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Using chitosan nanoparticles as drug carriers for the development of a silver sulfadiazine wound dressing

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ABSTRACT

Burn wounds environment favors the growth of micro-organisms causing delay in wound healing. The traditional treatment with antimicrobial creams offer inaccurate doses. In the present study, a dressing coated with silver sulfadiazine (SSD) loaded chitosan nanoparticles (CSNPs) for the controlled-release of SSD into burn wound to control bacterial growth was investigated. CSNPs were formulated with different concentrations of chitosan and CM-β-CD and loaded with SSD complexed in 1:1 molar ratio with CM-β-CD, CSNPs were assessed for their particle size, polydispersity index, morphology and association efficiency. The formula showing the best characteristics was selected for the preparation of SSD loaded CSNPs wound dressing through a padding process with/without the use of cross-linker. The dressing was characterized for its physical properties, in addition, FTIR, X-ray, SEM and *in vitro* release were used for characterization. The dressing was proven effective for the inhibition of the growth of Gram positive and Gram negative bacteria as well as candida on an infected wound.

1. Introduction

Wounds including those resulting from burns often provide a favorable environment for the colonization of micro-organisms which cause infection (bacterial or fungal) and in turn may delay wound healing. Consequently, in order to improve the opportunity for wound healing, it is important to create conditions that are unfavorable to micro-organisms and favorable for the host repair mechanisms. Treating an infection reduces the wound's bacterial burden, which has favorable effects on the dynamics of oxygen delivery and utilization within the wounds, it also diminishes the chronic inflammatory response and adjusts the tissue's capacity to respond to cell signaling and to develop sustained growth (Kirsner, Orstead, & Wright, 2001). Topical antimicrobial agents are believed to facilitate this process.

In bedsores and burn wounds, the wound stages are often divided into an infectious period, necrosis and agglutination period, proliferation period and epidermis formation period (Shigeyama et al., 2001; Shigeyama, 2004). Generally, formulations are selected based on the disease stage where the application period is a critical factor in the choice of the treatment formulation. Silver Sulfadiazine (SSD) is the drug of choice in the infectious period for combating the threat of bacterial infection and preventing wound sepsis (Salas Campos, Fernandes Mansilla, & Martinez de la Chica, 2005).

SSD has dual antibacterial effects. The free silver can react with both sulfhydryl groups of bacterial enzymes and DNA, and sulfadiazine can stop the synthesis of DNA by interrupting the production of folate (Adhya et al., 2014). However, the antibacterial effect of SSD is badly limited by its poor aqueous solubility (Dellera et al., 2014). Moreover, SSD has been shown to be cytotoxic *in vitro* toward fibroblasts and keratinocytes and consequently to retard wound healing *in vivo* (Rosen et al., 2015).

In addition, SSD is mostly present in a 1% cream. This cream dosage form exhibits a number of general side effects, including their inability to maintain effective drug concentrations for a prolonged period at moist wound surfaces due to their short residence time, their messiness causing inconvenience to patients Dobaria, Badhan, & Mashru, 2009) and according to its manufacturers the SSD cream causes discoloration of the wound bed (Gear et al., 1997), which, after several applications, interferes with judging wound status. Also, it shows low silver release levels which affects the drug's efficacy as the antimicrobial efficiency of silver ions depends directly on its concentration, which should not drop under the limit value required for minimal inhibition.

Over the past few years, there have been a rapid increase in the demand of silver dressings loaded with accurate doses of the drug sufficient to provide controlled and sustained bactericidal action.

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Dressings' advantages include the protection of the wound from physical damage and secondary infection, preventing wound contamination and peripheral channeling into the wound by bacteria, assisting in debridement, thermal insulation and ease of removal without causing any trauma to the wound (Boateng, Matthews, Stevens, & Eccleston, 2008; Wittaya-areekul & Prahsarn, 2006).

However, this is challenging as direct bonding of SSD on textile dressings is practically not possible as silver-based agents are not chemically bonded to textile fibres and in turn no specific drug concentration or release rate could be precisely expected from the dressing to the infected wound. In this case, encapsulation of SSD on a suitable hydrophilic nano-carrier that is then used to coat the dressing might prevent the cytotoxic effect of the drug and enhance the effective controlled application of SSD on the wound as it will lead to enhancing the SSD solubility and enabling uniform dispersion, embedment and controlled release of the drug from the prepared dressing (Agnihotri, Bajaj, Mukherji, & Mukherji, 2015).

During the past decades, chitosan has got great attention and been broadly applied in medical areas with respect to its excellent biological characteristics of bio-compatibility, absorptivity, non-hypersensitivity, biodegradability and wound healing (Jayakumar, Deepthy Menon, Manzoor, & Nair, 2010). Coating the cotton dressing with SSD-loaded chitosan nanoparticles is expected to allow the adherence of the dressing to the moist surfaces of the wound due to the bioadhesive property of chitosan (Dobaria et al., 2009) and thereby prolong contact time and subsequently offer prolonged and controlled SSD release.

