

# Peptide coated poly( $\epsilon$ -caprolactone) generates antifouling surfaces for medical devices

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## Abstract

*Medical devices inserted into an organism are suspected to the growth of bacteria on their surface to form biofilm. Biofilm can sometime resist antimicrobial treatments. Therefore, 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 by inhibiting 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( $\epsilon$ -caprolactone) (PCL). A polymer suitable for making biodegradable medical devices. Our results show 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 catheters, as well as of tissue engineering scaffolds.*

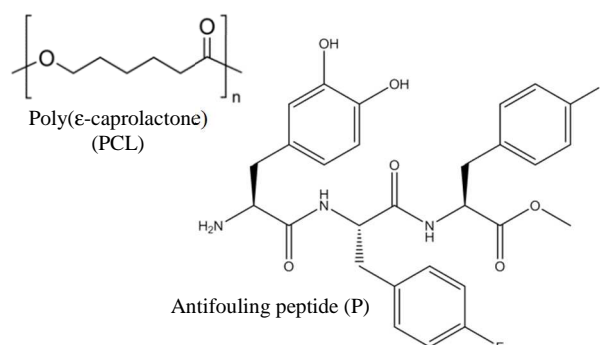
## 1. Introduction

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 [3].

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 [4]. 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 [5].

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 [6].

Poly( $\epsilon$ -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 [7]. 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 [8]. 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 [8,9]. In addition, the fluorine atom on each of the benzene rings provides the antifouling character to the peptide (see Figure 1).

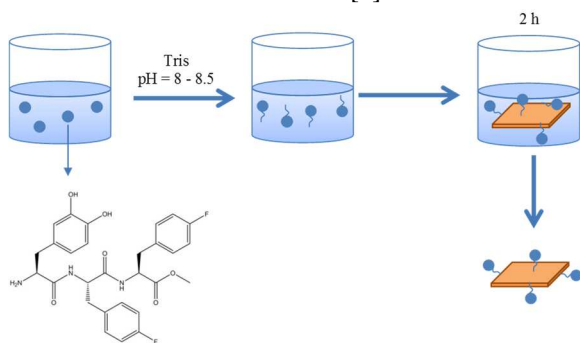


**Figure 1.** Chemical structures of PCL and Peptide

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. Coating of PCL films with antifouling peptide

PCL films with a thickness of 100  $\mu\text{m}$  were obtained by casting PCL from tetrahydrofuran (THF) solutions at room temperature. Then, square samples of PCL (1  $\text{cm}^2$ ) 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 Figure 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 [9].

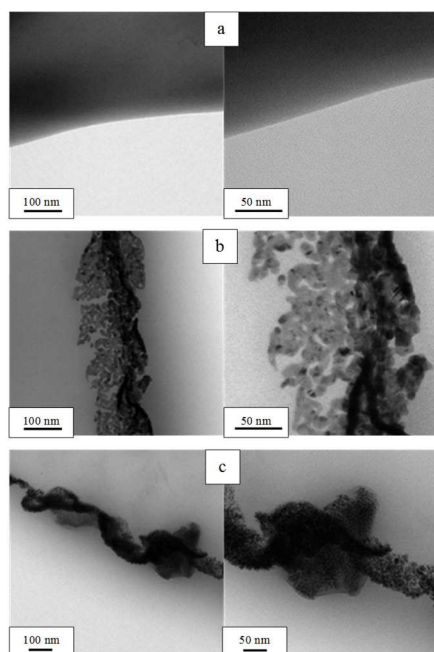


**Figure 2.** Schematic illustration of the spontaneous coating of the PCL films when they are immersed in the peptide solution

## 3. Surface characterization

### 3.1. Transmission Electron Microscopy (TEM)

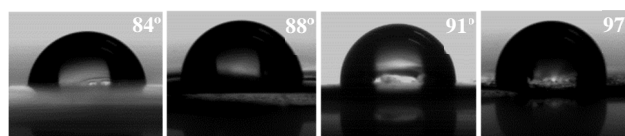
Transmission Electron Microscopy (TEM) images were recorded to analyze the coverage of the peptide on the polymeric surface (see Figure 3).



**Figure 3.** TEM images of the peptide on PCL films: (a) bare PCL, (b) PCL coated with a peptide solution of 0.1 mg/mL, and (c) with a peptide concentration of 0.2 mg/mL

### 3.2. Contact angle measurements

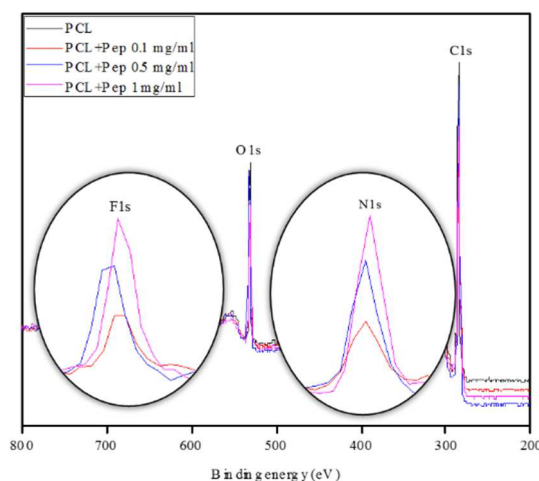
The bacterial adhesion on a surface is a key factor in biofilm formation. Actually, hydrophobic surfaces can reduce the contact with bacteria, limiting the bacterial attachment which forms the biofilm [10]. A surface is considered hydrophobic with a contact angle greater than  $90^\circ$ , whereas below  $90^\circ$  a surface is hydrophilic. With the aim of studying the hydrophobicity of the samples, contact angle measurements were performed onto surfaces of bare PCL and peptide-coated PCL with varying concentrations (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 Figure 4). The addition of fluorine atoms contained in the antifouling coating restricted the contact between the surface and water.



