Majalah Kedokteran Gigi Indonesia
Vol 7 No 3 – December 2021
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.62867

RESEARCH ARTICLES

Effects of sisal nanofiber addition to epoxy resin-based sealer on its antibacterial power against *Enterococcus faecalis*

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ABSTRACT

Sealer is one of root canal filler materials which has been developed and has an antibacterial agent to keep root canal sterile during and after an obturation process. This study aimed to find out the effect of sisal nanofiber addition to resin epoxy-based sealer on the antibacterial power against *Enterococccus faecalis* (*E. faecalis*) which is known as adaptive and potent bacteria which can be both aerobic and anaerobic. Sisal fibers were processed through many stages to make it nano sized (scouring, bleaching, neutralization, ultrasonification, and freeze-drying). Once nano-sized sisal fibers had been obtained, they were then mixed with sealer powder (AH26) in different concentrations: powder 0%, 0.25%, 0.5%, 0.75% and 1%. These concentrations were chosen based on preliminary research for reasonable contact angle measurement of sisal-sealer mixture. Antibacterial effect was examined using the diffusion method, each concentration was tested in 5 petri dishes which were planted with 1.5 X 10^8 CFU/ml *E.faecalis* bacteria. Each dish consisted of 5 holes (6 mm in diameter), each hole represented each concentration of nano sisal and sealer which were mixed until homogenous for 3 minutes before added to each hole. The dishes were then incubated for 48 hours at 37 °C. Inhibitory zones were measured, and analyzed using one-way ANOVA. The one-way ANOVA result showed that p=0.502 (p>0.05), meaning that the sisal nanofiber addition to epoxy resin-based sealer had no effect on *E.faecalis* inhibition. Thus, there was no effect of sisal nanofiber addition to epoxy resin-based sealer on *E. faecalis*.

Keywords: antibacterial; *Enterococcus faecalis*; epoxy resin-sealer; sisal nanofiber

INTRODUCTION

Dental caries is a problem which, if not treated promptly, can lead to pulp necrosis, requiring root canal treatment.[1] In root canal treatment, the methods, tools, and materials used always change and develop in a direction that allows patients and dentists to have several alternative options. One of the materials in root canal treatment that is still being researched and developed is sealer.

Sealers that are currently widely used are made from epoxy resin. Epoxy resins have been widely used to obtain better adaptation between fillers and restorative materials, which are currently mostly resin-based. The resin-based sealers currently on the market are of fairly good quality, but it is still possible to improve their physical properties, biocompatibility, radiopacity, long-term dimensional stability, and adhesion to dentin with the addition of a filler.[2]

Materials that can be used as fillers are natural fibers, for examples pineapple fiber, bamboo fiber, and sisal fiber. Sisal fibers are fibers derived from the Agave plants, namely *Agave Cantala* and *Agave Sisalana*.[3] Sisal contains alkaloids, flavonoids, saponins, and tannins which have an antibacterial effect.[4] Epoxy resin sealers also have antibacterial properties and are capable of achieving good penetration depths into the dentinal tubules in clinical situations.[5] However, epoxy resin sealers are highly toxic when mixed, yet the toxicity immediately decreases rapidly, allowing them to become sealers with the lowest toxicity compared to other sealers after 24 hours. The antibacterial content in the sealer is also very useful for keeping the root canal conditions sterile after obturation.[6]

The most common bacteria found in root canals are *Enterococcus faecalis (E. Faecalis)*. They are gram-positive bacteria that are resistant

Majalah Kedokteran Gigi Indonesia. December 2021; 7(3): 165 – 170
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

and anaerobic, so they are able to survive in root canals that are not reached by oxygen. Although the preparation, irrigation, and sterilization processes are able to remove most of these bacteria in the root canals, there may still be bacteria remaining and trapped in the dentinal tubules because these bacteria are able to form colonies and good adhesions to the protein surface and form biofilms on the dentinal walls.[7] These bacteria can penetrate into the dentinal tubules as deep as 600-1000 µm, while sodium hypochlorite is only able to penetrate at a depth of 60-150 µm. Sealer is a material that lasts for a long time in the root canal, so it is expected to be able to kill more *E. faecalis* bacteria[2] to keep it sterile during the last period of the root canal treatment.

MATERIALS AND METHODS

Ethical Clearance was obtained from the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada Number 00477 / KKEP / FKG-UGM / EC / 2020. Sisal fibers of their original size were processed to sisal nanofiber and mixed with epoxy resin-based powder by weight concentration. A total of 5 pieces of petri dishes were spread out with *E. faecalis* with a concentration of 1.5 X 10^8 CFU/ml. Each dish consisted of 5 wells with different concentrations of sisal sealers, namely 0%, 0.25%, 0.5%, 0.75%, and 1%. These concentrations were determined based on research on the acceptable sealer viscosity by measuring the contact angle of each concentration. The sisal fibers of their original size were processed into nano-sized fibers through three stages, namely alkalization, neutralization - bleaching, and sonication to break the fibers into nano-sized ones.

