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Development and characterization of cefazolin loaded zinc oxide nanoparticles composite gelatin nanofiber mats for postoperative surgical wounds

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ABSTRACT

Systemic antibiotic therapy in post-operative wound care remain controversial leading to escalation in levels of multi-resistant bacteria with unwanted morbidity and mortality. Recently zinc (Zn) because of multiple biophysiological functions, gain considerable interest for wound care. Based on our current understanding, the present study was designed with an intent to produce improve therapeutic approaches for post-operative wound management using composite multi-functional antibiotic carrier. The study involved the fabrication, characterization and pre-clinical evaluation of cefazolin nanofiber mats loaded with zinc oxide (ZnO) and comparing co-formulated mats with individual component, enable a side by side comparison of the benefits of our intervention. Minimum inhibitory concentration (MIC) of the drug, ZnO nanoparticles (ZnONPs) and drug-ZnONP mixture against *Staphylococcus aureus* was determined using micro dilution assay. The fabricated nanofibers were then evaluated for *in-vitro* antimicrobial activity and the mechanism of inhibition was predicted by scanning electron microscopy (SEM). Further these nanofiber mats were evaluated *in-vivo* for wound healing efficacy in Wistar rats. Study revealed that the average diameter of the nanofibers is around 200–900 nm with high entrapment efficiency and display sustained drug release behavior. The combination of ZnO and cefazolin in 1:1 weight ratio showed higher anti-bacterial activity of 1.9 ± 0.2 μg/ml. Transmission electron microscopy of bacterial cells taken from the zone of inhibition revealed the phenomenon of cell lysis in tested combination related to cell wall disruption. Further composite medicated nanofiber mats showed an accelerated wound healing as compared to plain cefazolin and ZnONP loaded mats. Macroscopical and histological evaluations demonstrated that ZnONP hybrid cefazolin nanofiber showed enhanced cell adhesion, epithelial migration, leading to faster and more efficient collagen synthesis. Hence the fabricated composite nanofiber mats have the potential to be used as a postoperative antimicrobial wound dressings.

1. Introduction

Although many advances have been made in the management of surgical wounds and various approaches including aseptic techniques, prophylactic antibiotics and laparoscopic surgery have been undertaken. The infection of surgical wounds and wound failure remain common complications of surgery [1]. A wound bed provides an ideal condition for the growth of microorganisms owing to its moist, warm and nutritious environment [2]. Thus an ideal formulation for post-operative wound management is desirable that possesses a broad spectrum of activity and is capable of controlling the bleeding, preventing infection and reduces the scarring. A standard wound dressing material should be non-toxic, have good permeability as well as skin flexibility, biocompatibility and biodegradability characteristics [3]. The traditional cotton-gauge dressings only provide a reliable initial mechanical protection, but absorbing the wound discharge which becomes a medium inviting the growth of pathogenic microflora. Therefore, instant care of skin wound is necessary for prevention of microbial infection and trans-epidermal water loss resulting in brisk regeneration of wound tissue [4].

In the recent past with advancement in the growing field of nanotechnology, there has been an increasing interest among the researchers for the utilization of metallic nanoparticles for wound healing applications due to their well-established antimicrobial properties [5–8]. The latest advances in nanotechnology have also enabled to fabricate nanofibrous constructs that possess architectural and morphological properties resembling the natural extracellular matrix [9,10]. In contrast to traditional bandages which do not adequately meet the requirements of wound care, fiber mats fabricated with electrospinning have a

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potential to provide an excellent platform for wound healing [11,12]. Consequently, the research involving the use of electrospun nanofiber mats that accelerate wound healing and prevent bacterial infections has gained momentum in the recent past [13,14]. Nanofibers produced by electrospinning process manifest high porosity levels [15], gas permeation and possess a high surface area to volume ratio. These properties lead to an enhancement in the rate of cell respiration, skin regeneration, moisture regeneration, removal of exudates and hemostasis [16].

In the current study, gelatin nanofibers loaded with an antimicrobial drug cefazolin and zinc oxide nanoparticles (ZnONPs) were fabricated to maximize the antibacterial efficiency and reduce the chances of resistance development in the microbes by employing multiple targeting approaches [17,18]. Cefazolin is among the drugs of choice in most of the surgical wound cases with the mechanism of inhibiting the cell wall synthesis by binding to specific penicillin-binding proteins (PBPs). Cefazolin due to its broad spectrum anti-microbial activity was selected for the current study. Further, cefazolin is considered to be safe and selected as the first line drug for the treatment of skin infections, systemic infection and wound care. Cefazolin has been shown to be very effective in treating *Staphylococcus aureus* infection which is most commonly observed in wound infection [19].

