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Research paper

Green synthesized nanosilver loaded silk fibroin gel for enhanced wound healing

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ABSTRACT

One of the ideal growth mediums for microbes especially bacteria is an open wound of host. Microbial infection of wounds has always presented challenges toward wound healing. It is believed that combination of cell growth promoting agent along with antimicrobial agent will prove fruitful in fast wound healing. Thus objective of the present work was to prepare nanosilver loaded silk fibroin (NSF) by *in situ* reduction of silver ions to silver nanoparticles (AgNPs) and further to evaluate its wound healing potential. The prepared NSF dispersion was characterized by UV spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction analysis (XRD), Transmission electron microscopy (TEM), silver content analysis and antibacterial activity against *Staphylococcus aureus*. Further, NSF was transformed into gel and compared to marketed topical gel (soframycin gel) for wound healing in male wistar rats. NSF gel had more pronounced wound healing than marketed formulation which is believed to be due to dual role played by NSF gel wherein silk fibroin acted as epidermal cell growth promoter and AgNPs acted as antimicrobial agent leading to faster percent wound closure. This investigation also proposes the need for such combinations of a cell growth promoting agent with antimicrobial agents in the treatment of wounds.

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1. Introduction

Wound healing, a common clinical entity contemporary to human beings is most often susceptible to microbial infection. The ultimate goal for wound healing is a speedy recovery with minimal scarring and maximal function [1]. Different approaches such as use of antimicrobials and cell growth promoting agents have been used for achieving the set goal.

Silk fibroin, a cell growth promoter has been used as wound healing agent [2]. Silk fibroin promotes the epithelization owing to its amino acid compositions which accelerates wound healing process. Further it has been reported that silk fibroin increase fibroblasts in the wounded tissue [3] which is indication of faster wound recovery. Silk fibroin as a wound healing material has several advantages as it is biodegradable, biocompatible and adheres to epidermal cells [4,5].

However, silk fibroin does not have anti-microbial activity. It is believed that anti-microbial activity will accelerate wound healing process since open wounds are most prone to the attack of variety of microbial agents. The presence of microbes in the wounds delays the wound healing process to a greater extent. The risk factors associated with microbes affecting wounds includes cellulittis, necrotizing subcutaneous infection, gas gangrene etc. Thus there is need to develop a formulation having dual role (i.e. cell growth promoting activity along with antimicrobial activity).

It is reported that upon reaching nanoscale, due to extremely small size, silver nanoparticles (AgNPs) exhibit remarkably unusual physicochemical properties and biological activities. AgNPs have shown great importance due to their broad spectrum antimicrobial activity. AgNPs have shown bactericidal activity against various strains of bacteria including *Salmonella*, *Staphylococcus and Pseudomonas* [6–8]. Most importantly, microbes fail to develop resistance against silver because it attacks a broad range of targets in the microbes [9]. The reports state that silver exhibits antibacterial activity owing to disruption of the cell wall which increases cell wall permeability and in turn depletes cell ATP affecting its viability. Cell death due to formation of free radicals by AgNP is another mechanism proposed to attribute their antimicrobial activity. Further, silver nanoparticles are reported to inactivate many important bacterial enzymes by interacting with their thiol groups.

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Silver, a soft acid interferes with the DNA replication of the bacterial cell by reacting with soft bases such as sulfur and phosphorus (e.g. DNA) present within them leading to cell death. Additionally according to reports the phosphotyrosine profile of bacterial peptides is altered by AgNP which in turn modulate the signal transduction in bacteria leading to the stoppage of growth [10,11]. The monodispersed AgNPs with narrow particle size distribution are more effective antibacterial agents, because of the high surface/volume fraction so that a large proportion of silver atoms are in direct contact with their environment [10].

Considering the wound healing activity of silk fibroin and broad spectrum anti-microbial activity of AgNPs, it is believed that the combination of silk Fibroin with AgNPs may be highly beneficial in wound healing process since the combination could act on wounds in two ways. Firstly, cell growth promoting action of silk fibroin proteins and second, broad spectrum anti-microbial action of AgNPs on the microbes affecting the wounds. Therefore in the present work AgNPs were green synthesized by *in situ* reduction of silver ions using silk fibroin solution. The prepared nanosilver loaded silk fibroin solution (NSF) was characterized by UV spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction analysis (XRD), Transmission electron microscopy (TEM), silver content analysis and antibacterial activity against *Staphylococcus aureus*. Further, NSF was formulated into gel and screened for wound healing activity in rats.

2. Experimental methods

2.1. Materials

Silk cocoons were obtained from Sericulture Institute and Training Centre, Pune. Litium bromide, carbopol 934 and sodium carbonate was purchased from Research Labs, Mumbai, India. Silver nitrate was purchased from Central Drug House (CDH), Mumbai, India. Cellophane Membrane was purchased from Hi-media labs Pvt. Ltd. Clinically isolated *S. aureus* strain was procured from National Chemical Laboratory (NCL), Pune, India.

