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The *Bombyx mori* L., Nanofibroin Has Potential for Composite Filler Restoration

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ABSTRACT

Bombyx mori L. nanofibroin has potential as an organic filler composite restoration because it has excellent mechanical and functional strength. One of the main requirements of the restoration material is to have good mechanical strength to wear resistance of dental restorations as well as tooth wear or antagonist restorations. The mechanical properties of microhardness are essential to study as a preliminary study in the new material. The aim of this study were to find out the nanofibroin potency as composite resin filler and to compare the microhardness of nanofibroin and nanofiller composites as gold standart. Methods: Nanofibroin particles were extracted from *Bombyx mori* L. cocoon by degumming, dissolving, dialysis and sonification. The nanofibroin filler weight was determined using volume fraction of natural fibers and mixed with an organic matrix. Three of sample groups were divided into nanofibroin composites, nanofiller composites (Z350 XT flowable 3M ESPE) as a positive control and composites without fillers as negative control. Microhardness was measured using a microhardness tester (Shimadzu, Japan). Data were analyzed using Kruskal Wallis test. Results: The nanofibroin composite have the highest microhardness (146.45), while nanofiller has a microhardness of (110.40 VHN) and the composite without filler has the lowest microhardness (31.55 VHN) and the statistical analysis showed $p>0.05$ level of significance. Conclusion. The *Bombyx mori* L. nanofibroin has potential for composite filler restoration eith better microhardness than positive control.

Keywords: *Bombyx mori L., composite, nanofibroin, nanofiller, microhardness*

1. INTRODUCTION

Composite resins have been developed rapidly over the past 5 decades, making them the choice for restoration materials. Composite resins in the dentistry field are matrix polymer materials that are reinforced with fillers, with the main components of composites including organic matrix polymers, inorganic filler particles, coupling agents, and space systems [1]. Composite resins have several advantages, including; (1) aesthetic, (2) requires a small amount of preparation, (3) low thermal conductivity, (4) can be used as an alternative to amalgam [2]. However, composite resin can shrink at the time of polymerization into microleakage causing secondary caries and composite resin resistance to decrease over time [3]. Silk has been widely known as a textile material even since many years ago, but there are things that can be studied and developed further on silk, especially from silkworms that are named *B. mori* L. The fibroin *B. mori* L. cocoon consists of two proteins namely fibroin and sericin [4]. Fibroin is a natural polymer consisting of 18 amino acid groups [5].

New challenges in developing nanofibroin as composite resin filler are glycine content in fibroin with high concentrations as an bacterial agent [6]. So there is a challenge to develop nanofibroin as a filler that can prevent the emergence of secondary caries. Fibroin has good mechanical properties with higher tensile strength compared to other natural fibers, and better hardness than other silk fibers [7], [8]. Fibroin of *B. mori* L., has been shown to have biocompatibility with pulp cells in humans [9].

Fillers in composite resins have several capabilities; reducing matrix's volume, improving mechanical properties, reducing shrinkage during polymerization, and preventing caries [10], [11]. Microhardness is one of the mechanical properties that needs to be tested on dental restorative materials. This study conducted a microhardness test as a preliminary study of new materials because microhardness can affect the resistance of restorative materials and wear on restorations and their antagonistic teeth [12]. This study's objective was to determine whether microhardness composite nanofibroin filler materials could be used as dental restorative material compared to the gold standard. The gold standard used is Z350 XT Flowable (3M ESPE) with a microhardness of 69.7-77.1 VHN which is equivalent to dentin microhardness (50-60 VHN) [13], [14]. Composite nanofibroin is expected to have good microhardness.

