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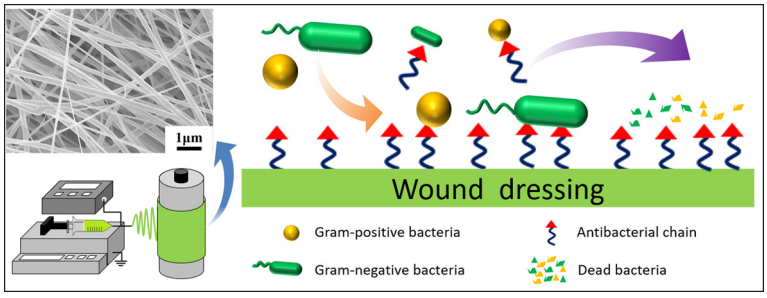
Regular Article

Long-acting and broad-spectrum antimicrobial electrospun poly (ε-caprolactone)/gelatin micro/nanofibers for wound dressing

Rui Shi [a,1], Huan Geng [b,c,1], Min Gong [b,c], Jingjing Ye [b,c], Chengai Wu [a], Xiaohua Hu [d], Liqun Zhang [b,c,*]

[a] Beijing Research Institute of Traumatology and Orthopaedics, Beijing 100035, PR China
[b] Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing 100029, PR China
[c] State Key Laboratory of Organic–Inorganic Composites, Beijing University of Chemical Technology, Beijing 100029, PR China
[d] Department of Burns and Plastic Surgery, Beijing Jishuitan Hospital, Beijing 100035, PR China

GRAPHICAL ABSTRACT



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ABSTRACT

Trimethoxysilylpropyl octadecyldimethyl ammonium chloride (QAS), which forms facile bonds with hydroxyl groups, acts as a cationic antibacterial agent. In this work, QAS was introduced into a polycaprolactone (PCL)/gelatin hybrid in increasing concentrations to fabricate a long-acting and broad-spectrum antimicrobial micro/nanofiber membrane as a novel wound dressing. The physical interactions and chemical bonding between QAS/PCL and QAS/gelatin were demonstrated by infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Measured water contact angle between the PCL-gelatin/QAS (PG-Q) nanofiber membranes suggested a hydrophobic surface, which has been shown to aid in removal of wound dressings. The mechanical strength of the membranes was sufficient to meet the clinical requirements. Furthermore, the 15% QAS (PG-Q15) and 20% QAS (PG-Q20) formulated nanofiber membranes showed a considerable increase in their bacteriostatic activity towards *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* (gram-negative) bacteria, suggesting a broad-spectrum bactericidal effect by the PG-Q membranes. The PG-Q membranes with various QAS formulations demonstrated little cytotoxicity. Therefore, the long-acting and broad-spectrum antimicrobial electrospun PG-Q micro/nanofibers membrane demonstrate potential efficacy as an antibacterial wound dressing.

1. Introduction

Skin acts as a barrier to pathogens and plays a key role in protective, immunological, thermoregulatory, and sensory functions [1]. Perforations in the skin barrier leave the body susceptible to

* Corresponding author.
E-mail address: zhanglq@mail.buct.edu.cn (L. Zhang).
[1] These authors contributed equally.

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wound infections, induced by microbiological contamination at the wound site. These bacterial infections represent a substantial burden that delay healing and can lead to increased patient morbidity and mortality [2]. Fabrication of an improved anti-infective wound dressing is a promising strategy for the advancement of wound care.

The application of wound dressings to cover the wound bed is typical in wound management. Wound dressings promote wound healing by providing a suitable environment for tissue regeneration via the removal of exudates, protection from fluid loss, and prevention from microbial infiltration [3]. Prompt treatment and covering of an exposed wound with wound dressings can promote re-epithelialization and wound healing [4]. However, the same dressing environment that is so beneficial for wound healing can also foster bacterial invasion and replication. Most antibacterial wound dressings contain various classes of antimicrobial agents, such as antibiotic drugs [5–7], silver nanoparticles [8–10], and/or quaternary ammonium salts [11]. The release of the antimicrobial agents to the surrounding milieu kills present bacteria. However, there exist several disadvantages associated with the local delivery of antimicrobial agents: (i) the mechanical properties of the dressings decrease due to damaged integrity of the dressings [12], (ii) time limited efficacy of antibacterial acivity [12], (iii) potential toxicity due to uncontrolled release [13], and (iv) the increasing risk of resistance to antibiotics [14].

