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Full Length Article

Effect of Cu Nanoparticle Addition to Antimicrobial Properties of Sericin-silica-fosfat Hydrogel as A Prospective Animal Wound Healing Biomaterial

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Abstract

The objective of this study was to determine the comparison of the inhibitory power of gram-positive and gram-negative pathogenic microbes of various sericin-silica-phosphate hydrogel compositions of various Ser/Si/P compositions made from *Bombyx mori* silk fiber filaments, rice husk ash silica, and cow bone waste hydroxyapatite. The production process of sericin-silica-phosphate-Cu hydrogel consists of stages (1) preparation of sericin gel from cocoon processing waste, (2) process of making sericin-silica-phosphate hydrogel by first making sericin hydrosol from sericin gel, silanol hydrosol from nanosilica, and calcium phosphate hydrosol from nanocalcium phosphate, then mixing them into sericin-silica-phosphate hydrosol and evaporating it to become sericin-silica-phosphate hydrogel and (3) making sericin-silicaphosphate-Cu hydrogel from sericin-silica-phosphate hydrogel with the addition of copper nanoparticles (CuNP) the result of green synthesis. G or the composition of sericin silica-phosphate = 50: 25: 25 has relatively the best anti-inflammatory properties among these hydrogels. Based on the linear equation in the calibration curve, the I50 of the sericin-silica-phosphate hydrogel is 1.895. All sericin-silica-phosphate hydrogel compositions have potential as antibacterial biomaterials and mass composition G or sericin-silica-phosphate composition = 50: 25: 25 has relatively the best performance in inhibiting gram-positive and gram-negative bacteria compared to other compositions. Composition P (mass ratio of the best Sr-Si-P: Cu-NPs = 90: 10 or 90G: 10 Cu-NPs) showed relatively the best anti-inflammatory and antibacterial performance. Based on the calibration curve, the anti-inflammatory I50 of the sericin-silica-phosphate-Cu hydrogel is 1.760 the best of the sericin-silica-phosphate hydrogel. Composition G or sericin-silica-phosphate composition has relatively the best performance in inhibiting gram-positive and gram-negative bacteria compared to other compositions. Composition P showed relatively the best anti-inflammatory and antibacterial performance.

Keywords: Cu nanoparticles; Antimicrobial; Hydrogel; Sericin-silica-phosphate; Biomaterials; Wound healing

Introduction

Animal healthcare is one of the keys in husbandry, especially the caring of the health of newborn livestock is currently taken high attention due to its importance in to increase the productivity of animal husbandry. Meanwhile, newborn livestock are very susceptible to injuries and wound healing is one of the main factors in the world of animal husbandry. Causes of wounds in livestock include impact or physical contact with sharp or hard objects, skin diseases such as scabies (Rezki *et al.* 2019), foot and mouth disease (FMD) (Bani and Asruddin 2022). Treatment or healing of wounds caused by these two diseases is of great concern to researchers because disease resistance makes complete healing difficult, so advances in wound healing drugs or biomaterials continue to be developed.

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Currently, nanomaterials have developed their use in livestock science, as summarized by Delir *et al*. (2022) namely as a material for delivering nutrients, health care, reproduction and breeding, improving meat quality, as well as increasing the safety and quality of milk. Nanomaterials which have biocompatibility and medical functions are often referred as medical nanobiomaterials, in which these medical biomaterials give beneficial for both humans and livestock. Nanobiomaterials have been used for a long time due to their superior biocompatibility, biodegradability, low toxicity, and low allergenicity, and produce degradation products that are less cytotoxic and more easily metabolized by host tissues (Han *et al*. 2022). Biomaterials for medical applications can be classified as organic biomaterials, inorganic biomaterials and organic-inorganic hybrid biomaterials. Organic and inorganic hybrid biomaterials are a type of biomaterial that has great potential, because the properties of the materials complement each other, resulting in synergy in the final product process (Saveleva *et al.* 2019). Organic-inorganic hybrid nanobiomaterials can be based on silica and metal-organic materials (Taylor-Pashow *et al*. 2010), for example, sericin-based inorganic-inorganic hybrid biomaterials combined with nanosilica and copper (Cu) nanoparticles. Sericin, a by-product of silk, has been recognized as an economical glycoprotein extracted during the silk degumming process in the textile industry and has medical properties including strong biocompatibility and biodegradability, in the form of nanoparticles it functions in the cancer drug delivery process, has the ability to increase efficacy drugs, have solubility and bio-diffusion that can increase cell stability, cell uptake, and endosomal release (Dan *et al.* 2022). The combination of sericin with nanosilica and nanophosphate can increase its biocompatibility and medical functionality, where bionanosilica from rice husk ash has high biocompatibility properties (Ghaferi *et al.* 2021) making it suitable for use in oncology therapy (Huang *et al*. 2020), cancer therapy (Li *et al*. 2019) and nanocalcium phosphate made from bone hydrocyapatite Cows also have functional medical properties such as being able to increase wound healing activity *in vitro* and having biocompatible properties that will be used for the development of biomaterials for wound dressings and implants (Han *et al.* 2022). Meanwhile, copper nanoparticles are nanomaterials that have antimicrobial and antiviral properties and have mechanisms such as ion release, reactive oxygen species (ROS), direct contact, and immunostimulant effects (Moorthy 2021).

