Laboratoire PPSM – UMR CNRS 8531

Photochimie et Photophysique Supramoléculaires et Macromoléculaires

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«Elaboration of new layer-by-layer (LbL) fluorescent thin films and their functionalization for the sensitive detection of bacteria»

Antibiotics have been used for the treatment of bacterial infections for over 70 years, saving millions of lives. The current antibiotic resistance crisis has been attributed to the overuse and misuse of these medications. Therefore, the prevention of infection transmission by the rapid and sensitive detection of antibiotic resistant strains is needed in managing this crisis. Fluorescent polymers show great potential for bacteria detection, because they are easy to functionalize, reproduce and graft. Compared with the methods used for bacterial detection in liquid, bacterial detection on a film surface is more convenient, easier to handle and is applied in devices that can be easily reused. The goal of my PhD work is to develop fluorescent and sensitive nanostructured polymer films on surfaces for bacterial detection.

Three types of BODIPY-based fluorescent polyelectrolytes (FPC) with different features were synthetized based on reversible addition-fragmentation transfer (RAFT) polymerization: relatively Short chains and Weak polyelectrolytes (SW FPCs), Short chains and Strong polyelectrolytes (SS FPCs) and Long chains and Weak polyelectrolytes (LW FPCs). FPC LbL films were fabricated on activated glass slides by means of electrostatic attraction. The photophysical and surface properties of FPC LbL fims were easily controlled by adjusting the deposition conditions.

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The following step aimed at increasing the films' sensitivity by using the metal-enhanced fluorescence (MEF) principle. A MEF based LbL film was prepared and tested for bacteria detection. Spherical gold nanoparticles (Au NPs) were synthesized and coated with poly(allylamine hydrochloride) (PAH). The LW FPC^- was selected as the fluorescent layer. Different films containing Au NPs and LW FPC^- were fabricated and the distance between the Au NPs and LW FPC- was adjusted by changing the numbers of layers with two oppositely charged polymers (PC^+ and PC^-). Both Au NPs/4 layers PCs/LWFPC^- and Au NPs/8 layers PCs/LWFPC^- surfaces indicated that E. coli can be detected by LW FPC^-.

The selectivity of LbL films was added by introducing an antibody on the surface of the film to provide specific recognition of a chosen bacterial strain. This LbL surface achieved a rapid, effective and specific detection of *E. coli* bacteria. The polyanion and polycation with a 4-dibenzocyclooctynol (DIBO) functional group were assembled on the activated glass slides and an anti-*E. coli* antibody containing an azide group was efficiently introduced on the surface in a single step based on the azide-alkyne cycloadditions (SPAAC) reaction. The number of *E. coli* captured on the surface was shown to be dependent on the amount of antibody on the surface. The anti-*E. coli* antibody surface showed significant selectivity for *E. coli*, compared with *B. subtilis*.

An alternative approach is to detect bacterial growth on thin LbL film by introducing pH sensitive fluorophore (fluorescein). The growth of bacteria is often associated with a decrease in pH of the growth medium due to a release of acidic metabolites. Different types of pH sensitive LbL film were prepared and tested for the detection of bacterial growth. Firstly, the synthesis of different functionalized polyanions (short and long chain of DIBO-PC^- and red fluorescent polymer) was carried out. Three types of pH sensitive surfaces containing fluorescein (DIBO-SWPC^-/fluorescein, DIBO-LW PC^-/fluorescein and ratiometric RFPC^-/fluorescein surfaces) were prepared based on the combination of LbL assembly and copper-free click chemistry. Finally, three pH sensitive surfaces were studied for bacteria growth detection. All the surfaces were shown to be biocompatible, the number of *E. coli* increased after several hours of incubation on each surface, as detected by brightfield microscopy imaging. The application for the fluorophore-dependent detection of bacterial growth remains to be developed.

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