International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Antibacterial Chitosan-Carboxymethyl Cellulose Composite Reinforced Propolis Sponge-like as Hemostatic Agent

Lale Rozykulyyeva[1], Prihartini Widiyanti[2], Suryani Dyah Astuti[2*]*

[1]*Mathematics and Natural Sciences Doctoral Program, Faculty of Science and Technology, Universitas Airlangga, 60115, East Java, Surabaya, Dr. Ir. H. Soekarno str., Indonesia*

[2]*Biomedical Engineering Study Program, Faculty of Science and Technology, Universitas Airlangga, 60115, East Java, Surabaya, Dr. Ir. H. Soekarno str., Indonesia*

Abstract

The development of antibacterial, economical, biosafe and effective materials for bleeding control are becoming increasingly important in the clinical field. Therefore, the study is to explore novel antibacterial composite-reinforced propolis prepared by crosslinking chitosan (CS), hydroxyethyl cellulose(HEC) and carboxymethyl cellulose (CMC) with various ratios following an improved vacuum freeze-drying method. The characterization composites were investigated by FTIR, water absorption capacity test, mechanical strength, antibacterial assay, and cytotoxicity test. The molecule interactions between CMC, HEC propolis and CS (as determined by FTIR analysis) significantly resulted in higher were evaluated for water absorption capacity and lower mechanical strength. Composite of CS/CMC/HEC/propolis presented a greater antibacterial activity against Staphylococcus aureus and Escherichia coli in comparison to CS/CMC without propolis.

Keywords: Antibacterial Hemostatic Composite, Chitosan, Propolis.

Full length article **Corresponding Author*, e-mail: *pwidiyanti@fst.unair.ac.id, suryanidyah@fst.unair.ac.id*

1. Introduction

Hemostasis plays an important role in all surgical procedures because of the complications of bleeding during and after surgery. In general, approximately 20% to 40% of deaths occurring after admission to the hospital involve massive bleeding following traumatic bodily injury and are potentially preventable through early medical and surgical intervention [1-3]. Severe traumatic hemorrhage and also reasons uncontrolled hemorrhage is a major cause of mortality. Interventions to control bleeding and hemostatic resuscitation have been shown to be useful in reducing mortality from hemorrhagic injuries. Management of hemostasis in situations of severe bleeding is not only dependent on the surgical team and also requires mitigating support, namely topical hemostatic agents [3-5]. The traditional method to control bleeding is the mechanically pressing of cellulose-based cotton gauze/ dressings on the bleeding place which absorbs and increases the concentration of coagulation factors [6-7]. The commonly used cellulose-based hemostatic materials have various disadvantages such as absorption delay and poor antibacterial effect.

Cellulose the other hand is not effective in bleeding control, because clotting depends on the coagulation process, that is, the hydrophilic properties of cellulose will not promote clot formation. Following coagulation cellulose sticks strongly to the injury, complicating removal and causing secondary bleeding and infection [5,8-10]. Materials to be hemostatic agents, need to make proper contact with the bleeding surface, promote rapid absorption of blood, keep the wound from infection and be easy to handle [3,5,11]. Hemostatic agents exist in various compositions and preparation forms which are formulated as powders, hydrogels, sponges, knitted, or nonwoven sheets [12-16]. Powder and hydrogel-type hemostatic agents can easily conform to shape, but are limited to uncontrolled bleeding and have difficulty in use [17]. Sponge-type hemostatic agents can absorb a larger amount of blood than others with clotting using pressure and can be removed easily [9,18-19]. Therefore, the development of antibacterial, economical, biosafe, and effective hemostatic materials for bleeding control is becoming increasingly important in the clinical field.

Inspired by hemostatic materials have been developed based on new strategies modified with chitosan for blood coagulation [5,20-21]. Therefore, the study is to explore a novel antibacterial sponge composed of natural materials; namely chitosan (CS), carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC) and propolis (bee resin) as the antibacterial drug. CS has been used for its hemostatic properties in that it binds to platelets and red blood cells to form a gel-like covering of blood vessels, CMC and HEC as a hydro fiber, and propolis is used for great antibacterial activity and has been widely used in pharmaceuticals, due to its biocompatibility, biodegradability, non-toxicity. Composite sponges with various ratios following an improved vacuum freeze-drying method. The characterization composites were investigated by FTIR, swelling ratio, tensile strength, antibacterial assay, and in vitro blood clotting performance.

