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RESEARCH ARTICLE

Effect of Bombyx mori silk-fiber volume on flexural strength of fiber-reinforced composite

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ABSTRACT

Dental glass fiber is one of dental synthetic fibers that are widely used in dentistry as a dental resin reinforcement, such as in dentin replacement material. The availability of glass fiber is limited in Indonesia because it must be imported and relatively expensive. Bombyx mori silk-fiber is one of the strongest natural fiber derived from silkworm cocoon processing. Silk-fiber is used in medical applications as a post-surgical sutures, scaffolds for tissue engineering and drug delivery. The purpose of this study was to evaluate the effect of Bombyx mori silk-fiber volume on the flexural strength of fiber-reinforced composite (FRC). We used Bombyx mori silk-fiber (Perhutani Pati, Central Java, Indonesia) and flowable composite (Charmfil flow, Denkist, Korea) in this study. The FRC samples were divided into 4 groups consisting of fiber volumes of 0%, 5%, 10% and 15% (n = 4). Tests of flexural strength were performed according to ISO 4049. The results were analyzed using one way ANOVA ($p<0.05$). The study showed that the means of the flexural strength (MPa) of Bombyx mori silk-fiber FRC for volume of 0%, 5%, 10% and 15% were 149.2 ± 5.5; 127.6 ± 3.8; 110.9 ± 3.5; 71.2 ± 4.2. One-way ANOVA test showed that the means of FRC flexural strength on the four groups' silk-fiber Bombyx mori volumetric were significantly different ($p<0.05$). This study concluded that Bombyx mori silk-fiber volumetric influences the flexural strength of fiber-reinforced composite. An increase in Bombyx mori silk-fiber volume decreases the flexural strength of FRC because there is a small gap due to the weak interfacial bonds between dental flowable composite and Bombyx mori silk-silk-fiber.

Keywords: Bombyx mori silk-fiber; flexural strength; volume

INTRODUCTION

Composite resin is a material most widely used in aesthetic dental restoration.[1] Since the first time dental composite resin was developed, a lot of effort has been made to improve its clinical performance.[2,3,4] Over the past few years, developing fiber-reinforced composite (FRC) has contributed to the dentistry which allows for adhesive, aesthetic and no-metal materials for dental restoration even for cases of molars.[5] FRC material is a hybrid material made from fiber reinforced polymer resins, combining the mechanical and physical properties of fiber with the physical properties of polymers.[6] Lately, short FRC was introduced as a composite resin for dental restorations which was used as a dentin replacement for high pressure areas, especially in molars. Laboratory mechanical test results showed that there were substantial improvements in bearing load capacity, flexural strength and fracture toughness of composite resin which was reinforced by short e-glass fiber fillers compared to composite resins with conventional fillers.[3,7,8] Short FRC is used as a bulk substructure for dentin replacement which will be covered by a layer of composite with filler particles.[9]

The availability of synthetic dental fibers such as glass fiber in Indonesia is still limited because they have to be imported and relatively expensive. One of the natural fiber alternatives that are easily available and relatively inexpensive is Silk-fiber from Bombyx Mori. Silk-fiber from Bombyx mori is produced by mulberry leaf silkworm *(Morus sp.)*. Silkworm grows and spins cocoons within six weeks. Each cocoon produces fiber about 914 meters long.[10] Central Java is one of the provinces in Indonesia that produces a lot of natural silk yarn, which was 2.15 tons in 2007. Natural silk development in Central Java is under the management of Perum Perhutani Unit I Central Java since 1965, together with the development of forest village communities.[11] Silk-fiber from Bombyx Mori is widely known in textile production,[12] while for medical applications Bombyx mori silk-fibers are used for post-surgical suture, scaffolds for tissue engineering and drug delivery.[13]

Silk fiber from Bombyx Mori is one of the strongest natural fibers that can be an alternative to synthetic fibers.[14] Mechanical strength of Bombyx Mori silk-fiber is comparable to that of e-glass fiber[15] and greater than those of other synthetic fibers, including kevlar.[16] Silk-fiber from Bombyx mori structurally consists of two protein components namely sericin and fibroin.[17] Sericin is a water-soluble glycoprotein like glue that binds fibroin, while fibroin is a structural protein of fibers that is not soluble in water.[13] Silk-fiber from Bombyx mori has properties that are suitable for application as biomaterials, including biocompatibility, low toxicity and non-irritation.[18] It also has good mechanical properties.[19]

