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RESEARCH ARTICLE

The effect of silk concentration on the flexural strength of FRC as a Bone Graft

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ABSTRACT

Bone fractures are mostly caused by trauma and disease. In the therapeutic process of bone healing which often meets some constraints, bone graft is mainly used to ensure that the healing process takes place. A fiber reinforced composite (FRC) is a popular bone graft material that is made to resemble bone properties. FRC is normally comprised of polymer matrix, hydroxyapatite filler, and fiber. Hydroxyapatite is a bioactive material widely used as a bone graft. Silk fiber is known as a reliable material to increase mechanical strength of the FRC. On this basis, this study aims to determine the effect of silk fiber concentration on the flexural strength of FRC. Fiber reinforced composite made of Bis-GMA/TEGDMA/UDMA resin (CharmFil®, DenKist, Korea), hydroxyapatite (Bioceramic Laboratory, DTMI UGM) and silk fiber (Perhutani, Pati) were divided into three groups. Each group contained different silk fiber concentrations which were 1%, 5% and 10%. The flexural strength test was performed with 3-point bending test according to ISO 10477. The result showed that FRC with silk fiber 1%, 5% and 10% respectively had flexural strength of 61.21 ± 8.43 MPa, 62.97 ± 3.92 MPa and 85.01 ± 7.71 MPa. The result of one way ANAVA analysis showed that mean of FRCs flexural strength were significantly different between one treatment group to another. Thus, it is conclusive that silk fiber has a significant effect on FRCs flexural strength. The addition of 10% concentration of silk fiber is proven to increase FRCs flexural strength.

Keywords: bone graft; fiber reinforced composite; flexural strength; silk fiber concentration

INTRODUCTION

Living tissues in the human body such as bone are very prone to injury or fractures. Bone fractures lead to a decreasing bone function and a degrading patients' quality life.[1] In the field of dentistry, bone fractures often occurs in the maxillofacial area caused by trauma, surgical procedures, resorption in periodontal disease,[2] and infections and tumors such as Ameloblastoma.[3] Generally, maxillofacial fractures occurs on the facial bone such as the mandible. Since its healing process is likely to be constrained by several interferences which may end up in failure requiring surgery and additional costs, it is necessary to use some substitute materials such as bone graft to help the bone healing process.[1]

Various types of bone grafts are distinguished by their origin such as auto graft (derived from the patient's bone), allograft (derived from the donor's bone) and xenograft (derived from the preserved animal bones). However, the bone graft is known to cause some disadvantages. For example, it is worthy to note that autograft has a limited area of tissue to be taken to fill the part with extensive damage and may lead to damage and death in donor tissue. In addition, allografts and xenografts can cause allergies and are prone to the risk of spreading the disease.[4] The deficiency of natural bone grafts increases the need for synthetic bone grafts. Through a good manufacturing process, synthetic bone grafts can provide some benefits including free risk of infection, strong resemblance to human bones, faster support of patient's mobilization, shorter postoperative care, reliable availability and quality and more predictable bone

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growth.[5] On this account, FRC is an alternative material that has been widely used as a synthetic bone graft.

FRC can be made to resemble bone characteristics by modifying its constituent components such as matrix, fiber number, fiber type, orientation and fiber length.[6] The popularly used polymer matrix in FRC is bis-glycidyl-A-dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) and urethandimethacrylate (UDMA).[7] However, the only drawback of the polymer matrix is that it has no bioactive properties,[8] so that it requires other additional materials to be used in bone graft applications.

Hydroxyapatite (HA) is one of the bioactive materials that has been widely used as a bone graft material[9] because it has bioactive properties, good osteoconductivity and has similar components with bone main minerals.[5] Most commercial HA produced by chemical synthesis such as solid state reaction, hydrothermal method, combustion preparation, sol-gel route, micro emulsion route, and mechano-chemical method. The chemical synthesis method produces a HA composition that is not homogeneous, requires a complicated process, and is highly costly. The disadvantages of commercial HA compel many experts to look for other alternative materials. Ox bone or bovine bone is an alternative source to be used as HA raw material. Hydroxyapatite derived from natural ingredients is known to have some advantages because of its abundant number and easy production process, which increases efficiency for massive production of HA.[10] HA has also been added as a filler to the polymer matrix with the aim of obtaining strong but still fragile restorative materials holding large loads.[8] The only drawback is that HA comes up with a low level of strength which may impede its application in the medical healing process.[11]

To overcome this drawback, it is possible to increase the mechanical strength of FRC by adding fiber.[12] Silk fiber is a natural polymer composed of sericin and fibroin proteins and is known to have high mechanical strength. Silk fiber mechanical strength comes from amino acids making up silk fiber protein. The dominant crystalline fibroin phase of amino acids such as glycine (Gly), alanine (Aly) and serine (Ser) form a b-sheet structure is the source of mechanical strength of silk fiber.[13] Thus, the addition of silk fiber is expected to produce FRC with better mechanical strength.

