Evaluation of Wound Healing Effect of Curcumin Loaded OPL Carbon Nanospheres Embedded Chitosan Membranes

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Abstract
Biowaste-derived carbon biomaterial scaffolds are being used for wound healing and are the focus of interest. Carbon nanospheres derived from oil palm leaves without any catalysts via pyrolysis were loaded with a traditional drug curcumin. The wound healing scaffolds were fabricated on the PP non-woven fabric support using chitosan as the biopolymer matrix. Prepared carbon nanospheres and the scaffolds were characterized using ATR-IR and FESEM techniques. The wettability of scaffolds was examined to ensure the feasible moisture absorption ability, in vitro drug release profile and in vitro antibacterial activity against two strains of bacteria. The in vivo wound healing feature of scaffolds was studied by excision wound model for MRSA infected wound. Measured wound contraction percentage and the bacterial count on wounds at regular time intervals proved that, the scaffold dressed with chitosan and curcumin loaded carbon nanospheres showed an efficient reconstruction of skin through histopathological investigations.

Graphical abstract

Keywords Carbon nanospheres · Wound healing · MRSA infection · Curcumin · Chitosan

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Introduction

Restoration of damaged tissue and its integrity is referred to as a wound healing process. Wound healing comprises a sequence of biochemical as well as physiological steps distributed in four phases [1, 2]. To name, firstly hemorrhage driven by vasoconstriction and complementing cascades, secondly, inflammation driven by vasodilation and activation of macrophage. Third one being proliferation involve epithelialization, angiogenesis and granular tissue formation. This phase also involves a provisional deposition of a matrix. The last one is remodeling phase, where, extracellular matrix deposition takes place in the well-organized network would form, formation of myofibroblasts and wound contraction are also involved in this phase [3]. The devastations at any phase of wound healing process would lead to an unhealable chronic ulcer [4].

Hence, well-planned management of wound healing and its related complications has become significant in recent years in order to increase the quality of life. Infection of wound is still a dispute for wound care professionals. Several strategies have been attempted to check the wound infections. Among them, practicing applicable dressings with enough potential to inhibit bacterial infiltration at the time of wound restoration is crucial [5].

Methicillin-resistant Staphylococcus aureus (MRSA) is the most conjoint infection distressing several thousands of patients every day [6, 7]. Anchoring antimicrobial negotiators to enhance the natural bactericidal efficiency of a few polymers is one of the best ways to combat the challenges associated with infected wounds [8, 9].

An advanced and ideal wound dressing should uphold the microenvironment and discourse all the limitations accompanied with nature and injury state of wound. The dressings must allow enough gaseous exchange and shelter the peri-wound skin against maceration. A balanced moisture and an efficiency to act as barriers for infections are the key requirements [10]. Along with which, the cell viability and cell proliferation is also an important parameter where the healthy cells must not be affected by the used drug material. Overall, the dressing must promote angiogenesis with less perfusion, immune cells modulation, invasion enhancement, fibroblasts migration and keratinocytes in the healing process along with treatment or prevention of infection [11–13].

Chitosan (Ch) is among the enormously used dressing materials which is known to accelerate the healing process [14, 15]. Stimulating the migration of mononuclear and polymorphonuclear cells and enhancing the reepithelialization and reconstruction of regular skin are the interesting functions of Ch [16]. Additionally, excess granulation tissue and formation of scar is also avoided, when chitosan is used [17, 18]. Koyano et al. has proved the fibroblasts growth by using poly vinyl alcohol/Ch hydrogel [19]. A polycationic nature of Ch in aqueous as well as acidic environments offer a bio-adhesive feature for Ch, which allows the researchers to explore it as biocompatible, biodegradable and antibacterial material [20, 21]. A film forming, spongy property of Ch and its interaction with electronegative components at the cell interfaces alter the permeability and seepage of intracellular components such as protein and electrolytes [22]. Chitosan dressing optimized by polyphosphate enhanced blood clotting, platelet adhesion and aided faster thrombin generation [23]. A composite with TiO2 and poly(N-vinylpyrrolidone) showed an excellent antimicrobial feature and better biocompatibility towards NIH3T3 and L929 fibroblasts [24].

