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Research Letter

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Biogenic silver nanoparticles as a more efficient contrivance for wound healing acceleration than common antiseptic medicine

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One sentence summary: A simple and facile way of application of biogenic silver nanoparticles for wound healing acceleration and suppression of wound infections.

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ABSTRACT

A simple and facile way of using biogenic silver nanoparticles (BSNP) (10–20 nm) was developed for wound healing acceleration and suppression of wound infections. The BSNP were formulated in an ointment base, and the study to accelerate the wound healing process was conducted in a rat. The pH of the BSNP ointment, pH 6.8 ± 0.5, lies in normal pH range of the human skin, with good spreadability and diffusibility. The wound closure rate, as a percentage, was highest at day 3 for a BSNP ointment-treated wound at 22.77 ± 1.60%, while in an untreated control the rate was 10.99 ± 1.74%, for Betadine 14.73 ± 2.36% and for Soframycin 18.55 ± 1.37%, compared with day 0. A similar pattern of wound closure rate was found at days 7 and 11. The antibacterial activity of BSNP was evaluated against wound-infection-causing bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* by the agar diffusion method. The total bacterial counts in the wound area were enumerated by the colony forming unit method. The lowest number of bacterial counts was found in the BSNP-treated wound compared with the other groups. BSNP treatment at 7.5% concentration enhanced migration of fibroblasts in a scratch assay. These findings reveal BSNP as an efficient contrivance for wound healing acceleration and as an eco-friendly alternative therapeutic antimicrobial agent.

Keywords: biogenic silver nanoparticles; wound healing acceleration; common antiseptic medicine; antibacterial activity; re-epithelialization

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INTRODUCTION

Development of nanomedicine is a rapidly emerging area because of the recent addition of nano-composites into a range of products and technologies (Rai, Nagaonkar and Ingle 2018). Recently, the application of nanoparticles in medicine has increased and been extended to the areas of molecular imaging (Kohl *et al.* 2011), drug delivery (Meng *et al.* 2010), treatment of cardiovascular diseases (Godin *et al.* 2010), wound healing (Tian *et al.* 2007), development of antimicrobial agents and medical devices (Rangari *et al.* 2010). Silver nanoparticles have peculiar and remarkable antimicrobial properties, which may be developed into substitute products against multidrug resistant microorganisms (Morones *et al.* 2005; Martinez-Gutierrez *et al.* 2010). Biogenic silver nanoparticles (BSNP) have been synthesized by reducing and modifying their surface with potent antimicrobial metabolites of *Trichoderma viride* (MTCC5661) to enhance their antimicrobial properties. It can demonstrate as a bio-inspired nano formulation which was urgently required to overcome the lacuna of presently available antimicrobial agents (Kumari *et al.* 2017). The antimicrobial potency of silver nanoparticles can avoid microbial infection in a wound and increase the rate of the wound healing process due to their anti-inflammatory and angiogenic activities (Marcato *et al.* 2015).

Wounds are the injured portion of tissue or skin that is repaired and regenerated by several cellular biochemical responses and a cascade of events (hemostasis, inflammation, proliferation) performed to regenerate tissue at the wound site (Urie *et al.* 2018). Hemostasis and inflammation processes are triggered by releasing platelets, blood components and clotting factor at the site of injury (Gandy *et al.* 2017), along with platelet-derived growth factors and transforming growth factor-beta (Chun *et al.* 2017). The immune response triggers the phagocytic cells, i.e. macrophages and neutrophils, that travel to the injured site. The foreign particles are scavenged by cytokines and reactive oxygen species released by phagocytic cells (Miron *et al.* 2017). The angiogenesis process is started by the proliferation of granulation tissue and fibroblasts are deposited over the site to form an extracellular matrix (Cianfarani *et al.* 2017). Although wound healing is a natural process, it is quite slow, and may leave scarring of the wound due to impaired healing and poor remodeling processess, leading turned to acute and chronic wounds (Omar *et al.* 2017). The healing process is also suppressed by pre-existing comorbidities like diabetes, immunosuppression, chronic peripheral vasculopathy and deposition of toxic materials in the wound and lack of proper metabolism (Tocco *et al.* 2012). Several preventive ointments are available for instant wound healing activity, but they have drawbacks like scar formation and slow rate of wound healing activity (Hussain *et al.* 2017; Yannas, Tzeranis and So 2017).

Advanced nanotechnology can provide an effective mechanism to cope with chronic wound conditions (Simões *et al.* 2018). BSNP gives new hope in the biomedical field by increasing surface area while having smaller particles size and long-time persistence to inhibit microbial infection (Agnihotri, Mukherji and Mukherji 2014 and Ovais *et al.* 2016). The lower concentration of silver nanoparticles exhibited possible wound healing activity along with improved bactericidal activity with low toxicity (Ambrožová *et al.* 2017).

In this study, BSNP synthesized from cell free filtrate of *T. viride* (MTCC 5661) was formulated as an ointment at a lower concentration. BSNP-containing ointment was evaluated as an effective biological contrivance for the acceleration of tissue regeneration and bactericidal activity at a wound site compared with common antiseptic medicines.

Table 1. Ingredients of BSNP ointment.

