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Understanding the mechanisms of interactions at interfaces between bacteria and materials: development of anti-adhesion and anti-biofilm surfaces

Compréhension des mécanismes d'interactions aux interfaces entre bactérie et matériaux : Elaboration de surfaces antiadhésives et anti-biofilm

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Scientific valorization

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2. Hage M, Akoum H, Chihib N-E, Jama C. 2021. Antimicrobial peptides coated stainless steel for fighting biofilms formation in food and medical fields. *Coatings.* (Publié) **https://doi.org/10.3390/coatings11101216**

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Understanding the mechanisms of interactions at interfaces between bacteria and materials: development of anti-adhesion and anti-biofilm surfaces

Abstract

The ambient operating environments in food and medical fields allows bacteria to adhere and develop on substrates, which results in the growth of resistant pathogenic bacterial biofilms. Indeed, the first stage of biofilm formation is the non-reversible adhesion of bacteria. Preventing and suppressing such adhesion is a passive strategy to inhibit the development of biofilms. These pathogenic structures are responsible for several foodborne diseases and nosocomial infections. Consequently, to combat this public health burden, one possible approach is the use of cold plasma technologies in coatings formulation. This work presents different factors influencing bacterial adhesion to a substrate. In addition, strategies for the development of passive coatings to prevent biofilm formation by cold plasma surface treatments are described as well as the anti-adhesive properties of the developed surfaces. General features of surface treatment, including the surface physicochemical changes and the use of cold plasma technologies, are also presented. In this context, a study was conducted to control, via cold plasma treatment of stainless steel, the persistent bacterium *Salmonella enterica*. Indeed, *Salmonella enterica* is responsible for several infections worldwide due to its persistence on abiotic surfaces in hospitals and food processing industries. Aiming to avoid the formation of *Salmonella enterica* biofilm, a surface modification process was carried out by the elaboration of a hydrophobic organosilicon coating from the monomer 1,1,3,3-tetramethyldisiloxane, mixed to oxygen, using a nitrogen flow microwave post-discharge plasma polymerization technique. The effects of cold plasma parameters on the coating properties, on the surface topography and on the adhesion of *Salmonella enterica* cells were investigated. The results revealed that surface topography influenced the rate of bacterial adhesion. Indeed, rough surfaces did not repel *Salmonella enterica* since the number of cells adhering to these surfaces varied from 30 ± 4 to 65 ± 4 bacteria per microscopic field. In contrast, smoother surfaces exhibited anti-adhesive behavior since the number of attached cells was close to zero on these coatings. A complementary approach to this passive strategy of anti-adhesive surface elaboration is the development of active surfaces. Emerging technologies for active and effective antimicrobial coatings are helping to address the challenge of eliminating pathogenic biofilms formed on materials used in medical and food processing environments. Stainless steel is a commonly employed material in these fields but it regrettably has insufficient bio-functional properties, which makes it susceptible to bacterial adhesion and biofilm generation. Therefore, in this thesis, a review of coatings developed by employing biocides and antimicrobial peptides (AMPs) grafted on stainless steel is presented. Moreover, a new active approach based on stainless steel coated with nisin, a common AMP accepted as a safe alternative to prevent pathogenic biofilms development, is developped. In this active strategy, stainless steel surfaces were functionalized by nisin which was grafted to the surface by either its carboxylic group or its amino group. The antimicrobial activity of the elaborated coatings was tested against *Listeria monocytogenes*, a dangerous pathogenic bacterium, with a high fatality rate. Indeed, the surfaces coated with nisin linked via its amino group exhibited a powerful antibacterial activity while the surface with nisin linked via its carboxyl group showed no antimicrobial effect. Surface property analyses provided a better understanding of the antibacterial effects, chemical and topographical characteristics of the treated surfaces, as well as the configuration and quantification of nisin.

Keywords: Biofilms; bacterial adhesion; cold plasma; 1,1,3,3-tetramethyldisiloxane; *Salmonella enterica*; Stainless steel; Coatings; Antimicrobial peptides, Biocides; Nisin*; Listeria monocytogenes*.

Compréhension des mécanismes d'interactions aux interfaces entre bactérie et matériaux : Elaboration de surfaces antiadhésives et anti-biofilm

