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RESEARCH ARTICLE

The effect of sisal *(Agave Sisalana)* nanofiber in epoxy resin sealer on root canal obturation's push-out bond strength

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ABSTRACT

Sisal nanofiber can be used as an additional filler to increase the adhesion strength of the resin epoxy sealer. The aim of this study was to observe the effect of sisal nanofiber addition to epoxy resin sealer on push-out bond strength of root canal obturation material against the root canals. Twenty-five mandibular premolars (n= 25) were prepared until file F3 and obturated using 0%, 0.25%, 0.5%, 0.75%, and 1% sisal nanofiber sealer. After the specimens were stored at incubator, they were horizontally sectioned at apical third. Root sections were tested with push-out technique, and observed under a stereo microscope to determine the failure type. Data were analyzed with one-way ANOVA and LSD pos hoc at 95% confidence level (α=0.05). A one-way ANOVA test revealed that the addition of sisal nanofiber in epoxy resin sealer had a significant effect on the push out bond strength of the obturation material against the root canals. The failure type was observed predominantly in the cohesive type and the lowest in the adhesive type. The addition of sisal nanofiber to the epoxy resin sealer could increase the push-out bond strength of the obturation material against the root canals. Epoxy resin sealers with the addition of sisal nanofiber at a concentration group of 0.75% resulted in the highest push-out strength of root canal obturation materials followed by 0.5% concentration group.

Keywords: epoxy resin sealer; push-out bond strength; sisal nanofiber

INTRODUCTION

The main goal of root canal treatment is to create an environment in the root canal system that allows the healing process to occur and maintains the health of the periradicular tissues. The obturation of the root canal system is an important phase of root canal treatment that aims to prevent leakage from the oral cavity and periradicular tissue into the root canal system.[1] Epoxy resin-based sealers have been widely used because they have radiopaque properties, low solubility, small shrinkage, antibacterial properties, and low toxicity.[2] The ability of a resin-based sealer to penetrate the dentinal tubules leaves a layer of sealer enriched with filler particles larger than the diameter of the dentinal tubules that couldn't penetrate the tubules dentin. This will result in a weak bond because the ratio of filler particles is too large in the sealer layer.[3] The addition of nanofillers to the sealer can help increase the bond strength between the sealer and the dentine of the root canals because the nano-sized fillers can penetrate the dentinal tubules with resin components.[4]

Nano-sized sisal fibers have been developed as a filler material for dental composite restoration material because sisal fibers have good mechanical properties. The use of nano-sized sisal fibers can increase the hardness of the restoration material and reduce the attachment of Streptococcus mutans colonies to the nanosisal composites.[5] Sisal nanofibers are classified into the type of nanocellulose material.[6] Epoxy nanocomposite resin with a 0.5% nanocellulose can improve its mechanical properties, which are tensile strength and compressive strength.[7] The aim of this study was to observe the effect of sisal nanofiber addition to epoxy resin sealer on push-out bond strength of root canal filling material against the root canals.

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MATERIALS AND METHODS

This was experimental, laboratory-based study and the procedure was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada, in accordance with Ethical clearance No.00416/KKEP/FKG-UGM/EC/2020. The preparation of nanofiber from sisal fiber was conducted through alkalization, bleaching, sonication and freeze-drying process at Nutrition Laboratory and Biotechnology Laboratoray, Universitas Gadjah Mada according to method by Sosiati et al.[8] Transmission electron microscopy (TEM) was applied to analyze the size of sisal nanofiber at Anorganic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada.

The FTIR was used to identify sisal nanofiber functional groups, epoxy resin sealer, and epoxy resin sealer with the addition of sisal nanofiber which was carried out at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada.

Sisal nanofiber sealer was prepared from AH-26 silver free epoxy resin sealer (Dentsply, Germany) powder added by sisal nanofiber and stirred with an epoxy resin paste with 3:1 ratio on mixing pad for 30 seconds using metal spatula. The mixing between resin epoxy sealer powder and sisal nanofibers was done at Biotechnology Laboratory, UGM. Sisal nanofiber sealer powder was prepared at various concentrations using sisal nanofiber as the filler, which is, 0.25%, 0.5%, 0.75%, and 1% (in weight).