Along with several composites, carbon-based nanomaterials/Ch membranes and hydrogels have been emerging in recent years due to their acceptable biological environment [25, 26]. Single and multi-walled carbon/Ch [27], carbon dots doped with cerium [28], carbon nanotubes and fullerenes [29], nitrogen-doped [30] and lanthanum doped carbon quantum dots [31] and many have been explored. Among various geometrical carbons, spherical-shaped ones are proved to have less toxicity and greater biocompatibility [32]. The high surface area of carbon nanospheres (CNS) owing to a greater ability to conjugate with biomolecules via feasible bonds, its affinity and stability towards chemicals are the vital contributions of CNS for bio-related applications [33].

Our previous study has elaborated on the usage of oil palm leaves-derived CNS [34] and sago bark-derived CNS [35] for cell imaging as well as targeted drug delivery towards cancerous cells. Another study from our group reported curcumin (Cur) loaded TiO2/Ch scaffolds for infected wound healing process [36]. These investigations have motivated the present work, wherein, oil palm leaves-derived CNS were loaded with curcumin and examined for its MRSA infected wound healing competence (in vivo and in vitro). Fabricated wound dressings were characterized in detail, wettability characteristics, in vivo drug release profile, in vitro toxicological test, antibacterial test against Escherichia coli and Staphylococcus aureus, in vivo wound healing ability and the MRSA infection control studies are elaborated.

Materials and Methods

Materials

Oil palm leaves were collected from local farms in Malaysia. Chitosan was gifted by SS membranes, Thailand. Curcumin
was obtained from Vidya Herbs, India. Polypropylene non-woven support was purchased from local shops of Bangkok. Chemicals such as ethanol, dimethyl sulfoxide (DMSO), phosphate buffer (PBS) were procured from Merck. An agar medium was obtained from Thermo Fischer Scientifics.

**Synthesis of CNS**

Collected oil palm leaves were cleaned; the midribs were separated and dried at 60 °C in an oven until they were moisture free (48 h). Restsch-ZM 200, Germany was used to mill the dried leaves and the milled product was sieved with a sieving machine of 62 µm to obtain a fine powder of uniform size. CNS was obtained by subjecting the biowaste powder to a pyrolysis process with the aid of a Nabertherm tube furnace. With 5 °C/min heating rate, the temperature reached 600 °C and the material was pyrolyzed for 2 h under nitrogen atmosphere. Dilute HCl (1 mM) was used to wash the slurry to get rid of impurities. More details of the synthesis and the morphology of CNSs can be obtained from [34].

**Loading of Cur to CNS**

The 100 mg of CNS was dispersed in ethanol of 50 mL and sonicated for 20 min. Cur was added to the mixture, sonicated and stirred for 15 min and 5 h, respectively. The mixture was kept undisturbed for 24 h, filtered and dried to get curcumin loaded CNS. The obtained product will be addressed as Cur/CNS for convenience.

**Wound Dressings Fabrication**

The Ch solution was obtained by dissolving 1 g of Ch in 1% acetic acid solution (100 mL). Four dressing membranes, one with only Ch on non-woven support (S/Ch), second one with Cur (200 mg) and Ch (S/Ch/Cur), third one with Ch and CNS (S/Ch/CNS) and the last one with Ch and 200 mg of Cur loaded CNS (S/Ch/Cur/CNS) were prepared. For the preparation of dressings, the homogeneous suspensions of respective dressings were casted on the polypropylene (PP) non-woven substrate. The membranes were dried in over at 60 °C and used further for in vivo and in vitro wound healing studies.

