Polysaccharide-capped silver nanoparticles impregnated-cream for the efficient management of wound healing

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Supplementary Data

S1. Stability Study

Stability testing aims to ensure that the cosmetic product maintains its intended physical, chemical, and microbiological quality, as well as functionality and aesthetics when stored under appropriate conditions. The stability of all formulations was checked for two weeks (measurements will be made on day 1, week 1, and week 2). The pH values, colour, physical appearance, and texture will be tested during the 2 weeks.

Accelerated stability studies

All the formulations were subjected to a 4-month-long protocol of accelerated stability testing conducted at 40 ± 2 ºC. Each formulation was examined for separation appearance, colour, or phase separation changes on consecutive days.

S2. Determination of Total Fatty Substance Content

Accurately weigh 2g of cream into a conical flask, and add 25 mL of dil. HCl, boil the contents until they areclear. Add the contents in a separating funnel. Add petroleum ether and shake well. Separate the aqueous phase. Filter the petroleum ether extracts through filter paper containing sodium sulphate, previously dried at a temperature of 90 ± 2 C. Distill off the petroleum ether and dry the material remaining in the flask to constant mass.

Total fatty substance percent by mass = 100 (M_1/M_2)

where M1 mass in g of residue and M2 mass in g of material taken for the test

S3. Test for Heavy metals

Weigh about 2 g of material in a crucible and heat it in a muffle furnace to ignite it at 600^{o}C to constant mass. Add 3 mL of dil. HCl and make up the volume to 100 mL. Filter the solution and transfer 25 mL of filtrate into Nessler's cylinder. Add 2 mL of dil. acetic acid and 1 mL of the standard lead solution to another Nessler cylinder and make up the volume with water to 25 mL. Add 10 mL hydrogen sulphide solution to each cylinder and makeup to 50 mL with water. Mix and allow to stand for 10 min. Compare the colour in two cylinders. If the colour in the sample is less than that of the standard solution, the sample will pass the test.

S4. Determination of Total Viable count after stability

Each cream formulation was accurately weighed at 1 gm. Emulsify the cream in a few drops of Tween 20, and further dilutions were prepared using 10 % DMSO.

S4.1. Serial dilution: All the samples were serially diluted in test tubes marked 10^{-1} to 10^{-5}. 9 mL of 10 % DMSO was taken in all test tubes. To the test tube marked 10^{-1}, 1 mL of the sample was added. From this dilution, 1mL of the sample was taken and added to 10^{-2} dilutions, and this10^{-5} dilutions were prepared.

S4.2. Pour plate method: Pour plate method was usually the method of choice for counting the colony-forming bacteria in a liquid specimen. This method uses a sterile pipette to place a fixed amount of inoculum (generally 1 mL) from a broth/sample in the centre of a sterile petri dish. Melted agar (approx. 15 mL) was then mixed well into the Petri dish containing the inoculum. After the solidification of the agar, the plate is incubated at 37°C for 24-48 hours to check the number of Colony Forming Units (CFU) in each formulation.

S5. Checking for the presence of pathogens

For the detection of pathogens like *E. coli, P. aeruginosa, S. aureus* and *C. albicans*, the cream suspension was inoculated on the surface of MacConkey Agar, Cetrimide agar, Baird Parker agar and SDA selective agar plates respectively. The plates were incubated for 72 h at 37^{o}C for bacterial growth and at 30^{o}C for *Candida*.