Ingredients	Amount
PEG 6000	0.5 g
White paraffin wax	0.5 g
Paraffin wax white soft	11.0 g
BSNP	1.0 ml
Total	13.0 g

MATERIALS AND METHODS

Materials

The BSNP used in this study was synthesized from a cell-free filtrate of *T. viride* (MTCC5661). The particles were spherical in size, 10–20 nm, with a polydispersity index of 0.239 ± 0.026 and zeta potential −26.36 ± 0.14 mV, reflecting their homogenous nature and longer stability. Details of their characterization and some other parameters have been described earlier by Kumari *et al.* (2017). The concentration of BSNP was adjusted to 0.02 mg/ml and sonicated for 2 min at 30% amplitude, particles were passed through a 0.22 μm syringe filter before use. Paraffin wax white soft and white paraffin wax were purchased from Qualigens Fine Chemical, Mumbai, India. Polyethylene glycol 6000 (PEG 6000) was purchased from SD Fine chemical Ltd. India. Betadine and Soframycin ointment were purchase from commercial sources. All other chemicals and reagents used were of analytical grad

Preparation of BSNP ointment

The ointment was prepared by mixing BSNP with paraffin wax white soft, white paraffin wax and PEG 6000, taken as an ointment base; the amount of each ingredient is given in Table 1. The base was thoroughly mixed until it turned into a fine paste. BSNP was added drop by drop into the mixture of base and mixed thoroughly by rotating the paste clockwise or anticlockwise at room temperature. Betadine and Soframycin were applied as standard drugs; they are commonly used for repairing wounds and cuts (Gershenfeld 1962).

Characterization of BSNP ointment

pH values

The pH of BSNP ointment was measured by digital pH meter (Bench top pH meter, inoLab-pH7310P). Ointment (1%) was dispersed in deionized water and vortexed throughly. The pH was measured three times after calibration of the pH meter (Panigrahi *et al.* 1997).

Solid content analysis

Total solid content was estimated by using the 'digital total dissolve solid meter'. Calibrated electrode was dipped in the formulation at 2 parts per thousand measurement range at room temperature.

Physical evaluation

Preliminary evaluation of BSNP ointment for homogeneity and visual appearance was carried out at room temperature (Panigrahi *et al.* 1997).

Spreadability

Spreadability of formulated BSNP ointment was determined by following the method of Wood, Catacalos and Lieberman (1963).

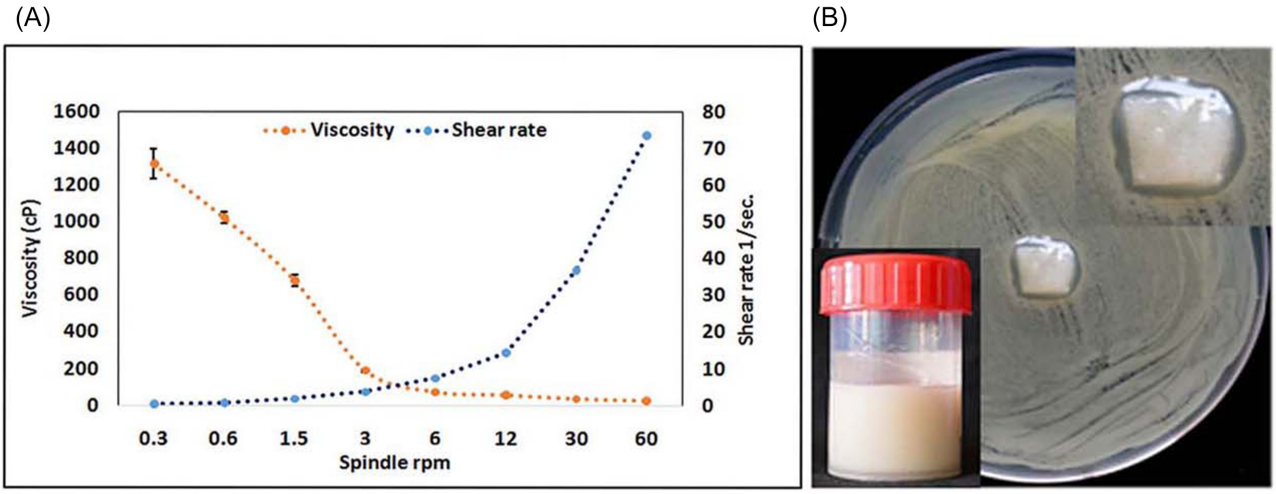

Figure 1. Characterization of BSNP ointment. Combo graph representation of viscosity (cP) and shear rate 1/s of BSNP ointment at different rpm (**A**). Agar diffusion assay of BSNP ointment. Left inset, the final form of BSNP ointment, and right inset, a magnified image of the zone of inhibition (**B**).

