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Regular Article

Fabrication of biocompatible and efficient antimicrobial porous polymer surfaces by the Breath Figures approach

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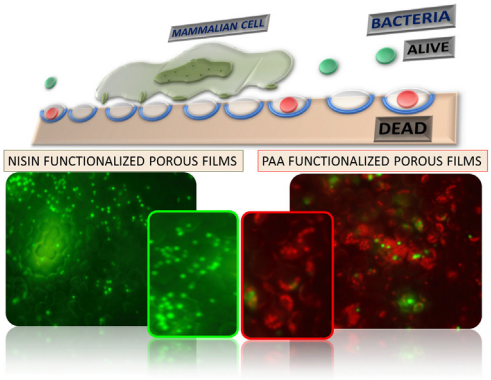
GRAPHICAL ABSTRACT



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ABSTRACT

We designed and fabricated highly efficient and selective antibacterial substrates, *i.e.* surface non-cytotoxic against mammalian cells but exhibiting strong antibacterial activity. For that purpose, microporous substrates (pore sizes in the range of 3–5 μm) were fabricated using the Breath Figures approach (BFs). These substrates have additionally a defined chemical composition in the pore cavity (herein either a poly(acrylic acid) or the antimicrobial peptide Nisin) while the composition of the rest of the surface is identical to the polymer matrix. As a result, considering the differences in size of bacteria (1–4 μm) in comparison to mammalian cells (above 10 μm) the bacteria were able to enter in contact with the inner part of the pores where the antimicrobial functionality has been placed. On the opposite, mammalian cells remain in contact with the top surface thus preventing cytotoxic effects and enhancing the biocompatibility of the substrates. The resulting antimicrobial surfaces were exposed to *Staphylococcus aureus* as a model bacteria and murine endothelial C166-GFP cells. Superior antibacterial performance while maintaining an excellent biocompatibility was obtained by those surfaces prepared using PAA while no evidence of significant antibacterial activity was observed at those surfaces prepared using Nisin.

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1. Introduction

Biomedical devices are today widely employed and have become indispensable for many different purposes. For instance, during the last decades vascular grafts, heart valves, knee implants and of course stents have been extensively employed to reestablish the quality of life of a great number of patients. Within the materials employed for implant fabrication, polymers are among the most extended materials and their use has been and is expected to steadily grow in the next years.[1] Among the advantages over other biomaterials polymers present interesting properties including biocompatibility, flexibility or bio-inertness.

However, an important remaining general issue in the use of polymeric but also metallic or ceramic materials for biological applications is the contamination of the material surface by microorganisms. This general problem touches many diverse areas including purification systems, food packaging, healthcare products and is particularly problematic in hospitals and in the handling of medical devices [2]. Although this is a common obstacle for all biomaterials, the situation becomes more serious when using polymeric biomaterials for long-term implants. In this case, device-related infections (DRIs) can be developed. These infections are difficult to detect and therefore when they are distinguished typically by tissue inflammation the damage is rather large and the implant needs to be replaced. In order to avoid these major problems two different strategies are available. On the one hand, DRIs can be lessen by taking measurements to reduce the risk of infection with prophylactic procedures (e.g. frequent replacement of the catheter) or improving the operation and sterilization techniques. However, these strategies usually delay the apparition of the infection but not prevent completely the microorganism contamination.

On the other hand, a large number of reports resort to the immobilization of antimicrobial compounds that are either covalently bonded (killing-on-contact substances) or embedded in the polymeric substrate and delivered to eradicate the microorganism [3]. In this case, major drawbacks include the partial efficiency combined with the toxicity of the antimicrobials employed. Finally, more recently, few studies have evidenced that surface topography at the micro/nanoscale can be also an interesting parameter for the fabrication of antibacterial/antifouling polymer surfaces [4]. While it is true that chemically/topographically mechanisms present a high bactericidal activity, aspects such as the period of activity or the eventual specificity against a particular bacterial strain still need further investigation. In summary, to the best of our knowledge none of the currently employed strategies are able to completely eliminate infections associated to polymeric biomaterials [5]. Moreover, the investigation of antibacterial activity of a particular surface needs to be combined with the analysis of their eventual toxicological effects.