**Characterization of Wound Dressings**

Before moving on to characterization of wound dressing materials, once the drug loading was confirmed, Cur, obtained CNS and Cur/CNS were characterized to confirm the loading of Cur in CNS using Brucker ECO-ATR-IR spectrophotometer. The CNS/Cur embedded Ch matrixe were further characterized by UV–Visible spectrophotometer and ATR-IR spectroscopy. The surface morphology of the prepared scaffolds was analyzed by Field Emission Scanning Electron Microscope (FESEM) JSM-700 1F model. The drug release studies of were investigated by Shimadzu 1800 UV–Visible spectrophotometer.

**Water Adsorption Studies**

As the balanced moisture content is important for wound dressings, the water adsorption ability of prepared dressing materials was estimated by considering 5 × 2 mm of the same. The membranes were dried in oven and were immersed in water for 24 h. Membranes were then blotted with blotting paper to remove excess water. The dry weight and wet weight of the membranes were noted to calculate the water uptake ability of dressing materials. The tests were done in triplicates and the average is considered.

**In Vitro Cytotoxicity Studies**

Formerly to zone inhibition test (antibacterial) for wound dressing scaffolds, cell viability test was performed for powdered samples (CNS and Cur-CNS) via MTT assay. To prepare stock solution, in a PBS solution, 5 mg/mL of MTT was dissolved and the formed formazan crystals were filtered and stored at −20 °C. A diluted MTT solution of ratio 1:10 and L929 cells were used for experimentations. In 96-well plate, 100 μL/1000 cells were incubated for 48 h, at 37 °C and under 5% carbon dioxide environment. Cells with just the media were deliberated as control ones and for others, the powder samples were injected to each plate, and the incubation was continued for 24 h. After removing the media, 100 μL MTT was injected to each wells and subjected to incubation for 2 h. The cells were further washed, gathered in DMSO and examined at 570 nm in UV spectrophotometer.

**In Vitro Drug Release Studies**

An in vitro drug release profile of S/Ch/Cur and S/Ch/Cur/CNS were performed via direct suspension method. Membranes of size 2 × 2 cm were immersed in a 200 mL of PBS solution with 10 mM concentration. The pH of the solution was 7.4 and incubated in shaker water bath at 37 °C. The experiment was performed for 14 days and the amount of drug released was analyzed every day. To calculate the drug release fraction, 2 mL of sample was taken out and the study samples were replenished by the same amount. 2 mL test sample was concentrated in centrifuge, the drug was homogenized in 10 mL of ethanol and evaluated for its concentration using UV–Visible spectrophotometer at 423 nm.
In Vitro Antibacterial Studies

The antibacterial test for prepared membranes was performed against one gram-positive and one-gram negative bacterial strains, *Escherichia coli* and *Staphylococcus aureus*, respectively. In a petri plate, the disc shaped membranes of 12 mm diameter were placed on LB agar medium inoculated with $10^5$ CFU/mL of cell concentration. The plates were refrigerated for 1 h for an appropriate diffusion and then incubated for 24 h at 37 °C. Inhibition zone for each membrane was measured using Vernier caliper.

In Vivo Wound Healing Studies

Details on Animals and Experimentation Protocols

Spargue dawley male rats of six weeks old and of 100–120 g of body weight were considered for the in vivo experiments. The rats were permitted to water with full access and a traditional chow diet. An alternative dark and light cycles of 12 h each was maintained. Xylazine and ketamine were used for anesthesia and the standard surgical techniques were followed. The randomized animals were teamed into four groups (Group I = Sham control; Group II = MRSA Infection control; Group III = Infection + S/Ch/CNS; Group IV = Infection + S/Ch/Cur/CNS) each with five animals. The guidelines given by Institutional animals’ ethics committee, SSCP/203/2019–20, Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, were followed for the conduction of experiments.