From this description, the organic-inorganic hybrid composite sericin-silica-phosphate-Cu (Ser-Si-P-Cu) in hydrogel form is believed to be a functional nanomaterial that has biocompatibility with spermatozoa and can be used as a wound healing medicine in livestock. Synthetic methods to produce organic-inorganic hybrid biomaterial compositions that have certain optimal medical functions are very necessary. Likewise, the composition of sericin-silica-phosphate (Ser-Si-P) hybrid biomaterials that have optimal medical functions needs to be researched by studying the effect of composition on their medical functions. However, before determining its medical function, the hybrid biomaterial that has been successfully synthesized needs to be characterized by physical and chemical properties related to its medical function such as its structure, particle size, porosity and microstructure. Thus, the formulation of the research problem is how to compare the inhibitory power of gram-positive and gram-negative pathogenic microbes of various Ser-Si-P compositions of various Ser/Si/P compositions made from *Bombyx mori* silk fiber filaments, rice husk ash silica, and waste hydroxyapatite cow bone.

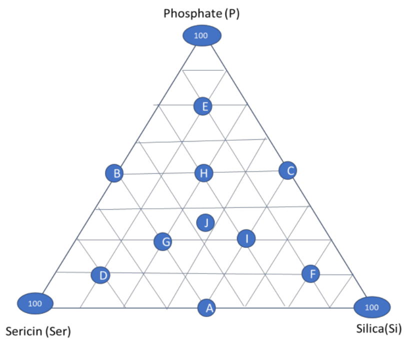
Materials and Methods

Experimental details and treatments

Experimental material: The research activity was carried out at the Universitas PGRI Kanjuruhan Malang and Universitas Pendidikan Ganesha, Singaraja, Bali Indonesia. Duration of research was 6 months (June-November 2023). Materials were used *Bombyx mori* silk cocoons produced by Pertenunan Artha Dharma (Bali, Indonesia) Na_2CO_3 (Pa, Merck), $CuSO_4$ (Pa, anhydrous, Merck), nanosilica from rice husk ash and nanocalcium phosphate from bovine bone waste as well as mango leaf extract produced by our research team in Inorganic Chemistry Laboratory, Chemistry Department of Universitas Pendidikan Ganesha (Bali, Indonesia).

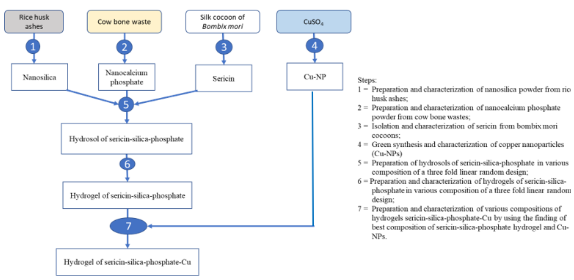
Treatments

The production process of Ser-Si-P-Cu hydrogels consisted of stages (1) preparation of sericin gel from cocoon processing waste, namely the process of degumming Bombyx mori silk cocoons, making nanosilica from rice husk ash, preparing nanocalcium phosphate from the hydroxyapatite isolation of bovine bone waste, and making copper nanoparticles (Cu-NPs) using green synthesis using natural dye extracts, (2) the process of making sericin-silica-phosphate hydrogel by first making sericin hydrosol from sericin gel, silanol hydrosol from nanosilica, and calcium phosphate hydrosol from nanocalcium phosphate, then mixing them into sericin-silica-phosphate hydrosol and evaporating it to become sericin-silica-phosphate hydrogel, with various three-axial compositions of the mixtures (Fig. 1a) and (3) making Ser-Si-P-Cu hydrogel from Ser-Si-P hydrogel with the addition of copper nanoparticles (Cu-NPs) resulting from green synthesis (Fig. 1b), by using the best composition of sericin-silica-phosphate and the green synthesized Cu-NPs with mass (g) composition Sr-Si-P: Cu-NPs = 100: 0 (K); 98: 2 (L); 96: 4 (M); 94: 6 (N); 92: 8 (O); 90: 10 (P), 85: 15 (Q); and 80: 20 (R), respectively. The hydrogel products of each step were characterized using SEM-EDS, anti-inflammation tests using DPPH method and anti-microbial tests.



Sample Code	Ser (g)	Si (g)	P (g)
A	50	50	0
B	50	0	50
C	0	50	50
D	75	12.5	12.5
E	12.5	12.5	75
F	12.5	75	12.5
G	50	25	25
H	25	25	50
I	25	50	25
J	33.3	33.3	33.3

(a)



(b)

Fig. 1: (a) Three-axial composition of sericin-silica-phosphate and **(b)** Steps of making Sr-Si-P-Cu hydrogels

