TẠP CHÍ KHOA HỌC & CÔNG NGHỆ ĐẠI HỌC DUY TÂN
DTU Journal of Science and Technology 02(39) (2020) 76-84

TRƯỜNG ĐẠI HỌC DUY TÂN
DUYTAN UNIVERSITY

pH-sensitive silkfibroin- based hydrogel for wound healing

Hydrogel nhạy pH từ fibroin tơ tằm dùng cho chữa vết thương hở

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(Ngày nhận bài: 21/10/2019, ngày phản biện xong: 25/10/2019, ngày chấp nhận đăng: 4/5/2020)

Abstract

In this research, we developed a novel sulfamethazine-based anionic copolymer and blended with silk fibroin to create a hybrid hydrogel platform as a potential wound healing material. The developed hybrid hydrogel system combines smart pH-sensitivity from synthetic, anionic copolymer poly (sulfamethazine lactide) (PSMLA) with outstanding biocompatibility from naturally derived silk fibroin. pH-sensitive hydrogel and blending system exhibited *in vitro* gelation with gel-state, covered physiological condition. Owing to the presence of lactide units, PSMLA hydrogel provided sufficient *in vivo* biodegradability. The subcutaneous implantation on *in vivo* animal models of the blending system accelerated the wound healing process greater than the parental PSMLA or silk fibroin hydrogels. This indicates the promising feasibility of the developed hybrid hydrogel for wound healing.

Keywords: Silk fibroin; pH-sensitive; wound healing; hydrogel.

Tóm tắt

Trong nghiên cứu này, chúng tôi đã phát triển một loại anion copolymer từ nhóm sulfamethazine và kết hợp với fibroin tơ tằm để tạo ra một hệ hydrogel lai sử dụng làm vật liệu giúp hồi phục vết thương hở. Hệ hydrogel lai này kết hợp tính nhạy pH thông minh từ anion copolymer poly (sulfamethazine lactide) (PSMLA) với sự tương thích sinh học vượt trội từ fibroin tơ. Cả hydrogel nhạy pH và hệ hydrogel kết hợp đều thể hiện khả năng tạo gel *in vitro*, ngay cả trong điều kiện sinh lý của cơ thể. Với sự tồn tại của nhóm lactide trong cấu trúc, hydrogel PSMLA thể hiện được khả năng phân hủy sinh học trong điều kiện *in vivo*. Việc sử dụng hệ gel kết hợp này lên vết thương hở trên động vật thí nghiệm đã đem lại khả năng phục hồi vết thương nhanh hơn là chỉ sử dụng hydrogel PSMLA hoặc hydrogel từ fibroin tơ. Điều này thể hiện tiềm năng ứng dụng của hệ vật liệu này cho việc điều trị vết thương hở.

Từ khóa: Fibroin tơ tằm; nhạy pH; hồi phục vết thương hở; hydrogel.

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1. Introduction

Recently, wound healing has gathered considerable attention from biomedical scientific community, besides other important research topics such as drug/protein delivery or bone regeneration. A wound can be described as a defect or break in skin due to physical, chemical or thermal damage [1]. While acute wounds are usually healable within a short time, chronic wounds would take a significantly longer time to fully recover [2]. The natural wound healing is a dynamic, intricate process which consists of four main phases including hemostasis, inflammation, proliferation and remodeling and involves parenchymal cells, extracellular matrix (ECM), blood cells and soluble mediators [3], [4]. In order to improve the healing rate, prevent microbial invasion as well as minimize scar formation, wound dressing materials are applied. The traditional gauze dressing materials only help to cover and conceal the wound, thus not so effective in case of severe wounds. Hence, novel advanced wound dressing materials are on significant demands [2]. A desirable wound dressing material would need to completely seal wound environment, create a moist environment and deliver biologics to stimulate or promote the cellular proliferation or migration and the production of ECM [5]. We have seen a great progress in development of wound dressing materials with a vast number of systems that have been studied and applied such as lyophilized wafers, hydrocolloids, hydrogels, wound healing film, wound healing foam, multi-layered dressings, electrospun nanofibers mats and scaffolds [1]. Hydrogels, which are three-dimensional, water-containing polymeric networks, can be considered as good candidates for wound dressing as they possess high biocompatibility, ability to mimic ECM, the formation of a barrier to prevent pathogens as well as create a hydrated environment to promote body's own healing process [6].