Résumé

L'environnement opératoire dans les domaines alimentaire et médical permet aux bactéries de se fixer et de se développer sur les surfaces, ce qui entraîne la formation de biofilms bactériens pathogènes et résistants. En effet, la première étape de la formation des biofilms est l'adhésion irréversible des bactéries. Prévenir et supprimer cette adhésion est une stratégie passive pour inhiber le développement des biofilms. Ces structures pathogènes sont responsables de plusieurs maladies d'origine alimentaire et d'infections nosocomiales. Par conséquent, pour lutter contre ce fléau de santé publique, une approche possible est l'utilisation des technologies plasma froid pour l'élaboration de revêtements sur différents matériaux. Ce travail présente les différents facteurs influençant l'adhésion bactérienne à un substrat. En outre, les stratégies d'élaboration de revêtements passifs visant à prévenir la formation de biofilms par des traitements de surface par plasma froid sont décrites ainsi que les propriétés antiadhésives des surfaces élaborées. Les aspects généraux du revêtement, y compris les modifications physicochimiques de la surface et l'utilisation des technologies par plasma froid, sont également présentés. Dans ce contexte, une étude a été menée dans le but d'inhiber l'adhésion de la bactérie pathogène *Salmonella enterica* à la surface de l'acier inoxydable, via son traitement par plasma froid. En effet, *Salmonella enterica* est responsable de plusieurs infections dans le monde en raison de sa persistance sur les surfaces abiotiques dans les hôpitaux et les industries agroalimentaires. Dans le but de limiter la formation du biofilm de *Salmonella enterica*, des revêtements organosiliciés à partir du monomère 1,1,3,3-tétraméthyldisiloxane, mélangé ou non à l'oxygène, ont été élaborés par polymérisation par plasma post-décharge micro-ondes d'azote. L'effet des paramètres du plasma froid sur les propriétés du revêtement, sur la topographie de la surface et sur l'adhésion des cellules *Salmonella enterica* a été étudié. Les résultats ont révélé que la topographie de la surface influençait de façon significative le taux d'adhésion des bactéries. En effet, les surfaces rugueuses n'ont pas inhibé l'adhésion de *Salmonella enterica* puisque le nombre de cellules adhérant à ces surfaces variait de 30 ± 4 à 65 ± 4 bactéries par champ microscopique. En revanche, un comportement anti-adhésif vis-à-vis de *Salmonella enterica* a été mis en évidence pour les surfaces plus lisses. En effet, le nombre de cellules attachées était proche de zéro sur ces revêtements. Une approche complémentaire à cette stratégie passive d'élaboration de surfaces anti-adhésives est le développement de surfaces actives. Les technologies émergentes de revêtements antimicrobiens actifs et efficaces permettent de relever le défi de l'élimination des biofilms pathogènes formés sur les matériaux utilisés dans les milieux hospitaliers et agroalimentaires. L'acier inoxydable est un matériau couramment utilisé dans ces domaines, mais il possède malheureusement des propriétés bio-fonctionnelles insuffisantes, ce qui le rend susceptible à l'adhésion bactérienne et au développement de biofilms. Dans ce contexte, cette thèse présente une revue des revêtements développés en employant des biocides et des peptides antimicrobiens (AMPs) greffés sur l'acier inoxydable. De plus, une nouvelle approche active basée sur l'acier inoxydable revêtu de nisine, un AMP commun accepté comme une alternative sûre pour prévenir le développement de biofilms pathogènes, est développée. Dans cette etude, des surfaces en acier inoxydable ont été fonctionnalisées par la nisine qui a été greffée à la surface soit via son groupe carboxylique ou via son groupe amino. L'activité antimicrobienne des revêtements élaborés a montré une grande éfficacité contre *Listeria monocytogenes*, une bactérie pathogène menaçante, avec un taux de mortalité élevé. En effet, les surfaces revêtues de nisine greffée via son groupe aminé ont montré une puissante activité antibactérienne tandis que la surface greffée avec la nisine liée par son groupe carboxyle n'a montré aucun effet antimicrobien. Les analyses des propriétés de surface ont permis de mieux comprendre les effets antibactériens, les caractéristiques chimiques et topographiques des surfaces traitées ainsi que la configuration et la quantification de la nisine.

Mots clés : Biofilms ; adhésion bactérienne ; plasma froid ; 1,1,3,3-tétraméthyldisiloxane ; *Salmonella enterica* ; acier inoxydable ; revêtements ; peptides antimicrobiens, biocides ; Nisine ; *Listeria monocytogenes.*

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GENERAL INTRODUCTION

The existence and the development of microbial species on various natural and artificial substrates, is a critical issue affecting various fields, in particular the food and healthcare fields (Galié et al. 2018). Actually, biofilm generation is a complicated process featured by a succession of steps. In the biofilm formation scheme, bacteria progress from a free-floating (planktonic) state where they operate as individuals, to a sessile state where they operate as communities (Stoodley et al. 2002). Adsorption, or reversible adhesion of bacteria, is the crucial first step in the construction of a biofilm on abiotic surfaces. This phase is favored by several non-covalent interactions. Indeed, when the bacteria reach a specific nanometric distance from the surface, the bacterial adhesion is stimulated by non-covalent forces (Bos et al. 1999). The resulting force of these interactions allows bacterial adsorption on the substrate. In addition, the environmental conditions around the bacteria can impact the characteristics of the bacteria and the surfaces, resulting in a modification of the bacterial adhesion behavior (Lecuyer et al. 2011). At this point, exopolymeric substances are produced by the bacteria which irreversibly attach to the surface. The adhering bacteria multiply and form micro-colonies while secreting an extracellular matrix composed of a variety of polysaccharides, nucleic acids, proteins and lipids. Then, the biofilm maturation phase leads to the growth of a mature and complex biofilm. The final step in the biofilm lifecycle is the detachment or dispersion of the bacterial cells from the biofilm and the colonization of new surfaces (Costerton et al. 1987). This step plays a key role in the dissemination of bacteria and the spread of infections.

