# Design of nanostructured coating to prevent biofilm formation on polycarbonate surfaces

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## ABSTRACT

Biofilms are surface-attached microbial organizations, where microbial species are enclosed in an exo-polysaccharides matrix(*1*). Biofilm formation represent issues in different fields, from industrial to biomedical where contamination of biomedical equipment as well as bio-corrosion of industrial equipment, biofouling, and cross-contamination of industrial products are often encountered(*2*)(3). In our project we are investigating technique to create nanostructured coatings on polycarbonate surfaces able to prevent bacterial adhesion and biofilm formation. More specifically, we are focusing our attention on a well-known antimicrobial peptide (Temporin L.) and are optimizing its grafting on surfaces by means of nano linkers bonding peptide to surface. Anti-biofilm efficiency of the nano-functionalized surfaces is evaluated considering two bacteria models: *P. fluorescens* (causing food contamination) and *S. epidermidis* (biomedical contamination) and compared with the case of the pristine surfaces.

## INTRODUCTION

Biofilm represents a common way of living of microorganisms colonies. Its formation is due to the interaction between bacteria and substrate, which is led by cell motility and physical conditions such as flow rate.

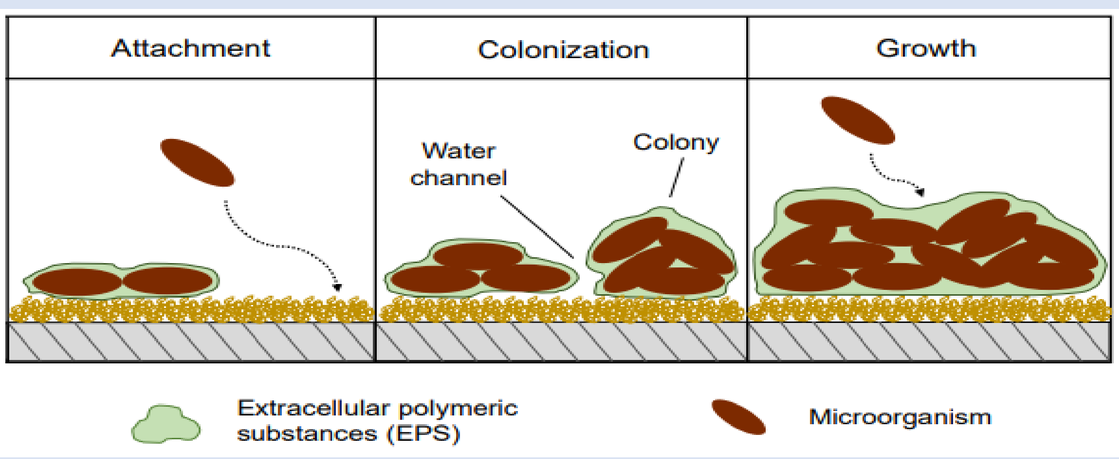



**Figure 1:** Biofilm formation on solid surface *(5)*

One of the most clinically and industrially relevant properties of biofilm communities is their tolerance to antibiotics and other common cleaning solutions. Biofilm removal/prevention from surfaces represents an important challenge.

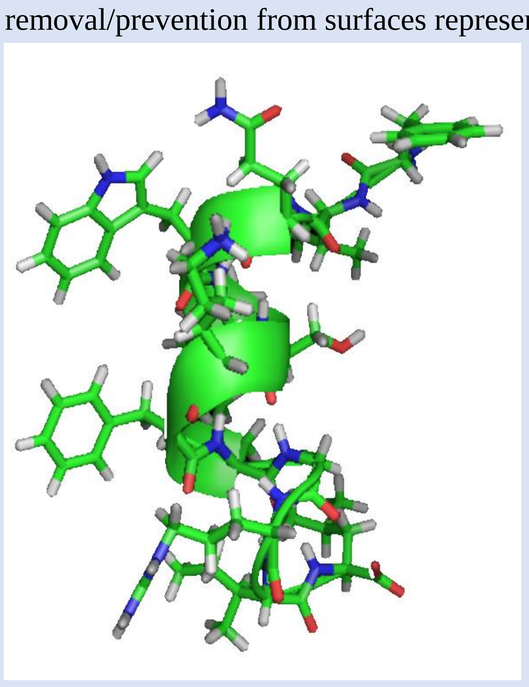
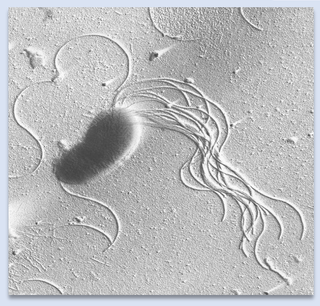