Naturally occurring polymers with their inherent excellent biocompatibility, biodegradability, low toxicity and non-allergenic nature are a promising platform to design hydrogel for wound healing. A vast number of natural polymers have been utilized for wound dressing materials, including hyaluronan, alginate, collagen and chitosan [7]–[10]. Compared to other biopolymers, silk fibroin (SF) extracted from *Bombyx mori* has recently received significant attention for wound dressing due to its inherent unique benefits including outstanding mechanical strength, equivalent biocompatibility degree with collagen, high water and oxygen uptake, low immunogenicity, tunable biodegradation together with the versatility in fabrication, modification and functionalization [11]. Especially, it has been confirmed the potential of SF film for skin repair and regeneration in human clinical trials [12]. Therefore, SF-based biomaterials, in original form, modified formulation or composite/blending system, have been introduced for wound healing application in numbers of previous research [13]–[19]. However, one noteworthy, intrinsic issue of SF-based hydrogel is the difficulty when using hydrogel at high SF concentration. The advantageous thixotropic (shear-thinning and self-healing) characteristic of SF cannot be applied at high concentration due to the very high viscosity of gel-state.

pH-sensitive hydrogels are representatives for "smart" hydrogel generation that provide the phase transition from sol-state into gel-state depending on environmental pH value [20]. This unique property provides the ease in storing and handling of materials at sol-state, as well as the *in situ*-forming ability after injection into wound site. Previously, there has been a

report of sulfamethazine-based, anionic pH-sensitive hydrogel as bio-inspired adhesive for wound healing use [21]. The developed DNA-bearing polyplex-loaded hydrogel system exhibited pH-responsive sol-gel transition, bioresorbability after 2 months, non-inflammation, bioadhesive capability and effectively sealed the wound and promoted tissue regeneration *in vivo*. Addressing the abovementioned issue of SF-based hydrogels, we suggest that the combination with sulfamethazine-based pH-sensitive hydrogel could be a potential solution. This approach would not only endow SF hydrogel with pH-sensitive *in situ*-gelling ability, equip pH-sensitive hydrogel with enhanced biocompatibility but could also make a synergistic effect to improve tissue regeneration. Herein, we report the synthesis of a novel sulfamethazine-based pH-sensitive polymer as well as the extraction of SF from *Bombyx mori* cocoon. The chemical composition of newly developed pH-sensitive polymer is confirmed by NMR spectroscopy. Furthermore, the sol-gel transition behavior of pH-sensitive or hybrid hydrogels is analyzed. *In vivo* biodegradation of pH-sensitive hydrogel is also determined. Finally, *in vivo* application on BALB/c mice is performed to evaluate the feasibility of the hybrid system for wound healing application.

2. Experimental

2.1. Materials

Polyethylene glycol (Mn = 2050 g/mol), dibutyltin dilaurate (DBTL, 95.0%), stannous octoate ($Sn(Oct)_2$, 95.0%), α-thioglycerol (TH, 97.0%), D,L-lactide (LA, 99.0%), 1,6-hexamethylene diisocyanate (HDI, 98.0%), sulfamethazine (SM, 99.0%), thiazolyl blue tetrazolium bromide (MTT, 98.0%), sodium carbonate anhydrous (Na_2CO_3, 99.5%), calcium chloride anhydrous ($CaCl_2$, 93%) and various anhydrous solvents used in the experiments, were obtained from the Sigma-Aldrich Co. (St. Louis, MO, USA). Acryloyl chloride was bought from the Tokyo Chemical Industries (TCI, Tokyo, Japan. *Bombyx mori* silkworm cocoons were kindly provided by Vietnam Sericulture Research Center. The remaining reagents were of analytical grade and utilized as received.

2.2. Preparation of silk fibroin solution and gel-state

In order to obtain aqueous solution of SF, the silkworm cocoons first underwent degumming process to remove sericin. 5g of dried *Bombyx mori* silkworm cocoons were cut into small pieces before being boiled in 2L of 0.05M Na_2CO_3 solution at 90^{o}C for 30 min, followed by rinsing with pure deionized water for three times. The cycle was carried out twice before drying resulting fibroin fibers at 60^{o}C for 24h. Ajisawa's reagent ($CaCl_2$:EtOH:Water with mole ratio of 1:2:8) was used to dissolve SF with final concentration of 15 wt% at 70^{o}C for 2h. Thereafter, the resulting solution was filtered, cooled down before pouring into dialysis membrane with molecular weight cut off of 3500 Da. Fibroin solution was dialyzed against excess amount of pure deionized water for 2 days with several changes of water at 1h, 3h, 6h, 12h, 24h and 36h to obtain pure aqueous fibroin solution. Final concentration of SF solution was determined by simply drying small amount of solution and measuring the remained weight. Gelation of SF aqueous solution was induced by ultrasonication at 50% amplitude (21W) for 30 s, followed by incubating in 37^{o}C water bath overnight [22].

