

RESEARCH ARTICLE

Polyvinyl alcohol–collagen–hydroxyapatite composite membrane derived from *Lates calcarifer* for alveolar socket preservation

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ABSTRACT

Alveolar socket preservation (ASP) requires an ideal barrier membrane that provides anti-bacterial activity, water contact angle (WCA) and swelling behavior to support bone regeneration. This study evaluated these characteristics in a Polyvinyl Alcohol-Collagen-Hydroxyapatite composite membranes derived from the scales of *Lates calcarifer*. The PVA-Col-HA CM was produced from the scales of *Lates calcarifer* through mixing, homogenization, casting, and drying and then divided into three groups: non-irradiated, 15 kGy irradiation, and 25 kGy irradiation. Antibacterial activity was assessed by disk-diffusion test and inhibition zone diameters were measured. Water contact angle was determined using a contact angle goniometer on both membrane surfaces. Swelling behavior was evaluated by immersing samples in phosphate-buffered saline for 60 minutes, followed by periodic weighing and calculation using a swelling formula. The anti-bacterial activity test showed a larger zone of inhibition in the 25 kGy group by 2mm on *Escherichia coli* and *Staphylococcus aureus*. One-way ANOVA test of WCA values showed significant differences ($p < 0.05$) among groups, with the 25 kGy group exhibiting the highest hydrophilicity. Analysis of swelling behavior using ANOVA and Kruskal-wallis test showed no significant differences ($p > 0.05$) among groups, but the linear graph shows that the 25kGy group displayed the lowest and most stable swelling profile. These findings suggest that the PVA-Col-HA CM exhibited favorable anti-bacterial activity, WCA, and swelling behavior, with optimal performance observed in the 25 kGy irradiation group.

Keywords: alveolar socket preservation; anti-bacterial activity; PVA-Col-HA composite membrane; swelling behavior; water contact angle

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INTRODUCTION

Dental implant placement is widely used to replace missing teeth after extraction and has demonstrated excellent long-term prognosis.¹ However, maintaining adequate bone volume remains critical for successful implant therapy. Tooth extraction consists of a series of physiological processes that can result in alveolar bone loss.² Numerous studies have shown that alveolar bone remodeling during post-extraction healing may result in a reduction of bone height and width of up to 40–60% within the first three months.³ Dental implant placement is widely used to replace missing teeth after extraction and has demonstrated excellent long-term prognosis.¹

Alveolar socket preservation (ASP) is a clinical procedure aimed at minimizing dimensional changes in alveolar bone following tooth extraction.⁴ One commonly used approach in ASP is the application of a barrier membrane, which is placed over the extraction socket to prevent the invasion of epithelial and connective tissue cells. Additionally, barrier membranes help maintain space for bone regeneration and selectively guide osteogenic cells toward the defect site.⁵ An effective barrier membrane should demonstrate different functional characteristics on each surface⁶ and maintain structural integrity between soft and hard tissues for an appropriate duration.⁵ The surface facing soft tissue should act

as an occlusive barrier, while the surface facing bone should promote regeneration.⁶

An ideal barrier membrane should be biocompatible, biodegradable, and possess some physical properties (including porosity, x-ray diffraction, swelling behavior, and water contact angle), mechanical properties (tensile strength, modulus of elasticity and elongation at break), and antibacterial activity.^{7,8} Antibacterial activity is necessary to prevent infection, which is a major cause of dental implant failure.⁹ In addition, antibacterial activity prevents biofilm formation and microbial growth without disrupting the balance between commensal and pathogenic microorganisms. Antibacterial properties of a membrane are required as bacteriostatic.¹⁰ Suitable physical and mechanical properties are required to avoid membrane collapse and improve the ease of handling and placement of a membrane.¹¹

Water contact angle is commonly used to assess the hydrophilic nature of membrane surfaces.¹² The hydrophilic nature and moderately rough surfaces are important factors for bone regeneration to enhance cell adhesion and proliferation.¹³ Swelling behavior is another important physical property, as excessive swelling may compromise space maintenance and allow soft tissue infiltration into the defect site.¹⁴ Therefore, barrier membranes with controlled and minimal swelling behavior are preferred to support effective bone formation.¹⁵

