Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2025 June; 58(2): 171–179

Original article

Pomegranate-peel-chitosan-gelatin composite: A hemostatic dental sponge with antibacterial enhancement

Lale Rozykulyyeva[1], Prihartini Widiyanti[2], Sirojuddin Kholil Muhammad[2], Suryani Dyah Astuti[2]
[1]Department of Mathematics and Natural Sciences, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Biomedical Engineering, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Effective post-tooth extraction bleeding management and the alleviation of patient discomfort hinge upon the careful choice and judicious application of suitable hemostatic agents.* ***Purpose:*** *In this study, we developed a biodegradable, porous hemostatic sponge composed of gelatin (GE) and chitosan (CS), enhanced by the incorporation of pomegranate peel extract (PE), which was designed for use in dental applications, with a focus on antibacterial properties and infection prevention.* ***Methods:*** *The sponge was synthesized using an environmentally friendly (green) foaming approach without a foaming agent and was fabricated by freeze-drying. The efficiency of the hemostatic sponge was evaluated using various tests, including structural analysis, mechanical strength, water absorption capacity, hydrophilicity, blood clotting time (BCT), in vitro antibacterial effectiveness, and biodegradability.* ***Results:*** *The calcium chloride–crosslinked CS-GE and PE-immersed (CS-GE-PE) sponges exhibited adequate tensile strengths, with CS-GE-PE at 0.776 ± 0.025 MPa. The CS-GE-PE sponge showed significant water absorption (927.1% ± 37.55%). Hydrophilicity was evident (contact angle: 45°) and decreased slightly with the addition of PE. The BCT was shorter for the CS-GE sponge (161 ± 9.644 s), and both sponges exhibited minimal hemolysis, indicating biocompatibility. The CS-GE-PE sponge exhibited slightly enhanced antibacterial properties.* ***Conclusion:*** *This study has successfully developed a composite sponge consisting of CS, GE, and PE that exhibits a balanced level of biodegradability, antibacterial and anti-inflammatory properties, and blood absorption properties that reduce clotting time. This innovative material has great potential for a wide range of clinical applications in dental procedures and wound care.*

Keywords: *antibacterial; dentistry; gelatin; hemostatic effect; medicine*
Article history: *Received 21 November 2023; Revised 18 April 2024; Accepted 3 May 2024; Online 20 March 2025*

Correspondence: Prihartini Widiyanti, Biomedical Engineering, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. Email: pwidiyanti@fst.unair.ac.id

INTRODUCTION

The prevention and treatment of tooth extraction bleeding (TEB) and the reduction of patient discomfort rely on selecting and applying suitable hemostatic agents.[1] Minimally invasive extraction techniques and the use of local hemostatic measures have proven effective in preventing bleeding.[2] Various hemostatic agents have been assessed for their effectiveness in post-TEB management. For example, clinical trials have demonstrated that a novel hemostatic agent known as Ankaferd Blood Stopper is highly effective in managing bleeding in patients with hemophilia.[3] Additionally, in patients undergoing oral anticoagulant therapy, the use of hemostatic agents, such as Collaplug®, has been shown to minimize postoperative bleeding and reduce pain following tooth extraction.[4]

In the domain of dental hemostats, sponge materials have emerged as superior alternatives to conventional dressings, such as surgical gauze. Dental sponges offer a range of advantages, including enhanced healing processes, eliminating the need for secondary surgeries to remove the sponge, and time-saving benefits due to their high hemostatic capacity. Moreover, their use has been associated with a reduction in postoperative complications related to tooth extraction, including pain, swelling, dry socket occurrence, and inflammation.[5–7] Our study focuses on addressing critical issues concerning the impact of hemostasis and hemostatic sponges on the healing process, particularly their anti-infective function. Excessive hemostasis without

DOI: 10.20473/j.djmkg.v58.i2.p171–179

anti-infective and antibacterial functions can negatively impact the healing process following tooth extraction and may lead to complications, such as dry sockets, hindering the healing process. Maintaining an appropriate balance between hemostasis and the body's immune response to infection is essential in patient management.[8,9]

The limitations of conventional hemostatic materials lie in their restricted hemostatic efficacy despite their admirable biosafety features. These materials frequently exhibit limited and insufficient hemostatic capabilities, failing to address the urgent need for effective and prompt bleeding control. Moreover, many conventional hemostatic sponges suffer from inadequate liquid absorption properties or rely on complex chemical synthesis techniques, hindering their practical application in clinical settings.[10–15] Taking all these factors into account, our research aims to develop a multifunctional hemostatic material that combines balanced biodegradability with antibacterial and anti-inflammatory properties. In this study, we formulated composite sponges of chitosan (CS), gelatin (GE), and pomegranate peel extract (PE) using a simple method that involved freeze-drying mixed solutions of CS and GE. To achieve a highly porous structure in the sponges, we utilized an environmentally friendly, "green" self-foaming approach, entailing the vigorous agitation of high-viscosity solutions containing amphiphilic GE, thereby eliminating the need for additional foaming agents. This technique not only facilitated foam formation but also contributed to the creation of a markedly porous architecture within the CS-GE-PE-based sponges. The incorporation of GE into the composite material serves multiple functions. GE is well-known for its hemostatic properties, which are crucial for the clotting process.[8] Moreover, it exhibits biocompatibility, making it suitable for medical applications and ensuring tolerability within the body.[5,16,17] Additionally, GE can entrap platelets and red blood cells (RBCs) within its matrix, thereby enhancing the hemostatic process.[5]

One promising alternative is the use of CS, a natural material known for its balanced hemostatic, antibacterial/anti-inflammatory properties.[8] The composite dental sponge reinforced with PE possesses naturally high antibacterial properties due to its phenolic content. By incorporating PE, we aimed to combat infections effectively and promote a healthier healing environment after tooth extraction, thereby minimizing complications, such as dry sockets. The antibacterial activity of PE against a range of bacteria, including *Bacillus subtilis, Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Enterobacter aerogenes,* and *Salmonella typhimurium*, has been investigated in dentistry.[18] The antibacterial and anti-inflammatory properties of this extract are attributed to its high phenolic content, including ellagic acid and gallic acid.[19] The dental sponges were dried using the freeze-drying method, and their functional groups were analyzed using Fourier transform infrared (FTIR) spectroscopy. Hemostatic performance was assessed through blood clotting time (BCT) in various tests, including fluid absorption capacity, mechanical strength, and in vitro antibacterial effectiveness. In this study, we developed a biodegradable, porous hemostatic sponge composed of GE and CS, enhanced by the incorporation of PE, which was explicitly designed for use in dental applications, with a focus on antibacterial properties and infection prevention.