FRC mechanical strength is one of the factors needed in bone graft applications. FRC used as a bone graft in the mandibular bone will experience a type of loading such as pull and press.[14] Therefore, it is necessary to test the mechanical strength of FRC. Flexural strength test is a test that includes compression and tensile loading on composites.[11] The composite properties reinforced by fiber are influenced by various factors. One of the composite properties such as flexural strength is influenced by the amount of fiber. When the fiber concentration is higher, the composite flexural strength increases.[12] On this basis, this study aims to determine the effect of silk fiber concentration on the flexural strength of FRC.

MATERIALS AND METHODS

This research has been approved by the ethical clearance No. 001020/KKEP/FKG/UGM/EC/2017. This laboratory experiment research was held at the Materials Laboratory of the Department of Mechanical and Industrial Engineering UGM and UGM Integrated Testing and Research Laboratory (LPPT) in the period of March-October 2017. The research stages are presented in Figure 1. Materials used in the research include bovine hydroxyapatite (Bioceramics Lab, UGM Department of Mechanical and Industrial Engineering), bombyx mori silk fiber (Perhutani/Indonesian Forest Enterprises, Pati), flowable composite (CharmFil®, DentKist).

Test materials were made with different concentrations of silk fiber, namely 1%, 5% and 10%. Control samples were made without silk fiber and HA additions by referring to Fransiska (2017) examining the flexural strength of composite Bis-GMA / UDMA / TEGDMA.[15] The study also indicated that 15% of silk fiber addition resulted in a decrease of flexural strength FRC.[15] This study used silk fiber of 2 mm in length.[16] Samples were made by mixing all the materials and stirring them manually for 5 minutes to obtain a homogeneous mixture.

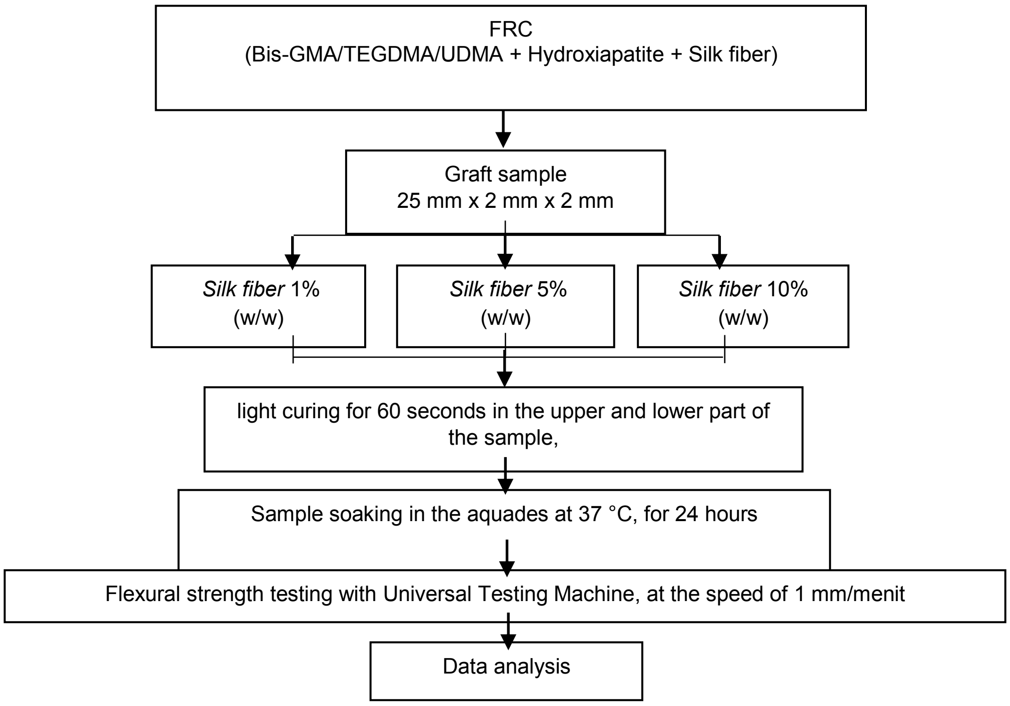

Figure 1. Research Scheme

Afterwards, the materials were poured into a mold measuring 25 mm x 2 mm x 2 mm.[17] Polymerization was carried out with the help of each LED light for 60 seconds at the top and bottom of the sample. Next, the samples were removed from the mold and trimmed with 1500 grit sandpaper. The samples were immersed for 24 hours in distilled water before flexural strength testing. Measurements were then made with a 3-point bending test using Universal Testing Machine with a speed of 1 mm/minute.[17] The results of the measurement data were then inputted into Equation 1. Afterwards, a one-way ANAVA statistical analysis was performed. The fractured FRC section was then analyzed using SEM to see the topography of the sample after the flexural test.