2.2. Methods

2.2.1. Green synthesis of nanosilver loaded silk fibroin solutions

Bombyx mori fibroin solutions were prepared according to previously published procedures [2]. Silver nitrate (10^{-2}–10^{-4} M) was added in solutions of silk fibroin (1–7% w/v). The prepared dilutions were kept at room temperature under light (40 W bulb, Philips) for 24 h. The solution showed i*n situ* reduction of silver ions to pale yellow colloidal solution containing AgNPs after 24 h [12]. The prepared solutions were investigated for the effect of pH in the range of 4–8.5.

2.2.2. Spray drying of colloidal solution

The optimized NSF and silk fibroin solution (SF) alone was then spray dried so as to obtain dry powder. Spray-drying was performed with a Mini Spray-dryer, Labultima (Mumbai, India) with inlet and outlet temperature of 150 °C and 100 °C respectively. The aspiration was maintained at 45 m^3/hr with a feed rate of 10 ml/min. The spray dried powders were recovered and stored at room temperature in desiccators and analyzed by FTIR (Jasco, V 5300, and Japan) and XRD.

2.2.3. UV–visible spectroscopy

UV–visible spectroscopy is the most common method used to confirm the formation of AgNPs, since nanosilver exhibit Surface Plasmon Resonance (SPR) at about 400 nm. All colloidal solutions along with SF dialysate were scanned between 200 to 800 nm.

2.2.4. Particle size analysis

Particle size analysis of the prepared nanoparticles (NSF) was performed using particle size analyzer (Malvern Mastersizer 2000 SM, UK) working on the principle of laser diffraction.

2.2.5. Fourier transform infrared spectroscopy

Infrared spectrum of spray dried powders of SF and NSF was obtained using fourier transform infrared spectrometer (JASCO FT/IR 4100, Japan). Samples (5–10 mg) were triturated with activated potassium bromide (KBr) and scanned in the range of 600–4000 cm^{-1}.

2.2.6. Transmission electron microscopy

Particle morphology of nanoparticles contained in NSF was analyzed using TEM (Philips model CM200). The instrument operated at an accelerating voltage of 200 kV. Samples for TEM analysis were prepared by placing drops of NSF on carbon-coated TEM copper grids followed by drying under the IR lamp.

2.2.7. X-ray diffraction analysis

The spray dried SF and NSF was subjected to XRD analysis. XRD patterns were recorded using Philips PW 1729 X-ray diffractometer (PW 1729, Philips, The Netherlands). The samples were irradiated with monochromatized CuKα radiation (1.542 A°) over 0°–70° diffraction angle (2θ).

2.2.8. Determination of silver content

The silver content of NSF was determined using Atomic Absorption Spectroscopy (AAS) (Varian spectrAA 220 spectrometer).

2.2.9. Antibacterial studies

Antibacterial studies were carried out on *S. aureus*. The samples were smeared on nutrient agar medium plates. The wells were made on the nutrient agar medium plates. Different dilutions of NSF and colloidal silver nanoparticulate solutions (SNs) containing silver in the range of 2–8 ppm were prepared and added to the well. The plates were then incubated at 37 °C for two days. The zone of inhibition was calculated with the help of Vernier caliper. SF was also tested likewise. The colloidal solution of silver nanoparticles (SNs) used for the antimicrobial study was prepared by chemical reduction of $AgNO_3$ solution 10^{-3} M using 1% w/v hydrazine hydride solution as a reducing agent [13].

2.2.10. Formulation of nanosilver loaded silk fibroin gel (NSF gel)

Carbopol 934 (0.7%w/v) was added to NSF containing 6% w/v silk fibroin along with 10^{-3} M $AgNO_3$ under stirring and pH was adjusted to 6.8–7.5 by addition of triethanolamine. The mixture was allowed to stand for an overnight to obtain nanosilver loaded silk fibroin (NSF) gel. Similar procedure was followed to prepare gel containing silk fibroin (SF, 6%w/v) and SNs respectively. Plain Carbopol gel was also prepared for comparison purpose.

2.2.11. Excision wound healing study in rats

Male albino wistar rats weighing 180–250 gm were used for wound healing study. Animals were kept in laboratory for 3–4 days for acclimatization, with free access to food and water. Six groups each containing 5 screened animals were prepared. The formulations used for a group were as follows.

Group I: Optimized Silk fibroin gel (SF gel), Group II: Optimized Nanosilver loaded silk fibroin gel (NSF gel), Group III: Silver nanoparticulate gel (SNs gel), Group IV: Carbopol gel, Group V: Positive Control (Soframycin gel), Group VI: Negative Control.

The animal study was approved by Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC) of Poona College of

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