

MODIFICATION OF GELATIN/CARBONATED HYDROXYAPATITE MEMBRANE WITH CHITOSAN TO IMPROVE THE TENSILE STRENGTH

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ABSTRACT

Membrane for guided tissue regeneration (GTR) is an interesting method for treating periodontitis cases. Synthesis of membranes for GTR based on gelatin and carbonated hydroxyapatite (CHA) modified by chitosan using the freeze-drying method has been successfully carried out. This study aims to obtain the optimum composition between gelatin and chitosan to obtain an effective combination as a membrane base material. The membrane was synthesized using a ratio of gelatin (G) and chitosan (CH) = 1:0, 1:0.5, 1:1, 1:1.5, 1:2 and 1:2.5. The addition of chitosan to gelatin and CHA is intended to improve the physical properties of gelatin to increase membrane stability, both in dry and wet conditions. The synthesized membranes were characterized using SEM-EDX, FTIR, XRD, tensile strength test, and water absorption test. The results of the tensile strength test showed that the membrane with a ratio of G: CH = 1:1 had good mechanical strength. It was indicated by the data of the highest tensile strength compared to other compositions. The SEM-EDX characterization results show that the membrane with the composition G: CH = 1:1 had a fairly hollow surface texture. It allows it to enter the water to have adequate absorption capacity. The absorption capacity of the membrane with a composition of G: CH = 1:1 was not much different from membranes with other compositions, while XRD data showed insignificant differences in crystallinity between membrane material compositions. The FTIR spectra all showed the typical adsorption bands of gelatin and chitosan as well as the formation of hydrogen bonds and electrostatic forces between gelatin and chitosan. The composition of G: CH = 1:1 proved to be more effective as a membrane material composition with the highest tensile strength, namely 1.34 ± 0.07 MPa (dry) and 0.06 ± 0.01 MPa (wet), and an adsorption capacity of $1486 \pm 109\%$.

Keywords: Membrane, GTR, Gelatin, Chitosan, CHA.

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INTRODUCTION

The need for treatment of periodontitis which continues to increase, encourages the development of various therapeutic methods to treat the disease. One method in treating periodontitis cases is to use a membrane as a GTR. The use of GTR in dentistry is currently more specific to its use as guided bone regeneration which is an effective method of regenerating alveolar bone and a means of managing localized defects associated with dental implant placement.¹ Guided bone regeneration is now an important part of implant dentistry because of its ability to restore shape, function, and aesthetics through an ideal placement of the implants.² In recent decades, both resorbable and non-resorbable membranes have attracted the interest of researchers and developed a lot. The use of gelatin and CHA as a membrane base material has been widely studied as an alternative therapy for periodontitis cases.^{3,4} The membrane is resorbable and does not require surgery after therapy is carried out. However, this type of membrane has a disadvantage, namely low tensile strength and the tendency to be easily degraded.⁵ Thus, further research is needed to obtain a membrane that has adequate mechanical strength and good stability.

Chitosan (CH) is potential material in the manufacture of membranes, but its use as a mixture of gelatin in membrane synthesis needs to be studied further. Therefore, this research will study the composition of gelatin and chitosan, which is effective as a membrane base material to obtain better mechanical strength and membrane stability and has advantages both in terms of biocompatibility, biodegradability, and suitable

adhesion between membranes and bone tissue as a drug carrier system. The use of gelatin, chitosan, and CHA has been investigated a lot, but research specifically studying the composition of gelatin and chitosan, which is effective as a membrane base material to increase the stability of the formed membrane, has not been carried out, so far. This study varied six different material compositions in manufacturing membrane potential for GTR.

EXPERIMENTAL

Material and Methods

The materials used in this study were gelatin, chitosan, carbonated hydroxyapatite, ethanol, and acetic acid.

Synthesis of Membranes

The membrane was synthesized using the freeze-drying method with the main precursors of gelatin, carbonate hydroxyapatite, and chitosan. Chitosan (CH) 2 grams were dissolved in 100 ml of 0.5 M (2% (v/v)) acetic acid solution. Gelatin (G) 2 grams were dissolved in 100 ml of demineralized water and stirred for 1 hour at 50 °C, in solution gelatin is added to the CH solution with moderate stirring (the volume ratio of CH and G is 1:0, 1:0.5, 1:1, 1:1.5, 1:2 and 1:2.5) and stirred for about 2 hours at 50 °C (Solution 1). Furthermore, the CHA solution was prepared by dissolving 0.25 grams of hydroxyapatite carbonate (CHA) in 10 ml of ethanol, then stirring for 2 hours at 50 °C called solution 2. Solutions 1 and 2 were mixed and stirred for 1 hour at a temperature of 50 °C. In the next step, the mixture was poured into a petri dish with a diameter of 60 mm and freeze-drying at -55 °C for 48 hours to obtain a porous polymer matrix, and the resulting membrane was characterized by SEM, FTIR, and XRD. Other properties, including water absorption and tensile strength, were tested.