2.3. Synthesis of sulfamethazine-based pH-sensitive copolymer

The sulfamethazine-based pH-sensitive copolymer was synthesized according to the scheme in **Figure 1**. In the first and second steps, sulfamethazine-acrylate (SMA) and sulfide-sulfamethazine (SSM) were synthesized following published protocols [21], [23]–[25]. Thereafter, ring-opening addition of LA to SSM in the presence of $Sn(Oct)_2$ as the initiator was carried out to synthesize SM-LA. Briefly, 0.2 mmol of $Sn(Oct)_2$, 10 mmol of SSM and 10 mmol of LA were added into a round-bottom flask and dried at 50^{o}C for 12h to remove moisture. Thereafter, 1,4-dioxane was added to dissolve reactants and the reaction was taken place at 110^{o}C with magnetic stirring. After 24h, the flask was cooled down and the product (SM-LA) was precipitated in an excess amount of diethyl ether before filtering and drying under vacuum for 48h. The last step is the polyaddition polymerization of SM-LA, PEG and HDI in the presence of DBTL as the initiator. Briefly, 1 mmol of PEG and 0.04 mmol of DBTL were added into two-neck round flask and vacuum-dried at 110^{o}C for 2h. Afterward, the temperature was reduced to 60^{o}C followed by addition of 3 mmol of SM-LA and drying for 1h. Then, tetrahydrofuran (THF) was added to dissolve reactant under nitrogen and the reaction was carried out at 62^{o}C for 5h with continuous stirring. The resultant copolymer (termed as PSMLA) was precipitated using diethyl ether in excess amount, filter and dried at room temperature under vacuum for 48h. The H-NMR spectra of PSMLA was obtained using a Varian Unity Inova 500NB spectrometer with an operation frequency of 500 Mhz and DMSO-d6 as solvent.

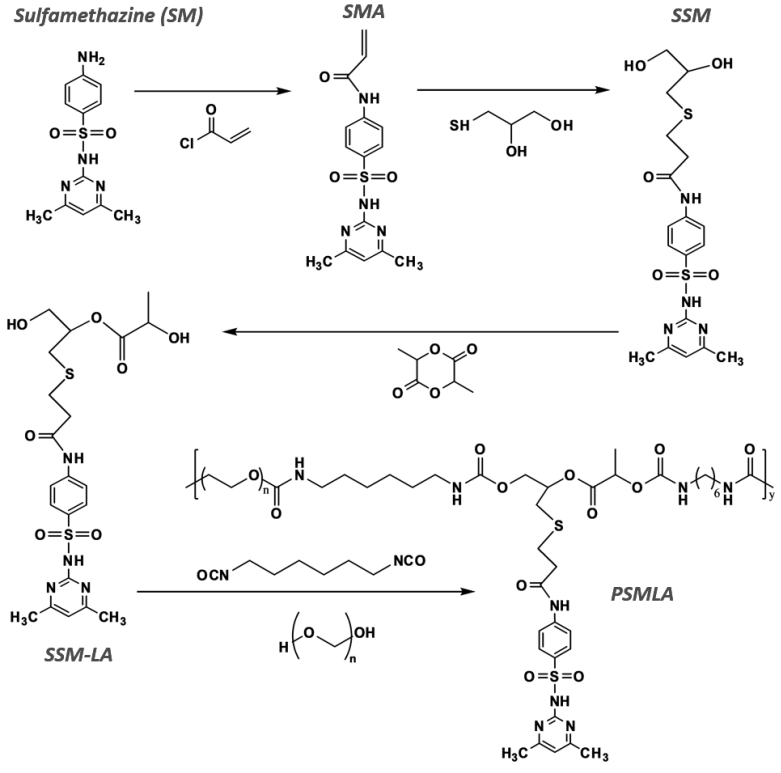

Figure 1. Schematic synthesis routes of SMA, SSM, SSM-LA monomers and PSMLA copolymer