FRC material can be called successful if the stress can be transferred properly from matrix to fiber. The mechanical properties of FRC are affected by fiber adhesion to the matrix as well as orientation, impregnation, position, length, volume and fiber composition.[20,21] One important mechanical characteristic is flexural strength. Flexural strength represents various types of forces that the teeth receive in the mouth during mastication.[22,23,24] A research conducted by Garoushi et al. (2006) showed that an increase in fiber volume fraction can increase the flexural strength of short FRC.[25]

The interest in using Bombyx Mori silk fiber for biological applications is increasing due to its mechanical properties and biocompatibility.[26] The use of silk-fiber in the dentistry is still rare, such as in post-surgical suture,[13] so that the application of Bombyx Mori silk-fiber in dentistry can still be explored. The purpose of this study was to determine the effect of Bombyx mori silk-fiber volume of 0%, 5%, 10%, and 15% on the flexural strength of fiber-reinforced composite.

MATERIALS AND METHODS

This study was experimental laboratories. The study was conducted at Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada and Engineering Material Laboratory, Faculty of Engineering, Universitas Gadjah Mada. The ethical clearance fot this study was obtained from the Ethics and Advocacy Unit, Faculty of Dentistry, Universitas Gadjah Mada No. 00917/KKEP/FKG-UGM/EC/2017.

Silk fiber from Bombyx mori (Figure 1) was obtained by boiling and spinning process (Perhutani Pati, Central Java, Indonesia). The Bombyx mori silk-fiber was cut using a scissor to 1 mm in size. Fiber was stored in a desiccator for 24 hours before application. The chopped fibers were weighed to determine the weight according to the volume fraction of the silk-fiber from Bombyx mori i.e. 5%, 10% dan 15%. The weighing of the fibers used a precision digital scale 0.0001 g (Mettler Toledo, Switzerland). There were a total of 16 FRC samples used in this study, divided into 4 groups consisting of fiber volume of 0%, 5%, 10% and 15% (n=4). The volume fraction was calculated by transforming the volume percent to weight using the following formula.[27]

$$Vf\,(\%) = \frac{Wf / rf}{Wf / rf + Wr / rr} \times 100\% \quad \text{....................... Formula 1)}$$

Where:

Vf(%) = Bombyx mori silk fiber volume (%)
Wf = Bombyx mori silk fiber weight (g)
rf = Bombyx mori silk fiber density (g/cm^3)
Wr = weight of resin matrix without fiber (g)
rr = resin matrix density (g/cm^3)

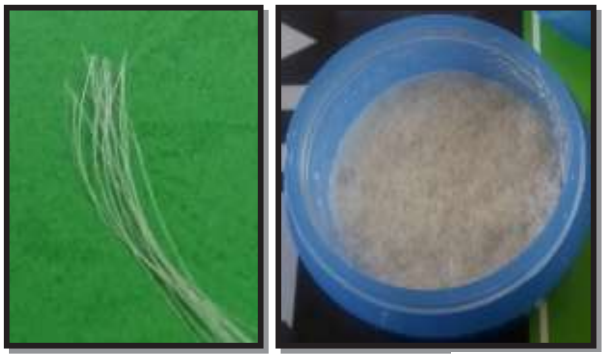

Figure 1. *Bombyx mori* silk fiber (left: strands, right: chopped fiber)

Figure 2. Scanning electron microscope (SEM) of Bombyx mori silk-fiber in FRC fracture surface (magnitude 50 μm)

