



Development of Silk Fibroin/Chitosan Sponge for Wound Dressing

Juthathip Phianphumipong^{1,2}, Rachasit Jeencham³, Yupaporn Ruksakulpiwat^{2,4}, and Piya-on Numpaisal^{2,5*}

¹ Biomedical Innovation Engineering Program, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

² Research Center for Biocomposite Material for Medical Industry and Agricultural and Food Industry, Nakhon Ratchasima 30000, Thailand

³ Faculty of Textile Industry, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand

⁴ School of Polymer Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

⁵ School of Orthopaedics, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

*Corresponding author: piya-on@sut.ac.th

Received 28 March 2025

Revised 9 March 2026

Accepted 17 March 2026

Abstract

Gauze is a traditional wound dressing. However, it often adheres to the wound and is not highly absorbent. To address the limitations of traditional dressings, biopolymers are commonly considered suitable materials for wound-dressing applications. Silk fibroin (SF) is an interesting polymer known for its good water absorption, but it has poor mechanical properties. In this study, chitosan (CS) was selected to improve the mechanical properties of SF. SF/CS sponges were prepared with glycerol as a plasticizer at different ratios of SF to CS: 100/0, 75/25, 50/50, 25/75, and 0/100 (w/w of dry substances) using a freeze-drying process. Their physicochemical properties were investigated, including chemical structure, morphology, mechanical properties, swelling ratio, water uptake, and porosity, as well as cell viability. According to the results, all SF/CS sponges had suitable pore sizes (28–61 μm). The sponges exhibited high water uptake (90–96%) and swelling ratios (900–2,100%). The addition of CS greatly enhanced the mechanical properties of the sponges. Notably, SF/CS sponges at ratios of 50/50, 25/75, and 0/100 showed increases in tensile strength (0.49–0.65 MPa) and elongation at break (71–190%). However, the 0SF/100CS sponge was found to have limitations due to its low porosity (<60%). In addition, the MTT assay confirmed that none of the SF/CS sponges exhibited cytotoxicity. In summary, SF/CS sponges at 50/50 and 25/75 ratios showed potential as biomaterials for wound dressings, offering advantages in water uptake, mechanical properties, swelling ratio, porosity, and non-cytotoxicity.

Keywords: Biopolymer, Natural polymer, Bioactive, Wound healing

1. Introduction

Among all human organs, the skin is the largest. It is susceptible to damage from various acute injuries and chronic diseases, including abrasion wounds, incised wounds, burn wounds, diabetic ulcers, and venous ulcers. Without appropriate treatment, these can be challenging to heal and may affect vital organs, potentially leading to serious complications or even death [1]. Wounds typically undergo a natural self-healing process. Wound care is a crucial aspect of medical practice and is present in every hospital. The primary goal of wound dressings is to promote optimal, rapid healing. A variety of materials are currently used for wound care, including gauze, film, hydrogel, hydrocolloid, sponge, and non-woven fibers. Gauze is one of the most commonly used dressings due to its low cost. However, it often adheres to the wound, lacks effective water absorption capabilities, and does not possess antibacterial properties. In wounds with excessive fluid production, it is crucial to use a dressing that effectively manages exudate while maintaining the proper moisture balance. The selection of dressings depends on the volume of wound exudate. Wounds characterized by moderate to high exudate, including large venous

ulcers, pyoderma gangrenosum, burn wounds, and blisters, benefit from dressings with strong absorption capabilities, which also help prevent maceration of the surrounding healthy tissue [2]. Although gauze may be widely used, it is not ideal for wounds that produce excessive fluid. To overcome the limitations of conventional dressings, researchers have explored and developed various enhanced alternatives. Effective wound dressings should regulate moisture levels, demonstrate excellent water absorption, permit gas exchange, and minimize the risk of bacterial infection [3]. Sponges are appropriate for wounds with high and moderate exudate, provided they do not stick to the wound bed or interfere with epithelialization [2]. As previously mentioned, to overcome the shortcomings of gauze, this research aims to develop a sponge-form wound dressing made from natural polymers that enhances water absorption for use in wounds with exudate. Silk has been used by humans for over 4,000 years [4]. Silk fibroin (SF) is a natural polymer, specifically a protein fiber extracted from silkworms and other insects that produce fibers [5]. Silk consists of two main components: fibroin, which forms the core of the fiber, and sericin, the protein that serves as the outer covering [7]. Numerous studies show that SF displays excellent biocompatibility, is non-irritating, non-polluting, non-toxic, and biodegradable [3]. Additionally, SF has been found to promote cell migration and proliferation, processes directly linked to accelerated wound healing [12]. Some studies also highlight the anti-inflammatory properties of SF peptides. For example, research by Kim et al. (2011) [13] demonstrates that these peptides exhibit anti-inflammatory activity in a mouse edema model of inflammation [14]. Due to these characteristics, SF is widely used in various fields, including biomaterials and tissue engineering. However, it has limitations, particularly low elasticity and flexibility, which may limit its application [3, 6]. To address these limitations, many researchers have developed SF composites by combining them with other materials, such as carboxymethyl cellulose and chitosan (CS), to improve their mechanical properties [6, 8].

CS is a natural biological material [9], a biopolymer composed of glucosamine and N-acetylglucosamine. It is derived from the deacetylation of chitin [10]. CS is widely used in pharmaceuticals, cosmetics, and wound dressings because of its properties, which support hemostasis, promote wound healing, and provide biocompatibility and biodegradability. Additionally, it has antibacterial properties and possesses excellent mechanical strength [11].