MATERIALS AND METHODS

CS (source: shrimp shells; degree of deacetylation: 75%; Himedia, India), calcium chloride ($CaCl_2$), acetic acid, ethanol 70% and 96%, GE (bovine skin) (Sigma Aldrich, USA), glycerol extra pure, NaOH, deionized (DI) water was purchased from CV Chemical Indonesia (Surabaya) and UD Scientific Source, and the medical gauze (as negative control) and GE-based commercial dental sponge (as positive control) were purchased at Kimia Farma Surabaya pharmacy.

The preparation procedures were conducted following the methodology outlined by Sun et al.[8] Initially, CS 3% underwent dissolution in a solvent comprising 0.5% acetic acid and distilled water at room temperature. This process, facilitated by magnetic stirring, aimed to create a homogeneous CS solution. Concurrently, GE was dissolved in distilled water under controlled conditions at 37°C, employing magnetic stirring to achieve a consistent GE solution. Following this, the CS and GE solutions were blended at a 1:1 mass ratio under low-speed mechanical stirring for 15 minutes. Upon achieving a homogenous CS-GE mixture, further enhancements were introduced to optimize the sponge's properties. The 2% solution of $CaCl_2$ was carefully added. As highlighted in recent studies, ionic crosslinkers, such as $CaCl_2$, offer superior biocompatibility compared to chemical alternatives, prompting the addition of $CaCl_2$ as an ionic crosslinker.[20,21] Simultaneously, glycerol 1% was added to the composite and incorporated to further optimize the sponge's mechanical properties, serving as a plasticizer to enhance flexibility and structural integrity.

The procedure of preparing aqueous PE followed the methodology described by Rozykulyyeva et al.[22] Following the extract preparation process, a solution of PE was prepared by dissolving the extract in distilled water, achieving a neutral pH of 7 through careful stirring for 15 minutes. Subsequently, the PE solution was carefully incorporated into the composite solution, with a precise addition of 3% aqueous extract introduced before the foaming process.

Subsequently, the composite solution underwent an intense foaming process, where vigorous agitation at 1,000 rpm using an electric mixer equipped with an egg beater induced the formation of a foamy consistency. This step, crucial for achieving the desired porous structure, laid the groundwork for subsequent freeze-drying. The samples were labeled accordingly, distinguishing between those containing the extract (CS-GE-PE) and those without (CS-

DOI: 10.20473/j.djmkg.v58.i2.p171–179

GE). Following the foaming process, the resultant foamy composite solution was carefully transferred into an acrylic petri dish and immediately subjected to rapid freezing at −80°C. The solidified composite was transferred to a freeze dryer and subjected to the lyophilization process for 24 hours at −55°C. The prepared samples were stored in sealed bags, facilitating subsequent characterization and analysis.

The identification of functional groups in GE, CS, glycerol, $CaCl_2$, PE samples, and the final product (dental sponge) was carried out via attenuated total reflection FTIR spectroscopy in the range of 1,200–800 cm^{-1}, using a model spectrophotometer FTIR (Percin Elmer Spectrum One, USA) and potassium bromide powder as reference materials.

The tensile strength characterization for assessing the tensile strength provides valuable insights into the structural properties of the prepared sponge in comparison to commercial alternatives, as highlighted in previous research.[23] Moreover, tensile strength testing serves the purpose of determining the maximum tension the sponge can withstand before breaking, offering practical implications for surgical procedures.[24] Tensile strength test observations of the sponge composite were carried out, followed by the analysis of its tensile strength using a universal testing machine adhering to the ASTM D882–91 standard method, with a 100 kg load cell and a speed of 10 mm/min. Each sample was measured securely and held in place using clamps at both ends. The reported results represent the average of three replicates.[25]

The water absorption capacities of the composite sponge were measured in DI water. Each sponge sample (approximately 1 × 1 cm^2 in size) was weighed after soaking in 20 mL of DI water. The solution was soaked for 60 min, removed from DI water, and filtered to remove excess water from the surface. The increase in wet sample weight, which represents the amount of water absorbed by the sponge, was calculated. The wet weight of the samples (Wt) was measured immediately. Water absorption capacity (Q) is calculated using the following equation:

$$Q = (Wt - Wo) / Wo \times 100\ (\%),$$

where Wt is the wet weight of the sponge after swelling and Wo is the initial weight of the sponge.[26] The water contact angle test assesses material surface hydrophilicity via drop analysis, deriving a wettability indicator value from the angle formed by the liquid droplet on the surface. Wettability, indicative of intermolecular interactions, is observed when a liquid spreads along a solid surface, with variations attributed to different liquid–solid interactions.[27,28] The water contact angle (θ) was measured at room temperature; at each measurement, a drop of distilled water was placed on the surface of the sponge, and then an image was taken.[29]

An antibacterial assay of CS-GE and CS-GE-PE sponge composites using negative and positive controls was carried out using the disc diffusion method, with *E. coli* and *S. aureus* as bacterial strains and gentamicin as positive control. First, each sponge composite was placed in a centrifuge tube sterilized by ultraviolet (UV) light irradiation. Each piece of the paper disc was dipped in a sponge sample and placed in the middle of a culture dish. After incubation for 24 hours at 37°C, the diameter of the clear circle zone was measured and recorded for analysis.

During the BCT experimental setup, sponge samples were prepared in separate tubes, each containing a sponge of dimensions 10 × 10 × 10 mm. Next, 1 mL of fresh blood was placed in the tube and placed in an incubator at 37°C. A positive control tube containing medical gauze and a commercial sponge was prepared in parallel. A chronometer was used to carefully document the clotting time of the tubes. The vial was rotated for 1 minute and mounted vertically on a laboratory bench. The bottle was inverted every minute until the sponge sample completely stopped flowing, and the time was recorded. All experimental and control groups were run in triplicates (n = 3).[30,31] Visual inspection of whole blood adhesion properties in sponge samples were carried out with 200 µL of whole blood from healthy sheep mixed with a 3.8% sodium citrate solution. Next, the prepared blood mixture was carefully dripped onto each sponge sample; the size of each sponge was 1–1.5 cm.[32] The samples were immersed in a phosphate-buffered saline (PBS) solution, shaken, and then photographed to capture the resulting outcomes.