Supplementary Table 1: Analytical Parameters

Sl.No.	**Test**	**Specification**
01	Appearance	Opaque, homogenous cream
02	Color	Characteristic
03	pH	6.00 – 7.50
04	Spreadability	Diameters measured
05	Stability	Need to be stable at RT, 4^{o}C and 45^{o}C
06	Microbial test; 1. Total count, CFU/gm	 <100

Supplementary Table 2: Organoleptic properties of formulated creams

	Base Cream – 1	SNP@PSP Cream - 2	SNP@PSP Cream – 3	SNP@PSP Cream - 4
Appearance	Opaque homogenous cream	Opaque homogenous cream	Opaque homogenous cream	Opaque homogenous cream
Colour	White	Light yellow	Light brown	Light brown

Supplementary Table 3: Stability checking of all cream formulations

	Appearance	**Colour**	**pH**
Base cream - 1	No separation of phases. Homogenous 5cream	White	8.49
SNP@PSP cream – 2	No separation of phases. Homogenous cream	Yellow	7.7
SNP@PSPcream – 3	No separation of phases. Homogenous cream	Yellow	7.4

SNP@PSPcream – 4	No separation of phases. Homogenous cream	Light brown	7.4

Supplementary Table 4: Weight loss analysis of SNP@PSP doped cream

Sample	Temperature	Initial weight (wt of Petri dish-wt of cream) (gm)	Final weight (wt of Petri dish-wt of cream) (gm)	% of Weight loss
SNP@PSP Cream	4°C	33.21	33.04	0.51
	45°C	37.93	36.92	2.92
	Room temperature	36.92	36.52	1.08

Supplementary Table 5: BIS analysis of SNP@PSP incorporated cream

Sl. No:	**Test**	**Results**	**Specifications**
1	pH	7.6	IS 6608: 2004, Reaff. 2019, Annex B
2	Total Fatty substance	7.03	IS 6608: 2004, Reaff. 2019, Annex B
3	Total residue	37.43	IS 6608: 2004, Reaff. 2019, Annex B
4	Thermal stability	Stable	IS 6608: 2004, Reaff. 2019, Annex B
5	Heavy metal (as Pb)	<20 ppm	IS 6608 2 004, Reaff. 2019, Annex B
6	Arsenic (as As_2O_3)	<2ppm	IS 6608: 2004, Reaff. 2019, Annex B

7	Yeast and mold count	<10cfu/g	IS 14648: 2011, Reaff. 2016
8	*E. coli*	Absent/g	IS 14648 :0 11, Reaff. 2016
9	*S. aureus*	Absent/g	IS 14648: 2011, Reaff. 2016
10	*P. aeruginosa*	Absent/g	IS 14648: 2011, Reaff. 2016
11	*C. albicans*	Absent/g	IS 14648:2 011, Reaff. 2016
12	Total Viable Count	<10cfu/g	IS 14648: 2011, Reaff. 2016

Supplementary Table 6: Measurement of zone diameter in Disc diffusion method

Organism	Positive control	Base Cream 1	SNP@PSP Cream 2	SNP@PSP Cream 3	SNP@PSP Cream 4
S aureus	45 ± 5 mm	9 ± 3 mm	18 ± 4 mm	20 ± 3 mm	22 ± 3 mm
C albicans	32 ± 3 mm	-	15 ± 3mm	18 ± 2 mm	21 ± 3 mm

Supplementary Table 7: Measurement of the zone diameter in the Well plate method

Organism	positive control	SNP@PSP Cream 2	SNP@PSP Cream 3	SNP@PSP Cream 4
S aureus	43 ± 2 mm	19 ± 2 mm	21 ± 3 mm	23 ± 4 mm
C albicans	42 ± 3 mm	18 ± 2 mm	20 ± 2 mm	21 ± 3 mm

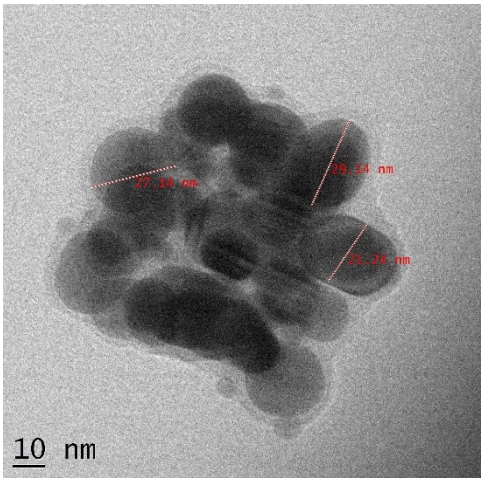
Supplementary Table 8: Microscopic appearance of wound tissues in H & E staining

SL. No	**Group**	**Microscopic appearance**
1	Control	The dermis shows focal collections of lymphocytes and fibroblasts, and the surrounding area shows dense fibrosis. The deep dermis shows scattered inflammatory infiltrates.
2	Silverex cream	Dermis shows scattered lymphocytic infiltrates, fibroblasts and appendages; the surrounding area shows dense fibrosis.

