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Characterization and Evaluation of Silk Sericin-Based Hydrogel: A Promising Biomaterial for Efficient Healing of Acute Wounds

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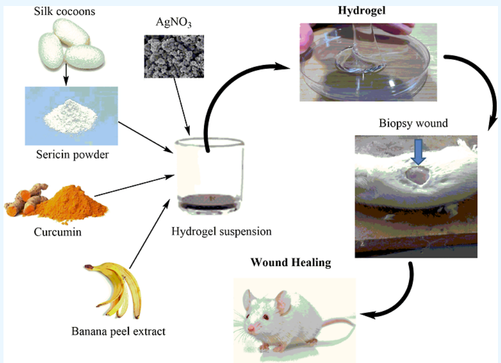
ABSTRACT: The present study was aimed to prepare the potent silk sericin-based hydrogels in combination with plant extracts (curcumin and banana peel powder) and silver nanoparticles (AgNPs) to accelerate the acute wound healing process. Experimental excision wounds were created in mice by biopsy puncture, and the wound healing potential of silk sericin (2%)-based hydrogel and its combinations with curcumin (2%), banana peel powder (2%), and AgNPs (2%) was estimated by calculating the percent wound contraction, healing time, histology of skin tissues, and different biochemical tests. The results showed that the mice treated with sericin-based hydrogels showed significantly ($P < 0.001$) high percent wound contraction as compared to negative control, and wounds were healed in 11 days. The histological evaluation also showed that wounds covered with hydrogels were healed more than the uncovered wounds. Furthermore, the results of biochemical tests revealed that the treatment groups showed a significant ($P < 0.001$) decrease in the serum level of pro-inflammatory cytokines (IL-6). A significant ($P < 0.001$) increase in anti-inflammatory cytokines (IL-10) and anti-oxidant enzymes was observed in treatment groups. The highest wound healing potential was observed by sericin-based hydrogel containing banana peel powder, leaving behind the commercially available ointment polyfax (positive control). It can be concluded that the silk sericin-based hydrogels in combination with plant extract and AgNPs can be used as natural biomaterials in wound dressing for the rapid healing of acute wounds.



INTRODUCTION

Wounds are caused by any damage to the tissue of the skin such as trauma, bacterial infection, or underlying medical conditions.[1,2] Wounds are classified into two types such as chronic and acute.[3] Healing of wounds is a biological progression, which consists of four phases, that is, homeostasis, inflammation, proliferation, and remodeling.[4,5] To heal the wounds, wound dressing must be of the following qualities; it must absorb excess exudate from the skin, have minimal side effects, be non-allergenic and non-adherent, have antimicrobial activity, and can maintain moisture.[6−8] Dry dressings such as cotton and bandages cannot maintain a moist environment and can cause secondary trauma upon removal.

Despite the large number of wound dressings available today, there is a dire need to improve wound dressing's performance. Nowadays, different synthetic and natural polymers are used to fabricate the wound dressings, which ensures maximum recovery and wound healing.[1] Hydrogels are the main type of wound dressing used for wound healing.[9] It is made up of natural or synthetic material which consists of three dimensional networks.[10] The structure of hydrogel is formed by the hydration in an aqueous environment. A hydrogel has the ability to hold and absorb water and also minimizes the hazard of secondary trauma. This attribute of hydrogel assists in wound healing.[11]

Natural products-based hydrogels are preferred due to their biocompatibility and superior therapeutic potentials.[12,13] Moreover, natural products have high antioxidant potential that makes them a perfect candidate for wound management.[14−16] Silk, a functional biomaterial synthesized by silkworms in the form of proteins, possesses the therapeutic potential against acute and diabetic wounds.[17] Silk sericin is an excellent biomaterial with extraordinary properties, which make it a good candidate to be used in wound dressing. Sericin is obtained from silkworm cocoon, which is a hydrophilic protein and comprises 25−30% portion of cocoon. Two natural macromolecular proteins such as sericin and fibroin are produced from silkworms, *Bombyx mori*.[18,19] Sericin is made up of 18 amino acids.[20] Sericin consists of high

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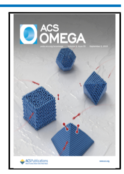

molecular and granular proteins, which give it gelatin-like and adhesive features. Due to the presence of different hydroxyl groups, it can absorb a large amount of water on skin.[21] It mainly consists of serine amino acid (30%), which gives the skin natural moisturization.[22]

Sericin has different properties such as UV light protection, antioxidant, anticancer, and antibacterial.[23,24] Silk sericin has good biocompatibility, hydrophilicity, excellent affinity for biomolecules, and biodegradability.[25] Sericin has mitogenic and cytoprotective effects on keratinocytes and fibroblasts as well as it attracts them to the skin for tissue repair and skin development.[26] Topical application of sericin-based wound hydrogel accelerates deposition of collagen and skin tissue re-epithelization to promote the wound healing process.[27] Due to its immunomodulatory and reactive oxygen species (ROS) clearance capacities, sericin serves as the main active biological substance in wound dressings.[28] Nevertheless, sericin cannot be used independently for making hydrogels because it has an amorphous nature and poor mechanical strength.[29] Amines, hydroxyl, and carboxyl groups are the polar side chains of sericin that make it possible to crosslink with other biomaterials which in turn enhances its mechanical performance.[19] Copolymerization, crosslinking, and blending with some other biomaterials help to decrease the brittleness of sericin.[30]