2. MATERIALS AND METHODS

The extraction stage of fibroin to become nanofibroin is divided into 5 processes [15], [16]: the degumming process, to eliminate of non-biocompatible sericin by using alkaline which is Sodium Carbonate. Dissolution process, to dissolve fibroin by mixing Lithium Bromide and heated in a 60°C oven for 4 hours. Dialysis and centrifugation processes; carried out for 48 hours in Milli-Q water to get pure fibroin. Sonification process; to break down fibroins into nano-sized using a sonifier. The lyophilization process (dry freeze); to get nanofibroin filler. The volume dan weight of nanofibroin filler are determined by the following formula [17]:

$$v_f = [\rho_f . w_f/(\rho_f . w_f + \rho_m . w_m*]$$

Description:
vf = fiber volume fraction (%)

ρm = matrix density (g/cm3)

ρf = fiber density (g/cm3)

wm = weight of matrix (g)

wf = weight of fiber (g)

The calculation shows the weight of filler with a volume of 63.3% (equal to the number of positive control fillers) is 14.37g. Nanofibroin filler was mixed with BisGMA, TEGDMA, Champorquinon (Sigma-Aldrich, USA) until homogeneous then casted with 3x6 mm casts of 4 pieces. Samples were polymerized with LED light cure and then labeled N1. Gold standard (Z350 XT Flowable) and negative controls (Matrix without filler) were printed in 4 pieces and polymerized. Microhardness test was performed with a microhardness tester (SHIMADZU, Japan). Samples of nanofibroin composites and nanofiller composites were analyzed using Scanning Electron Microscope (SEM) to determine the filler's charge and morphology. Silk fibers and fibroins are analyzed to determine the surface condition.

3. RESULTS

Based on the results of Scanning Electron Microscope (SEM) examination (Fig. 1) it can be seen that the sonification process to break down fibroins into nano-sized (fig. 1.D) can cause the appearance of surface roughness that plays a role when bonding with the matrix. This study analyzed silk fibers, fibroins, nanofibroin composites, and nanofiller composites under the SEM as shown in Figure 1.

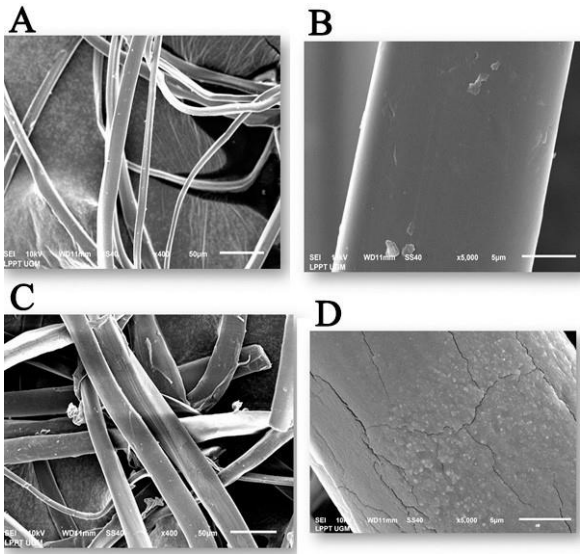
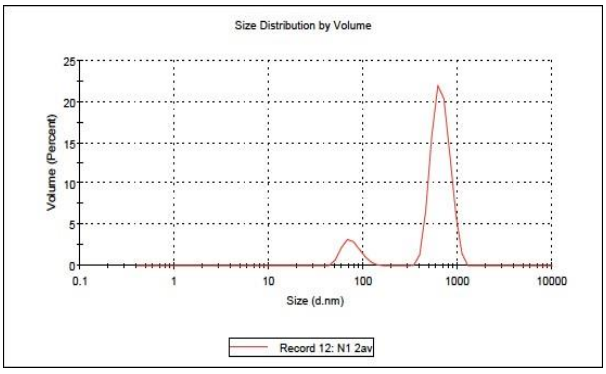


Figure 1. 400x magnification of fibroin (A) and 5000x magnification of fibroin (B). 400x magnification of nanofibroin (C) and 5000x magnification of nanofibroin (D).