2. Materials and methods

Sodium Carboxymethyl Cellulose (CMC-Na) (Wealthy Co., Ltd, China, Shrimp shell Chitosan (degree of deacetylation>75%) (Himedia, India), Hydroxyethyl Cellulose (HEC) (Kello Cel tm, China), Calcium Chloride, Acetic Acid, Ethanol 70%, were purchased from Chemical Indonesia (Surabaya). Raw Propolis was purchased from the Kelanceng Bee Farm, Kediri, East Java and extracted according to the procedure reported by Sharaf et al. Propolis extraction from Trigona bee colonies was carried out by ethanol extraction method. The propolis sample was mixed with 70% pure ethanol at a ratio of 1:10 (w/v) and shaken using a shaker at 300C for 48 hours. Filtered to remove debris and dirt using Whatman filter paper. Dry in a rotary evaporator. Extracted propolis is stored at 200C for further use [21]. Composite sponges were prepared with 1% (w/v) chitosan dissolved in 1% (v/v-100 mL) acetic acid (pH 5-6), 2% (w/v) CMC and 2% (w/v) HEC dissolved in distilled water (pH 7). Avoiding the formation of CMC and HEC clumps by slowly adding them to the water with magnetic stirring and stirring for 1 hour at 35^0C. After the solution is completely dissolved, the pH value of all solutions is adjusted to 1 with hydrochloric acid. Then, the two solutions were mixed together slowly and stirred until homogeneous and prepared at different ratios, and added 1 mL of propolis aqueous ethanolic solution to the propolis-based sample (3:1, 1:1, 2:2:2, 3:2:2). and 3:1p and 1:1p-for antibacterial testing), and are named CMC/CS3, CMC/CS1, CMC/CS/HEC2, CMC/CS/HEC3, CMC/CS3p, and CMC/CSp. The composite was placed in a smoke cupboard for 12 hours to remove acetic acid. The air bubble-free composite was poured into a Petri dish, then frozen at -280C for 24 hours and followed by freeze-drying at -40^0C to obtain a sponge composite. The samples were then stored in closed plastic bags in a dry place at room temperature. Characterization of CS-CMC-HEC composite previously carried out for propolis liquid extract to identify the phytochemical components according to previous studies [22]. The functional groups of CMC, HEC, CS, Propolis and composites were studied with FT-IR spectra of different materials obtained on a spectrometer (Bruker, Germany). Spectra were taken over the wavenumber range 600-4000 cm^{-1}, with a scanning resolution of 4 cm^{-1}, and each sample was scanned 15 times [9]. Sponge composite was carried out, followed by analysis of its tensile strength measured by Universal Testing Machine (UTM), (Shimadzu, Japan) and expressed in MPa. The membrane was cut into 2 × 6 cm^2 dimensions and given a load of 5 N with a movement speed of 5 mm/minute [23]. The swelling ratio (%) was determined according to the method reported by Zhou et al [24]. Each sponge sample was cut into a rectangular shape (approximately 1 × 1 cm^2 in size), and the dry weight (W_0) of each sample was weighed before testing. Then, the dry samples were immersed in Phosphate Buffered Saline (PBS) with a pH value of 7.4 and incubated at 37^0C for 10 minutes until it reaches the saturated swelling ratio. At different time intervals (10 minutes, 30 minutes, 60 minutes, 80 minutes, and 100 minutes), the samples were removed and the water was carefully cleaned with filter paper to remove the excess water on the surface of the sponge and the wet weight of the sample (Wi) was immediately measured. The swelling ratio is calculated using the following formula:

$$\text{Swelling ratio (\%)} = (Wi - W_0)/W_0 \times 100\% \quad (1)$$

Composite sponges CMC3/CS1, CMC/CS1, CMC3/CS3p, CMC/CSp and using Chloramphenicol 30 µg as negative control and positive control. It was carried out using the agar diffusion method using *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 bacteria strains, with density of 1.5 x 10^8 CFU/mL. Each piece of sponge sample was placed in the center of the culture dish and then incubated for 24 hours at 37˚C, the diameter of the clear circle zone was measured and recorded for analysis [25]. The complete blood clotting assay is used to evaluate the hemostatic ability in vitro. The prepared samples were cut into rectangles with a diameter of 9 mm placed in culture dishes and warmed at 37°C for 10 minutes. A volume of 100 µL of recalcified whole blood solution (0.2 m $CaCl_2$, 10 mm in blood) was dropped onto the surface of the sample. All culture plates were incubated at 37°C and 50 rpm to allow interaction between blood and material. After 5 minutes, 10 mL of deionized water (DI) was gently added to release the blood bonds without disturbing the clot and then incubated on a shaking table at 37°C and 50 rpm for 5 minutes [18]. The whole blood clotting time of the samples was tested as previously described. Briefly, 2mL of whole blood (containing 10% sodium citrate) was added to a 1.5mL polypropylene tube containing 10 mg of each sample, and then 60 µL of $CaCl_2$ solution (0.25 M) was added to the tube. The tube is inverted every 15 seconds and the blood clots each time. Five replicates were performed for each group [18,26].

3. Results and Discussions

The phytochemical analysis of ethanolic aqueous extract obtained from raw propolis revealed the presence of triterpenoids, flavonoids, furanoids, sugars, quinones, tannins, and phenols as summarized in Table 1. Previous study by Priyadarshini et al., Bloomfield Hills, USA. propolis contains of triterpenoids, flavonoids, furanoids, sugars, coumarins, quinines, tannins, phenols and acids which are beneficial in the immune system [22]. The presence of more phytochemical groups in propolis provides synergistic effects in many biological applications including antibacterial effects [27]. The active components of polyphenols and flavonoids have been identified as chemicals that have anti-atherosclerotic, anti-inflammatory and anti-angiogenic properties, as well as cardioprotective, vasoprotective, antioxidant and antibacterial properties [28-30].