Polyvinyl alcohol is used with sericin to prepare hydrogels because it increases its mechanical property.[31] Carboxymethyl cellulose (CMC) is another polymer, which is an acid derivative of cellulose and water soluble cellulose ether. It has various biomedical applications as an antioxidant due to its biodegradability, low cost, and non-toxicity.[32] It absorbs excess exudate in the vicinity of wounds, acts as a cell carrier, and helps in the control delivery of drugs.[33] CMC has the advantage of blending with other polymers and forming hydrophilic films, which are compatible with skin, mucous membrane, and bone.[34]

The aforementioned designated wound healing hydrogel properties can be further enhanced by blending them with natural bioactive agents. Curcumin is a natural polyphenolic group which is extracted from the plant *Curcuma longa* rhizome. It is a natural herbal product, which has great potential for wound healing because it contains bioactive agents with numerous properties such as anti-inflammatory, anti-oxidant, anti-HIV protease, and anti-bacterial activity.[35,36] Peels of unripe banana contain sodium, calcium, copper, potassium, zinc, iron, and phosphorus. The extract of unripe banana peel endorses/incorporates thymidine into cellular DNA, which stimulates the proliferation of cells. Peels of unripe banana contain a flavonoid known as leucocyanidin, which stimulates the proliferation of cells and ultimately helps in wound healing.[37]

Nowadays, to increase the antimicrobial activity of hydrogels, different types of nanoparticles are used. Nanomedicine has a widespread use in making different types of metal nanoparticles such as Ag, platinum, and gold. Out of all these nanoparticles, silver nanoparticles due to their surface plasmon resonance have gained much more attention in nanomedicine.[38,39] Moreover, silver nanoparticles have also gained a lot of attention due to their antimicrobial activity and low tendency to develop resistance in the treatment of wound healing.[40,41] Sericin acts as a reducing agent for the fabrication of silver nanoparticles.[42]

■ MATERIAL AND METHODS

Extraction of Sericin from Cocoons. Freshly woven silkworm cocoons were obtained from Sericulture Wing, Forestry Wildlife and Fisheries Department, Punjab, Pakistan. Silk sericin was obtained by cutting the cocoons into small pieces after washing them with distilled water. Then, 10 g of pieces of cocoons were added in 100 mL of distilled water and autoclaved for 30 min at 120 °C. The solution was allowed to cool at room temperature for 1 h. The filter paper was used to filter the sericin solution. Fibroin remained in the filter cake, while the sericin passed through the filter paper.[7] To obtain the powder of sericin, the solution was lyophilized at−20 °C.[43]

Extraction and Preparation of Plant Extracts. Fresh banana peels were taken, washed, sterilized with 70% ethanol, dried in an oven for 48 h at 70 °C, and subsequently ground into fine powder. Then the powder was added to 70% ethanol and left at room temperature for 48 h; after that, the entire slurry was filtered with Whatman no. 1 filter paper (Whatman 1442-125 125 mm no. 42 Ashless Filter Paper Circles). A rotatory evaporator was used to evaporate the ethanol. The powdered extracts (0.2 g) of banana peel extract and curcumin were added to 10 mL of distilled water separately to prepare 2% extract of both banana peel extract and curcumin.

Preparation of Different Types of Hydrogels. Sodium carboxy-methyl-cellulose (2% w/v) and poly vinyl alcohol (PVA) (2% w/v) solutions (10 mL each) were prepared in distilled water to form a homogenous solution. Then, 2% of sericin was prepared by dissolving 0.2 g lyophilized sericin powder in 10 mL of distilled water at 80 °C to form a homogenous solution. Then, the sericin solution (10 mL) was poured into a mixture of Na-CMC and PVA solutions (20 mL) with continuous stirring for 20 min to promote the blending of sericin and PVA. The solution was poured into Petri plates (Standard 90 mm Petri dishes). SS/PVA/Na-CMC hydrogel was prepared after repeated freeze and thaw cycles to form the stable sericin 2% hydrogel.[7] Likewise, different types of hydrogels (sericin 2% + AgNPs 2% hydrogel; sericin 2% + BPP 2% hydrogel; sericin 2% + curcumin 2% hydrogel) were prepared by mixing (2% w/v) solutions of AgNPs, banana peel powder extract, and curcumin with SS/PVA/Na-CMC solution. After repeated freeze−thaw cycles, hydrogels were formed. Then the hydrogel solutions were stored in a refrigerator (9188WBLVS R).

Characterization of Hydrogels. *FTIR.* The chemical nature of silk sericin-based hydrogels was determined by Fourier transform infrared spectroscopy (FTIR). This technique was used to detect the functional groups present in the prepared hydrogels. The range of spectrum was fixed between 4000 and 400 cm^{-1}.