However, the control of the first stage of biofilm formation is a crutial step in the fight against dangerous infections. Indeed, eliminating the bacterial adhesion is a passive approach to prevent the growing of biofilms that are responsible for many food-borne diseases and nosocomial infections. Consequently, to fight this public health threat, researchers have applied cold plasma technologies in the design of coatings. This dissertation summarizes various parameters

governing the bacterial adhesion to a substrate. Furthermore, passive coating strategies for preventing biofilm formation by cold plasma treatments were outlined by a description of anti-adhesive designed substrates. The general properties of surface treatment, including physicochemical modifications and the application of cold plasma technologies, were also presented. In this perspective, a research was carried out to limit the adhesion, via cold plasma treatment of stainless steel, the persistent bacterium *Salmonella enterica*. *Salmonella enterica* is the cause of several worldwide infections due to its persistency on abiotic surfaces in hospitals and food processing industries. In order to prevent the development of *Salmonella enterica* biofilm, a surface treatment was performed via the formulation of organosilicon coatings from the monomer 1,1,3,3-tetramethyldisiloxane, mixed or not to oxygen, using a nitrogen flow microwave post-discharge plasma polymerization technique. The effect of cold plasma parameters on the coating characteristics, surface topography, and *Salmonella enterica* cell attachment was studied. The findings showed that the surface topography affected the bacterial adhesion rate. In fact, rough surfaces did not repulse *Salmonella enterica* since the number of cells attached to these surfaces varied from 30 ± 4 to 65 ± 4 bacteria per microscopic field. In opposition, smoother surfaces displayed an anti-adhesive behavior as the number of adhered cells was almost nil on these coatings. A complementary strategy to this passive anti-adhesive approach is the elaboration of active surface approach. Emerging technologies for effective and active antimicrobial coatings on materials used in medical and food sectors are helping to overcome the challenge of pathogenic biofilm persistance. Stainless steel is a commonly used material in these fields, but it regrettably has poor bio-functional characteristics, rendering it vulnerable to bacterial adhesion and biofilm generation. Hence, in this manuscript, a review of coatings using biocides and antimicrobial peptides grafted onto stainless steel is presented. The manuscript also presents a study investigating nisin based coating of stainless steel, nisin is a common AMP accepted as a safe alternative to inhibit the

growth of pathogenic biofilms. In order to develop an antibacterial active approach, stainless steel surface was functionalized using nisin, which was grafted to the surface either by its carboxyl group or by its amino group. The antimicrobial effectiveness of the developed coatings was tested against *Listeria monocytogenes*, a pathogenic bacterium with a high mortality rate. The results showed that the surfaces coated with nisin linked by its amino group revealed a significant antibacterial activity while the surface grafted with nisin linked by its carboxyl group did not show any antimicrobial activity. Surface property analyses provided an understanding of the antibacterial effect, the chemical and topographic characteristics of the treated surfaces, as well as the configuration and quantification of nisin.

In this context, the first goal was to figure out the existing strategies of plasma treatments already carried out to fight passively against biofilm formation, via bacterial repulsion. In the first chapter of this PhD, the principles of biofilm formation and studies to limit its formation were analyzed and described. The second chapter highlights the strategies carried out to fight actively against biofilm formation by killing the attached bacteria. A review of the main stainless steel coated surfaces with several biocidal molecules, focusing on antimicrobial peptides, including the bacteriocin nisin is presented. In the third chapter, the antiadhesive properties of TMDS-based cold plasma coatings against *Salmonella enterica* adhesion and biofilm formation are presented and their behavior agnaist *Salmonella enterica* adhesion is discussed highlighting the chemical properties of the coatings. Another aim was to analyse and test the chemical and biological properties of a nisin-coated stainless steel surfaces for fighting actively biofilm formation. This part corresponds to the fourth chapter of this PhD.

CHAPTER: I

LITERATURE REVIEW – Part 1

Cold plasma surface treatments to prevent biofilm formation in food industries and medical sectors

Cold plasma surface treatments to prevent biofilm formation in food industries and medical sectors

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Abstract

Environmental conditions in food and medical fields enable the bacteria to attach and grow on surfaces leading to resistant bacterial biofilm formation. Indeed, the first step in biofilm formation is the bacterial irreversible adhesion. Controlling and inhibiting this adhesion is a passive approach to fight against biofilm development. This strategy is an interesting path in the inhibition of biofilm formation since it targets the first step of biofilm development. Those pathogenic structures are responsible for several foodborne diseases and nosocomial infections. Therefore, to face this public health threat, researchers employed cold plasma technologies in coating development. In this review, the different factors influencing the bacterial adhesion to a substrate are outlined. The goal is to present the passive coating strategies aiming to prevent biofilm formation via cold plasma treatments, highlighting antiadhesive elaborated surfaces. General aspects of surface treatment, including physico-chemical modification and application of cold plasma technologies were also presented.

Key points

- *Factors surrounding pathogenic bacteria influence biofilm development.*
- *Controlling bacterial adhesion prevents biofilm formation.*
- *Materials can be coated via cold plasma to inhibit bacterial adhesion.*

Keywords Biofilm; Cold plasma; Antiadhesive; Surface treatment.

