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Development of Wound Healing Scaffold using Curcumin Longa

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Abstract—*Wounds being inescapable events of life and can cause physical disabilities which mainly arise due to physical, chemical or microbial agents. To maintain normal anatomical structure and junction an efficient management of wound is very important for its healing. Many herbs have proved to possess significant healing properties in different types of wounds. Usage of certain herbs, which possess antiseptic, astringent, anti-inflammatory, anti-microbial and bio-stimulator property can also enhance the rate of wound healing. These herbs increases the rate of tissue healing by providing different essential substances, required at various steps of regeneration. Curcumin, the main component in Turmeric is a non-toxic, highly promising natural antioxidant compound. Curcumin helps in inhibiting the growth of germs, harmful microbes and bacteria. Ultrasonic bath or sonicator was used for making Curcumin nanoparticles. Nanocurcumin which was formed was freely dispersible in water whereas turmeric and Curcumin cannot be dissolved in water. Zwitterionic Chitosan, A derivative of Chitosan wih low molecular weight was used along with curcumin nanoparticles which 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.The antimicrobial activity of Curcumin showed the best results at 400mg/ml concentration when tested against four bacteria i.e. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis. To protect burns skin and other wounds from infections and also to regenerate tissue, SALM-CS with blend of Curcumin Nanoparticles, formulation in form of gauze fabric bandage (scaffolds) could show rapid tissue regeneration, non allergic, biocompatible and antibiotic activity in vivo level.*

1. INTRODUCTION

Wounds being inescapable events of life and can cause physical disabilities which mainly arise due to physical, chemical or microbial agents. To maintain normal anatomical structure and junction an efficient management of wound is very important for its healing. Understanding wound healing today involves much more than simply stating that there are three phases: inflammation, proliferation, and maturation. Tissue repair and wound healing is complex processes that involve inflammation, granulation, and remodelling of the tissue [7].

Healing power of many plants has been used since ancient times. Plants like Aloe vera, neem, tulsi, *curcumin,* eucalyptus oil, tree oil, ginger and many more have a long history of use as a therapeutic agent with many reported medicinal properties. Amongst its therapeutic properties many of these have shown anti-inflammatory activity immune stimulatory activity and cell growth stimulatory activity and help to fight against a variety of infections.

Curcumin is a highly potent, nontoxic; bioactive agent found in turmeric and has been known for centuries as a household remedy to many ailments. In India, curcumin plays a pivotal role in Ayurvedic, Unani, Siddha and Tibetian system of medicines. The most well known medicinal action of is its use as a powerful anti-inflammatory, the effectiveness of which is comparable to pharmaceutical medicines. However, it also acts as an alternative analgesic, antimicrobial, anti-inflammatory, anticarcinogenic, anti-allergic, antioxidant, antiseptic, antispasmodic, astringent, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary. [4-6]

The only disadvantage that it suffers is of low aqueous solubility and poor bioavailability, as its retention time in the body is limited due to its rapid systemic elimination. To overcome this shortcoming, nanoparticles of curcumin (nanocurcumin) were developed and found to have a much greater dispersion in water in the absence of any surfactants against *Staphylococcus aureus*, *Bacillussubtilis*, *E.coli*, *Pseudomonas aeruginosa*, *etc.*

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 [5].

2. MATERIALS AND METHODS

Materials

Curcumin: Extracted from the rhizome part of the turmeric, 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 *curcumin* nanoparticles.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.

Developing Curcumin Nanoparticles

To make the nanoparticles of curcumin 5mg of curcumin was dissolved in 15ml distilled water in a phalcon. It was kept for 1- 1½ hours in an ultrasonic bath or sonicator for agitation of the particles.

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.[1] 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).

Preparation of scaffold using SALM-CS matrix and curcumin nanoparticles

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 and curcumin nanoparticles were 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. 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. 1). 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.