SEM. Surface morphology of sericin based hydrogels was analysed by using scanning electron microscope (SEM) at the Centre of Advanced Studies in Physics (CASP), Government College University, Lahore. For SEM analysis, the samples were prepared by lyophilizing the hydrogels.

Rearing of Mice. The Swiss albino mice weighing around 25−30 g were used as the experimental model. They were reared in standard plastic cages in the Animal House of the Department of Zoology, Government College University Lahore. Standard laboratory conditions, i.e., 20−22 °C temperature, 12 h light and dark cycle, and 45−65% humidity were maintained for the animals. All the animals were fed with water and a standard animal diet in the cage. The animal

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experiment was carried out in accordance with the guideline of the ethical committee. Pathogen-free and healthy mice were taken for the experiment. Animals were acclimatized for 1 week prior to the study. The study was conducted after getting permission from the University ethical committee (Vide letter no. GCU-IIB-827: Dated 21st January, 2021), and all the experiments were performed during day time.

Creation of Skin Wound. After 1 week of acclimatization, the mice were randomly divided into 6 groups, with 5 mice in each group. There were two control groups, i.e., negative control in which saline solution (0.9%) was used to wash wounds and positive control in which clinical ointment (polyfax) was used. The remaining four groups were treated with sericin based hydrogels. Mice of all groups were anesthetized with intraperitoneal injections of a solution containing xylazine, ketamine, and saline in the ratio of 4:1:15 (μL) prior to the experiment. Electrical shaving machine was used for shaving the surgical area. The skin was disinfected with 70% alcohol. Two full thickness excision wounds were created on the dorsum of each mice by using a 6 mm biopsy punch device. These surgical interventions were carried out under sterile conditions. For each mice, all the surgical procedures were of 15−20 min. Treatment was applied on all animals once a day from post wounding day 1 till the complete healing of wound. Skin irritation, body weight, and skin color were observed and recorded daily.

Percent Wound Contraction. The mice with excision wounds were subjected to their respective treatments from the 1st day till complete wound healing. The hydrogels were evenly applied on the surface of the wounds daily. The wound margins were traced on a transparent graph paper at 2 days intervals after the creation of wound. Measurements were continuous until thorough repair of the wound. The healed wound area was calculated after 2 days of intervals. The contraction was termed as percent wound contraction at the wound site and the process of reepithelialization was observed after complete wound healing.

The rate of healing as percent wound contraction was measured by using the following formula

$$\text{Wound contraction (\%)} = (S0 - S/S0) \times 100\%$$

In the abovementioned equation, $S0$ is the original wound area and S is the wound area after applying the hydrogel.[20]

Histological Evaluations. On the day 6 and 12, the skin tissues of the wound area were taken and the central portion of tissue was fixed by using formalin (10% v/v) (pH = 7). Alcohol was used for dehydration and xylene for clearance. After slicing the tissues into 5 mm thin microtome, stains such as hematoxylin and eosin were used. After preparing the histological slides, photographs were taken and examined histologically under a light microscope using low power magnification[7]

Measurement of Anti-Oxidants Level. The blood of mice from each respective group was obtained through cardiac puncture on post-surgery 5th and 11th day. Blood was centrifuged for 10 min at 4000 rpm, and serum was separated. The level of different antioxidants, i.e., SOD, GSH, and GSH-Px in serum was determined by using biochemical kits. Manufacturer's instructions were used to perform this assay. GSH concentration was measured in mg/L, SOD concentration in U/mL, and GSH-Px concentration in U/L. All the experiments were completed in quintuplicates to confirm the observations.

Estimation Anti-Inflammatory (IL-10) and Pro-Inflammatory Cytokines (IL-6). Blood samples of mice from each group were collected on post wounding days 5 and 11. An enzyme-linked immunosorbent assay (ELISA) kit (Mouse interleukin 10 ELISA Kit; cat. no. E0022Mo; standard curve range: 5−2000 pg/mL; sensitivity: 2.48 pg/mL; size: 96 wells) was used to estimate the anti-inflammatory (IL-10) and pro-inflammatory cytokines (IL-6) level. Manufacturer's instructions were used to perform this assay. Cytokine concentration was measured in pg/mL, and the graph was plotted for standard. All the experiments were completed in quintuplicates to confirm the observations.

Statistical Evaluations. For statistical analysis, normality of the data was assessed first, by using Shapiro−Wilk test. One-way ANOVA was carried out in order to compare the control and treatment groups, followed by Tukey's post-hoc test using SPSS software (version 28.0.0.0). All the data was presented as ± SEM.

■ RESULTS

FTIR. The FTIR analysis of the dried hydrogels showed different peaks, which confirmed the presence of different functional groups in the hydrogel. The FTIR peaks at 3200−3550 cm^{-1} showed the presence of the O−H functional group in all samples. These peaks are the confirmation of hydroxyl groups of water content. Amide I, amide II, and amide III are the characteristics of sericin content in the hydrogel. The peak at 1600−1700 cm^{-1} showed the presence of amide I of sericin. Amide II was identified at 1510−1580 cm^{-1}, while the peak at 1200−1350 cm^{-1} confirmed the presence of amide III. Three characteristic bands of FTIR of all four types of dried hydrogels are the confirmation of PVA functional groups such as the peak at 2840−3000 cm^{-1} confirmed the presence of (C−H) groups, and (C−O) stretching showed at peak 1085−1150 cm^{-1} and (C−H) bending at 830−833 cm^{-1}.