The FTIR spectra results of the chitosan peak showed a characteristic absorption band at 1636 cm^{-1} corresponding to amide-I. The increased peak at 1615 cm^{-1} corresponds to the N-H vibration of the amide group [31]. The hydrogen-bonded O-H stretching of the phenolic compound phenolic hydroxyl group is found at about 3200-3500 cm^{-1}. In previous studies, both types of chitosan showed an adsorption band around 3400 cm-1, which indicates the presence of -OH overlapping with -NH groups [23,31]. The absorption band around 1024.91 cm^{-1} corresponds to the C-O-C ether functional group for CMC. The peaks around 897 cm^{-1} and 1160 cm^{-1} in the spectrum refer to the saccharide structure, and the C-O-C ether functional group was found to be around 1024 cm^{-1} for CMC [32]. Occording to Wang et al. the intensity of the new absorption peak varies with the degree of carboxymethyl substitution, indicating that carboxymethylation has proceeded well [33]. The peak at 1636 cm^{-1} (CMC-CS-Propolis) according to Wang et al. is due to the overlap of C-O vibrations of carboxylic groups on CMC and N-H bending vibrations on CS [31,33]. Regarding the absence of new vibrations at the interaction between CS and CMC, it was observed by Wang et al. and Stiglic et al. that this interaction does not involve chemical bonding but indicates electrostatic interactions between oppositely charged chitosan and CMC [31,34]. The FTIR spectra for HEC found around 993.08-1587.19 cm^{-1}, which are attributed to O-H stretching vibrations, C-H stretching vibrations of methylene group, C-H deformations, and C-O stretching vibrations, respectively [35]. Furthermore, spectra samples showed the absorption characteristic peaks of CMC, CS and HEC, demonstrating the successful preparation of sponges. The band around 1014.19 cm^{-1} and 1359.79cm^{-1}, which can be assigned vibration of ether C-O, which is also enhanced that the increase in saturated C–H groups [36].

While the characteristic functional group of propolis extract showed absorption bands at around 1265.56 cm^{-1} corresponding to C-O groups of polyols e.g., hydroxy flavonoids of the propolis extract. Previous studies reported intense bands between 1300 and 1000 cm-1 are C-O stretching and C-OH bending derived from alcohols, ethers, esters, and carboxylic acids, which are functions found mainly in phenolic acids and flavonoids found in propolis extracts [37]. The majority of FT-IR bands are characteristic of triterpenoids, flavonoids, furanoids, sugars, coumarins, quinine, tannins, phenols, and acids contained in the aqueous extract of propolis [38-40]. The tensile strength of composite sponges with different compositions is shown in Figure 1. CMC/CSp sponge with low CMC concentration showed the lowest tensile strength value of 4.9 MPa with increasing CMC content CMC/CS3p composite showed the best tensile strength (14.8 MPa). While the CMC/CS/HEC2 composite showed a tensile strength result of 12.4 MPa. Recent study by Zhu et al., developed cross-linked composite of CMC and HEC by vacuum freeze-drying method showed tensile strength of 15.2 MPa. According this study the composite surface with irregular porous structure and interlaced fibrous features improved the tensile strength of the composite [20]. Further research indicates that the increased tensile strength of crosslinked CS/CMC is related to the combination of chitosan and crosslinked CMC [23].

Wang et al. suggested that the tensile strength of CMC-based composites is due to the excellent compatibility and strong electrostatic interaction between the molecules [41]. Hu et al. also reported that with the addition of CMC, the tensile strength of the composite membrane increased from 2.06 to 45.56 MPa [42]. Lan et al. reported tensile strenght of CS/SA/CMC composite was 32.10 MPa and related with crystallinity of chitosan and also the quantity of CMC [43]. An important feature of the hemostatic effect of topical hemostatic agents is rapid absorption to accumulate blood cells and clotting factors [44]. The swelling ratio test results of all sponges were rapidly absorbed within the first 5 minutes and the curve stabilized after 60 minutes is shown in Figure 2. Sponges with high CMC content and with HEC content, observed significantly increased water adsorption capacity. The composites CMC/CS/HEC3, CMC/CS/HEC2 and CMC/CS3 absorbed immediately after being dripped with water. This is expected to have better blood absorption characteristics and consequently increase the blood clotting rate. On the other hand, CMC/CSp and CMC/CS1 composites exhibited low swelling ratio. Swelling measurements observed by Khabibi et al that chitosan and CMC blend membranes have the highest water absorption compared to chitosan [23]. Tang et al. reported that the swelling ratio increased rapidly with increasing CMC content [45]. According to previous studies CMC fibers exhibit a certain water holding capacity as the highly hydrophilic carboxyl groups of CMC act as water absorbents to fill the pores, and swell while absorbing water rapidly leading to a high swelling ratio. Reported that the swelling ratio decreases with an increase in chitosan content. This can be explained that the chitosan chains can entangle themselves easily by hydrogen bonding in a high chitosan concentration solution, leading to reduced space [9,45].

The whole blood clotting model was used to evaluate the in vitro hemostatic properties (Fig. 3). After rinsing with water, there was an increase in the color of the water due to hemolysis in the control gauze group, indicating that blood was difficult to clot on the gauze. There were more blood clots were formed on the surface of the commercial sponge and gauze than on the sponge. In the other hand, in the CMC/CS/HEC2 sample, the CMC/CS/HEC3 blood actually coagulated in the clear and clear water. This indicates that the blood can clot well on the sponge, and the hemostatic sponge further shortens the blood clotting time. The in vitro hemostatic properties of the samples were evaluated by measuring the blood clotting time. Four selected samples were achieved blood coagulation formation after adding blood for a certain time. Furthermore, the blood clotting time of CMC/CS/HEC2 sponge was 150 ± 15 seconds and CMC/CS/HEC3 was 175 ± 15 seconds. The sponges approaching the medical gauze of the positive control group (270 ± 15 seconds) were CMC/CS3 (250 ± 15 seconds) and CMC/CS1 significantly faster than the medical gauze (220 ± 15 seconds). Similar results were reported by Xie et al with a blood clotting time of 180 ± 15 seconds from sponge hemostatic [19]. The hemostatic sponge developed by Zhu et al. completed hemostasis within 3.11 minutes [21]. This was probably due to the sponge having a unique fine nanofiber structure, which greatly increased the surface area and exposed more amino groups to the blood.