Currently, numerous reports describe the use of silk fibroin/chitosan (SF/CS) blends for wound dressings. However, due to the various forms of SF/CS, such as nanofibers [15], films [16], and hydrogels [17], limitations in water absorption remain. Film-based materials typically possess dense structures with limited porosity, which restrict fluid uptake [30]. Although hydrogels can absorb water, their high intrinsic water content may compromise mechanical stability under conditions of excessive exudate [30]. Nanofibrous mats, despite their high surface area, often exhibit small pore sizes and limited thickness, which may constrain total fluid absorption capacity [16]. In contrast, sponge-type dressings feature a three-dimensional interconnected macroporous structure [5], facilitating efficient capillary-driven fluid absorption and exudate retention [30]. This architecture promotes uniform fluid distribution while preserving structural integrity. Therefore, the aim of this research is to develop SF/CS sponges specifically designed for wound-dressing applications, emphasizing high water absorption to manage exudate wounds while also satisfying the necessary mechanical and physical properties. Additionally, the study compares the properties of these sponges with those of an ideal wound dressing to ensure they meet established criteria. This research involves the preparation of SF/CS sponges in various ratios using a freeze-drying process. The chemical interactions between SF and CS are examined using Fourier-transform infrared (FTIR) spectroscopy. Furthermore, the sponges' morphology, swelling properties, water uptake, porosity, and mechanical properties are characterized, and the cytotoxicity of the SF/CS sponges is evaluated.

2. Materials and methods

2.1 Materials

Silk cocoons of *Bombyx mori* (Luang Saraburi) were sourced from Huai Thalaeng, Nakhon Ratchasima. CS, with over 90% deacetylation and a mean molecular weight of 890 kDa, was purchased from Marine Bio Resources Co., Ltd (Samutsakhon, Thailand). Sodium carbonate (Na_2CO_3 , analytical grade), calcium chloride (CaCl_2 , laboratory grade), and glycerol (analytical grade) were sourced from KEMAUS (Cherrybrook, N.S.W. 2126, Australia). Acetic acid and ethanol were purchased from Carlo Erba Reagents (Milano, Italy). Sodium hydroxide (NaOH , analytical grade) was purchased from QReC (New Zealand). SnakeSkin dialysis tubing with a molecular weight cut-off (MWCO) of 10,000 Daltons was sourced from Thermo Scientific Inc. (Illinois, USA).

2.2 Methods

2.2.1 Preparation of silk fibroin solution

Silk fibroin (SF) was obtained following the procedure reported by Ajisawa [18]. Briefly, the cocoons of *Bombyx mori* were cut into small pieces and degummed by boiling in a solution of 0.02 M Na₂CO₃ at 100 ± 2 °C for 30 minutes to remove sericin. The fibers were then rinsed with distilled water and dried overnight at 60 °C. The degummed silk fibers were dissolved in a mixed solution of CaCl₂, ethanol (CH₃CH₂OH), and water (H₂O) in a molar ratio of 1:2:8 at 70 °C for 4 h and then filtered to remove any undissolved components, resulting in an SF solution. To remove impurities, the SF solution was dialyzed in distilled water for 72 h using SnakeSkin dialysis tubing with a 10,000 MWCO. After dialysis, the solution was centrifuged twice for 20 minutes at 4 °C and 9,000 rpm to remove residual impurities.

2.2.2 Preparation of silk fibroin/chitosan sponges

A CS solution was prepared by dissolving 2% (w/v) CS in 2% (v/v) acetic acid. Then, 2% (w/v) SF was added to the solution to create mixtures with varying SF-to-CS ratios of 100:0, 75:25, 50:50, 25:75, and 0:100. Each SF/CS mixture was combined with 30 PHR glycerol as a plasticizer and homogenized using a magnetic stirrer. The SF/CS solution was transferred into a silicone mold and frozen at -20 °C overnight, then lyophilized for 48 h. The dried specimens were subsequently removed from the silicone mold and immersed in 90% ethanol for 1 h to induce crystallization (β-sheet formation), followed by immersion in 1M sodium hydroxide and rinsing with distilled water until neutral pH was obtained. The sponges were then further lyophilized for 48 h (Gamma 2-16 LSC plus, CHRIST).

2.2.3 Thickness measurements

The thickness of the SF/CS sponges was determined using a thickness gauge. Measurements were taken from five random positions on each sample, and the average value was calculated to represent the sponge thickness [19].

2.2.4 Fourier-transform infrared spectroscopy (FTIR)

An FTIR spectrophotometer (Bruker Tensor 27, Billerica, MA, USA) was used to examine the chemical interactions between SF and CS. All sample spectra were measured at a 4 cm⁻¹ resolution within the wave range of 4000–400 cm⁻¹ [19].

2.2.5 Morphology

Field-emission scanning electron microscopy (FESEM; Carl Zeiss Auriga, Oberkochen, Germany) with gold coating was employed to examine the morphology of the SF/CS sponges. The pore size of the sponge was determined by randomly selecting 100 pores from the SEM images and utilizing image analysis software (ImageJ). Each sample was tested in triplicate to ensure repeatability [20].

2.2.6 Swelling ratio and water uptake ratio

The SF/CS sponges were sectioned into 2.5 × 2.5 cm pieces, and their dry weight (W_d) was recorded. Subsequently, the sponge samples were submerged in 10 mL of phosphate-buffered saline (PBS) solution at room temperature. After a certain period, the sponges were carefully removed, placed on tissue paper to remove any excess solution, and then weighed again (W_w). The water uptake ratio was determined using the following Equation (1):

$$\text{Water uptake ratio (\%)} = \frac{W_w - W_d}{W_d} * 100\% \quad (1)$$

The procedure was repeated at intervals of 1, 2, 4, 8, 12, 16, 20 and 24 h. The swelling ratio was then calculated using the following Equation (2):

$$\text{Swelling ratio (\%)} = \frac{W_w - W_d}{W_d} * 100\% \quad (2)$$

The values presented represent the averages calculated from three separate measurements. ($n = 3, \pm \text{SD}$) [10, 20].

