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Light-activated polymethylmethacrylate nanofibers with antibacterial activity

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ABSTRACT

The creation of an antibacterial material with triggerable properties enables us to avoid the overuse or misuse of antibacterial substances and, thus, prevent the emergence of resistant bacterial strains. As a potential light-activated antibacterial material, polymethylmethacrylate (PMMA) nanofibers doped with silver nanoparticles (AgNPs) and *meso*-tetraphenylporphyrin (TPP) were prepared by electrospinning. TPP was chosen as an effectively reactive oxygen species (ROS) producer. Antibacterial tests on *Staphylococcus epidermidis* (*S. epidermidis*) and *Enterococcus faecalis (E. faecalis)* showed the excellent light-triggerable antibacterial activity of the doped materials. Upon light irradiation at the wavelength corresponding to the TPP absorption peak (405 nm), antibacterial activity dramatically increased, mostly due to the release of AgNPs from the polymer matrix. Furthermore, under prolonged light irradiation, the AgNPs/TPP/PMMA nanofibers, displayed enhanced longevity and photothermal stability. Thus, our results suggest that the proposed material is a promising option for the photodynamic inactivation of bacteria.

1. Introduction

The formation of pathogenic bacterial colonies in hospitals is a significant problem [1]. Applications of hard and periodic antibacterial treatment by UV light can particularly solve this problem, but cannot be considered as a universal solution. Alternatively, antibacterial coatings with passive release of drugs lose their activity and can induce the formation of antibacterial resistance due to the permanent presence of antibacterial compounds in environment [2]. Therefore, the creation of protective surfaces with antibacterial properties with a triggered release of antibacterial substances is an option in the field of material engineering for medical application. These surfaces must be responsive to the "mild" stimuli, to avoid the evacuation of patients during sanitation procedure. From this point of view, light-activated antibacterial materials seem to be very promising [3].

In the field of light-activated antibacterial materials there are two main strategies: 1) fabrication of materials, which undergo photoisomerisation upon to irradiation [4]; 2) fabrication of materials which generate reactive oxygen species (ROS) upon light exposure (photosensitizers) [5]. The former include azobenzene-containing polymers undergoes *cis*-trans isomerization [6]. The consequence of such a phase transition could be, for example, formation of pores in the material, which lead to the release of antibacterial molecules. Another example is reversibly wettability switching upon irradiation, preventing the formation of antibacterial film. The second approach uses the photosensitizers such as porphyrins, semiconductor quantum dots, graphene, graphene oxide, metal nanoparticles and metal nanoparticles oxides [7–10]. The advantage of using photosensitizers is a non-selective action on the bacterial membrane that diminishes the risk of developing bacterial resistance [11,12].

Among photosensitizers *meso*-tetraphenylporphyrin (TPP) has been reported as an effective ROS producer, which inactivates bacterial membranes upon light irradiation [13–17]. TPP-loaded polymeric materials exhibit significant antibacterial activity upon light irradiation. Felgenträger et al. [18] showed the photodynamic inactivation of *Staphylococcus aureus* TPP covalently bonded onto polyurethane. Mosinger et al. reported about antibacterial activity of polystyrene and gelatin nanofibers loaded with TPP [19].

Nanofibers are effectively used as a carrier of anticancer and antimicrobial agents due to high surface-to-volume ratio. Electrospinning provides a simple method for fabrication nanofibers from plenty of materials including polymers. In this method a high voltage is applied between a polymer droplet (solution or melt) flowing out from the nozzle (spontaneously or controlled by the syringe pump) and collector. At a threshold value of high voltage, repulsive electrostatic forces on the surface of a droplet overcome surface tension forces. A droplet of polymer solution distorts, elongates into cone shape (so-called Taylor cone) and jet is formed. Polymer jets are collected on the target and solvent evaporates during the deposition. The advantage of this method is the possibility to adjust of the process parameters (the nature of the polymer, applied voltage, tip to collector distance, flow

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rate, solution concentration and conductivity, volatility of solvent, humidity) i.e. to produce fibers with specified characteristics [20–23].

The fabrication of antibacterial polymer nanofibers is frequently focused on the combination of degradable polymers with metal nanoparticles [24–27]. The main disadvantage of such materials is an irreversibility of release process that restricts control of the release kinetics of antibacterial substances. Therefore, much attention must be paid to the design of stimuli-responsive nanofibers with controlled and triggerable antibacterial effect [28,29]. TPP and other porphyrins also have significant drawback, because of the limited stability upon irradiation. Upon light exposure irreversible photo-oxidative degradation of porphyrins occurs and materials lose antibacterial activity [30].

Herein, we reported about the PMMA nanofibers doped with TPP and silver nanoparticles (AgNPs). PMMA is insoluble in water polymer, which has a potential as cargo of antibacterial agents. PMMA prevents aggregation of AgNPs through the interaction between the carboxylate groups of PMMA with silver [31]. The TPP/AgNPs combination was chosen to promote AgNPs release by heating the polymer due to light absorption by TPP, to protect TPP against photobleaching [32] and to enhance thermal stability of nanofibers. Nanofibers were prepared by electrospinning and demonstrated light-triggered antibacterial properties. The activation of the material is provided by LED-irradiation with wavelength 405 nm. The triggering is achieved through turning-on/off of the light source.