Introduction

The presence and growth of bacterial species on many natural and synthetic surfaces leading to the formation of biofilms, is a major problem affecting different fields, especially food and medical sectors (Abdallah et al. 2014a ; Ciofu et al. 2015 ; Galié et al. 2018). In a matter of fact, the biofilm formation is a complex process characterized by a succession of steps already described by different works (Stoodley et al. 2002; Donlan and Costerton 2002; Abdallah et al. 2014b). In the biofilm formation system, the bacteria switch from a free floating (Planktonic) state where they function as individuals, to a sessile state where they function as communities. Adsorption, or reversible adhesion of bacteria is the first and essential step in biofilm formation on abiotic surfaces. The adherent bacteria in this step are not all initiated to be in the differentiation mechanism leading to biofilm formation, and many can actually escape from the surface and return to the planktonic lifestyle (Stoodley et al. 2002; Khelissa et al. 2019). This phase is reversible and promoted by numerous non-covalent interactions. Indeed, when microorganisms reach a certain distance from the surface (between 2 and 50 nm), bacterial adhesion is induced, by non-covalent forces, such as Van der Waals, acid-base and electrostatic interactions. The resulting force of these interactions allows bacterial adsorption on the support. Moreover, environmental conditions surrounding the bacteria, like temperature, pH and organic matter may influence the bacterial and surface properties leading to a modification in the bacterial adhesion behavior. At this stage, exopolymeric substances (EPS) are secreted by bacteria that become irreversibly attached to the surface. Adhered bacteria multiply and form microcolonies while secreting an extracellular matrix containing a mixture of polysaccharides, nucleic acids, proteins and lipids. Then, the biofilm maturation process leads to development of a mature and complex biofilm. The final step of a biofilm lifecycle is the detachment or dispersion of bacterial cells from the biofilm and colonizing new surfaces. This step has a key role in the dissemination of bacteria and the spread of infections (Fig. 1).

These persistent pathogenic structures, are responsible for a variety of nosocomial infections and foodborne illnesses (Abdallah et al. 2014a; Veerachamy et al. 2014). Moreover, the plans of disinfections carried out by hospitals and industrials do not remove completely the biofilms formed on the equipment, especially the resistant ones (Kostakioti et al. 2013). These plans aiming to restrain the biofilm formation, have a negative environmental footprint and an important economic impact on these fields (Pace et al. 2006). Indeed, it's of significant relevance to find solutions to get rid of bacterial contamination and biofilm formation. The development of surfaces that limit the formation of biofilms is an aim that researchers and industrials have been trying to reach. Several investigations have been carried out to elaborate effective, harmless and stable antiadhesive and antimicrobial coatings in order to prevent biofilm structuration. In the passive approach aiming to prevent biofilms formation, surfaces have an antiadhesive property towards pathogenic microorganisms. In this approach, the surface's chemical and physical aspects modification is investigated. The resistance to bacterial adhesion is owed, on those films, to the interactions between bacteria and modified surface. The surface properties are adjusted to inhibit bacterial adhesion mechanisms. Indeed, physical properties like surface wettability, roughness and surface charge are adapted according to the desired characteristics (Rodrigues 2011; Guo et al. 2016). One of the possible way to develop these modified surfaces is cold plasma treatments (Saulou et al. 2012).

Cold plasma treatment is a valuable coating technology since it imparts a homogenous and stable surface modification. It also permits researchers to tailor the functionalization of the surface according to the properties needed. Regarding the prevention of biofilm formation, surfaces elaborated by plasma can act passively with antiadhesive character towards bacteria, and actively with antimicrobial properties depending on the molecules and parameters used in the coating elaboration (Chan 1993; Saulou et al. 2012). In this review, the plasma technology for coating elaboration is highlighted. A special attention is given to the developed surfaces

with antiadhesive property for its importance as a first step in preventing biofilm formation. Indeed, this research presents the cold plasma coating strategies, for biofilm formation prevention, as a passive approach.

