ORIGINAL ARTICLE

Antibacterial Properties of Hydrogel Membranes Infused with Liquid Smoke on Growth Inhibition of *Staphylococcus aureus*

A.A. Sutadi Saputra, Tyas Prihatiningsih, Oedijani Santoso

Department of Dental Medicine, Faculty of Medicine, Universitas Diponegoro, 50275 Tembalang, Semarang, Central Java, Indonesia

ABSTRACT

Introduction: *Staphylococcus aureus* is the most common bacteria infecting chronic wounds. Wound dressing are available in various physical forms including hydrogels. However, hydrogels are expensive and contain synthetic materials, so natural materials such as liquid smoke infusing to the hydrogels will be improve its composition and needed as antibacterial wound dressing. This study analysed the difference in antibacterial activity between a hydrogel base and hydrogel membranes infused with liquid smoke to inhibit the growth of *S. aureus*. **Methods:** This study was a true laboratory experiment with a post-test only control group design. Twenty-four samples were divided into four concentration groups of 0%, 8%, 12% and 16%. The parallel streak method (AATCC 147-2004) was used for antibacterial testing. Data were analysed using Kruskall-Wallis and Mann-Whitney tests. **Results:** The average clear zone widths of inhibition of *S. aureus* at 0%, 8%, 12% and 16% were 0.013 mm, 0.416 mm, 1.191 mm and 1.625 mm respectively. The Mann-Whitney test indicated significant difference in the width of the inhibition zone between 0% and 12% and 16%, and between 8% and 16% ($p<0.05$). The hydrogel membrane infused with liquid smoke at a concentration of 12% was the lowest concentration that inhibited *S. aureus*. **Conclusion:** Hydrogel membranes infused with liquid smoke had antimicrobial properties against growth inhibition of *S. aureus*.

Keywords: Antibacterial Properties, Hydrogel Membranes, Liquid Smoke, *Staphylococcus aureus*

Corresponding Author:
A.A. Sutadi Saputra, BDent
Email: aasutadisaputra@students.undip.ac.id
Tel: +6281227802589

INTRODUCTION

Staphylococcus aureus is the most common bacteria causing pyogenic infections. These bacterial infections usually occur with typical signs such as inflammation, necrosis and abscess (1). As many as 60% of chronic wound cases are related to *S. aureus* and Pseudomonas infections (2-3).

Wound treatments using wound dressing are growing rapidly. More than 500 types of artificial wound dressings are available in the form of hydrogels, films, hydrocolloids, calcium alginates, absorbent dressings, antibacterial and hydrophobic antimicrobial dressings (4). Hydrogels are polymer wound dressings with soft, flexible, and non-irritating characteristics. Hydrogels release drugs or other compounds for the wound healing process (5-6). Hydrogels are produced from natural or synthetic polymers or a combination of both to produce the best material properties (7).

Hydrogel membranes have been synthesized from a combination of synthetic polymers such as polyvinyl-alcohol (PVA) and natural polysaccharides like chitosan and starch. PVA is one of the oldest synthetic polymers most often used as a cover for hydrogel dressings (8-9). Chitosan is a natural polysaccharide with biodegradable, biocompatible characteristics, that is also capable of forming a film (10). In addition, starch is a natural polymer with large cross-linking properties through hydroxyl groups (-OH) (11).

The hydrogel membranes used for wound dressings must have antimicrobial properties to protect against wound infection (8). Natural antibacterial agents have fewer side effects and are more effective than commercial antibiotics (12). One of the natural antibacterial with the potential for wound healing is liquid smoke (13). Liquid smoke is produced from the pyrolysis and condensation of smoke from the burning of coconut shells (14-15). It is known to have considerably high polyphenol contents. Polyphenol is an antioxidant, with antibacterial properties (15).

Hydrogel preparations in dental medicine can be developed as candidate antimicrobial transport systems to treat oral mucosal ulcerations, such as angular cheilitis that is a common oral mucosa pathology characterized by erythema, fissures, and crusting at 1 or both labial commissures due to infection from *S. aureus* (17). This study analyzed the difference in antibacterial activity between hydrogel membranes without infused liquid smoke and hydrogel membranes infused with liquid smoke against of *S. aureus* growth.