Quaternary ammonium salts have broad-spectrum and potent antimicrobial activities and can effectively inhibit the growth of gram-positive bacteria, gram-negative bacteria, yeast, and fungi [15]. Trimethoxysilylpropyl octadecyldimethyl ammonium chloride (QAS; known as DOW CORNING® 5700 antimicrobial agent), is a novel antibacterial agent containing a siloxane group bound to an antibacterial quaternary ammonium via a long-chain alkyl [16]. QAS was approved by the Environmental Protection Agency (EPA Registration Number 64881-1) and the Food and Drug Administration as a modifier of medical devices in the late 1970s [17,18]. QAS can be easily bound to polymer chains by reacting the siloxane groups on QAS with hydroxyl groups on the matrix, endowing the matrix with an antibacterial property. Two possible antibacterial mechanism of QAS were reported: (i) the positively charged quaternary nitrogen on the QAS has an electrostatic attraction to the negatively charged bacterial membrane, which leads to bacterial membrane rupture due to the non-uniform surface charge distribution [19]; (ii) the hydrophobic long-chain alkyl groups can integrate into the bacterial membrane and change the physicochemical property of the bacterial cell membrane, inducing the leakage of bacterial content and inactivation of the bacteria [19]. Regardless of specific mechanism, QAS effects a change in the cell membrane permeability. This alteration of membrane permeability damages the membrane integrity and results in leakage of cellular contents, which is referred to as a contact-killing mechanism [19]. As a robust antibacterial agent, QAS has been widely researched and is currently used in textiles, for medical sterilization, and incorporated in package materials [18,20–22].QAS has been bound to silica nanoparticles, with excellent observed antibacterial performance [23], as well as adsorbed to a carbohydrate matrix via an adsorption-curing process to yield an antimicrobial micro-fibrillated cellulose [24]. One study incorporated a non-silicon derived quaternary ammonium salt into an electrospun nanofiber system to yield an antibacterial membrane [25]; however, there was no observed reaction between the quaternary ammonium salt and the matrix material. To date there have been no reported studies on the incorporation of the organosilicon quaternary ammonium salt in electrospun nanofibers.

Electrospinning is the most straightforward and efficient method to fabricate a nano/micro scale fiber membrane. Generally, electrospinning of nanofiber scaffolds allows for a high surface area-to-volume ratio, interconnected pore structure, and high porosity, which are also needed to allow wound dressings to promote hemostasis, gas permeation, moisture retention, and removal of exudates. Previously, a series of electrospun PCL/gelatin (PG) nanofiber membranes were developed with antibiotics for guided tissue regeneration/guided bone regeneration (GTR/GBR) implants [5,26,27]. Although the drug-loaded PCL/gelatin membranes demonstrated bactericidal activity, acceptable degradation performance, and cell compatibility, the membranes possessed a limited drug release period and drug resistance was still observed [28]. QAS was believed to yield a longer-lasting antibacterial electrospun membrane. There are two potential mechanisms of bactericidal activity for PG-Q membranes. Before the degradation of gelatin, the bacteria are killed mainly via contact with the membrane' surface. However, once infection occurs, the bacteria produce proteolytic enzymes which can degrade gelatin. The breakdown of the matrix will release QAS from the gelatin, resulting in higher antibacterial activity and increased contact with the bacterial cells.

For the current study, the QAS-functionalized PCL-gelatin hybrid nanofiber was fabricated to develop a novel wound dressing with broad-spectrum and long-term contact antibacterial properties. The membrane morphology, surface hydrophilicity, interactions between QAS and polymer matrix, antibacterial activity, and cytotoxicity were investigated. The current work is expected to provide valuable insight into the development of antibacterial wound dressing.

2. Materials and methods

2.1. Materials

QAS was purchased from Fluorochem Ltd. (99.5% purity, trade name: DC5700, United Kingdom) and was used without any further purification. Poly (ε-caprolactone) (PCL: Mn = 70–90 kDa, 99.0% purity), gelatin, and 3-(4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) (MTT, 99.5% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without any further purification. 2,2,2-Trifluoroethanol (TFE) solvent (99.8% purity) was purchased from Aladdin Industrial Corporation (Ontario, CA, USA); Dulbecco's modified Eagle's medium (DMEM, 99.9% purity), fetal bovine serum (99.9% purity), 0.05% Trypsin EDTA, and phosphate buffer saline (PBS, 99.9% purity) were obtained from Gibco (ThermoFisher Scientific, Waltham, MA, USA). Methanol (99.9% purity) was obtained from Alfa-Aesar Chemical (ThermoFisher Scientific). L929 fibroblast cell lines were kindly donated by Jishuitan Hospital.

2.2. Preparation of electrospun membranes

Based upon previous studies [29,30], the mass fraction of PCL should be at least 60% w/w to provide adequate strength and ductility, yet possess a slow degradation rate and a hydrophobic surface that reduces the adhesiveness of fibroblasts. However, the fraction of PCL in the matrix cannot be too high, as gelatin provides the bonding substrate for the QAS. Balancing these factors led to the usage of a 70:30 (w/w) PCL/gelatin ratio.

PCL-gelatin (PG) was prepared by blending 6% w/w PCL/TFE and 6% w/w gelatin/TFE at a ratio of 70:30 (w/w). QAS was added to the PG solution at various mass ratios. Membranes with QAS contents of 5% w/w, 10% w/w, 15% w/w, and 20% w/w were labeled as PG-Q5, PG-Q10, PG-Q15, and PG-Q20, respectively. All solutions were magnetically stirred at 90 °C for 12 h. The solution was fed at a rate of 1.3 mL/h by a syringe pump. The collector with a rotating rate of 300 rpm was located at a distance of 20 cm from the needle. Optimized high voltage current (8–20 kV) was applied between the