Dental Journal
Majalah Kedokteran Gigi

Research Report

Effects of alkalisation and volume fraction reinforcement of *Bombyx mori* silk fibre on the flexural strength of dental composite resins

Dyah Anindya Widyasrini and **Siti Sunarintyas**
Department of Dental Biomaterial,
Faculty of Dentistry, Universitas Gadjah Mada
Yogyakarta – Indonesia

ABSTRACT

Background: *Composite resins are widely used in dentistry to restore dental caries. Recently, short fibre-reinforced composite (FRC) resins have been widely used for high-stress areas, especially in posterior teeth. Bombyx mori silk fibre is under research to reinforce dental composite resin as it has good mechanical properties.* **Purpose:** *This study aims to obtain the effects of alkalisation and silk fibre volume fraction on the flexural strength of FRC.* **Methods:** *Bombyx mori silk fibres were obtained from Perhutani, Pati, Indonesia. Samples were divided into two alkalisation groups (4% and 8%). Alkalisation of the silk fibres was conducted through the scouring process in NaOH, hydrolysis (30% H_2SO_4) and drying. Silk fibres were then reinforced in a resin matrix. The samples were subdivided based on the fibre volume fraction reinforcements, which were 0%, 5%, 10% and 15%. Each group of samples consisted of three specimens (n = 3). Flexural strength was measured using a universal testing machine. Data were analysed by two-way ANOVA (p < 0.05) and post-hoc least significant difference test (p < 0.05).* **Results:** *The results showed the flexural strength (MPa) means of the 4% alkalisation group were 169.31 ± 54.28 (0%), 76.08 ± 43.69 (5%), 107.86 ± 40.61 (10%) and 101.99 ± 10.61 (15%). The flexural strength (MPa) means of the 8% alkalisation group were 169.31 ± 54.28 (0%), 82.62 ± 22.41 (5%), 111.07 ± 32.89 (10%) and 153.23 ± 23.80 (15%). Statistical analysis by ANOVA indicated that the fibre volume fraction affected the flexural strength of composite resins.* **Conclusion:** *It can be concluded that the volume fraction of silk fibre increases the flexural strength of composite resins, although the strength is not as high as a composite resin without fibres. However, the alkalisation percentage did not affect the flexural strength of composite resins, and there was no interaction between alkalisation percentage and fibre volume fraction with the flexural strength of composite resins.*

Keywords: *alkalisation; composite resins; flexural strength; silk fibre; volume fraction*

Correspondence: Siti Sunarintyas, Dental Biomaterial Department, Faculty of Dentistry, Universitas Gadjah Mada, Sekip Utara Yogyakarta 55281 Indonesia, E-mail: sunarintyassiti@ugm.ac.id

INTRODUCTION

Oral and dental health are inseparable parts of overall body health. To date, caries pose a major problem in dental health throughout the world and cause significant tooth decay. Dental restorative materials are needed to prevent or repair tooth decay.[1] Composite resins are the most preferred restorative material due to their good biocompatibility, aesthetic properties, affordable costs and mechanical bonding to the tooth structure.[2] However, there are some problems concerning composite resin restorations, such as inadequate mechanical properties, high water absorption, shrinkage after polymerisation and poor wear resistance when used.[3,4]

Recently, short fibre-reinforced composite (FRC) has been introduced as a restorative material of composite resins for bearing high-stress areas, especially in molars. The results of laboratory mechanical tests show that there are substantial improvements in load-bearing capacity, flexural strength and fracture toughness in composite resins that are reinforced with short e-glass fibre fillers compared to conventional fillers.[5]

The availability of synthetic dental FRC resin for restorations in Indonesia is still limited and more expensive than regular composite resin because it must be imported from another country. As an alternative to using synthetic fibres to reinforce composite resin as a restorative material, silk fibre, taken from the *Bombyx mori* silkworm cocoon, is used. *Bombyx mori* silk fibre is one of the strongest natural fibres.[6] Previous studies have shown that the silk fibre of the *Bombyx mori* silkworm cocoon has a higher tensile strength than glass fibres or other organic synthetic fibres and has good resilience and elasticity.[7] Widely used in biomedical applications, *Bombyx mori* silk fibres have shown not only good mechanical properties but also excellent biocompatibility and low immunogenicity.[6] Another study shows that up to 100 μg/ml of extract from the silkworm cocoon of Cricula triphenestrata is not cytotoxic to gingival fibroblast cells.[8]