The wettability of the sealer was evaluated using a contact angle. The contact angle is the resultant between the adhesive strength of the sealer and the substrate and the cohesive strength of the sealer.[9] The sealer that had been stirred was checked to see its flowability until it could be lifted above 1.5 - 2.5 cm above the glass plate until it broke, then 0.3 ml was placed on the glass object. After 3 minutes and 10 minutes from the start of mixing the sealer, a photo of the sealer was taken. The measurement of the contact angle was carried out on the photos via a computer using Corel Draw software and the contact angle modified image J.[10]

Samples of twenty-five mandibular premolars were prepared at the root canal, and the crown was cut using a diamond disc bur to leave 14 mm of the root. K-file number 15 was inserted up to the apical foramen section with a working length of 13 mm. The root canals were prepared by the crown-down technique using ProTaper (Dentsplay) in clockwise reaming movements. The ProTaper consists of shaping files (X1, X2 and X3). The preparation was started using X1 file and then X2 file with a working length of 13 mm, and completed using X2 file until a working length of 13 mm was reached. The apical gauging of root canal foramen was measured using the last same K-file numbers of 30. Each root canal file was irrigated with a maximum of 2 ml of 2.5% sodium hypochlorite irrigation solution using a Max-I-Probe irrigation needle (Dentsply, Maillefer, North America) and 17% ehylenediaminetetraacetic acid (EDTA) alternately. Subsequently, the root canals were flooded with sodium hypochlorite for 5 minutes, 17% EDTA for 1 minute, and then irrigated again using saline and dried using paper points.

The samples were divided randomly into five treatment groups of five teeth each. Group I was obturated with 0% sisal nanofiber sealer, group II was obturated with 0.25% sisal nanofiber sealer, group III was obturated with 0.5% sisal nanofiber sealer, group IV was obturated with 0.75% sisal nanofiber sealer, and group V was obturated with 1% sisal nanofiber sealer. After the specimens were stored in an incubator at 37 °C for seven days, specimens were then horizontally sectioned in apical third with 2 mm thickness. Root sections were tested with push-out technique at a crosshead speed of 1 mm/min using a universal testing machine from the apical to the coronal direction until bond fracture occurred. The maximum load applied to filling material before fracture was recorded in newtons and converted to megapascals (MPa) according to the following formulation:[11]

Push-out bond strength (MPa) = N/A
N = maximum load (N)
A = adhesion area of root canal filling (mm^2)

The adhesion surface area of each section was calculated as: (p r1 + p r2) 9 L, where L = √ (r1 – r2)2 + h2; p = 3.14, r1 and r2 = smaller and larger radii, respectively, and h = thickness of the slice in mm. Data were analyzed with one-way ANOVA and LSD pos hoc at 95% confidence level (α=0.05).

After obtaining data that was normally distributed and homogeneous, it was continued with one-way Anova parametric test to determine the effect of adding sisal nanofibers in epoxy resin sealers on the attachment of root canal obturation materials and continued with the post hoc LSD test.

A stereo microscope was examined to determine the type of failure of the attachment of the root canal filling material. Each sample was evaluated under a stereo microscope at a 40x difference. The results obtained are included in the following categories: type I is the adhesive failure between the dentine and the sealer, type II is a cohesive failure in the root canal filling material, and type III is a combination of adhesive and cohesive failure (the dentine surface is partially covered with the sealer).

RESULTS

Observation of sisal fibers that have been nano-sized was conducted using TEM (Transmission Electron Microscopy). Figure 1 showed the sisal nanofibers observed via TEM. The image was analyzed with the ImageJ application, and showed that sisal nanofibers measure 7-20 nm in diameter and 150 – 1000 nm in length.

