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Elaboration of highly hydrophobic polymeric surface — a potential strategy to reduce the adhesion of pathogenic bacteria?

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ABSTRACT

Different polymeric surfaces have been modified in order to reach a high hydrophobic character, indeed the superhydrophobicity property. For this purpose, polypropylene and polystyrene have been treated by RF or μwaves CF_4 plasma with different volumes, the results were compared according to the density of injected power. The effect of pretreatment such as mechanical abrasion or plasma activation was also studied. The modified surfaces were shown as hydrophobic, or even superhydrophobic depending of defects density. They were characterized by measurement of wettability and roughness at different scales, i.e. macroscopic, mesoscopic and atomic. It has been shown that a homogeneous surface at the macroscopic scale could be heterogeneous at lower mesoscopic scale. This was associated with the crystallinity of the material. The bioadhesion tests were performed with Gram positive and negative pathogenic strains: *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Hafnia alvei*. They have demonstrated an antibacterial efficiency of very hydrophobic and amorphous PS treated for all strains tested and a strain-dependent efficiency with modified PP surface being very heterogeneous at the mesoscopic scale. Thus, these biological results pointed out not only the respective role of the surface chemistry and topography in bacterial adhesion, but also the dependence on the peaks and valley distribution at bacteria dimension scale.

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1. Introduction

Bacterial adhesion to solid surfaces is known to occur in many environments including medico-hospital sector, food industry, packaging area. When pathogenic bacteria are involved, the consequences of such phenomena are often problematic. They can range from hygiene problems to human infections and lead to significant public health problems [1,2]. Among the materials most frequently used, polymers are major components, especially polypropylene (PP) and polystyrene (PS) often used in the packaging (soft packages or hard ones such as storage tanks, tubs showers …), in medical devices (heart valves, suture materials, syringes or dialysis systems ….). Thus, the development of new strategies to reduce the bacterial contamination of these polymeric surfaces and then ensuring their hygienic status remains a fundamental scientific, technological and industrial challenge.

Hydrophobicity and roughness of solid surfaces were shown to be involved in bacterial adhesion. Consequently, it may be presumed that the modification of these polymeric surfaces based on these two characteristics, will affect their ability to the bacterial contamination. Among the possible strategies, the concept of highly to superhydrophobic surface appears to be relevant [3–5]. This concept of highly to superhydrophobic synthetic surfaces is quite new, since it has been demonstrated in the mid-1990s [6–10]. Such surfaces are characterized by water contact angles particularly high (greater than 120°) which can reach values up to 160–170° (so-called superhydrophobic surfaces). These values are mostly induced by two factors, hydrophobicity and roughness. The latter parameter could be ranged from several hundred micrometers to a few nanometers [3–5]. Potential applications of these original surfaces so-called self-cleaning surfaces were demonstrated in the automotive, aerospace or civil engineering. In the biological field, the interest of these new surfaces is still little explored. While several studies have shown positive effect of these new surfaces on protein adsorption [11], and osteoblast cell proliferation [12]; very few have been dedicated to their impact on bacterial adhesion [13]. However, Tang et al. [14] have shown a decrease in the adhesion of a strain of *Staphylococcus aureus* on superhydrophobic titanium. But, in the same time, an increase in the adhesion of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on very hydrophobic PLA is reported [15]. What would be the behavior of other polymers such as PP and PS? In our knowledge and so far, no study concerning the impact of highly to superhydrophobic polymeric surfaces (such as surface-treated PP and PS) on the adhesion of pathogenic bacteria have been yet performed.

Therefore, in this work, various simple methods leading to highly or superhydrophobic PP and PS surfaces are described. These new surfaces have been characterized at different scales using complementary

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techniques of analysis and of imaging. Finally, the impact of these surface modifications on the adhesion of different kinds of pathogenic bacteria is evaluated by *ex vivo* tests.

2. Materials and methods

2.1. Materials

The polypropylene substrates (PP, $\Theta_{H20}=98°$) were manufactured by the company EUDICA (Annecy, France), the polystyrene plates (PS, $\Theta_{H20}=72°$) were purchased from Goodfellow (ST313120). All samples are cleaned with an absolute ethanol solution under supersonic stirring, then dried under a laminar flow hood. The plasma atmospheres are composed of CF_4 gas (purity > 99.985%, Messer), O_2 or Ar (purity > 99.995%, Air Liquide).