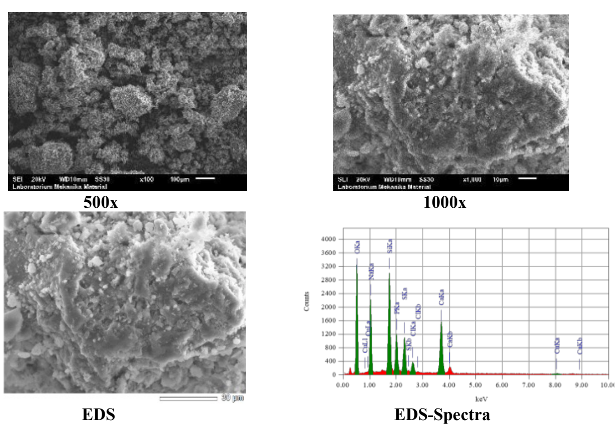


Fig. 2: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition K

Zone inhibition of anti-bacterial tests were conducted by using agar disc diffusion method, utilizing pathogenic strains of Escherichia coli (gram negative) ATCC 25922 and Streptococcus aureus (gram positive) ATCC 25923 (Avanti Research, commercially available at Tokopedia). As negative control groups of discs were agar only added by a drop the *E. coli* and *S. aureus* strains, meanwhile the experimental groups of discs contained agar and a drop the *E. coli* and *S. aureus* strains, then added with 0.5% w/v of Ser-Si-P as well as 0.5% m/v Ser-Si-P-Cu hydrogels respectively. The positive control groups of discs contained agar and a drop the *E. coli* and *S. aureus* strains, then added with 0.5% w/v Streptomycin and 0.5% w/v Ampicillin. The testing was replicate three times.

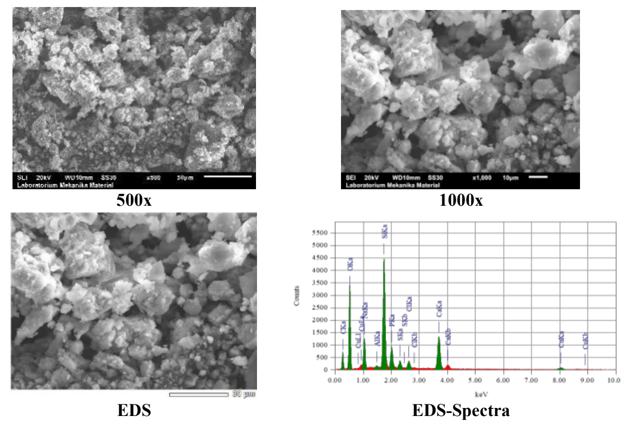


Fig. 3: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition L

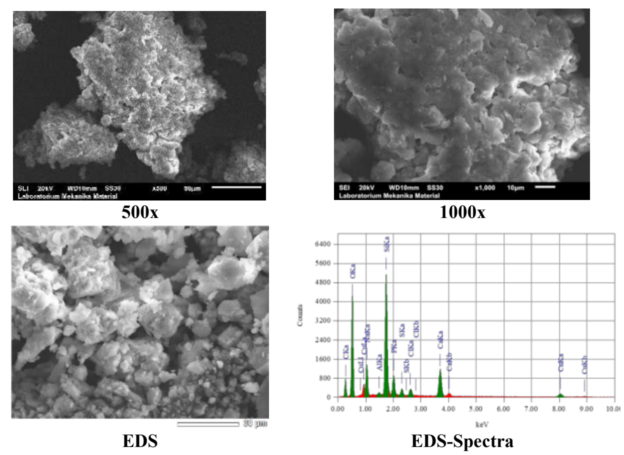


Fig. 4: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition M

Data analysis

Parameters measured were SEM-EDS, anti-inflammation tests using DPPH method and anti-microbial tests. The collected data were statistically treated for Analysis of Variance (ANOVA) using data analysis of excel and if there is a significant influence then proceed with the Least Significant Differences test (LSD-test).

Results

Results of making sericin-silica-phosphate-Cu hydrogel

Microstructural characterization of hydrogels to see the distribution of constituent particles, aggregation and surface porosity can be studied using the SEM-EDS instrumentation method. The results of scanning at various magnifications for each composition of the sericin-silica-phosphate-Cu hydrogel and EDS analysis to determine the distribution of the elements Si, Cu, P, O are presented in Fig. 2 to 9. The SEM-EDS results show that samples of P, Q and R compositions have homogeneous and porous surface