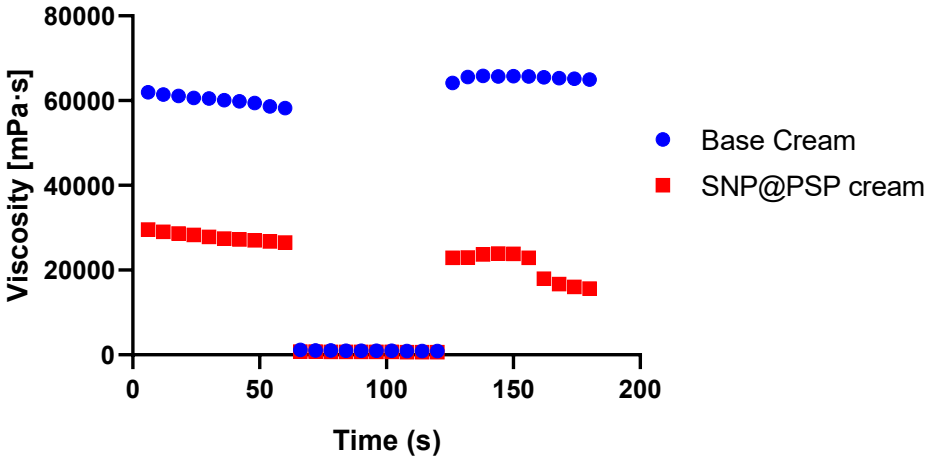
	treated	
3	SNP@PSP cream treated	Skin shows epidermis with Re-epithelialization. The epidermis shows scattered inflammatory infiltrates, fibroblasts and thin-walled capillaries in a fibrocollagenous stroma. The deep dermis shows moderate inflammatory infiltrates.
4	Base cream treated	Sub epithelium shows dense fibro collagenous stroma, appendages and scattered inflammatory infiltrates.

Supplementary Table 9: Reaction of wound tissues in Cox 2 and Veg F immune histochemistry

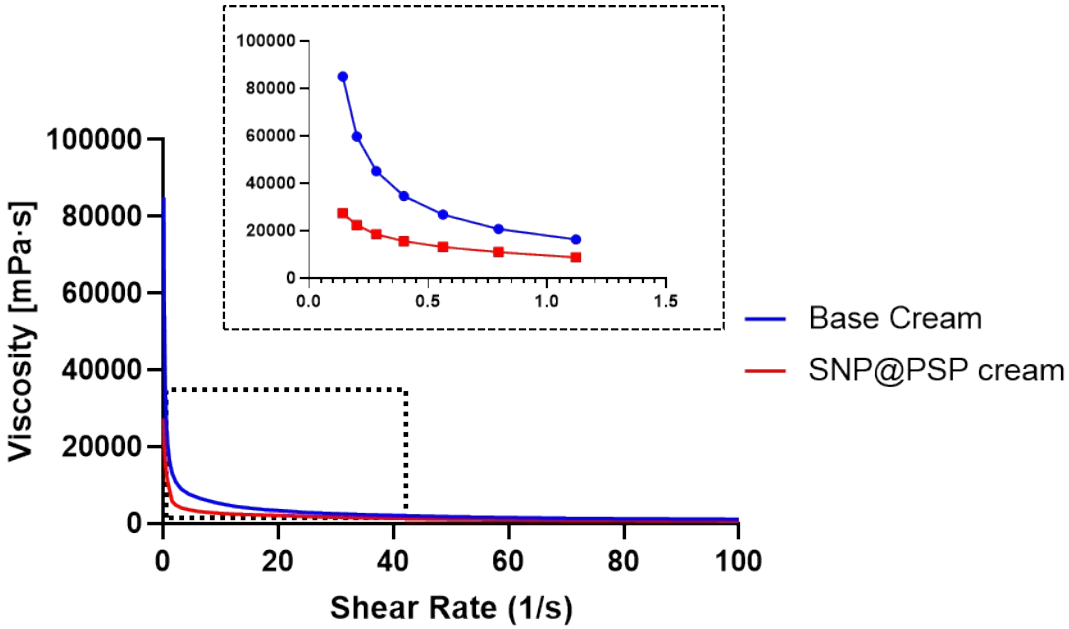
SL. No:	**Sample**	**COX 2**	**Veg F**
1	Control	Immunoreactive (2+) Nuclear positivity (+)	Negative (0)
2	Silverex treated	Immunoreactive (1+) Focal Nuclear positivity (+)	Negative (0)
3	SNP @ PSP Cream tre	Immunoreactive (1+) Nuclear positivity (+)	Negative (0)
4	Base cream	Immunoreactive (1+) Nuclear positivity (+)	Negative (0)