2.4. Sol-gel transition behavior

In order to determine the sol (flow)-gel (non-flow) phase transition behavior of PSMLA as well as the blending system at different pHs, the vial inverting method was applied. The 15 wt% PSMLA solution was prepared by dissolving dried PSMLA with phosphate buffered saline (PBS) at pH 9.0 (by adding NaOH). The blending solution was prepared simply by mixing solution of 15wt% PSMLA with SF solution 2 wt%. The pHs of resulting solutions were adjusted to predetermined values using 5M NaOH and 5M HCl in prior to stabilizing at 4^{o}C overnight. After stabilization, 5mL vials containing 0.5 mL of samples were put into a temperature-controlled water incubator. The temperature was increased slowly from 2^{o}C to 60^{o}C with temperature interval of 2^{o}C and stabilization time of 10 min. The gel-state was determined whenever the sample cannot flow after inverting and keeping for 30 s.

2.5. In vivo gelation and biodegradation

In order to evaluate the biodegradability of PSMLA, *in vivo* experiments on male Sprague-Dawley (SD) rats were performed. The 5-6 weeks old rats were cared and handled according to the National Institutes of Health (NIH) guidelines as well as Sungkyunkwan Pharmacy School's regulation. 200 mL PSMLA 15 wt% solution at pH 9.0 was subcutaneously injected onto SD rats after appropriate anesthetizing. To check the formation of gel-state after injection of PSMLA solution, the rat was sacrificed and the injection site was observed after cutting with an operation scissor. Moreover, to address the biodegradability of PSMLA, the gels at 10 min, 1 week, 2 weeks, 4 weeks and 7 weeks after injection were removed from skin site and frozen-dried for 3 days before measuring remained weight.

2.6. Wound healing efficacy

Male SD rats were anesthetized while the hair on target skin site was gently removed. Thereafter, a sterilized surgical knife was used to create full-thickness incisions with a diameter of 1 cm on the center of SD rat back. Thereafter, four rats were received injection of 0.1 mL of samples including PBS solution at pH 7.4 for the control group), PSMLA 15wt%, SF 2wt% and blending system onto surface of the wound incisions. The wound healing efficacy was observed at day 0, 1, 2, 3, 5, 7, 10 post-wounding and photographs were taken at a constant distance using a digital camera.

3. Results and discussion

3.1. Synthesis of PSMLA

The pH-sensitive PSMLA copolymer was prepared via a four-step route schemed in **Figure 1**. Double bond-bearing SM-A was synthesized by acrylating the amine end-group of sulfamethazine in the first step. Thereafter, SM-A was reacted with α-thioglycerol in a Michael addition reaction to endow two hydroxyl end groups. The resulting derivative was modified with LA to incorporate the hydrolytic biodegradation property. Finally, a urethane-forming reaction between HDI, SMLA and PEG was carried out to generate PSMLA copolymer. In ^{1}H-NMR spectrum of synthesized PSMLA (**Figure 2**), peaks at 3.30 (l), 1.34 (m), 1.21 (n) ppm indicates protons from methylene groups of HDI units [23]. Besides, peak at 3.60 ppm (o) was assigned for characteristic methylene group of PEG. The appearance of LA unit was confirmed via peak of methine proton at 5.05 ppm (j) [26]. Furthermore, the presence of sulfamethazine group was determined by peaks at 7.95 (c) and 7.75 (d) ppm [23]. Overall, characteristic peaks in H-NMR spectrum have confirmed the successful synthesis of PSMLA which consists of anionic pH-sensitive SM, hydrophilic PEG, biodegradable LA and enzymatic-degradable urethane links.

