Comparison of b*ombyx mori l.* Fibroin coatings on the mechanical properties of collagen membranes

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ABSTRACT

Background: *Most of dental implants are accompanied by guided bone regeneration (GBR) procedures. Collagen membrane which is the most widely used type of membrane in the GBR procedure has the disadvantage of not having antimicrobial properties, old bone formation, easy to tear,and soft so that it does not maintain the dimensions of the augmentation area. Bombyx mori L. fibroin has good mechanical properties and has the potential to be used as a pharmaceutical material that is biocompatible, antimicrobial, and stimulates bone growth. The aim of this study was to analyze the effect of Bombyx mori L. fibroin coating on the mechanical properties of collagen membranes.*
Method: *The samples consisted of the control group (group A) and the treatment group, the collagen membrane was coated using the Bombyx mori L dip-coating fibroin technique, namely group B (1 time coating process), group C (2 times coating process) and group D (3 times the coating process). Statistical analysis used one-way ANOVA (p<0.05).*
Result: *The results showed that the average tensile strength of the negative control group was 2.62 MPa, the 1-coated group was 4.51 MPa, the 2-coated group was 5.45 MPa, and the 3-coated group was 1.41. The significance value of ANOVA was 0.000, the post hoc test results showed that there were significant differences in all testgroups with a significance level of 0.005*
Conclusion: *It is concluded that coating Bombyx mori L. fibroin on collagen membranes increases the physicomechanical properties of tensile strength.*

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INTRODUCTION

The prevalence of tooth loss based on the results of the national health research, is 51.4%. (1). Replacement of missing teeth is using a denture that aims to restore the function of mastication,phonation, providing facial muscle support in supporting appearance and maintaining the remaining teeth (2). Based on how to wear a denture consists of a removable denture and a fixed denture. According to Zitzmann *et al,* (3) the number of users of fixed dentures ranges from 31%-45% of all denture users. The number of dental implant users has increased sharply, from 0.7% in 2000 to 5.7%in 2016 (4). The advantages of using dental implants are comfortable use, long wearability, excellent aesthetics, biocompatible with oral cavity tissues (5).

Research shows that more than 50% of dental implant installations require a processus alveolaris growth procedure. One of the procedures for growing the processus alveolaris is guided bone regeneration *(GBR*). (6,7). GBR is a bone regeneration procedure using barrier membrane with *bone graft* material. The application of GBR can increase the success of dental implant treatment up to 93.8% (8).

Based on the ability of absorption by the body, the barrier membrane used in the GBR procedure consists of 2 types, namely non-resorbable and resorbable membrane. Non resorbable membrane is a membrane material that cannot be absorbed by the body. Resorbable membrane is amembrane material that can be absorbed by the body. Examples of this material are polycaprolactone (PCL), chitosan membrane, poly(d,l-lactic acid) (PDLLA) and collagen membrane (7). The most widely used barrier membrane material is collagen membrane (9). *The* disadvantages of this material are that it does not have antimicrobial properties, long bone formation, easy to tear, and softso that it does not maintain the dimensions of the augmentation area (10).

Bombyx mori L. is a widely cultivated species of silkworm. This species has great potential to be utilized in the health field, including as a medicine for bronchial asthma, diabetes, hepatitis, hypertension, antimicrobial, antihistamine, and bone growth spur (11,12). *Bombyx mori* cocoons have macromolecular proteins in the form of fibroin that have biocompatible, osteoinduction, anti-microbial, anti-inflammatory characteristics (13-15). Fibroin has good mechanical properties and accelerates the process of bone formation (16). It also has the ability to increase the tensile strength of medical devices in orthopedic procedures (17).

Fibroin derived from silkworms has the potential to be used as a pharmaceutical material applied by *coating* (18). Silk fibroin can be formed into thin nanoscale coatings. The thickness of this fibroin layer reaches 10 nanometers (19). Based on the description above, the author intends to conduct research on the comparison of *Bombyx mori L.* fibroin coating on the mechanical properties of collagen membranes.

MATERIALS AND METHODS

The research is laboratory experiment. The sample consisted of control group (groupA) and treatment group of collagen membrane coated using *dip-coating* process of *Bombyx mori L.* fibroin, namely group B (1 time *coating* process), group C (2 times coating process) and group D (3 times *coating process)*.

