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Wound Healing by Multifunctional Natural Matrix

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Abstract—*Wound healing is a biological process related to the general phenomenon of tissue regeneration. Wound healing process, is a series of interdependent and overlapping stages, in which a variety of cellular matrix components act together to re-establish the integrity of damaged tissue and replacement of lost tissue. Plants have been a source of medicinal compounds and play an important role in maintaining human health since ancient times. In Ayurveda many diseases and infections are healed using natural herbs.*

Chitosan has a number of commercial and biomedical uses. Chitosan has high molecular weight and thus is not soluble in water. A derivative, SALM-CS or Zwitterionic Chitosan (ZWC) was developed that has low molecular weight than chitosan, by using Succinic anhydride. This

derivative (ZWC) is water soluble and is an affective nanoparticles delivery system for drug. Prepared Zwitterionic Chitosan biomaterial was confirmed structure by1H NMR studies, further along with plant extracts which contains ayurvedic medicinal compounds showed good biocompatibility by MTT test and haemostatic activity. Matrix blends of different ratios were showed good antibiotic for wound pathogens along with MRSA pathogen, which was killed completely within 12 hrs by CFU test. In microscopic studies, it was showed that ≤200 nm size nono spheres were identified along with mesh like scaffold textures with porosity, and is suitable for regeneration of tissue in in-vivo for cell interaction and cell migration after cell proliferation.

To protect burns skin and other wounds from infections and also to regenerate tissue, SALM-CS with blend of tulsi extract by ayurvedic bhasma approach, formulation in form of gauze fabric bandage (scaffolds) could show rapid tissue regeneration, non allergic, biocompatible and antibiotic activity in vivo level

1. INTRODUCTION

Wound Management is gaining importance in present times, with demand of advance technology in development of Medical devices and Medical textile fields. Number of research works are going on to utilize the important properties of natural available products in wound healing. Ayurvedic ‘Bhasms’(Alternative traditional medicine) application as the Ehono- nanomedicine for exploitation of our traditional knowledge, to develop efficient wound therapies, is also fast gaining popularity.

In Ayurveda and Unani system,*Ocimum sanctum L***.** (Tulsi) has been used for thousands of years for its diverse healing properties. In these traditional medicines, herb extracts with unique metallic preparation are important tools for curing the diseases. Similarly, recent research findings showed that tulsi herb extracts is a good source for the preparation of nanoparticles of silver metal and can be used in ethano-nanomedicine.[12] It shows synergistic interactions of many different active phytochemicals along with wound healing, anti-inflammatory etc.reported the antimicrobial activity of silver nanoparticles (10-25nm size) that was synthesized by chemical method. Nano based silver particles are being used effectively for their antibiotic action in post-surgery effectively. Preparation of the nanoparticles synthesis by green chemistry is more active, biologically accepted. Moreover, the process is cost effective too. Although, the activity of the nanoparticle depends on different physical parameters, hence we need to apply synergetic properties for development of wound healing dressings along with other factors.

Chitosan, as a cationic natural polymer, has been widely investigated as an antimicrobial agent for preventing and treating infections owing to its intrinsic antimicrobial properties, and also its ability to effectively deliver extrinsic antimicrobial compounds into the infected area. Many factors present in the chitosan molecule or its environment can influence the antimicrobial properties, such as the molecular weight, DDA and the ionic strength and pH [10].

The glucose amino glycan constituents of the wound tissue play an important role in giving structure and strength to newly formed collagen in granulating the tissue of the healing wound. Chitosan provides amino groups which in turn can be made available to the fibroblasts that proliferate under the action of interlukin-1 for incorporation into hyaluronate and glucose amino glycan. Thus guiding ordered deposition of collagen leading to wound healing [11].