The alkalization process (scouring) was carried out by placing 5 grams of sisal fiber which had been cut into 300 ml of 6% w / v NaOH solution (the immersion ratio was 1 gram of fiber to 60 ml of solution), heated continuously at a temperature of 100 °C using a hotplate stirrer for 3 hours with stirring using a magnetic stirrer. The solution was added every 1 hour until it was equal to the initial volume, namely 300 ml. This procedure was repeated in triplicate to remove the lignin and cellulose fiber content.

The alkalized fibers were neutralized by immersing them in 1% CH_3COOH solution then rinsed with running distilled water. The pH was then measured using a pH meter. The neutralization process was complete once the neutral pH was reached. The bleaching process was carried out by heating the sisal fibers in a solution of 1% NaOH and 3% H_2O_2 at a temperature of 70 °C for 2 hours continuously and stirring with a magnetic stirrer. This process was also repeated in triplicate. The ultrasonication process was carried out by placing the sisal fibers into distilled water, and sonication was performed at 750 Watt, 20 kHz, and an amplitude of 39% for 2 hours. This process aimed to break down the sisal fibers into nano size. The sisal powder was then dried using a freeze dryer for 24 hours, resulting in thin flakes resembling nano-sized sisal powder (analyzed using *TEM*, in dispersion of 100-200 nanometer, ImageJ observed that the nano-sized sisal was 15-30 nanometer in diameter and 100-300 nanometer in length).

Sisal nanofiber sealers were obtained by mixing AH 26 sealer (AH 26 sealer consists of material powder and resin liquid which were needed to be mixed to be a paste) powder with sisal nanofiber powder. For the manufacture of sisal sealer with a concentration of 0.25%, 5 mg of sisal nanofiber was mixed with 1995 mg of sealer powder, resulting in powder with a total weight of 2000 mg, then stirred by ultrasonic with a circular motion until homogeneous. Likewise, the calculation and preparation of the nanofiber sisal sealer powder with other sisal percentages (according to Table 1) were done. The mixing was done using a low ratio, by considering the results of the contact angle and consistency tests.

E. faecalis bacteria were cultured and diluted according to the Mc. Farland 0.5 i.e., 1.5 x 10^8 CFU / ml. The diluted bacterial suspension was smeared with a sterile cotton swab on MHA media on 5 petri dishes, then a 6 mm diameter well was made of 5 wells with the same distance in each petri dish

Table 1. Weight of sisal nanofiber and epoxy resin sealer powder in each group (0%, 0.25%, 0.5%, 0.75%, and 1%)

Groups (concentration)	Sisal nanofiber weight (mg)	Epoxy resin sealer powder weight (mg)
I (0%)	0	2000
II (0.25%)	5	1995
III (0.5%)	10	1990
IV (0.75%)	15	1985
V (1%)	20	1980

that had been smeared with bacteria (Figure 1). According to the predetermined concentrations, the sisal sealer powder was mixed with the resin (115 mg of sisal nanofiber powder and 57.5 mg of resin were stirred homogeneously and left for 3 minutes until the polymerization process took place) and put into uniformly sized wells (the wells were made using the same size punch, and the mixture was placed using a small spatula until the wells were fully filled) that had been marked / labeled that represented each concentration. The petri dishes were left at room temperature for 2 hours to pre-diffuse the test materials. Incubation was carried out for 48 hours at 37 °C in an incubator.

The inhibition zone was observed as a transparent clear zone (as shown in Figure 3) around the well. The zone of this thin circular area was measured by the same operator to avoid bias, using a digital caliper with an accuracy of 0.05 mm. The measurements were made from three locations, then the mean drag zone was calculated and analyzed (Figure 2).

RESULTS

The results of this study showed a thin transparent circular area around the well. This indicates that the bacteria on this zone did not thrive well compared to outer zone. This means that the growth was hampered by the agent in the well.