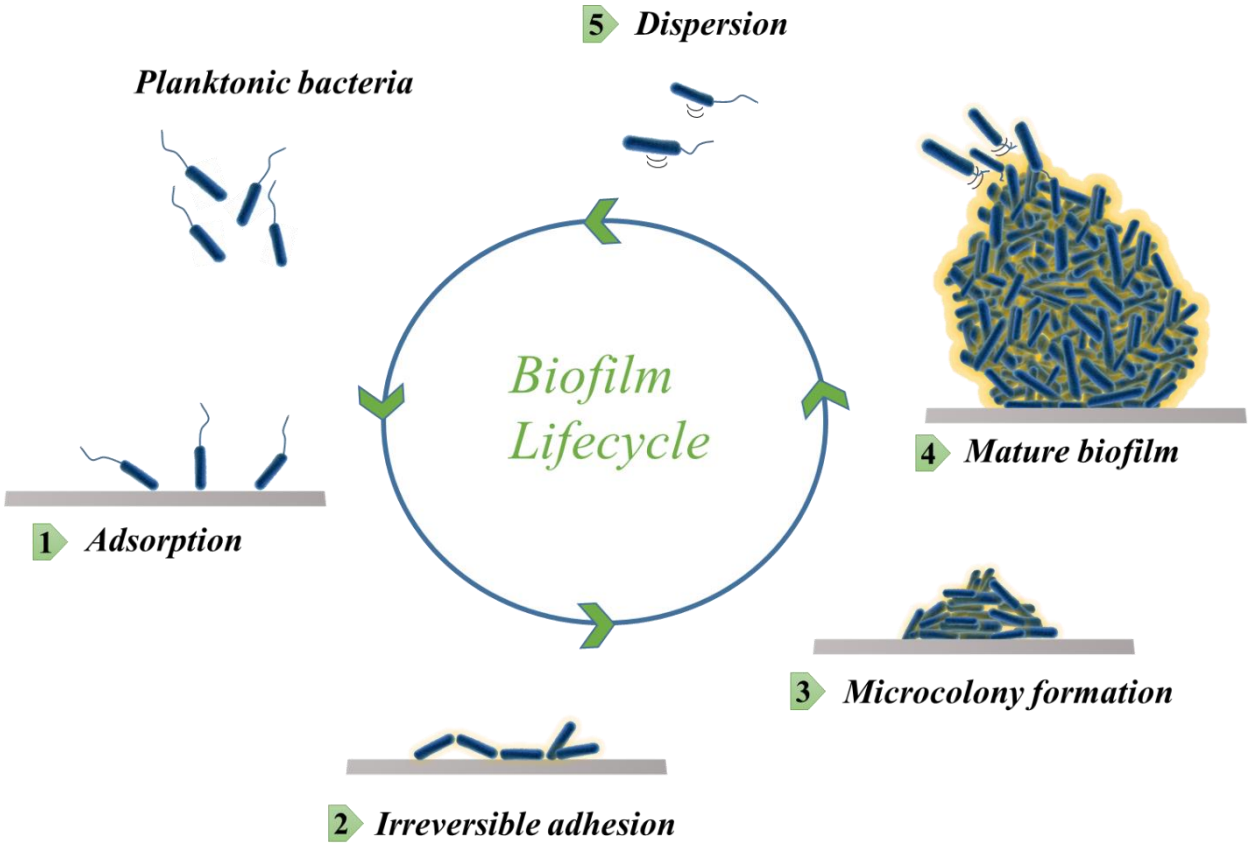

Figure 1 *Biofilm lifecycle. Step 1) adsorption or reversible attachment of bacteria to the surface. Step 2) irreversible adhesion due to the production of eps. Step 3) microcolony formation. Step 4) maturation of biofilm structure. Step 5) dispersion and detachment of bacteria from the biofilm to regain the planktonic stage or recontaminate other surfaces*

Factors influencing the bacterial adhesion to a substrate

The factors influencing the initial adhesion of bacteria to substrates involves multiple parameters. The adhesion factors are linked to the microorganism, the target surface and the surrounding environment (An and Friedman 1998). The relative impact of these characteristics

depends on the microbial strain studied (Katsikogianni and Missirlis 2004). However, these factors must be carefully considered in order to develop effective strategies in the prevention of microbial colonization.

The most significant factors influencing this adhesion were discussed hereafter and summarized in Figure 2.