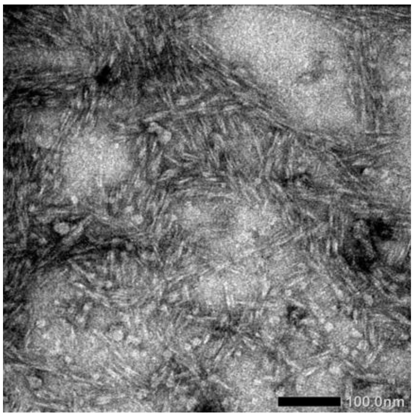

Figure 1. TEM image of sisal nanofibers with diameter of 7-20 nm and length ranging from 150-1000 nm

The FTIR test results on the synthesized sisal nanofiber indicate that the alkalization and bleaching did not damage the chemical bonds that makeup cellulose, the -CH2 bonds, C-O bonds, C-H bonds and -OH bonds. The loss of the C = O bond proves that the noncellulose component in the synthesized sisal nanofiber has been reduced during the scouring, bleaching, and ultrasonic processes similar to the results of the research by Kargarzadeh.[12]

The FTIR analysis of epoxy resin sealers (AH 26) at 914 cm-1 plays a role in the formation of the C-O groups in the oxirane group. According to González et al. the main characteristic of epoxy monomers is the oxirane functional group, which is a three-membered ring formed between two carbon atoms and oxygen which is absorbed in a wavelength of ~ 914 cm-1.[13]

FTIR test results on epoxy resin sealer without additional sisal nanofiber and epoxy resin sealers with additional sisal nanofibers showed that both graphs have almost the same absorption area. The wideband at the 3433 cm-1 peaks is the absorption area of the hydroxy group (-OH) which is almost the same as the wideband at 3448 cm-1, as found in the FTIR test results of the epoxy resin sealer, which indicates a possible interaction between the epoxy resin sealers and sisal nanofibers in the area. This can indicate that the addition of sisal nanofibers has the potential as a filler in the epoxy resin sealers.

The results of the measurement of the contact angle in all concentration groups showed that the contact angle was <90°. This demonstrates that the addition of sisal nanofiber to the epoxy resin sealers does not change the wetting ability of the epoxy resin sealers. An adhesive material has a contact angle of <90°, so it has the ideal wetting ability that can flow, fill the gaps between the gutta percha and root canal walls, and penetrate the dentin surface of the root canal, thereby increasing the adaptability of sealers and root canal walls without any porosity between the surfaces.[14]

The mean and standard deviation of the push-out bond strength of root canal obturation materials with epoxy resin-based sealer without addition and with the addition of sisal nanofibers in several concentration groups can be seen in Table 1. The results of the one-way ANOVA test (Table 1) showed that the addition of sisal nanofiber in the epoxy resin siler had a significant effect on the push-out bond strength of the root canal obturation material (p<0.05).

Based on LSD test (Table 2), it can be seen that the push-out bond strength of obturation material with the addition of sisal nanofiber in the epoxy resin-based sealer in the 0.5% and 0.75% concentration groups in the epoxy resin sealers was significantly different (p <0.05) compared to other sample groups. However, there was no significant difference between the groups that were obturated using 0% sisal nanofiber sealer and those that were obturated using 0.25% sisal nanofiber sealer and between groups that were obturated using 0% sisal nanofiber sealer and those that were obturated using 1% sisal nanofiber sealer. The concentration group of 0.75% (mean 8.25 ± 0.982) had the highest push-out strength of obturation root canal material followed by the 0.5% concentration group (mean 6.42 ± 0.913).

Table 3 showed the distribution of the percentage of the mode of push out bond strength failure analysis and the most common failure mode was mixed failure among the group. Specimen sample of every type push out bond strength failure was analyzed by stereomicroscope shown in Figure 2.

Table 1. Mean and standard deviation (SD) of the push-out bond strength of root canal obturation materials with epoxy resin-based sealer without additives and additional sisal nanofibers in several concentrations in Megapascal (MPa)

Obturation material	n	Mean ± standard deviation
Resin epoxy based sealer + sisal nanofiber (0%)	5	3.68 ± 1.334
Resin epoxy based sealer + sisal nanofiber (0.25%)	5	4.59 ± 1.075
Resin epoxy based sealer + sisal nanofiber (0.5%)	5	6.42 ± 0.913
Resin epoxy based sealer + sisal nanofiber (0.25%)	5	8.25 ± 0.982
Resin epoxy based sealer + sisal nanofiber (0.25%)	5	3.87 ± 0.875

Table 2. Result of LSD pos hoc test of the push-out bond strength of root canal obturation materials with epoxy resin-based sealer without additives and additional sisal nanofibers in several concentrations in Megapascal (MPa).

