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Chitosan-PVP-nano silver oxide wound dressing: *In vitro* and *in vivo* evaluation

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ABSTRACT

The main aim of this work was to prepare wound healing material with chitosan, poly vinyl pyrrolidone (PVP), silver oxide nanoparticles. The prepared chitosan, chitosan-PVP-nano silver oxide (CPS) films were characterized for their thermal behaviour, morphological properties, mechanical properties, antibacterial properties and wound healing properties. The CPS film found higher antibacterial activity because the materials both chitosan as well as silver oxide poses good antibacterial activity. L929 cell lines were for cytotoxicity study and Adult male albino rats (140–180 g) were used for wound healing study. The prepared film has more wound healing property than of cotton gauge, 100% chitosan and other reported chitosan based dressings.

1. Introduction

The wound healing is a very complex and dynamic process that involves three main phases, which are distinct, sequential and overlapping such as inflammation, proliferation and tissue remodelling. Wound dressings are used to protect from external environment, maintain moist environment around the wound, facilitate the wounds healing and short the recovery period.

Chitosan has many advantageous biological properties in the application as a wound dressing, namely biocompatibility, biodegradability, hemostatic activity, anti-infectional activity and property to accelerate wound-healing [1–7]. Chitosan influence positively in to various steps of complex wound healing process. Chitosan will gradually depolymerize into N-acetyl-D-glucosamine, which initiates fibroblast proliferation, assists in ordered collagen deposition and stimulates increased levels of natural hyaluronic acid synthesis at the wound site [8]. It was reported to have hemostatic activities through activation of platelets [9] and in wounds treated with chitosan an increased infiltration of inflammatory cells and a higher angiogenic activity promoting wound healing, has been observed [10]. Ueno et al. reported that chitosan accelerates creation of type III collagen and results in the increase of granulation in wound healing in dogs [11].

Natural and synthetic polymers blending results a new desirable material with improved mechanical property and reduced cost. PVP is a synthetic polymer has good compatibility, good transparency and used as a blood plasma expander [12] and as a vitreous humor substitute [13]. The blends of chitosan and PVP have already studied for antibacterial activity [14], biocompatibility [15] and biomedical applications [16]. NMR and FTIR analyses proved that hydrogen bonding was present between the chitosan and PVP [17–20].

Silver is one of the most powerful antiseptic materials available naturally and possess low toxicity towards mammalian tissue. Over decades many ways silver used in the medical therapy. The antibacterial property of silver nanoparticles has been studied by a number of microbiologists. Silver compounds have been used to treat burns, wounds and infections [21]. It has long been known that silver ions can be bacteriostatic as well as bactericidal [22]. Wei et al. [23] found that compared to pure chitosan film, silver impregnated films showed both fast and long-lasting antibacterial effectiveness toward *Escherichia coli* and chitosan-based silver nanoparticles have a dual mechanism of action for antibacterial activity, *viz.*, the bactericidal effect of silver nanoparticles and cationic effects of chitosan. Lu et al. [24] prepared a novel wound dressing composed of nano-silver and chitosan and found that silver impregnated dressings can promote wound healing and combat infection, and also decrease the risk of silver absorption in comparison with nano-silver and chitosan dressings. A number of wound dressings developed using silver (Acticoat™, 3M™ Tegaderm™, Bactigrass®, SilvaSorb®, Fucidin®, AQUACEL

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Ag, ALLEVYN* Ag, PolyMem® Silver) have been approved by the US Food and Drug Administration (FDA).

In a human clinical study, wound dressing containing NSPs promoted the healing of chronic leg ulcers by not only reducing bacteria numbers in the wound bed, but by decreasing inflammatory response as well [25]. Nymark et al. [26] prepared polyvinylpyrrolidone-coated silver nanoparticles and studied Genotoxicity in BEAS 2B cells. The cells were exposed to various doses (0.5–48 μg/cm^2 corresponding to 2.5–240 μg/ml). They found that the lack of induction of chromosome damage by the PVP-coated AgNPs is possibly due to the coating which may protect the cells from direct interaction with the AgNPs, either by reducing ion leaching from the particles or by causing extensive agglomeration of the nanoparticles, with a possible reduction of the cellular uptake. Jena et al. [27] prepared chitosan coated silver nanoparticles and tested antibacterial activity and cytotoxicity. No significant DNA damage was observed in cells treated with chitosan coated silver nanoparticles at 3 ppm and DNA damage, was observed at the concentration of 20 ppm.

Lu et al. [24] prepared wound dressing composed of nanosilver and chitosan. The silver nanocrystalline chitosan dressing treated group found efficient in wound healing. After observing these results we have tried to investigate feasible effect of easily available three principal modern wound materials: chitosan, PVP, silver oxide nanoparticles and find whether the ternary combination has advantage over their individual and binary strength than the previous researchers.