The hemolysis ratio of the samples was tested in vitro at various concentrations. After the sample was immersed in the saline solution and incubated at 37°C, 200 µL of the fresh whole blood was added to the sample suspension (1 mL) and incubated for 1 hour at 37°C. The mixture was then centrifuged at 2,000 rpm for 10 minutes. The absorbance of the supernatant was measured at a 545 nm wavelength using a UV-visible spectrophotometer. Normal salt solution and DI water were designated as the negative and positive controls, respectively. After repeating the same experiment three times, the following equation was used to calculate the hemolysis rate (HR%):

$$HR\% = ODs - ODn / ODp - ODn \times 100,$$

where ODn, ODp, and ODs are abbreviations for the absorption lines of normal salt mixed with distilled water samples, mixed with samples, and samples, respectively.[8]

In the degradation test, each sample was cut into 10 × 10 mm squares. The initial weight of each sample (W_0) was then recorded. The prepared samples were placed into 50-mL beakers, whereupon 25 mL of a PBS solution with a pH of 7.4 was added. Subsequently, the sponges underwent degradation in a PBS solution at a constant temperature of 37°C for various durations. Following immersion for 1, 3, 5, 7, and 12 days, the samples were individually retrieved from the solution; the samples were then weighed after removing excess surface water using a filter paper, as per Kayabolen et al.[33] To ensure consistent samples throughout the degradation process, the PBS solution was renewed to maintain experimental integrity, and a standard pH meter calibrated at 37°C was employed to determine the pH of the samples. Finally, the weight of the dried samples (Ww)

DOI: 10.20473/j.djmkg.v58.i2.p171–179

and the percentage of material degradation were calculated using the following formula:[34]

$$\text{Mass ratio } (\%) = W_f / W_o \times 100.$$

The experiments were conducted in triplicate for statistical robustness, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, CA). The significance of differences among groups was determined using analysis of variance, followed by a student's *t*-test, and $P < 0.05$ was considered statistically significant.

RESULTS

FTIR testing aims to determine the chemical groups in dental sponges and the materials used. After testing the dental sponge and its composition materials, the following results were obtained (Figure 1). The infrared FTIR spectra characterization confirming cross-linking with $CaCl_2$ in the CS-GE sponge and CS-GE-PE is presented in Figure 1A. Specifically, a broad band observed at 3337 cm^{-1} in the sponge spectrum indicated NH stretching, OH stretching, and intermolecular hydrogen bonding. Additionally, a slight shift in the Amide I peak from 1,636 to 1,640 cm^{-1} suggested an interaction between CS-GE and PE, which is indicative of bond formation. Moreover, PE exhibited a peak at 989 cm^{-1}, positively correlating with its antioxidant properties, identified as a CO-stretching functional group of alcohol and phenol. The FTIR spectra showed that the sponges crosslinked with CaCl2 displayed a significant rightward shift at 1,613 cm^{-1}.

The dental sponges manufactured within this investigation demonstrated markedly elevated tensile strength under dry conditions (Figure 2A). Specifically, the values recorded were 0.776 ± 0.025 MPa for CS-GE and 0.722 ± 0.009 MPa for CS-GE-PE. In contrast, the medical gauze demonstrated the highest tensile strength among the materials assessed due to several factors, with a recorded value of 9.913 ± 11.660 MPa. Conversely, the commercial sponge exhibited a comparatively lower tensile strength of 0.418 ± 0.052 MPa.

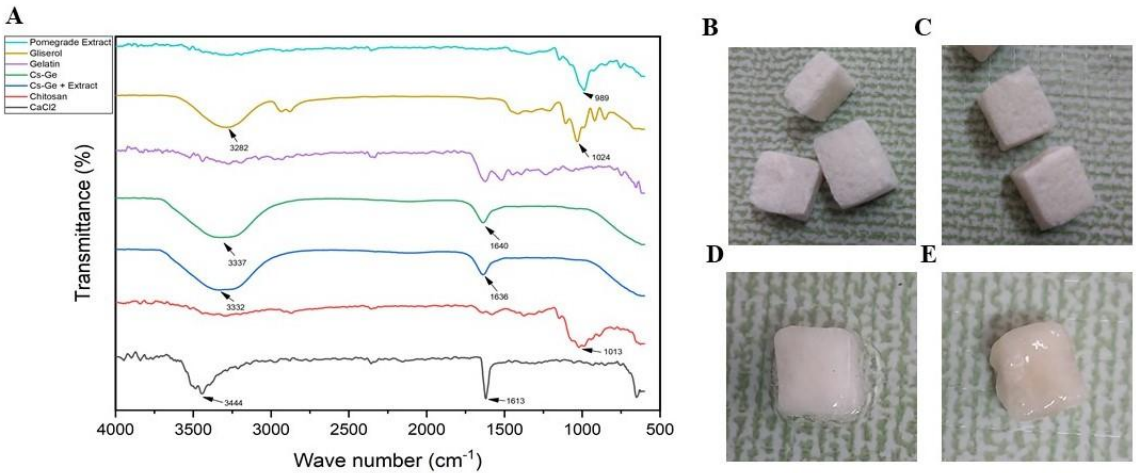

Figure 1. (A) FTIR spectra results of CS-GE, CS-GE-PE, CS, GE, and PE. Images before (B and C) and after (D and E) water absorption of the CS-GE (B and D) and CS-GE-PE (C and E) sponges.

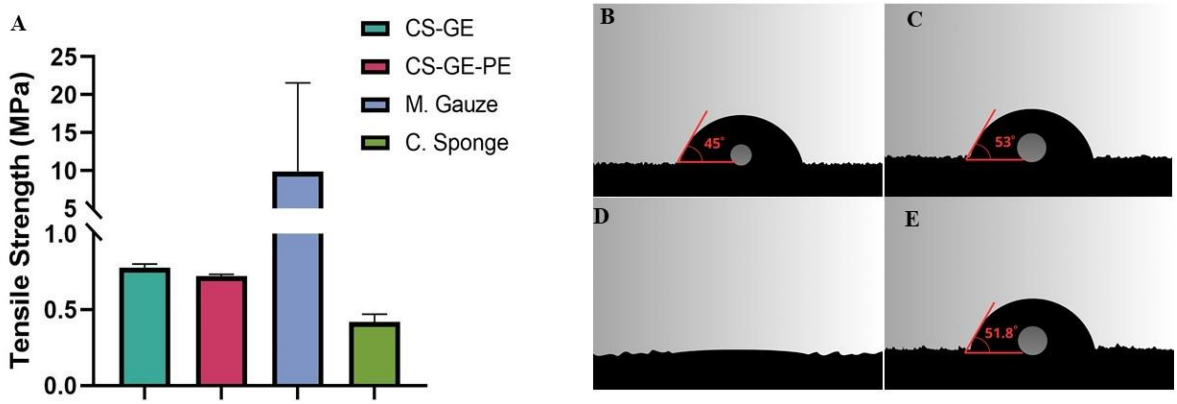

Figure 2. (A) Mechanical characterization of hemostatic sponges. Tensile strength of CS-GE, CS-GE-PE, medical gauze, and commercial sponge. (B–E) The water contact angle test of the samples. Photographs of water droplets on (B) CS-PE, (C) CS-GE-PE, (D) medical gauze, and (E) commercial sponge. The mean values ± SD for n = 3 are shown.

