Materials Science and Engineering C 70 (2017) 311–316

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Light-induced antibacterial activity of electrospun chitosan-based material containing photosensitizer

A.N. Severyukhina [a,*], N.V. Petrova [a], A.M. Yashchenok [a], D.N. Bratashov [a,b], K. Smuda [c], I.A. Mamonova [d], N.A. Yurasov [a], D.M. Puchinyan [a,d], R. Georgieva [c,e], H. Bäumler [c], A. Lapanje [a,f], D.A. Gorin [a,b]

[a] Institute of Nanostructures and Biosystems, Saratov State University, 410012 Saratov, Russia
[b] Department of Nano- and Biomedical Technologies, Saratov State University, 410012 Saratov, Russia
[c] Institute of Transfusion Medicine, Charité-Universitätsmedizin, 10117 Berlin, Germany
[d] Institute of Traumatology and Orthopedics, 410002 Saratov, Russia
[e] Department of Medical Physics, Biophysics and Radiology, Medical Faculty, Trakia University, 6000 Stara Zagora, Bulgaria
[f] Josef Stefan Institute, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 23 August 2016
Accepted 3 September 2016
Available online 7 September 2016

Keywords:
Antibacterial material
Photosensitizer
Electrospinning
Antimicrobial photoactivation
Chitosan
Staphylococcus aureus

ABSTRACT

Increasing antimicrobial resistance requires the development of novel materials and approaches for treatment of various infections. Utilization of photodynamic therapy represents an advanced alternative to antibiotics and metal-based agents. Here, we report the fabrication of electrospun material that possesses benefits of both topical antimicrobial and photodynamic therapies. This material combines chitosan, as a biocompatible polymer, and a second generation photosensitizer. The incorporation of photosensitizer doesn't affect the material morphology and its nearly uniform distribution in fibers structure was observed by confocal Raman microscopy. Owing to photosensitizer the prepared material exhibits the light-induced and spatially limited antimicrobial activity that was demonstrated against *Staphylococcus aureus*, an important etiological infectious agent. Such material can be potentially used in antibacterial therapy of chronic wounds, infections of diabetic ulcers, and burns, as well as rapidly spreading and intractable soft-tissue infections caused by resistant bacteria.

1. Introduction

Currently, one of the crucial tasks of antibacterial therapy is the development of novel approaches that will not involve antibiotics for either curing or preventing infections. In order to solve this problem the researchers have extensively investigated antibacterial materials containing various compounds. Among the options is the use of metal-based materials (e.g. Ag nanoparticles). However, release of even low concentrations of metals from such materials can support occurrence and long living presence of antibiotic resistant genes in bacterial populations due to induction of co-transfer and gene fixation of both, namely metal and antibiotic resistance genes, within bacterial cells [1].

Utilization of photodynamic therapy (PDT) represents much more advanced alternative for the treatment of infectious diseases [2,3]. This method is based on the combined use of a photosensitizing agent, namely a photosensitizer, and irradiation by light of specific-wavelengths in the presence of oxygen. This interaction leads to the production of reactive oxygen species (ROS) [3–5]. The antimicrobial action of ROS production affects multiple targets within microbial cells, thereby bacteria will not readily develop resistance against PDT. Among ROS singlet oxygen is highly reactive and plays a major role in photodynamic inactivation of pathogens. It has a short half-life in biological systems as well as a short radius of action, leading to localized response [6].

Additionally, this therapy is advantageous due to its practical use that is based on two components: i) accumulation of a photosensitizer in target cells or tissue and ii) illumination of the lesion in a spatially controlled manner. PDT has also been utilized very efficiently against multi-antibiotic resistant strains [3,7].

Although PDT has been increasingly used in biomedical applications, the current treatment approaches are in general relied on applying liquid solution of a photosensitizing agent on the infected surface following by light irradiation. Thus, a number of drawbacks such as uncontrollable release and diffusion of the photosensitizer along with relatively high drug consumption, limit PDT from being one of the well-recognized and widespread therapies. To address these problems a variety of macromolecular nanocarrier platforms such as liposomes, polymeric nanoparticles, and micelles have been investigated for systemic delivery of photosensitizers [8]. However, for efficient topical antimicrobial therapy, PDT should meet several criteria such as high and sustained concentration of antimicrobial substance (AS) at the site of infection, limited total amount of AS, limited potential for systemic absorption and toxicity of AS [9]. PDT methods currently used can be improved according to the above mentioned criteria by the

* Corresponding author.
E-mail address: severyuhina_alexandra@mail.ru (A.N. Severyukhina).

http://dx.doi.org/10.1016/j.msec.2016.09.005
0928-4931/

impregnation of wound dressing with a photosensitizer. However, the right material and method of its production should be correctly chosen.

Recent studies have been focused on chemical bonding of AS to textiles [10–12]. Therefore, it may be possible to incorporate the photosensitizer directly into the polymer as a comonomer [13].

Due to its biocompatibility, biodegradability, large surface area to volume ratio, high porosity, sorption capacity, vapor permeability and feasibility to be functionalized and derivatized, nanofibrous mats produced from biopolymers are frequently used as wound dressings and scaffolds in tissue engineering [14,15]. For mass production of one-by-one continuous polymer nanofibers, electrospinning is one of the most promising fabrication methods due to simplicity of this technique, its low cost and high throughput [16,17]. Additionally, it is a very robust method, which works with almost any soluble polymer and any other fiber-forming solutions as it has been demonstrated through using various polymers with potential biomedical, environmental, and antibacterial applications in recent years [18,19]. Most importantly, different additives could be incorporated into fibers structure during electrospinning [20–29].