Barrier membranes can be made from synthetic or natural polymers. Natural polymers are generally considered safer, more biocompatible, and more biodegradable than synthetic polymers. However, they often have poorer mechanical properties. In contrast, synthetic polymers are usually detrimental to newly growing tissues, so a blend of natural and synthetic polymers in a particular ratio combines both advantages.¹⁶

The Polyvinyl Alcohol-Collagen-Hydroxyapatite Composite Membrane (PVA-Col-HA CM) from the scales of *Lates calcarifer* has active ingredients derived from natural and synthetic polymers. Collagen and hydroxyapatite are obtained from natural materials, particularly white snapper (*Lates calcarifer*) scales, while polyvinyl alcohol

serves as a synthetic polymer. Previous studies have demonstrated that PVA-Col-HA CM derived from *Lates calcarifer* scales can enhance fibroblast proliferation,¹⁷ maintain a stable chemical structure during 30 days of storage in three different media, demonstrate the highest tensile strength value in the 25 kGy irradiation group, and yield an ideal pore size, and ideal degradability for applications in soft and hard tissue engineering.¹⁸

As part of the requirements for an ideal barrier membrane, it is necessary to research antibacterial activity and key physical properties of the membrane, including water contact angle and swelling behavior. Antibacterial activity is essential to prevent infection, which is a major cause of implant failure. Furthermore, the effectiveness of a composite membrane placed within the defect region is strongly influenced by the ideal water contact angle and swelling behavior as they determine its ability to function optimally as a barrier membrane and ASP.^{19,20} Therefore, this study aimed to evaluate the antibacterial activity, water contact angle, and swelling behavior of the PVA-Col-HA composite membrane to assess its suitability as an ideal barrier membrane.

MATERIALS AND METHODS

This study received ethical exemption from the Health Research Ethics Committee, Faculty of Dentistry, Universitas Trisakti (No. 638/S1/KEPK/FKG/3/2023). The PVA-Col-HA composite membrane used in this study (patent number IDP000070254) consisted of polyvinyl alcohol (molecular weight 72,000; Merck-Schuchardt OHG, Hohenbrunn, Germany), collagen, and hydroxyapatite derived from white snapper (*Lates calcarifer*) scales through chemical hydrolysis. The membrane was produced at the National Research and Innovation Agency (BRIN), South Jakarta.

Polyvinyl alcohol, collagen, and hydroxyapatite were mixed using a hotplate magnetic stirrer (607102795, IKA, Breisgau, Germany), followed by homogenization using a homogenizer (0003593000, IKA, Breisgau, Germany). The homogeneous mixture was poured into sterile

containers and dried at room temperature for approximately two weeks. After drying, the PVA–Col–HA composite membranes were cut into dimensions of 1.5×1 cm, with a thickness of 0.7 cm, and divided into three groups: non-irradiated (0 kGy), 15 kGy irradiation, and 25 kGy irradiation, in accordance with ISO 11137 standards.

For antibacterial testing, PVA–Col–HA composite membrane samples (patent number IDP000070254) derived from *Lates calcarifer* scales were cut into circular discs with a diameter of 0.7 cm. A 50 mL bacterial suspension was prepared using sterile distilled water (786-1711, G-Bioscience, USA), and tryptic soy agar (TSA; 236950, Difco™, Sparks, USA) was prepared as the growth medium. The samples were placed onto Petri dishes (632.492.003.100, Anumbra, Czech Republic) and incubated at 37 °C in an incubator (8402851, Heraeus, Hanau, Germany) for 24 hours. The diameter of the inhibition zone was then measured using a ruler.²¹

Water contact angle measurements were performed using a contact angle goniometer (Easy Drop K100, Krüss GmbH, Hamburg, Germany). PVA–Col–HA composite membrane samples derived from *Lates calcarifer* scales were cut into dimensions of 1.5×1 cm and placed on a glass slide secured with double-sided tape. A single drop of distilled water was applied to the membrane surface using a micropipette, and the contact angle was measured immediately. Measurements were repeated, and the average value was calculated.²²

The swelling behavior of the polyvinyl alcohol–collagen–hydroxyapatite composite membrane (PVA–Col–HA CM; patent number IDP000070254) derived from *Lates calcarifer* scales was evaluated in three groups: non-irradiated (0 kGy), 15 kGy irradiation, and 25 kGy irradiation. For each group, membrane samples were cut to a size of 1.5×1 cm. Each sample was weighed prior to immersion to determine its initial dry weight.

