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Bacterial cellulose-zinc oxide nanocomposites as a novel dressing system for burn wounds

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ABSTRACT

Bacterial cellulose possesses physical and mechanical properties of an ideal wound dressing material but lack of antimicrobial activity limits its biomedical applications. Therefore, in current study, the inherent wound healing characteristics of bacterial cellulose and antimicrobial properties of zinc oxide nanoparticles were combined. The reinforcement (impregnation) of zinc oxide nanoparticles into bacterial cellulose sheets was confirmed through various characterization techniques. The antimicrobial capacity of bacterial cellulose-zinc oxide nanocomposites was tested against common burn pathogens. The *in-vivo* wound healing and tissue regeneration of the nanocomposites was investigated in burn BALB^c mice model. Characterization techniques confirmed the successful impregnation of nanoparticles into bacterial cellulose. Bacterial cellulose-zinc oxide nanocomposites exhibited 90%, 87.4%, 94.3% and 90.9% activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Citrobacter freundii*, respectively. Bacterial cellulose-zinc oxide nanocomposites treated animals showed significant (66%) healing activity. The histological analysis revealed fine tissue regeneration in composites treated group. These findings suggest that bacterial cellulose-zinc oxide nanocomposites could be a novel dressing material for burns.
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1. Introduction

Significant advances in the field of biomaterials have revolutionized the area of medicine, surgery and health care. Natural polymers have gained a great interest and are considered as the potential candidates for biomedical applications particularly for wound care and tissue engineering. Severe injuries like burns badly alter the anatomic structure and function of the skin. Burn injury is the most painful form of trauma and over 300,000 people die worldwide every year due to burns related complications with 90% of these deaths occurring in lower middle income countries (Peck, 2011). First degree burns usually heals without any complication while partial and full thickness burns are more complex and a significant clinical challenge to deal with (Pham, Greenwood, Cleland, Woodruff, & Maddern, 2007). In case of burns the altered anatomic structure and function of skin leads to excessive fluid loss and infectious complications like impetigo, cellulitis and invasive bacterial infections (Weinstein & Mayhall, 2003).

Major complications in treatment of burns may arise due to large exposure area of injury, longer stay of patients in burn units, (Latenser et al., 2007) contamination of wound from external environment, air, water and hands of health care workers (Mehta et al., 2014) while non-availability of modern treatment and poor management make it the major health crises. Topical antimicrobial compositions and ointments are lesser effective treatments in case of burns. Proper burn therapy requires an effective wound closure material that should create optimal environment for epidermal regeneration and provide barrier against chronic wound infection and water loss (Latarjet, 1995) Traditional dressings like gauze and cotton wool are widely used for healing of wounds but these materials are usually dry in nature and may adhere to the wound surface and causes discomfort and pain on removal. These traditional dressings are now replaced by modern and better wound care products synthesized from biocompatible natural polymeric compounds like collagen, chitosan, elastin, alginates, and hyaluronic acid (Boateng, Matthews, Stevens, & Eccleston, 2008) that can release bioactive constituents and aids the healing process. Bacterial cellulose (BC) is one of such promising class of

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biopolymers that is produced by some bacterial genera belonging to *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* species (Ullah, Santos, & Khan, 2016). BC possesses novel physical and chemical properties like high biocompatibility, hydrophilicity (Backdahl et al., 2006) microporosity, transparency and nontoxicity that make it an ideal wound care system and skin substitute (Gelin et al., 2007). However, it does not possess any inherent antimicrobial activity to prevent infections (Dahman, 2009). The versatile biomedical characteristics of BC can be exploited for fabricating a novel dressing system by incorporating different active molecules like antimicrobials to enhance its wound healing properties (Maneerung, Tokura, & Rujiravanit, 2008). The use of nanoparticles and nanocomposites for diagnostic and biomedical purposes revolutionized the field of medicine. Recently, nanomaterials are being frequently used as novel antibacterial agents against infectious pathogens (Wahid, Khan, Shehzad, Ul-Islam, & Kim, 2014). Interaction of nanoparticle kills the microbes by disrupting the integrity of cell membrane (Huh & Kwon, 2011) and cellular respiration (Thill et al., 2006) It also has the ability to produce lethal reactive oxygen species (ROS) inside the bacterial cell (Wan et al., 2011) BC composite films reinforced with some nanoparticles have also shown antibacterial and biocompatible properties *in vitro* (Ullah, Wahid, Santos, & Khan, 2016).

In the current study, for the first time, the *in vivo* wound healing potential of BC-zinc oxide (BC-ZnO) nanocomposites was assessed in burn mice model. The antimicrobial potential was studied against various pathogens that have role in burns complication. BC-ZnO nanocomposites successfully inhibited the growth of the tested pathogen and showed a remarkable healing and tissue regeneration properties in animal models.

