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Antibacterial effects of electrospun chitosan/poly(ethylene oxide) nanofibrous membranes loaded with chlorhexidine and silver

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Abstract

To prevent percutaneous device associated infections (PDAIs), we prepared electrospun chitosan/poly(ethylene oxide) (PEO) nanofibrous membrane containing silver nanoparticles as an implantable delivery vehicle for the dual release of chlorhexidine and silver ions. We observed that the silver nanoparticles were distributed homogeneously throughout the fibers, and a fast release of chlorhexidine in 2 days and a sustained release of silver ions for up to 28 days. The antibacterial efficacy of the membranes against *Staphylococcus aureus* showed that the membranes exhibited an obvious inhibition zone upon loading with either chlorhexidine (20 μg or more per membrane) or $AgNO_3$ (1 and 5 wt% to polymer). Furthermore, long-term antibacterial effect up to 4 days was verified using membranes containing 5 wt% $AgNO_3$. The results suggest that the membranes have strong potential to act as an active antibacterial dressing for local delivery of antibacterial agents to prevent PDAIs.

Key words: Electrospinning; Chitosan; Controlled release; Chlorhexidine; Silver nanoparticles

Percutaneous medical devices, such as external fixation devices, urinary catheters, peritoneal dialysis catheters, bone-anchored hearing aids, voice prostheses and intravenous catheters for hemodialysis, have become indispensible in clinical practice.[1–3] These external devices, however, are particularly susceptible to bacterial infections due to their penetration through the skin, the body's primary defence against infection. The open space created around the implant provides an entrance for bacteria migrating into the body and subsequently colonizing on the implanted medical devices followed by formation of a biofilm.[4,5] Within the biofilm, organisms are protected from a matrix consisting of self-produced extracellular polymeric substances, which enables the bacteria less susceptible to host defense mechanisms and to the treatment of antibiotics.[2,5] Consequently, the development of such infections may result in life-threatening complications, which yields high morbidity and mortality rates, and high costs to the health care system.[6] Although decades of research, including hospital and patient hygiene measures and development of antimicrobial coatings, have been done, no ubiquitously accepted clinical solution to address the problem of the percutaneous device associated infections (PDAIs) has been forwarded.

Instead of treating the PDAIs, development of methods or techniques to prevent PDAIs is of enormous value. Recently, the use of an effective chlorhexidine (CHX)-impregnated dressing at the exit-site of catheters with weekly replacement was reported.[7] This dressing had high efficiency both in reducing colonization of bacteria on the catheter and in the reduction of the catheter-related

Abbreviations: PDAIs, percutaneous device associated infections; PEO, poly(ethylene oxide); CHX, chlorhexidine; SEM, scanning electron microscopy; TEM, transmission electron microscopy; AgNPs, silver nanoparticles; *S. aureus*, *Staphylococcus aureus*; RP-HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectroscopy; FBS, fetal bovine serum; HFFs, human foreskin fibroblasts; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CFU, colony-forming units.

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Table 1
Experimental groups.

Group	Amount of $AgNO_3$ (wt% to polymer)	Amount of chlorhexidine (μg per membrane)
1	–	–
2	0.1	–
3	1	–
4	5	–
5	–	20
6	–	60
7	–	100
8	0.1	60
9	1	60
10	5	60

bloodstream or central nervous system infections. Therefore, the development of active antibacterial dressing, which can deliver antimicrobial agents locally in a sustained manner, can be an efficient approach for the prevention of PDAIs.

Antimicrobial agents involve both antibiotics and antiseptics,[8] which are chemical substances that prevent the growth and development of the microorganisms. Nevertheless, compared with antiseptics, antibiotics are only effective against bacteria and have a higher risk to develop antibacterial resistance.[9,10] In fact, drug-resistant pathogens are emerging dramatically, leading to strongly increased global healthcare cost.[11,12] Unfortunately, the development rate of antimicrobial resistance exceeds the development rate of new drugs by far.[12] Furthermore, clinical therapy based on single drug is often not sufficiently effective to counteract infections, which has stimulated the development of combinatorial multidrug approaches to combat infections more effectively.[12–14] To this end, a combination of antiseptics can be an effect method to kill bacteria without inducing antibacterial resistance.