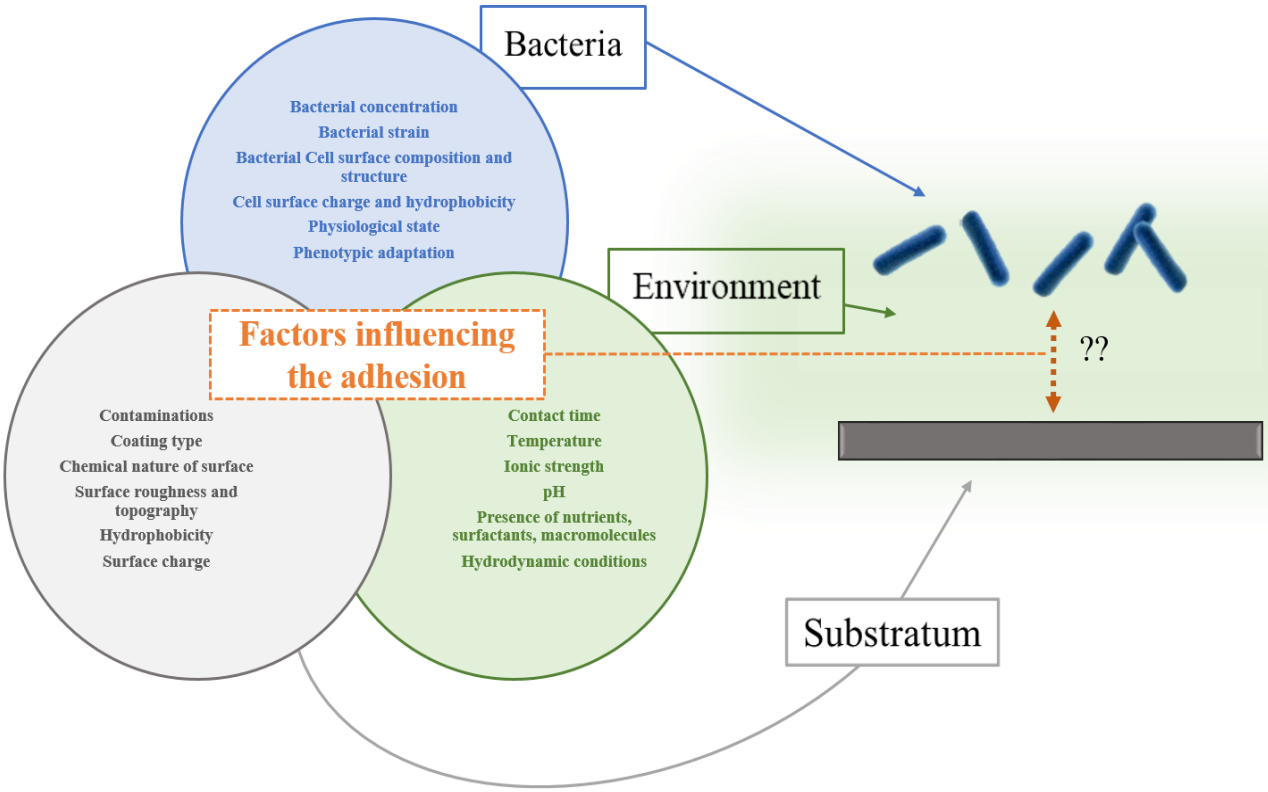

Figure 2 *Factors influencing bacterial adhesion on a substratum*

A solid surface is systematically covered with a layer of organic contamination because of the air pollution (Corn 1961). After being cleaned, surfaces such as glass, plastics or metallic materials are prone to re-contaminate themselves in order to acquire a thermodynamically stable state. During manufacturing processes, material surfaces might be contaminated by micro-particles. Since materials are frequently in contact with other material types, drugs or foods during production processes (Bohinc et al. 2016). This contamination may affect the bacterial

adhesion to the substrate. Indeed, the presence and accumulation of organic soil on surfaces affects its surface roughness and the bacterial adhesion behavior (James et al. 2017; Verran & Boyd 2001). A material adsorption with macromolecules like organic and inorganic compounds is called the “conditioning film”. Moreover, the surface modification by grafting molecules via multiple coating techniques, using classical chemistry or plasma technology, permits the elaboration of various coating types. All those reactions result in a significant modification of the physical and chemical characteristics of the support like its roughness, hydrophobicity and charge. It influences positively or negatively microbial adhesion. In addition, when microorganisms are detached from a surface by mechanical stress, the constituents of their membrane might remain adhered to the surface and promote other microbial attachment (Donlan 2002; Lorite et al. 2011; Chouirfa et al. 2019).

The chemical type of surface encountered by the microorganisms strongly influence the development of the biofilm, more specifically the first stage of bacterial adhesion. For example, Verheyen et al. (1993) showed that *Staphylococcus aureus* adheres more preferentially to the metal 316L steel than to the polymeric surface poly(L-lactide) due to the differences in chemical composition and polarity of these surfaces. Moreover, a study by Alam & Balani (2017), investigated the adhesion force of *Staphylococcus aureus* on different biomaterial surfaces. The UHMWPE surface (ultra-high molecular weight poly ethylene) showed a weak adhesion force (~ 4 nN) whereas stainless steel showed strong adhesion force (~15 nN) owing to their surface roughness and surface energy.

Surface roughness is one of the most discussed parameters influencing the bacterial adhesion. Indeed, it seems that microbial adhesion can be impacted positively or negatively, depending on the bacterial size and on the surface topography that includes several parameters like the width/depth of the "micro-cracks" and the presence of stripes. Thus, the presence of cracks and "Micro-cracks" increases the contact area and can promote adhesion mechanisms by protecting

bacteria from hydrodynamic shear stress and chemical disinfection agents. In fact, a study by Dantas et al. (2016) analyzed the relationship between the bacterial adhesion and surface roughness of Acrylic Polymethyl Methacrylate substrates. This investigation showed that the increase in surface roughness of the samples was directly related to an increase in bacterial adhesion of *Streptococcus sanguinis.* On the same wave, Hage et al. (2021) studied a plasma-modified stainless steel by organosilicon monomer 1,1,3,3-tetramethyldisiloxane mixed with oxygen, using a nitrogen flow microwave post-discharge plasma polymerization process. The influence of cold plasma parameters on coating characteristics, coated surface structure and attachment of *Salmonella* Enteritidis cells was investigated. The results demonstrated that the surface structure affected the rate of bacterial adhesion. Indeed, rough coatings did not repel *Salmonella* Enteritidis as the numbers of adhered cells on these surfaces ranged from 30 ± 4 to 65 ± 4 bacteria per microscopic field. However, the smoother coatings exhibited an anti-adhesive nature as the number of adhered cells was almost nil on these surfaces. In addition, Whitehead et al. (2005) showed that titanium surfaces, with holes of similar or larger size than those of the bacteria *S. aureus* (diameter ~ 0.5-1 µm) and *Pseudomonas aeruginosa* (diameter ~ 1-3 µm), as well as *Candida albicans* yeast cells (diameter ~ 2 µm), offer a better adhesion. For other authors, roughness has no influence on biofilm initiation and it inevitably grows after a period of time (Vanhaecke et al. 1990; Rodriguez et al. 2008). In addition, Flint et al. (2000) observed no correlation between arithmetic roughness of AISI 304L stainless steel surfaces (Ra between 0,5 and 3,3 µm) and the attachment of heat-resistant *staphylococci*. However, they have shown an interesting adhesion for a value of Ra equal to 0.9 µm, suggesting a trapping of microorganisms linked to their size. Moreover, according to other studies, the increase of surface roughness reduces the contact surface between the substrate and the microorganism when its size is higher than the surface roughness, promoting cell detachment (Boulangé-