Chitosan and its derivatives have low toxicity and hence can be used over wide range of applications present in pharmacological applications. Chitosan is non-soluble in water and has high molecular weight. To overcome this disadvantage, it was reported that low molecular Chitosan conjugated with succinic anhydride showed zwitterions function for non-toxic, biocompatibility and drug delivery. They also reported that ZWC has potential application in nanoparticle drug delivery system. Their Pre-clinical studies also indicated that chitosan has various biological activities.[13]

Considering the above knowledge, tulsi-NanoAgs, Low molecular chitosan- zwittorion have used in the present study for analysis of antibacterial activity for further applications of advance wound scaffolds and wound management.

2. MATERIALS AND METHODS

2.1 Materials

Ultra pure silver nitrate (assay 99.8%) was purchased from Micro Photo Film Ambala Cantt. The Lanoline anhydrous IP from Rolex Lanoline Limited Products Mumbai, Chitosan from Himedia Laboratory Mumbai, Active Charcoal, Muller Hilton Agar and other reagents and solvents were of reagent grade.

Common human pathogenic bacterial clinical isolates *Escherichia coli (E. coli)* and *Staphylococcus aureus (S.aureus)* were used for assessment of antimicrobial activity of synthesized silver nanoparticles using *Ocimum sanctum* (Tulsi). Growth and maintenance of bacterial strains was done using nutrient agar. The suspension culture was prepared using nutrient broth. Muller Hilton Agar was used for assessing antimicrobial activity.

2.3 Preparation of non-allergenic Lanolin

The brown color lanoline paste is dissolved in Hexane and this is passed through a column of silica gel. The solution thus obtained after filtration is heated to evaporate the solvent.

2.4 Preparation of Ocimum sanctum nanoparticles

25 g of fresh Ocimum sanctum (Tulsi) leaves were mixed with 100 ml of distilled water and boiled for 5 minutes and the extract was filtered and was stored at 4ºC.

5ml each of Tulsi leaf extract and $AgNO_3$ aqueous solution was mixed and left for couple of hours. For studying the maximum effectiveness, AgNPs in different concentrations were made.[2,3]

2.5 Synthesis of succinic anhydride-conjugated low molecular weight chitosan (SALM-CS)

Low Molecular Chitosan was first dissolved in 1% acetic acid and freeze-dried. 200mg of Lyophilized solid was dissolved in 30 mL deionized (DI) water. 140mg Succinic anhydride was added as solid to the 400mg chitosan solution in varying quantities, over 5 min under vigorous stirring. After 1 hour of reaction, the pH of the reaction mixture was adjusted between 8 & 9 with 0.2 N NaHCO3.[4] After overnight reaction at room temperature under stirring, the reaction mixture was dialyzed against water using film having molecular weight cut-off: 3500. The pH was then adjusted between 10 &11 with 1 N NaOH. The purified succinic anhydride-conjugated low molecular weight chitosan (SALM-CS) was freeze-dried and stored at -20°C. In order to confirm the molecular composition of derivative formed, a Nuclear Magnetic Resonance (NMR) was done on the derivative. The NMR spectrum of the derivative was studied and compared with the NMR spectrum of the chitosan that were used. The proton signals were recorded at 70ºC. δ (ppm): 5.41(m),5.13(m),4.41-4.2 (br), 3.71(t), 2.58(s).

2.6 Preparation of scaffold using SALM-CS matrix and herbal extract

A 5% solution of SALM-CS in distill water was made. This solution was used to prepare matrix on the scaffolds and with the help of pipette the herbal extract was poured in different concentrations.

3. RESULTS

SALM-CS or Zwitterionic Chitosan (ZWC), has low molecular weight than the chitosan and is water soluble and affective nanoparticles delivery system for drug.

The Nuclear Magnetic Resonance (NMR) study of ZWC (Fig. 2) was done along with the chitosan control sample (Fig. 1). After analyzing the NMR spectrum of both the samples, it was concluded that the derivative has same molecular structure as original chitosan.