The metal mold sized 25 x 2 x 2 mm^3 was marked at a height of 0.5 mm for placing of resin and fiber, then the mold was placed on the glass plate. The flowable composite was injected into the mold until full for the sample group FRC fiber 0%, while the group with the addition of fiber (volume of 5%, 10%, and 15%) was injected to the marker limit (0.5 mm).[28] Fiber that had been mixed with flowable composite (ChamFill flow Denkist, Korea) by hand mixing with figure-eight motions was inserted into the mold, then the flowable composite was reinjected until the entire fiber surface was covered with resin and the mold was fully loaded. The FRC surface was covered with a celluloid strip followed by irradiation of the upper part of the sample using light curing unit perpendicular to the sample. Irradiation was continued at the bottom of the sample in the same way. After irradiation was completed the sample was removed from the mold and the excess was polished with 320 grit sandpaper (ISO 4049:2000).[29]

The sample was immersed in 20 ml distilled water on a 50 ml conical tube which had a perforated lid using a sewing needle. A thread was inserted through the hole in the lid of the conical tube and then fastened to the sample so that the length of the thread in the conical tube reached 80 mm. The remaining thread was affixed to the conical tube lid with insulation. The sample was immersed in distilled water stored in an incubator at 37 °C for 24 hours (ISO 4049:2000).[27] The sample was removed from the conical tube and dried using a tissue for 10 seconds and left in the air for 15 seconds.

The three point bending test was done using a universal testing machine (Tokyo testing machine, Japan) (ISO 4049:2000).[27] The flexural strength test was done by placing a sample on the device with a support at each of both ends. The load was applied to the center until the sample fractured. The distance used was 20 mm (L). After loading until the sample fractured, a number appeared on the monitor (F) which was the maximum stress that the sample could receive. The data obtained were calculated using the following formula (ISO 4049:2000):

$$\sigma = (3F.L) / 2\, b.h^2 \quad \text{………………. Formula 2)}$$

where:
σ = flexural / transverse strength (N/mm^2 or MPa)
F = maximum load given before fracture of the sample (N)
L = distance between the two supports (mm)
b = sample width (mm)
h = sample thickness (mm)

The water absorption data were analyzed statistically by performing normality test using saphiro-wilk test and homogenity test using levene's test. Furthermore, a parametric analysis was conducted using one way ANOVA to see the effect of volume variation of Bombyx mori silk-fiber on the flexural strength of fiber-reinforced composite, with a significance level of $p<0.05$. After that, LSD post hoc test was done to see the magnitude of the average difference between each group. The scanning electron microscopy (SEM) test was performed after the study to look at the interfacial bonds between the Bombyx mori silk-fiber with the dental resin matrix.

RESULTS

The results of the study on the effect of Bombyx mori silk-fiber volume on fiber-reinforced composite

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flexural strength can be seen in Table 1. Table 1 shows that the lowest mean of the flexural strength was found in FRC fiber 15% and the highest in FRC fiber 0%. In general, there is a tendency of a decreasing flexural strength in all the FRC groups with different volumes. Shapiro-Wilk test for the flexural strength data in all the groups showed statistical values with p> 0.05. It can be concluded that the research data were normally distributed. Homogeneity test for the flexural strength data showed a statistical value of 1.47 (p = 0.27). With a significance value of p> 0.05. It can be concluded that the data were homogeneous.

One-way ANOVA test results (Table 2) show the value of F = 234.71 (p <0.05). Based on this, it can be concluded that fiber volume had a significant effect on the flexural strength of FRC. The data were tested post hoc using the LSD test (Table 3). The results of the post hoc LSD analysis on FRC flexural strength with different fiber volumes data showed significant differences (p <0.05) in all the groups. The scanning electron microscope (SEM) test results showed 6-8 μm small gap between the Bombyx mori silk-fiber and the dental resin matrix (Figure 2).

Table 1. Mean and standard deviation of FRC flexural strength (MPa) with different fiber volumes

Groups	Flexural strength (MPa) Mean ± SD
FRC fiber 0%	149.16 ± 5.49
FRC fiber 5%	127.59 ± 3.77
FRC fiber 10%	110.91 ± 3.46
FRC fiber 15%	71.25 ± 4.21

DISCUSSION

The results indicated that the flexural strength of FRC was influenced by the percentage of fiber volume, with a tendency to decrease flexural strength along with increased fiber volume. This result is in line with Dhakal's research, et al[29] and Ho, et al,[30] showing that flexural strength decreases by increasing the volume fraction of fiber due to the weak interfacial matrix resin fiber bonding.