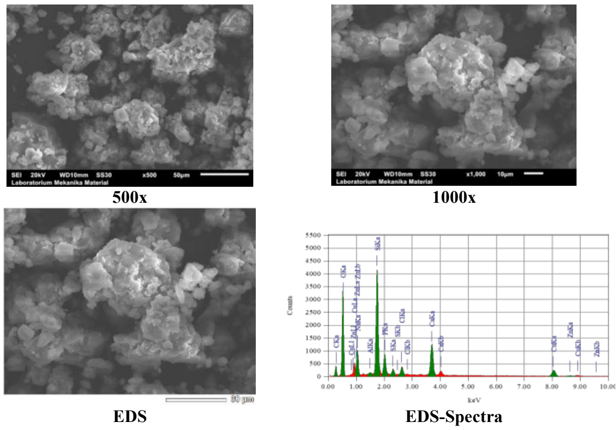


Fig. 5: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition N

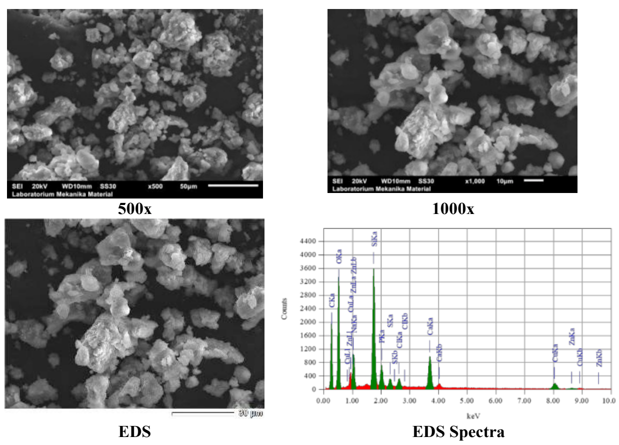


Fig. 6: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition O

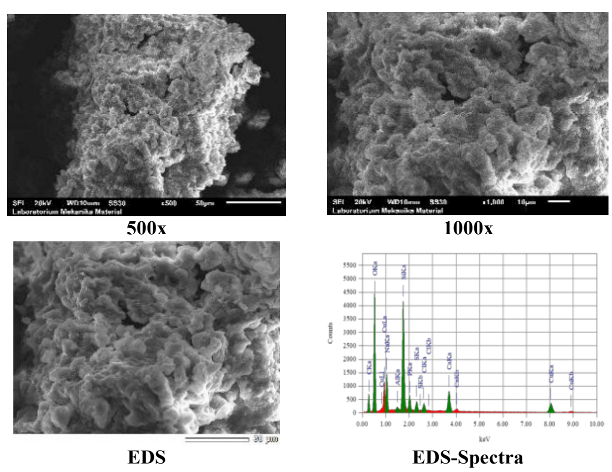


Fig. 7: SEM-EDS micrograms of sericin-silica-phosphate-Cu hydrogel composition P

contours with evenly distributed aggregation. Fig. 10 shows the FTIR spectra of the samples of sericin-silica-phosphate-Cu hydrogels of various compositions which are coded as K, L, M, N, O, P, Q and R.

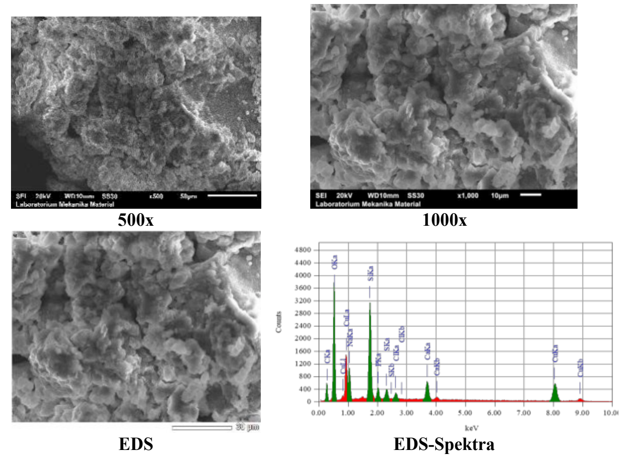


Fig. 8: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition Q

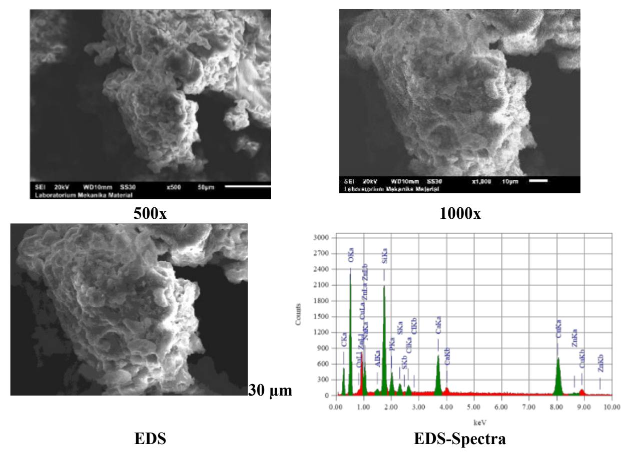


Fig. 9: SEM-EDS microgram of sericin-silica-phosphate-Cu hydrogel composition R

Results of DPPH and bacterial inhibition testing

DPPH testing and bacterial inhibition zone diameter data of Ser-Si-P hydrogels were reported on Fig. 11-13, meanwhile the statistical testing results were shown on Table 1-4. Results of the one-way ANOVA on Ser-Si-P hydrogels with P value of *0.000* (Table 1) showed that the compositions of sericin, nanosilica and nanocalcium phosphate significantly affected the % inhibition of DPPH and from LSD test (Table 2) showed that the composition of G from those hydrogels is the best hydrogel in term of its lest % inhibition of DPPH. Results of the two-way with replication ANOVA on bacterial inhibition zone diameter of Ser-Si-P hydrogels with P value of *0.000* (Table 3) indicated that the compositions of sericin, nanosilica and nanocalcium phosphate significantly influenced the anti-bacterial properties (against gram positive and gram negative) of Ser-Si-P hydrogels and from LSD test (Table 4) showed that the composition G from those hydrogels had the best ability against tested bacteria.

Table 1: One-way ANOVA results of DPPH testing on Ser-Si-P hydrogels

Source of Variation	SS	df	MS	F	*P* value	F crit
Sample Code	10412.31	9	1156.92	264.23	*0.00*	2.39
Error	87.57	20	4.38			
Total	10499.88	29				

Table 2: LSD test results of DPPH testing on Ser-Si-P hydrogels

Sample Code	% Inhibition (Average ± Standard Deviation)
A	51.38 ± 0.81^b
B	53.94 ± 2.15^b
C	96.22 ± 0.09^f
D	50.56 ± 1.37^b
E	91.46 ± 0.18^e
F	90.44 ± 0.23^e
G	45.30 ± 0.32^a
H	80.67 ± 0.70^d
I	84.05 ± 0.70^d
J	76.58 ± 5.95^c

Table 3: Two-way with replication ANOVA results of bacterial inhibition zone diameter of Ser-Si-P hydrogels