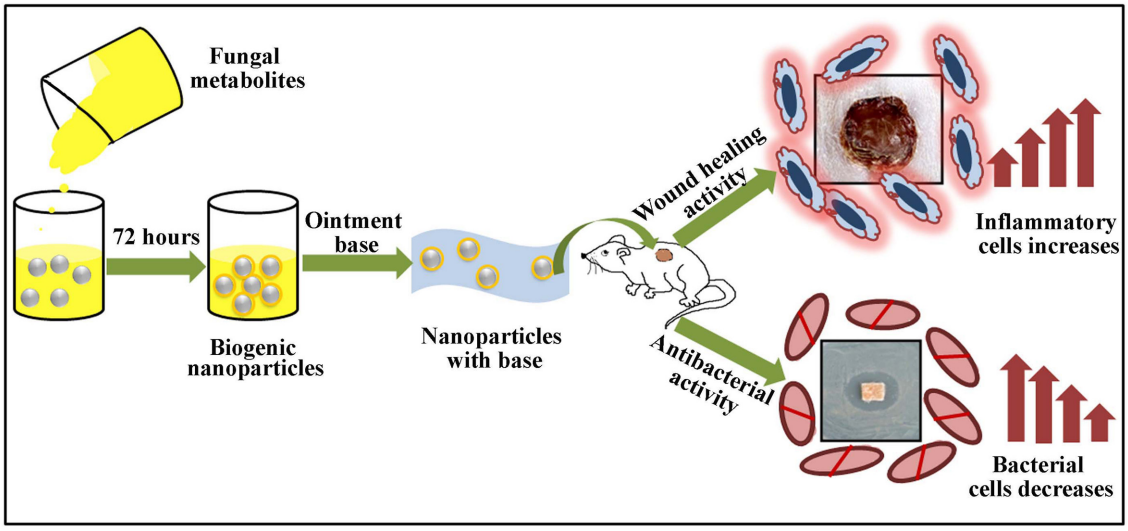

Figure 2. Schematic representation of BSNP synthesis and topical application as an ointment.

Ointment was placed between two glass slides and a 100 g weight was put on the upper slide for 5 min to spread the ointment uniformly. Thereafter, a 50 g weight was tied to the upper slide for complete separation of both slides. The time was recorded in seconds and calculated by using the formula ($S = M.L/T$). Where S = spreadability, M = weight tied to upper slide, L = length of glass slides and T = time in seconds taken to separate the slides from each other.

Rheological evaluation

Viscosity is the major rheological parameter required for a topical formulation to evaluate the rate of diffusion; it was measured with a Brookfield viscometer V6.5RV, spindle SSA31 (Brookfield Engineering Laboratories, Middleboro, MA). The test was performed at 37°C. The spindle of the viscometer was rotated at 0.30, 0.60, 1.50, 3.00, 6.00, 12.00, 30.00 and 60.00 rpm. The viscosity and stress rate were recorded at each rotation rate (Kim *et al.* 2003).

Agar diffusion assay

Agar diffusion assay was performed for measurement of the diffusion properties of BSNP ointment. *Staphlococcus aureus* bacterial culture grown overnight was spread on a nutrient agar plate and an 8 mm square sterilized piece of cotton cloth loaded with ointment was placed in the centre of the plate. Thereafter, the plate was put in an incubator overnight at 37°C. The experiment was performed in triplicate.

Stability of the BSNP ointment

Stability of the BSNP ointment was evaluated by changes in physical parameters including color, phase separation and odor. Formulated ointment was divided into different glass vials and kept at 4° C, 25° C and 40°C for 60 days to observe the changes in all the physical parameters described above (Şahiner, Pekel and Güven 1999).

EXPERIMENTAL DESIGN

Animals

Sprague Dawley (SD) rats (body weight 140–180 g) were purchased from the animal house of CSIR-Central Drug Research Institute, Lucknow, India. They were kept in the animal house at CSIR-National Botanical Research Institute, Lucknow, India. SD rats were allowed to acclimatize to the new environment for a week at temperature 25 ± 2° C and relative humidity 45–56%. The light and dark cycle was maintained as 12 h and 12 h, respectively. Standard feed pellets and water were provided to each animal in equal amount. The animal care and handling was strictly followed according to NIH guidelines given for rats and laboratory animals (Hill 1986). Ethical approval was obtained from the Institutional Animal Ethical Committee before the experiment (Notification no. 1732/GO/Re/S/13/CPCSEA).

Wound excision model

Twenty four healthy male SD rats were divided into four groups with weights in the range 140–180 g as described by Moraal *et al.* (2012). The six rats in each group were housed in individual-compartment plastic cages containing sterile paddy husk bedding. Excision wound model experiment was performend with divided four groups of rats. The hair of each rat was trimmed using a normal hair trimmer and rats were anesthetized by 2 min inhalation of 40% diethyl ether. Under anesthetic, rats were placed dorsal sice up and the excision area was cleaned with 70% ethanol; a 0.8 ± 0.1 cm area of skin was measured and parallel excised by surgical skin punch. Rats were left with exposed skin in atmospheric conditions. Each animal was kept individually in a plastic cage with proper marking for all groups such as: control (without any treatment), Betadine, Soframycin as common antiseptic ointment, and BSNP ointment. All the treatments were applied on rats till 14th day from next day of excision.

Effect of BSNP ointment on wound closure rate

The excision wound area was measured by measuring scale on days 0, 1, 3, 7, 11 and 14. Percentage of progressive changes in wound area were determined by using the following equation. Images of the wounds were captured by digital camera on the same day as wound area measurement.

$$\text{Wound closure rate (\%)} = (1 - A/Ai) \times 100$$

Where, Ai is the initial wound area and A is the wound area after a fixed measured time interval (Fayemi *et al.* 2018). Body weight and behavior of each animal were monitored on every wound area measurement day.

Histological study

Tissue from the wound area was collected at days 3 and 7 post-wounding day and washed with 1X phosphate buffered saline (PBS), then transferred into 4% formalin fixing reagent for 24 h. The formalin fixed tissues were processed through increasing grades of alcohol [70, 80, 90%, and absolute alcohol) and cleaned with ethanol xylene (1:1)]. Further, tissues were treated with pure xylene and embedded in paraffin wax at 65°C overnight. Thereafter, molds were prepared and stored at 4°C until use. Five-micron thin sections were cut by using a semi-automatic motorized microtome (Lyzer, model no: LT-151-5). Sections were deparaffinised with xylene and rehydrated through decreasing grades of ethanol (100, 95 and 70%). They were then washed with water and stained by haematoxylin and eosin stain (Horobin and Kiernan 2002). The haematoxylin is a basic dye and stains the nuclear region of the cells. The acidic nature of the nuclear region provokes binding of haematoxylin and stains to a dark purple color that reflects the particular morphology of the stained area. The basic nature of the remaining area of tissue is stained by the eosin counter stain (Gartner and Hiatt 2007).