MATERIALS AND METHODS

Samples

This study was true experimental research with a post-test only control group design. *S. aureus* (ATCC 25923) was obtained from Microbiology Laboratory, Faculty of Medicine, Diponegoro University, was divided into four groups: a control group and three liquid smoke treatment groups. The liquid smoke was grade 1 prepared from coconut shells and the concentrations in the hydrogel membranes were 0%, 8%, 12%, and 16%. Each group consisted of six samples. Our research was not determine the antimicrobial of liquid smoke alone as positive control because this was determine from another study. The calculation basis of the concentration liquid smoke :

$$CLQ \; : \frac{FLQ \times BV}{TV}$$

Where CLQ is the concentrations of liquid smoke in hydrogel membrane (T), FLQ is the first concentrations of liquid smoke, BV is the basis hydrogel volume (mL) and TV is the total volume (mL).

Antibacterial Test

One loopful of sub-cultured streak bacteria from freshly grown culture was streaked to transfer in the Mueller-Hinton Agar plate. The test specimen was gently pressed transversely across the agar surface, and the hydrogel membrane was applied on the streak in as many as six lines with a distance of 1 cm each. The number of replicates were six from each group. The specimens were incubated at 37°C for 20 h. The plate was examined for interrupted growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of zone of inhibition along the streak on either side of the test specimen was calculated using the following equation based on ISO AATCC 147-2004 (16):

$$W = \frac{T - D}{2}$$

Where W is the width of the clear zone of inhibition in mm; T the width of the test specimen and clear zone in mm; and D is the width of the test specimen in mm.

Statistical Analysis

This study used the Cronbach's alpha reliability test and the results were tested for normality. The differences between the control and treatment groups were evaluated with the non-parametric Kruskal-Wallis and Mann-Whitney tests.

RESULTS

The Average Inhibited Width of the Hydrogel Membrane as an Indicator of *S. aureus* Growth

The effects of hydrogel membranes infused with liquid smoke on growth inhibition of *S. aureus* were illustrated by the average growth inhibited width zone of *S. aureus* (Figure 1). For C and D, the bacterial culture was not wet, but the membrane hydrogel melted again when incubated at 37 C for 24 hours so that the hydrogel zone was not uniform. The results of inhibited width at a concentration of 16% (1.625 mm) greater than at concentrations of 12% (1.191 mm), 8% (0.416 mm) and 0% (0.013 mm), as shown in Table I and Figure I. Based on a descriptive analysis, it can be stated that the growth inhibited zone of *S. aureus* increased with the increase in liquid smoke concentration.

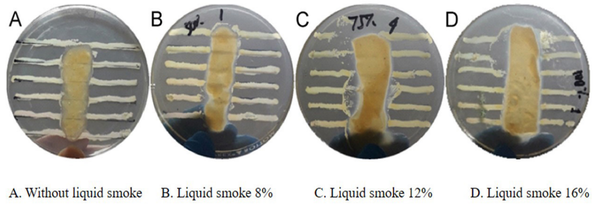

Figure 1: Growth inhibition of *S. aureus* by the hydrogel membranes

Table I: Average inhibited width of the hydrogel membrane

Groups	n	Mean (mm)	SD (mm)
Control	6	0.013	0.033
I	6	0.416	0.513
II	6	1.191	1.328
III	6	1.625	0.332

Note:
A. Control group (hydrogel base membrane); B. Treatment I group (hydrogel base membrane infused with liquid smoke 8%);
C. Treatment II group (hydrogel base membrane infused with liquid smoke 12%); D. Treatment III group (hydrogel base membrane infused with liquid smoke 16%)

The Cronbach's alpha reliability test was used to determine the level of observer confidence in the inhibitory width measurements. The value from three observers was 0.932 (0≥0.60) so it was concluded that the observational data were reliable and consistent. The Kruskal-Wallis test was performed to evaluate the differences between the control and treatment groups, and a significant difference in the *S. aureus* inhibition width was observed among the treatment groups (p 0.001). The Mann-Whitney test was conducted to determine the differences between two treatment groups within all samples. The results of the Mann-Whitney test are described in Table II.