Phosphate-buffered saline (PBS; Merck KGaA, Darmstadt, Germany) was prepared, and samples were immersed for up to 60 minutes at predetermined time intervals. After immersion, excess surface liquid was gently removed using filter

paper (WHA1004150, Merck KGaA, Darmstadt, Germany), and the samples were weighed using an analytical balance (GR200, A&D Company Limited, Tokyo, Japan). Swelling behavior was calculated using the following formula:²²

$$\text{Swelling Behavior (\%)} = \frac{(W_r - W_1)}{W_1} \times 100$$

where W_0 is the initial dry weight and W_t is the weight at time t .

Statistical analysis was performed using SPSS version 23. Data normality was assessed, and because both normally and non-normally distributed data were identified, one-way ANOVA and Kruskal–Wallis tests were applied accordingly. Post hoc tests were conducted when statistically significant differences were observed.

RESULTS

The results of the antibacterial activity test on the PVA–Col–HA CM from the scales of *Lates calcarifer* in the non-irradiated (0 kGy), 15 kGy, and 25 kGy irradiation groups on *E. coli* and *S. aureus* bacteria showed that all three groups had antibacterial effects. The 25 kGy group was found to have the largest inhibition zone, measuring roughly 2mm, compared to the other two groups. The figure also shows that the inhibition zone possessed by *E. coli* bacteria had a more apparent zone than that of *S. aureus* bacteria (Figure 1).

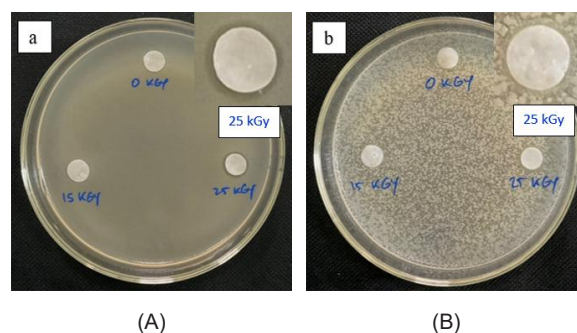


Figure 1. Antibacterial activity of PVA–Col–HA composite membrane derived from *Lates calcarifer* scales evaluated using the disk-diffusion method against (A) *Escherichia coli* and (B) *Staphylococcus aureus*. Groups represent 0 kGy, 15 kGy, and 25 kGy irradiation treatments. Larger inhibition zones indicate stronger antibacterial effects

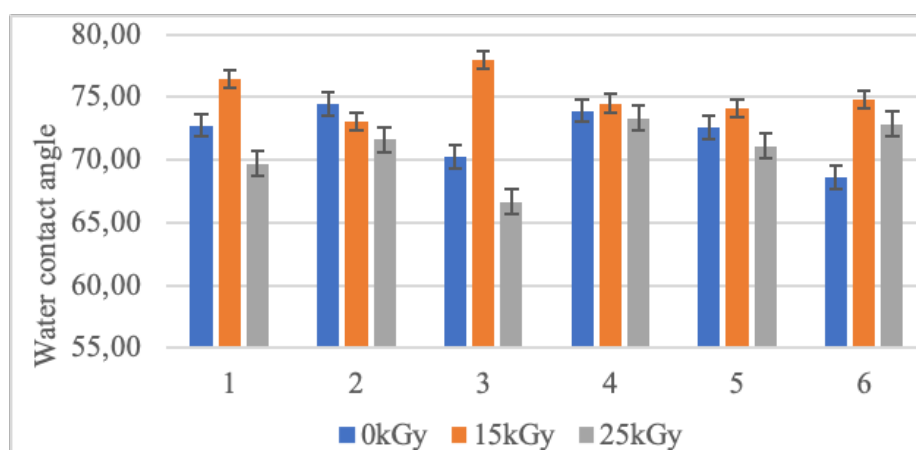


Figure 2. Water contact angle (WCA) measurements of PVA-Col-HA composite membrane from the scales of *Lates calcarifer*. Groups represent 0 kGy, 15 kGy, and 25 kGy irradiation. Numbers 1-6 indicate replicate measurements for each group. Lower WCA values indicate greater hydrophilicity