RESULTS AND DISCUSSION

Morphological Analysis

Membrane pores are an essential characteristic as they support fluid uptake and promote infiltration and fibroblast adherence. SEM analysis of the synthesized membrane can be seen in Fig.-1. The membrane surface tends to be hollow and has good inter-pore connectivity but is irregular, allowing for water absorption. The addition of the chitosan composition did not significantly affect the change in the surface texture of the membrane. Based on the results of the composition analysis in Table-1, the EDS spectrum of the membrane shows the characteristics of the elements of gelatin and chitosan: carbon (C), oxygen (O), while CHA: calcium (Ca).

Table-1: Membrane Composition

Membrane	Rate of Mass (%)			
	Carbon	Nitrogen	Oxygen	Calcium
G/CH/CHA (G:CH=1:0)	42.73	29.65	27.23	0.39
G/CH/CHA (G:CH=1:0.5)	39.60	25.98	34.10	0.32
G/CH/CHA (G:CH=1:1)	39.31	24.44	35.77	0.48
G/CH/CHA (G:CH=1:1.5)	36.51	22.89	37.06	0.54
G/CH/CHA (G:CH=1:2)	40.33	22.22	36.62	0.83
G/CH/CHA (G:CH=1:2.5)	40.41	21.93	36.57	1.09

Functional Groups and Crystallinity

The FTIR spectra of membranes are shown in Fig.-2 (left). The presence of gelatin is shown in the absorbance bands of amide around 3448 cm⁻¹ (O-H and N-H stretching), 1635cm⁻¹ (C=O and N-H stretching), 1558 cm⁻¹ (C-N stretching and N-H bending), and 1223 cm⁻¹ (N-H stretching and C-N bending).⁶⁻⁹ The addition of chitosan results in a widening and a slight shift of the absorbance band of gelatin from 3448 cm⁻¹ to 3387 cm⁻¹ for G:CH = 1: 1, indicating the formation of intermolecular hydrogen bonds between gelatin and chitosan.^{8,9} The decrease in the intensity of C=O and N-H stretching of the composite membrane demonstrates that the addition of chitosan can cause a reduction in the content of gelatin helices.^{6,10} The change of the N-H stretching and C-N bending intensity in the composite membrane with the volume ratio change of gelatin to chitosan indicates that the amino group of chitosan interacts mainly with the carboxyl group of gelatin through the formation of electrostatic interaction.^{6,11,12} The shift of the

gelatin absorbance band from 1558 to 1550 cm^{-1} (in G: CH = 1: 1) indicates the formation of a hydrogen bond involving the -NH group of gelatin.⁸