DOI: 10.20473/j.djmkg.v58.i2.p171–179

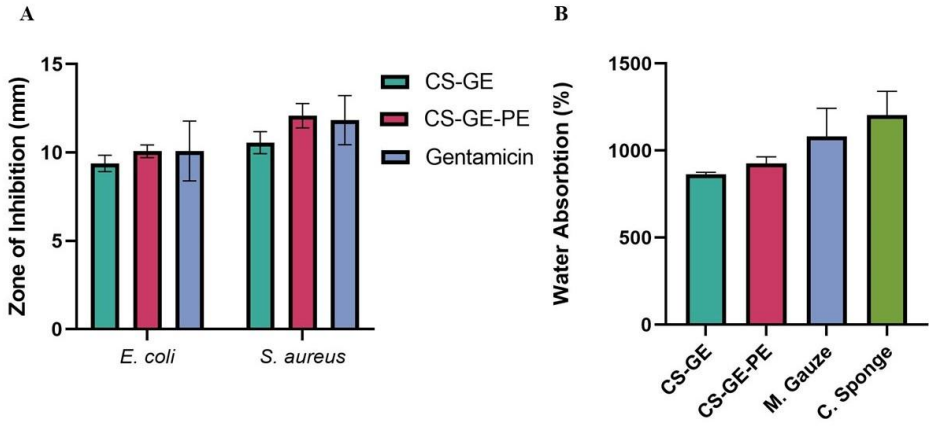

Figure 3. (A) The antibacterial activity was assessed via the disc diffusion assay, with the inhibition zone diameter measured for CS-GE, CS-GE-PE sponges, and gentamicin, employed as the control. (B) Water absorption test results for CS-GE, CS-GE-PE, medical gauze, and commercial sponge samples. Error bars, mean ± SD for n = 3.

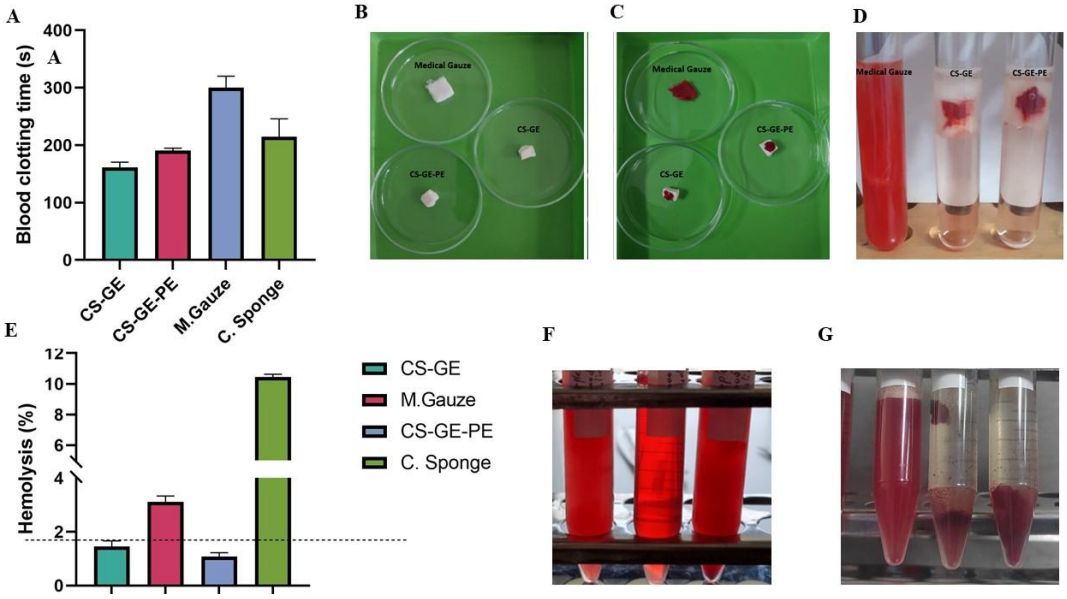

Figure 4. (A–D) Hemolytic effect of hemostatic materials. (A) Result of BCT test. (B) Photographs of the appearance of the sponges before the adhesion properties treatment. (C) After blood absorption. (D) RBCs' adherence to the surface of the materials. (E) In vitro hemolysis assay results. (F) Samples incubated at 37°C for 1 hour were centrifuged. (G) The hemolysis ratios were determined (n = 3). Error bars, mean ± SD.

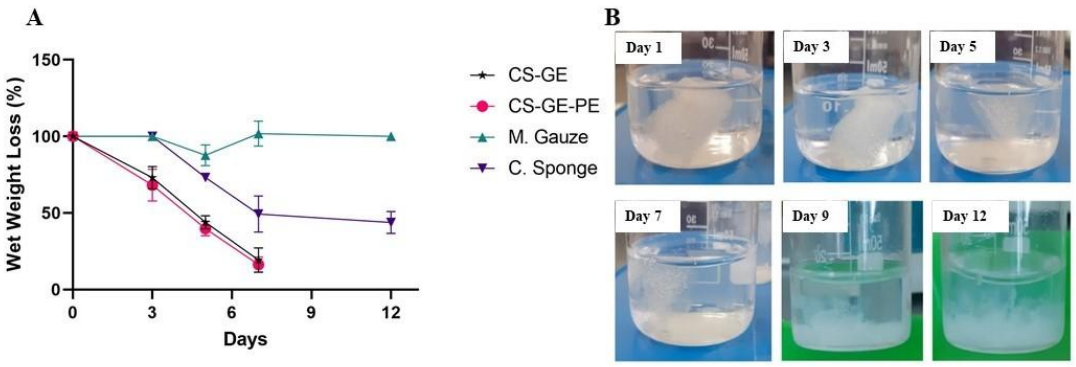

Figure 5. Degradation performance. (A) Degradation in PBS. Error bars, mean ± SD. (B) Images of the sponges corresponding to degradation into PBS after 1, 3, 5, 7, 9, and 12 days.