Source of Variation	SS	df	MS	F	*P* value	F crit
Sample	13.88	1	13.88	736.38	*0.00*	4.04
Columns	2356.75	11	214.25	11368.58	*0.00*	1.99
Interaction	10.19	11	0.93	49.13	*0.00*	1.99
Within	0.90	48	0.02			
Total	2381.72	71				

Table 4: LSD test results of bacterial inhibition zone diameter of Ser-Si-P hydrogels

Sample Code	Inhibition Zone Diameter (Average ± SD) mm
SA-A	3.62 ± 0.05^e
SA-B	1.02 ± 0.06^a
SA-C	1.15 ± 0.10^a
SA-D	6.61 ± 0.29^j
SA-E	3.13 ± 0.14^d
SA-F	3.06 ± 0.17^d
SA-G	7.89 ± 0.14^l
SA-H	3.27 ± 0.13^d
SA-I	4.14 ± 0.08^f
SA-J	4.43 ± 0.10^h
SA-Amp	18.72 ± 0.40^o
SA-Strep	17.11 ± 0.15^n
EC-A	3.12 ± 0.08^d
EC-B	2.11 ± 0.07^b
EC-C	2.54 ± 0.11^c
EC-D	6.91 ± 0.03^k
EC-E	4.08 ± 0.04^f
EC-F	3.96 ± 0.08^f
EC-G	9.25 ± 0.11^m
EC-H	5.17 ± 0.06^i
EC-I	4.20 ± 0.04^g
EC-J	4.24 ± 0.07^g
EC-Amp	20.18 ± 0.04^p
EC-Strep	18.91 ± 0.12^o

DPPH testing and bacterial inhibition zone diameter data of Ser-Si-P-Cu hydrogels were reported on Fig. 14-16, meanwhile the statistical testing results were shown on Table 5-8. Results of the one-way ANOVA on Ser-Si-P-Cu hydrogels with *P* value of *0.000* (Table 5) showed that the Cu-NPs addition in various compositions to Ser-Si-P

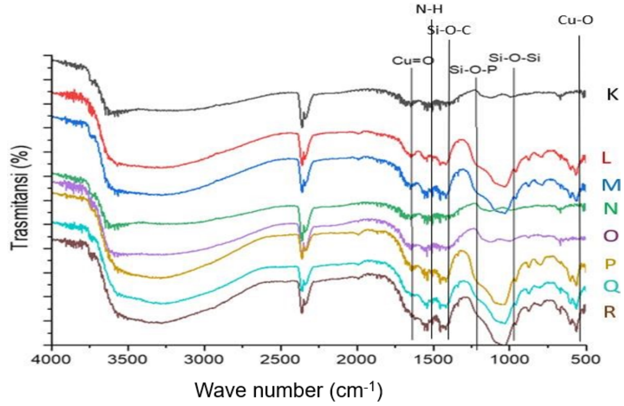


Fig. 10: FTIR spectra of sericin-silica-phosphate-Cu hydrogel

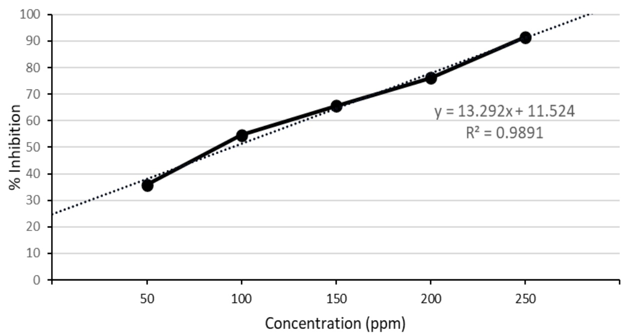


Fig. 11: Control solution calibration curve

hydrogel of G composition gave a significant effect on the % inhibition of DPPH and from LSD test (Table 6) showed that the compositions of O, P, Q and R from those hydrogels are the four best hydrogels in term of their lest % inhibition of DPPH. Results of the two-way with replication ANOVA on bacterial inhibition zone diameter of Ser-Si-P-Cu hydrogels with *P* value of *0.000* (Table 7) indicated that the Cu-NPs addition in various compositions to Ser-Si-P hydrogel of G composition gave a significant influence on the anti-bacterial properties (against gram positive and gram negative) of Ser-Si-P-Cu hydrogels and from LSD test (Table 8) showed that the compositions of P, Q and R from those Ser-Si-P-Cu hydrogels had the three best ability against tested bacteria. Considering of the both testing results and the efficiency of using Cu-NPs amount, the P composition of Ser-Si-P-Cu hydrogel is considered the best composition of the hydrogels as anti-inflammation and anti-bacterial properties.

Discussion

Based on the comparison of micrograms and EDS spectra graphic data, it can be seen that the most homogeneous distribution is composition G. Anti-inflammatory testing which refers to anti-oxidation due to free radicals or other oxidants has been carried out using the DPPH method and using standard vitamin C as a control at wavelength. 517 nm using UV-vis Spectroscopy instrumentation.