Table 2. Antibacterial activity of BSNP.

Pathogenic bacteria	Zone size (mm)
S. aureus	12.25 ± 0.05
P. aeruginosa	18.74 ± 0.95
E. coli	27.75 ± 1.25

Assessment of antibacterial activity

The bacterial population in the wound area was enumerated by the colony forming unit (CFU/ml) method. Samples of wound area were taken by cotton swab at days 3 and 7 and inoculated in 0.85% sterile saline. Serially diluted samples were spotted on nutrient agar plates and incubated overnight at 28°C. Antibacterial activity of BSNP against wound-infection-causing bacteria such as *Pseudomonas aeruginosa* (ATCC15692) and *S. aureus* (ATCC33591) was observed, and details of their mechanism of action was as described earlier by Kumari *et al.* (2017). Briefly, bacterial culture grown overnight was spread on a nutrient agar plate. Then, 100 µl of BSNP (0.02 mg/ml) was added on an 8 mm square sterile cotton disc kept in centre of the plate. The plates were incubated for 24 h at strain-specific growing temperature (28 and 37°C, respectively). Zone size was closely monitored around the cotton disc. The antibacterial sensitivity test against *Escherichia coli* was also done by same method.

In vitro scratch assay

Scratch assay was performed following the protocol of Liang, Park and Guan 2007 with some modifications. Briefly, mouse fibroblast cells were seeded at a density of 1×10^5 cells/mL with Dulbecco's Modified Eagle's Medium (DMEM) in 60 mm dishes and grown for 24 h at 37°C in a CO_2 incubator. Thereafter, cell monolayer was scratched in a straight line with a p200 pipet tip. The scratched cells were removed by washing the dish with PBS thrice. DMEM was added to the control dishes again, while DMEM supplemented with 5 and 7.5% BSNP was added to the BSNP-treated dishes. The dishes were incubated for 12 h at 37°C in a CO_2 incubator. After 12 h, the cells were visualized with a FLUOVIEW FV3000 confocal laser scanning microscope (Olympus, Japan) under 10X magnification and analysed using ImageJ Software (National Institute of Health, USA). The experiments were performed in triplicates, and at least 10 different readings per plate were noted down to calculate the % migration rate.