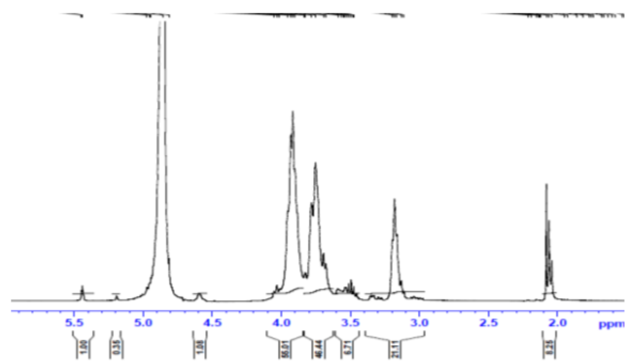


Fig. 1: NMR study of control Chitosan sample

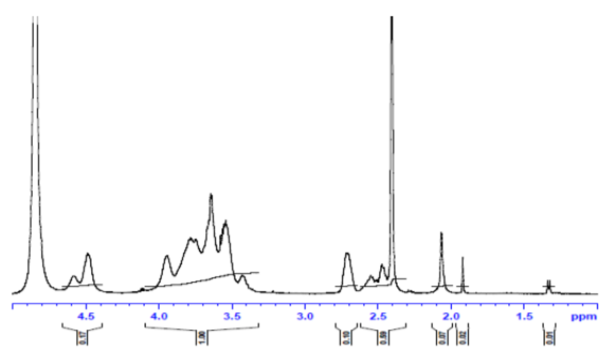


Fig. 2: NMR study of ZWC sample

In order to find out the morphology of the ZWC, scaffold prepared with the solution of ZWC in distill water was observed under Scanning Electronic Microscope (SEM) (Fig. 3). It was noticed that the ZWC had a white jelly with porous matrix form. Thus, it can be said that ZWC helps in transdermal drug delivery.

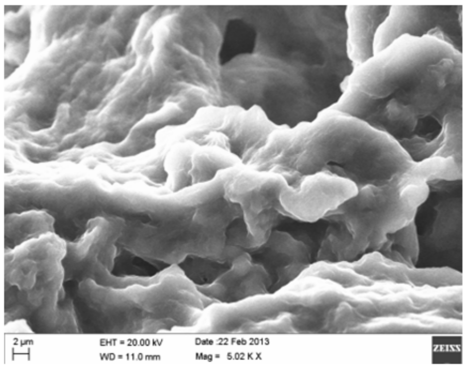


Fig. 3: ZWC matrix viewed under SEM at 2µm (5.02KX)

As Tulsi (*Ocimum sanctum*) exhibited a reasonably good antimicrobial activity, hence silver nanoparticles (AgNPs) were synthesized using Tulsi leaf and extract solution. Through this extraction method, an attempt was made to combine the inherent antimicrobial activities of silver and Tulsi for enhanced antimicrobial activity.

The scaffold coated with these AgNPs were analyzed under SEM and it was observed that AgNPs of size 53.35nm were formed and was good, biocompatible for medical application (Fig. 4), hence confirming the formation of AgNPs.