Table 1. Water contact angle test value, kruskal-wallis p-value, and mann whitney post-hoc test of PVA-Col-HA CM from the scales of *Lates calcarifer*

Group	Repetition	Mean \pm SD	p value
0 kGy	6	72.08 \pm 4.23 ^a	p = 0.006*
15 kGy	6	75.14 \pm 3.80 ^{ab}	
25 kGy	6	70.86 \pm 3.30 ^a	

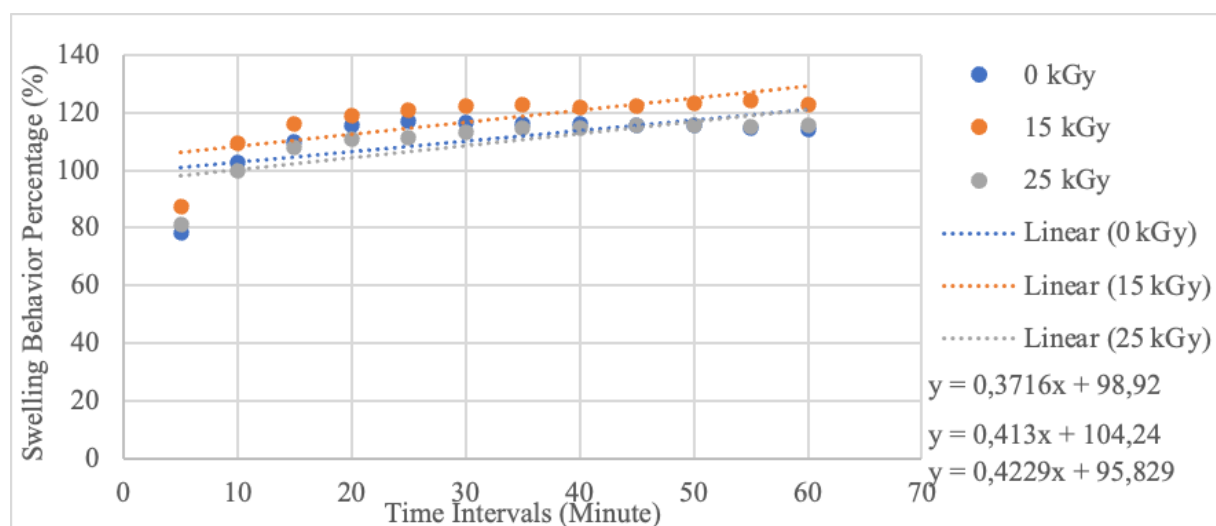


Figure 3. Swelling behavior of PVA-Col-HA composite membrane from the scales of *Lates calcarifer* during 60 minutes of immersion in phosphate-buffered saline (PBS). The graph compares three irradiation groups (0 kGy, 15 kGy, and 25 kGy), showing changes in swelling percentage over time. Lower and more stable swelling indicates a more favorable membrane performance.

Water contact angle (WCA) measurements of the PVA-Col-HA CM derived from *Lates calcarifer* scales showed values below 90° on both left and

right surfaces across all irradiation groups (0 kGy, 15 kGy, and 25 kGy), which indicated hydrophilic surface characteristics (Figure 2). The Kruskal-

Table 2. Swelling behavior values and one-way ANOVA p-values of PVA–Col–HA composite membranes derived from *Lates calcarifer* scales.