The results of this study showed that an increase in Bombyx mori silk-fiber volume decreases the flexural strength of FRC. This may be due to a weak bonding between fiber and matrix. The strength of FRC is a direct indicator of the strength of the interfacial bond, because the stress will be transferred through the interfacial bonding between matrix and fiber.[31,32] Scanning electron microscopy (SEM) tests were carried out after the research to look at the interfacial bonding between Bombyx mori silk-fiber and dental resin matrix. SEM test results showed 6-8 μm small gap between Bombyx mori silk-fiber and dental resin matrix at a volume of 5%, 10%, and 15%.

This study used Bombyx mori silk-fiber from Perhutani Pati, Central Java, Indonesia. Bombyx mori silk-fiber in this company is processed using boiling method in a boiling machine at 80 ^{0}C for 15 minutes, then silk-fiber spun.[11] Boiling is one of the stages of degumming. Degumming is the process of eliminating sericin.[32] Degumming affects the mechanical properties of Bombyx mori.[33] This study did not modify other surfaces on the silk-fiber, and no compatibilizer, coupling agent or other bonding

Table 2. Summary of one way ANOVA of flexural strength of FRC with different fiber volumes

	Sum of Squares	Df	Mean Square	F	p
Between Groups	13023.09	3	4341.03	234.71	0.000
Within Groups	221.94	12	18.49		
Total	13245.04	15			

Table 3. Summary of LSD test of flexural strength of FRC with different fiber volumes

FRC Groups	FRC fiber 0%	FRC fiber 5%	FRC fiber 10%	FRC fiber 15%
FRC fiber 0%		21.56*	38.25*	77.90*
FRC fiber 5%			16.68*	56.34*
FRC fiber 10%				39.65*
FRC fiber 15%				

*= significant differences (p<0.05)

agent was added, because undesirable chemical applications were excessive. Boiling technique cannot eliminate all sericin,[34,35] therefore residual sericin is still found on the surface of the fiber core.[36]

The hydrophilic sericin blocks the matrix-fiber bonding, causing a poor interfacial matrix-fiber bonding.[30] The remaining sericin layer on the surface of the fiber absorbs some of the energy when the stress is applied to the FRC but does not pass completely to the fiber core, causing deformation of the material.[37] The weak interfacial bonding will limit the transfer of stress, so that in the part that contains fiber will form a non-reinforcing cavity which results in a decrease in mechanical strength. Fiber that does not bind well to the FRC matrix will reduce the number of areas filled with matrix, thus becoming damaged parts which can reduce the effectiveness of cross sectional areas so that mechanical strength is weak.[38,39]

Sericin on silk fiber must be removed by proper degumming techniques so that the Silk-fiber from Bombyx mori becomes more hydrophobic. The use of coupling agents is also recommended to provide a good interfacial bonding between silk-fiber and dental resin matrix so that mechanical strength increases, so that silk-fiber can be expected to be an alternative in the manufacture of FRC in dentistry especially as a dentin replacement material. This is because Bombyx Mori silk fiber is one of the strongest natural fibers, easily obtained and relatively inexpensive.

According to ISO 4049 (2000), a restorative material is considered to meet the initial requirements if the flexural strength of the test sample is ≥ 50 MPa.[28] The results of the study regarding the flexural strength showed the lowest mean in the 15% FRC fiber group that is 71.25 MPa and the highest in the 0% fiber FRC group which is 149.15 MPa. As a comparison, short FRC with glass fiber brand EverX posterior, GC, Japan has a flexural strength of 201.08 MPa.[40] This shows that all FRC sample groups produce flexural strength, meaning that they are in accordance with ISO 4049 (2000), but when compared to EverX posterior, GC, Japan the flexural strength of Bombyx Silk-fiber FRC memory is much lower.

CONCLUSION

The conclusion of this study is the volume of Bombyx mori silk-fiber influences the flexural strength of FRC. Increased volume of Bombyx mori silk-fiber decreases the flexural strength of fiber-reinforced composites. This is thought to be because there is a small gap due to a weak interfacial bonding between dental resin matrix and fiber which decreases flexural strength.

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