AgNP formation did not affect the peaks of amide of sericin, which showed that structure was not changed. There was a shifting of the N−H vibrational band from 1517 to 1526 cm^{-1}, which was the indication of blending of PVA with sericin, which slightly alters the original structure of sericin. The characteristic band of CMC was at 800 and 1200 cm^{-1} corresponding to the C−O−C and C−O bending and stretching of C−O, C−H of glycosidic linkage, which were shifted due to the incorporation of sericin. It confirmed the hydrogen bonding and side chain reactions between CMC and sericin.

The functional group extracted from the unripe banana peel powder was also detected by infrared spectroscopy analysis, in which peaks were obtained at 1600 cm^{-}1, which indicated the presence of C═C, which is a functional group of the aromatic ring of the tannins/phenol group. Spectra were also observed at 1320 and 1340 cm^{-1}, which was a characteristic of the presence of deformation of the C−O−H angle of phenol resulting from tannins ring. Stretch in the peak at 1340 cm^{-1} showed the presence of C−O of the C-ring of flavonoids. The characteristic peak of curcumin at 856−1508 cm^{-1} was due to the C−O−C and C═O vibrations. This was also slightly shifted due to physical cross linking as shown in Figure 1.

SEM. Scanning electron microscopy (SEM) showed cross section micrographs of the all four types of hydrogels made up of sericin, PVA, CMC in combination with AgNPs and plant extract, i.e., banana peel powder and curcumin. No separation of components and boundaries was observed, indicating a good

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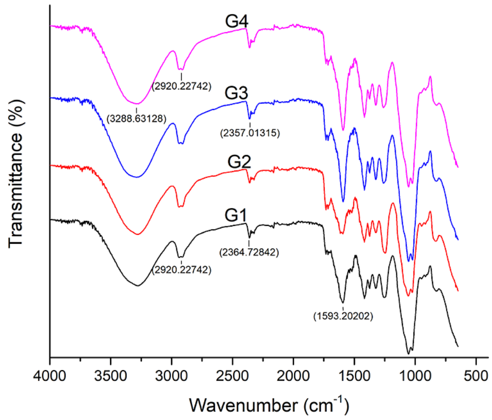

Figure 1. FTIR spectra of (G1) sericin-based hydrogel; (G2) sericin-based hydrogel loaded with AgNPs; (G3) sericin-based hydrogel loaded with banana peel powder; (G4) sericin-based hydrogel loaded with curcumin.

compatibility of components present in the hydrogel. The hydrogel containing only PVA, CMC, and sericin showed a uniform structure. The hydrogel formed of pure sericin showed some irregularities, which were the indications of residues or impurities from the raw material used from sericin extraction, which were persisted in the hydrogel film. Particles indicated the presence of AgNPs in the hydrogel. Precipitates and discontinuities were present in sericin + banana peel powder and curcumin hydrogels, which was characteristic of the presence of compounds from banana peel powder extract in the hydrogel, which structurally changed the sericin matrix (Figure 2).

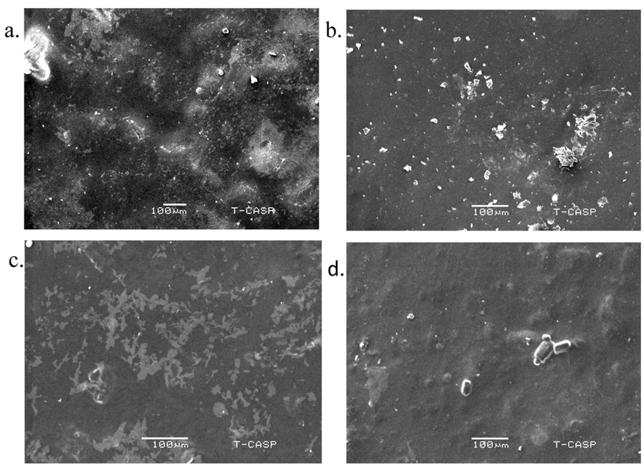

Figure 2. SEM analyses images of (a) sericin-based hydrogel; (b) sericin-based hydrogel loaded with AgNPs; (c) sericin-based hydrogel loaded with banana peel powder; (d) sericin-based hydrogel loaded with curcumin.

Evaluation of Wound Contraction. The healing region of the excision wounds in control groups (negative control; saline solution: positive control; Polyfax) and treatments groups (sericin 2%), (sericin 2% + AgNPs 2%), (sericin 2% + banana peel powder 2%), and (sericin 2% + curcumin 2%), is represented as percent wound contraction in (Table 1; Figure 3).