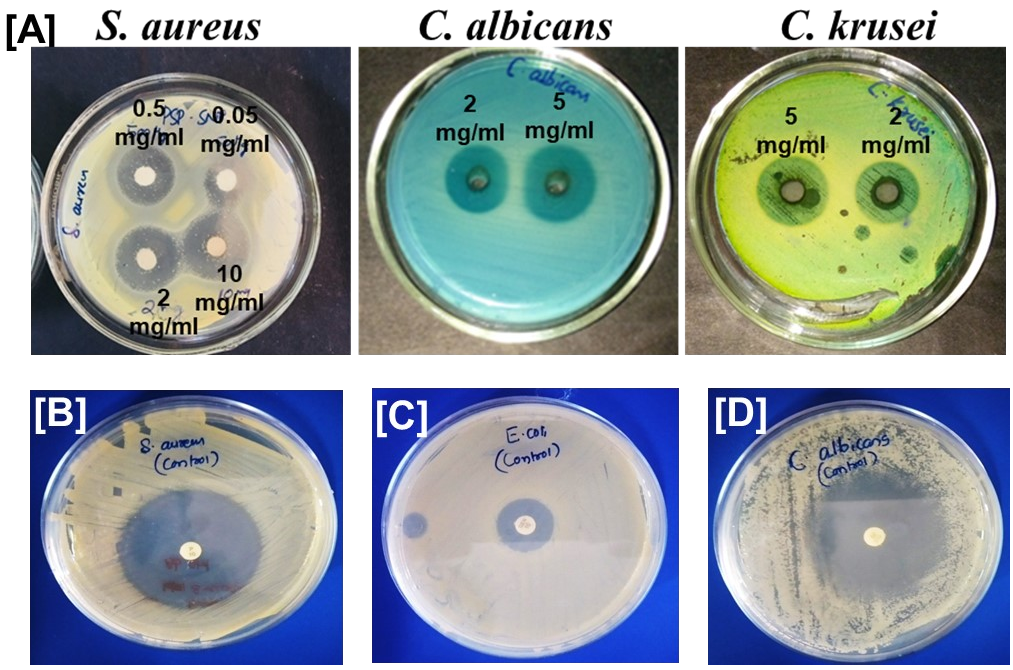
Supplementary Figure 1: TEM image of SNP@PSP