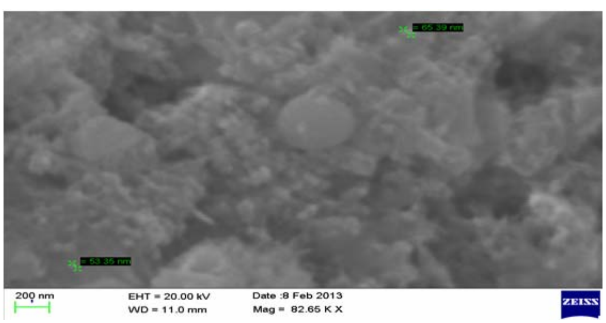
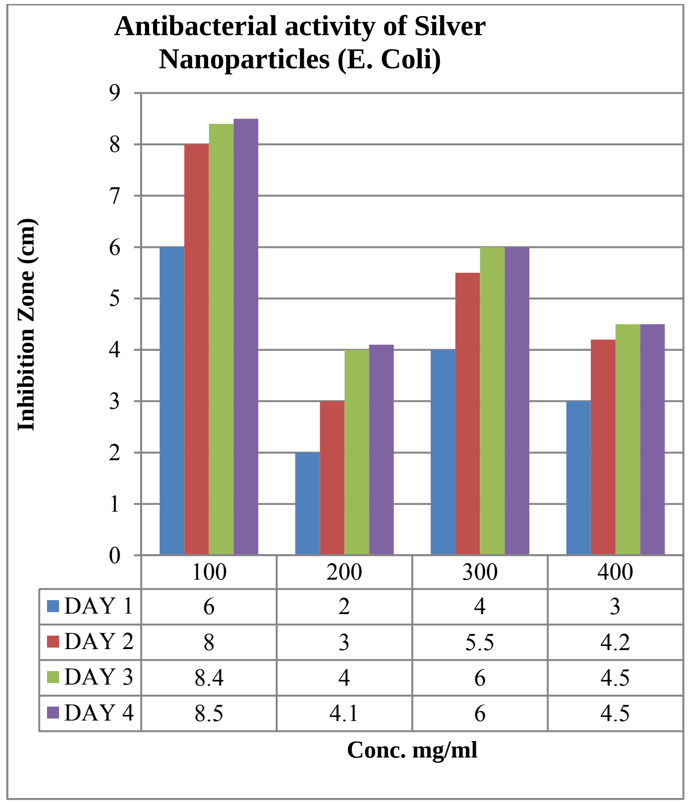
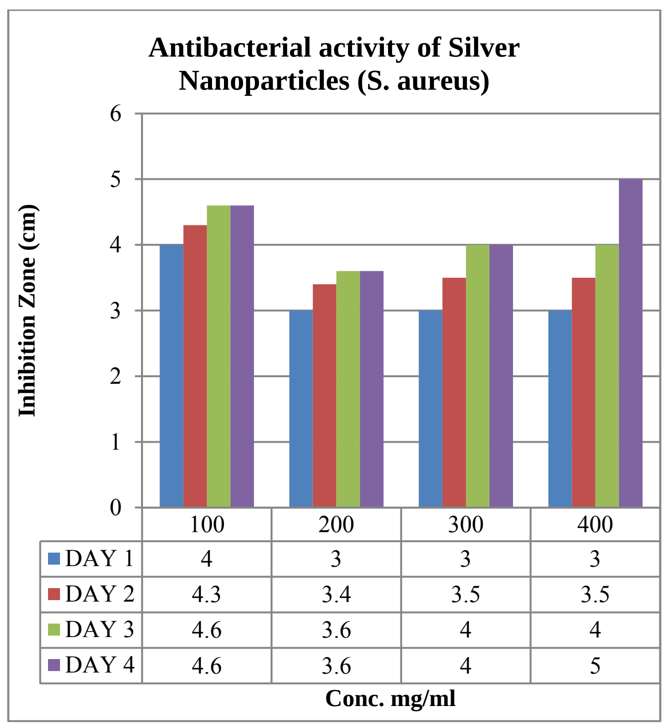


Fig. 4: AgNPs under SEM at 200 nm (82.65 KX)

The mixture of Hexane, Active Charcoal, Non-allergenic Lanoline and Glycerin was applied on the scaffold in form of primary coating and was further coated with ZWC solution. Different concentrations of AgNPs, ranging from 100 mg/ml to 400 mg/ml, were applied separately. To test the antimicrobial property and to fix the optimal concentration, these prepared samples were tested against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) Fig. 5 and Fig. 6 respectively. Their respective zone of inhibitions were measured, for four consecutive days, and shown in the Fig. 7. The coated fabric exhibited antimicrobial activity till the end of third day. The final coated fabric was also sent to an independent NABL accredited Laboratory for testing. There also the coated fabric resulted in zero colony formation units (cfu) after 1 hour, in the case of bacteria including MRSA and fungi.





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