Group		LSD post hoc test
Sisal nanofiber sealer 0%	Sisal nanofib*er* sealer 0.25%	0.184
	Sisal nanofiber sealer 0.5%	0.001*
	Sisal nanofiber sealer 0.75%	0.000*
	Sisal nanofiber sealer 1%	0.768
Sisal nanofiber sealer 0.25%	Sisal nanofiber sealer 0.5%	0.012*
	Sisal nanofiber sealer 0.75%	0.000*
	Sisal nanofiber sealer 1%	0.295
Sisal nanofiber sealer 0.5%	Sisal nanofiber sealer 0.75%	0.012*
	Sisal nanofiber sealer 1%	0.001*
Sisal nanofiber sealer 0.75%	Sisal nanofiber sealer 1%	0.000*

*): significantly different (p<0.05)

Table 3. Percentage type of push-out bond strength failure of root canal obturation materials without addition and with the addition of sisal nanofibers in epoxy resin-based sealers in various concentrations

Group	Type of Failure		
	Tipe I	Tipe II	Tipe III
I	1	3	1
II	0	4	1
III	1	2	2
IV	0	4	1
V	I	3	1
Total	3(12%)	16 (64%)	6 (24%)

(A) (B) (C)

Figure 2. (A) The results of a stereo microscope examination with 40x magnification on a specimen with type I failure or adhesive failure, showing that the root canal walls are clean of sealers; (B) type II failure or cohesive failure indicates that the entire root canal wall is covered by sealers; (C) type III failure or a combination of adhesive and cohesive failure depicts that part of the root canal wall is covered by sealers

DISCUSSION

TEM (Transmission Electron Microscopy) observations resulted in sisal nanofibers with diameter of 7 - 20 nm and length ranging from 150 - 1000 nm. Sisal nanofibers are classified into cellulose nanofibers, which are cellulose-based nanomaterials with flexible fiber networks that have a diameter of 4 - 20 nm and a length of > 1 μm so that they have a high surface aspect ratio.[15] Natural cellulose is composed of microfibrils which have advantages in several mechanical properties.[16] Cellulose nanofibers show an amorphous phase and crystalline cellulose area and have a large surface area, making them a good filler to achieve more effective reinforcement in a polymer matrix.[17] Various studies using cellulosic nanofibers have shown that fibers with smaller and longer diameter sizes exhibit stronger reinforcing effects.[18] The pure cellulose component extracted from sisal fiber in this study is an important component as a reinforcing material needed to increase the attachment strength of the obturation material of the root canal. Natural cellulose has advantages in several potential mechanical properties as a composite reinforcing agent.[16]

The chemical bond between the epoxy resin sealers and sisal nanofiber was shown by the results of the Fourier transform infrared spectroscopy (FTIR), namely the similarity of the absorption area pattern in the hydroxy group (-OH) wavelength in the form of a wideband at a wavelength of ~ 3400 cm -1. Neelakantan et al.[19] stated that a wide band of ~ 3400 cm -1 is an -OH group of epoxide-amine polymer epoxy resin sealers, which are associated with intramolecular and intermolecular hydrogen bonds. Epoxy resins contain several polar functional groups, which can interact with the -OH groups on the surface of the sisal nanofiber via hydrogen bonding.

In endodontics, the contact angle formed by the siler (liquid) to the surface of the dentin (solid) or gutta percha is very important.[14] In this study, all sisal nanofiber sealers in all concentration groups had the the contact angle measurement of <90°. However, sisal nanofiber was added to several concentration groups to generate good flowability as a way to wet the root canal dentin and carry the sisal nanofiber component through the dentinal tubules and fill the area microporosity in dentin, which has an irregular structure. This is due to the good interaction between sisal nanofibers and the epoxy resin matrix.

One-way ANOVA test showed that there was an effect of sisal nanofiber addition in epoxy resin sealer on the push-out bond strength of the obturation material of the root canals. This research showed that the addition of sisal nanofiber to the epoxy resin sealer with a concentration of 0.5% had an effect on increasing the push-out strength of the obturation material of the root canal, with the optimum value at a concentration of 0.75%. These results are consistent with the research of Saba et al. that the addition of nanocellulose at concentrations of 0.5% and 0.75% at the time of making nanocomposites using an epoxy matrix can improve the mechanical properties of a nanocomposite.[20] This occurs because of the good interface adhesion between the nanocellulose and the epoxy matrix, and the absence of agglomeration.