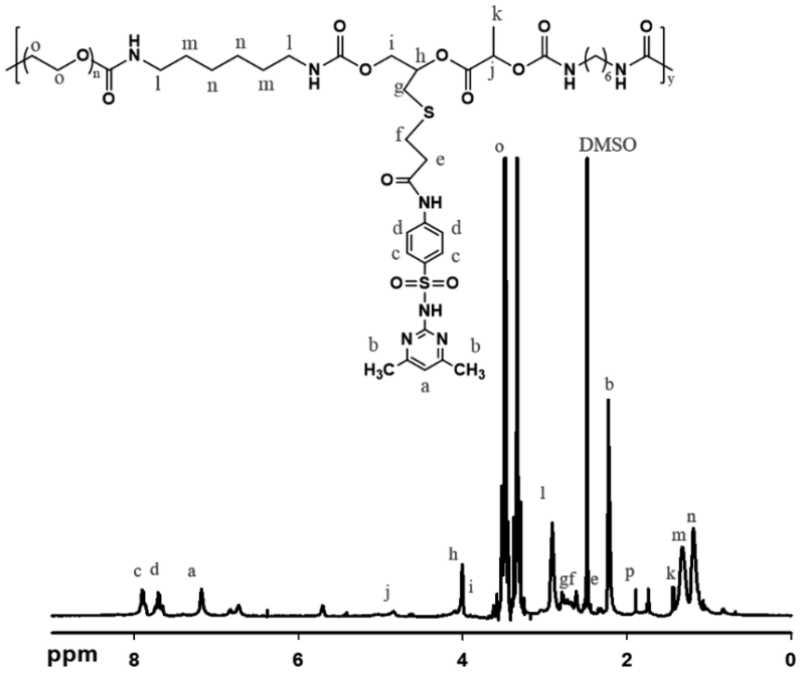

Figure 2. H-NMR spectrum of the synthesized copolymer PSMLA

3.2. In vitro sol-gel phase transition

The sol-to-gel phase transition of PSMLA as well as PSMLA-SF blending system were studied using the tube inverting method. As shown in **Figure 3**, the sol-gel transition from flowing state into the non-flowing state was driven by pH. The aqueous solution of PSMLA 15wt % appeared as the sol-state at high pH (>7.8) due to the ionization of anionic pH-sensitive sulfamethazine moiety, which made copolymer more hydrophilic. However, when lowering down environmental pH, the sulfamethazine group is deionized, which led to the increase of hydrophobicity of PSMLA copolymer. Hence, the enhanced micelle formation will be occurred due to the increase of interaction between hydrophobic segments of PSMLA. With the hydrophilic bridges between these micelles, three-dimensional network of hydrogel was formed and created the non-flowing gel-state at low pH. The upper gel-to-sol transition temperature at neutral and low pH is due to the dehydration of PEG chains as well as the breaking of hydrogen bonding between urethane links of PSMLA blocks. Herein, the gel-state region covered body condition (37ºC, pH 7.4), which indicates the gelability of PSMLA aqueous solution after injecting into physiological environment. Finally, by adding SF 2wt % into PSMLA solution, the resulting blending system exhibited narrower gel-region. This could be explained that the long-chain, large molecular weight of silk fibroin molecules perhaps hindered the bridging between PSMLA micelles, thus loosened the interaction of hydrogel network. Nevertheless, the gel-state region still included the physiological condition, which also proved the potential for use in *in vivo* experiments. *In vitro* gelation figures also demonstrated the transition of PSMLA and PSMLA-SF solutions from transparent free-flowing sol-state at pH 8.9, room temperature into turbid non-flowing gel-state at physiological condition, indicated the pH-responsive gelation ability after injecting into body.

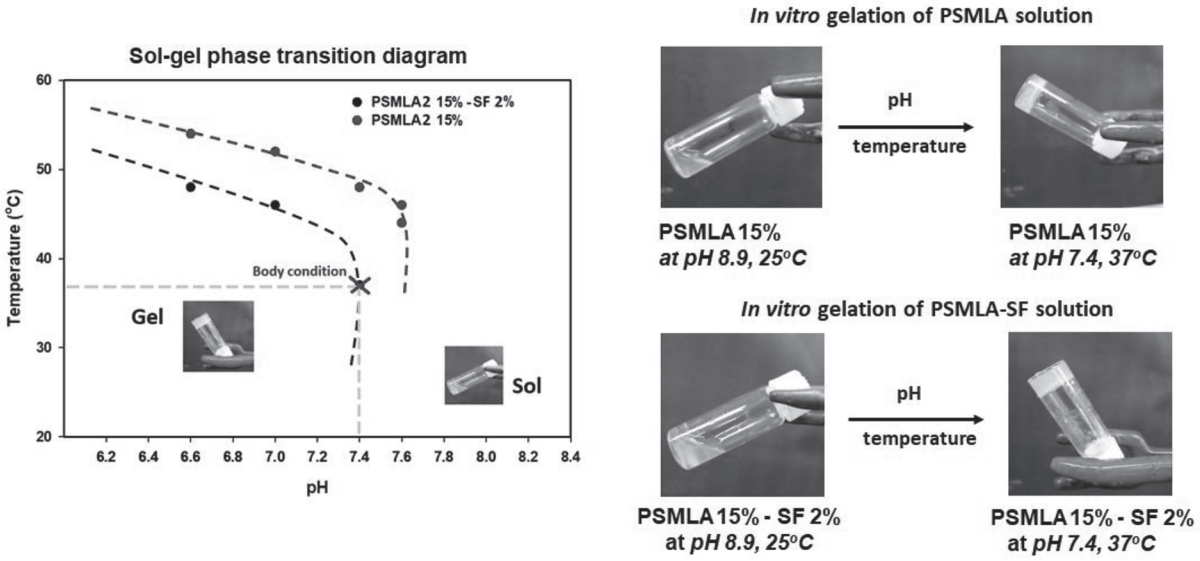

Figure 3. Sol-gel phase transition and in vitro gelability of PSMLA and PSMLA-SF aqueous solutions