Among the various types of antiseptics, silver nanoparticles (AgNPs) are particularly attractive thanks to their broad-spectrum antibacterial activity. As a consequence, AgNPs have been widely used in various medical products.[15,16] More recently, it has been demonstrated that silver ions (Ag^+) are the active bactericidal agents that are released from AgNPs, and the antibacterial activity of AgNPs could be controlled by modulating Ag^+ release through manipulation of oxygen availability, particle size as well as the shape of AgNPs.[17] Besides AgNPs, CHX is another commonly used antiseptic, which has both bacteriostatic and bactericidal properties against gram -positive and -negative bacteria.[18] Recent studies showed that the combination of CHX with silver reduced the incidence of catheter infections and prolonged the antibacterial efficacy against a wide range of clinically significant potential pathogens.[19,20]

To achieve the dual delivery of Ag^+ and CHX, a suitable drug carrier is required for the fabrication of antibacterial dressing. More recently, chitosan and chitosan-based nanofibrous matrices have gained interest due to their large specific surface area, high drug encapsulation efficiency, superior biocompatibility, and ease of processing by using the electrospinning technique.[21–23] Furthermore, chitosan is a stabilizing material that can protect AgNPs from oxidization,[24] while chitosan itself has intrinsic antibacterial properties.[23,25]

Based on the aforementioned, the objective of this work was to develop a chitosan-based nanofibrous antibacterial dressing containing AgNPs for the dual release of Ag^+ and CHX. We hypothesized that the most effective approach to prevent PDAIs would involve a fast release of CHX to achieve a powerful initial bactericidal effect and avoid the development of bacterial resistance, followed by a sustained release of Ag^+ to prevent any latent bacterial infections. To this end, we electrospun chitosan/poly(ethylene oxide) (PEO) in an acetic acid solution containing silver nitrate ($AgNO_3$) to obtain uniform and defect-free nanofibers embedding AgNPs. Additionally, we post-loaded CHX onto the nanofibrous membrane by a diffusion method and then investigated the release of CHX and Ag^+ in deionized water. Finally, we examined the antibacterial effect of the membranes loaded with different amount of CHX and $AgNO_3$ and the long-term antibacterial effect of the membrane loaded with 5 wt% of $AgNO_3$ using the zone of inhibition test against *Staphylococcus aureus* (*S. aureus*), which is one of the most common pathogens associated with PDAIs.[1]

Methods

Electrospinning of nanofibers containing AgNPs

Chitosan (degree of deacetylation = 90%, molecular weight = 200-400 kDa, Heppe Medical Chitosan) and PEO (molecular weight = 900 kDa, Sigma-Aldrich®) were mixed at a weight ratio of 75:25 and dissolved in 35 v/v% acetic acid solution to form a 3 w/v% polymer solution. Subsequently, 0.1, 1 and 5 wt% (to polymer) of $AgNO_3$ (Boom BV, the Netherlands) were added and the mixture was stirred overnight before electrospinning. The electrospinning setup was described previously.[26] The electrospinning process was conducted at 27 kV and the polymer solution was ejected at a flow rate of 4 ml/h controlled by a syringe pump (BD scientific Inc., USA), while the distance between the spinneret and collector was fixed at 15 cm. The electrospun membranes were then punched into disks with a diameter of 11–12 mm, a surface area of approximately 1 cm^2 and a thickness of approximately 135 μm. The punched disks were cross-linked by glutaraldehyde (25 wt% in water) vapor for 2 h and then left in a fume hood overnight to remove the residual glutaraldehyde. Thereafter, the samples were stored in a desiccator at room temperature for further use.

Characterization of electrospun fibers

The electrospun disks were sputtered coated with gold (approximately 10 nm in thickness) to make the nanofibers conductive and the morphology of the disks was observed by scanning electron microscopy (SEM, JEOL 6301) at an accelerating voltage of 10 kV. Diameter distributions of the fibers were determined from the SEM micrographs obtained at random locations using the software Image J (National Institutes of Health, Bethesda, USA). One hundred fibers per membrane were analyzed. The formation of AgNPs was examined by transmission electronic microscopy (TEM, JEOL 1010) and TEM-energy dispersive X-ray spectroscopy (TEM-EDX, JEOL 2100 TEM with