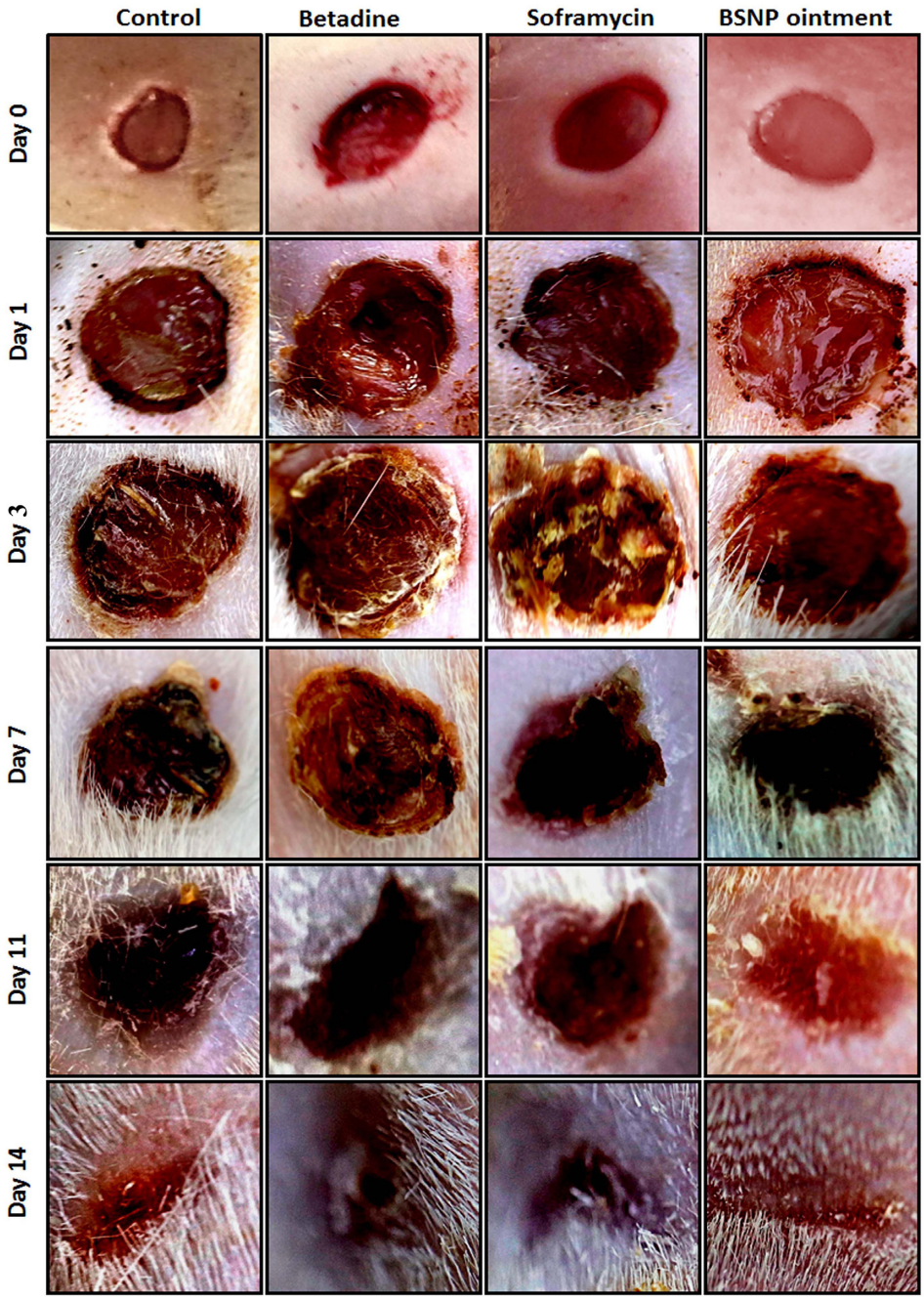

Figure 3. Representative images of wound healing at days 0, 1, 3, 7, 11 and 14. Topical administration of different wound dressing materials: untreated control, Betadine, Soframycin and BSNP ointment.

Antioxidant examination

The 2,2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of BSNP was estimated by following the method of McDonald *et al.* (2001) with some modifications. The results were expressed in percentage of radical scavenging activity. Briefly, 0.5 mM of DPPH methanolic solution was mixed with various concentrations of BSNP (25, 50, 100 150 μl/ml). The reaction mixture was mixed well and incubated for 30 min in the dark. Thereafter, the absorbance of the mixture was read at 517 nm. The DPPH radical scavenging activity (%) was calculated by using the formula: scavenging (%) = (1−absorbance of sample/absorbance of control) × 100.

Statistical analysis

All the experiments were performed at least three times and represented in terms of means ± SE in histograms. ANOVA single factor was used for assessing wound closure rate (%) difference between the treated and untreated experimental groups. Differences were calculated statistically when $^{*}P < 0.05$, $^{**}P < 0.01$ and

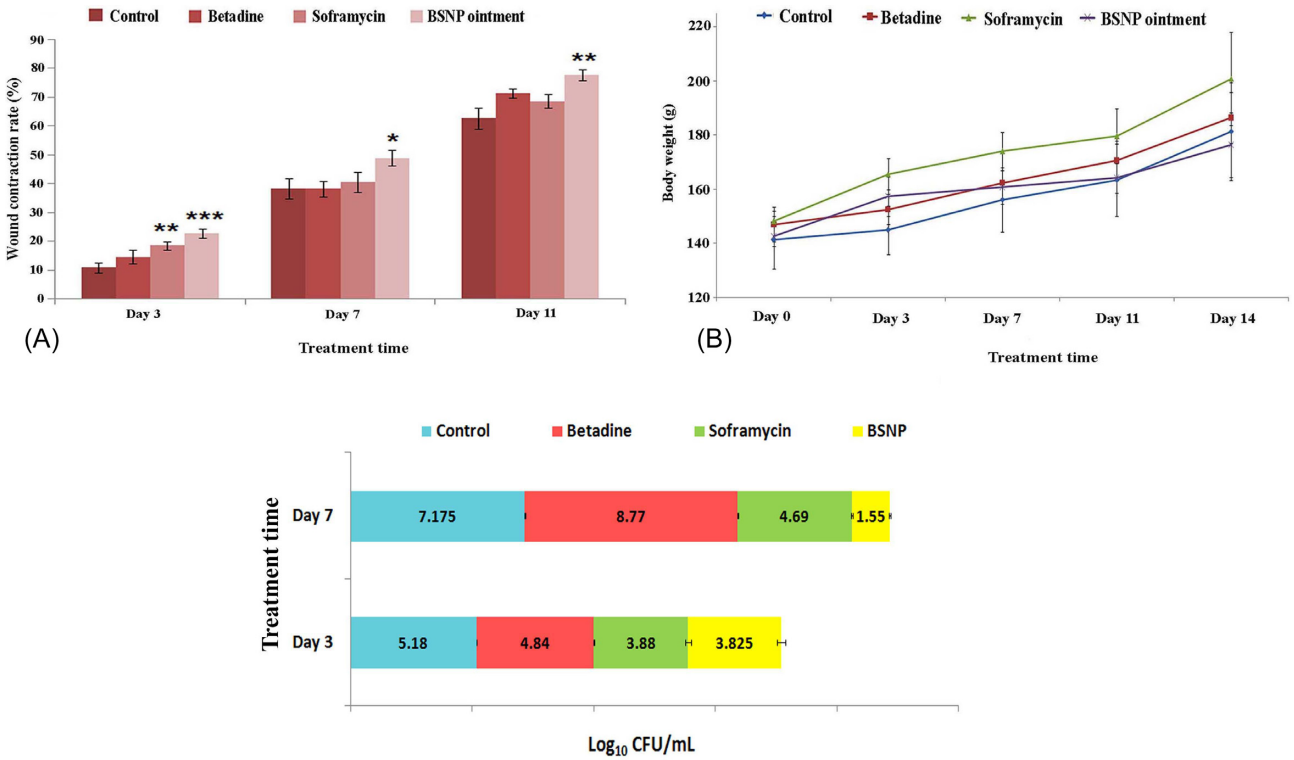

Figure 4. Wound contraction rate (%) at days 3, 7 and 11 of wound area treated with different wound dressing materials: untreated control, Betadine, Soframycin and BSNP ointment. The data are represented as mean ± standard deviation from six independent experiments. Statistically significant (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$) (**A**). Body weight (g) of SD rats at days 0, 3, 7, 11 and 14. The data are represented as mean ± standard deviation from six independent experiments (**B**). Enumeration of total bacterial population in wound area at days 3 and 7. Values represented in $\log_{10}$ CFU/ml (**C**).