At present there are a few reports dedicated to electrospun materials containing photosensitizers [30–35]. However, these materials contain either non-biocompatible polymers, or utilize first generation photosensitizers. In that respect, chitosan is among the most frequently used polymers mainly because of its biocompatibility and biodegradability. This polymer and its derivatives have been widely investigated as antimicrobial materials against various target organisms including algae, bacteria, yeasts and fungi [36–39].

Phthalocyanines used in our study represent second-generation photosensitizers (SGP) with the enhanced light absorption in the near-infrared (NIR) spectral region (therapeutic window) where light has its maximum depth of penetration into the tissue.

Therefore, our motivation and consequently the novelty of this study are in the creation of the electrospun fibers based on combination of biocompatible polymer, namely chitosan, and second-generation photosensitizer (SGP). For the first time, here we reported the use of electrospinning method to prepare this novel material combining chitosan, together with SGP, especially for its usage in topical antimicrobial PDT applications.

To reach our goal we followed three specific criteria: (i) to produce the material loaded with hydrophilic and clinically tested SGP, (ii) to characterize the properties of this material in view of its production optimization and (iii) to test potency of this new material to use it in PDT against bacterial infections.

2. Material and methods

2.1. Materials

Chitosan (degree of deacetylation, 85%; MW, 200 kDa), polyethylene oxide (PEO; MW, 1000 kDa) were purchased from Sigma-Aldrich.

Photosens was kindly supplied by the Institute of Organic Intermediates and Dyes (Moscow, Russia). This photosensitizer is a mixture of sulfonated aluminum phthalocyanines with different degrees of sulfonation (n = 2, 3, and 4). It exhibits a 675-nm absorbance peak and can induce the generation of reactive oxygen species at the energy of 100 J/cm^2.

Reagents for in vitro experiments, if not otherwise indicated, were purchased from Sigma-Aldrich.

2.2. Electrospinning process

The detailed preparation of solutions and the electrospinning procedure itself were described in our previous work [40].

Briefly, solutions with different proportions of chitosan/PEO/PS (88/7/5, 90/7.5/2.5, 91/8/1, and 93/7/0) were prepared.

A laboratory device for textile electrospinning, NanoSpider NS 200 (Elmarco, Czech Republic), was utilized to produce fibers from the prepared polymer solutions. The polymer solutions were electrospun using a four-wire rotating electrode and a stationary wire electrode. The distance between the spinning electrode and the collecting electrode was 150 mm. The applied voltage was set to 80 kV.

2.3. Measurements of fiber size

Scanning electron microscopy (SEM) images of the nanofibers were obtained with SEM MIRA\\LMU 2, microscope (Tescan, Czech Republic) at a 20-kV acceleration voltage. Before the measurement, all the samples were sputtered with gold by Emitech K450X setup (Quorum Technologies, UK). SEM images were postprocessed with Atlas Image Processing software. The diameters of the fibers were obtained from three independent sets of 100 random measurements for each sample. Thereafter, the size distribution, average diameter, and standard deviation of the fibers were statistically analyzed with the Origin program (OriginLab Corporation). An independent *t*-test was applied to compare the mean fiber diameters, in which the $p < 0.05$ values were considered statistically significant.

2.4. Fiber spectroscopic analysis

Diffuse reflection spectra were acquired in triplicate by using a scan UV–Visible spectrophotometer (UV-2500, Shimadzu) equipped with an integrating sphere assembly (ISR-204A).

We analyzed the uniformity of distribution of the photosensitizer agent by confocal Raman microscope (Renishaw inVia, UK) equipped with a diode-pumped 785 nm NIR laser for excitation. Laser power was below 60 mW and controlled by a neutral optical density filter. The laser beam was focused through a 50× (Leica N PLAN, NA = 0.5) microscope objective. The spectra were acquired with a thermoelectrically cooled CCD detector optimized for near IR (with spectral resolution of about 1 cm^{-1}). All the spectra were collected by using WiRE software V4.1 (Renishaw, UK).

2.5. Antimicrobial PDT activity of newly developed material

The antibacterial activity of chitosan/PEO/PS composite material against *Staphylococcus aureus* was defined by using the modified agar disk diffusion method (Fig. 1). The experiment was conducted in triplicate to ensure its reproducibility.

Sterile solid nutrient agar (NA) medium (20 ml) solidified in the Petri dishes (2R = 7.5 cm) was used. We inoculated the surface of NA by spreading the 100 μl suspension of freshly grown overnight *Staphylococcus aureus* (ATTC 25923) cultures containing approximately 10^3 bacterial cells on the medium. Strain *Staphylococcus aureus* ATCC 25923 was kindly provided by Saratov Institute of Traumatology and Orthopedics (Saratov, Russia). After that, four disks (with diameter d = 1 cm) were disinfected in 70% alcohol, two of pure chitosan scaffolds and two disks of PS loaded scaffolds (with 5% w/w of PS; 10 mm in diameter), respectively, were placed on the surface of inoculated solid media and incubated at 37 °C for 2 h, aiming to release PS from fibers. Following this incubation period two out of four disks, one of the pure chitosan disks and one of the PS-loaded, were irradiated with a 675 nm light emitting diode (Polironik, Moscow, Russia) for 10 min at the power density of 80 mW/cm^2. The area of the light spot on the plate surface was about 28 cm^2. The samples that were not exposed to irradiation were considered as a control.

After the light irradiation, plates were incubated for 24 h at 37 °C to obtain confluent growth of bacteria on the solid media surface. To evaluate the antimicrobial efficiency we measured the size of growth inhibition zones observed as complete absence of bacterial confluent growth. The results were expressed as arithmetic means with standard deviations.