Petermann et al. 1997). The surface roughness parameter effect on bacterial adhesion is frequently associated with the surface wettability which is a very important parameter.

The hydrophobicity and surface free energy of a material are recognized to influence bacterial adhesion (Quirynen et al. 1994; Subramani et al. 2009). The non-specific physico-chemical interactions are constituted of Van der Waals forces, electrostatic and acid-base interactions, which characterize the surface free energy of a substratum (Grivet et al. 2000). Results from several investigations that relate surface wettability to bacterial adhesion are conflicting (Grivet et al. 2000; Oh et al. 2018). However, it is known that according to the bacterial adhesion thermodynamic model, hydrophobic bacteria preferentially colonize hydrophobic substrates and vice versa (Mabboux et al. 2004; Wassmann et al. 2017). A recent study investigated the effects of surface texture and roughness on the bacterial adhesion. *Staphylococcus aureus* adhesion behavior, towards a bio-ceramic joint implants with different roughness grades (Ra 205–1.1 nm) and surface texture (uniform and unidirectional textures), elaborated via polishing technologies, was studied. The results showed that when the surface roughness reduces from the sub-micron scale to the nano-scale level, the surface state gradually changes from hydrophobic to hydrophilic. In this case, it turns unsuitable for the adhesion of *Staphylococcus aureus* which is hydrophobic. Moreover, the anchoring points for bacterial adhesion gradually disappear, and then the bacterial-surface bonding strength weakens. It was concluded that the preparation of a smooth surface and the elimination of unidirectional surface textures can inhibit the initial adhesion of *Staphylococcus aureus* on those surfaces, reducing the occurrence of implant-related infections (Lu et al. 2020). Another study aimed to investigate bacterial adhesion on different ceramic and titanium surfaces, and analyzed the relationship between surface hydrophobicity and surface roughness defining the predominant factor for bacterial adhesion on each material. Results showed that the variations in surface roughness did not show any differences in the adhesion of *Staphylococcus epidermidis*. However, higher surface

roughness showed an increase in *Streptococcus sanguinis* adhesion. In contrast, for *Staphylococcus epidermidis*, the bacterial adhesion rates detected were higher on the hydrophobic surfaces than on the hydrophilic surfaces but not for *Streptococcus sanguinis*. The adhesion potential of *Streptococcus sanguinis* was higher on the ceramic surfaces than on the titanium surfaces while no such preference was detected for *Staphylococcus epidermidis.* Indeed, both surface wettability and roughness can impact the adhesion behavior of bacteria on biomaterials. In this context, the predominant factor is dependent on the bacterial species (Wassmann et al. 2017).

Several studies have established that the surface charge of materials plays an important role during cell adhesion (Behrens and Grier 2001; Palmer et al. 2007; Choi et al. 2017). A recent study demonstrated the relationship between the surface charge and the bacterial adhesion. Indeed, Guo et al. (2018) elaborated layer by layer films via branched polyethylenimine and synthesized polyanions bearing either alkylcarboxylic or poly (ethylene glycol) side chains. This development was carried out with control over wettability and surface charge parameters. Adhesion test results showed that the *Escherichia coli* and *Staphylococcus aureus* adhesion was guided by surface charge and wettability.

Several factors linked to the microorganism properties influences the bacterial adhesion. Investigations showed that when the microbial concentration increase, the number of adhered cells gets higher, until the surface is completely covered (Piette and Idziak 1992). In addition, the presence of primary microorganisms colonizing a surface can facilitate the occurrence of other microorganisms (Beloin et al. 2008). This phenomenon, known as "co-aggregation" has been highlighted in a study of the oral cavity presenting adhered bacteria to the teeth (Whittaker et al. 1996). Other studies have demonstrated the existence of this cooperation in food, agriculture and biomedical sectors (El-Azizi and Khardori 1999). The biochemical composition and the architecture of the bacterial cell surface (presence of proteins, fimbriae, flagella,