Creation of Wound (Excision Wound Model)

A cocktail of xylazine and ketamine of 10 and 70 mg/kg body weight were injected to the experimental animals for which the dorsal skin was shaved. A wound of known area (2 cm$^2$) was made under aseptic environments on the thoracic region. The stress and pain for the experimental animals was reduced by the administration of analgesic. For wound healing, the dressings fabricated in the study were positioned in such way that, the active components of the membranes faced the wound. With an aid of crepe band, the dressings were wrapped and the rats were housed in above mentioned conditions. The restoration of skin on the day of wound excision, after one week (7th day) and two weeks (14th day), was measured by analyzing the boundaries of wound. A percent of wound contraction and the epithelization was examined by taking histograms for the last day of the study.

Presence of Bacteria on the Wound

On the wound contraction measurement days, day 0, 7 and 14, the wounds were swabbed with cotton, sliced and placed in a sterile physiological saline (1 mL). The mixture was vortexed and the bacterial cells were released to saline. From the suspension, 100 μL was pipetted, added to agar plate and incubated for bacterial growth (16 h, 37 °C). The colonies of MRSA were counted for further studies.

Measurement of Wound Contraction and Analysis of Histograms

The size of the wound was measured by tracing the wound on the transparent paper on day 0, 7 and 14. The traced wound boundaries were then shifted to graph sheet of 1 mm$^2$ to calculate the size of the wound. With an aid of wound size on zeroth day, the contraction in the wound was calculated by Eq. 1. For histograms, the sectioned biopsies of wound area on the last day (14th day) were fixed in formalin solution of 10%. The collected samples were implanted in paraffin wax and haemotoxylin-eosin staining was done. The samples were examined using light microscope.

\[
\text{% Wound contraction} = \left( \frac{\text{Initial wound size on zeroth day} - \text{Specific day wound size}}{\text{Initial wound size on zeroth day}} \right) \times 100
\]  

(1)

each with five animals. The data were stated in mean ± SEM. The data were analyzed using a software GraphPad Prism 5.0, San Diego, USA. The statistical assessment were done between the groups treated with drugs and control ones. Tuki annova method was applied for the same. The bacterial wound count and contraction of wound were examined by same method and multiple range test of Bonferroni’s applied for post hoc investigations.

Statistics

The data were taken in triplicates, wherever necessary. The results are stated in mean ± SEM. The data were analyzed using a software GraphPad Prism 5.0, San Diego, USA. The statistical assessment were done between the groups treated with drugs and control ones. Tuki annova method was applied for the same. The bacterial wound count and contraction of wound were examined by same method and multiple range test of Bonferroni’s applied for post hoc investigations.
Results and Discussion

Material Characterization

The detailed characterization of synthesized CNS was given in supplementary information, SI (Fig. 1 of SI). A spherical form with uniform size distribution with a diameter of 35–45 nm was observed for CNS and is epitomic for drug loading. The EDAX spectra showed maximum percentage of carbon, 82% and minimal amounts of oxygen and calcium. Obtained spherical shape was also verified by TEM micrographs. In the next step, the Cur loaded CNS was characterized using UV–Visible spectra and ATR-IR, the obtained results are provided in Fig. 2 and Fig. 3 of SI. The UV–visible absorbance peak for Cur alone was obtained at 435 nm and the same peak is observed for CNS loaded with Cur.

The ATR-IR spectra and FESEM micrographs of scaffolds i.e., nonwoven fabric (support), chitosan coated membrane, CNS loaded chitosan membrane and CNS/Cur loaded chitosan membrane are provided in Figs. 1 and 2, respectively. For substrate (polypropylene non-woven fabric), ATR-IR peaks at 2929, 1716 and 970 cm⁻¹ were assigned to CH₃, C = O and C–O–C groups of PP [37]. A peaks from 1000 to 1600 cm⁻¹ attributes to the oxygen containing functionalities of chitosan [38] for S/Ch sample. The peaks at 1066 and 1028 cm⁻¹ are attributed to C–O stretching vibrations. A peak at 1153 cm⁻¹ was due to the C–O–C bridge asymmetric bending of chitosan [39]. For S/Ch/Cur, the overlapped stretching frequencies for alkene C= C and C=O is observed at 1628 cm⁻¹. A peaks in the range of 3200–3500 cm⁻¹ are attributed to O–H groups, aromatic stretching of C=C is at 1427 cm⁻¹. A high intensity band at 1512 cm⁻¹ is for the mixed vibrations including v (C=O) in-plane bending vibration around aliphatic δ CC–C, = O, δ CC–H in-plane bending vibrations of aromatic carbon of keto and enol configurations and v CC bonds of the same of curcumin [40]. A peaks at 1100 and 1560 cm⁻¹ of S/Ch/CNS are attributed to N–H bending of primary amines and C–N stretching of aliphatic amine. Peaks for C–OH for the cellulose-derived carbon framework were observed at 3400–3600 cm⁻¹. For the final wound dressing material which is composed of peaks from non-woven PP substrate, chitosan, curcumin and CNS were overlapped and very slight shifts were observed.