Combination of cefazolin and ZnONPs for antimicrobial and wound healing activity in a biocompatible and biodegradable polymer is the novel aspect of this study. Zinc oxide (ZnO) is another established moiety to deal with wound healing. Cefazolin alone is not recommended in surgical cases where there is possibility of any kind of contamination. Zinc (Zn) deficient wounds typically experience slower healing times [20,21]. ZnONPs serve as a sustained ionic Zn source best suited for topic wound application by enhancing re-epithelialization, decreasing inflammation and bacterial growth [22,23]. Reactive oxygen species (ROS) generation, a key related with the semiconductor nature of ZnONPs, which can improve cell adhesion, proliferation and cell migration through growth factor mediated pathways [24,25]. Zn being the cofactor of metaloprotein plays an important role in the regeneration of extracellular matrix. Moreover Zn also acts as regulator for auto debridement and keratinocyte migration, both of which are essential for wound repair. Release of Zn ions from zinc-oxide nanoparticles follows a biphasic process. Initially Zn undergoes hydration in presence of biological fluids to form hydrated zinc oxide. Next, the hydrated zinc oxide is highly sensitive towards the phosphate ions at pH 7, resulting a slow and steady release of Zn ions due to degradation of ZnONPs [26]. Gelatin (a long fibrous non-immunogenic protein) was chosen as the polymer of choice owing to its merits like biological origin, good swelling property, biocompatibility, non-immunogenic and biodegradability under normal physiological conditions and commercial availability at low cost [27,28]. Gelatin is also known to hamper the loss of fluid due to exudation resulting in the enhancement of its wound healing properties [29,30]. Moreover, it can also accelerate wound healing because of the anti-inflammatory nature of one of its amino acid glycine [31]. Present study was designed to fabricate medicated nanofiber composite of ZnONPs and cefazolin to provide better postoperative wound care solution.

2. Experimental details

2.1. Materials

Cefazolin sodium was obtained as a gift sample from Nectar Life Sciences (Chandigarh, Punjab, India). Gelatin (from porcine skin, Type A) and 2,2,2-trifluoroethanol, Muller–Hinton agar were purchased from HiMedia Laboratories (Mumbai, India). Zinc chloride and glutaraldehyde were obtained from Sigma Aldrich (St. Louis, MO, United States of America). All other chemicals used in this study were of analytical grade. *S. aureus* freeze dried culture was procured from IMTECH Chandigarh.

2.2. Synthesis and characterization of ZnONPs

ZnONPs were synthesized by zinc chloride reduction method with little modification of earlier published method [32]. Briefly, 2.5 g of zinc chloride was taken in double neck round bottom flask and dissolved in 50 ml of distilled water by magnetic stirring. The temperature of the round bottom flask was raised to 90 °C by using hot plate. In the meantime, 1 g of sodium hydroxide was dissolved in 50 ml of distilled water in a separate beaker. 8 ml of sodium hydroxide solution was added to the zinc chloride solution with the help of glass rod while touching the walls of the flask under a constant stirring environment (500 rpm). Reaction was allowed to proceed for 2 h after the complete addition of sodium hydroxide solution. After the completion of reaction, solution was allowed to settle and the supernatant was removed. The settled material was washed five times with distilled water. After washing, the powder is allowed to dry in oven at 70 °C for 1 h. Synthesized ZnONPs were characterized for UV-absorption spectrum (UV 1700 Shimadzu, Japan), particle size, zeta potential and polydispersity index (Zeta sizer, Bechman Coulter, UK), X-ray Powder Diffractometry (Bruker AXS D8 Advance Diffractometer) and morphology (JSM-6510, JEOL, USA), scanning electron microscope (SEM) and Hitachi H-7500 transmission electron microscope (TEM), USA operating at 100 kV [33].

2.3. Fabrication of nanofibers mats

Plain gelatin nanofiber, cefazolin loaded nanofiber, ZnONP loaded nanofiber and both cefazolin and ZnONP nanofibers were fabricated. Gelatin solution (10% w/v) in 2,2,2-trifluoroethanol was used as the base material for the fabrication of gelatin nanofibers using electrospinning unit ESPIN-NANO (Super ES2, IIT Kanpur) [34]. Passive loading (adding the active moiety in the gelatin solution prior to electrospinning) was used for the fabrication of above mentioned nanofibers.

2.3.1. Electrospinning solution preparation

For the fabrication of cefazolin nanofibers, 1% w/v solution of cefazolin was prepared in (10% w/v) gelatin solution and the mixtures were stirred at room temperature for 3–4 h to obtain homogenous solutions appropriate for electrospinning. In similar way, fabrication of ZnONP nanofibers was done with 0.3% w/v solution of synthesized ZnONPs was prepared in (10% w/v) gelatin solution. For the fabrication of both cefazolin and ZnONP loaded gelatin nanofibers, a homogeneous 10% w/v gelatin solution containing 1% cefazolin and 0.3% ZnONPs was prepared. All the prepared solutions were sonicated using a bath sonicator for 10 min prior to electrospinning.

2.3.2. Electrospinning conditions

For electrospinning process, the polymer solution to be electrospun was filled within a 10 ml syringe with a needle size 24 gauge of inner diameter 0.311 mm and the distance between the needle tip and the aluminum foil collector was kept 15 cm. The syringe was loaded in a syringe pump and dispensed at a flow rate of 800 μl/h, at an applied voltage of 10 kV. The nanofibers were collected on the stationary plate and were vacuum dried at least for 72 h to remove the residual solvent before further use.

2.3.3. Glutaraldehyde based nanofiber crosslinking

Cross linking was carried out by placing each of the fabricated nanofiber with a supporting aluminum foil in a sealed desiccator (5 l) containing 20 ml of 50% aqueous glutaraldehyde solution in a petri dish (90 mm). These gelatin based nanofiber mats were subjected to crosslinking in the glutaraldehyde vapor environment for 48 h at room temperature. Subsequent to cross linking, the samples were exposed in fuming hood for 2 h followed by a post-treatment at 100 °C for 1 h to remove residual glutaraldehyde and partially enhance the crosslinking [35].