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Antifouling Poly(ε-Caprolactone) Surfaces For Medical Devices

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Abstract **-** Medical devices inserted into an organism are suspected to the growth of aggregated bacteria on their surface termed biofilm. Biofilm can sometime resist antimicrobial treatments. Thus, implant-associated infections cannot always be treated in an effective way with antibiotics, and in the majority of cases, the only way to fight the infection is to remove the implant. This event poses a public health problem, being crucial to find new strategies to face this serious issue. A promising approach to prevent biofilm formation on medical devices is preventing the adhesion of bacteria to the surface using a coating that avoids bacterial attachment on surfaces, i.e., an antifouling coating. In this work, we used a fluorinated tripeptide that prevent biofilm formation to coat the biopolymer poly(ε-caprolactone) (PCL) that is suitable for making biodegradable medical devices. Our results shows that PCL coated with this tripeptide reduced the amount of bacteria by ~50% when compared to bare PCL. This newly developed PCL can be useful for the formation of tracheal stents, as this biodegradable polymer is suitable for long term applications due to its slow degradation rate.

Keywords**:** Poly(ε-Caprolactone) (PCL), Biofouling, Peptide, Self-Assembled Coating, Surface Properties.

1. Introduction

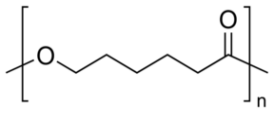
Invasive medical devices are widely used for diagnostic and therapeutic purposes in most medical specialties. Infectious risk is one of the most frequent complications related to the use of medical devices such as orthopedic or cardiac prostheses, and vascular or urinary catheters [1]. Bacteria colonize the surface of the foreign material forming a well-defined network called biofilm, which is extremely resistant to antibiotics [2]. Hence, the replacement of the contaminated device is often the only way to treat the infection. It is a proven fact that medical device-related infections are a public health concern and an economic burden.

Biofilm formation is an important strategy of bacteria to survive in adverse environmental conditions. This process consists of different stages: reversible attachment, irreversible attachment, microcolony formation, maturation and dispersion [1]. Therefore, when a medical device is contaminated with bacteria, the microorganisms must adhere to the implant enough time so that the settlement is irreversible to form the biofilm [3]. Once adhered, microorganisms duplicate and develop as microcolonies over the entire surface. That being said, a promising strategy for avoiding infections on implants is the development of antifouling surfaces that prevent the initial bacterial adhesion [4].

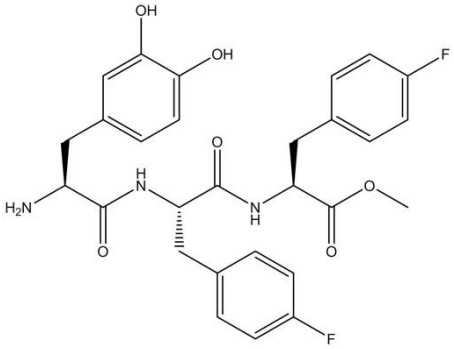
Here, we combined the versatility of a biodegradable material with a new antifouling coating that will reduce the attachment of bacteria on the surface. The main advantage of using biodegradable medical devices is that they do not need to be removed after finishing their service, as they can be absorbed or excreted by the body. In this way, the tissue surrounding can return to its original state, and a follow-up surgery is avoided. Consequently, the combination of a biodegradable polymer with an antifouling coating will be a great advance in materials for making medical devices that prevent infections.

Poly(ε-caprolactone) (PCL) is a semicrystalline polyester widely used in biomedical applications due to its biodegradability, biocompatibility, and good mechanical properties. Its degradation can last from several months to years, making it suitable for long-term biomedical applications [5]. On the other hand, the antifouling compound under study consists on a low-molecular weight tripeptide, which design allows its spontaneous adsorption onto any kind of substrate, as well as the creation of surfaces with anti-adhesive properties [6]. Due to the insertion of the amino acid 3,4-dihydroxyl-L-phenylalanine (DOPA), a key compound in the formation of mussel adhesive proteins, the peptide has the ability to attach

to different surfaces [7]. In addition, the fluorine atom on each of the benzene rings provides the antifouling character to the peptide (see Fig. 1).



Poly(ε-caprolactone) (PCL)



Antifouling peptide (P)

Fig. 1: Chemical structures of PCL and Peptide.

Taking everything into consideration, the coating of PCL with a new rationally designed antifouling peptide would lead to the development of innovative antifouling materials for medical applications.

2. Results and discussion

2.1. Coating of PCL films with antifouling peptide

PCL films with a thickness of 100 μm were obtained by casting PCL from tetrahydrofuran (THF) solutions at room temperature. Then, square samples of PCL (1 cm^2) were immersed into an alkaline aqueous solution of the peptide at different concentrations (0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL, and 4 mg/mL) for 2 hours (see Fig. 2). It should be noted that when the solution reached a pH around 8.5, the initially transparent solution changed to a suspension composed by floating white aggregates. Afterwards, the coated films were rinsed extensively with distilled water and dried in a vacuum oven. The catechol groups of the peptide enabled its immobilization on the PCL surface by a simple dipping process under alkaline conditions [8].