FESEM micrographs of all prepared scaffolds are provided in Fig. 3. The fiber nature of nan-woven fabric was evident from Fig. 3(a). The coating of chitosan, S/Ch and curcumin, S/Ch/Cur (Fig. 3b and c) masked the fiber nature of substrate material, indicating the coating of them on the polypropylene fabric. The micrographs of S/Ch/CNS and S/Ch/Cur/CNS showed the aggregated lumps on the fabric surface. The inset zoom in pictures provided for both the samples highlight the incorporation of CNS (nanospheres). It is also noted that, the nanosphere nature of CNS remained same even after loading of Cur. The adhesion of chitosan and the composite matrix on the PP matrix was also observed by FESEM cross sectional images, which are presented in Fig. 4 of SI.

Wettability Test for Wound Dressings

Water flux is an important parameter for wound dressing materials, the material must be able to absorb water and maintain the feasible moisture conditions. Though the required moisture content for the same is unknown, water wettability of the prepared dressings is measured and the results with respect to water adsorption of dressings with CNS, Cur/CNS and Cur are compared. Calculated water wettability in percent is demonstrated in Fig. 3. The dressing Ch showed the water uptake of 255.24 ± 10.66%, it increased to 384.19 ± 14.70% for the one with CNS. Water uptake was about 362.78 ± 18.83% for S/Ch/Cur material and 475.37 ± 8.98% for the dressing which contained CNS loaded with Cur. Chitosan is hydrophilic in nature and was able to absorb enough amount of water. With the addition of CNS, which are porous in nature, the water uptake increased. The porous nature of carbon nanospheres and the functionalities of curcumin synergistically contributed to water adsorption and hence wound dressing S/Ch/CNS/Cur showed 475.37 ± 8.98%. The water adsorption proved that, S/Ch/CNS/Cur material possessed a great ability to adsorb enough moisture and support wound healing process by adsorbing the moisture content produced during the progression of wound.
MTT Assay

Dose dependent cell viability is a significant factor for the wound healing applications [41, 42]. Though many plant-based extracts and several compounds are studied for various biological applications, the cytotoxic effects of the same on cell type of interest are neglected sometimes. Nevertheless, the trend of examining the cell viability has fallen in place. Such responses for considered L929 cell lines on treating with Cur and Cur/CNS were investigated and the percent cell
viability is given in Fig. 4. From ancient times, curcumin has proven its efficacy in wound healing, though the cell viability was neglected. However, recent research is considering cell proliferation assay as crucial component [43, 44]. In present study, the percent cell viability for the Cur treated cells was of 99.82 ± 0.32% at lower concentrations (25 μg/mL) and it reached to 95.29 ± 0.73% for 200 μg/ml. For Cur/CNS, the values were 99.25 ± 0.56% and 93.03 ± 0.24% at lower and higher concentrations, respectively. The obtained values clearly confer the feasibility of Cur loaded CNS for the wound healing applications.