A study conducted by Fransiska et al.[9] shows that an increase in the *Bombyx mori* silk fibre volume fraction in composite resins increased water absorption and decreased flexural strength. The study found that a small gap was allegedly due to weak interfacial bonds between the resin matrix and the *Bombyx mori* fibre. This induced the water to penetrate easily, reducing flexural strength. Sericin, which was still attached to the surface of the *Bombyx mori* fibre, was ostensibly the cause of the bad bond between the resin matrix and the fibre.[9] In addition, sericin has been shown to induce allergic and immunological reactions, so it must be removed before use in biological applications.[10] Sericin can dissolve in water and can be removed by a thermochemical process known as degumming.[11] During the degumming process, sericin is hydrolysed, and amide bonds with long protein molecules are broken into smaller fractions, then dispersed and dissolved in solutions containing alkali, soap or synthetic detergents.[12] In order to overcome the damage that occurs during the degumming process, it is necessary to research the effects of alkalisation, which aims to remove sericin and impurities in the flexural strength of composite resins. This study aims to reveal the effects of alkalisation and the addition of a volume fraction of *Bombyx mori* silk fibre on the flexural strength of composite resins as dental filling material.

MATERIALS AND METHODS

Bombyx mori silk fibres were obtained from Perhutani, Pati, Central Java, Indonesia; the silk fibres had been reeled and spun from the *Bombyx mori* silkworm cocoon. UDMA was obtained from Esstech Inc. (Essington, Pennsylvania). BisGMA, TEGDMA, champorquinone (CQ) and CEMA were obtained from Sigma-Aldrich Chemical (Darmstadt, Germany). NaOH, CH$_3$COOH and other chemicals used at analysis levels were obtained from Sigma-Aldrich Chemical (Darmstadt, Germany).

Bombyx mori silk fibres were alkalised based on the method developed by Mohammed and Dauda,[13] with modifications. *Bombyx mori* silk fibres were alkalised by cutting silk fibres to 2 mm long using stainless steel scissors and grouping them into two groups based on the alkalisation percentage. Afterwards, the scouring process was performed by soaking the silk fibres in 250 ml of NaOH solution in a beaker glass at 65ºC for three hours on a hotplate stirrer. The concentrations of NaOH solution used were 4% and 8%. The silk fibres were rinsed with distilled water, then neutralised with 500 ml of 2% CH$_3$COOH solution in a beaker glass for two hours at 65ºC on a hotplate stirrer. The fibres were then rinsed using distilled water until the litmus paper showed a neutral pH. The fibres were left to dry at room temperature.[13] Drying was continued until it reached a constant fibre weight.

The results of the 4% and 8% silk fibre alkalisation were mixed with a composite resin matrix mixture until they were homogeneous, according to Sunarintyas et al.[14] (BisGMA 67%, TEGDMA 30%, UDMA 1%, CQ 1%, CEMA 1%). The results of each percentage of silk fibre alkalisation were then subdivided into four groups based on the silk fibre volume fraction. The volume fractions of the silk fibres used were 0%, 5%, 10% and 15%.[9] The samples of the silk FRC for flexural strength tests were made according to ISO 4049 (2009). Three (n = 3) samples were made for each volume, so there were eight groups of samples. Afterwards, the composite was put into a mould measuring 25 mm x 2 mm x 2 mm until it was fully loaded. The composite surface was covered with a glass slide, and the top of the sample was illuminated using a light-curing unit perpendicular to the sample from a distance of 2 mm. The tip diameter of the light-curing unit was 8 mm, while the sample length was 25 mm. The illumination was divided into three parts, each of which was illuminated for 20 seconds; non-illuminated parts were covered with aluminium foil. After the illumination was complete, the sample was removed from the mould.

Flexural strength was tested with a three-point bending test using a universal testing machine (Tokyo Testing Machine, Japan), according to ISO 4049.[15] The test was performed by putting the sample on a supporting board with a support distance of 20 mm (L), then the sample was loaded in the middle until it broke. Afterwards, the monitor screen displayed a number (F), which was the maximum pressure that the sample received. Furthermore, the measurement data obtained were entered into the following equation:[15]

$$\sigma = (3F.L) / 2 \, b.h^2$$

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

Normality (Shapiro–Wilk) and homogeneity (Levene's test) tests were performed on the data obtained from the results of the flexural strength test. Furthermore, to test

the hypothesis, a two-way ANOVA test was performed to reveal the effects of alkalisation and the addition of the volume fraction of *Bombyx mori* silk fibre on the flexural strength of composite resins with a significance level of $p < 0.05$. Furthermore, the data were analysed using the post-hoc least significant difference (LSD) test to see the average difference between each group.

