Development of antibacterial surfaces by photo-grafting of bioactive biosourced products

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It is nowadays admitted that any microorganism (yeast, bacteria, virus) favors a life in a fixed form (called sessile) rather than in suspension (called planktonic). In most cases, this leads to the formation of a biofilm allowing bacteria to persist and resist to an unfavorable environment. A biofilm is defined as a bacterial aggregate attached to a surface and enclosed in a self-produced exopolymeric gangue. It has been shown that this "way of life" makes bacteria up to 1000 times more resistant to antibiotic or biocide treatments than their planktonic counterparts[1]. This formidable resistance makes the fight against bacterial contamination of surfaces very problematic. In this context, prevention seems preferable to any treatment. Among these prophylactic strategies, one consists in preventing the adhesion of bacteria on surfaces through the development of non-stick surfaces, and another in killing (or damaging) bacteria through the development of biocidal surfaces.

Since bacterial adhesion to surfaces is a prerequisite for the formation of biofilms[2], the development of surfaces that prevent this phenomenon seems logical to fight bacterial contamination. Based on the parameters influencing the adhesion of bacteria on surfaces (e.g., surface topography, surface charges, surface chemistry,...), many strategies to elaborate this type of surfaces have been developed[3-6] such as micro-structured surfaces (mimicking shark skin), low surface energy materials, negatively charged surfaces or hydrophilic surfaces. However, the use of only one of these approaches does not completely prevent bacterial adhesion. The combination of several of these approaches is inevitable.

Concerning biocidal surfaces, two approaches for immobilizing antimicrobial agents on the surface are possible[7]. The first is a non-covalent approach which has the advantage of combining a contact and release action. However, it has the major disadvantage of continuous enrichment of the organism with the inhibitor and consequently the risk of promoting the emergence of multi-resistant bacteria. The second is a covalent approach which has the advantage of avoiding the depletion of the material in inhibitor but can lead to a loss of activity following the deposition of the cellular material of the first dead bacteria.

Recently, in the framework of Yuzhen Lou's thesis (CSC funding), we have shown the possibility of obtaining antibacterial surfaces by the covalent approach using an original immobilization strategy by photo-grafting[9-10]. However, these surfaces presented, in some cases, a decrease of the antibacterial activity in time for the reason evoked previously. Moreover, the compounds used in this thesis, that are quaternary ammoniums, present a toxicity and their use is thus limited in certain applications.

In order to combine the advantages of each approach, we propose in this thesis project (i) to covalently immobilize antibacterial compounds by photo-grafting and (ii) to introduce a pH-sensitive link of ester or hydrazone type allowing the diffusion of the antibacterial molecule only during a bacterial contamination. Indeed, local variations of pH (i.e. acidification) at the surface occur during a bacterial contamination[11]. In addition, in order to address the toxicity of classical antibacterial molecules, natural antibacterial molecules such as essential oils and antimicrobial peptides (AMPs) will be used. Moreover, these molecules have the advantage over antibiotics and quaternary ammoniums of inducing few resistance mechanisms while presenting a broad spectrum of activity.

In a first step, the functions necessary for the anchoring on surfaces by photo-grafting as well as the pH-sensitive link will be introduced on the structure of the various antibacterial molecules. All the modifications will be analyzed by the spectroscopic techniques available in the PBS laboratory (UV-visible, FTIR, NMR).

Then, the antibacterial molecules will be immobilized on the surfaces by a photo-grafting reaction based on the work of the thesis of Yuzhen Lou. The surfaces will be characterized, by contact angle to evaluate the wettability, by atomic force microscopy (AFM) and by Fourier Transform Infrared (FT-IR) X-ray photoelectron spectroscopy (XPS) to analyze the chemical composition of the surfaces. The diffusion kinetics of active molecules as a function of pH will also be studied.

Finally, the antimicrobial activity of the surfaces will be evaluated against S. aureus and P. aeruginosa, two opportunistic pathogenic bacteria commonly involved in bacterial contamination of surfaces. This will be evaluated by enumerating the number of viable bacteria in the solution but also adhered to the surface. Biocidal activity will be determined by confocal microscopy and/or flow cytometry using the LIVE/DEAD® BacLightTM kit to discriminate between bacteria with an intact membrane and those with a damaged membrane. In addition, the basal cytotoxicity of the modified surfaces will be evaluated using L-929 cells. For this purpose, the LIVE/DEAD® BacLightTM kit will be used to examine the cell viability, 24/48h after seeding the cells on the modified surfaces.

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