3.3. In vivo gel biodegradation

Biodegradation is pivotal property for *in vivo* application of biomaterials. To determine the biodegradability of newly synthesized PSMLA, we performed subcutaneous injection on SD rats. As presented in **Figure 4**, the aqueous solution of PSMLA 15% exhibited obvious gel formation within 10 min. Thereafter, the gel size was reduced gradually throughout the examined time period, which clearly suggests the *in vivo* degradability of PSMLA gel. After 7 weeks, the remaining gel weight was 20% compared to initial dried weight. With the presence of lactide unit, the degradation of PSMLA could be partly driven by hydrolysis of ester bonds. Moreover, the urethane links among copolymer chains could also be slowly degraded by enzymatic mechanism. The result from PSMLA suggests that the blending system would also present *in vivo* biodegradation since SF has been widely known as bio-degradable material [27].

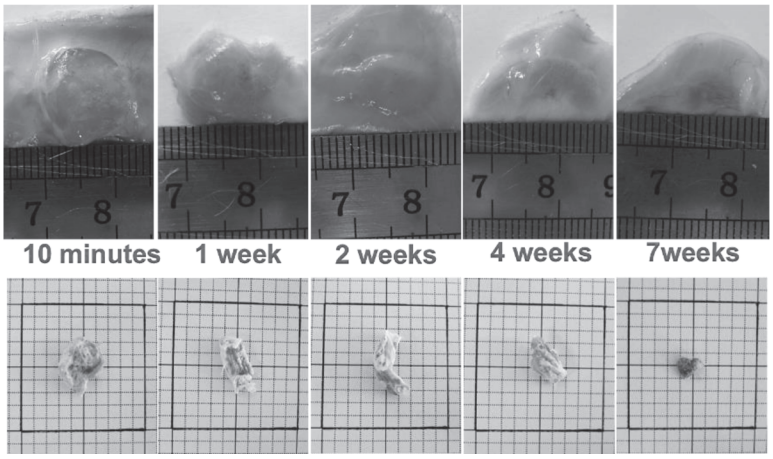

Figure 4. In vivo degradability of PSMLA hydrogel

3.4. Wound healing efficacy

The *in vivo* preclinical application of the PSMLA hydrogel and blending system as wound dressing materials was evaluated and compared against SF hydrogel as well as PBS solution (control group). The wound healing

efficacy was monitored at predetermined times including 10 min, 1d, 2d, 3d, 5d, 7d and 10d. As clearly observed in **Figure 5**, the wound treated with hydrogels provided a better overall healing rate compared to control group which used PBS solution. Interestingly, the healing effect of animal model treated by PSMLA 15 wt% gel was somehow comparable to SF 2wt % gel. SF hydrogel clearly presented superior efficacy over PSMLA hydrogel since the much lesser concentration of SF was used. This could be explained that the protein nature of SF has contributed much to help the growth of endothelial cells in proliferation and migration phases of the wound healing process. However, PSMLA still held visible potential over the control group. This could be due to the bioadhesive property that made a positive effect in preventing the invasion of surrounding microorganisms into wound-site. Finally, the combination of PSMLA 15wt% and SF 2wt% provided synergistic effect, as proved by smaller of wound site at day 7 and day 10 of blending system-treated animal. The hybrid system has combined the strong bioadhesive property and pH-sensitivity from PSMLA copolymer with the superior biocompatibility and non-immunogenicity by SF. Indeed, the pH-sensitive from PSMLA has endowed the system with ease in handling and injection ability at the sol-state. Moreover, the self-assembly mechanism of SF can further strengthen the *in vivo* gel via β-sheet transition. This valuable result definitely would unravel the promising feasibility of the developed pH-sensitive SF-based blending hydrogel for the treatment of various types of wounds.