The positively charged amino groups tend to interact with negatively charged platelets, causing increased platelet aggregation to form a blood clot [19,46]. The quick initial absorption of blood is very important for the hemostatic agent to achieve hemostasis in a very short time. CMC can form a soft gel-like "pseudoclot" around the injury which acts as a barrier to occlude blood flow while it can also activate the intrinsic coagulation pathway to accelerate the process of blood clotting [47-48]. The antibacterial test results of the hemostatic sponge against E. coli bacteria from the CMC/CS1 sample showed an inhibition zone of 11.53 mm while CMC/CS3 showed an inhibition zone of 10.32 mm. The results of samples with propolis CMC/CSp with inhibition zone of 11.21 mm and CMC/CS3p 8.44 mm inhibition zone (Fig. 4). However, the antibacterial effect against S. aureus bacteria from CMC/CS1 sponge showed an inhibition zone of 11.53 mm and CMC/CS3 inhibition zone of 10.32 mm.

The inhibition zone against S. aureus bacteria on CMC/CSp propolis-based samples was 11.21 mm and CMC/CS3p had 7.87mm inhibition zone (Fig. 4). From the antibacterial test results of the composite sponge against *E. coli* and *S. aureus* bacteria, it can be seen that the inhibition zone. However, our supervision incubated all samples for 5 days and, it was observed that there no bacterial colonies in the inhibition zone or in the sample area. CMC and CS-based sponges developed by Cai et al., reported the ability to restrain the growth of *Escherichia coli* with a 4.96 mm antibacterial circle diameter. The research antibacterial properties of the samples were attributed to the effect of active components of polyphenols and flavonoids from propolis and chitosan which has natural antibacterial activity due to which it neutralizes the negative electrons on bacteria with its positively charged amino radical (-NH_2) group [33S].

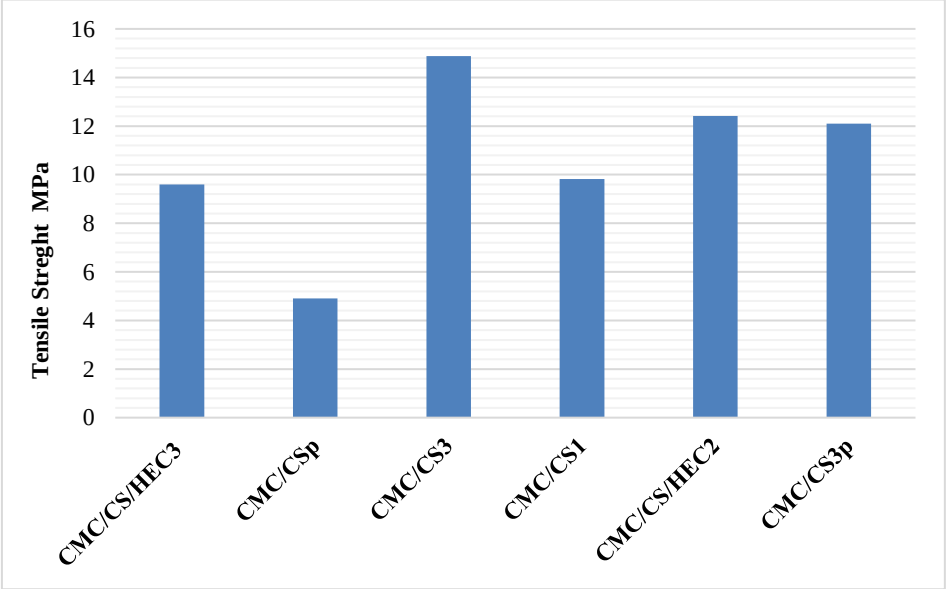

Figure 1. Tensile strength of CMC/CS3, CMC/CS1, CMC/CS/HEC2, CMC/CS/HEC3, CMC/CS3p and CMC/CSp sponges.