The results indicate that the hydrogel membrane without infused liquid smoke and that infused with 8% liquid smoke did not have antibacterial properties. In contrast, the hydrogel membrane infused with 12% and 16% liquid smoke had antimicrobial activity. The 12% concentration was the lowest concentration that

Table II: Mann-Whitney test results

Treatment Groups	*p value*
Liquid smoke 0% against liquid smoke 8%	0.074
Liquid smoke 0% against liquid smoke 12%	0.003*
Liquid smoke 0% against liquid smoke 16%	0.003*
Liquid smoke 8% against liquid smoke 12%	0.199
Liquid smoke 8% against liquid smoke 16%	0.006*
Liquid smoke 12% against liquid smoke 16%	0.150

Note: *) significant difference: $p \leq 0.05$

inhibited *S. aureus* and the 16% concentration was the optimum concentration.

DISCUSSION

S. aureus cause many soft tissues infections and consequently interrupts the wound healing process. It has been reported that *S. aureus* is the most common reason for clinical infections (18). The *S. aureus* samples in the present study were treated with PVA, chitosan and starch in hydrogel base membranes infused with liquid smoke from coconut shells.

The hydrogel based PVA, chitosan and starch samples exhibited no bacterial growth under the sample contact area except in the first repetition. In contrast, various concentrations of hydrogel base infused with liquid smoke inhibited the growth of *S. aureus*. The average width of the zone of inhibition along a streak on either edge of the test specimen was calculated.

The results of the difference test subsequently revealed a significant difference between the treatment groups ($p \leq 0.05$). The data of this study agree with previous research that the higher concentration of liquid smoke affected the growth of *S. aureus*. This result is consistent with research conducted by Kailaku et al., who reported an increased concentration of liquid smoke from coconut shells of 25%, 50%, and 75% increased the inhibitory effect on *S. aureus* growth (16). According to Adhiasari, the minimum inhibitory concentration and the minimum bactericidal concentration values of liquid smoke against *S. aureus* are 25% and 50% respectively (19). Based on these data, the first concentrations of liquid smoke used in this study were 50%, 75%, and 100%, whereas the last concentrations of liquid smoke after becoming incorporated in the hydrogel membrane were 8%, 12%, and 16%.

Another study by Anisah revealed that applying liquid smoke to *S. aureus* causes the surface of the bacteria cell wall to become rough and uneven (20). Liquid smoke contains active antibacterial compound, such as phenols, acidic acids, and carbonyls (21). However, the two different studies above that reported on the activity of liquid smoke against *S. aureus* did not infuse the liquid smoke with hydrogels membrane, therefore the comparison might not be accurate and it becomes a limitation in this research.

Polyphenols are bioactive molecules related to their molecular structure; phenolic compounds have the capacity to link with proteins and bacterial membranes to form complexes through their hydroxyl groups or the phenolic ring (22). Phenolic compounds, such as phenol, 2-methoxylphenol (guaiacol), 3,4 dimethoxyl phenol, and 2-methoxy-4-methyl phenol are prominent in coconut shell liquid smoke and play a major role in the antibacterial activity (23).

Liquid smoke inhibits the growth of Gram negative and Gram positive bacteria (24). *S. aureus* is Gram positive bacterium that tends to be more sensitive to antimicrobial compared to the cell wall structure of Gram negative bacteria (25). The cell walls of Gram positive bacteria tend to be thinner because they do not have an outer membrane making it easier for hydrophobic compounds to penetrate the cell (26). Phenols and their derivatives are bacteriostatic because they coagulate the amine functional (-NH) and sulfhydryl (-SH) groups of bacterial proteins (20). Phenols interact with bacterial cells through absorption, resulting in hydrogen linking. Proteins form weak links at low levels and immediately decompose followed by penetration of phenols into the cell causing protein denaturation. Phenol causes protein coagulation and membrane lysis (24).