DOI: 10.20473/j.djmkg.v58.i2.p171–179

The evaluation of fluid absorption capacity in CS-GE and CS-GE-PE samples serves as a crucial parameter in assessing their hemostatic potential. The CS-GE demonstrated a water absorption capacity of 863.9% ± 12.28%, whereas the CS-GE-PE variant demonstrated a higher capacity of 927.1% ± 37.55%, as depicted in Figure 3B. In contrast, the gauze and commercial sponge displayed absorption capacities of 1,080% ± 163.7% and 1,204% ± 136.8%, respectively. This variance observed in fluid absorption capacity—when compared to the control groups—can be attributed to structural variances within the CS, GE, $CaCl_2$, and pomegranate extract solution chains. Despite this, the nonsignificant difference in fluid absorption capacity between CS-GE and CS-GE-PE suggests that both possess relatively similar characteristics.

In our study, contact angles of 45° and 53° were observed for the CS-GE and CS-GE-PE samples, respectively, whereas the gauze and commercial sponge samples displayed angles of 0° and 51.8°, respectively (Figure 2B-E). Notably, in the medical gauze, upon contact, water droplets swiftly spread and infiltrated the surface due to its inherent hydrophilicity and capillary structure, as depicted in Figure 2D. These results highlight the high hydrophilicity of medical gauze, attributed to its loose open weave structure, which facilitates rapid liquid absorption.

The antibacterial effectiveness of the hemostatic samples was evaluated against *E. coli* and *S. aureus* through the disc diffusion assay. CS-GE-PE exhibited antimicrobial activity, with inhibition zones of 10.070 ± 0.364 mm for *E. coli* and 12.082 ± 0.691 mm for *S. aureus*, whereas CS-GE demonstrated inhibition zones of 9.381 ± 0.464 mm for *E. coli* and 10.556 ± 0.622 mm for *S. aureus*. Gentamicin, used as a standard antibiotic, exhibited inhibition zones of 10.083 ± 1.695 mm against *E. coli* and 11.830 ± 1.383 mm against *S. aureus*. These findings suggest that CS-GE-PE may possess enhanced antimicrobial properties compared to CS-GE, demonstrating the efficacy of gentamicin against the tested bacterial strains.

The clotting efficacy of the sponges was assessed by the clotting time. The blood-clotting properties of the samples—as corroborated by the clotting time analysis—are presented in Figure 4A. Notably, the CS-GE sponge exhibited the shortest clotting time at 161 ± 9.644 seconds, surpassing that of the control medical gauze and commercial sponge at 300 ± 20 and 214.3 ± 31.56 seconds, as well as the CS-GE-PE group at 190.7 ± 4.04 seconds. The next experiment confirmed the superior blood adhesion properties of GE–CS and CS-GE-PE compared to gauze, and the sponges appeared darker (Figure 4B-C) owing to increased RBC adhesion. The presence of hemoglobin outside the materials (Figure 4D) post-immersion in PBS and shaking exhibited a redder color for gauze, indicative of elevated hemoglobin concentration that reflects the extent of RBC adhesion. Lower hemoglobin concentrations outside the GE-CS and CS-GE-PE sponges signified higher RBC adhesion.

In order to comprehensively explore the hemolytic effects, we conducted a meticulous assessment of the rate of RBC lysis upon sponge exposure. The study evaluated the hemolysis rates for the resulting sponge, positive control (DI water), and negative control (normal saline). Hemolysis tests were conducted on CS-GE, CS-GE-PE, gauze, and commercial sponge samples to ascertain their blood compatibility. CS-GE demonstrated a notably low hemolysis rate of approximately 1.453% ± 0.207%, as depicted in Figure 4E-G. In contrast, the hemolysis rate for CS-GE-PE decreased to 1.087% ± 0.134%, while those of gauze and commercial sponges were 3.114 ± 0.220% and 10.46% ± 0.174%, respectively.

In the degradation test conducted in PBS, illustrated in Figure 5, significant observations emerged. Over time, both the size and weight of the remaining fragments in the sponge samples notably decreased, indicating a degradation process. Initially, the sponge retained its cubic form and transformed into a transparent cubic gel on the first day. From days 3 to 5, partial degradation of the sponge's structure occurred while maintaining transparency. On the day 5, the CS-GE sponge exhibited a degradation rate of 43.897% ± 4.256%, and the CS-GE-PE sponge showed a rate of 39.788% ± 4.596%. However, by the day 7, the CS-GE sponge exhibited a degradation rate of 19.359% ± 7.912%, and the CS-GE-PE sponge demonstrated a rate of 16.310% ± 4.954%, with both beginning to break into small pieces or disintegrate, precluding accurate measurement beyond this point. Visual monitoring of the degradation process revealed the transformation of the CS-GE and CS-GE-PE sponges into small pulps, with approximately 90% of the sponge degraded by the day 10. Finally, on the day 12, the sponge material degraded. Notably, the commercial sponge exhibited a degradation rate of 43.818% ± 7.142% within 12 days, whereas the gauze did not show any degradation. This variation underscores the influence of different materials on the degradation process in the prescribed PBS environment.

DISCUSSION

This research effort aimed to develop degradable sponges with both hemostatic and antibacterial properties using self-foaming and freeze-drying techniques.. First, CS and GE were mixed to create a foam solution, as described by Sun et al.[8] The self-foaming process was performed without the use of additional agents, and the resulting solution was lyophilized to produce exceptionally lightweight and highly porous sponges.[8] The strength of dental sponges must conform to the proposed biomedical standards. By utilizing glycerol as a plasticizer and carefully selecting the concentration of $CaCl_2$ as a crosslinker, we aimed to achieve an optimal balance between mechanical strength and flexibility in the GE sponge while ensuring its biocompatibility. Additionally, factors such as elongation at break, influenced by filler content, softening agent, and the

DOI: 10.20473/j.djmkg.v58.i2.p171–179

presence of air cavities, further underscore the complexity of mechanical property optimization.[20,21]

This strategic approach allows for the customization of the sponge's mechanical properties to meet specific surgical requirements. Medical gauze exhibits higher tensile strength than sponges because it is made of materials such as cotton fibers, which inherently possess strong mechanical properties. These materials are engineered to withstand tension and provide support, making them suitable for applications requiring high tensile strength.[35] Contact angle measurement presents a straightforward and accessible technique for assessing the wettability of a solid–liquid interface. A contact angle value below 90° denotes hydrophilicity.[36] The CS-GE dental sponges exhibited the highest tensile strength, measuring 0.776 MPa. However, previous research on CS-based materials reported a range of tensile strength values, typically between 0.07 and 0.11 MPa. Specifically, studies involving GELITA-SPON and GE sponges have documented values of 0.080 and 0.093 MPa.