2.2.7 Porosity measurement

Porosity of the sponges was assessed through alcohol displacement with ethanol [21]. The samples were submerged in 2 mL of ethanol for 24 h [22] and then removed and weighed. The porosity was calculated using the following Equation (3):

$$\text{Porosity (\%)} = \frac{W_2 - W_1}{\rho V} * 100\% \quad (3)$$

Where: W_1 = Weight before immersing in ethanol

W_2 = Weight of the samples following immersion in ethanol

ρ = Density of alcohol

V = Known volume of ethanol use

2.2.8 Mechanical properties

To assess the tensile properties, SF/CS specimens, each measuring 1 cm in width and 10 cm in length, were prepared. These specimens were subjected to testing using a universal testing machine (Stable Micro System TA.XT plus C Texture Analyzer), operating at a crosshead speed of 10 mm/min with a 5 kN load cell and a 30 mm gauge length, all conducted at room temperature. Average values of tensile strength, Young's modulus, and elongation at break were obtained from five specimens ($n = 5$).

2.2.9 Cytotoxicity study

The SF/CS sponges were cut and placed into 96-well plates. The 96-well plates were then sterilized by exposing them to ultraviolet (UV) light for 30 minutes. The cytotoxicity was evaluated by determining the viability of human fibroblast cells following a 24-hour incubation in the SF/CS sponge extract. An MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was performed on days 1, 3, and 7 to evaluate cell viability. Cells were seeded in 96-well plates containing culture medium (DMEM high glucose supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamax). 10 μ L of MTT solution was added to each well, followed by 90 μ L of culture medium. Plates were gently swirled to ensure uniform MTT distribution. Following a two-hour incubation at 37 °C with 5% CO₂, the formation of purple formazan crystals was observed using an inverted microscope (ZEISS, Germany). Each well's MTT solution was gently aspirated, and 100 μ L of dimethyl sulfoxide (DMSO; PAN BIOTECH) was introduced to dissolve the formazan crystals. Plates were gently swirled and shaken at low speed for 10–15 minutes using a shaker (FINEPCR, Korea). The microplate reader (TECAN, Switzerland) was used to record absorbance at 570 nm, with a reference wavelength of 630 nm. As shown in Equation (4), cell viability (%) was calculated by measuring and comparing the absorbance of sponge-cultured cells with that of the control.

$$\text{Cell viability (\%)} = \frac{\text{O.D. of treatment}}{\text{O.D. of control}} \times 100 \quad (4)$$

2.2.10 Statistical analysis

Statistical analyses were performed using one- or two-way ANOVA as appropriate, followed by Tukey's post hoc test. Welch's ANOVA with the Games–Howell test was used when variance homogeneity was not met. Data are presented as mean $\pm$ SD, with $p < 0.05$ considered significant.

3. Results and discussion

The SF/CS sponges, with a thickness of 5 ± 1 mm, were successfully fabricated using the freeze-drying method. These sponges are robust, non-brittle, and can be handled without deformation, making them ideal for further evaluation of their mechanical, physicochemical, and cytotoxic properties.

3.1 Fourier-transform infrared spectra

The FTIR spectra of the 100SF/0CS, 75SF/25CS, 50SF/50CS, 25SF/75CS, and 0SF/100CS sponges are shown in Fig. 1. The positions of the absorption bands indicate the conformation of silk protein. The 100SF/0CS sponge exhibited absorption bands at 1622 cm^{-1} (amide I, C=O stretching) and 1511 cm^{-1} (amide II, N-H bending and C-H stretching), corresponding to the β -sheet conformation, indicating the crystalline portion. Furthermore, the amide III absorption band was observed at 1230 cm^{-1} (N-H stretching and C=O bending), indicating a random-coil conformation (amorphous region) [23, 24, 25]. This shows that the SF sponge consists of both β -sheet and

random-coil structures. The 0SF/100CS sponge exhibited absorption bands at 1648 cm^{-1} (C = O stretching), 1582 cm^{-1} (NH_2 stretching), 1151 cm^{-1} (C-O-C stretching), and 1065 and 1035 cm^{-1} (C-O stretching) [20, 26].

The SF/CS sponges exhibited shifts in the peaks of both SF and CS, with the intensity and positions of the amide I, amide II, and amide III peaks slightly altered depending on the SF/CS blending ratios. For example, the spectra of 25SF/75CS revealed that the amide I, II, and III peaks of SF shifted from 1622 to 1629 cm^{-1} , 1511 to 1532 cm^{-1} , and 1230 to 1260 cm^{-1} , respectively. The shift in the SF/CS peaks indicates good compatibility, suggesting an interaction between the SF and CS molecules.