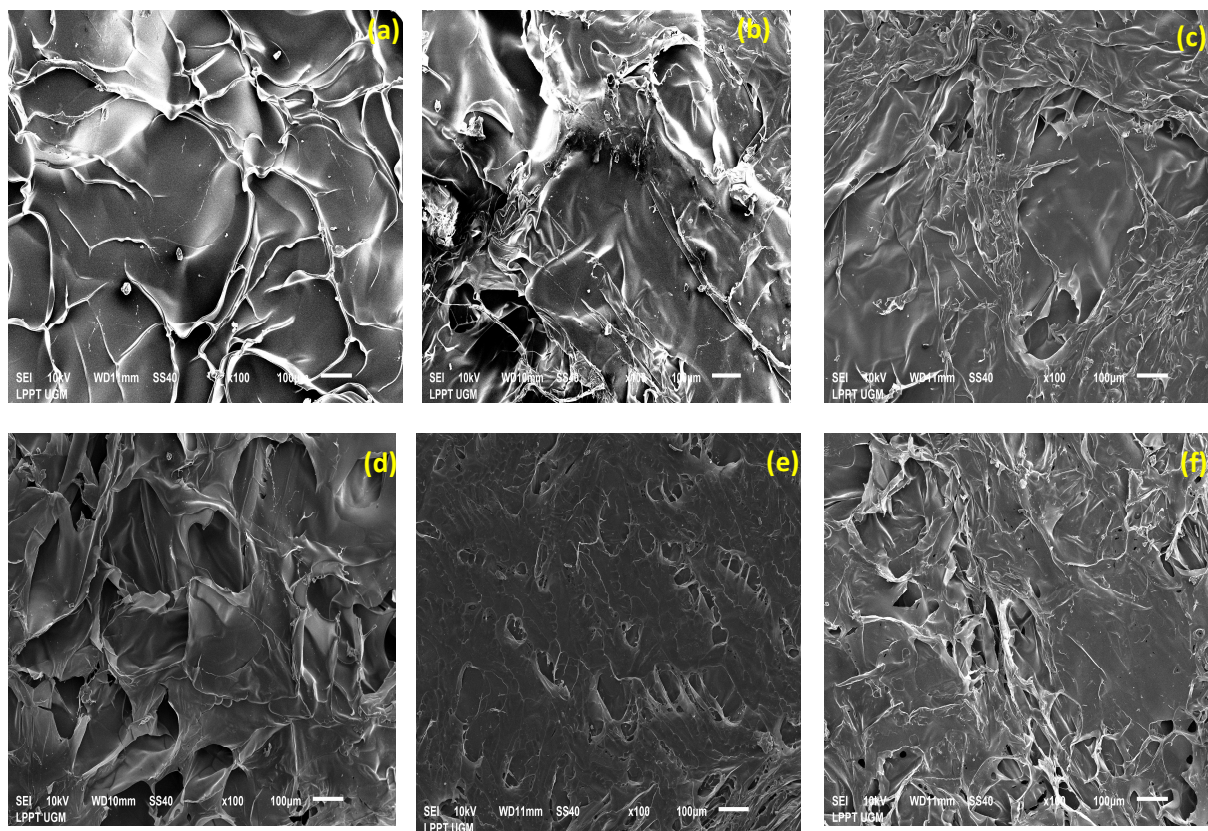


Fig.-1: SEM Images of Membranes (a) G:CH=1:0 , (b) G:CH=1:0.5, (c) G:CH=1:1, (d) G:CH=1:1.5, (e) G:CH=1:2, (f) G:CH=1:2.5

XRD analysis of the synthesized membrane is shown in Figure-2 (right). The diffraction pattern of gelatin is shown at $2\theta = 7.78^\circ$ and $2\theta = 19.18^\circ$.¹³ The chitosan is shown a diffraction pattern at $2\theta = 10.88^\circ$ and 22.14° (ICDD # 00-039-1894), which indicates the presence of crystal structure.¹⁴ The diffraction peak intensity of the composite membrane at about $2\theta = 7.78^\circ$ decreases with the addition of the chitosan. It indicates that the triple helix content in the membrane composite decreased.¹⁵ The composition of chitosan increasingly inhibits the formation of triple-helical structures due to the interaction between gelatin and chitosan.¹⁶ The peak around $2\theta = 11.22^\circ$ (b, c, d, e, and f) indicates that the mixture of chitosan-gelatin is soluble at the molecular level.¹⁵ The presence of the CHA compound is shown at $2\theta = 25.82^\circ$, 31.74° , and 39.98° according to ICDD # 09-432.¹⁷

Tensile Strength

The membrane tensile strength was measured in dry and wet conditions. Figure-3a shows that the ratio of gelatin and chitosan composition = 1:1 produces optimal tensile strength. It applies to both membranes in dry and wet conditions. It can be concluded that the tensile strength of the membrane in both dry and wet conditions shows similar effect to the chitosan addition. The tensile strength in the dry state of the membrane is as follows: the ratio of gelatin and chitosan of 1:0 is 0.3 ± 0.04 MPa, 1:0.5 is 0.7 ± 0.05 MPa, 1:1 is 1.34 ± 0.07 MPa, 1:1.5 is 0.42 ± 0.08 MPa, 1:2 is 0.34 ± 0.08 MPa, and 1:2.5 is 0.32 ± 0.09 MPa. The tensile strength in a wet state is successively as follows: the ratio of gelatin and chitosan of 1:0.5 is 0.034 ± 0.005 MPa, 1:1 is 0.06 ± 0.01 MPa, 1:1.5 is 0.009 ± 0.003 MPa, 1:2 is 0.008 ± 0.003 MPa, and 1:2.5 is 0.006 ± 0.004 MPa. It shows that chitosan in a certain composition can increase the tensile strength, but too much chitosan added will decrease the tensile strength in both dry or wet conditions of the membrane.