2. Materials and methods

2.1. Materials

Chitosan (79% deacetylated) was obtained from Central Institute of Fisheries Technology (CIFT, Cochin). PVP, Silver nitrate ($AgNO_3$), trisodium citrate were purchased from Sigma–Aldrich. Nutrient agar and nutrient broth were obtained from Himedia, Mumbai. The test strain, *E. coli* (MTCC 739, Gram −ve) and *Staphylococcus aereus* (MTCC 3160, Gram +ve) were obtained from IMTECH, Chandigarh. Cell line, L929 (mouse fibroblast, NCCS, Pune, India) was used. Distilled water was used as solvent throughout the experiments.

2.2. Preparation of CPS film

Silver oxide nanoparticles were prepared by sodium citrate reduction of $AgNO_3$ [28]. In brief, after the addition of trisodium citrate (1%, w/v) into $AgNO_3$ solution, black precipitates was obtained. The precipitates were filtered and rinsed with distilled water. A mixture (1:1, v/v) of chitosan and PVP was prepared. The above mixture was divided in three parts. 0.001 mg, 0.010 and 0.100 mg of silver oxide nanoparticles were added respectively. The resulting solutions were cast on a ceramic plate in a dust free environment and left to complete dried in air at room temperature for 48 h. Then it was kept in vacuum to complete dry and kept the CPS film for further usage.

2.3. Characterization of CPS film

The structure of CPS and chitosan film were analysed by FTIR (PerkinElmer RX1 FTIR spectrophotometer model). Thermal stability of chitosan and CPS films were investigated using thermo gravimetric analysis (TGA) (PerkinElmer Pyris 6). The surface morphology of CPS films were analysed by scanning electron microscope (SEM, JEOL Model JSM-6390LV) and Transmission electron micrographs (TEM) with Technai 30 G2 S-Twin high resolution transmission electron microscope operating at 300 kV. X-ray diffraction patterns were analyzed by Rigaku X-ray diffractometer. The X-ray source was Ni-filtered Cu Kα radiation (40 kV, 30 mA; λ = 1.54060 Å).

2.4. Swelling test

The swelling test was performed by immersing a pre weighed dry sample in PBS solution with 7.4 pH at a given interval. Highly swollen samples weight was taken after removing excess of water using filter paper. Same experiment was repeated for three times, and the average value was considered as swelling ratio. The degree of swelling was calculated from the following formula

$$DS = \left[\frac{W_w - W_d}{W_d}\right] \times 100$$

In the above formula W_w and W_d are the weights of wet and dry dressing material respectively. The pH values was precisely checked by a pH-meter (Decibel DP510) previously standardized with buffer solutions of pH 4 and 9.2.

2.5. Mechanical properties

The mechanical properties of the CPS film was determined by using a universal testing machine (Model 1185, Instron, USA) with across head speed of 5 mm/min under 10 Hz at 23 °C. Dressing material thickness (mm) was determined using a screw gauge and verified with vernier calliper.

2.6. Antimicrobial assessment

A well-known agar diffusion method was used to determine the antimicrobial effects of films on bacterial strains [29]. At first agar solution (nutrient agar 2.8 g in 100 mL water) and broth media (nutrient broth 1.3 g in 100 mL water) were sterilized by autoclaving and then agar plates were prepared. The suspensions of bacterial cultures in the range of 10^7–10^8 CFU/mL were made and spread over agar plates to obtain uniform growth. The solution was placed on the well of agar plates. The incubation was continued for 18 h at 37 °C and inhibition zone was measured to determine antibacterial efficacy of 0.001 mg, 0.010 and 0.100 mg of silver oxide nanoparticles and one well is left as it is (control).

2.7. Cytotoxicity test

Cell viability of the CPS films were evaluated by indirect cytotoxicity test using Alamar blue [30,31]. The cytotoxicity test of samples were done according to ISO 10993-5. The L929 cells [32] are commonly used model cell type for studying cytotoxic effect of samples. L929 cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 50 IU ml^{-1} penicillin, 50 μg ml^{-1} streptomycin (Invitrogen, CA, USA) at 37 °C in a humidified 5% CO_2 atmosphere. L929 cells were seeded in to 96 well plates at a seeding density 1 × 104 cells/well and incubated for overnight. The samples were sterilized with UV, Triplicates of each sample were taken and incubated in serum containing media for 24 h at 37 °C. 100 μl of the media from each sample was taken and transferred into each well. The cells were then incubated for 1, 3 and 7 days using Alamar blue assay (Invitrogen, USA). The optical density was measured at 570 nm with 620 nm set as the reference wavelength using a micro plate spectrophotometer (Biotek Power Wave XS, USA).