In this work, we combine the advantages of silver sulfadiazine as an effective antimicrobial and antifungal drug with the advantages of chitosan nanoparticles as drug carrier systems and effective fabric coating material to present a novel antimicrobial silver sulfadiazine dressing.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Mallinckrodt, USA [M.W = 400,000, degree of deacetylation 95%]. Silver sulfadiazine was a kind gift from El Nasr Pharmaceutical Company, Cairo, Egypt. Glutaraldehyde (25% solution), Sigma-Aldrich, USA. Glyoxal (40% solution), Sigma-Aldrich, USA. Glacial acetic acid 99.8% was obtained from El Nasr Pharmaceutical Chemicals Co., Egypt. CM-β-CD was purchased from Sigma–Aldrich, USA. Other chemicals and solvents were of analytical grade.

2.2. Methods

2.2.1. Formulation of chitosan nanoparticles;

Aqueous solutions of CM-β-CD (1 to 12 mg/ml) were added to acetic acid solutions of CS (pH 4.6) (0.1–0.3% w/v) and stirred for 24 h using a magnetic stirrer. NPs were obtained spontaneously due to ionotropic gelation which involves the interaction between the positively charged amino groups of CS and the negatively charged CM-β-CD (Ammar, El-Nahhas, Ghorab, & Salama, 2012). After the preparation of the NPs, three different systems were identified: clear solution, opalescent dispersion and aggregates. In this step we intended to determine the suitable conditions for the formation of CS NPs using CM-β-CD as cross linking agent. ***Sixty*** CS-CM-β-CD nanoparticle preparations were performed with each repeated thrice to check the results.

2.2.2. Formulation of SSD-CM-β-CD loaded CS NPs

For the association of SSD to the different nanoparticle systems, SSD was dissolved in the CM-β-CD phase in 1:1 molar ratio based on our previous work (Hegazy, Sharaf, El-Feky, & El-Shafei, 2013). Nanoparticles were then prepared as described under 2.2.1.

2.2.3. Quality control of CS NPs by transmission electron microscopy (TEM)

One drop of each sample was deposited on a film- coated 200-mesh copper specimen grid and allowed to stand for 10 min after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungestic acid and allowed to dry for 5 min before examination. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoparticles droplets.

2.2.4. Determination of CSNPs association efficiency

The nanoparticles were separated from the aqueous suspension medium by ultracentrifugation at 20,000 rpm for 30 min. The amount of SSD loaded into the CSNPs was calculated as the difference between the total amount used to prepare the nanoparticles and the amount found in the supernatant. The SSD association efficiency was calculated as follows:

$$\text{Association efficiency} = \frac{\text{Total amount of SSD - Free SSD}}{\text{Total amount of SSD}} \times 100$$

Drug analysis was carried out using HPLC.

2.2.5. Preparation of the wound dressing

In this study, Egyptian cotton guaze samples were used as the base of the dressing and the best method for coating the gauze dressing with the SSD loaded CSNPs was further studied.

2.2.5.1. Treatment of gauze with SSD loaded CSNPs using padding process. Cotton fabric samples were padded with SSD loaded CSNPs using laboratory padder at 85% wet pick up. The padded fabrics were subjected to drying at 80 °C for 5 min and cured at 150 °C for 3 min. The treated fabric samples were subjected to washing in distilled water, dried and then placed in a desiccators till further study.

2.2.5.2. Treatment of gauze with SSD loaded CSNPs using padding process in presence of cross-linker. The cotton gauze samples were padded in CSNPs solution loaded with SSD using pad-dry-cure process as follows: fabric samples were impregnated for 5 min in pad baths containing a specified amount of SSD loaded CSNPs equivalent to 1% SSD per each $5 \times 5\,cm^2$ wound dressing and 5% owb of two different cross-linkers (glutaraldehyde and glyoxal) with their compatible catalysts. Samples were then padded through laboratory padder. Then drying followed by curing in the thermo-fixation oven at 85 °C, for 5 min and 150 °C for 3 min respectively, were carried out. After curing, the fabric samples were rinsed with warm water for 15 min and dried at room temperature.

2.2.6. Physical characterization of the dressings

2.2.6.1. Weight. The basic weight of the dressing before treatment was measured by averaging the weights of three samples with fixed dimensions. Weight add-ons were determined after conditioning the samples and comparing initial weight (before treatment) and final weight (after treatment).

2.2.6.2. Thickness. The basic thickness of the dressing before treatment was measured by averaging the thickness of ten samples with fixed dimensions. Average thickness was re-determined after conditioning the samples and comparing initial thickness (before treatment) and final thickness (after treatment).