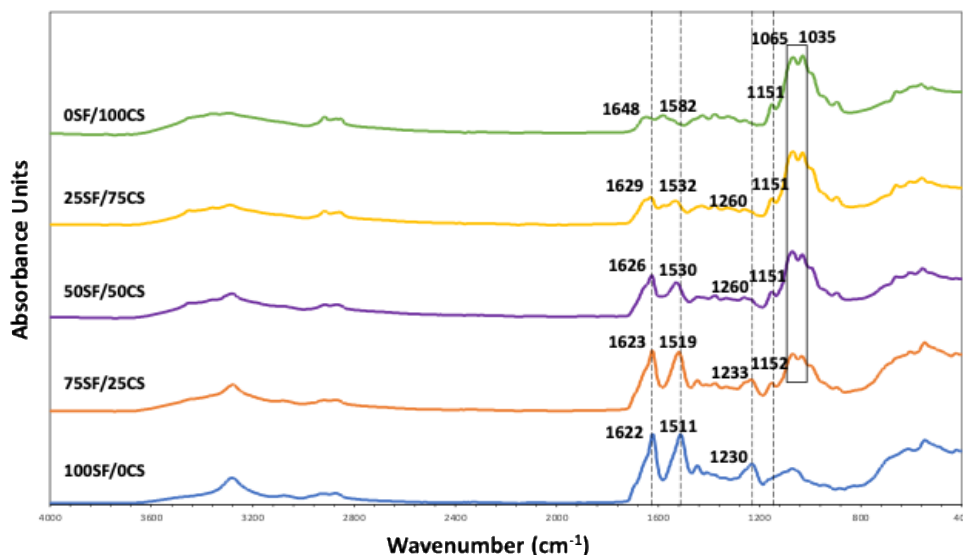


Figure 1 FTIR spectra of 100SF/0CS, 75SF/25CS, 50SF/50CS, 25SF/75CS, and 0SF/100CS sponges.

3.2 Silk fibroin/chitosan sponge morphology and pore size

The SF/CS sponges had pore sizes ranging from 28–61 μm , as shown in Fig. 2. The pore size of the sponges significantly increased with increasing SF content ($p < 0.05$). SEM images of cross-sections of SF/CS sponges at various ratios (Fig. 3) revealed that all SF/CS sponges were highly microporous with interconnected channels. The SF/CS sponges demonstrated a larger pore size compared to 0SF/100CS. In general, fibroblasts can cover pores measuring 50 μm or less [27]. These results indicate that all SF/CS sponges have suitable pore sizes for wound-dressing applications.

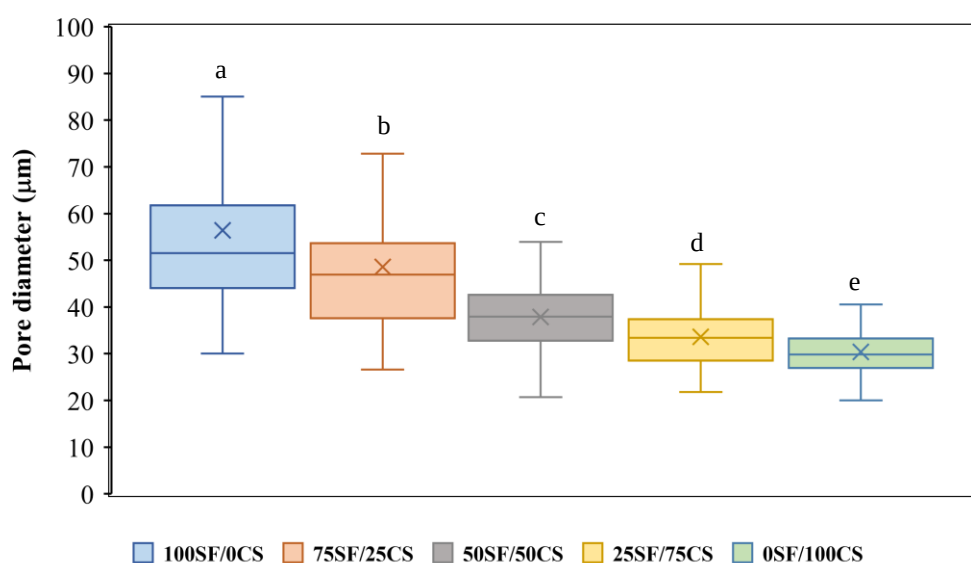


Figure 2 Pore diameters of SF/CS sponges. Different letters indicate statistically significant differences among groups ($p < 0.05$).