**Figure 2:** Temporin L NMR structure. (www.rcsb.org)

- Antimicrobial peptides (AMPs) represent good candidates to conventional antibiotics due to their low propensity to induce bacterial resistance(*4*).
- The AMP Temporin-L (TL) effect was investigated on *Pseudomonas fluorescens* biofilm both in static and dynamic conditions.
- Investigations were performed using Confocal Laser Scanning Microscopy and Time-Lapse Microscopy and were quantified via image analysis.
- Preliminary results shows that TL could be a candidate for the creation of nanostructured antibiofilm surfaces
- The design of nanocoated antibiofilm structures can be useful in a wide range of biomedical and industrial applications.

## MATERIAL AND METHODS



**BACTERIAL STRAIN:** *Pseudomonas fluorescens* NCDO 2085, AR 11 strain;
**CULTURE MEDIUM:** A minimal salts medium (C-source; N-source; oligo-elements; Mg/Ca-salts; water);
**OPERATIVE CONDITIONS:** Biofilms were cultivated at 30°C, pH 7, in aerobic conditions;

**Figure 3:** Scanning electron micrograph of *P.fluorescens (6)*

### EXPERIMENTAL SETUPS FOR BIOFILM MORPHOLOGY

The antimicrobial properties of TL were investigated for biofilms cultivated under in-flow conditions, following an already validated approach *(2)*. Briefly, a continuous experimental set up made of a commercial microfluidic chamber (17 mm × 1 mm × 0.1 mm, Ibidi Cell in Focus, µ-Slide VI 0.1, Ibidi GmbH). The Ibidi Cell was stained and placed under CLSM station.