Nanofibroin fillers were measured using a Particle Size Analyzer Zetaziser (Malvern, UK) measuring 672.1nm as much as 87.4% and 76.97 nm as much as 12.6%. The measurement results can be seen in table 1. Nanofibroin composites have amorphous filler morphology, whereas nanofiller composites have mostly spherical filler morphology (fig. 2.H), which can affect the filler loading in the composite.

Table 1. Size distribution by volume of nanofibroin *B. mori* L., was 672.1 nm (87.4%) and 76.97 nm (12.6%) of total volume



The following table is the microhardness calculation table of nanofibroin composite samples compared to the positive control group (composite nanofiller) and negative control (matrix composite without filler). Nanofibroin composites with 63% filler volume have an average microhardness of 146.45 VHN which is higher than nanofiller composites that have 63.3% filler volume with an average microhardness of 110.40 VHN. Compared with the two samples, the composite without

filler had the lowest average microhardness of only 31.55 VHN.

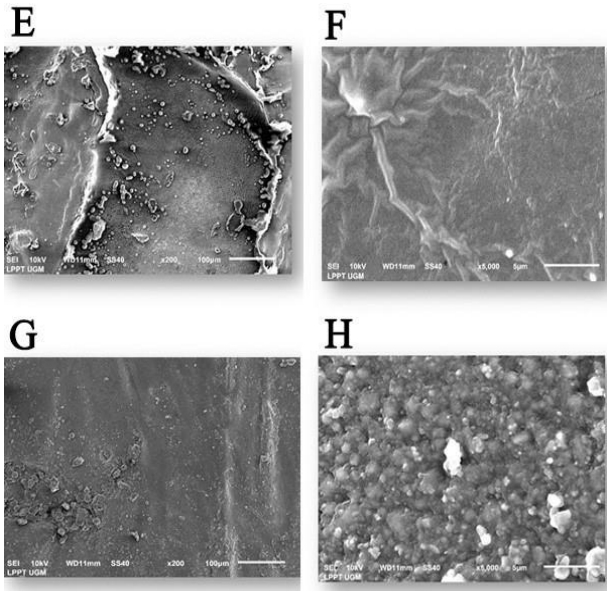


Figure 2. Nanofibroin composite (E and F), 400x magnification of nanofibroin composite (H) and 5000x magnification of nanofibroin composite with amorphous morphology (F). Nanofiller composite (G and H) 400x magnification of nanofiller composite (G) and 5000x magnification of nanofiller composite with spherical filler morphology (H).

Table 2. Microhardness (VHN) average value of nanofibroin, nanofiller composite and matrix resin

Sample	Particle size of filler (nm)	Volume of filler (%)	Weight of filler (%)	Average of VHN
Nanofibroin	672.1 76.97	63%	78%	146.45
Nanofiller	20 (Si) 4-11 (Zr)	63.3%	78.5%	110.40
Matrix Resin	-	0%	0%	31.55

4. DISCUSSION

B. mori L., fibroin is made from natural protein fibers which includes semicrystalline polymers consisting mainly of crystalline β-sheets, micro-void, and amorphous structures [18]. The crystalline domain is a hydrophobic domain and is embedded in an amorphous structural area which is a hydrophilic area and can hold moisture. The crystalline's main structure is the polypeptide chain which is mainly made of aminoglycan acid (Gly) and alanine (Ala), and the adjacent chains are bound together by strong hydrogen bonds in the anti- parallel arrangement to form β-sheets. Strong interactions between chains contribute to the superior strength of silk fibroin. The formation and strength of hydrogen bonds are very important to determine the stability and strength of crystalline β-sheets [19].