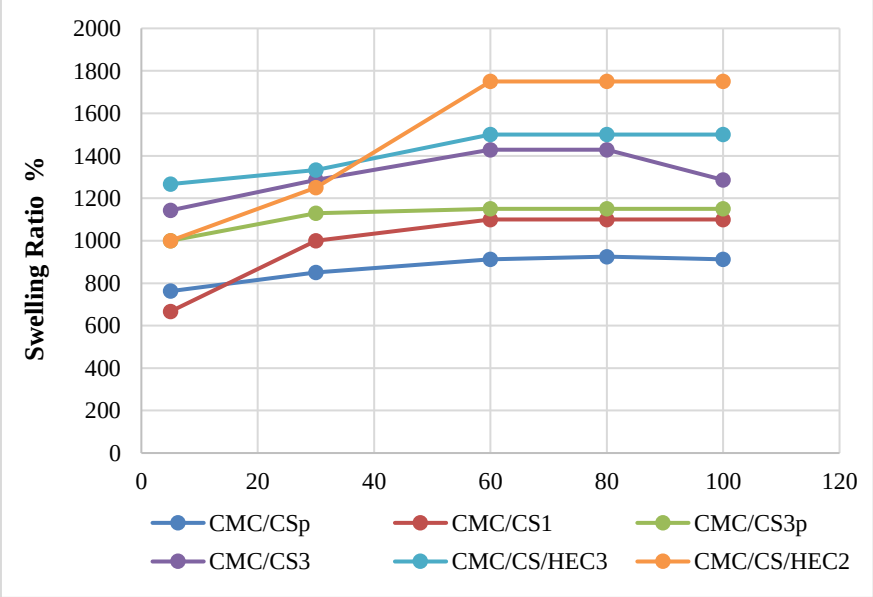

Figure 2. Swelling Ratio of CMC/CS3, CMC/CS1, CMC/CS/HEC2, CMC/CS/HEC3, CMC/CS3p and CMC/CSp sponges.

.

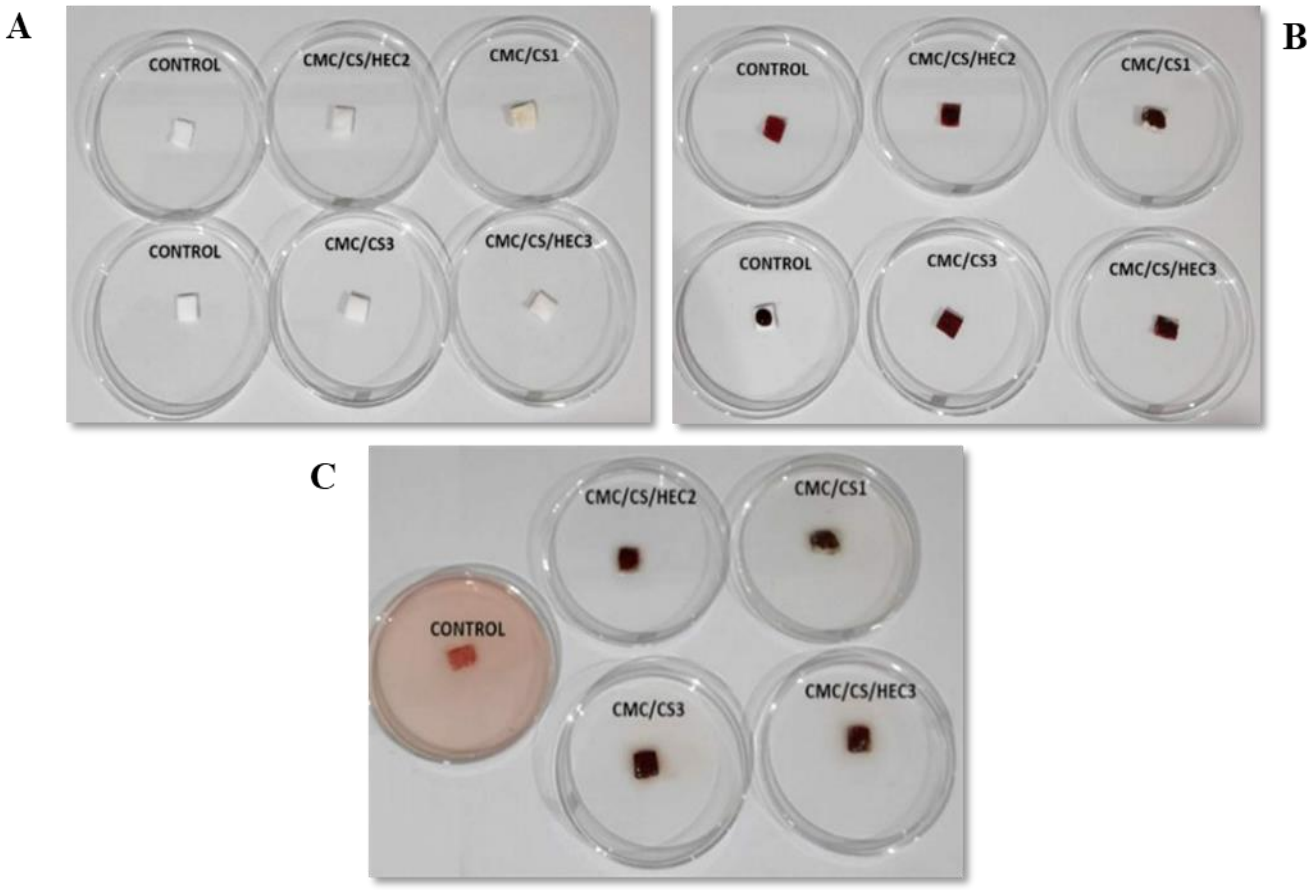

Figure 3. In vitro hemostatic evaluation of samples, a whole-blood clotting model. A) Samples, B) Blood added samples, C) Rinsed by water.