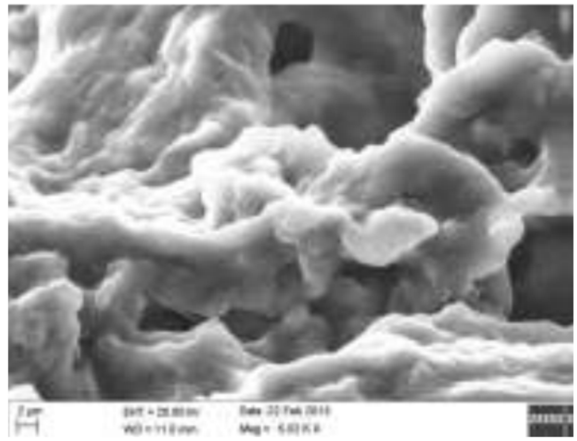


Fig. 1: ZWC matrix viewed under SEM at 2µm (5.02KX)

It was studied while reviewing the literature that the active ingredient in turmeric is curcumin. Curcumin is a highly potent, nontoxic, bioactive agent. It is a highly promising natural antioxidant compound and has important disease fighting substances and has a wide spectrum of biological functions and exhibits anti-inflammatory and antimicrobial properties.

The only disadvantage that it suffers is of low aqueous solubility and poor bioavailability. The insolubility of curcumin in water restricts its use to a great extent which can be overcome by the synthesis of curcuminin nanoparticles.

Unlike turmeric or curcumin, that does not dissolve in water, nanocurcumin (Fig. 2) formed was found to be freely dispersible in water in the absence of any surfactants

Fig. 2: Nanocurcumin

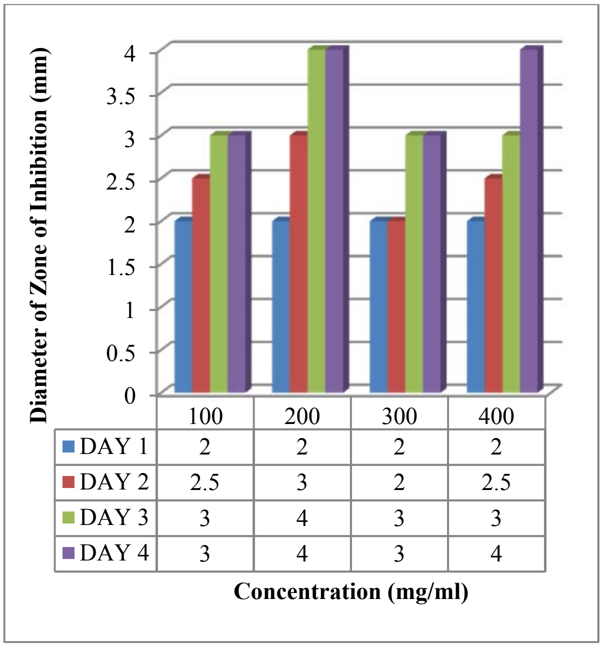


Fig. 3: Antimicrobial activity of nanocurcumin against *E. coli* by Agar Diffusion Method

On Day 1 the fabric coated with nanocurcumin, of concentration ranging between 100 mg/ml and 400 mg/ml, when tested against *Escherichia coli* by Agar Diffusion method, exhibited a zone of inhibition having diameter of 2mm (Fig. 3) for all the concentrations.

On Day 2 the fabric coated with 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 mg/ml of nanocurcumin showed the zone of inhibition having diameter of 2.5mm, 3mm, 2mm and 2.5mm respectively.

Day 3 showed that the diameter of zone of inhibition, in the case of concentration of 200 mg/ml, was 4mm and rest all the three concentrations showed the zone of inhibition of 3mm each.

Day 4 showed that there was no increase in the diameter of zone of inhibition in all four cases.

Thus the optimal concentration of nanocurcumin against *Escherichia coli* was concluded as 200 mg/ml.

On Day 1 the fabric coated with nanocurcumin, of concentration ranging between 100 mg/ml and 400 mg/ml, when tested against *Staphylococcus aureus*(Figure:4) by Agar Diffusion method, exhibited a zone of inhibition having diameter of 2mm for all the concentrations.