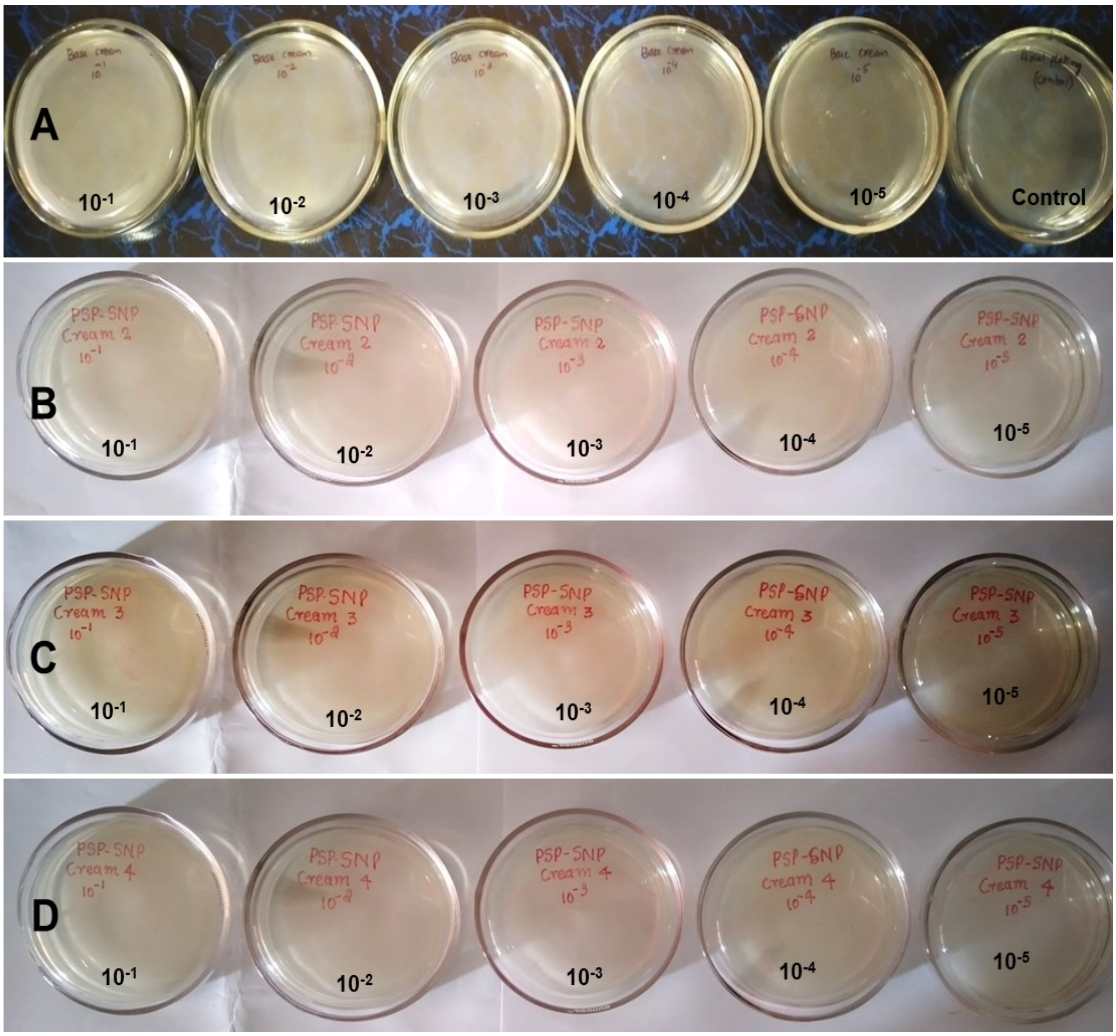
Supplementary Figure 2: Rheological behaviour of Base Cream and SNP@PSP Cream using thixotropic behaviour analysis