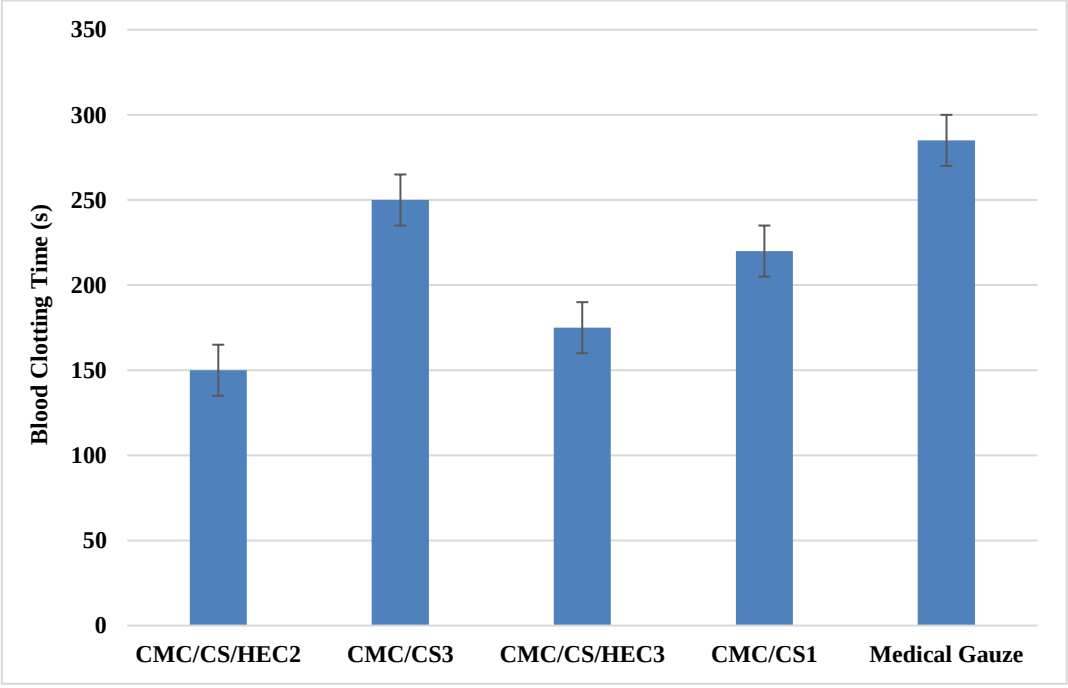

Figure 4. Clotting time of sponges CMC/CS3, CMC/CS1, CMC/CS/HEC2, CMC/CS/HEC3 and medical gauze.

Table 1. Qualitative phytochemical analysis of aqueous extract of propolis.

Phytochemical Parameters	Yes (+) or No (-)
Acids	+
Flavonoids	+
Phenols	+
Quinones	+
Sugars	+
Tannins	+
Triterpenoids	+

4. Conclusions

CMC/CS/HEC/propolis-based composite sponges as hemostatic materials were successfully prepared by crosslinking followed by freeze-drying process. In addition, low pH showed intermediate homogenization distribution than high pH. Based on the results, it is recommended that the synthesized hemostatic composites are required to improve the mechanical characters adding plasticizers and further developed to be applied and used as hemostatic agents in the future.

Acknowledgment

The authors acknowledge and would like to thank the financial support for this study received from the PTN/LLDIKTI [Project Number = 0008046902] Indonesian Government.

References

[1] S. Alven, & B. A. Aderibigbe. (2020). Chitosan and cellulose-based hydrogels for wound management. International journal of molecular sciences. 21 (24): 9656.

[2] J. C. Salamea-Molina, A. N. Himmler, L. I. Valencia-Angel, C. A. Ordoñez, M. W. Parra, Y. Caicedo, ... & J. C. Puyana. (2020). Whole blood for blood loss: hemostatic resuscitation in damage control. Colombia Médica. 51 (4).

[3] J. B. Holcomb, B. C. Tilley, S. Baraniuk, E. E. Fox, C. E. Wade, J. M. Podbielski, ... & PROPPR Study Group. (2015). Transfusion of plasma, platelets, and red blood cells in a 1: 1: 1 vs a 1: 1: 2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. Jama. 313 (5): 471-482.

[4] B. M. Pereira, J. B. Bortoto, & G. P. Fraga. (2018). Topical hemostatic agents in surgery: review and prospects. Revista do colegio brasileiro de Cirurgioes. 45.

[5] G. H. Kim, J. N. Im, T. H. Kim, G. D. Lee, J. H. Youk, & S. J. Doh. (2018). Preparation and characterization of calcium carboxymethyl cellulose/chitosan blend nonwovens for hemostatic agents. Textile Research Journal. 88 (16): 1902-1911.

[6] D. A. Hickman, C. L. Pawlowski, U. D. Sekhon, J. Marks, & A. S. Gupta. (2018). Biomaterials and advanced technologies for hemostatic management of bleeding. Advanced Materials. 30 (4): 1700859.

[7] Y. Li, F. Niu, X. Zhao, C. H. Yap, & Z. Li. (2021). Nonwetting Nanostructured Hemostatic Material for Bleeding Control with Minimal Adhesion. Advanced Materials Interfaces. 8 (23): 2101412.

[8] Y. Wang, P. Zhou, D. Xiao, Y. Zhu, Y. Zhong, J. Zhang, ... & Z. Mao. (2019). Chitosan-bound carboxymethylated cotton fabric and its application as wound dressing. Carbohydrate polymers. 221: 202-208.

[9] X. Lu, X. Li, J. Yu, & B. Ding. (2022). Nanofibrous hemostatic materials: Structural design, fabrication methods, and hemostatic mechanisms. Acta Biomaterialia.