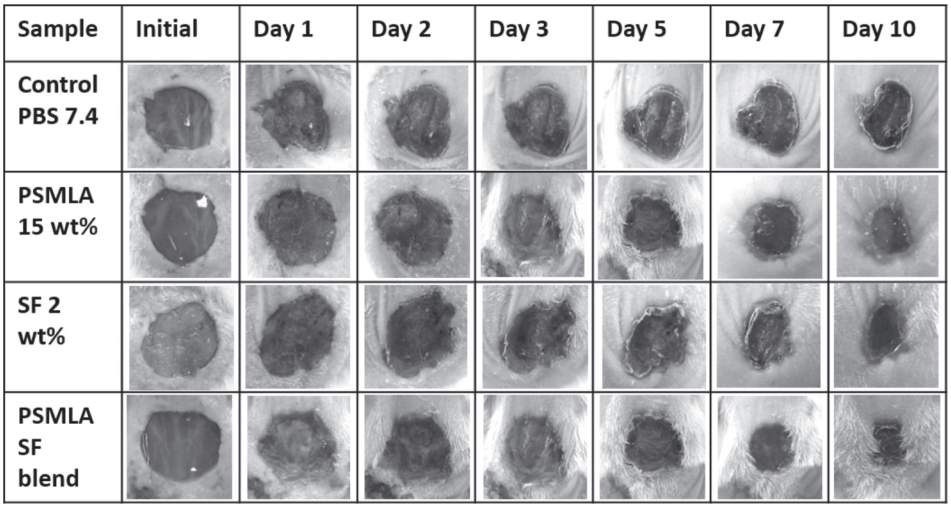

Figure 5. Wound healing efficacy after in vivo application on mice models

4. Conclusion

Herein, we introduced the novel pH-sensitive, *in situ*-forming blending hydrogel from SF and newly synthesized PSMLA copolymer. The blending system represented the sol-to-gel transition with the gel region covered body condition. *In vivo* biodegradation of PSMLA hydrogel was confirmed on rat models with 20% of remaining weight after 7 weeks. Finally, the blending system showed a synergistic effect that accelerated the wound healing process more than solely utilization of PSMLA or SF hydrogels. This evidence undoubtedly revealed the potential of the developed system as wound dressing materials.

References

[1] N. Mayet *et al.*, "A comprehensive review of advanced biopolymeric wound healing systems," *J. Pharm. Sci.*, vol. 103, no. 8, pp. 2211–2230, 2014.

[2] J. Boateng and O. Catanzano, "Advanced Therapeutic Dressings for Effective Wound Healing - A Review," *J. Pharm. Sci.*, vol. 104, no. 11, pp. 3653–3680, 2015.

[3] L. E. Dickinson and S. Gerecht, "Engineered biopolymeric scaffolds for chronic wound healing," *Front. Physiol.*, vol. 7, no. AUG, 2016.

[4] S. Das and A. B. Baker, "Biomaterials and nanotherapeutics for enhancing skin wound healing," *Front. Bioeng. Biotechnol.*, vol. 4, no. OCT, pp. 1–20, 2016.

[5] R. Z. Murray, Z. E. West, A. J. Cowin, and B. L. Farrugia, "Development and use of biomaterials as wound healing therapies," *Burn. Trauma*, vol. 7, no. 1, pp. 1–9, 2019.

[6] E. A. Kamoun, E. R. S. Kenawy, and X. Chen, "A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings," *J. Adv. Res.*, vol. 8, no. 3, pp. 217–233, 2017.

[7] Bp. S. Thomas, "Alginate dressings in surgery and wound management – part 1," *J. Wound Care*, vol. 9, no. 2, pp. 9–13, 2000.

[8] I. Bano, M. Arshad, T. Yasin, M. A. Ghauri, and M. Younus, "Chitosan: A potential biopolymer for wound management," *International Journal of Biological Macromolecules*, vol. 102. pp. 380–383, 2017.

[9] S. Chattopadhyay and R. T. Raines, "Review collagen-based biomaterials for wound healing," *Biopolymers*, vol. 101, no. 8. pp. 821–833, 2014.

[10] C. Longinotti, "The use of hyaluronic acid based dressings to treat burns: A review," *Burn. Trauma*, vol. 2, no. 4, p. 162, 2014.

[11] M. Farokhi, F. Mottaghitalab, Y. Fatahi, A. Khademhosseini, and D. L. Kaplan, "Overview of Silk Fibroin Use in Wound Dressings," *Trends Biotechnol.*, vol. 36, no. 9, pp. 907–922, 2018.