Bombyx mori L. fibroin solution with a concentration of 25 mg/mL was made by mixing

50 mg/mL fibroin 20 mL diluted using distilled water until the volume reached 40 mL. A 20 mL beaker glasswas filled with fibroin *Bombyx mori L.* 25 mg/mL concentration solution as much as 13 mL. Collagen membrane measuring 2x1.5 cm was placed inside the closed beaker glass containing fibroin solution. Collagen membranes were arranged in such a way that all collagen membrane surfaces were submerged. The glass beaker that had been covered with aluminum foil was inserted in a sonicator thathad been filled with distilled water until half the height of the cup was submerged. The sonicator was turned on at a frequency of 20-60 Hz, the soaking process lasted for 12 minutes. The collagen membrane was then removed and dried at room temperature for 24 hours. The coating process was repeated for groups C, and D.

Tensile strength test was conducted using a universal testing machine. Collagen membrane samples measuring 2 cm long, 1.5 cm wide, 0.1 cm thick that had been coated with *Bombyx mori L.* fibroin solution were placed on the longitudinal side between the plates on the universal testing machine. Load was applied to the sample. The vertical compressive force along the sample produced tensile stress perpendicular to the vertical plane passing through the center of the sample plate. Fracture occurred along the vertical plane. Statistical analysis using *one-way ANOVA ($p<0.05$).*

RESULTS

Table 1. Tensile strength measurement results between groups

Group	Mean (MPa)	s.d.
Negative control	2,62	0,56
1 time coating	4,51	0,24
2 times coating	5,45	0,55
3 times coating	1,41	0,62

Based on the results of tensile strength measurements between groups, it is known that the order of the largest to smallest tensile strength is the 2 times coating group, 1 time coating, negative control group and 3 times coating group.

Table 2. Normality test results (Saphiro-wilk) and homogeneity test (Levene)

Group	Sig.	Description	Homogeneity
Negative control	0,361	normal distribution	0.116 (homogeneous distribution)
1 time coating	0,139	normal distribution	
2 times coating	0,785	normal distribution	
3 times coating	0,422	normal distribution	

All groups were known to be normally distributed so that they continued with the *oneway* ANOVAtest with a significance level of $p <0.05$. The results of the *oneway* ANOVA test showed a sig value. 0.000 so that there are significant differences between test groups. To determine the difference betweeneach group, the least significant difference (LSD) post hoc test was conducted.

Table 3. Results of *post hoc least significant difference* (LSD) test

LSD	Negative control	1 time coating	2 times coating	3 times coating
Negative control		0,000	0,000	0,001
1 time coating			0,005	0,000
2 times coating				0,000
3 times coating				

Based on the results of the post hoc test, it is known that all test groups have significant differences in tensile strength test results.

DISCUSSION

The results showed significant differences between the negative control group and the treatment group of *Bombyx mori L.* fibroin coating on collagen membrane. The tensile strength of the treatment group was greater than the negative control group. This occurs because the polymer structure of fibroin binds to collagen. Fibroin has the same components as collagen membrane. These components are glycine polypeptides (20) making it easier for bonding to occur.

The structural strands between the fibroin polypeptide and the collagen membrane bind with hydrogen bonds. The bonding between these two materials causes an increase in the tensile strength of the collagen membrane as it is affected by fibroin which has better physicomechanical properties. The superior physicomechanical properties of fibroin are due to the presence of H-chains and L-chains. H-chains form discrete sheet crystals that serve as the main mechanical structure component while L-chains play a complementary mechanical role. (20). The results of this study are comparable to research stating that fibroin can increase the mechanical strength of acrylic plates in the orthopedic field. (17).

The 3 times coating group showed a decrease in tensile strength compared to the 1 time and 2 times coating groups, even the average value of the tensile strength of this group was smaller than the negative control group. This can occur because the bond between fibroin and collagen membrane has reached a saturation point. After exceeding the culmination point, the brittle fibroin will dominate the mechanical characteristics of the collagen membrane. So that the elastic modulus of the collagen membrane decreases and then followed by a decrease in the tensile strength of the collagen membrane.

The greatest tensile strength was achieved in the 2 times coating group. This condition can occur because the treatment of 2 times coating reached the peak of maximum bonding between fibroin and collagen membrane.

CONCLUSION

It is concluded that *Bombyx mori L.* fibroin coating on collagen membrane improves the mechanical properties of tensile strength. The greatest tensile strength was achieved in the 2 times coating group.

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