The FTIR observations to those reported elucidated interesting physicochemical alterations in the sponges crosslinked with $CaCl_2$, affecting the polymer chains. As substantiated by earlier investigations, the crosslinking of polymer chains imparts insolubility to materials in aqueous environments.[7,37–39] We underscore the significance of this discovery, as such crosslinking plays a pivotal role in modulating the balance of the degradation behavior of the sponges.

The study demonstrated that the sponges created exhibited desirable biomechanical characteristics, making them suitable for a range of medical applications, such as dental procedures and wound care. The observed high tensile strength values indicate their robustness and resilience under challenging conditions.[40] Moreover, the incorporation of glycerol as a plasticizer enhanced its mechanical strength without eliciting cytotoxic effects, while carefully selecting the appropriate concentration of $CaCl_2$ as a crosslinker augmented the strength of the CS-GE sponge while maintaining its biocompatibility.[20,21,40]

These findings underscore the significant absorbency and swelling capacity of the sponge, which are crucial attributes for its efficacy in hemostatic applications.[41] According to the results of our experiment, the sponge proved capable of absorbing up to −9 (927%) times its weight. Previous research reported the absorbency of materials such as CS sponges, which can absorb up to 10 times their weight,[42] and GE-based hemostatic agents that can swell up to 35 (3500%) times their weight.[23] Direct comparisons with existing literature on dental sponges are limited. However, our study focuses specifically on dental surgical sponges, which possess a balanced absorbency capacity to be useful in clinical contexts. Specifically, crosslinking mechanisms play a significant role in determining the liquid-absorption capacities and internal porous structure of sponges.[8] Incorporating PE into the CS-GE matrix did not have a detrimental effect on the crosslinking state of the material. However, it may have influenced the physical crosslinking of the CS and GE chains, resulting in a slight enhancement of its liquid absorption capacity compared to CS-GE.

The wettability of gauze used in daily dental applications significantly influences its capacity to absorb blood fluids, adsorption of proteins, and adhesion of blood cells. Previous studies indicated that materials exhibiting water contact angles ranging from 40° to 70° facilitate cellular adhesion.[43] Considering the incorporation of GE as a hydrophilic polymer with a porous structure, the samples exhibited contact angles within the hydrophilic range. While the hydrophilic and porous nature of materials is advantageous in various biological contexts, it also raises concerns regarding bacterial proliferation.

Ordinarily, the wounded areas may become infected with a substantial quantity of bacteria. When bacterial infections occur at injury sites, they result in inflammation, which delays the healing process and presents a substantial risk to human health. Therefore, hemostatic materials that effectively reduce pathogen counts, alleviate inflammation, and expedite wound healing processes are crucial for practical applications.[36] The results showed antibacterial efficacy; sponge samples were evaluated against common Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria using the disc inhibition zone method. The antibacterial properties of sponges are influenced by the antibacterial capability of CS, with a mechanism involving electrostatic interactions between cationic CS chains and negatively charged cytoplasmic membranes, subsequently inhibiting membrane transport.[44,45] In particular, a slightly enhanced antibacterial effect was observed following the incorporation of PE.[18,22] This is due to the ability of free phenols to enter bacterial cells and disrupt their metabolic processes, whereas immobilized PE may be ineffective in inhibiting bacterial growth.

Bleeding clotting time serves as an indicator of the activation of the intrinsic pathway within the coagulation cascade and provides insight into the effectiveness of procoagulation agents in reducing clotting time.[42] The formation of aggregated erythrocytes and platelets at the bleeding site plays a crucial role in initiating coagulation and is essential for controlling bleeding. CS promotes platelet aggregation, with a positively charged CS surface being more conducive to hemostasis.[46] GE, which swells upon blood absorption, creates a soft "pseudo-clot" barrier that obstructs blood flow. This barrier subsequently serves as a substrate for the formation of dense fiber clots, expediting the blood clotting process.[47] These results indicate that the incorporation of GE effectively mitigated the presence of several anionic clotting factors associated with the CS component. This interaction is believed to be the primary mechanism underlying the enhanced hemostatic properties observed in CS-GE compared with commercial dental sponges.

Hemocompatibility is desired for hemostatic dental sponges, which may come in direct contact with blood flow.[48] A hemolysis test was conducted to assess the

DOI: 10.20473/j.djmkg.v58.i2.p171–179

hemolysis ratios of CS-GE and CE-GE-PE. This evaluation was conducted following the guidelines outlined in standard ISO 10993-4, wherein a hemolysis rate below 2% indicates substance biocompatibility.[23] As a natural material, GE and CS have demonstrated excellent biocompatibility in numerous biomedical applications.[5] Our data indicated that the CS-GE-PE sponges exhibited good hemocompatibility compared to commercial hemostatic materials and gauze.

Incorporating rapidly biodegradable hemostatic agents can mitigate secondary tissue damage, alleviate local stress, and obviate the need for the subsequent removal of non-degradable materials. This approach holds promise for reducing recovery times and enhancing the quality of patient care.[23] The degradation profile of the developed sponge was assessed over 2 weeks. The findings from our degradation testing highlighted the efficacy of the developed sponge, demonstrating a degradation rate of 12 days compared to the 4 to 6 weeks typically required by commercial alternatives, such as SPONGOSTAN™. The biodegradability of the developed dental sponge contributes to the advancement of hemostatic materials, providing insights into its biodegradation and potential clinical applications. The outcomes of the short-term degradation testing conducted over 2 weeks, as well as the absence of in vivo biocompatibility evaluations, indicated the necessity for extended assessments and animal studies to substantiate the clinical applicability of the sponge.

In conclusion, this study successfully developed a multifunctional hemostatic material—a CS-GE-PE composite sponge—with balanced biodegradability and antibacterial and anti-inflammatory properties. Utilizing environmentally friendly techniques, including self-foaming and freeze-drying, we created highly porous structures conducive to hemostasis. The addition of CS enhanced hemostatic properties, while PE bolstered antibacterial efficacy. These findings highlight the potential of CS-GE-PE sponges for dental procedures and wound care, offering versatile clinical applications. Future research should focus on optimizing performance, assessing long-term biocompatibility, and scaling up production for widespread clinical use. Therefore, future research should focus on optimizing sponge properties, conducting in vivo trials, and examining its efficacy in diverse clinical settings.