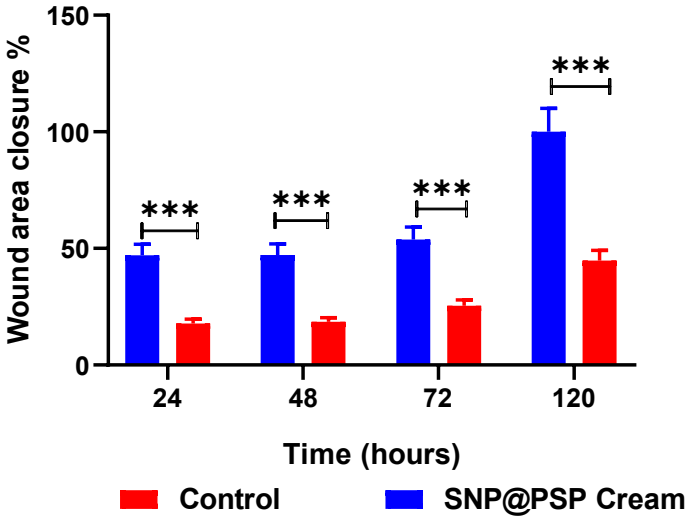
Supplementary Figure 3: Rheological behaviour of Base Cream and SNP@PSP Cream using viscosity analysis. Inset shows the viscosity difference after the addition of SNP@PSP in base cream at lower shear rates



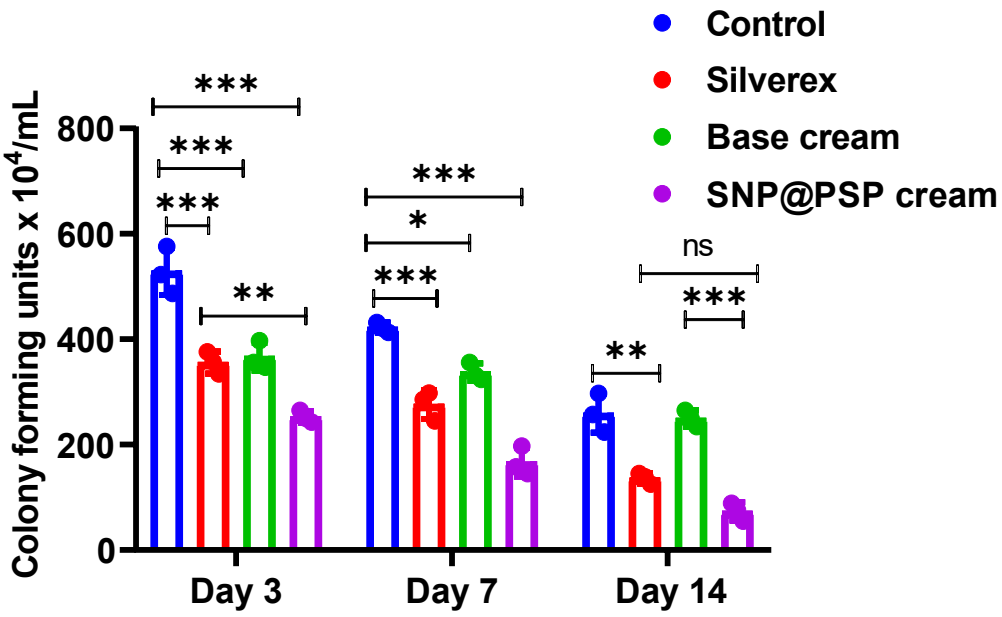
Supplementary Figure 4: (A) Antimicrobial activity of cream formulations on Muller Hinton agar plates- zone of inhibition of susceptible test organisms (S. aureus, C. albicans and C. krusei) (B) Positive control plates for S. aureus, (C) E. coli and (D) C. albicans with Penicillin, gentamycin and Fluconazole sensitivity discs respectively



Supplementary Figure 5: Spread plate assay for determining the microbial contamination of (A) Base cream (B) SNP@PSP cream -1 (C) SNP@PSP cream-2 (D) SNP@PSP cream-3



Supplementary Figure 6: In vitro percentage wound area closure of cells treated with control and SNP@PSP cream. Results were expressed as mean ± SD (n = 3)



Supplementary Figure 7: Total viable counts of microbes (Colony forming units x 10^4/mL) from the wound area of mice in different treatment groups at different time points