The results of the research on the inhibition zone of sisal nanofiber sealers as in Figure 3 are shown in Table 2, as follows:

Table 2 above shows that the mean inhibition zone ranged from 6.58 - 7.33 mm. To test whether

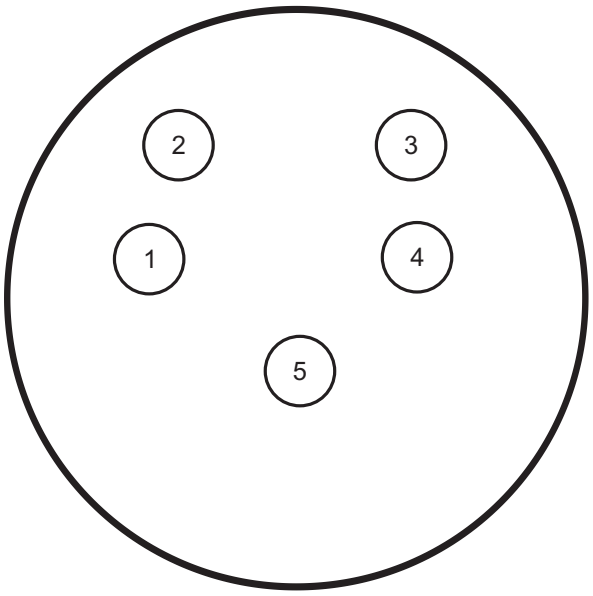
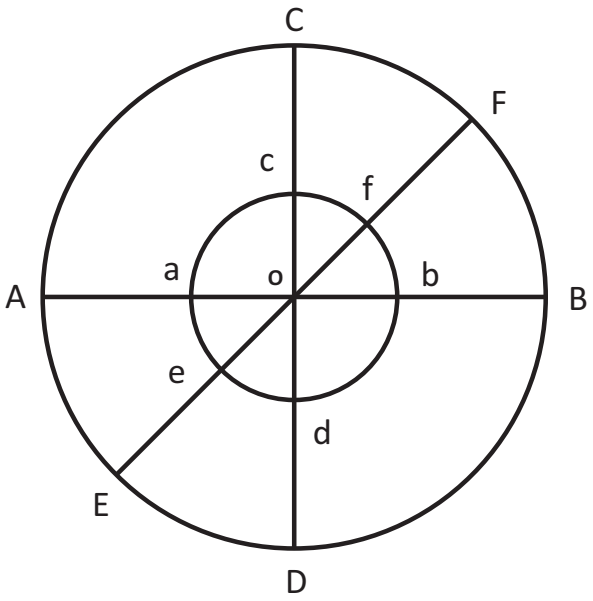


Figure 1. The position of the well on a petri dish containing MHA where the wells were numbered 1 = 0%, 2 = 0.25%, 3 = 0.5%, 4 = 0.75%, and 5 = 1%



$$\frac{½ (AB-aWb) + ½ (CD-cd) + ½ (EF-ef)}{3}$$

Where:
Point O: The center of the pit
Lines A-B, C-D, E-F: the formed inhibition zone
Lines a-b, c-d, e-f: diameter of the pit
Measurement I: (AB-ab) / 2
Measurement II: (CD-cd) / 2
Measurement III: (EF-ef) / 2

Figure 2. Measurement of the inhibition zone of sisal nanofiber sealers against *E.faecalis* taken from 3 places in each well

Majalah Kedokteran Gigi Indonesia. December 2021; 7(3): 165 – 170
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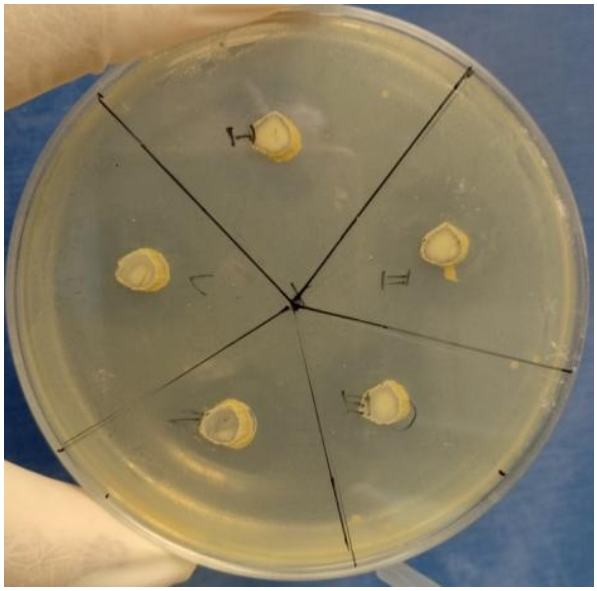

Figure 3. Observation of the inhibition zone that was found around the well, as a transparent clear zone on the media surrounding the well containing sisal nanofiber sealers

there was an effect of adding sisal nanofiber on the anti-bacterial power of epoxy resin sealers against *E. faecalis*, a one-way ANOVA test was performed (Table 3).

The value of p = 0.502 (p>0.05) indicated that the addition of sisal nanofiber had no effect on the anti-bacterial power of epoxy resin sealers against *E. faecalis* bacteria.