In Vitro Drug Release Studies

In vitro drug release is crucial as far as nanoparticles are considered, due to its dependency on many features such as (1) diffusion of the drug through the nanomaterials, (2) diffusion via hydrated pores within the nano materials, and (3) due to the cleavage of bonds in case of degradable ones. In present work, case one and two are expected. The results of in vitro drug release studies are provided in Fig. 5. The water uptake studies have clearly demonstrated the maximum water affinity of the final wound dressing scaffold i.e., S/Ch/Cur/CNS, which would lead to the diffusion of Cur through water filled pores of CNS. But, with S/Ch/Cur, the release of drug is directly from the polymer matrix, allowing the faster diffusion and hence greater drug release of nearly 62.3% on the day one itself. Almost all the drug is released within 5 days and it attained the saturation level. For S/Ch/Cur/CNS, first day drug release was minimal and is about 35.2%, the gradual release is observed for the next 9 days owing to the existence of CNS. In the absence of CNS, a rapid drug release was observed as it is directly diffused into PBS solution due to the bulk polymer degradation. Whereas, in the presence of CNS, though the bulk polymer degradation occurs as of S/Ch/Cur, the drug release was quite slower as it was entrapped in CNS [45].

In Vitro Antibacterial Activity

In vitro antibacterial activity was performed for the membrane samples, including bare nonwoven fabric and chitosan membrane. The zone of inhibition test was carried out and the pictures are provided in Fig. 6. For both gram + ve (Fig. 6a) and gram – ve (Fig. 6b) bacteria, the zone of inhibition for nonwoven fabric (M0) was negligible. For membrane loaded with Cur (M1), the values were

![Fig. 5 In vitro drug release studies for S/Ch/Cur and S/Ch/Cur/CNS](image)

![Fig. 6 Camera pictures of Zone of Inhibition study for all prepared membranes](image)

| Dressing : Details |
|--------------------|
| M0 : Control (S) |
| M1 : S/Ch/Cur |
| M2 : S/Ch |
| M3 : S/Ch/CNS |
| M4 : S/Ch/Cur/CNS |

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6.1 ± 0.2 and 10.2 ± 0.1 mm, for membranes with Ch alone (M2), 7.21 ± 0.1 and 6.9 ± 0.4 mm, for membranes with S/Ch/CNS (M3), 8.3 ± 0.9 and 11 ± 0.8 mm and for S/Cur/CNS loaded membranes (M4), 8 ± 0.6 and 11 ± 1.0 mm, for gram + ve and gram − ve bacteria, respectively. Curcumin being natural polyphenolic flavonoid offers several biological applications. For antibacterial agents, the bacterial cytotoxicity assessed by the stability and congress of FtsZ protofilaments is a key component [46]. The inhibition of congress dynamics of the FtsZ in Z-ring by curcumin is uttered to suppress the cell proliferation [47]. The interaction of carbon with bacterial membrane is found to destroy the cell membrane resulting in antimicrobial nature [48, 49]. In present work, a feasible antimicrobial feature is confirmed for all four membranes S/Ch, S/Ch/Cur, S/Ch/CNS and S/Ch/Cur/CNS, except nonwoven fabric, conferring the further use of these membranes for in vivo applications.

**In Vivo Wound Healing Studies**

In vivo wound healing studies were performed for four groups, sham control, infection control, S/Ch/CNS and S/Ch/Cur/CNS. The studies were not conducted for S/Ch and S/Ch/Cur as they were detailed in our previous studies [36]. The representative camera pictures of assessment of wound healing characteristics for the membranes are presented in Fig. 7 for day 0, 3, 7, 10 and 14. The respective calculated % wound contraction is provided in Fig. 8. For sham and infection control at day 3, the wound contraction was just 4 and 3.85% whereas, for S/Ch/CNS and S/Ch/Cur/CNS, it was 10.65 and 14%. For day 7, sham control and S/Ch/CNS showed nearly same % wound contraction. However, infection control showed 28.4% and S/Ch/Cur/CNS showed 39.5% of wound contraction. At day 10, S/Ch/Cur/CNS gave a highest wound contraction and was of 76% and the control groups showed ~ 45%. At day 14, the wound contraction was maximum and almost healed. Control groups still possessed ~ 25% of wound, S/Ch/CNS groups showed ~ 11% wound and our material of interest S/Ch/Cur/CNS possessed 4.5% of wound.