$$\sigma = \frac{(3PL)}{2bd^2} \qquad (1)$$

Description σ = flexural strength (MPa), P = maximum load given before broken (N), L = distance between the two buffers (mm), b = sample width (mm) and d = sample thickness (mm).

RESULTS

The results of flexural strength testing of FRC with different percentages of fiber weight were presented in Table 1 and Figure 2. In general, the flexural strength increases along with the increasing concentration of silk fiber. In addition, 10% concentration of silk fiber FRC is proven to have the highest flexural strength, while one percent of silk fiber FRC is noted to have the lowest flexural strength.

SEM observations revealed that FRC fault sections and different silk fiber distributions were seen in each sample (Figure 3). 1% of FRC silk fiber indicated the far distance of fiber and uneven distribution as compared to silk fiber FRC at 5% and 10%.

Table 1. Average and standard deviation of FRC flexural strength (MPa) with different fiber percent weight.

Group	Flexural Strength (MPa) Average ± SD
FRC *silk fiber* 1%	61,21 ± 8,43
FRC *silk fiber* 5%	62,97 ± 3,92
FRC *silk fiber* 10%	85,01 ± 7,71

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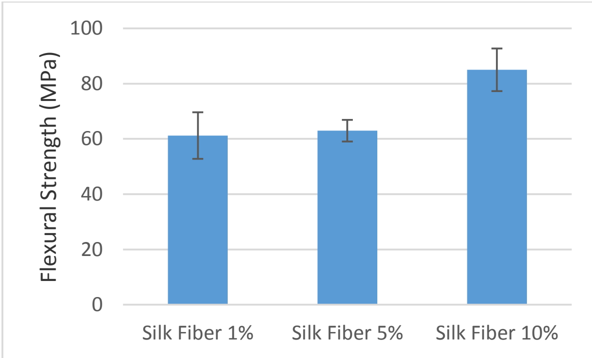

Figure 2. Comparison of flexural strength of FRC with different silk fiber concentrations.

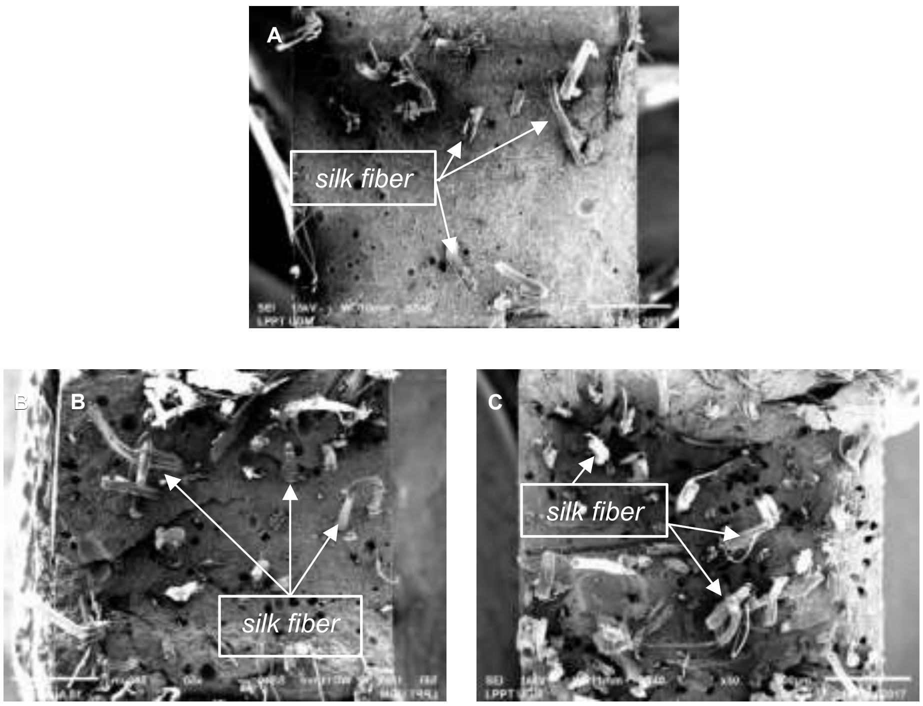

Figure 3. SEM results in FRC fracture parts, A. 1% silk fiber FRC, B. 5% silk fiber FRC, and C.10% silk fiber FRC Arrows indicate the silk fiber found in FRC.