2. Materials and methods

2.1. Materials

Bacteriological peptone and nutrient agar media were purchased from Oxoid Ltd. Dextrose was acquired from Daejung, Korea. Zinc nitrate tetrahydrate ($Zn(NO_3)_2{\cdot}4H_2O$) and sodium hydroxide were obtained from Sigma Aldrich Chemicals Company, St Louis, MO, USA. Sodium dihydrogen phosphate was acquired from Merck, Darmstadt, Germany. Yeast extract powder was obtained from HiMedia Laboratories Mumbai India and citric acid was purchased from Riedel-deHaen, Germany.

2.2. Synthesis of ZnO nanoparticles

ZnO nanoparticles were synthesized from aqueous solutions of sodium hydroxide (NaOH) and zinc nitrate tetrahydrate ($Zn(NO_3)_2{\cdot}4H_2O$) through a previously reported method (Moghaddam, Nazari, Badraghi, & Kazemzad, 2009). In short, stock solutions of 0.9 M NaOH and 0.45 M $Zn(NO_3)_2{\cdot}4H_2O$ were prepared. NaOH solution was heated at 50 °C and the same volume of $Zn(NO_3)_2{\cdot}4H_2O$ solution was added drop-wise at vigorous magnetic stirring. After complete mixing, ZnO nanoparticles were allowed to precipitate that were subsequently separated and purified by repeated washing with water and ethanol.

2.3. Synthesis of bacterial cellulose films

Hestrin–Schramm (HS) growth medium was used for *Gluconacetobacter xylinum* culture. This medium was composed of 2% glucose, 0.5% peptone, 0.5% yeast extract, 0.27% Na_2HPO_4, 0.115% citric acid dissolved in double distilled water and was autoclaved. The preculture for BC synthesis was prepared by inoculating *G. xylinum* colonies in 50 ml media and kept at 150 rpm and 30 °C for 24 h in a shaking incubator (WY 100 orbital shaking incubator).

BC sheets were prepared in sterilized plastic rectangle containers (20 cm × 20 cm) by pouring 300 ml HS media in each container along with 25 ml pre-culture and placed in a static incubator at 30 °C for 7 days. Resulting BC sheets were then processed by washing with distilled water followed by autoclaving in 3 M NaOH solution at 120 °C for 15 min in order to disrupt and dissolve the microbial cells. The sheets were then subsequently washed with and stored in distilled water at 4 °C for further use.

2.4. Synthesis of BC-ZnO nanocomposites

Aqueous suspension of ZnO nanoparticles (1%) was prepared in distilled water with continuous stirring for 30 min to form a homogenous mixture. Then BC-ZnO nanocomposites were prepared by immersing bacterial cellulose films into suspension of ZnO nanoparticles and mixed in shaking incubator at 50 °C and 150 rpm for 24 h. The BC nanocomposites were lyophilized (TELSTAR CRYODOS −50) at −50 °C for 10 h.

2.5. Characterization of the ZnONP and BC-ZnO nanocomposites

Synthesis of nanoparticles and composite were confirmed through field emission scanning electron microscope (FE-SEM), X-Ray Diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). The SEM images of the freeze-dried BC and BC-ZnO nanocomposites were obtained using a Hitachi S-4800 and EDX-350 (Horiba) FE-SEM (Tokyo Japan). Samples were fixed onto a brass holder and coated with osmium tetra oxide (OsO_4) by a VD HPC-ISW osmium coater (Tokyo Japan) prior to FE-SEM observation. XRD patterns of the nanoparticles and nanocomposite were recorded on an X-Ray diffractometer (X'Pert-APD PHILIPS, Netherland), radiation was Cu Kα with a wavelength of 1.54 Å. The X-ray generator tension and current were 40 kV and 30 mA, respectively. The angle of scanning was varied from 10 to 70°. The crystallinity indices of BC and BC-ZnO composites were determined from the integrated areas of crystalline and amorphous phases, as reported earlier (Focher et al., 2001). FT-IR spectra of the dried BC and nanocomposites were recorded with a Perkin Elmer FTIR spectrophotometer (Spectrum GX & Autoimage, USA), Spectral range: 4000–400 cm^{-1}; Beam splitter: Ge coated on KBr; Detector: DTGS; Resolution: 0.25 cm^{-1} (step selectable).

2.6. Antimicrobial assay of BC-ZnO composite films

Agar disc diffusion assay was performed to evaluate the antimicrobial activity of composites against common pathogens involve in burn wound infection. Three species of Gram negative bacteria, *Escherichia coli* (*E. coli*), *Citrobacter freundii* (*C. freundii*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and a Gram positive bacterium *Staphylococcus aureus* (*S. aureus*) were selected for the current study. Discs of BC-ZnO composite and BC of 20 mm was cut through a sharp metal cutter. Silver sulfadiazine (SD) was used as standard drug (positive control). Discs of SD were prepared by coating it on sterilized filter paper of the same size as BC-ZnO composite discs. The discs were placed over agar plates containing lawn of selected bacterial strains and incubated at 37 °C for 24 h. Zones of inhibition were measured to determine the antimicrobial capability of BC-nanocomposites. The percent inhibition was determined by using the formula

$$\text{Percent inhibition} = \left(\frac{\text{Zone of inhibition of testsample (mm)}}{\text{Zone of inhibition of standard drug (mm)}}\right) \times 100$$

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