The bond that occurs between the matrix and the nanofibroin filler is a mechanical bond. The bond can be formed due to the degumming process using alkaline, because alkalinization can increase surface roughness, so that bonding occurs between the surface of the filler and the matrix (fig. 1.D) [20]. Ultrastructured composite resin which includes filler particles and the chemical composition of the composite resin has an influence on its microhardness [21]. Microhardness can also be determined by the type and number of fillers and the composition of the matrix used in the material being tested [22]. This may be the background of why microhardness possessed by *B. mori* L., nanofibroin composites and composites of Z350 XT Flowable (3M ESPE) nanofiller have quite difference in filler type and size (table 2). Nanofiller was made from anorganic materials and the size range are 4-11nm for zirconia and 20nm for silica, on the other hand, nanofibroin was made from anorganic material and the size is ranging from 672.1 to 76.97nm. Other factors that can affect the microhardness of composite resins are morphology and filler loading in composite resins, because composites that have spherical filler morphology (fig. 2.H) such as nanofiller composites have a higher filler loading than composites with fillers with amorphous morphology such as nanofibroin composites (fig. 2.F) [23], [24].

5. CONCLUSION

The *B. mori* L., nanofibroin composite has potential as filler in composite restoration materials. Researchers get better microhardness results on nanofibroin composites that have a filler volume comparable to nanofiller composites (gold standard).

AUTHORS' CONTRIBUTIONS

First authors make substantial contributions to conception and design, analysis and interpretation of data; Second author participate in drafting the article or revising it critically for important intellectual content. Third author give final approval of the version to be submitted and any revised version.

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REFERENCES

[1] R. L. Sakaguchi, J. M. P. PhD, and J. F. PhD, *Craig's Restorative Dental Materials: Elsevier Ebook on Vitalsource*. Elsevier Health Sciences, 2018.

[2] J. B. Summitt, *Fundamentals of Operative Dentistry: A Contemporary Approach*. Quintessence Pub., 2006.

[3] K. Khosravi, S.-M. Mousavinasab, and M. S. Samani, "Comparison of microleakage in Class II cavities restored with silorane- based and methacrylate-based composite resins using different restorative techniques over time," *Dental Research Journal*, vol. 12, no. 2, p. 7, 2015.

[4] T.-T. Cao and Y.-Q. Zhang, "Processing and characterization of silk sericin from Bombyx mori and its application in biomaterials and biomedicines," *Materials Science and Engineering: C*, vol. 61, pp. 940–952, Apr. 2016, DOI: 10.1016/j.msec.2015.12.082.

[5] M. K. Sah and K. Pramanik, "Regenerated Silk Fibroin from B. mori Silk Cocoon for Tissue Engineering Applications," *International Journal of Environmental Science and Development*, pp. 404–408, 2010, DOI: 10.7763/IJESD.2010.V1.78.

[6] W. I. Abdel-Fattah, N. Atwa, and G. W. Ali, "Influence of the protocol of fibroin extraction on the antibiotic activities of the constructed composites," *Progress in Biomaterials*, vol. 4, no. 2–4, pp. 77–88, Dec. 2015, doi: 10.1007/s40204-015-0039-x.

[7] Z. Shao and F. Vollrath, "Surprising strength of silkworm silk," *Nature*, vol. 418, no. 6899, pp. 741–741, Aug. 2002, DOI: 10.1038/418741a.

[8] H. A. Tuan, S. Hirai, Y. Tamada, and S. Akioka, "Preparation of silk resins by hot pressing Bombyx mori and Eri silk powders," *Materials Science and Engineering: C*, vol. 97, pp. 431–437, Apr. 2019, DOI: 10.1016/j.msec.2018.12.060.

[9] S. Puspita, M. H. Soesatyo, S. Sunarintyas, and E. Mulyawati, "The fibroin (Bombyx mori L.) is cytocompatible with human primary pulp cells," Yogyakarta, Indonesia, 2019, p. 020018, DOI: 10.1063/1.5098423.

[10] K. J. Anusavice, *Phillips' Science of Dental Materials: Middle East and African Edition*. Elsevier Health Sciences, 2003.

[11] G. Markovic and P. M. Visakh, "Polymer blends," in *Recent Developments in Polymer Macro, Micro and Nano Blends*, Elsevier, 2017, pp. 1–15.