DISCUSSIONS

Technological advances have enabled humans to engineer various materials according to their wish. Technology also allows humans to combine various materials to produce new materials with superior properties. One of these materials is FRC which has been used as a bone graft.[18] The FRC in this study was made by combining bombyx mori silk fiber, hydroxyapatite, and Bis-GMA / TEGDMA / UDMA based resins. Based on data analysis, it is revealed that there was an increase in flexural strength as the silk fiber content increased in FRC. One factor to influence the mechanical strength of FRC is the concentration of fiber. Low concentrations can cause uneven distribution of fiber, making some parts of FRC not supported by fiber.[19] Fiber distribution on 1% of silk fiber FRC resulted in uneven distribution of fiber and far distance between fibers. Based on SEM results, it can be seen that the higher the concentration, the closer the distance between fibers.

During the loading process, the matrix will receive pressure at first which will be channeled to the fiber. The load received by FRC can occur on the part of the matrix that is not supported by fiber. The load that occurs in that area cannot be channeled to the fiber and is borne by the matrix which is the weakest part of FRC, thereby reducing mechanical strength.

Reinforced materials such as silk fiber has mechanical strength derived from the structure of the β -sheet contained in fibroin. Amino acids such as glycine, alanine, serine, tyrosine, valine and threonine can be organized to form β -sheet structures through hydrogen bonds, hydrophobic and van der Waals bonds.[20] Increasing silk fiber concentrations will increase the number of amino acids that make up the β -sheets to provide mechanical strength to FRC.

One of the factors to influence the regeneration and bone remodeling is mechanical stimulation.[21] Hard materials such as metal can absorb pressure and prevent the mechanical stimulation in the bone, resulting in a process of bone resorption. Bone tissue replacement material must be designed to have mechanical strength that matches the bone. The highest flexural strength in this study was 85.01 MPa resulted from FRC with 10% of silk fiber. The flexural strength was still below the flexural strength of the compact part of the mandibular bone at 159.8 MPa.[22]

The flexural strength of FRC in this study was lower than that of FRC as revealed by several other studies. Aitasalo et al. (2014) examined FRC for craniofacial defects consisting of bioactive glass, Bis-GMA / TEGDMA and E-glass fiber matrices that were coupled agent.[23] The results showed that FRC had flexural strength of 420 MPa and high fracture resistance.[23] Research on FRC as a bone defect reconstruction material by Hautamäki et al. (2008) produced flexural strength of 320 MPa. FRC used PMMA as matrix and E-glass fiber impregnated with PMMA.[24] Research on flexural strength of composite resin Bis-GMA / UDMA/TEGDMA without additional silk fiber and hydroxyapatite led to the amount of 149,156 MPa, which was higher than that of the test group.[15] Addition of other materials to FRC requires homogeneous mixing to produce high mechanical strength. Composite Bis-GMA/UDMA/TEGDMA without silk fiber and HA are factory-made products that have gone through a manufacturing process in such a way that all materials can be mixed homogeneously to be used as dental fillings.

The flexural strength of FRC in this study was below a polymer-based commercial bone graft. Cortoss® is a bone graft consisting of 33% Bis-GMA and 67% bioactive glass.[25] Boynd et al. (2008) stated that Cortoss® has a flexural strength of 96 MPa.[26] High flexural strength is likely caused by the mixture between polymers and fillers using mixing/extruder tools[27] to mix the material homogeneously. In contrast, the material mixing in this study was done manually which might result in non-homogeneous mixture.

The results showed that silk fiber is a potential material to reinforce FRC for bone grafts. However, it is necessary to conduct further research to obtain FRC with a homogeneous mixture and optimal silk fiber concentration to obtain flexural strength that matches the mandibular bone. The use of coupling agents can be considered as one way to get higher flexural strength. Furthermore, it is also vital to conduct a research on the ability of FRC to be degraded because the bone graft material is expected to degrade as the bone regeneration process takes place.

CONCLUSION

Silk fiber concentration is proven to affect the flexural strength of FRC. FRC with 10% silk fiber content has the highest flexural strength of 85.01 MPa as compared to the concentration of silk fiber at 1% and 5%. However, the flexural strength of FRC is still below the flexural strength of the mandibular bone of 159.8 MPa.

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