**Figure 4.** 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

### 3.3. X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) revealed the presence of nitrogen and fluorine atoms on the PCL surfaces and verified the presence of the peptide (see Figure 5 and Table 1).



**Figure 5.** XPS spectra of bare PCL (black), PCL coated with a peptide solution of 0.1 mg/mL (red), 0.5 mg/mL (blue), and 1 mg/mL (pink)

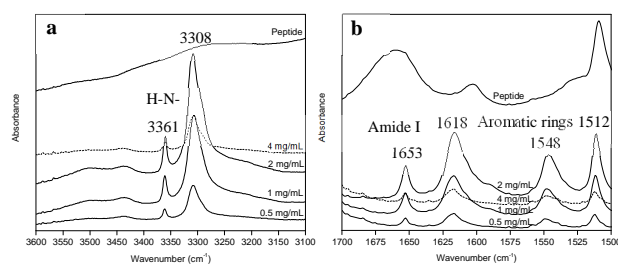
PCL+Pep	0 mg/mL	0.1 mg/mL	0.5 mg/mL	1 mg/mL
% C	80.8	77.7	75	72.2
% O	19.2	17.6	18.2	17.2
% N	-	3	4.2	6.4
% F	-	1.7	2.6	4.2

**Table 1.** Surface elemental composition of PCL films coated with different concentrations of the peptide determined by XPS

The atomic percentage of N and F became higher as the concentration of peptide increased.

### 3.4. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR)

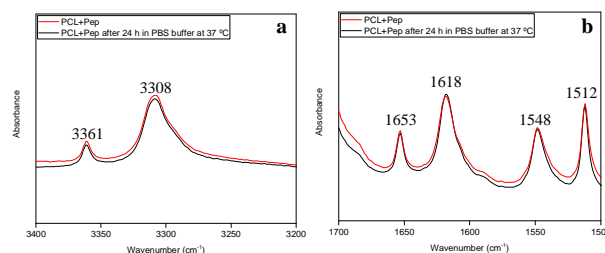
Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy also verified the presence of the peptide on the PCL surface. The spectra focused in regions between 3200-3400  $\text{cm}^{-1}$  and 1500-1700  $\text{cm}^{-1}$  where PCL does not show any band [11].



**Figure 6.** ATR-FTIR spectra of a bare peptide, PCL coated with a peptide solution of 0.5 mg/mL, 1 mg/mL, 2 mg/mL, and 4 mg/mL. (a) N-H stretching region, and (b) amide I and aromatic rings zone

After coating, new bands appeared at 3361 and 3308  $\text{cm}^{-1}$  (see Fig. 6a). These bands can be assigned to the N-H stretching vibrations of the peptide, indicative of the presence of itself on the polymer. In the 1500-1700  $\text{cm}^{-1}$  region, the bands at 1653 and 1618  $\text{cm}^{-1}$  indicates  $\alpha$ -helix and  $\beta$ -sheet structures of the amide I in the peptide, whereas the bands at 1548 and 1512  $\text{cm}^{-1}$  represent the aromatic rings (Fig. 6b) [12]. We concluded from the analysis that the optimal concentration of peptide solution for PCL coating was 2 mg/mL (see Figure 6).

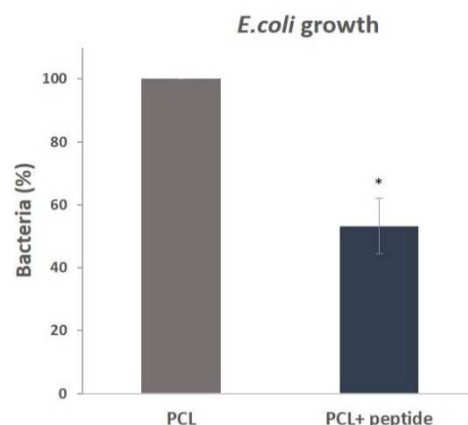
In addition, ATR-FTIR showed that the coating is stable in 0.1M PBS solution at 37 °C for 24 h, as the characteristic bands (1512, 1548, 1618, 1653, 3308 and 3361  $\text{cm}^{-1}$ ) remained the same after one day under these physiological conditions (see Figure 7). This time is enough to resist the initial adhesion of biomolecules and bacterial cells on surfaces, since the first step of biofilm formation (reversible attachment) takes from minutes to hours. In this way, the bacterial accumulation and biofilm formation is prevented.



**Figure 7.** ATR-FTIR spectra of PCL film coated with a peptide solution of 2 mg/mL before immersing the film into 100 mL of PBS buffer at 37 °C (red), and after 24 h into the buffer solution and dried into a vacuum oven (black). (a) N-H stretching region, and (b) amide I and aromatic rings zone

## 4. Biofilm formation on PCL surfaces coated with antifouling peptide

Bare and peptide-coated PCL films (PCL+P) were incubated overnight at 37 °C 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. The statistical difference between samples was tested by t-test at a confidence level of 95% ( $p < .05$ ). That being said, a reduction of 47 % was observed in the amount of bacteria on the surface when compared to bare PCL (Figure 8).



**Figure 8.** Normalized amount of *E. coli* grown on bare and peptide-coated PCL surfaces

## 5. 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. TEM, contact angle, XPS and ATR-FTIR verified the presence of the coating on PCL. Furthermore, the coating was stable under physiological conditions for 24 hours, time enough to interfere with the biofilm formation. The peptide-coated PCL reduced the amount of *E. coli* by 47 % on average.

Taken together, this work provide an additional advantage to a biomaterial such as the PCL.

## Acknowledgments

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