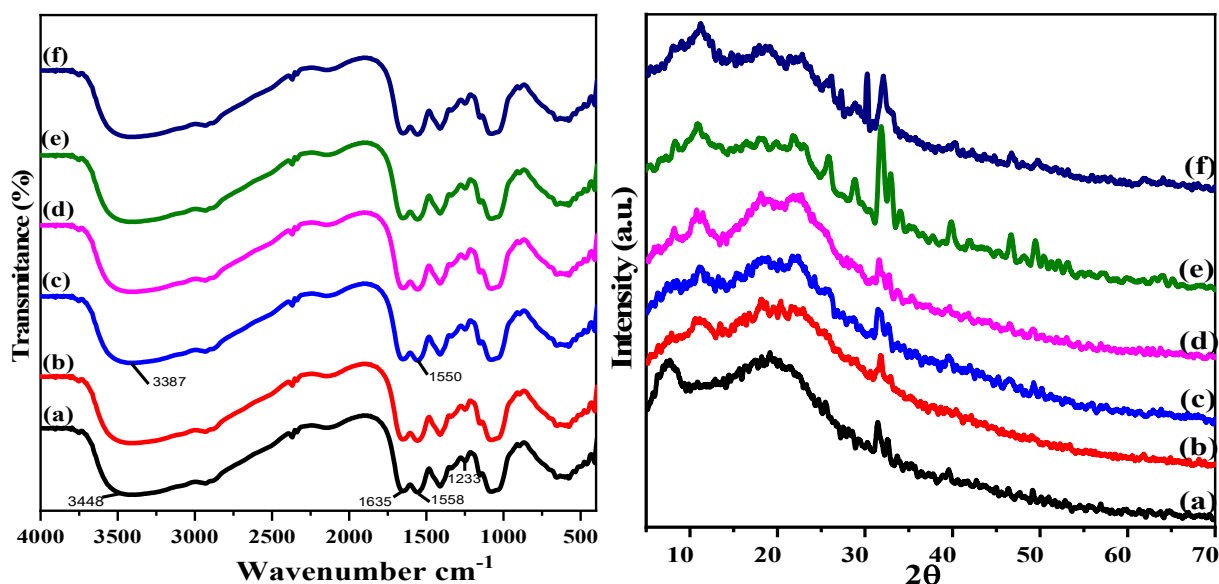


Fig.-2: FTIR Spectra (left) and XRD Patterns (right) (a) G:CH=1:0, (b) G:CH=1:0.5, (c) G:CH=1:1, (d) G:CH=1:1.5, (e) G:CH=1:2, (f) G:CH=1:2.5