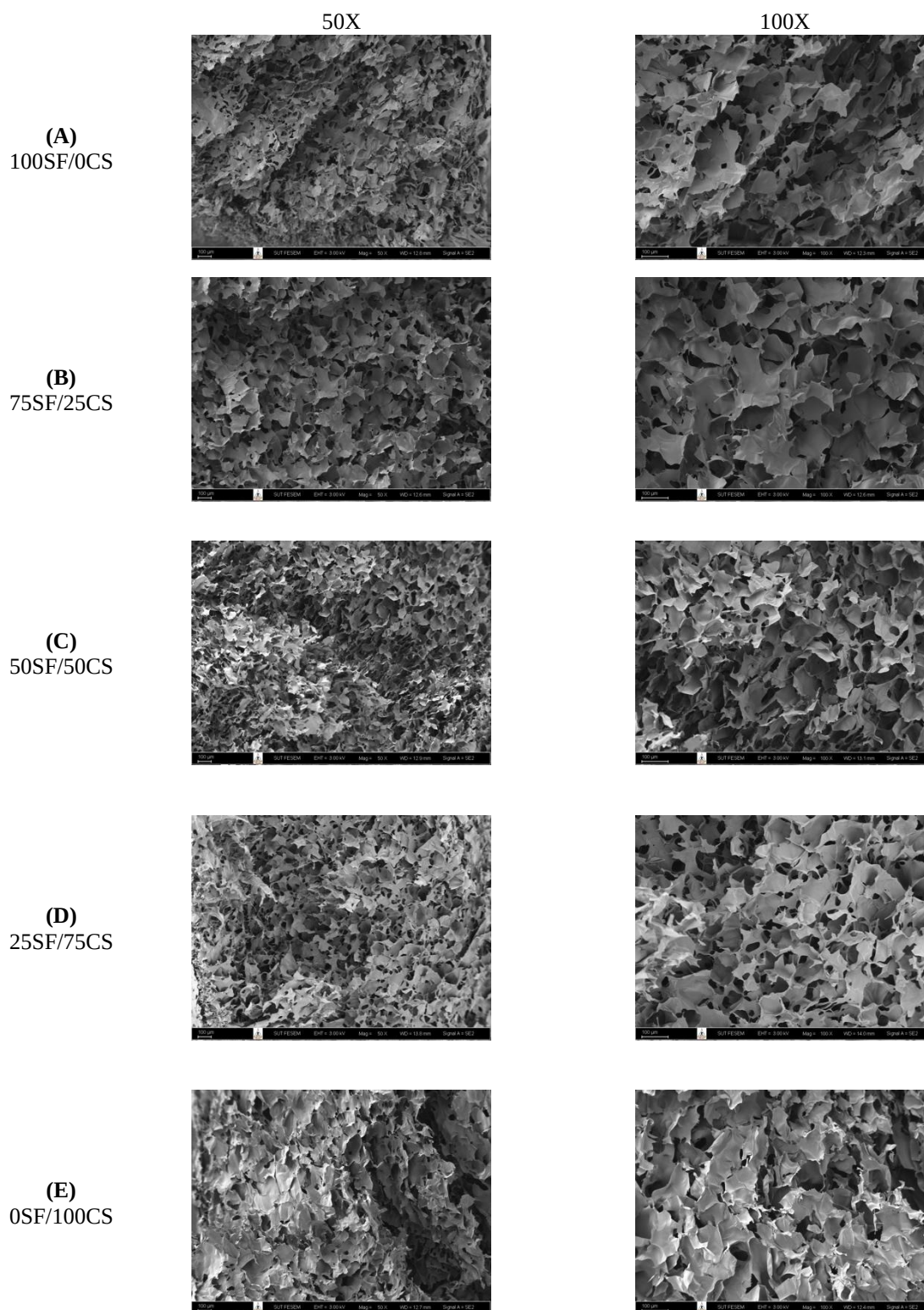


Figure 3 Morphology of SF/CS sponges, fabricated by various ratios: (A) 100SF/0CS, (B) 75SF/25CS, (C) 50SF/50CS, (D) 25SF/75CS, and (E) 0SF/100CS at different magnifications (50x and 100x).

3.3 Swelling ratio, water uptake ratio, and porosity

Water uptake, swelling capacity, and porosity are crucial parameters for wound-dressing performance, as they directly affect the effectiveness of wound healing and recovery. An optimal wound dressing should effectively absorb excess exudate from the wound bed. Materials with high water absorption capacity and hydrophilic

properties facilitate the diffusion of nutrients, bioactive molecules, cells, and waste products [28]. According to the ideal wound-dressing requirements, the swelling ratio, water absorption, and porosity should typically fall within the ranges of 100–900%, >70%, and 60–90%, respectively [9, 28]. All SF/CS sponges exhibited high swelling ratios (900–2100%), as shown in Fig. 4. Statistical analysis at the equilibrium time (24 h) revealed significant differences among all compositions ($p < 0.05$), with swelling capacity decreasing progressively as CS content increased (100SF/0CS > 75SF/25CS > 50SF/50CS > 25SF/75CS > 0SF/100CS). The statistical analysis was performed exclusively at the equilibrium time point (24 h), and no statistical comparisons were conducted at earlier time points. This trend indicates that increasing CS content reduces the water-holding capacity of the scaffold. Similarly, water uptake values ranged from 90–96%, and one-way ANOVA demonstrated significant differences among compositions ($p < 0.05$) (Table 1). The gradual reduction in water uptake with increasing CS content was consistent with swelling behavior, suggesting that polymer composition strongly influences fluid absorption properties. Porosity values ranged from 55–83%. Statistical analysis also confirmed significant differences among groups ($p < 0.05$), with higher SF content associated with greater porosity. Notably, the 0SF/100CS sponge exhibited significantly lower porosity than the other compositions, which may explain its reduced swelling performance. The observed decreases in swelling ratio and water uptake with increasing CS content may be attributed to reduced pore size and a denser polymer network, limiting water penetration and retention. Larger and more interconnected pores facilitate greater fluid absorption, explaining the superior performance of SF-rich compositions. Overall, all SF/CS sponges—except the 0SF/100CS formulation—demonstrated swelling behavior, water uptake, and porosity within or exceeding the desirable range for wound-dressing applications, highlighting their suitability for effective exudate management.