Minute	Mean ± Standart Deviation			p value ANOVA
	0 kGy	15 kGy	25 kGy	
5	78.47 ± 18.05	87.39 ± 17.68	81.04 ± 15.80	p = 0.660
10	102.87 ± 16.38	109.41 ± 14.60	99.92 ± 10.58	p = 0.505
15	109.95 ± 16.41	116.09 ± 11.93	107.75 ± 8.53	p = 0.515
20	115.67 ± 17.49	118.83 ± 11.35	110.86 ± 6.23	p = 0.555
25	116.95 ± 16.49	121.04 ± 11.88	111.33 ± 5.96	p = 0.408
30	116.65 ± 14.90	122.29 ± 11.96	113.34 ± 4.69	p = 0.408
35	115.85 ± 15.55	122.87 ± 11.82	114.4 ± 4.43	p = 0.420
40	115.84 ± 15.10	121.9 ± 8.74	114.5 ± 4.42	p = 0.442
55	114.78 ± 15.52	123.98 ± 9.17	115.26 ± 4.94	p = 0.281
60	114.01 ± 16.33	122.89 ± 9.48	115.44 ± 5.21	p = 0.369

Table 3. Swelling behavior test value, p-value kruskal-wallis of PVA–Col–HA CM from the scales of *Lates calcarifer* scales

Minute	Mean ± Standart deviation			p value kruskall-wallis
	0 kGy	15 kGy	25 kGy	
45	115.56 ± 16.31	122.06 ± 8.92	115.52 ± 4.34	p = 0.366
50	115.37 ± 15.41	123.19 ± 9.27	115.53 ± 5.17	p = 0.191

Wallis test revealed a statistically significant difference among groups ($p = 0.006$). Post hoc Mann–Whitney analysis showed significant differences between the 0 kGy and 15 kGy groups ($p = 0.030$) and between the 15 kGy and 25 kGy groups ($p = 0.002$), while no significant difference was observed between the 0 kGy and 25 kGy groups ($p = 0.476$). Based on the Shapiro–Wilk test, the data were found to be normally distributed ($p > 0.05$) in the 0 kGy and 25 kGy groups, while the 15 kGy groups showed that the data were not normally distributed ($p < 0.05$). The results of mean + standard deviation in each group of PVA–Col–HA CM from the scales of *Lates calcarifer* are presented in Table 3. The Kruskal–Wallis test revealed a significant difference ($p = 0.006$) in each group of PVA–Col–HA CM from the scales of *Lates calcarifer*. The Mann–Whitney post hoc test showed significant results between the 0 kGy and 15 kGy groups ($p = 0.030$) and between the 15 kGy and 25 kGy groups ($p = 0.002$), while no

significant difference was observed between the 0 kGy and 25 kGy groups ($p = 0.476$).

The swelling behavior test showed that all PVA–Col–HA CM groups indicated relatively low swelling percentages over the 60-minute immersion period. Among the three groups, the 25 kGy irradiation group demonstrated the lowest and most stable swelling profile compared with the 0 kGy and 15 kGy groups (Figure 3).

Normality testing using the Shapiro–Wilk test showed that swelling data were normally distributed ($p > 0.05$) from the 5th minute to the 60th minute, except the 45th and 50th minutes ($p < 0.05$). One-way ANOVA test results revealed that there were no statistically significant differences in swelling behavior among the three irradiation groups ($p > 0.05$) (Table 2).

Similarly, the Kruskal–Wallis test applied to non-normally distributed data also showed no significant differences among groups ($p > 0.05$) (Table 3).

DISCUSSION

Alveolar socket preservation is a procedure performed to preserve alveolar bone volume for successful implant placement after tooth extraction. One commonly used ASP method is the application of membrane barriers.⁴ A barrier membrane is placed over the defect area to prevent cell invasion of the gingival epithelium and connective tissue and maintain space for soft and hard tissue regeneration.^{5,11} In addition, a barrier membrane with antibacterial activity is required to prevent infection, which is one of the major causes of dental implant failure.⁹ Barrier membranes must possess suitable physical and mechanical properties to avoid the collapse of the membrane and increase the ease of handling and placement of a membrane.¹¹