[12] N. Nasoohi, M. Hoorizad, and S. F. Tabatabaei, "Effects of Wet and Dry Finishing and Polishing on Surface Roughness and Microhardness of Composite Resins," *J Dent (Tehran)*, vol. 14, no. 2, pp. 69–75, Mar. 2017.

[13] D. A. Abuelenain, E. A. A. Neel, and A. Al-Dharrab, "Surface and Mechanical Properties of Different Dental Composites," p. 6, 2015.

[14] N. Shakila, A. Ali, and S. Zaidi, "Micro Hardness of Dental Tissues Influenced by Administration of Aspirin During Pregnancy," *International Journal of Morphology*, vol. 33, no. 2, pp. 586–593, Jun. 2015, DOI: 10.4067/S0717-95022015000200028.

[15] D. N. Rockwood, R. C. Preda, T. Yücel, X. Wang, M. L. Lovett, and D. L. Kaplan, "Materials fabrication from Bombyx mori silk fibroin," *Nature Protocols*, vol. 6, no. 10, pp. 1612–1631, Oct. 2011, DOI: 10.1038/nprot.2011.379.

[16] R. C. Preda, G. Leisk, F. Omenetto, and D. L. Kaplan, "Bioengineered Silk Proteins to Control Cell and Tissue Functions," in *Protein Nanotechnology*, vol. 996, J. A. Gerrard, Ed. Totowa, NJ: Humana Press, 2013, pp. 19–41.

[17] A. M. Hussein, K. V. Sharma, R. A. Bakar, and K. Kadirgama, "The Effect of Nanofluid Volume Concentration on Heat Transfer and Friction Factor inside a Horizontal Tube," *Journal of Nanomaterials*, vol. 2013, pp. 1–12, 2013, DOI: 10.1155/2013/859563.

[18] S. Chen, M. Liu, H. Huang, L. Cheng, and H.-P. Zhao, "Mechanical properties of Bombyx mori silkworm silk fibre and its corresponding silk fibroin filament: A comparative study," *Materials & Design*, vol. 181, p. 108077, Nov. 2019, DOI: 10.1016/j.matdes.2019.108077.

[19] Y. Cheng, L.-D. Koh, D. Li, B. Ji, M.-Y. Han, and Y.-W. Zhang, "On the strength of β-sheet crystallites of *Bombyx mori* silk fibroin," *Journal of The Royal Society Interface*, vol. 11, no. 96, p. 20140305, Jul. 2014, DOI: 10.1098/rsif.2014.0305.

[20] M. Ho, H. Wang, K. Lau, J. Lee, and D. Hui, "Interfacial bonding and degumming effects on silk fibre/polymer biocomposites," *Composites Part B: Engineering*, vol. 43, no. 7, pp. 2801–2812, Oct. 2012, DOI: 10.1016/j.compositesb.2012.04.042.

[21] R. J. Scougall-Vilchis, Y. Hotta, M. Hotta, T. Idono, and K. Yamamoto, "Examination of composite resins with electron microscopy, microhardness tester and energy dispersive X-ray microanalyzer," *Dental Materials Journal*, vol. 28, no. 1, pp. 102– 112, 2009, DOI: 10.4012/dmj.28.102.

[22] J. Tchorz, R. Doll, M. Wolkewitz, E. Hellwig, and C. Hannig, "Microhardness of Composite Materials With Different Organic Phases in Deep Class II Cavities: An *In Vitro* Study," *Operative Dentistry*, vol. 36, no. 5, pp. 502–511, Oct. 2011, DOI: 10.2341/10325-L.

[23] K.-H. Kim, J. L. Ong, and O. Okuno, "The effect of filler loading and morphology on the mechanical properties of contemporary composites," *The Journal of Prosthetic Dentistry*, vol. 87, no. 6, pp. 642–649, Jun. 2002, DOI: 10.1067/mpr.2002.125179.