Percent Wound Contraction at Various Days. The results of the excision wound model after the topical administration of different types of hydrogels indicated a significant difference between negative control and treatment groups at day 3 ($F_{5,24}$ = 24.974; $P < 0.001$), day 9 ($F_{5,24}$ = 245.031; $P < 0.001$), and day 11 ($F_{5,24}$ = 102.289; $P < 0.001$). At day 9, positive control (Polyfax) differs non-significantly from T1 (sericin 2%) and T2 (sericin 2% and AgNPs 2%). However, percent wound contraction at day 9 differs significantly between T3 (sericin 2% and banana peel powder 2%) and T4 (sericin 2% and curcumin 2%) in comparison with positive control (Polyfax).

Results of Tukey's test showed that T3 (sericin 2% and banana peel powder 2%) was highly significant from positive control (Polyfax). However, T1 (sericin 2%) and T2 (sericin 2% and AgNPs 2%) differ non-significantly from positive control (Polyfax). There was no significant difference between T3 (sericin + banana peel powder 2%) and T4 (sericin + curcumin) but a higher rate of wound closure was observed in mice treated with (sericin 2% + banana peel powder 2%) hydrogel in T3 from the day 9 to 13, which were 93.2 and 100%, respectively. It was comparable with the standard ointment (Polyfax) (91.6 and 100%) from day 9 to 13 (Figure 3; Table 1).

Histological Analysis. To better evaluate the effect of different types of hydrogels on the healing process of wounds, a histological study was performed on skin tissue taken from mice at post wounding day 5 and 11 (Figure 4). Hematoxylin & Eosin staining and examination of tissues taken from mice of negative control showed the uneven formation of new epidermis, and inflammatory cells were also observed at day 5. The wound was not completely re-epithelialized, and adipose tissue was observed at day 11. Histology of tissues from T1 (sericin 2%) indicated re-epithelialization in mice wound at post wounding day 5. However, it was observed that inflammatory cells were present and there was not complete epithelialization at the post wounding day 11. The histological evaluation of T2 (sericin 2% and AgNPs 2%) showed regeneration of the epidermal tissues, and re-construction of the skin tissue at post wounding day 5. Moreover, there was no ulceration and the surface of epidermis became even at day 11.

H&E staining of the T3 (sericin 2% and banana peel powder 2%) also affirmed the formation of new epidermis at day 5. While at day 11, histological evaluation confirmed the development of new blood vessels, which ensured the remodeling of the dermis of the skin. Moreover, a bundle of dense collagen and hair follicles in the animals was also detected. T4 (sericin 2% and curcumin 2%) also showed that there was regeneration of the new epidermis at day 5. The enhancement of collagen in the granulation tissue of the wounds was also observed at day 10. Histology of polyfax treated mice tissues (positive control) indicated the collagen filaments at day 5 and formation of thin epithelium at post day 11.

Production of Anti-Inflammatory (IL-10) and Pro-Inflammatory Cytokines (IL-6). IL-6 levels at post-surgery day 5 was found to be significantly increased in mice treated with sericin 2% + banana peel powder extract 2%, while this level was decreased at post-surgery day 11(5th day: 38.4 ± 2.96648 pg/mL; 11th day: 41.2 ± 2.26716 pg/mL). There was an increase in pro-inflammatory cytokines IL-6 level at post-surgery day 11 in negative control group (5th day: 86.8 ± 2.51796 pg/mL; 11th day: 92.4 ± 2.61916 pg/mL) comparison to positive control in which a decrease in IL-6

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Table 1. Percentage Wound Contraction Area (mm^2) of Control and Treatment Groups[a]

days	negative control	positive control	sericin 2%	sericin 2% + AgNPs 2%	sericin 2% + BPP 2%	sericin 2% + curcumin 2%	ANOVA
day 3	$7.998^{a} \pm 1.225$	$13.894^{a} \pm 0.808$	$13.652^{a} \pm 4.129$	$17.252^{a} \pm 2.839$	$36.662^{b} \pm 1.844$	$32.664^{b} \pm 1.159$	($F_{5,24} = 24.974$; $P < 0.001$)
day 5	$14.662^{a} \pm 1.137$	$21.33^{ab} \pm 0.424$	$29.32^{bc} \pm 3.224$	$31.658^{c} \pm 3.148$	$48.096^{d} \pm 1.826$	$42.164^{d} \pm 2.630$	($F_{5,24} = 29.229$; $P < 0.001$)
day 7	$25.83^{a} \pm 1.179$	$33.662^{b} \pm 0.972$	$42.66^{cd} \pm 2.097$	$41.664^{c} \pm 2.040$	$61.662^{e} \pm 1.726$	$49.998^{d} \pm 1.955$	($F_{5,24} = 52.876$; $P < 0.001$)
day 9	$32.898^{a} \pm 1.265$	$47.486^{b} \pm 0.836$	$49.96^{b} \pm 2.033$	$50.23^{b} \pm 0.957$	$85.984^{d} \pm 0.612$	$62.53^{c} \pm 0.393$	($F_{5,24} = 245.031$; $P < 0.001$)
day 11	$39.996^{a} \pm 1.178$	$57.162^{b} \pm 2.549$	$61.3^{b} \pm 1.44$	$59.93^{b} \pm 1.743$	$86.928^{c} \pm 0.424$	$79.666^{c} \pm 1.855$	($F_{5,24} = 102.289$; $P < 0.001$)

[a]Values are expressed as mean ± SEM ($n = 5$). Values with similar and different superscripts in each row show non-significant and significant differences respectively.