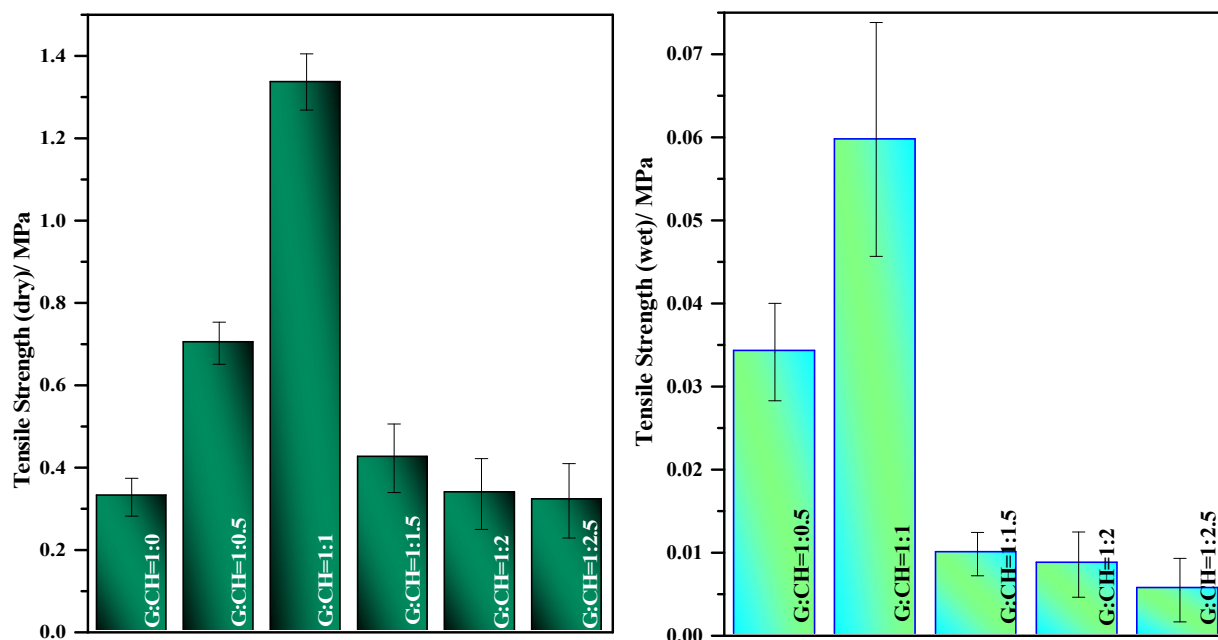


Fig.-3: Tensile Strength of Membrane in Dry Condition (Left) and Wet Condition (Right)

Water Absorption Test

The ability to absorb water is an important property because it can increase the membrane's pore size and surface area/volume to act as a promising drug carrier. The increase in water uptake also affects the exchange of nutrients/waste from the cultural media. However, while the water absorption behavior increases cell adhesion, it can decrease the membrane's mechanical properties.¹⁸ The effect of chitosan addition on the absorption capacity of membranes made of gelatin and chitosan has been investigated in PBS pH 7.4 for 24 hours at 37 °C. Figure-4 presents the degree of water absorption (WA) of all membranes. The membranes made of gelatin, chitosan, and CHA showed high water absorption ability. The water absorption capacity of the membrane increases with the increase in the composition of chitosan as a

constituent of the membrane, causing more significant membrane swelling. It is related to the hydrophilic nature of chitosan to PBS solution. The functional groups of chitosan, which are hydrophilic and responsible for water absorption, are the amine ($-\text{NH}_2$) and hydroxyl ($-\text{OH}$) groups in the repeating unit, making the polymer dissolve in an aqueous acid solution and produce a hydrogel that is springy in water¹⁹. In other words, the more chitosan as the component of the membrane, its absorption capacity will increase.

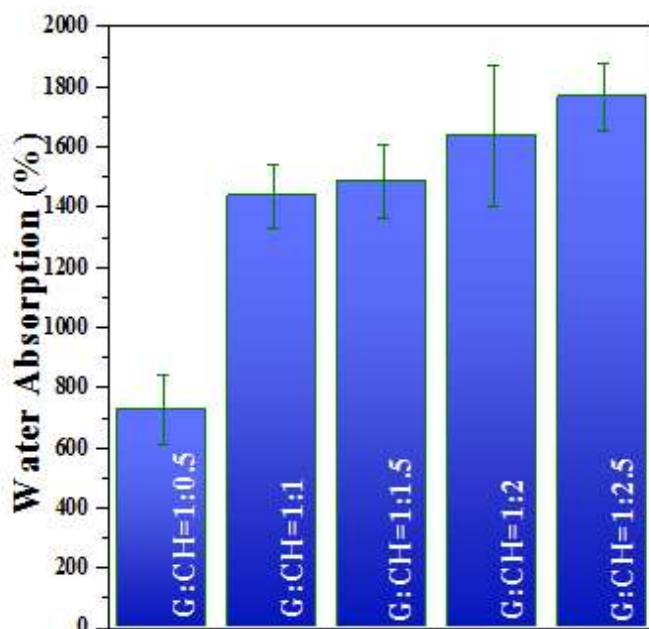


Fig.-4: Water Absorption (WA) of Membrane Samples

CONCLUSION

This research focuses on studying the composition of gelatin and chitosan, effective as the basic ingredients of membranes to increase membrane stability. The ratio G:CH = 1:1 is an effective composition in obtaining a membrane with optimal stability. In more detail, the composition of G:CH = 1:1 proved to be more effective by considering the tensile strength of 1.34 ± 0.07 MPa in dry conditions and 0.06 ± 0.01 MPa in wet conditions, while the adsorption capacity was $1486 \pm 109\%$. This composition makes it possible to obtain a membrane that can be used as a drug delivery system.

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