Table 5: One-way ANOVA results of DPPH testing on Ser-Si-P-Cu hydrogels

Source of Variation	SS	df	MS	F	*P* value	F crit
Sample Code	296.16	7	42.31	82.79	*0.00*	2.66
Error	8.18	16	0.51			
Total	304.34	23				

Table 6: LSD test results of DPPH testing on Ser-Si-P-Cu hydrogels

Sample Code	% Inhibition (Average ± Standard deviation)
K	45.27 ± 0.39^d
L	44.29 ± 0.15^c
M	43.11 ± 0.24^c
N	40.90 ± 0.41^b
O	36.78 ± 0.99^a
P	36.83 ± 0.88^a
Q	36.73 ± 1.26^a
R	36.68 ± 0.58^a

Table 7: Two-way with replication ANOVA results of bacterial inhibition zone diameter of Ser-Si-P-Cu hydrogels

Source of Variation	SS	df	MS	F	*P* value	F crit
Sample	21.85	1	21.85	1188.62	*0.00*	4.08
Columns	819.49	9	91.05	4952.64	*0.00*	2.12
Interaction	1.62	9	0.18	9.78	*0.00*	2.12
Within	0.74	40	0.02			
Total	843.69	59				

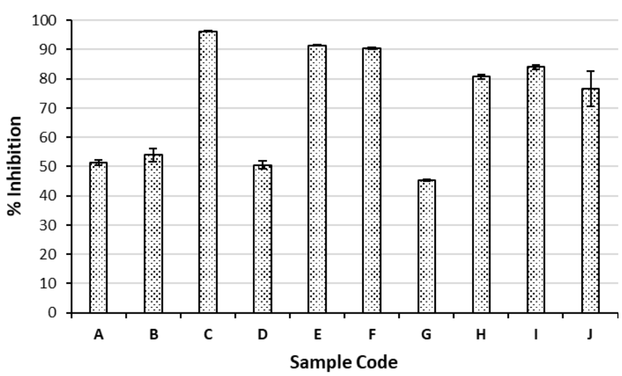


Fig. 12: % DPPH inhibition of sericin-silica-phosphate hydrogel composition A-J

The DPPH test results for the sericin-silica-phosphate hydrogel system (Fig. 11) as a calibration graph to determine the linear equation for the level of inhibition or % inhibition. Based on this graph, the level of inhibition for each sample of the A-I hydrogel composition can be obtained (Fig. 12). Statistical testing by using one-way ANOVA (Table 1) and continued with LSD test (Table 2) shows that the composition of sericin, nanosilica and nanocalcium phosphate gives a significant influence on the % DPPH inhibition with P value of 0.000 (Table 1) and the best composition of Ser-Si-P hydrogels in terms of % DPPH inhibition is G composition (sericin: nanosilica: nanocalcium phosphate = 50: 25: 25).

Based on the meaning of the % inhibition value, the smaller the inhibition value, the stronger the antioxidant

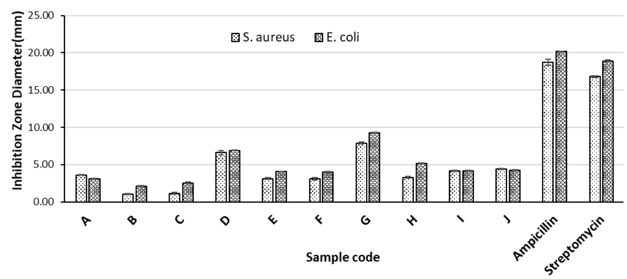


Fig. 13: Comparison graph of the bacterial inhibitory power of sericin-silica-phosphate hydrogels of various compositions

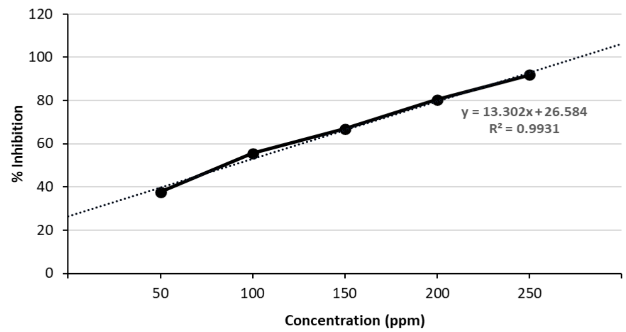


Fig. 14: Control solution calibration curve

properties of the material or the better the anti-inflammatory properties, then the G composition or sericin-silica-phosphate composition = 50: 25: 25 can be obtained which has the relatively best anti-inflammatory properties among them. the hydrogel. Based on the linear equation in the calibration curve, the I50 of the sericin-silica-phosphate hydrogel is 1.895.

Testing the inhibitory power of gram-positive and gram-negative bacteria is one of the tests to determine the effectiveness of hydrogel as a candidate for functional medical biomaterials, especially as a candidate for wound healing drugs. Anti-bacterial testing was carried out using the method of measuring the inhibitory power against gram-positive bacteria *Streptococcus aureus* and gram-negative *Escherichia coli* with positive control antibiotics Ampicillin and Streptomycin. The test results are summarized and presented as a graph in Fig 13. Based on the data in this graph, it was found that all sericin-silica-phosphate hydrogel compositions have the potential as antibacterial biomaterials and the G composition or sericin-silica-phosphate composition = 50: 25: 25 has relatively the best performance. the inhibitory power of gram-positive and gram-negative bacteria compared to other compositions. This finding is supported by the statistical testing. Two-way with replication ANOVA testing on bacterial inhibition zone diameter of Ser-Si-P hydrogels results *P* value of *0.000* (Table 3) that means the compositions of sericin, nanosilica and nanocalcium phosphate give significantly influence on the anti-bacterial properties (against gram positive and gram negative) of Ser-Si-P hydrogels and from LSD test (Table 4) shows that the composition G from those hydrogels has the best ability against tested bacteria.