Wound closure was lesser for sham and infection controls and was greater for the one with Cur and CNS. In early wound healing days (3rd day), the wound closure was almost similar. Cur is known to reduce the inflammation and induce cell proliferation leading to the damage tissue reconstruction. Cur is proven to typically act as an antioxidant since free radicals are deliberated to be the major reasons for inflammation thru wound healing course [50]. The contribution of Cur in every step of wound closure mechanism is elaborated by many researchers. In the first step itself, Cur suppress the inflammation by scavenging the reactive oxygen species. Gopinath et al., have shown the in vitro antioxidant ability of Cur which was dispersed in collagen matrix via lipid peroxidation process [51]. Gadankar et al., in the in vivo study, demonstrated the suppression of keratinocytes and fibroblasts damage induced by 

H₂O₂ [52]. In fibroblasts proliferation step, curcumin at its optimal dosage enhances the infiltration of fibroblasts [53]. The next step is of granulation of tissues, where a protein marker hydroxyproline is measured which confers the collagen synthesis. Gopinath and group also worked on this process and showed the involvement of Cur in the granulation of tissues which further increases the re-epithelialization and provides the basal backing for the migration of epithelial cells which would further help the wound closure [51]. As the wound healing process progress, apoptosis plays a crucial step in eliminating the undesirable inflammatory cells. Though, the exact mechanism is unclear, the functioning of Cur in this step is demonstrated [53]. The biocompatibility of the CNS was elaborated by one of our co-author, wherein CNS was loaded with a florescent dye and was used for cell imaging and drug delivery purpose [34]. The proven antibacterial effect of CNS and the chitosan have facilitated the synergistic effect and accelerate the wound healing process.

**Histopathological Investigations**

To understand the reconstruction of skin structure, histograms play a key role. Histograms of skin tissues from considered four groups at day 14, are provided in Fig. 9. Histology of Group I skin showed normal histo-architecture with normal epithelization (Fig. 9a) and infection control, Group II skin showed partial reepithelization as highlighted with an arrow in Fig. 9b and focal epidermal hyperplasia. Group III skin tissue showed granulation with neo angiogenesis as highlighted with an arrow in Fig. 9c and diffused epidermal hyperplasia. Group IV, treated with S/Ch/Cur/CNS wound dressing showed a normal histology which is in accordance with Group I histogram. The histograms confirm that, the reconstruction of skin tissue was with norma architecture for Group IV, proving the potency of considered dressing as the best wound healing material.

**Conclusions**

A successful fabrication of Ch-based Cur loaded CNS on PP non-woven support was confirmed. The spherical nature of CNS remained same even after loading of Cur which is evident from FESEM images. Embedded drug in the CNS framework resulted in feasible drug release pattern. Porous CNS and rich functionalities of Cur together contributed to the feasible water adsorption by the scaffolds and the wound dressing S/Ch/CNS/Cur showed a maximum wettability of 475.37 ± 8.98%. Nearly 93–99% cell viability was observed.
from MTT assay proving the non-toxic nature of prepared scaffolds. The inhibition of cell proliferation by Cur and the destruction of cell membrane by carbon material resulted in good antimicrobial activity for Cur loaded CNS. Nearly 96.5% wound contraction was observed for our material of interest on the 14th day of study and the regain of skin structure was confirmed by histogram showing normal reepithelization and granulation.

Fig. 7 Photographic pictures of wound contraction for considered groups at 0, 3, 7, 10 and 14 days
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Declarations

Conflict of interest  The authors have no relevant financial or non-financial interests to disclose.

Fig. 8  Percentage (%) wound contraction for Group I, II, III and IV at 3, 7, 10 and 14 days

Fig. 9  Histograms of skin tissue for a Group I; b Group II; c Group III and d Group IV taken at 14th day of the study
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