In this manuscript, an alternative strategy to produce efficient antimicrobial surfaces improving the major drawbacks of previous designs is reported. The concept relies on the different sizes of bacteria (1–5 μm) in comparison with eukaryotic cells (>20 μm). Providing micrometer size surface features, in this case pores with the appropriate diameters above the size of bacteria and below the size of eukaryotic cells, we aim to selectively favor the contact between the antimicrobial polymer and bacteria located inside of the pores while limiting the contact with cells.

We propose the use of micrometer size porous surfaces in which the inner part of the pore is decorated with poly(acrylic acid) (PAA) [6] and an antimicrobial peptide, *i.e.* Nisin immobilized inside the pores, widely accepted for its excellent antimicrobial behavior for comparative purposes [7–9]. Thus, this approach combines simultaneously an appropriated surface chemistry and the microstructuration [13,14]. The Breath Figures (BFs) approach allow us to finely tune both aspects can be finely tuned, i.e. the fabrication of microporous interfaces with precisely defined chemical composition inside/outside the pores as well as the control over the pore sizes and distribution. This methodology is a widely employed strategy and several reviews have been recently reported covering both the chemical and physical aspects of porous film formation [10–12,15]. In terms of applications, porous films obtained using the Breath Figures methodology have the potential to be employed in tissue engineering applications since they can either support cell adhesion and proliferation [16–29] or exactly the opposite, *i.e.* improve the adhesion and act as barriers [30,31]. However, the systems explored up to date for bioapplications has been limited to few chemically different polymeric materials [32,33] and investigations combining microorganisms and cells are rare [31].

2. Experimental section

2.1. Materials

The monomers, *t*-butyl acrylate (tBA) (Sigma-Aldrich, 98%) and styrene (St) (Sigma, Aldrich, 99%), required purification by first dried over calcium hydride and distilled using vacuum techniques. The following list of compounds were used as received: 2,2′-bipyridyl (bipy) (Sigma-Aldrich, 99%), phenylethyl bromide (PhEBr) (Sigma-Aldrich, 97%), N,N,N′,N″,N″,-pentamethyldiethylenetriamine (PMDETA) (Sigma-Aldrich, 99%), Copper (I) bromide (CuBr) (Sigma-Aldrich, 98%) and other solvents. Coupling reactions were carried out using N-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma-Aldrich – USA). The polystyrene-*b*-poly(acrylic acid) diblock copolymers were prepared following the procedures already reported by our group [39].

2.2. Characterization

Scanning electron microscopy (SEM) micrographs were obtained using a Philips XL30 microscope working at a voltage of 25 kV. In order to improve the visualization, the porous surfaces were metallized with gold-palladium (80/20).

Information about the pore dimensions (average diameter and distribution) was obtained using the image analysis software (ImageJ, http://rsb.info.nih.gov/ij/).

The surface analysis by Confocal Raman Microscopy was performed using a WITec Alpha 300 RA (Ulm, Germany). This instrument uses a Nd:YAG laser (wavelength of 532 nm) and an output power of 10 mW output power. Moreover, two different gratings of 600 and 1800 grooves/line and a 100× objective (N.A: 0.95) were employed. This equipment is coupled to a piezoelectric stage that allows recording the images point by point with each 100 nm. Moreover, an optical fiber of 25 μm in diameter permits a spatial resolution less than 300 nm. To analyze the spectra and to make all the calculations and to build the Raman images, the software Witec Project Plus was employed.

ATR-FTIR spectra were recorded in a FTIR spectrometer Spectrum One of Perkin-Elmer. Using ATR with and internal reflection elements diamond/ZnSe the region analyzed correspond to the 2 μm depth.

2.3. Preparation of the antimicrobial mirocoporous (honeycomb films)

A high molecular weight polystyrene (80 wt%) was blended with a 20 wt% of the different block copolymers and dissolvent in chloroform to reach a polymer concentration of 30 mg/ml. The