[10] Y. Wang, Y. Zhao, L. Qiao, F. Zou, Y. Xie, Y. Zheng, ... & S. Yang. (2021). Cellulose fibers-reinforced self-expanding porous composite with multiple hemostatic efficacy and shape adaptability for uncontrollable massive hemorrhage treatment. Bioactive Materials. 6 (7): 2089-2104.

[11] H. He, W. Zhou, J. Gao, F. Wang, S. Wang, Y. Fang, ... & H. Liu. (2021). A super-efficient and biosafe hemostatic cotton gauze with controlled balance of hydrophilicity/hydrophobicity and tissue adhesiveness.

[12] Y. Wu, F. Wang, & Y. Huang. (2018). Comparative evaluation of biological performance, biosecurity, and availability of cellulose-based absorbable hemostats. Clinical and Applied Thrombosis/Hemostasis. 24 (4): 566-574.

[13] H. Kaptan, & M. Ayaz. (2016). New Haemostatic Agent and Application Tool for Neurosurgery.

[14] Y. Wang, D. Xiao, H. Yu, Y. Zhong, L. Zhang, X. Sui, ... & Z. Mao. (2023). Composite hydrogel based oxidated sodium carboxymethyl cellulose and gelatin loaded carboxymethylated cotton fabric for hemostasis and infected wound treatment. International Journal of Biological Macromolecules. 224: 1382-1394.

[15] S. E. D. Al-Mofty, A. H. Karaly, W. A. Sarhan, & H. M. E. Azzazy. (2022). Multifunctional hemostatic PVA/chitosan sponges loaded with hydroxyapatite and ciprofloxacin. ACS omega. 7 (15): 13210-13220.

[16] F. Cheng, Y. Wu, H. Li, T. Yan, X. Wei, G. Wu, ... & Y. Huang. (2019). Biodegradable N, O-carboxymethyl chitosan/oxidized regenerated cellulose composite gauze as a barrier for preventing postoperative adhesion. Carbohydrate polymers. 207: 180-190.

[17] H. He, C. Sun, Y. Weng, H. Huang, P. Ni, Y. Fang, ... & H. Liu. (2022). Catechol modification of non-woven chitosan gauze for enhanced hemostatic efficacy. Carbohydrate Polymers. 286: 119319.

[18] A. A. Oun, & J. W. Rhim. (2020). Preparation of multifunctional carboxymethyl cellulose-based films incorporated with chitin nanocrystal and grapefruit seed extract. International journal of biological macromolecules. 152: 1038-1046.

[19] X. Xie, D. Li, Y. Chen, Y. Shen, F. Yu, W. Wang, ... & X. Mo. (2021). Conjugate electrospun 3D gelatin nanofiber sponge for rapid hemostasis. Advanced healthcare materials. 10 (20): 2100918.

[20] K. Zhang, X. Bai, Z. Yuan, X. Cao, X. Jiao, Y. Li, ... & X. Zhang. (2019). Layered nanofiber sponge with an improved capacity for promoting blood coagulation and wound healing. Biomaterials. 204: 70-79.

[21] X. Zhu, J. Wang, S. Wu, T. Liu, G. Lin, B. Shang, ... & J. Wang. (2022). Biological Application of Novel Biodegradable Cellulose Composite as a Hemostatic Material. Mediators of Inflammation.

[22] S. M. Sharaf, S. E. D. Al-Mofty, E. S. M. El-Sayed, A. Omar, A. S. Abo Dena, & I. M. El-Sherbiny. (2021). Deacetylated cellulose acetate nanofibrous dressing loaded with chitosan/propolis nanoparticles for the effective treatment of burn wounds. International Journal of Biological Macromolecules. 193: 2029-2037.

[23] P. Andal, S. Tamilselvy, & P. Indra Priyatharesini. (2018). Green synthesis of silver nanoparticles from carrot. Research Journal of Pharmacy and Technology. 11 (7): 2757-2760.

[24] K. Khabibi, D. Siswanta, & M. Mudasir. (2021). Preparation, Characterization, and In Vitro Hemocompatibility of Glutaraldehyde-Crosslinked Chitosan/Carboxymethylcellulose as Hemodialysis Membrane. Indonesian Journal of Chemistry. 21 (5): 1120-1131.

[25] M. Zhou, J. Liao, G. Li, Z. Yu, D. Xie, H. Zhou, ... & R. Zhang. (2022). Expandable carboxymethyl chitosan/cellulose nanofiber composite sponge for traumatic hemostasis. Carbohydrate Polymers. 294: 119805.

[26] V. C. Nguyen, V. B. Nguyen, and M. F. Hsieh. (2013). Curcumin-loaded chitosan/gelatin composite sponge for wound healing application. International Journal of Polymer Science.

[27] M. N. Sundaram, V. K. Kaliannagounder, V., K. Selvaprithiviraj, M. Suresh, R. Biswas, A. K. Vasudevan, ... & R. Jayakumar. (2018). Bioadhesive, hemostatic, and antibacterial in situ chitin–fibrin Nanocomposite gel for controlling bleeding and preventing infections at mediastinum. ACS Sustainable Chemistry & Engineering. 6 (6): 7826-7840.

[28] A. E. Akca, G. Akca, F. T. Topçu, E. Macit, L. Pikdöken, & I. Ş. Özgen. (2016). The comparative evaluation of the antimicrobial effect of propolis with chlorhexidine against oral pathogens: An in vitro study. BioMed research international.