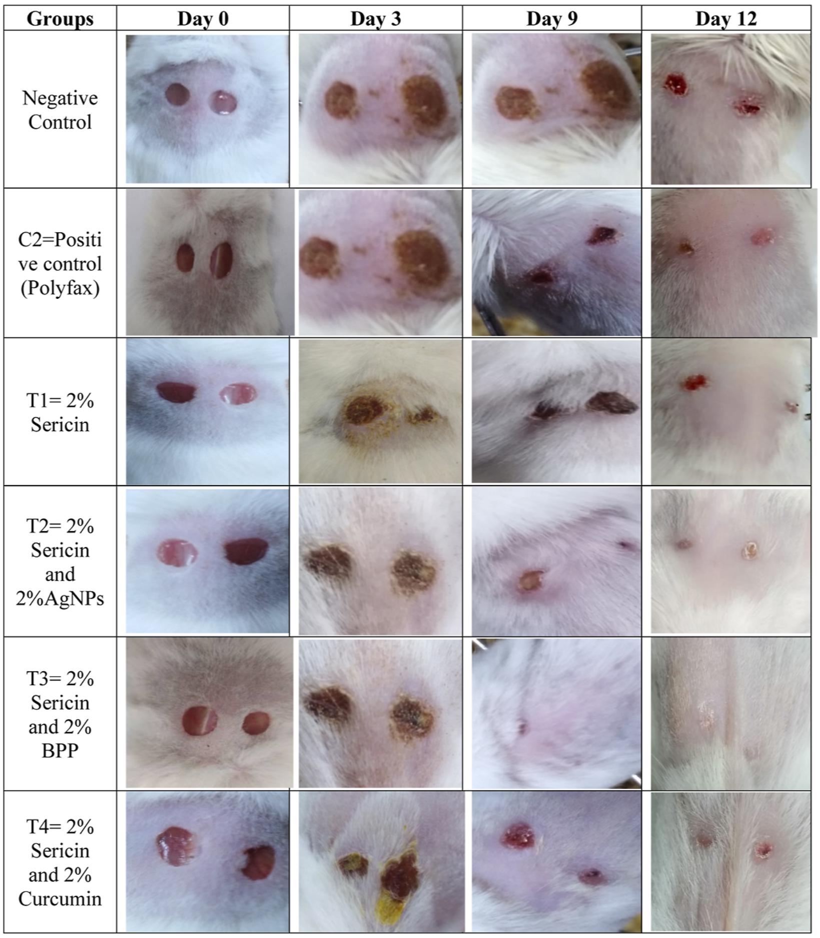

Figure 3. Wound healing process in different mice at post wounding day 3, 9, and 11. C1 = negative control (saline solution); C2 = positive control (Polyfax); T1 = 2% sericin; T2 = 2% sericin and 2% AgNPs; T3 = 2% sericin and 2% BPP and T4 = 2% sericin and 2% curcumin.

was observed at post surgery day 11 (5th day: 66.4 ± 2.5219 pg/mL; 11th day: 71 ± 2.46982 pg/mL) (Figure 5).

The levels of IL-10 at post-surgery day 5 and 11 are tabulated as mean ± SEM (Figure 5). After wound induction, levels of anti-inflammatory cytokines (IL-10) in mice of group T3 was significantly higher (5th day: 499.6 ± 7.71103 pg/mL; 11th day: 515.2 ± 6.98140 pg/mL) and found to be lower in the negative control group (5th day: 320.4 ± 12.01915 pg/mL; 11th day: 371.6 ± 9.92270 pg/mL) than that of positive control (Polyfax) (5th day: 415.4 ± 17.94882 pg/mL; 11th day: 452.6 ± 14.00214 pg/mL).

Antioxidant Property. The in vivo potential of antioxidants showed that there was significantly increased level of superoxide dismutase (SOD), glutathione activity (GSH), and GSH-Px in comparison to negative control ($P < 0.001$). SOD prevents the formation of free radicals, while GSH performs the detoxification of free radicals by the scavenging process. GSH also acts as a co-substrate in glutathione peroxidase-catalyzed reduction (GPx) (Figure 6).

■ DISCUSSION

Hydrogels with different combinations are considered to be an excellent candidate for acute wound healing.[30,35−37] In the present study, the potential of silk sericin-based hydrogels and its combinations with banana peel extract, curcumin, and silver nanoparticles for the acceleration of acute wound was investigated. Characterization results of hydrogels established the potential of hydrogels as an efficient wound dressing. The indication of different peaks confirmed the presence of functional groups and surface morphology confirmed the accurate formation of different hydrogels due to uniformity and good compatibility of components.