2. Experimental

2.1. Materials

Polymethylmethacrylate (PMMA, 15 kDa), anhydrous *N*-methyl-2-pyrrolidone (NMP, 99.5%), silver nitrate ($AgNO_3$, 99.9999%) were obtained from Sigma Aldrich and used without further purification. Chloroform (99.5%) was purchased from PENTA. *Meso*-Tetraphenylporphyrin (TPP, 99.0% purity) was supplied from Frontier Scientific. Mueller-Hinton agar (MHA) were prepared as described by producer (Oxoid, CM0337) and sterilized in the autoclave.

2.2. Fabrication of AgNPs/TPP/PMMA nanofibers

2.2.1. Solution preparation

PMMA was dissolved in 0.9 g of chloroform to obtain 35.7% (*w*/w) solution; 0.05 g of $AgNO_3$ was dissolved in 0.3 g of NMP and placed for 2 h in the dark; 0.0064 g of TPP was dissolved in 0.3 g of chloroform. The AgNPs colloid was obtained by reduction of $AgNO_3$ with NMP during 2 h at the ambient temperature in the dark (the solution became characteristic yellow) [33–35]. Finally, all solutions were mixed together and placed in the glass syringe with 0.8 mm needle.

2.2.2. Electrospinning

The electrospinning was performed using a vertical set up containing a syringe pump (NE-300, New Era Pump Systems, Inc.) with a 0.01 ml h^{-1} feeding rate, 40 mm tip-to-collector distance and potential 14 kV. The metal needle tip was clamped to the positive electrode of high voltage power supply (Chargemaster CH30P SIMCO, USA). The AgNPs/TPP/PMMA nanofibers were collected for 15 min on glass substrates placed on the aluminum foil. Obtained nanofibers were finally dried overnight under vacuum in order to remove any residual solvent. The glasses with nanofibers were carefully separated from aluminum foil before antibacterial tests. The TPP/PMMA nanofibers were also prepared by electrospinning. The polymer solution was prepared in a similar way (0.5 g PMMA was dissolved in 0.9 g of chloroform, the same amount of TPP dissolved in chloroform and NMP was added).

2.3. Film fabrication

The films were prepared by the spin-coating at 1000 rpm for 15 s on rounded glass substrates with 10 mm diameter. Then obtained samples were immediately placed onto a hot plate (60 °C) and kept up to solvent evaporation. The preparation of the Ag/TPP/PMMA solution was the same as for electrospinning.

2.4. Sample irradiation and characterization

The samples treatment was performed with Light-Emitting Diode light source (110 mW power output, 405 nm center wavelength and the diameter of light spot 10 mm). Alternatively, 405 nm emitting laser system with 50 mW output (ThorLabs ITC 4001), collimated to 4 mm spot diameter was used.

Confocal images of the samples surfaces were taken using the optical microscope Lext OLS 3100 (Olympus Corporation, Shinjuku, Tokyo, Japan). Transmission electron microscopy (TEM) was performed on a JEOL JEM-1010 transmission electron microscope, with SIS MegaView III digital camera (Soft Imaging Systems, acceleration voltage 80 kV) For TEM observation composite nanofibers were collected on carbon-coated copper grids. The scanning electron microscopy (SEM) (HITACHI S 4700) was used for study morphology and distribution of the nanofibers. Samples were sputtered with 50 nm gold layer to make them conducting, and the SEM images recorded at 5000× magnification.

The detection of singlet oxygen was performed by spectrophotometric method, using potassium iodide. The samples were placed into 3 ml of 1 M KI solution and exposed to light for 3 h, 8 h, 20 h. UV–Vis spectra were measured using Spectrometer Lambda 25 (Perkin-Elmer), indicated coloration due to I^- reduction under the ROS influence [36].

2.5. Antibacterial tests

The antibacterial properties were tested against two environmental Gram-positive bacterial strains: (i) *Enterococcus faecalis (E. faecalis)* and (ii) *Staphylococcus epidermidis (S. epidermidis)*. Experiments were performed with and without (dark control) light irradiation. The effect of light irradiation was taken into account by irradiation of bacteria without sample (light control). As a dark control, the AgNPs/TPP/PMMA nanofibers were placed onto agar plates previously colonized by bacteria (1 ml of bacteria suspension containing 5×10^7 bacteria cells in all cases) and remained in contact for 3 h under the dark condition. Experiments were performed at ambient temperature of 25 °C. Another samples were placed onto agar plates and then irradiated for 3 h with light (405 nm), to evaluate the effect of light on antibacterial activity. After samples removing bacterial strains were incubated overnight at ambient temperature in the dark. The final evaluation of antibacterial properties was performed on the basis of the inhibition zones size.

3. Results and discussion

Fig. 1A shows SEM images of as spun Ag/TPP/PMMA nanofibers (Fig. 1A). SEM images show relatively broad distribution of nanofibers diameter, from 100 nm to 2.7 μm (determined on ≥10 SEM images from randomly selected 10 places from each image using image analysis software). The nanofibers were obtained after careful optimization of experimental conditions (see experimental part), where both, the electrospinning procedure and NMP amount, which is necessary to reduce $AgNO_3$ were taken into account. Chloroform was chosen for electrospinning of PMMA, due to relative low value of surface tension and good volatility. The presence of NMP is necessary to induce the silver ions reduction, but the quality of resulted nanofibers worsens because of poor volatility of NMP. The appearance of the beads along fibers and their broad diameter distribution (polythickness) can be attributed to the NMP presence.