[29] A. Oryan, E. Alemzadeh, & A. Moshiri. (2018). Potential role of propolis in wound healing: Biological properties and therapeutic activities. Biomedicine & pharmacotherapy. 98: 469-483.

[30] M. L. Vică, M. Glevitzky, D. M. Tit, T. Behl, R. C. Heghedűş-Mîndru, D. C. Zaha, ... & S. Bungău. (2021). The antimicrobial activity of honey and propolis extracts from the central region of Romania. Food Bioscience. 41: 101014.

[31] Y. Alan, E. Atalan, N. Erbil, O. Bakir, Z. Orman, & P. Kanik. (2014). Antimicrobial Activity of Honey and Propolis Collected In Muş and Bitlis Region. Muş Alparslan University Journal of Science. 2 (1): 221-229.

[32] J. Wang, & H. Wang. (2022). Tolerant chitosan/carboxymethyl cellulose@ calcium composite films on nylon fabric for high-flux water/oil separation. Carbohydrate Polymers. 294: 119832.

[33] B. Cai, T. Zhong, P. Chen, J. Fu, Y. Jin, Y. Liu, ... & L. Tan. (2018). Preparation, characterization and in vitro release study of drug-loaded sodium carboxy-methylcellulose/chitosan composite sponge. PLoS One. 13 (10): e0206275.

[34] F. J. Wang, F. S. Lu, M. Cui, & Z. Q. Shao. (2015). Biocompatible microcapsule of carboxymethyl cellulose/chitosan as drug carrier. Advanced Materials Research. 1118: 227-236.

[35] A. Dobaj Štiglic, R. Kargl, M. Beaumont, C. Strauss, D. Makuc, D. Egger, ... & T. Mohan. (2021). Influence of charge and heat on the mechanical properties of scaffolds from ionic complexation of chitosan and carboxymethyl cellulose. ACS Biomaterials Science & Engineering. 7 (8): 3618-3632.

[36] I. Ayouch, I. Kassem, Z. Kassab, I. Barrak, A. Barhoun, J. Jacquemin, ... & M. El Achaby. (2021). Crosslinked carboxymethyl cellulose-hydroxyethyl cellulose hydrogel films for adsorption of cadmium and methylene blue from aqueous solutions. Surfaces and Interfaces. 24: 101124.

[37] N. Sun, T. Wang, & X. Yan. (2017). Synthesis and investigation of a self-assembled hydrogel based on hydroxyethyl cellulose and its in vitro ibuprofen drug release characteristics. RSC advances. 7 (16): 9500-9511.

[38] C. da Silva, A. Prasniewski, M. A. Calegari, V. A. de Lima, & T. L. Oldoni. (2018). Determination of total phenolic compounds and antioxidant activity of ethanolic extracts of propolis using ATR–FT-IR spectroscopy and chemometrics. Food Analytical Methods. 11: 2013-2021.

[39] C. Castro, F. Mura, G. Valenzuela, C. Figueroa, R. Salinas, M. C. Zuñiga, ... & C. Delporte. (2014). Identification of phenolic compounds by HPLC-ESI-MS/MS and antioxidant activity from Chilean propolis. Food research international. 64: 873-879.

[40] E. K. Soltani, R. Cerezuela, N. Charef, S. Mezaache-Aichour, M. A. Esteban, & M. M. Zerroug. (2017). Algerian propolis extracts: Chemical composition, bactericidal activity and in vitro effects on gilthead seabream innate immune responses. Fish & shellfish immunology. 62: 57-67.

[41] J. Cao, L. Q. Peng, L. J. Du, Q. D. Zhang, & J. J. Xu. (2017). Ultrasound-assisted ionic liquid-based micellar extraction combined with microcrystalline cellulose as sorbent in dispersive microextraction for the determination of phenolic compounds in propolis. Analytica chimica acta. 963: 24-32.

[42] B. Wang, X. Yang, C. Qiao, Y. Li, T. Li, & C. Xu, (2018). Effects of chitosan quaternary ammonium salt on the physicochemical properties of sodium carboxymethyl cellulose-based films. Carbohydrate polymers. 184: 37-46.

[43] D. Hu, H. Wang, & L. Wang. (2016). Physical properties and antibacterial activity of quaternized chitosan/carboxymethyl cellulose blend films. LWT-Food Science and Technology. 65: 398-405.

[44] W. Lan, L. He, & Y. Liu. (2018). Preparation and properties of sodium carboxymethyl cellulose/sodium alginate/chitosan composite film. Coatings. 8 (8): 291.

[45] H. Khoshmohabat, S. Paydar, H. M. Kazemi, & B. Dalfardi. (2016). Overview of agents used for emergency hemostasis. Trauma monthly. 21 (1).

[46] H. Tang, H. Chen, B. Duan, A. Lu, & L. Zhang. (2014). Swelling behaviors of superabsorbent chitin/carboxymethylcellulose hydrogels. Journal of Materials Science. 49: 2235-2242.

[47] R. Jayakumar, M. Prabaharan, P. S. Kumar, S. V Nair, & H. J. B. A. Tamura. (2011). Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnology advances. 29 (3): 322-337.

[48] G. H. Kim, J. N. Im, T. H. Kim, G. D. Lee, J. H. Youk, & S. J. Doh. (2018). Preparation and characterization of calcium carboxymethyl cellulose/chitosan blend nonwovens for hemostatic agents. Textile Research Journal. 88 (16): 1902–1911.