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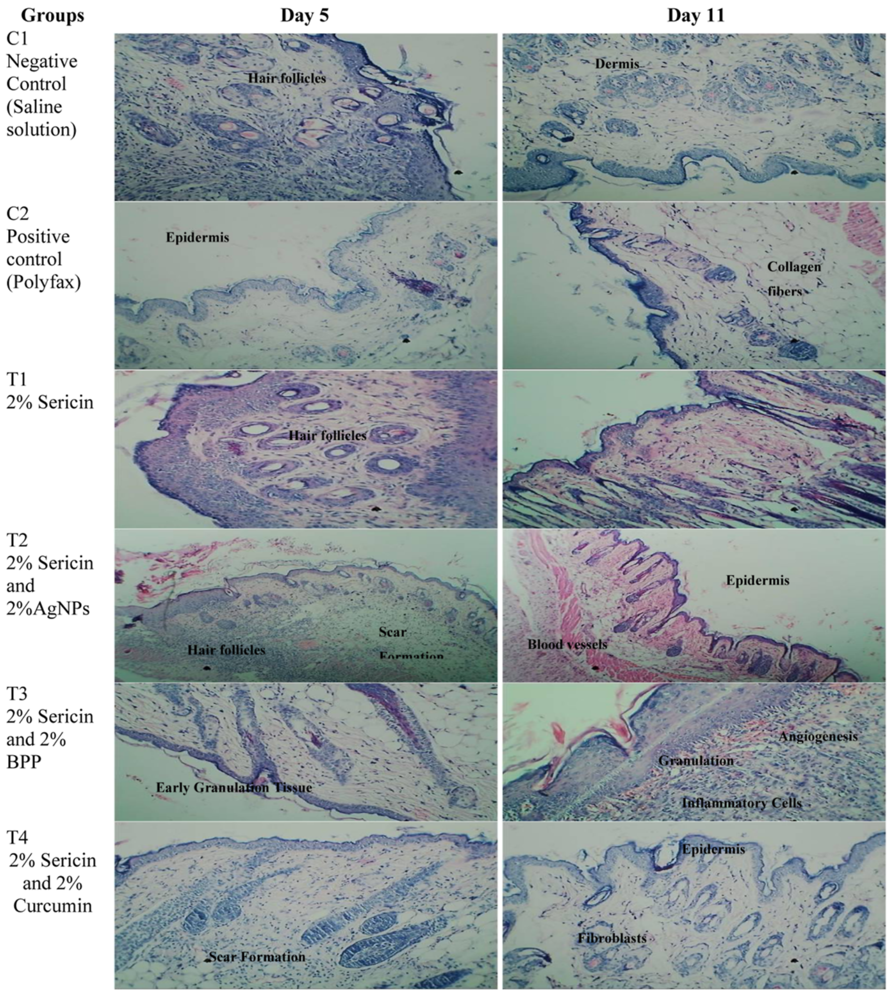

Figure 4. Comparison of histological evaluation of mice treated with saline solution (negative control) at post-wounding day 5 and 11.

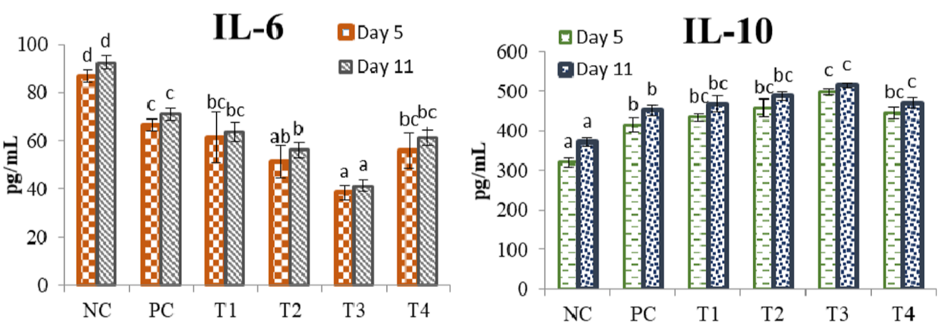

Figure 5. Levels of pro-inflammatory cytokines IL-6 and anti-inflammatory cytokines IL-10 in the blood serum of mice at post surgery day 5 and 11. Note: The columns with similar and different superscript show non-significant and significant differences respectively.

The results of this study indicated that silk sericin-based hydrogels in combination with AgNPs and different plant extracts, that is, banana peel powder and curcumin fasten the healing process with no allergic reaction because these hydrogels were prepared by the physical method without the addition of any chemicals. The wounds treated with hydrogel, that is, silk sericin (2%) and banana peel powder extract (2%) showed 86% healing in 11 days, while wounds treated with silk sericin (2%) and curcumin (2%) showed 80% healing in 11 days as compared to wounds treated in positive control 57%. Various studies have already proved the wound healing potential of silk sericin in wound care and management field.