***$P < 0.001$ were significantly different from the control. 'Statistical package for the social sciences' software was used for the significant difference in the *in-vitro* scratch assay, represented by different letters of the alphabet, according to Duncan's multiple comparison test ($P < 0.05$).

RESULTS AND DISCUSSION

Physicochemical property of BSNP ointment

The pH of the BSNP ointment lies in the normal pH range of the human skin (6.8 ± 0.5). This pH range of the ointment may help in maintaining skin quality and cannot adversely affect the skin (Pattanayak *et al.* 2011).

Total solid content 3.33 ± 1.03 ppm in BSNP ointment was estimated by using a total dissolved solids meter. In preliminary physical evaluations, the BSNP ointment appeared light brown in color and no phase separation was observed at 4, 25 and 40°C.

The formulated BSNP ointment showed the ability to spread evenly for topical delivery of the drug. The average value of spreadability observed was S = 24.35 ± 2.64 g.cm/s, which indicated the normal semisolid behavior of the formulations. In a previous report, similar results were observed by Pattanayak *et al.* (2011). The spreadability and rheological behavior of the formulations showed the drug delivery efficiency of the ointment. Viscosity (cP), the major rheological parameter, was determined at lower to higher rotation (rpm) of the viscometer spindle, when the rotation of the viscometer spindle increased, viscosity gradually reduced, while the shear rate was gradually increased, and vice versa. Viscosity (cP) data has been represented in a combo graph with shear rate 1/s. (Fig. 1A). The lowest viscosity value (20.16 cP) of BSNP ointment was recorded at 60.00 rpm with 73.38 shear rate 1/sec at 37°C. As per a previous report, lower viscosity of a semi-solid formulation may directly influence the transdermal diffusion rate of the drug (Ueda *et al.* 2009). Furthermore, the agar diffusion assay also supported the diffusion efficacy of BSNP ointment by showing the zone around the applied area of ointment due to inhibiting the growth of *S. aureus* (Fig. 1B). In this study, all the ointment characterization parameters such as spreadability, rheological behavior and agar diffusion assay revealed that BSNP ointment retained the better diffusible property and it may be an effective topical ointment for acceleration of the wound healing process.

Wound contraction rate

Formulation and topical management of BSNP is briefly illustrated in Fig. 2. BSNP-containing ointment significantly accelerated wound healing activity in the rat as compared to untreated control and wounds treated with common antiseptic ointment, i.e. Betadine and Soframycin (Fig. 3A). The wound closure rate of the BSNP-treated wound area was 22.77 ± 1.60% as compared to day 0 untreated control, while closure rates observed at day 3 for the other groups were control 10.99 ± 1.74%, Betadine 14.73 ± 2.36% and Soframycin 18.55 ± 1.37%. In addition, % of the wound contraction rate, including days 7 and 11, is graphically represented in (Fig. 4A) (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Topical application of BSNP ointment has not shown any significant adverse effect on weight of treated and untreated control rats (Fig. 4B); a previous study by Krausz *et al.* (2015) also supported this result.

The process of wound contraction is made up of several primary inflammatory stages such as epithelialization, granulation and collagenation, followed by development of fibroblast proliferation and collagen fiber (Gong, Li and Wang 2018). However, BSNP proficiently upgraded the promotion of primary inflammatory processes. BSNP has effective wound healing ability relative to other common antiseptic drugs that are used for wound repair.

In vitro antibacterial activity of BSNP and enumeration of total bacterial population in the wound area

Wounds are usually infected by bacterial colonization, emanating either from the normal flora of the skin or the outside environment, that causes tissue damage and suppresses the healing process. Previous results have proven BSNP to be a more effective antibacterial bio-agent than its chemical counterparts, described earlier by Kumari *et al.* (2017). Moreover, selection of BSNP for this study was based on its strong antibacterial efficacy against wound infections caused by *S. aureus* (ATCC 33 591) and *P. aeruginosa* (ATCC15692). Furthermore, antibacterial sensitivity results against *E. coli* were also observed, and a significant zone of inhibition was recorded against three selected pathogenic bacterial strains of 12.25 ± 0.05, 18.74 ± 0.95 and 27.75 ± 1.25 mm in diameter respectively, as shown in Table 2. *S. aureus* is a well-known pathogenic microbe that causes direct infections in skin and wound areas. *P. aeruginosa* also has been reported as opportunistic and causing long-term infection in skin and surgical wound areas (Stover *et al.* 2000). Similarly, *E. coli* is the commonest pathogen found at original sites of infection, in the bloodstream and in wound areas of the body (Jarvis and Martone 1992). Antibacterial activity of BSNP ointment was also evaluated by enumeration of the total bacterial population in the wound area on days 3 and 7. Log CFU/ml values were calculated for bacterial colonies grown for 24 h on nutrient agar plates. A gradual reduction was found in the bacterial population of a BSNP-treated wound swab sample as compared with untreated control and all other groups. The BSNP ointment treated wound samples had a $\log_{10}$ CFU/ml value of 3.825 ± 0.17, untreated control 5.18 ± 0.05, Betadine 4.84 ± 0.023 and Soframycin 3.88 ± 0.12, while at day 7, BSNP ointment treated wound samples had a $\log_{10}$ CFU/ml value of 1.55 ± 0.02, untreated control 7.17 ± 0.02, Betadine 8.77 0.01 and Soframycin 4.69 ± 0.04 (Fig. 4C).