Barrier membranes with antibacterial and bone regeneration capacities are needed for clinical use.¹⁹ Bacterial adhesion to the surface of medical materials can result in infection and even material or surgical failure.²³ Effective antibacterial activity can reduce the occurrence of infection caused by exposed membranes and promote osteogenesis and soft tissue healing.²⁴ In this study, the PVA-Col-HA CM from the scales of *Lates calcarifer* exhibited antibacterial activity in all groups (0 kGy, 15 kGy, and 25 kGy). The 25 kGy groups had a larger inhibition zone diameter against *E. coli* and *S. aureus* bacteria than the other two groups. This finding is in line with previous studies on agarose/gelatin/hydroxyapatite study, which showed that an increase in irradiation dose in the 25 kGy groups resulted in greater inhibition zone in the antibacterial activity test when compared to the 0 kGy and 15 kGy groups. The enhanced inhibition zone may be due to the increased dissolution of polymer chains upon irradiation.²⁵ In this study, it was also found that *E. coli* bacteria had a more apparent inhibition zone than *S. aureus*, which had a slightly cloudier inhibition zone.²⁶ The observed antibacterial activity may also be related to the presence of hydroxyapatite, which has been reported to possess antibacterial properties.²⁷

Water contact angle is commonly used to assess the hydrophilic nature of membrane

surfaces.¹² Hydrophilic properties are essential for biological reactions at the bone-implant material interface, including osteoblast adhesion, and the material surface's ability to adsorb serum proteins.¹³ Contact angle values below 90° indicate hydrophilic properties, whereas values above 90° indicate hydrophobic surfaces.²⁸ In this study, the PVA-Col-HA CM composite membrane from the scales of *Lates calcarifer* was included in the hydrophilic membrane category, with the 25 kGy irradiation group showing the greatest hydrophilicity. This finding is in line with other studies on polycaprolactone/PEG/BGs membranes where the contact angle value obtained also showed a hydrophilic category.²⁹ Mann-Whitney post hoc test demonstrated a significant difference ($p < 0.05$), with the 25 kGy groups having a lower contact angle value and the most hydrophilic compared to the other two groups, the non-irradiated and 15 kGy groups. Similar trends have been reported in studies on polypyrrole (PPy) biomaterials, where increasing gamma irradiation doses enhanced surface hydrophilicity due to increased surface oxygenation.³⁰ This is consistent with this study, where the 25 kGy dose had a lower contact angle value than the 15 kGy dose.

Swelling behavior is one of the crucial characteristics of the barrier membrane because the space occupied by the membrane is limited, and thus excessive swelling behavior is not preferred. Excessive swelling behavior can compress the nerves around the damaged area, potentially causing discomfort and compromising the function of the barrier membrane. Thus, a barrier membrane with low swelling behavior is preferred.^{15,31} In all three doses, the linear graph showed that the 25 kGy irradiation group indicated the most stable behavior from the 35th to the 60th minute. Also, 25 kGy is a commonly used standard dose according to ISO11137, which serves as an international standard for the sterilization of medical products.³²

Furthermore, membranes irradiated at 25 kGy showed a lower swelling percentage than 15 kGy. This is in line with previous studies on swelling behavior of SS/PVA hydrogels, which revealed

a decrease in swelling behavior with increasing irradiation dose. At higher doses, polymer chain mobility is inhibited by the formation of tighter structures and more cross-links, thereby reducing water penetration.³³ Similarly, another study on chitosan/PVA hydrogels have shown that irradiation at 25 kGy enhances polymer cross-linking, forming a more robust network with higher resistance to expansion, thus reducing swelling behavior.³⁴

This study is limited to in-vitro evaluation of antibacterial activity, water contact angle, and short-term swelling behavior, without assessing long-term degradation, mechanical properties, or cell responses. Future studies should include in-vivo testing, extended degradation analysis, and mechanical evaluation to further support the clinical potential of the PVA-Col-HA composite membrane.

CONCLUSION

This study reveals that the PVA-Col-HA composite membrane from the scales of *Lates calcarifer* demonstrated antibacterial activity, favorable water contact angle and controlled swelling behavior. Among the observed research groups, the 25 kGy irradiation group exhibited the most ideal performance across all evaluated parameters compared with the other groups.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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