2.2. Cold plasma treatment

The small volume radiofrequency (RF: 13.56 MHz, vol. ≈1 L, so-called small reactor) and μwaves (433 MHz, vol. ≈0.7 L) cold plasma reactors previously described in [3–5] were designed in the laboratory while the large one (RF: 13.56 MHz, vol. ≈60 L, so-called large reactor) corresponds to a RF apparatus (Manufacturer, ISYTECH, Lannion, France) located in an ISO 7 clean room. All of them are composed of three main parts: a processing chamber, a pumping system and a RF or μwaves generator. The experiment is performed by optimizing the parameters influencing the treatment: gas nature, discharge power (*P*), gas flow (*F* in sccm), pressure (*p* in mbar) and duration (t in min) of treatment. With the μwaves plasma, the substrate can be moved in or out from the plasma volume. The *d* (in cm) corresponding to the distance between the bottom of the excitatory source and the sample was fixed at 4 cm. The substrate was introduced into the reactor chamber. After reaching the ultimate pressure of the chamber, the gas was introduced into the chamber at chosen parameters. After certain duration, the cold plasma was turned off and the vacuum was broken down to atmospheric pressure. Contact angle measurements were just after the plasma treatment or 3 days after, incompressible delay between surface treatment and the biological test. All treated samples sent for biological test were kept in dark and under vacuum.

2.3. Surface characterization

2.3.1. Multiscale contact angle measurements

Just after the plasma treatment, the contact angle measurements were run on a standard goniometer from RAME-HART.inc (model: 100-00-230) at IMMM and a goniometer from DATA PHYSICS (model: OCA20) at CTTM. Deionized ultrapure water was used as standard liquid. For each tested substrate, the value of the contact angle was the average of several measurements made using three drops of each liquid (2 μL of water).

Picodroplets measurements at INRA-AgroParisTech were realized with a “picoLiter goniometer” (Goniometer DSA100M, Krüss, Palaiseau, France). This apparatus deposed a droplet of deionized distilled water (300 pL) by a piezo dosing unit. Contact angles were monitored during 2 s through a fast CCD camera with ×4 zoom and an objective of microscope (×20). An automatic procedure for generating drops, image acquisition and mapping of sample was used to perform a mapping of surfaces (typically 5 mm × 5 mm and one drop every 0.25 mm).

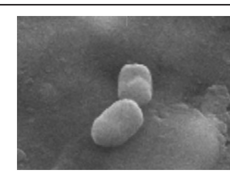
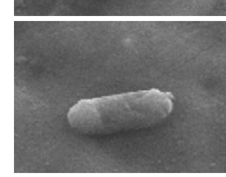
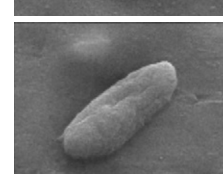
2.3.2. Multiscale surface roughness measurement

The surface roughness was measured with a stylus profilometer (M2 Perthometer, Mahr, Palaiseau, France). R_a, R_z and R_{max} are parameters for evaluating the roughness and correspond respectively to the arithmetic mean of all values of the roughness profile, the average roughness depths of five successive assessment areas and the most important roughness within the evaluation length.

2.3.3. Surface topography measurement

Surface topography of the substrates was characterized by Atomic Force Microscopy (AFM) in contact mode (PicoSPM, Molecular Imaging, ScienTec, Palaiseau, France) operating under air at 22 °C. For these experiments, we used a cantilever (silicon nitrides gold-coated oxide-sharpened, ScienTec, Palaiseau, France) with a spring constant of ~0.38 N m^{-1}. Topographic images were acquired at a scanning rate of 1 line/s and for 512 lines/image.

Table 1
Characteristic of the chosen strains.

Gram	Strain	Origin	BHM code	Hydrophobie scale	Typical picture
Positive	*Listeria monocytogenes* Serovar 1/2	CIP 103574	Lm 152P	Hydrophilic	
Positive	*Listeria monocytogenes* Serovar 4b	CIP 103575	Lm 155P	Highly hydrophilic	
Negative	*Hafnia alvei*	ENSIA Massy	Ha 510N	Hydrophilic	
Negative	*Pseudomonas aeruginosa*	ATCC 15442	Pa 588P	Highly hydrophilic	

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