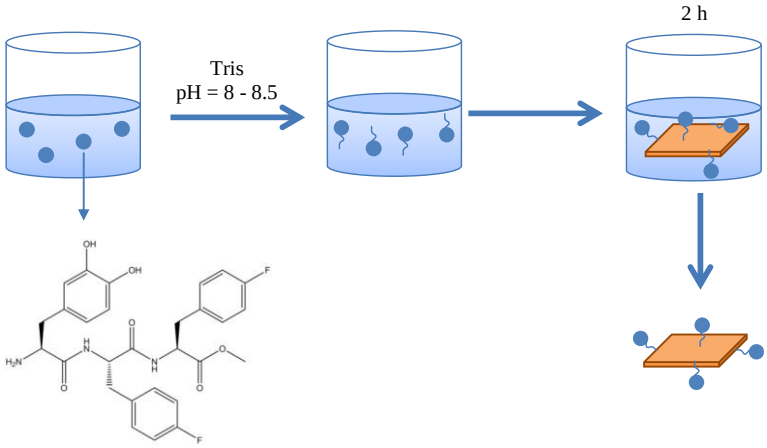


Fig. 2: Schematic illustration of the immersion process of the PCL films in the peptide solution.

2.2. Surface Characterization

The bacterial adhesion on a surface is a key factor in biofilm formation. Hence, hydrophobic surfaces can reduce the contact with bacteria, limiting the bacterial attachment which forms the biofilm [9]. A surface is considered hydrophobic with a contact angle greater than 90º, whereas below 90º a surface is hydrophilic. With the aim of studying the hydrophobicity in the samples, contact angle measurements were performed onto surfaces of bare PCL and PCL coated into different peptide

solutions (0.5 mg/mL, 1 mg/mL and 2 mg/mL), using a Drop Shape Analyzer. The modified substrates exhibited a slight increase in the contact angle, and therefore, a slight increase in hydrophobicity as the peptide concentration increased (see Fig. 3). The addition of fluorine atoms contained in the antifouling coating restricted the contact between the surface and water. Therefore, the less contact results in a lower chance of bacteria to attach to the polymer [9].

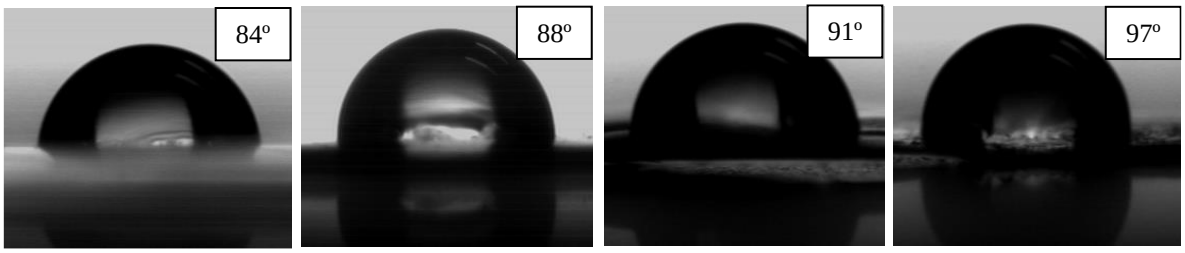


Fig. 3: Contact angle measurements of PCL, and superficially modified PCL surfaces after dip coating in different peptide solutions: (a) PCL, (b) PCL coated with a peptide solution of 0.5 mg/mL, (c) 1 mg/mL, and (d) 2 mg/mL.

3. Biofilm Formation on PCL Surfaces Coated with Antifouling Peptide

Bare and peptide-coated PCL films (PCL+P) were incubated overnight at 37 ºC in inoculums of Escherichia coli, in order to assess the bacterial attachment. Then, bacteria attached during the incubation were removed from the surfaces, diluted and cultured on LB agar plates. After the incubation of the plates, the number of colonies formed was counted (Fig. 4). The statistical difference between samples was tested by t-test at a confidence level of 95% ($p < .05$). That being said, it was observed a reduction of 47 % in the amount of colonies formed on the surface when compared with bare PCL.

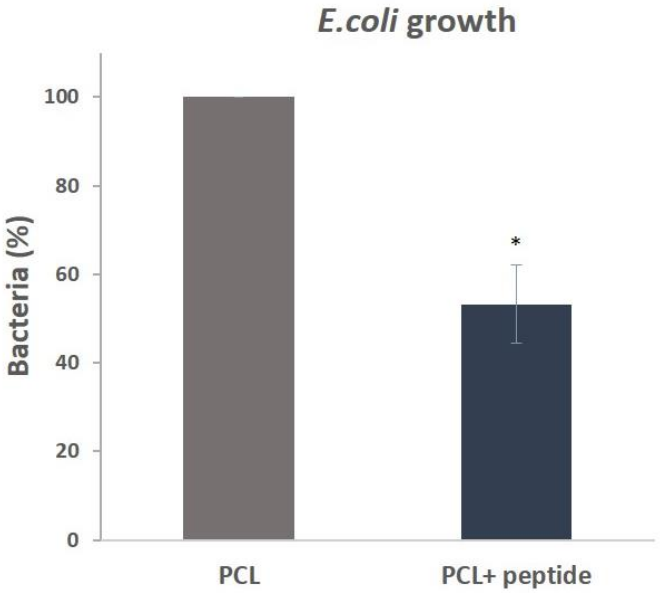


Fig. 4: Number of *E. coli* colonies grown on bare and peptide-coated PCL surfaces.

4. Conclusion

Bacterial adhesion to surfaces and subsequent biofilm formation are a leading cause of chronic infections. In this work, a synthetic tripeptide that interferes with the first step of biofilm formation coated films of PCL. The coating forms spontaneously by self-assembly while the amino acid DOPA acts as a glue and attach the peptide to PCL. This is a much simpler strategy compared to other surface coupling methods that require extensive surface modifications and complex reaction steps. The peptide-coated PCL reduced the amount of *E. coli* by 47 % on average. Taking together, this work provides an additional advantage to a biomaterial such as the PCL.

Acknowledgements

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