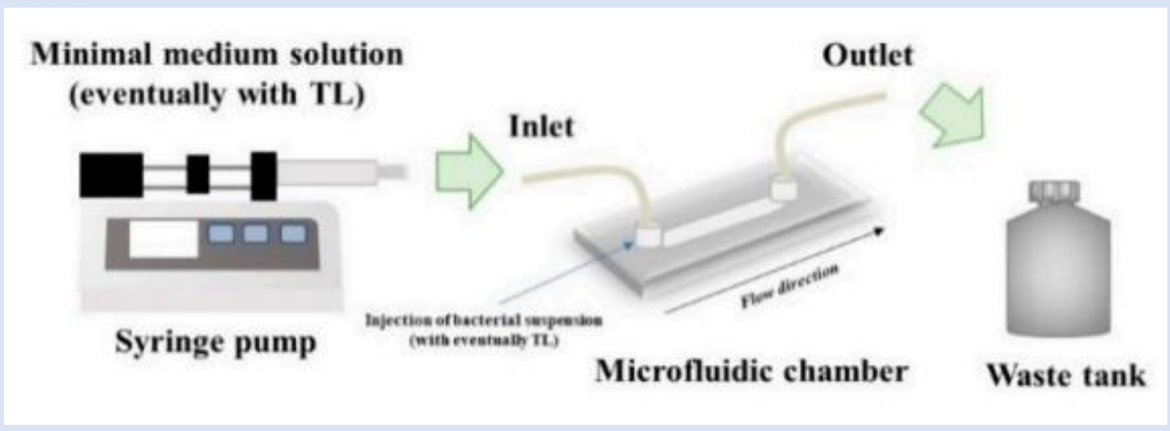



**Figure 4:** Schematic representation of the experimental set up *(4)*

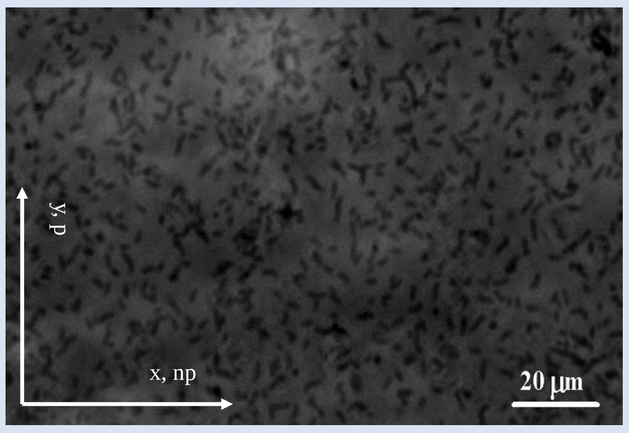


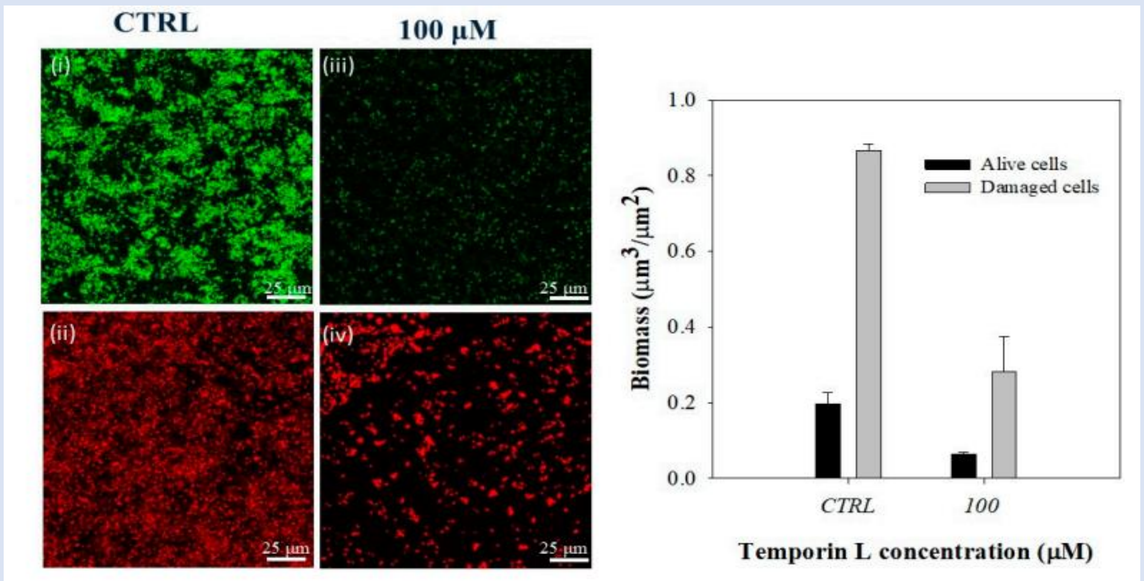

**Figure 5:** Example of Time lapse acquisition

### BIOFILM CHARACTERIZATION

- Migratory behaviour of cells observed by Time Lapse microscopy using Persistent Random Walk model *(7)*
- Images of the interested region taken at different intervals over a given period of time.
- Density of adhesion and cell tracking and migration of bacteria analyzed by Image Pro Plus.

## Results and next steps

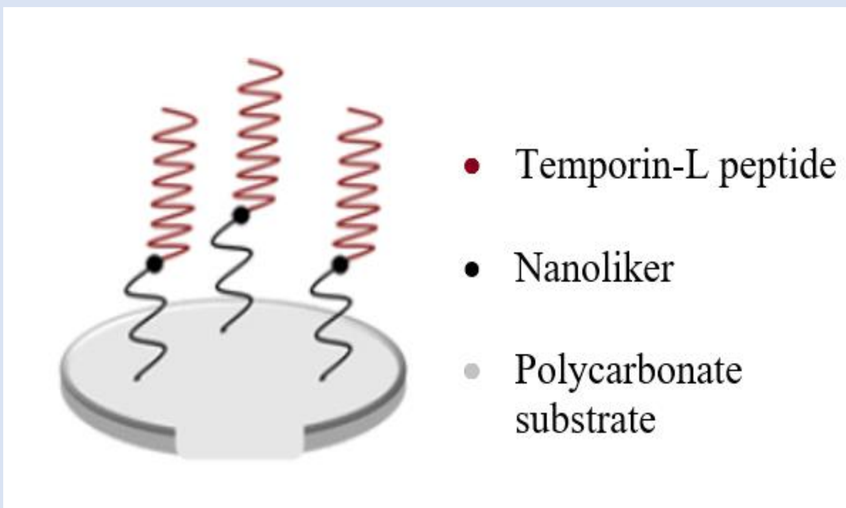
### TEMPORIN-L ANTIBIOFILM ACTIVITY




CLSM images of 3 days-old biofilms cultivated under the action of TL (100 µM. Biofilms were double-stained with the LIVE/DEAD. The alive and dead cells ratio was measured as 0.23 in the control, and 0.22 in the TL treated sample confirming the antibiofilm activity

### DESIGN OF TEMPORIN-L COATING

The next step of the experimental campaign is the creation nanostructured coatings on polycarbonate surfaces, using Temporin-L peptides.




The interaction between biotin and streptavidin can be useful for Temporin-L and nano linker binding.
Polycarbonate substrate functionalization is under studying using oxygen cold plasma treatment.
Further investigation are needed

**Figure 6:** Schematic representation of a nanocoated surface

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