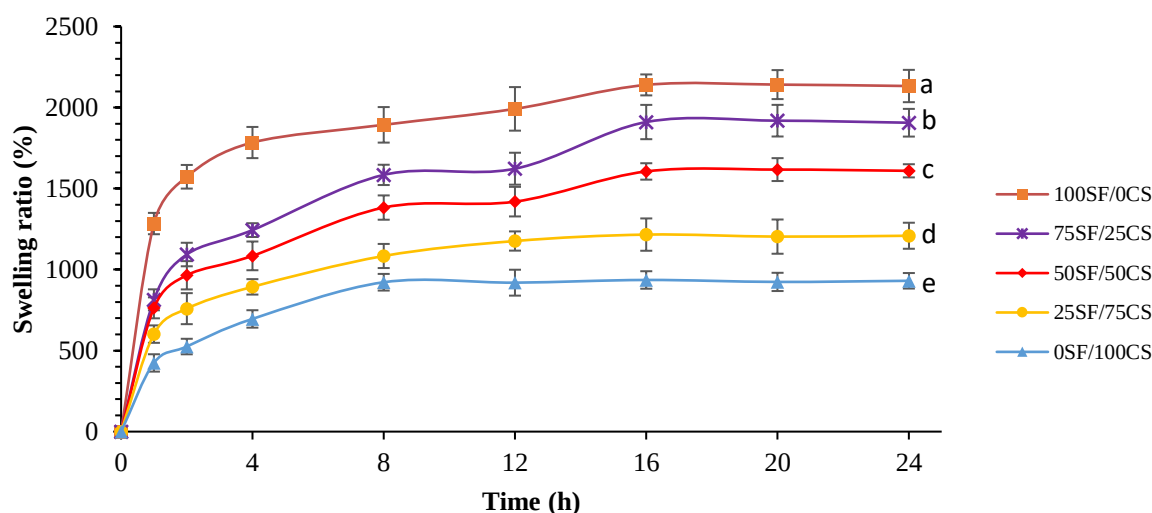


Figure 4 Swelling ratio of SF/CS sponges, prepared with different blending ratios of SF and CS, evaluated at various time intervals (1, 2, 4, 8, 12, 16, 20, and 24 h). Different letters indicate significant differences among compositions at the equilibrium time point (24 h) ($p < 0.05$).

Table 1 Water uptake ratio and porosity of SF/CS sponges

Samples	Water Uptake Ratio (%)	Porosity (%)
100SF/0CS	95.51 ± 2.20 ^a	83.24 ± 3.86 ^a
75SF/25CS	94.98 ± 1.49 ^{ab}	72.07 ± 3.89 ^b
50SF/50CS	92.90 ± 3.20 ^{bc}	66.89 ± 2.28 ^{bc}
25SF/75CS	92.20 ± 1.42 ^{abc}	63.01 ± 2.34 ^{cd}
0SF/100CS	90.03 ± 1.17 ^c	55.26 ± 1.80 ^d

Data are presented as mean ± SD ($n = 3$). Different superscript letters indicate statistically significant differences among groups ($p < 0.05$).

3.4 Mechanical properties

A wound dressing must be both flexible and durable, ensuring it remains stable and strong enough to consistently cover the wound throughout the healing process [28, 29]. The ultimate tensile strength, elongation at break, and Young's modulus of the SF/CS sponges are summarized in Table 2. Statistical analysis revealed significant differences among the different compositions in tensile strength, elongation at break, and Young's modulus ($p < 0.05$). The 100SF/0CS sponge exhibited the lowest tensile strength (0.036 MPa), elongation at break (9.87%), and Young's modulus (3.71 MPa). As the CS content increased, the tensile strength and elongation at

break gradually increased from 0.036 MPa to 0.650 MPa and from 9.87% to 190.83%, respectively. However, the Young's modulus of the SF/CS sponge was highest at a 25/75 ratio, followed by 50/50, 75/25, 0/100, and 100/0. The maximum ultimate tensile strength, elongation at break, and Young's modulus of Kaltostat (a commercial dressing made from natural polymer calcium alginate) were reported to be 0.04 MPa, 68%, and 0.24 MPa, respectively [30]. These results indicate that all SF/CS sponges meet the requirements for ultimate tensile strength and Young's modulus. Nevertheless, elongation at break was found to be suitable for wound-dressing applications in SF/CS sponges with ratios of 50/50, 25/75, and 0/100.

Table 2 Mechanical properties of SF/CS sponges

Samples	Ultimate Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
100SF/0CS	0.036 ± 0.003 ^a	9.87 ± 0.54 ^a	3.71 ± 0.90 ^a
75SF/25CS	0.310 ± 0.010 ^b	45.01 ± 2.48 ^b	6.87 ± 0.15 ^{ab}
50SF/50CS	0.490 ± 0.030 ^c	71.59 ± 3.43 ^c	7.19 ± 0.07 ^{ab}
25SF/75CS	0.560 ± 0.050 ^c	83.03 ± 11.30 ^c	7.94 ± 0.13 ^b
0SF/100CS	0.650 ± 0.020 ^d	190.83 ± 9.86 ^d	5.11 ± 0.15 ^c

Data are presented as mean ± SD (n = 5). Different superscript letters within the same column indicate statistically significant differences (p < 0.05).

3.5 Cytotoxicity

The cell cytotoxicity test is an important process for evaluating certain chemicals and materials prior to use in medical applications to determine their potential to destroy living cells [31]. Fibroblasts are the host cells in the skin and connective tissue, playing a crucial role in wound healing, tissue repair, and overall maintenance of the extracellular matrix. The cytotoxicity test ensures that products or materials intended for human use are safe and helps reduce the risk of side effects or harm. Additionally, it can help minimize the need for animal experimentation. The viability of fibroblasts on SF/CS sponges was assessed using the MTT assay. Fibroblast viability was evaluated after incubation in the medium extracted from the SF/CS sponges. The fibroblast viability was assessed at 1, 3, and 7 days, with values of approximately 80–96%, 85–109%, and 70–94%, respectively (Fig. 5). Two-way ANOVA revealed significant effects of time and sample composition, with no significant interaction between the two factors, indicating that the effect of composition on cell viability was consistent across time points. Post hoc comparisons further showed that significant differences among compositions were observed at day 7 (p < 0.05), with no significant differences detected on days 1 or 3. The cell viability of SF/CS sponges was lower than that of the control group at all time points, except for the SF/CS sponge with a ratio of 0/100, which showed higher viability than the control group on day 3. The observed results may be due to the uneven distribution of seeded fibroblasts on the sponges and overconfluence by day 7. Nevertheless, cytotoxicity tests were conducted in accordance with ISO 10993-5 to evaluate cytotoxic potential. The developed material must demonstrate at least 70% cellular viability. In summary, the SF/CS sponges were found to be non-cytotoxic, as evidenced by cell viabilities greater than 70% [31, 32].