RESULTS

Figure 1 presents the results of the research into the effects of alkalisation and volume fraction of *Bombyx mori* silk fibre on the flexural strength of composite resins. Figure 1 shows that the lowest flexural strength was seen in composite resins with a 5% silk fibre volume fraction, and the highest value was in composite resins without fibre additions (0% silk fibre volume fraction) in both the 4% and 8% alkalisation groups. In composite resins with the addition of silk fibre, th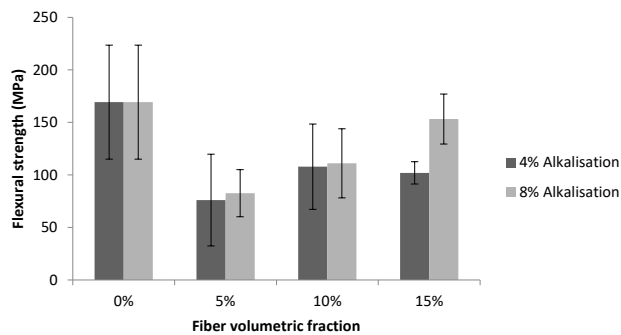ere was an increase in the flexural strength value along with the addition of the fibre volume fraction in the two alkalisation groups.

The results of the two-way ANOVA test indicated the effects of adding fibre volume fraction on flexural strength ($p = 0.007$), but the percentage of fibre alkalisation did not significantly affect the flexural strength of silk fibre composite resins ($p = 0.344$). Based on the statistical analysis, there was no interaction between the percentage of alkalisation and fibre volume fraction and the flexural strength of composite resins ($p = 0.626$). The results of observations with a scanning electron microscope (Figure 2) showed a small gap between the fibre and the matrix in the 8% alkalisation group specimen. Furthermore, the data obtained were tested post-hoc using the LSD test Table 1). The post-hoc LSD test results indicated that there were significant differences between the flexural strength values of the composite resins with 0% and 5% volume fractions in both alkalisation groups. Meanwhile, the flexural strength values of composite resins with 0%, 10% and 15% fibre volume fractions were not significantly different.
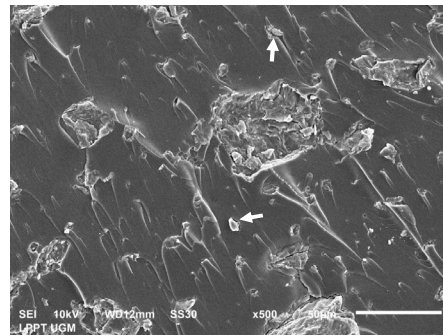

Figure 1. Mean and standard deviation of flexural strength of silk fibre composite resins.

Figure 2. The results of an SEM photograph on a silk fibre composite resin in 8% alkalisation with a magnification of 500x. There are small gaps between the fibre and the matrix (white arrow).

Table 1. Summary of LSD test of fibre volume fraction of 4% alkalisation group on the flexural strength of silk fibre composite resin

Volume fibre fraction	0%	5%	10%	15%
0%		93.23000*	61.45333	67.32000
5%			31.77667	25.91000
10%				5.86667
15%				

*= Significantly different (p <0.05)

Table 2. Summary of LSD test of fibre volume fraction of 8% alkalisation on the flexural strength of silk fibre composite resin

Volume fibre fraction	0%	5%	10%	15%
0%		86.69333*	58.24000	16.07333
5%			28.45333	70.62000*
10%				42.16667
15%				

*= Significantly different (p <0.05)

DISCUSSION

The results of the research indicated that the greatest flexural strength in the two alkalisation groups was in the 0% volume fraction treatment, while the lowest flexural strength was shown in the 5% silk fibre volume fraction. This may be due to the absence of fibre additions (0% volume fraction), causing no gaps to form in the matrix (homogeneous matrix without fibre additives). In composite resins with the addition of fibres, especially fibres that are not perfectly clean, there is a gap between the matrix and fibres, which happens because there is still a layer of sericin and other impurities that have not been completely removed from the silk fibres through the alkalisation process. The presence of a sericin layer can prevent physical contact or chemical attachment between the fibres and the matrix, reducing the mechanical properties of the silk fibre composite resins.[16] The small gap between the fibres and the matrix shown in Figure 2 might be due to imperfect adhesion due to sericin remaining in the fibres. Fibre-matrix attachment is another important factor affecting the mechanical properties of short-FRC. The gap formed between the fibre and the matrix is like a cavity without reinforcement, reducing the composite's overall strength. Poor interfacial attachment between the silk fibre and polymer matrix dominates other factors that might influence the decrease in mechanical strength.[16] This shows that a better sericin cleaning process is needed so that the sericin does not interfere with the fibre's attachment to the matrix. In addition, a coupling agent can be added to improve the interfacial attachment between silk fibres and the matrix. The addition of coupling agents to composites made from natural fibres can improve mechanical properties by up to 61% because they can increase the bond between organic and inorganic materials.[17] With the addition of a coupling agent, it is expected that the load transfer to the fibre will be more effective, increasing mechanical strengths, one of which is flexural strength.