Histological study

Histological study of wound area at days 3 and 7 revealed more and well organized fibroblast, collagen fibres and inflammatory cells (neutrophils, macrophages and lymphocytes) in BSNP treated wound tissue compared with other groups (Fig. 5). Cells were morphologically differentiated by light microscope (100X). The fibroblast cells were elongated and dark purple in color; a similar observation was described by Amin *et al.* (2015). Moreover, the macrophages had an irregular, round shape while neutrophils were circular and had dark spots in the cells due to multilobulated nuclei. The collagen of skin tissue stained by eosin counter stain showed a dark pink colour; similar results were highlighted by Dantas *et al.* (2014) and Udhayakumar *et al.* (2017). Conclusively, enhanced collagen appear in granulation tissues with other bio-molecules that may organize the wound healing process (Kwan *et al.* 2011). The wound healing process contributing by inflammatory and fibroblast cells participated in temporary repair and regeneration process. Multiple chemotactic signals generated from wound site which are recruited the neutrophils and macrophages from circulating blood (Martin 1997).

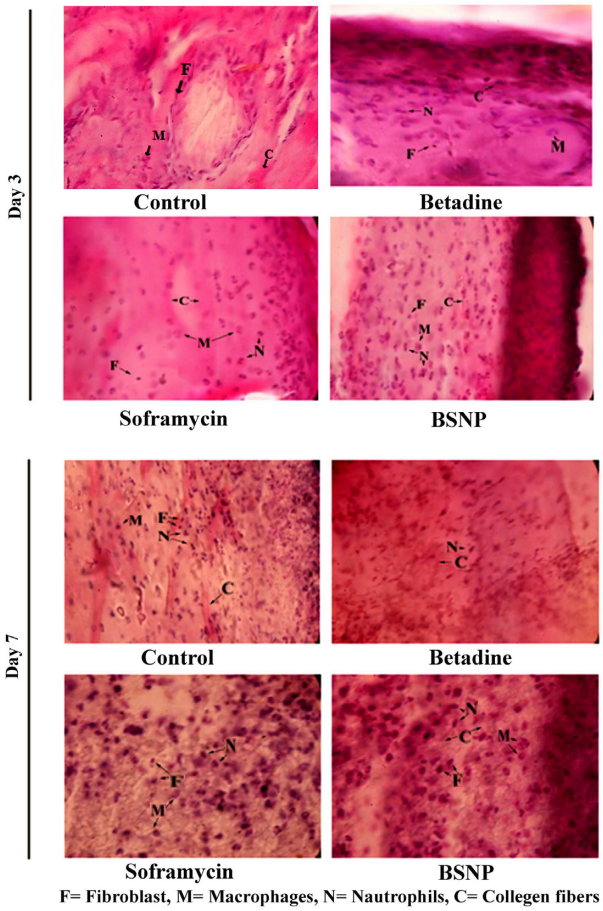

Figure 5. Histological images of the wound tissues at days 3 and 7 after the treatment with different wound dressing materials: untreated control, Betadine, Soframycin and BSNP ointment.

So, these evaluations support that BSNP had an evident effect on accelerating the wound healing process compared with other antiseptic medicine commonly using for wound repair.

BSNP increase cell migration *in vitro*

In vitro scratch assay revealed the wound healing potential of BSNP. After 12 h of incubation with 5 and 7.5% BSNP, a concentration-dependent increase in wound healing was observed (Fig. 6A). The concentration used in ointment preparation (7.5%), showed 68.92 ± 1.9% migration of fibroblast cells in comparison to 32.47 ± 7.8% migration in control (Fig. 6B). Scratch assay is a convenient method to show the wound healing potential of drugs or molecules by enhancing cell–cell and cell–extracellular matrix (ECM) interaction (Liang, Park and Guan 2007). Previously, You *et al.* (2017) and Moniri *et al.* (2018) have shown wound healing by silver nanoparticle-loaded collagen/chitosan scaffolds caused by increasing fibroblast migration, macrophage activation and alteration in gene expression. In this study, BSNP at 5 and 7.5% concentrations were well compatible with fibroblast cells and corroborated the *in vivo* results.

DPPH activity

BSNP showed significant DPPH scavenging activity: the % inhibition of DPPH radical observed at different concentrations of BSNP (25, 50, 100 and 150 μl/ml) were 13, 22.92, 50.53 and 66.45