The acidity of liquid smoke also provides antimicrobial properties. The antibacterial activity of phenols increases when mixed with acidic compounds (20). The principle of bacterial inhibition by organic acids is that a non-dissociated acid penetrated the bacterial cell wall to disrupt normal cell physiology (27). Furthermore, the carbonyl mechanism involves penetration through the bacterial cell membrane and inactivating enzymes in the cytoplasm. This affects the overall pH and forms an acid that does not dissociate resulting in disturbed metabolism (21). Nevertheless, liquid smoke can be used as an oral medicine treatment, although further research in this area is necessary. Hydrogel preparations in dental medicine have been developed as candidate antimicrobial transport systems to treat oral mucosal ulcerations, such as AC in the form of an oral mucoadhesive hydrogel patch.

CONCLUSION

We conclude that the hydrogel membrane without infused liquid smoke did not have antibacterial properties while the hydrogel membranes infused with liquid smoke had antibacterial properties based on the width of the inhibition zone of *S. aureus*.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Research and Technology, Republic of Indonesia for its funding of the Student Creativity Program, Ltd. Multifunctional Liquid Smoke (ACM) Semarang for providing liquid smoke, and all researchers for their contributions to this investigation.

REFERENCES

1. Jawertz, Melnick, Adeberg's. Mikrobiologi Kedokteran Edisi 23 Penerjemah Geo F. Brooks, Janet S. Butel, dan Stephen A. Morse. Jakarta: Penerbit Buku Kedokteran EGC. 2005.
2. Rhoads DD, Cox SB, Rees EJ, Sun Y, Wolcott RD. Clinical Identification of Bacteria in Human Chronic Wound Infections : Culturing vs. 16S Ribosomal DNA Sequencing. BMC Infectios Diseases. 2012;12(321):1–8. https://doi.org/10.1186/1471-2334-12-321
3. Cardona AF, Wilson SE. Skin and Soft-Tissue Infections : A Critical Review and the Role of Telavancin in Their Treatment. Clinical Infentious Diseases. 2015;61:69–78. https://doi.org/10.1093/cid/civ528
4. Cristina A, Gonzalez DO. Wound Healing - A Literature Review. Anais Brasileiros de Dermatologia. 2016; 5: 614–20. https://doi.org/10.1590/abd1806-4841.20164741
5. Jagur-grodzinski J. Polymeric Gels and Hydrogels for Biomedical and Pharmaceutical Applications. Poymers Advanced Technology. 2010;21:27–47. https://doi.org/10.1002/pat.1504
6. Singh A, Peppas NA. Hydrogels and Scaffolds for Immunomodulation. Advenced Materials. 2014;1–12. https://doi.org/10.1002/adma.201402105
7. Ahmed EM. Hydrogel : Preparation, Characterization and Applications : A Review. Journal of Advanced Research. 2015;6(2):105–21. https://doi.org/10.1016/j.jare.2013.07.006
8. Kamoun EA, Kenawy ES, Chen X. A Review on Polymeric Hydrogel Membranes for Wound Dressing Applications : PVA-Based Hydrogel Dressings. Journal of Advanced Research. 2017;8(3):217–33. https://doi.org/10.1016/j.jare.2017.01.005
9. Kamoun EA, Chen X, Mohy MS, Kenawy ES. Crosslinked Poly (Vinyl Alcohol) Hydrogels for Wound Dressing Applications : A Review of Remarkably Blended Polymers. Arab Journal of Chemistry. 2014; 8 (1): 1-14. https://doi.org/10.1016/j.arabjc.2014.07.005
10. Celebi H, Kurt A. Effect of Processing on the Properties of Chitosan/Cellulose Nanocrystal Films. Carbohydrate Polymers. 2015: 133 (20): 284-93. https://doi.org/10.1016/j.carbpol.2015.07.007