In the 8% alkalisation group, the flexural strength increased from the 5% to the 15% volume fraction. Meanwhile, in the 4% alkalisation group, the flexural strength increased from the 5% to the 10% volume fraction but slightly decreased in the 15% volume fraction. The increased value in the flexural strength of the composite resins might be due to the addition of silk fibre as a reinforcement material. *Bombyx mori* silk fibre is a natural fibre that has good mechanical strength. Silk fibre has high tensile strength, good flexibility and resistance to compression strength, making it suitable to be used as a composite material to bear the load.[4] The mechanical strength of *Bombyx mori* silk fibre is equal to e-glass fibre and is greater than that of other synthetic fibres, including Kevlar.[18] The mechanical strength of *Bombyx mori* silk fibre is obtained from the β-sheet structure, which is formed by a repeated sequence of the amino acids glycine, alanine and serine (G-A-G-A-G-S) in the crystalline phase of fibroin.[10] The addition of a volume fraction of silk fibres increases the amount of silk fibre in the composite composition, thereby increasing the mechanical strength of the composite resins.

The results of the two-way ANOVA indicated the effects of fibre volume fraction on flexural strength (p = 0.007). The effectiveness of fibre reinforcement is affected by the amount of fibre in the composite resin. An increase in fibre content increases mechanical strength.[19] This is consistent with the results of this research; the flexural strength of composite resins generally increased in line with the addition of silk fibre in composite resins. However, statistically, the difference in the percentage of fibre alkalisation indicated that there was no significant effect on the flexural strength of the composite resin (p = 0.344). This might be due to the degumming procedure using alkalisation being poorly performed so that the results of the two alkalisation percentages did not show any differences. In the degumming process with alkali, the non-covalent fibroin bond in a silk fibre is modified, making the fibre swell. The swelling effect of the fibre is due to the difference in osmotic pressure arising between the fibre and the solution, forming protein salts. Furthermore, sericin degrades into sericin peptides or hydrolysed sericin.[12,20] The degumming process of silk fibres using NaOH is widely used, but this process results in strong irritation to the fibroin content of silk fibre.[12] Degumming using alkali may result in a damaged structure of the silk fibres, poor stretch quality and lack of shine.[21] Therefore, it is necessary to use a more optimal and safer degumming procedure when removing sericin to maintain the physical and mechanical properties of fibroin. In addition, proper degumming procedures are needed to increase fibre roughness, thus increasing attachment strength. This is consistent to Ho et al.'s[16] statement on the interaction between the fibre and matrix being supported by the existence of the mechanical interlocking obtained from fibre surface roughness, providing weak adhesion.

Based on the results of the post-hoc test, it can be seen that there was an increased value in the flexural strength of composite resin with 5%, 10% and 15% fibre volume fractions in the 8% alkalisation group. These results indicate that fibre has the potential to increase the mechanical strength of composite resins, although the results are not as good as the composite resins with a 0% fibre volume fraction. Also, the results of the post-hoc LSD test indicated that the flexural strength of composite resins with 0%, 10% and 15% fibre volume fractions did not differ significantly (Tables 1 and 2), especially in the 8% alkalisation group. This indicates that degumming using 8% NaOH has better cleaning potential than using 4% NaOH, although it was not perfect. In addition, according to ISO 4049[15] restorative material is deemed to have met the initial requirements if the flexural strength of the test sample is more than 80 MPa. All samples in the 8% alkalisation group had an average flexural strength greater than 80 MPa.

Based on the results of the research, it can be concluded that the fibre volume fraction increases the flexural strength

of composite resins, although they are not as strong as the composite resin without fibres. Silk fibre has the potential to increase the mechanical strength of composite resins; however, the optimum volume fraction of silk fibre to be added in the composite resins still needs to be found. Meanwhile, the percentage of silk fibre alkalisation does not affect the flexural strength of composite resins

ACKNOWLEDGEMENT

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