Table 8: LSD test results of bacterial inhibition diameter zone of Ser-Si-P-Cu hydrogels

Sample Code	Inhibition Zone Diameter (Average ± SD) mm
SA-K	7.90 ± 0.12[a]
SA-L	8.16 ± 0.18[b]
SA-M	8.48 ± 0.04[c]
SA-N	8.76 ± 0.12[d]
SA-O	9.32 ± 0.04[e]
SA-P	9.93 ± 0.10[f]
SA-Q	9.96 ± 0.20[f]
SA-R	9.99 ± 0.04[f]
SA-Amp	18.66 ± 0.12[j]
SA-Strep	17.16 ± 0.02[i]
EC-K	9.29 ± 0.07[e]
EC-L	9.50 ± 0.06[e]
EC-M	9.90 ± 0.01[f]
EC-N	10.15 ± 0.11[g]
EC-O	10.19 ± 0.12[g]
EC-P	10.77 ± 0.11[h]
EC-Q	10.78 ± 0.26[h]
EC-R	10.80 ± 0.25[h]
EC-Amp	20.03 ± 0.25[l]
EC-Strep	18.97 ± 0.17[k]

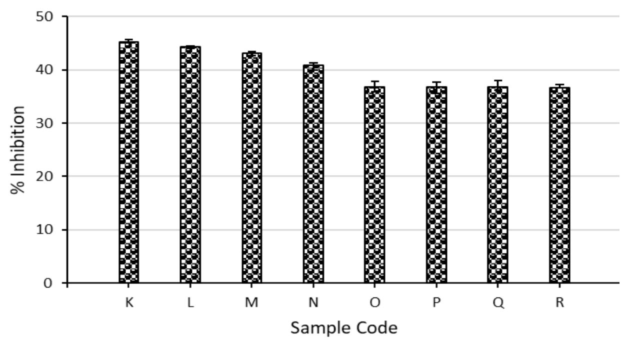


Fig. 15: % DPPH inhibition of sericin-silica-phosphate-Cu hydrogel composition K-R

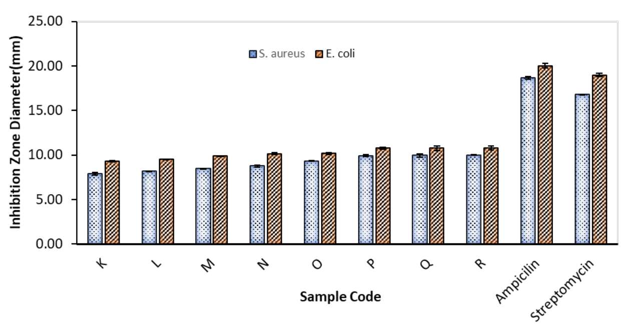


Fig. 16: Comparison graph of the bacterial inhibitory power of sericin-silica-phosphate-Cu hydrogels of various compositions

The characterization of functional groups and chemical bonding phenomena in the sericin-silica-phosphate-Cu hydrogel was analyzed using an infrared spectroscopy instrument combined with the Fourier Transform method or abbreviated as FTIR. The result of the FTIR spectra from samples of sericin-silica-phosphate-Cu hydrogel compositions of K to R is presented in Fig 10. Based on Fig. 10, the attachment of sericin is represented by the elements C, H, O and N as the main constituent of protein compounds with phosphate being represented characteristically by P and silica are represented by Si and Cu nanoparticles, so the interactions C-O-P, P-O-Si, C-O-Si, N-O-P, Si-O-Si, H-O-P, H-O-Si are bonds which are an indication of the bond between the components that make up the hydrogel. These interactions cause a shift in the Si-O, C-O, O-H, P-O wave numbers. Based on Fig. 10, a shift has occurred in the related functional groups, this indicates that there is cross-linking between sericin and silica and with phosphate and copper particles.

The results of DPPH testing and bacterial inhibition (Fig. 14-16) show that the P composition (the mass ratio of the best Sr-Si-P: Cu-NPs = 90: 10 or 90G: 10Cu-NPs) has relatively the best anti-inflammatory and antibacterial performance. Based on the calibration curve, the anti-inflammatory I50 of the Ser-Si-P-Cu hydrogel is 1.760 is better than that of the Ser-Si-P hydrogel with I50 of 1.895, because of the smaller the I50 value the better of the anti-inflammation properties of the hydrogels. This finding is supported by the one-way ANOVA analysis continued by LSD testing on % DPPH of Ser-Si-P-Cu hydrogels that showed the Cu-NPs addition on G composition of Ser-Si-P hydrogels giving a significant effect with P value of 0.000 (Table 5) on their inflammation properties and there are four compositions of O, P, Q and R (Table 6) of the Ser-Si-P-Cu hydrogels having least % DPPH reduction. Two-way with replication ANOVA analysis on bacterial inhibition zone diameter of Ser-Si-P-Cu hydrogels resulted P value of 0.000 (Table 7) that means the Cu-NPs addition in various compositions to Ser-Si-P hydrogel of G composition has a significant effect on the anti-bacterial properties (against gram positive and gram negative) of Ser-Si-P-Cu hydrogels and LSD test (Table 8) resulting the compositions of P, Q and R from those Ser-Si-P-Cu hydrogels have the highest inhibition against tested bacteria. Considering of the testing results DPPH, anti-bacterial assays and efficiency of using Cu-NPs amount, the P composition of Ser-Si-P-Cu hydrogel is considered the best composition of the hydrogels as anti-inflammation and anti-bacterial properties.