11. Zhang J, Sun X. Mechanical Properties of Poly (Lactic acid)/Starch Composites Compatibilized by Maleic Anhydride. Biomacromolecules 2004;1446–51. https://doi.org/10.1021/bm0400022
12. Mukhtar S, Ghori I. Antibacterial Activity of Aqueous and Ethanolic Extarcts of Garlic, Cinnamon and Turmeric Against Escherichia Coli ATCC 25922 and Bacillus Subtilis DSM 3256. International Journal of Applied Biology Pharmaceutical Technology. 2012;3(2):131–6. Avalaible online: www.ijabpt.com
13. Tarawan VM, Mantilidewi KI, Dhini IM, Radhiyanti PT, Sutedja E. Coconut Shell Liquid Smoke Promotes Burn Wound Healing. Journal of Evidance-Based Integrative Medicine. 2017;22(38):436–40. https://doi.org/10.1177/2156587216674313
14. Kasim F, Fitrah AN, Hambali E. Aplikasi Asap Cair pada Lateks. Jurnal PASTI. 2015;IX(1):28–34.
15. Kailaku S, Syakir M, Mulyawanti I, Syah A. Antimicrobial Activity of Coconut Shell Liquid Smoke. IOP Conference Series: Materials Science and Engineering. 2017;1–6.
16. Pinho E, Magalhres La, Henriques Ma, Oliveira R. Antimicrobial activity assessment of textiles: standard methods comparison. Ann Microbiol. 2011;61:493–498 DOI 10.1007/s13213-010-0163-8
17. Perez M, Gandara P, Garcia A, Blanco A, Pineiro S, Chamorro C, Lorenzo AI. Hyaluronic Acid Dermal Fillers in the Management of Recurrent Angular Cheilitis : A Case Report. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2019;129:20-25 https://doi.org/10.1016/j.oooo.2019.06.026
18. Carvalho A, Waitman F, Silva DO, Telleria J, Moura V, Prieto T, et al. Tetracycline Hydrochloride-Loaded Electrospun Nano Fibers Mats Based on PVA and Chitosan for Wound Dressing. Materials Science and Engineering C. 2017;77:271–81. http://dx.doi.org/10.1016/j.msec.2017.03.199
19. Adhiasari R, Santoso O, Ciptaningtyas VR. Pengaruh Asap Cair Berbagai Konsentrasi Terhadap Viabilitas Staphylococcus aureus. Jurnal Kedokteran Diponegoro. 2019;8(1):420–7.
20. Anisah K. Analisa Komponen Kimia dan Uji Antibakteri Asap Cair Tempurung Kelapa Sawit (Elaeis guineensis Jacq.) pada Bakteri Staphylococcus aureus Dan Pseudomonas aeruginosa [undergraduate thesis]. Jakarta. UIN Syarif Hidayatullah Jakarta. 2014.
21. Milly PJ. Antimicrobial Properties of Liquid Smoke Fractions [master's thesis]. Georgia. University of Georgia; 2003.
22. Zongo C, Savadogo A, Somda MK, Koudu J, Traore AS. In Vitro Evaluation of the Antimicrobial and Antioxidant Properties of Extracts from Whole Plant of Alternanthera pungens H.B. & K. and Leaves of Combretum sericeum G. Don. International Journal Phytomedicine. 2011;3:182–91.
23. Zuraida I, Mulawarman U, Sukarno S, Budijanto S. Antibacterial Activity of Coconut Shell Liquid Smoke (CS-LS) and Its Application on Fish Ball Preservation. Int Food Res J. 2011;18:405–10.
24. Pellisari F, Grosmann M, Yamashita F, Pineda EA. Antimicrobial, Mechanical, and Barrier Properties

of Cassava Starch - Chitosan Films Incorporated with Oregano Essential Oil. Journal of Agricultural and Food Chemistry. 2009;57:7499–504.
25. Dinar YW. Aktivitas Antibakteri Ekstrak Etanol Kayu Secang (Caesalpinia sappan L.) Terhadap Staphylococcus aureus ATCC 25923, Shigella sonnei ATCC 9290, dan Escherichia coli ATCC 25922 [undergaduate thesis]. Surakarta. Universitas Muhammadiyah Surakarta; 2012.
26. Reuk-ngam N, Chimnoi N, Khunnawutmanotham N, Techasakul S. Antimicrobial Activity of Coronarin D and its Synergistic Potential with Antibiotics. Biomed Research International. 2014;2014:1-8
27. Sumpono. Uji Aktivitas Antioksidan dan Antibakteri Asap Cair Tempurung Kelapa Sawit. Semin Nasional Pendidikan Sains. 2018/10/27.