[12] W. Zhang *et al.*, "Silk Fibroin Biomaterial Shows Safe and Effective Wound Healing in Animal Models and a Randomized Controlled Clinical Trial," *Adv. Healthc. Mater.*, vol. 6, no. 10, pp. 1–16, 2017.

[13] H. W. Ju *et al.*, "Silk fibroin based hydrogel for regeneration of burn induced wounds," *Tissue Eng. Regen. Med.*, vol. 11, no. 3, pp. 203–210, 2014.

[14] X. Li, Y. Liu, J. Zhang, R. You, J. Qu, and M. Li, "Functionalized silk fi broin dressing with topical bioactive insulin release for accelerated chronic wound healing," *Mater. Sci. Eng. C*, vol. 72, pp. 394–404, 2017.

[15] P. Thangavel, B. Ramachandran, R. Kannan, and V. Muthuvijayan, "Biomimetic hydrogel loaded with silk and l-proline for tissue engineering and wound healing applications," *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, vol. 105, no. 6, pp. 1401–1408, 2017.

[16] S. Kanokpanont, S. Damrongsakkul, J. Ratanavaraporn, and P. Aramwit, "An innovative bi-layered wound dressing made of silk and gelatin for accelerated wound healing," *Int. J. Pharm.*, vol. 436, no. 1–2, pp. 141–153, 2012.

[17] P. Uttayarat, S. Jetawattana, P. Suwanmala, J. Eamsiri, T. Tangthong, and S. Pongpat, "Antimicrobial electrospun silk fibroin mats with silver nanoparticles for wound dressing application," *Fibers Polym.*, vol. 13, no. 8, pp. 999–1006, 2012.

[18] Z. X. Cai *et al.*, "Fabrication of chitosan/silk fibroin composite nanofibers for Wound-dressing Applications," *Int. J. Mol. Sci.*, vol. 11, no. 9, pp. 3529–3539, 2010.

[19] A. Vasconcelos, A. C. Gomes, and A. Cavaco-Paulo, "Novel silk fibroin/elastin wound dressings," *Acta Biomater.*, vol. 8, no. 8, pp. 3049–3060, 2012.

[20] Q. V. Nguyen, D. P. Huynh, J. H. Park, and D. S. Lee, "Injectable polymeric hydrogels for the delivery of therapeutic agents: A review," *Eur. Polym. J.*, vol. 72, pp. 602–619, 2015.

[21] T. M. D. Le, H. T. T. Duong, T. Thambi, V. H. Giang Phan, J. H. Jeong, and D. S. Lee, "Bioinspired pH- and Temperature-Responsive Injectable Adhesive Hydrogels with Polyplexes Promotes Skin Wound Healing," *Biomacromolecules*, vol. 19, no. 8, pp. 3536–3548, 2018.

[22] X. Wang, D. N. Rockwood, D. L. Kaplan, T. Yücel, M. L. Lovett, and R. C. Preda, "Materials fabrication from Bombyx mori silk fibroin," *Nat. Protoc.*, vol. 6, no. 10, pp. 1612–1631, 2011.

[23] V. H. G. Phan, T. Thambi, M. S. Gil, and D. S. Lee, "Temperature and pH-sensitive injectable hydrogels based on poly(sulfamethazine carbonate urethane) for sustained delivery of cationic proteins," *Polymer (Guildf).*, vol. 109, pp. 38–48, 2017.

[24] Q. V. Nguyen *et al.*, "A novel sulfamethazine-based pH-sensitive copolymer for injectable radiopaque embolic hydrogels with potential application in hepatocellular carcinoma therapy," *Polym. Chem.*, vol. 7, no. 37, pp. 5805–5818, 2016.

[25] Q. V. Nguyen, M. S. Lee, J. S. Lym, Y. Il Kim, H. J. Jae, and D. S. Lee, "PH-Sensitive sulfamethazine-based hydrogels as potential embolic agents for transcatheter vascular embolization," *J. Mater. Chem. B*, vol. 4, no. 40, pp. 6524–6533, 2016.

[26] N. K. Singh, Q. V. Nguyen, B. S. Kim, and D. S. Lee, "Nanostructure controlled sustained delivery of human growth hormone using injectable, biodegradable, pH/temperature responsive nanobiohybrid hydrogel," *Nanoscale*, vol. 7, no. 7, pp. 3043–3054, 2015.

[27] C. Holland, K. Numata, J. Rnjak-Kovacina, and F. P. Seib, "The Biomedical Use of Silk: Past, Present, Future," *Advanced Healthcare Materials*, vol. 8, no. 1. 2019.