REFERENCES

1. Hanafiah OA, Hanafiah DS, Syaflida R. The effect of 3% binahong leaf extract gel on the wound healing process of post tooth extraction. Dent J. 2021; 54(2): 57–62.
2. Caliskan M, Tukel H, Benlidayi E, Deniz A. Is it necessary to alter anticoagulation therapy for tooth extraction in patients taking direct oral anticoagulants? Med Oral Patol Oral y Cir Bucal. 2017; 22(6): e767–73.
3. Kazancıoğlu HO, Çakır O, Ak G, Zülfikar B. The effectiveness of a new hemostatic agent (ankaferd blood stopper) for the control of bleeding following tooth extraction in hemophilia: A controlled clinical trial. Turkish J Hematol. 2013; 30(1): 19–24.
4. Abdelaziz M, Shaaban R, Abdelhalim S, Sadaka M. Effect of Collaplug® on the healing of extraction sockets in patients under oral anticoagulant therapy (clinical study). Alexandria Dent J. 2015; 40(2): 166–72.
5. Irfan NI, Mohd Zubir AZ, Suwandi A, Haris MS, Jaswir I, Lestari W. Gelatin-based hemostatic agents for medical and dental application at a glance: A narrative literature review. Saudi Dent J. 2022; 34(8): 699–707.
6. Arteagoitia M, Barbier L, Santamaria J, Santamaria G, Ramos E. Efficacy of amoxicillin and amoxicillin/clavulanic acid in the prevention of infection and dry socket after third molar extraction. A systematic review and meta-analysis. Med Oral Patol Oral y Cir Bucal. 2016; 21(4): e494-504.
7. Shakiba-Marani R, Ehtesabi H. A flexible and hemostatic chitosan, polyvinyl alcohol, carbon dot nanocomposite sponge for wound dressing application. Int J Biol Macromol. 2023; 224: 831–9.
8. Sun Y, Miao T, Wang Y, Wang X, Lin J, Zhao N, Hu Y, Xu F-J. A natural polyphenol-functionalized chitosan/gelatin sponge for accelerating hemostasis and infected wound healing. Biomater Sci. 2023; 11(7): 2405–18.
9. Assinger A, Schrottmaier WC, Salzmann M, Rayes J. Platelets in sepsis: an update on experimental models and clinical data. Front Immunol. 2019; 10: 1687.
10. Liu R, Dai L, Si C, Zeng Z. Antibacterial and hemostatic hydrogel via nanocomposite from cellulose nanofibers. Carbohydr Polym. 2018; 195: 63–70.
11. Zhang S, Li J, Chen S, Zhang X, Ma J, He J. Oxidized cellulose-based hemostatic materials. Carbohydr Polym. 2020; 230: 115585.
12. Yang X, Liu W, Li N, Wang M, Liang B, Ullah I, Luis Neve A, Feng Y, Chen H, Shi C. Design and development of polysaccharide hemostatic materials and their hemostatic mechanism. Biomater Sci. 2017; 5(12): 2357–68.
13. Liu J-Y, Hu Y, Li L, Wang C, Wang J, Li Y, Chen D, Ding X, Shen C, Xu F-J. Biomass-derived multilayer-structured microparticles for accelerated hemostasis and bone repair. Adv Sci. 2020; 7(22): 2002243.
14. Liang Y, Li Z, Huang Y, Yu R, Guo B. Dual-dynamic-bond cross-linked antibacterial adhesive hydrogel sealants with on-demand removability for post-wound-closure and infected wound healing. ACS Nano. 2021; 15(4): 7078–93.
15. Xu Z, Tian W, Wen C, Ji X, Diao H, Hou Y, Fan J, Liu Z, Ji T, Sun F, Wu D, Zhang J. Cellulose-based cryogel microspheres with nanoporous and controllable wrinkled morphologies for rapid hemostasis. Nano Lett. 2022; 22(15): 6350–8.
16. Takagi T, Tsujimoto H, Torii H, Ozamoto Y, Hagiwara A. Two-layer sheet of gelatin: A new topical hemostatic agent. Asian J Surg. 2018; 41(2): 124–30.
17. Herliana H, Yusuf HY, Laviana A, Wandawa G, Cahyanto A. Characterization and analysis of chitosan-gelatin composite-based biomaterial effectivity as local hemostatic agent: A systematic review. Polymers (Basel). 2023; 15(3): 575.
18. Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. J Food Sci Technol. 2014; 51(12): 4132–7.
19. Ferrazzano GF, Scioscia E, Sateriale D, Pastore G, Colicchio R, Pagliuca C, Cantile T, Alcidi B, Coda M, Ingenito A, Scaglione E, Cicatiello AG, Volpe MG, Di Stasio M, Salvatore P, Pagliarulo C. In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic bacteria. Biomed Res Int. 2017; 2017: 1–7.
20. Amiruddin, Muh. Agus Syamsur Rijal, Dewi Melani Hariyadi. Effect of CaCl2 crosslinker concentration on the characteristics, release and stability of ciprofloxacin hcl-alginate-carrageenan microspheres. J Farm Ilmu Kefarmasian Indones. 2023; 10(3): 312–23.
21. Doustdar F, Olad A, Ghorbani M. Effect of glutaraldehyde and calcium chloride as different crosslinking agents on the characteristics of chitosan/cellulose nanocrystals scaffold. Int J Biol Macromol. 2022; 208: 912–24.
22. Rozykulyyeva L, Astuti SD, Zaidan AH, Pradhana AAS, Puspita PS. Antibacterial activities of green synthesized silver nanoparticles from Punica granatum peel extract. In: AIP Conference Proceedings. 2020. p. 060012.

