spectrum Analyzer in the range of Pure Tone Average / PTA (the threshold of human hearing) is capable of receiving the intensity of the incoming sound up to 100 dB and forward 85 dB into the acceptable range of intensities at normal tympanic membrane in normal hearing. [11]

3.4. Cytotoxicity Test result

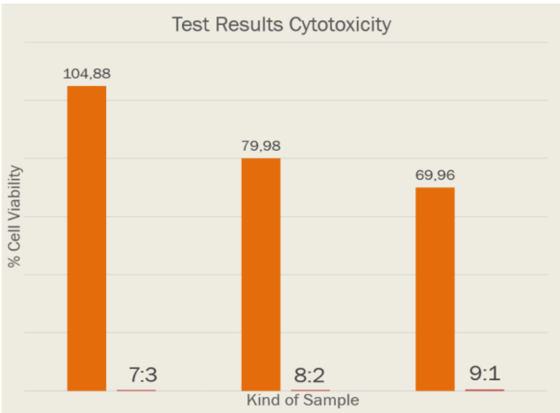

Figure 4. Cytotoxicity test result

The results showed that the cytotoxicity assays (%) of the cell viability in the variations of 7: 3.8: 2, and 9: 1 were 104.88%, 79.98%, and 69.96% respectively. The results obtained indicated that the viability (%) of all samples was more than 50% which means non-toxic [8].

4. Conclusion
Synthesis of Colagen-Kitosan Composite with the addition of glycerol could be obtained by mixing collagen and chitosan with glycerol and printing it in accordance with the dimension of the human tympanic membrane.

The test results showed that the samples had a tensile modulus of elasticity values in the range of 30-60 MPa. The result of Sound Absorption Coefficient Test was showed capable of receiving intensity of the incoming sound up to 100 dB and forwarding 85 dB into the acceptable range of intensities at normal tympanic membrane in normal hearing. Cytotoxicity test results showed that the cell viability (%) was more than 50% which indicates that the samples were not toxic.

Acknowledgment
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References

[1] WHO, 2007 SEA-Deafness-10 Distribution: General Situation Review and Update on Deafness Hearing Loss and Intervention Programmes
[2] Feenstra L, Kohn FE and Feyen J 1984 The concept to fanartificial tympanic membrane Clinical Otolaryngology 9 (1):215-220
[3] Begum AA, Radhakrishnan R and Nazeer KP 2011 Study ofstructure-property relationship on sulfuric acid crosslinked chitosan membranes *Malaysian Polymer Journal* 6 (1):27-38
[4] Mao L, Imam S, Gordon S, Cinelli P and Chiellni E 2002 Extruded cornstarch glycerol polyvinyl alcohol blends mechanical properties, morphology, and biodegradability *Journal of Polymers and the Environtment* Vol 8 (4): 205-211
[5] Smith and Brian C 2011 Fundamentals of Fourier Transform Infrared Spectroscopy Washington: CRC Press
[6] Purwanti A 2010 Analisis kuat tarik dan elongasi plastik kitosan terplastisasi sorbitol *Jurnal Teknologi* Volume 3 Nomor 2 Desember 2010 99-106
[7] Robert Oldfield 2006 Measuring Absorption Coefficient: "*Improved Membrane Absorbers*" University of Salford Salford: UK 41-56
[8] ATCC 2011 MTT Cell Proliferation Assay ATCC® 30 -1010K
[9] Fay J, Puria S, Decraemer WF and Steele C 2005 Three approaches forestimating the elastic modulus of the tympanic membrane *Journal of Biomechanics 38*: 1807–1815
[10] Caldarelli, David D and Ruth S Campanella Ear World Book Online Americas Edition 26 May 2003.
[11] UIUC Physics 406 Acoustical Physics of Music 2016