On Day 2 the fabric coated with 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 mg/ml of nanocurcumin showed the zone of inhibition having diameter of 2mm, 2.5mm, 2.5mm and 3mm respectively.

Day 3 showed that the diameter of zone of inhibition, in the case of concentration of 200 mg/ml and 400 mg/ml, was 3.5mm each and rest all the two concentrations showed the zone of inhibition of 3mm each.

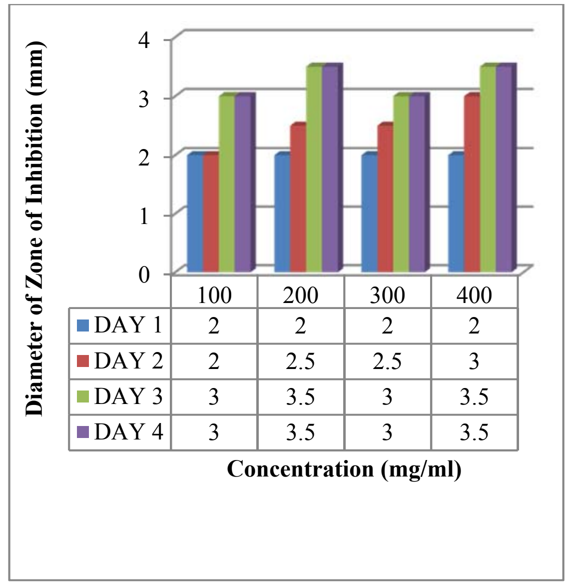


Fig. 4: Antimicrobial activity of nanocurcumin against *S. aureus* by Agar Diffusion Method

Day 4 showed that there was no increase in the diameter of zone of inhibition in any of the concentrations.

Thus, 200 mg/ml concentration of nanocurcumin was finalized to be the optimum concentration that inhibits the growth of *Staphylococcus aureus*.After optimizing the individual concentrations of nanocurcumin i.e. 100 mg/ml and 200mg/ml respectively a final bandage was made by coating the gauze fabric in the following sequence:

- Primary coating consisting of active charcoal and glycerin in Hexane.
- ZWC
- Nanocurcumin of 200 mg/ml concentration

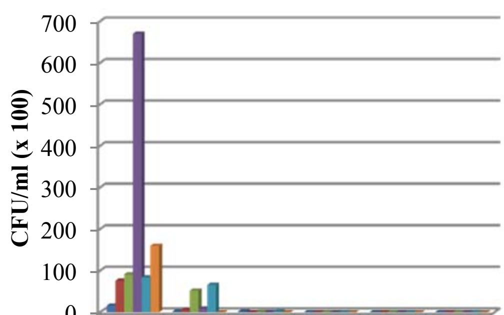
Active charcoal is known to have antimicrobial property and helps in removing foul smell that is generated in an infected wound. Glycerin helps in absorbing wound secretion and maintains an optimal moist wound environment. Further it inhibits the growth of bacteria and fungi; it forcefully extracts the water from the thriving bacterial cells around the injury, and thus facilitates wound healing process.

The final coated fabric was tested against following bacteria and fungi at the Analytical Division of ARBRO Pharmaceuticals Ltd, New Delhi, (an NABL accredited laboratory).

- Escherichia coli
- Staphylococcus aureus
- Pseudomaonas aeruginosa
- *Methicillin Resistant* Staphylococcus Aureus (MRSA)
- Candida albicans
- Aspergillus niger

The Colony Forming Unit per milliliter (CFU/ml) was recorded at following time intervals

- 0 hr
- 0.5 hour
- hour
- 12.0 hours
- 24.0 hours
- 48.0 hours



	0	0.5	1	12	24	48
Staphylococcus aureus	16	2	1.8	0	0	0
Escherichia coli	76	6	0	0	0	0
Pseudomaonas aeruginosa	91	52	0.3	0	0	0
MRSA	670	9	0.32	0	0	0
Candida albicans	84	66	1.6	0.04	0	0
Aspergillus niger	160	0.25	0.12	0	0	0

Time in hours

Fig. 5: Antimicrobial activity of bandage against various bacteria and fungi

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