DOI: 10.20473/j.djmkg.v58.i2.p171–179

23. Sharifi S, Maleki Dizaj S, Ahmadian E, Karimpour A, Maleki A, Memar MY, Ghavimi MA, Dalir Abdolahinia E, Goh KW. A biodegradable flexible micro/nano-structured porous hemostatic dental sponge. Nanomaterials. 2022; 12(19): 3436.
24. Nasruddin, Susanto T. Composites of natural rubber, carbon black, and kaolin sodium bicarbonate content for sponge application. IOP Conf Ser Mater Sci Eng. 2019; 509(1): 012094.
25. Pan S, Li Y, Tong X, Chen L, Wang L, Li T, Zhang Q. Strongly-adhesive easily-detachable carboxymethyl cellulose aerogel for noncompressible hemorrhage control. Carbohydr Polym. 2023; 301: 120324.
26. Cai B, Zhong T, Chen P, Fu J, Jin Y, Liu Y, Huang R, Tan L. Preparation, characterization and in vitro release study of drug-loaded sodium carboxy-methylcellulose/chitosan composite sponge. Mishra YK, editor. PLoS One. 2018; 13(10): e0206275.
27. Jahan N, Ibne Mahbub MS, Lee B-T, Bae SH. In Vivo and In Vitro Investigation of a Novel Gelatin/Sodium Polyacrylate Composite Hemostatic Sponge for Topical Bleeding. J Funct Biomater. 2023; 14(5): 265.
28. Wang J, Chen Y, Xu Q, Cai M, Shi Q, Gao J. Highly efficient reusable superhydrophobic sponge prepared by a facile, simple and cost effective biomimetic bonding method for oil absorption. Sci Rep. 2021; 11(1): 11960.
29. Singh S, Nwabor OF, Sukri DM, Wunnoo S, Dumjun K, Lethongkam S, Kusolphat P, Hemtanon N, Klinprathum K, Sunghan J, Dejyong K, Lertwittayanon K, Pisuchpen S, Voravuthikunchai SP. Poly (vinyl alcohol) copolymerized with xanthan gum/hypromellose/sodium carboxymethyl cellulose dermal dressings functionalized with biogenic nanostructured materials for antibacterial and wound healing application. Int J Biol Macromol. 2022; 216: 235–50.
30. Behrens AM, Sikorski MJ, Li T, Wu ZJ, Griffith BP, Kofinas P. Blood-aggregating hydrogel particles for use as a hemostatic agent. Acta Biomater. 2014; 10(2): 701–8.
31. Zhao Y-F, Zhao J-Y, Hu W-Z, Ma K, Chao Y, Sun P-J, Fu X-B, Zhang H. Synthetic poly(vinyl alcohol)–chitosan as a new type of highly efficient hemostatic sponge with blood-triggered swelling and high biocompatibility. J Mater Chem B. 2019; 7(11): 1855–66.
32. Wang Y, Zhou P, Xiao D, Zhu Y, Zhong Y, Zhang J, Sui X, Feng X, Xu H, Mao Z. Chitosan-bound carboxymethylated cotton fabric and its application as wound dressing. Carbohydr Polym. 2019; 221: 202–8.
33. Kayabolen A, Keskin D, Aykan A, Karslıoglu Y, Zor F, Tezcaner A. Native extracellular matrix/fibroin hydrogels for adipose tissue engineering with enhanced vascularization. Biomed Mater. 2017; 12(3): 035007.
34. Wang Y, Zhao Y, Qiao L, Zou F, Xie Y, Zheng Y, Chao Y, Yang Y, He W, Yang S. Cellulose fibers-reinforced self-expanding porous composite with multiple hemostatic efficacy and shape adaptability for uncontrollable massive hemorrhage treatment. Bioact Mater. 2021; 6(7): 2089–104.
35. Fang H, Li D, Xu L, Wang Y, Fei X, Tian J, Li Y. A reusable ionic liquid-grafted antibacterial cotton gauze wound dressing. J Mater Sci. 2021; 56(12): 7598–612.
36. Monfared-Hajishirkiaee R, Ehtesabi H, Najafinobar S, Masoumian Z. Multifunctional chitosan/carbon dots/sodium alginate/zinc oxide double-layer sponge hydrogel with high antibacterial, mechanical and hemostatic properties. OpenNano. 2023; 12: 100162.
37. Ramasubramaniam S, Govindarajan C, Nasreen K, Sudha PN. Removal of cadmium (II) ions from aqueous solution using chitosan/starch polymer blend. Compos Interfaces. 2014; 21(2): 95–109.
38. Haider A, Waseem A, Karpukhina N, Mohsin S. Strontium- and zinc-containing bioactive glass and alginates scaffolds. Bioengineering. 2020; 7(1): 10.
39. Kimi M, Chong CJ. Facile preparation of chitosan-alginate crosslinked with calcium chloride hydrogel as sustained release fertilizers. 2024. p. 1–24.
40. Hao Y, Zhao W, Zhang L, Zeng X, Sun Z, Zhang D, Shen P, Li Z, Han Y, Li P, Zhou Q. Bio-multifunctional alginate/chitosan/fucoidan sponges with enhanced angiogenesis and hair follicle regeneration for promoting full-thickness wound healing. Mater Des. 2020; 193: 108863.
41. Mahmoudi A, Ghavimi MA, Maleki Dizaj S, Sharifi S, Sajjadi SS, Jamei Khosroshahi AR. Efficacy of a new hemostatic dental sponge in controlling bleeding, pain, and dry socket following mandibular posterior teeth extraction—A split-mouth randomized double-blind clinical trial. J Clin Med. 2023; 12(14): 4578.
42. Zhang Y, Guan J, Wu J, Ding S, Yang J, Zhang J, Dong A, Deng L. N-alkylated chitosan/graphene oxide porous sponge for rapid and effective hemostasis in emergency situations. Carbohydr Polym. 2019; 219: 405–13.
43. Su C, Zhao H, Yang H, Chen R. Stearic acid-modified starch/chitosan composite sponge with asymmetric and gradient wettability for wound dressing. ACS Appl Bio Mater. 2019; 2(1): 171–81.
44. Bal-Ozturk A, Karal-Yilmaz O, Akguner ZP, Aksu S, Tas A, Olmez H. Sponge-like chitosan-based nanostructured antibacterial material as a topical hemostat. J Appl Polym Sci. 2019; 136(19).
45. Cao S, Bi Z, Li Q, Zhang S, Singh M, Chen J. Shape memory and antibacterial chitosan-based cryogel with hemostasis and skin wound repair. Carbohydr Polym. 2023; 305: 120545.
46. Xia Y, Yang R, Wang H, Li Y, Fu C. Application of chitosan-based materials in surgical or postoperative hemostasis. Front Mater. 2022; 9(994265).
47. Xie X, Li D, Chen Y, Shen Y, Yu F, Wang W, Yuan Z, Morsi Y, Wu J, Mo X. Conjugate electrospun 3D gelatin nanofiber sponge for rapid hemostasis. Adv Healthc Mater. 2021; 10(20): 2100918.
48. Zhang Y, Wang Y, Chen L, Zheng J, Fan X, Xu X, Zhou G, Ullah N, Feng X. An injectable antibacterial chitosan-based cryogel with high absorbency and rapid shape recovery for noncompressible hemorrhage and wound healing. Biomaterials. 2022; 285: 121546.

DOI: 10.20473/j.djmkg.v58.i2.p171–179