The designed hydrogel prototype is well suited to the needs of biomaterials that have superior biocompatibility, biodegradability, low toxicity, and low allergenicity, and produce degradation products that are less cytotoxic and more easily metabolized by host tissues (Han *et al.* 2022). Biomaterials for medical applications can be classified as organic biomaterials, inorganic biomaterials and organic-inorganic hybrid biomaterials. Organic and inorganic hybrid biomaterials are a type of biomaterial that has great potential, because the properties of the materials complement each other, resulting in synergy in the final product process (Saveleva *et al*. 2019). Organic–inorganic hybrid nanobiomaterials can be based on silica and metal–organic materials (Taylor-Pashow *et al*. 2010) such as sericin-based inorganic–inorganic hybrid biomaterials combined with nano-silica and copper (Cu) nanoparticles.

Sericin, a by-product of silk, has been recognized as an economical glycoprotein extracted during the silk degumming process in the textile industry and has medical properties including strong biocompatibility and biodegradability, in the form of nanoparticles it functions in the cancer drug delivery process, has the ability to increase efficacy drugs, have solubility and bio-diffusion that can increase cell stability, cell uptake, and endosomal release (Dan *et al.* 2022). The combination of sericin with nanosilica and nanophosphate can increase its biocompatibility and medical functionality, where bio-nanosilica from rice husk ash has high biocompatibility properties (Ghaferi *et al.* 2021) making it suitable for use in oncology therapy (Huang *et al*. 2020), cancer therapy (Li *et al*. 2019) and nanocalcium phosphate made from bovine bone hydroxyapatite also have functional medical properties such as being able to increase wound healing activity *in vitro* and having biocompatible properties that will be used for the development of biomaterials for wound dressings and implants (Han *et al*. 2022). Meanwhile, copper nanoparticles are nanomaterials that have antimicrobial and antiviral properties and have mechanisms such as ion release, reactive oxygen species (ROS), direct contact, and immunostimulant effects (Moorthy 2021).

Various naturally-derived hydrogels have similar function with sericin-silica-phosphate-Cu hydrogel for wound healing, namely collagen-based, elastin and fibrin, gelatin and silk fibroin, polysaccharide-based, bioactive, and smart-responsive hydrogels as reviewed by Pham *et al*. (2025). Critical attributes of an ideal wound dressing and wound healing are (1) moisture for supplying moisture to the dry wounds and absorbing moisture and exudates from wet wounds, (2) absorption that is controlling the level of wound exudate and preventing tissue maceration by effectively absorbing excess fluid permeability, (3) permeability that is facilitating gaseous exchange (water vapor, O_2, CO_2) to the wound bed to accelerate cellular activity, (4) protection that means preventing microbial infections that could impede the wound healing process and prolong its duration, (5) transparency for enabling clinicians to visualize the wound and monitor the healing process, (6) mechanical robustness, that is mimicking the structure of native skin while being rigid enough to allow fixation on the wound, (7) flexibility and elasticity for adapting to body movements and minimizing patient discomfort and pain during application and dressing replacement, (8) adhesiveness, that is providing good adhesion to healthy skin without sticking to the wound itself, facilitating easy removal after re-epithelialization, and preventing secondary injuries in the newly formed tissue, (9) biocompatibility, that is minimizing the risk of immune reactions or side effects, (10) safety, that is ensuring the absence of toxic substances that could cause damage or result in dire consequences as well as ensuring that products resulting from degradation are safe and follow the normal metabolic pathway, (11) cost-effectiveness, that is supplying a cost-effective wound dressing solution and (12) availability, that is widely available to all patients and healthcare centers, as reviewed by Ribeiro *et al*. (2024). In terms of those twelve attributes, the sericin-silica-phosphate-Cu hydrogel seemly fulfills them. Furthermore, the hydrogel is also prospectively used as a candidate of biomaterials for bio-ink in the 3D-bioprinting purposing for tissue engineering because of its biocompatibility, anti-microbial and anti-inflammatory properties. Hence, further investigation for fulfilling all requirements as biomaterial for bio-inks is needed.

Conclusion

Composition G or sericin-silica-phosphate composition with mass ratio = 50: 25: 25 has relatively the best performance in inhibiting gram-positive and gram-negative bacteria compared to other compositions. The addition of Cu-NPS to sericin-silica-phosphate hydrogels affects their anti-inflammation and anti-microbial properties. Composition P (the mass ratio of the best Sr-Si-P: Cu-NPs = 90: 10 or 90G: 10Cu-NPs) showed relatively the best anti-inflammatory and antibacterial performance. Based on the calibration curve, the anti-inflammatory I50 of the sericin-silica-phosphate-Cu hydrogel is 1.760 is better than that of sericin-silica-phosphate hydrogel with I50 of 1.895. Thus, it can be emphasized that Cu-NPs addition to sericin-silica-phosphate hydrogels gives a significant effect on the inflammation and anti-bacterial properties.

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Author Contributions

EDK and IWK: Designed, coordinated the study, collected data. AK and CFK: analyzed data. NAJ and KMS: reviewed the final version of the manuscript. All authors have read, reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability

The data will be available on a fair request to the correspondence author.

Ethics Approval

This article does not contain any studies involving human or animal subjects.

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