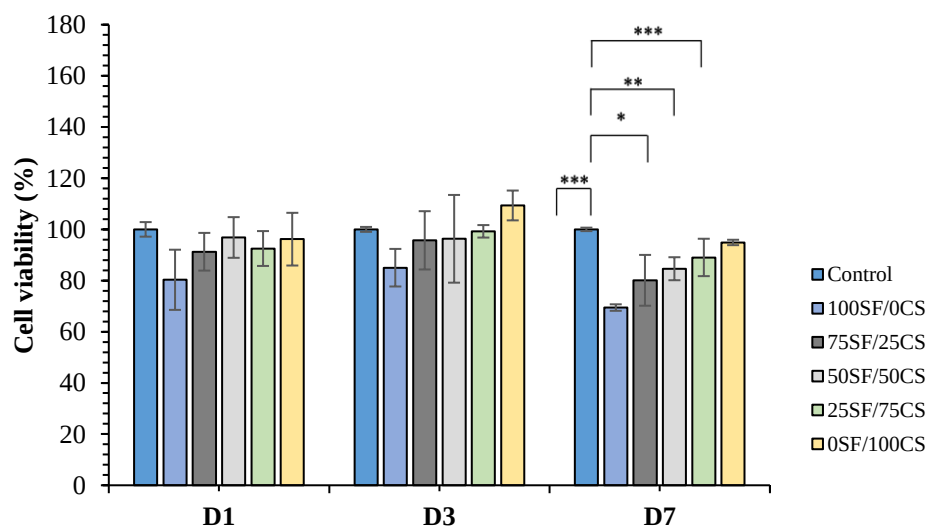


Figure 5 Fibroblast viability after incubation in media extracted from SF/CS sponges for 1, 3, and 7 days. Data are presented as mean ± SD (n = 3). Asterisks indicate statistically significant pairwise comparisons among all groups (*p < 0.05, **p < 0.01, ***p < 0.001).

4. Conclusion

SF/CS sponges were fabricated using a freeze-drying process, with a thickness of 5 ± 1 mm. Higher SF content led to larger pore sizes, higher swelling ratios, greater water uptake, and higher porosity. Except for 0SF/100CS, all sponges demonstrated excellent water absorption, making them suitable for exudate wounds. CS significantly enhances the mechanical properties of the sponges. SF/CS sponges with 50/50 and 25/75 ratios show great potential for wound dressings due to their physicochemical properties and biocompatibility. These optimized sponges hold promise for further in vitro antibacterial studies and in vivo investigations into inflammatory responses and wound healing.

5. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, San Francisco, CA, USA) to assist with language editing and proofreading. The authors subsequently reviewed and edited the content and take full responsibility for the accuracy and integrity of the published work.

6. Acknowledgments

The authors would like to thank Mr. Narongrit Srikaew for his technical assistance with cell culture studies and cytotoxicity testing. The authors also acknowledge Suranaree University of Technology (SUT) for the scholarship, the Research Center for Biocomposite Materials for the Medical Industry and Agricultural and Food Industry, and the School of Polymer Engineering for their support.

7. Author contributions

Conceptualization, P.-o.N. and Y.R.; methodology, J.P., P.-o.N.; validation, P.-o.N. and R.J.; formal analysis, J.P.; investigation, J.P.; resources, P.-o.N.; data curation, J.P.; writing—original draft preparation, J.P.; writing—review and editing, P.-o.N.; visualization, J.P.; supervision, P.-o.N., Y.R., and R.J.; project administration, P.-o.N., and Y.R.; funding acquisition, P.-o.N. All authors have read and agreed to the published version of the manuscript.

8. Conflicts of interest

The authors have no conflict of interest to declare.

9. References

- [1] Namviriyachote N, Muangman P, Chinaronchai K, Chuntrasakul C, Ritthidej GC. Polyurethane-biomacromolecule combined foam dressing containing asiaticoside: fabrication, characterization and clinical efficacy for traumatic dermal wound treatment. *Int J Biol Macromol*. 2019;131:1010–1019.
- [2] Dabiri G, Damstetter E, Phillips T. Choosing a wound dressing based on common wound characteristics. *Adv Wound Care*. 2016;5(1):32–41.
- [3] Yin J, Fang Y, Xu L, Ahmed A. High-throughput fabrication of silk fibroin/hydroxypropyl methylcellulose (SF/HPMC) nanofibrous scaffolds for skin tissue engineering. *Int J Biol Macromol*. 2021;183:1210–1221.
- [4] Rockwood DN, Preda RC, Yücel T, Wang X, Lovett ML, Kaplan DL. Materials fabrication from Bombyx mori silk fibroin. *Nat Protoc*. 2011;6(10):1612–1631.
- [5] Sun W, Gregory DA, Tomeh MA, Zhao X. Silk fibroin as a functional biomaterial for tissue engineering. *Int J Mol Sci*. 2021;22(3):1499.
- [6] Feng YF, Li XF, Zhang Q, Yan SQ, Guo Y, Li M, et al. Mechanically robust and flexible silk protein/polysaccharide composite sponges for wound dressing. *Carbohydr Polym*. 2019;216:17–24.
- [7] Promnil S, Ruksakulpiwat C, Numpaisal PO, Ruksakulpiwat Y. Electrospun poly(lactic acid) and silk fibroin based nanofibrous scaffold for meniscus tissue engineering. *Polymers (Basel)*. 2022;14(12):2435.
- [8] Kweon H, Ha HC, Um IC, Park YH. Physical properties of silk fibroin/chitosan blend films. *J Appl Polym Sci*. 2001;80(7):928–934.
- [9] Fan Y, Lu Q, Liang W, Wang Y, Zhou Y, Lang M, et al. Preparation and characterization of antibacterial polyvinyl alcohol/chitosan sponge for wound dressing. *Eur Polym J*. 2021;157:110619.
- [10] Sionkowska A, Planecka A. Preparation and characterization of silk fibroin/chitosan composite sponges for tissue engineering. *J Mol Liq*. 2013;178:5–14.