DISCUSSION

The results of this study showed there was no effect of adding sisal nanofiber to the epoxy resin sealer. The results of this study are different from the results of research conducted by Nugroho[8] which showed that sisal has an antibacterial activity against *S. mutans* because it contains alkaloids. This is because the research conducted by Nugroho used composite resins that do not contain antibacterial properties, so the addition of sisal nanofiber can clearly show its antibacterial effect. Nugroho added sisal nanofiber in a large concentration, namely 60%, to the composite resin, thus resulting in significant changes in physical and chemical properties. The addition of a large concentration of sisal nanofibers indeed increases the antibacterial power of the resin, but in the context of epoxy resin sealers, such addition will also change the physical characteristics of the epoxy resin sealers. The addition of nanofiber with a concentration above 2% from the results of this study was proven to increase the contact angle, which means that it will decrease the density and adhesion of the sealer to the surface.

There was no significant effect of the sisal addition to the sealer since the pure sealers had antibacterial components, each of which with antibacterial properties, so the difference in the width of the inhibition zone between the pure sealer and the sealer with the sisal addition was not significant. Epoxy resin-based sealers contain Bismuth oxide, Methenamine, Silver, and Titanium dioxide. Methenamine is a material used for the polymerization of resin sealers and when the polymerization process takes place, methenamine will release formaldehyde. The setting time of

Table 2. Mean inhibition zone of sisal nanofiber sealers against *E. faecalis* in 5 sample groups (0%, 0.25%, 0.5%, 0.75%, and 1%)

Concentration	n	Mean	Std Deviation
0%	5	6.58	0.636916
0.25%	5	6.99	0.639512
0.50%	5	7.33	0.275154
0.75%	5	7.08	0.630985
1%	5	6.95	0.905595

Table 3. One-way nanofiber sisal sealer pathway in 5 sample groups (0%, 0.25%, 0.5%, 0.75%, and 1%)

Sample	Sum of Square	df	Mean Square	F	Sig.
Between Group	1.457	4	0.364	0.864	0.502
Within Group	8.434	20	0.422		
Total	9.892	24			

this sealer is 9-15 hours; this sealer releases formaldehyde up to 48 hours after mixing.[9] Formaldehyde is a strong bactericide, as well as toxic to tissues. Formaldehyde will continue to decrease until it disappears after 48 hours, therefore, the bactericidal properties of this sealer work well within 48 hours in the beginning, and at the same time are also toxic to the tissue at the beginning of its application, so intrusion of the sealers into the periapical tissue must be avoided. AH26 sealer used in this study also contained silver. Many studies have shown that silver is an antibacterial agent and is widely used as a disinfectant mixture.

The process of setting the epoxy resin sealer increased the pH of this sealer, Joseph[10] also stated the same thing regarding the addition of sisal to epoxy resin which would cause alkaline properties. This certainly changes the original properties of the sisal fiber and also the epoxy resin itself to be rigid and to produce a strong composite material. This condition also affects bacterial growth. *E. faecalis* bacteria are resistant to all conditions, but changes in pH that occur in the near future will also affect and inhibit their growth rate. Within 24 hours, the pH of the epoxy resin-based sealer will significantly change to neutral.[11] Pasril[7] in his research also explained the effect of high pH (alkaline) in $Ca(OH)_2$ which makes this material effective in killing *E. Faecalis* bacteria. Another reason why the addition of the sisal nanofiber in this study had no effect on the anti-bacterial power of the nanofiber sisal was the fact that the sisal nanofiber added was in a low concentration. The addition of sisal nanofiber in a high concentration will affect the viscosity and density of sealers which will actually reduce their mechanical properties, but very small concentrations of sisal will not be able to significantly increase their antibacterial power against *E. faecalis*.

Based on the literature review, it is known that sisal contains alkaloids, flavonoids, saponins, and tannins which have antibacterial properties. A bleaching process, which is carried out when making nano-sized sisal, removes tannins. Tannins are one of the pigments contained in sisal fibers that must be removed through the bleaching process to make nano-sized sisal fibers. Tannins are antibacterial by deactivating bacterial adhesin, inhibiting enzyme action, and inhibiting protein transport to the cell sheath.[12] Tannin toxicity damages bacterial cell membranes and forms metal ion complex bonds from tannins which play a role in tannin toxicity. With the loss of tannins as a pigment component in nano-sized sisal, there is also a reduction in one of the anti-bacterial components in sisal of this size.

CONCLUSION

The addition of sisal nanofiber with a concentration of 0.25%, 0.5%, 0.75%, and 1% had no effect on the antibacterial power of epoxy resin-based sealers against *Enterococcus faecalis* bacteria.

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