The results of wound healing potential of silk sericin and banana peel powder are comparable with a previous study reported by Tamri et al. (2016).[44] Rabbit was used as the animal model in previous study that showed maximum healing in 15 days. In the current study, it has been recorded that wounds have healed faster (11 days) by using hydrogels of sericin and banana peel extract, but mice were used as an experimental animal. It is well reported that silver nanoparticles are biocompatible and possess antimicrobial activities.[43] Therefore, hydrogels used in this study were incorporated with silver nanoparticles, as compared to Ai et al. (2019) and Li et al. (2020), who used ZnO-based nanoparticles and Ag/

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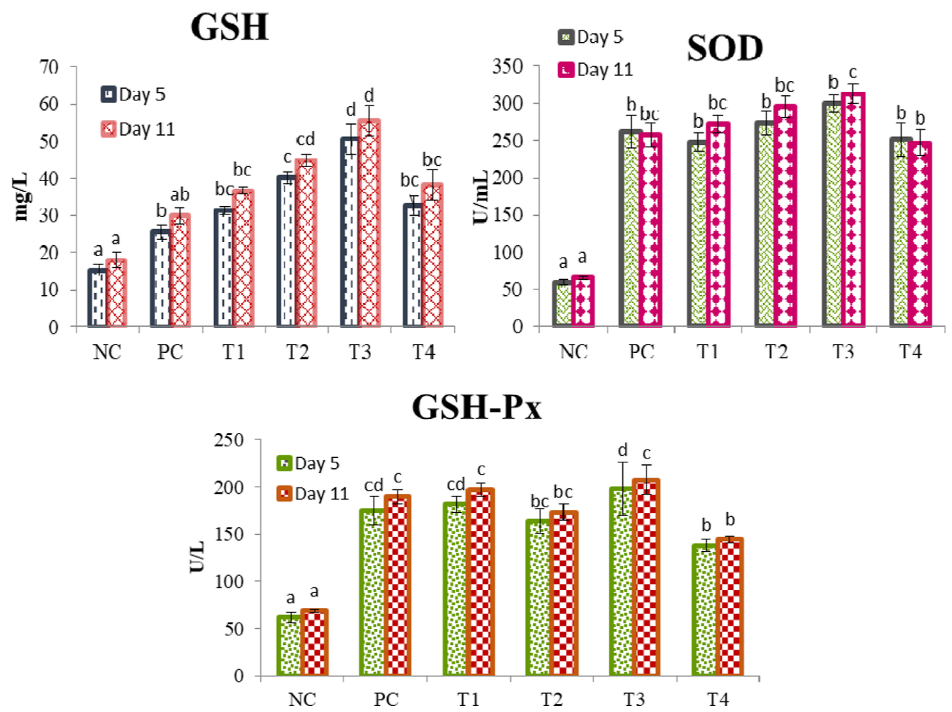

Figure 6. Anti-oxidants levels of GSH, SOD and GSH-Px in the blood serum of mice at post surgery day 5 and 11. Note: The columns with the similar and different superscript show non-significant and significant differences respectively.

ZnO hybrid nanoparticles, respectively, in sericin-based hydrogels and biofilms.[46,47] He et al. (2017b) also prepared sericin-based hydrogel containing silver nanoparticles (AgNPs) by using the UV-assisted green synthesis method; however, in this study, the sericin-based hydrogels were prepared by the freeze−thaw method.[45] Furthermore, Tao et al. (2021) also prepared sericin-based hydrogel wound dressing by using calcium ions as the crosslinking agents.[48]

The histology of treatment groups in the current study also showed the increase in fibroblasts cells, collagen deposition, and angiogenesis after the treatment of wounds with sericin in comparison to the positive control group (polyfax). Moreover, Wang et al. (2018) and Sapru et al. (2019) found that sericin has mitogenic and cytoprotective effects on keratinocytes and fibroblasts by attracting them to skin for tissue repair and accelerate deposition of collagen to promote wound healing efficiently.[26,27]

Due to its various actions such as immunomodulatory capacity and ROS clearance, sericin serves as a main active biological substance in wound dressing.[28] The current study has also showed high levels of anti-oxidant enzymes such as SOD and GSH, which prevents the free radical generation and helps in ROS clearance in the case of sericin-based hydrogel treatment. The present study also reported anti-inflammatory potential of sericin-based hydrogels, indicated by decreased levels of pro-inflammatory cytokines and high level of anti-inflammatory cytokines. Deenonpoe et al. (2019) also reported the anti-inflammatory potential of silk sericin.[49]

Rosida et al. (2014) reported that methoxyl and hydroxyl groups in curcumin bind with proteins of microbes and then kill them. It also causes the production of TGF-β, which helps in the fast healing of wound. It has a higher concentration of phenolic groups and also different antibacterial and anti-oxidants compounds.[50] Franco et al. (2017) evaluated that banana peel contains different enzymes associated with antimicrobial activity such as polyphenol oxidase, tannin, saponins, and flavonoids.[51] Polyphenol oxidase helps in relieving pain and swelling.[52] Chabuck et al. (2013) stated that banana peel also has fatty acids, vitamin A, vitamin C, and Vitamin B6.[53] Thus, association of PVA-based sericin hydrogel and unripe banana peel might be a great substitute for inexpensive biomaterials used for wound dressings.

CONCLUSIONS

The results of this study suggest that silk sericin-based hydrogels in combination with AgNPs and plant extracts promote healing of wounded skin; however, the underlying mechanisms of skin repair of such hydrogels requires more depth experimental evaluation. Furthermore, the current study will provide a foundation, upon which further wound healing experimentations of sericin-based hydrogels can be conducted along with clinical trials.

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Notes

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