In this study, sisal nanofibers addition at sealer resin epoxy at 0.5% and 0.75% concentration acted as a barrier against crack propagation, which caused the strength needed to release the sisal nanofiber sealer from dentin to be greater when the push-out test was carried out. A homogeneously dispersed filler in an adhesive material serves as a barrier against crack propagation which will cause crack deflection.[21] At a concentration of 0.75% sisal nanofiber plays an optimal role as a barrier against crack propagation, which can initiate a failure to adhere to the interface between the sealer and the dentin. At optimal concentrations, well dispersed nanocellulose in the polymer matrix can increase the interlocking bond between the filler and the matrix, thereby increasing interface adhesion.[22]

In this study, the addition of sisal nanofibers in epoxy resin sealer at a concentration of 0.25% did not increase the push-out bond strength of the obturation material, which was probably due to the insufficient amount of sisal nanofiber needed as a barrier against crack propagation, so the strength needed to remove obturation material was not much different compared to the group without the addition of sisal nanofibers.

A filler can be distributed homogeneously if there is good inter-surface interaction and adhesion between the filler and the epoxy resin matrix.[23] The epoxy resin matrix which can wet sisal nanofiber will increase the high interface interaction and reduce the sisal fiber to nanofiber which can facilitate the fusion or adhesion of the filler with the matrix so that it can improve the mechanical properties and structural properties of the composite. In this study, good adhesion between sisal nanofiber and epoxy resin matrix was also obtained from the scouring (alkalization) process and bleaching using 6% NaOH and 3% H_2O_2 during the process of making sisal nanofibers. The alkalization treatment causes better penetration of the matrix into the fibers, because the surface of the fiber becomes cleaner from dirt and lignin layers. Another effect due to alkaline treatment is that the fiber surface becomes rougher and more porous, making it easier for the matrix to be absorbed by the fiber.[24]

The addition of nanofiber in the epoxy resin sealer, which possibly penetrates the dentinal tubules measuring 1.5 - 2 μm, also plays a role in increasing the adhesion of the epoxy resin sealer with the addition of sisal nanofibers. This study used the sisal nanofiber at the size of 7 - 20 nm to allow penetration into the sized dentinal tubules. Fillers that are able to penetrate into the dentin tubule together with the resin component will increase the strength of the sealer adhesion to the root canal wall.[25]

The absence of an increase in the push-out bond strength of obturation materials in the 1%

concentration group is probably due to the sisal nanofiber agglomeration which decreases the overall interface bond between the matrix and the filler, thereby reducing the potential strengthening effect of a nanomaterial. The existence of a weak interface bond between the epoxy resin matrix and the filler will result in micro voids and porosity in a nanocomposite so that stress concentration causes premature failure when applying tensile/compressive stresses.[20]

The examination using a stereo microscope to see the type of failure that occurred in specimens type II failure (cohesive failure) resulted in the highest failure of 64% in all treatment groups. This might be due to the high strength of the sealer nanofiber sisal bond to the dentin. However, a research by Tedesco et al. revealed that there was no correlation between failure patterns and sealer penetration of the root canal dentinal tubules.[26] This highlights that the adhesion strength of a sealer does not only depend on the penetration of the sealer against the dentin. Adhesion to root canal dentin is a complex process because it involves many factors, namely the physical and chemical properties of a sealer, the surface energy of the dentin, the surface tension of the sealer, the wetting ability of the sealer, the cleanliness of the root canal surface, as well as the stress that occurs due to differences in thermal expansion coefficients and changing dimensions during setting.[27]

CONCLUSION

There is an effect of sisal nanofiber addition in epoxy resin sealer on the push-out strength of root canal obturation materials. The push-out bond strength of the root canal obturation material with the addition of sisal nanofiber in the epoxy resin sealer was the highest in the 0.75% concentration group followed by the 0.5% concentration group. The addition of sisal nanofibers groups with concentrations of 0.25% and 1% resulted in the same push-out strength of root canal obturation materials as the epoxy resin sealer group without sisal nanofiber addition (control).

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