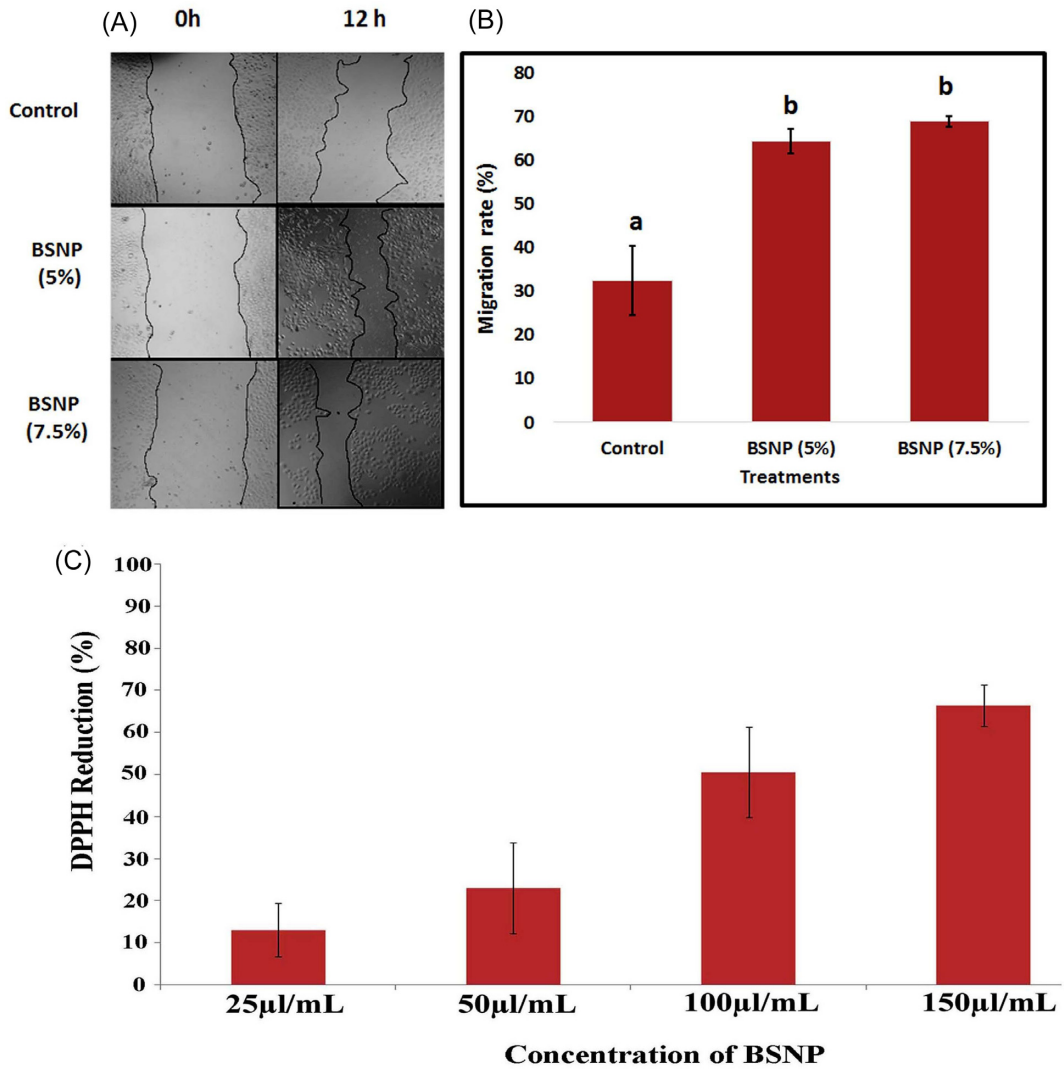

Figure 6. *In vitro* scratch assay: BSNP increases migration of fibroblast cells after 12 h of incubation (**A**), % migration rate of cells in the presence of 5 and 7.5% of BSNP in comparison to control (**B**), and DPPH radical scavenging capacity of BSNP (**C**). 'Statistical package for the social sciences' software was used for the significant difference in the *in-vitro* scratch assay, represented by different letters of the alphabet according to Duncan's multiple comparison test ($P < 0.05$).

respectively (Fig. 6C). Previous studies by Kharat and Mendhulkar (2016) and Priya, Geetha and Ramesh (2016) also observed antioxidant activity of biologically synthesized silver nanoparticles. Results of these studies strongly recommend the application of BSNP as a natural antioxidant for health preservation against degenerative diseases.

Stability of BSNP ointment

There was no evidence found for physical and chemical instability up to 40°C, while at 55°C, a slight phase separation in the ointment was observed. This observation indicated a good combination between BSNP and ointment base, thus resulting in better thermal stability of the formulated ointment. A study of Fayemi *et al.* (2018) had also described an earlier report for the thermal stability and a good interaction between polyacrylonitrile nanofibers with 0.5 g of moringa leaf extract.

CONCLUSION

Herein, an environmentally friendly approach was demonstrated for acceleration of wound healing activity by BSNP synthesized from *T. viride* filtrate. Our findings reveal a simple, low cost, high commercial value and eco-friendly proposition for replacement of many common wound healing ointments and as a substitute for antimicrobial resistant drugs used in ointment preparation. Various biomedical applications such as antimicrobial, antioxidants and wound healing property of *T. viride* metabolites involved in the synthesis of BSNP potentially promoted to the application of metallic nanoparticles. Simultaneously, BSNP accelerated tissue regeneration and re-epithelialization. *In vitro* scratch assay revealed the wound healing potential of BSNP at the concentration used in the ointment preparation. After clinical trials and investigating the possible adverse effects of BSNP ointment, it can be used for treating and covering infection-sensitive wounds such as normal, diabetic or burns wounds. Conclusively, the BSNP-containing formulation provides great antimicrobial activity against the opportunistic bacteria of wound infections.

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AUTHOR CONTRIBUTIONS

A.M. and C.S.N. designed the work and proofread the manuscript. V.P.G., S.P. and M.K. carried out the experimental work and contributed in the manuscript writing. S.K.P. helped in animal handling and A.T. helped in the revision work. C.V.R. provided the animal house facility. M.S. provided the facility for rheological analysis and R.K. contributed to data interpretation.

Conflict of interest. None declared.

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