Investigating the Antibacterial Property of Silver Nanoparticles in Alginate Wound Dressings

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Abstract

Introduction: Nanosilver is a nanotechnology product with antimicrobial property. It can improve efficiency, reduce cost and increase antimicrobial durability and performance of wound dressing.

Objective: This research intended to introduce a dressing that accelerated and improved the wound healing process by keeping the wound area moist and simultaneously prevented colonization by microorganisms and wound infection.

Materials and Methods: Silver nanoparticles were trapped in the pores of calcium alginate sheets, and particle loading on films took place. When this dressing is put on a wound, the silver ions are released in the wound area. After loading the silver nanoparticles; their presence in the calcium alginate structure was confirmed by FE-SEM micrographs. Wounds (2 × 2 cm2) were made on rats' bodies and images were taken of the wound healing process on the 1st, 7th, and 14th days to study the effect of the dressing on tissues of living organisms.

Results: Characterization of silver nanoparticles was carried out by the DLS test and Zeta potential measurement, in which the values of 61.4 nm and -20.83 mV were obtained, respectively. Antibacterial properties were studied and cell toxicity tests were performed to determine the suitable concentration. Results indicate that the optimum concentration was 100µg/l (or 17.5µg per unit area of the alginate film). Using the ImageJ software, the surface areas of the wounds were calculated, and histological studies were also conducted on wound healing. Moreover, on the 7th and 14th days, samples were taken of them to carry out histopathological investigations, and changes in skin cell shape were studied.

Conclusion: The results of this study suggestthat the dressing accelerates simultaneously the process of wound healing and prevents wound infection and postponement of its repair.
Conflict of interest: non declared

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Extended Abstract

Introduction: Wound dressing is a suitable protective coating on the wound. It has features such as creating a humid environment, being impermeable to pathogens and incoherence [1]. Calcium alginate is a blood blocking agent and is widely used in dressings because of its abundance, relatively low cost, good biocompatibility and simple jelly-forming mechanism [2].

In this study, the antibacterial agent and the alginate polymer was examined together, and the appropriate concentration of nano-silver for use in dressing was determined, antibacterial release assay and cytotoxicity test were performed and finally, the effect of dressing on the living tissue was investigated.

Objective: The dressing was introduced as the accelerating and improving factor of the wound healing process. The purpose of this study is to use silver nanoparticles instead of common antibiotics to create antimicrobial activity and also to use a natural polymer such as alginate instead of traditional sterile dressings to expedite and improve the wound healing process.

Materials and Methods:

Dressing preparations

Calcium alginate films prepared by Kitotek was cut to 5 cm by 5 cm. After determination of the amount of adsorption by each sheet, a number of calcium alginate sheets were immersed for a sufficient time in a solution of colloidal silver nanoparticles prepared from nanoside at a concentration of 50 to 4000 ppm. Sheets were frozen in freezer for 24 hours after removal from the solution [4].

Morphology of dressing layers: The morphology of calcium alginate sheets before and after loading nanoparticles on them was investigated by field emission electron microscopy.

Evaluation of antibacterial properties: The lyophilized E. coli ATCC 25922 was prepared from Bahar Afshan Company. Antimicrobial dilution methods (MICs) and disk diffusion were used to evaluate antibacterial properties.

Measurement of release of silver nanoparticles from dressing: For this purpose, dressings measuring 5 cm by 5 cm were placed in an Erlene containing phosphate buffered saline solution (PBS) at pH 7.4.

Animal experiments: To evaluate the clinical effect of dressing on wound healing, an animal model study was performed and compared with conventional dressing. Twenty adult male Wistar rats, weighing 200 to 250 g were used in this study. All rats had free access to standard pelleted water and food during the study and exposure was 12 hour dark and 12 hour light. During the study the laboratory temperature was about 22 to 25 ° C. Anesthesia protocols and analgesic procedures were performed in accordance with the guidelines for the protection of laboratory animals of the European Union.

Histomorphometric evaluation: To assess the extent of shrinkage, the formation of cover tissue and overall wound healing until closure, digital photos of the wounds were taken on days 1,7,14 after wound healing.

Finaly,Pathological parameters in sections were evaluated by light microscope for evaluation of wound healing quality.

Results: The characterization of silver nanoparticles was performed by the Zero-potential dynamic diffraction method and was 61.4 nm and -20.83 mV, respectively. Antibacterial properties and cytotoxicity tests were performed and the results showed in the optimum concentration of 100 µg / l or 17.5 µg / l alginate film surface area. Also, on the 7th and 14th days of the wound, biopsy was done for histopathological studies and changes of skin cell shape were studied.

Evaluation of average dressing absorption in solution

The results showed that the rate of absorption was not significantly different over time. During the experiment it was observed that the alginate sheet absorbs the solution at the exact moment it is placed in the solution. Alginates are composed of large hydrophilic molecules, so alginates tend to bond with water [5]. The reason for the rapid uptake of water is the hydrophilicity of alginate molecules. Hydrophilic dressing is desirable because it absorbs the secretions of the wound by being placed on the wound and thus, facilitates the wound healing process, as well as keeping the environment moist. The average weight of dry sheet was 0.797 g.

Cytotoxicity test: Cell viability was assessed by MTT assay and the opacities were read by spectrophotometer. Within 48 hours, the dressing containing nanoparticles at concentrations less than 50 g / ml was 100% viable, and also for the concentrations of 200 g / ml and 100% g / ml 72% and 85%, respectively. It was observed that these values could be acceptable for the percentage of cell viability in the vicinity of silver nanoparticles.

Histomorphometric evaluations

In this evaluation, the rate of wound healing was calculated on the first, seventh, and fourteenth days and the two groups were statistically compared. Changes in wound area were significant for the experimental group

between days 7 and 14. Therefore, in the second week of the experiment, the wound healing process in the experimental group occurred at a higher rate.

Histopathological evaluation: Five factors indices have been considered to evaluate the wound healing process, with four levels of progression for each of these five factors. Therefore, the results of the histopathology were somewhat poorly reported. On the 14th day, the epidermis was fully reconstructed and a horny layer was formed. There was a marked inflammatory reaction. Collagen filaments were also found in the dermis with significant thickness. The tissue was vascular with many fibroblasts.

Conclusion: Calcium alginate sheet was considered as a layer of dressing and loaded with antibacterial agent of silver nanoparticles. As a result, a dressing is created that enhances the wound healing process simultaneously, prevents wound infection and delays wound healing. FE-SEM photos confirm the loading and show a somewhat uniform distribution of alginate particles. The antibacterial property of the dressing was evaluated by the minimum inhibitory concentration method and it was found that concentrations higher than 100 µg / ml exhibit antibacterial activity in the dressing. The dressing causes toxicity at concentrations above 200 µg / ml. The release efficiency of silver nanoparticles in PBS release medium was about 80%. The effect of dressing on the wound healing process in the living body (rat) was investigated and it was found that the presence of contracted wound dressing and epithelialization was evident. There will certainly be no risk in dynamic conditions.

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