- [11] Tuwalska A, Grabska-Zielińska S, Sionkowska A. Chitosan/silk fibroin materials for biomedical applications—A review. *Polymers (Basel)*. 2022;14:1343.
- [12] Patil PP, Reagan MR, Bohara RA. Silk fibroin and silk-based biomaterial derivatives for ideal wound dressings. *Int J Biol Macromol*. 2020;158:11–20.
- [13] Kim DW, Hwang HS, Kim DS, Sheen SH, Heo DH, Hwang GJ, et al. Effect of silk fibroin peptide derived from silkworm *Bombyx mori* on the anti-inflammatory effect of Tat-SOD in a mice edema model. *BMB Rep*. 2011;44(12):787–792.
- [14] Rodriguez-Nogales A, Lozano-Pérez AA, Aznar-Cervantes SD, Algieri F, Garrido-Mesa J, Garrido-Mesa N, et al. Effect of aqueous and particulate silk fibroin in a rat model of experimental colitis. *Int J Pharm*. 2016;511(1):1–9.
- [15] Khosravimelal S, Chizari M, Farhadhosseiniabadi B, Moosazadeh Moghaddam M, Gholipourmalekabadi M. Fabrication and characterization of an antibacterial chitosan/silk fibroin electrospun nanofiber loaded with a cationic peptide for wound-dressing application. *J Mater Sci Mater Med*. 2021;32(9):104.
- [16] Mohebian Z, Tajmohammadi I, Yavari Maroufi L, Ramezani S, Ghorbani M. A novel Aloe vera-loaded ethylcellulose/hydroxypropyl methylcellulose nanofibrous mat designed for wound healing application. *J Polym Environ*. 2022;30(3):867–877.
- [17] Xu Z, Chen T, Zhang K, Meng K, Zhao H. Silk fibroin/chitosan hydrogel with antibacterial, hemostatic and sustained drug-release activities. *Polym Int*. 2021;70(10):1235–1243.
- [18] Akiyoshi A. Dissolution of silk fibroin with calcium chloride/ethanol aqueous solution. *J Seric Sci Jpn*. 1998;67:91–94.
- [19] Poonguzhali R, Basha SK, Kumari VS. Synthesis and characterization of chitosan-PVP-nanocellulose composites for in vitro wound dressing application. *Int J Biol Macromol*. 2017;105:111–120.
- [20] Li DW, Lei X, He FL, He J, Liu YL, Ye YJ, et al. Silk fibroin/chitosan scaffold with tunable properties and low inflammatory response assists the differentiation of bone marrow mesenchymal stem cells. *Int J Biol Macromol*. 2017;105:584–597.
- [21] Buyana B, Aderibigbe BA, Ray SS, Ndinteh DT, Fonkui YT. Development, characterization, and in vitro evaluation of water soluble poloxamer/pluronic-based wound dressing. *J Appl Polym Sci*. 2019;136(48):48745.
- [22] Ngece K, Aderibigbe BA, Ndinteh DT, Fonkui YT, Kumar P. Alginate-gum acacia based sponges as potential wound dressings for exuding and bleeding wounds. *Int J Biol Macromol*. 2021;172:350–359.
- [23] Zhang H, Li L, Dai F, Zhang H, Ni B, Zhou W, et al. Preparation and characterization of silk fibroin as a biomaterial with potential for drug delivery. *J Transl Med*. 2012;10:117.
- [24] Shetty GR, Rao BL, Asha S, Wang Y, Sangappa Y. Preparation and characterization of silk fibroin/hydroxypropyl methyl cellulose (HPMC) blend films. *Fibers Polym*. 2015;16(8):1734–1741.
- [25] Shang S, Zhu L, Fan J. Intermolecular interactions between natural polysaccharides and silk fibroin protein. *Carbohydr Polym*. 2013;93(2):561–573.
- [26] Jeenchan R, Sutteerawattananonda M, Tiyafoonchai W. Preparation and characterization of chitosan/regenerated silk fibroin films as a biomaterial for ophthalmic drug delivery. *Int J Appl Pharm*. 2019;11(5):275–284.
- [27] Salem AK, Stevens R, Pearson RG, Davies MC, Tendler SJ, Roberts CJ, et al. Interactions of 3T3 fibroblasts and endothelial cells with defined pore features. *J Biomed Mater Res*. 2002;61(2):212–217.
- [28] Morgado PI, Aguiar-Ricardo A, Correia IJ. Asymmetric membranes as ideal wound dressings: an overview on production methods, structure, properties and performance relationship. *J Membr Sci*. 2015;490:139–151.
- [29] Liu L, Hu D, Xu G, Shou L, Yao J. Fabrication and evaluation of polyurethane-based asymmetric membranes. *J Mater Sci*. 2013;48(5):1902–1910.
- [30] Minsart M, Van Vlierberghe S, Dubruel P, Mignon A. Commercial wound dressings for the treatment of exuding wounds: an in-depth physico-chemical comparative study. *Burns Trauma*. 2022;10:tkac024.
- [31] Jeenchan R, Tawonsawatruk T, Numpaisal PO, Ruksakulpiwat Y. Reinforcement of injectable hydrogel for meniscus tissue engineering using cellulose nanofiber from cassava pulp. *Polymers (Basel)*. 2023;15(9):2092.
- [32] Pacheco MS, Kano GE, de Almeida Paulo L, Lopes PS, de Moraes MA. Silk fibroin/chitosan/alginate multilayer membranes as a system for controlled drug release in wound healing. *Int J Biol Macromol*. 2020;164:479–487.