exopolymers, peptidoglycan in Gram-positive bacteria, and lipopolysaccharides in Gram-negative bacteria) contribute to the adhesion of microorganisms to the substrates. For example, the fimbriae contain a high proportion of hydrophobic amino acids, which leads to the establishment of hydrophobic interactions with the material (Donlan 2002). The flagella allow the bacterium to be mobile and play an important role in the early stages of adhesion by counteracting electrostatic repulsion forces (Pratt and Kolter 1998). Lipopolysaccharides (LPS), are present in the wall of Gram-negative bacteria, and more specifically the carbohydrate part (O antigen) of these LPS, give the cell hydrophilic properties. As a result, mutants of *Pseudomonas fluorescens* unable to produce LPSs, adhere in greater numbers to hydrophobic substrates (Williams and Fletcher 1996). The teichoic acids, specific components of Gram-positive bacteria, affects their adhesion mechanism since they give the cell a negative surface charge. Indeed, Gross et al. (2001) demonstrated that a mutant of *Staphylococcus aureus*, whose teichoic acids do not contain D-alanine, was unable to adhere to the polystyrene, due to the increased negative surface charge compared to the D-alanine-containing strain. Surface proteins, frequently referred to as "adhesins", are also strongly involved in the bacterial adhesion to surfaces via hydrophobic interactions (Flint et al. 1997). For example, Cucarella et al. (2001) identified in *Staphylococcus aureus* a protein called BAP ("Biofilm Associated Protein"). They have shown that the bacteria producing this protein strongly adhered to plastic surfaces (polystyrene, polyvinyl chloride) while mutants BAP-deficient adhered poorly to the two tested surfaces. Moreover, several studies showed that polysaccharides, present on the bacterial surface, are involved in their initial attachment. Polymers excreted by bacteria (EPS) induce a reinforcement of adhesion to the support, making it irreversible (Atabek and Camesano 2007). Moreover, regarding physico-chemical properties of the material affecting the adhesion, the hydrophobicity and the surface charge of the cell wall play also a major role in the adhesion mechanism (Palmer et al. 2007). Indeed, those properties are linked to the composition of the

cell surface, that are influenced by the growth rate and the physiological state of the bacterial strain. The adhesion of a bacterial strain to a receptor substrate permits the development of a different perception of its environment (Kimkes and Heinemann 2020). Specific genes are then over- or under-expressed according to the new bacterial needs. Thus, the genes coding for flagella are inhibited, since the microorganism turned to the sessile state (Kuchma and O'Toole 2000). Otherwise, the expression of genes involved in quorum sensing or EPS production or parietal proteins increases (Prigent-Combaret et al. 1999; O'Toole et al. 2000).

Environmental characteristics affect directly the bacterial adhesion to a substrate. The increase in contact time between the microorganism and the support induces a reinforcement of established linkages (Nejadnik et al. 2008).

The temperature of the surrounding environment influences microbial colonization, because growth temperature is maximal for a so-called optimal temperature, specific to each microorganism. In addition, numerous studies have proven the influence of the ionic strength of the medium on microbial adhesion, through the electrostatic interactions established between the microorganism and support (Bos et al. 1999; Poortinga et al. 2002).

The pH of the surrounding environment has an influence on bacterial growth and on their surface physico-chemical properties. The pH value has also an impact on the surface charge of the substrate, especially in the case of metals such as stainless steel, for which the oxidation state depends on the pH (Palmer et al. 2007). The presence of surfactants in the bacterial environment modifies the solid/liquid and microorganism/liquid interfaces, influencing cell adhesion and detachment, as noted by McEldowney and Fletcher (1986). Moreover, the presence of nutrients, such as carbon and nitrogen, affects the bacterial metabolism and has an influence on the bacterial surface properties, thus on the initial adhesion (Strevett and Chen 2003; Nitschke and Silva 2018). Hydrodynamic conditions influence bacterial adhesion.

Indeed, when the flow regime is laminar or slightly turbulent, the boundary layer at the material/liquid interface is thick and the adhesion of microorganisms depends on their ability to penetrate it according to their mobility and size and the flow velocity. Moreover, depending on the Brownian motion, gravity or convection movements can favor the initial adhesion of microorganisms. Otherwise, when the regime is turbulent, the numerous eddies can facilitate contact between bacteria and surface. However, the decrease in the thickness of the hydrodynamic boundary layer reduces the interaction time between the cell and the substrate. Indeed, the establishment of weak bonds hinders the irreversible attachment of the bacteria (Donlan 2002; Palmer et al. 2007; Nejadnik et al. 2008). In conclusion, microbial adhesion on surfaces is a multifactorial phenomenon constantly evolving over time. It involves many different parameters linked to the substrate, the microorganisms and the suspending medium. Controlling all of these parameters is a challenge, for both industrial and biomedical fields, in which the microbial colonization of surfaces is at the origin of particularly negative impacts.

Cold plasma technologies

The treatment of surfaces with plasma techniques permitted to develop surfaces according to desired properties. Plasma has been defined as a gas that is partially or fully ionized into charged particles and neutral molecules (Moreau et al. 2008). It is regarded as the fourth state of matter and obtained when gases are excited into energetic states by radiofrequency, microwaves, or electrons from a hot filament discharge (Bogaerts 1999; Chu et al. 2002). Concerning the types of plasmas, they're divided into two main categories: Thermal plasmas and Non-thermal plasmas also called atmospheric cold plasma, cold atmospheric plasma or simply cold plasma (Mandal et al. 2018). The thermal and cold plasmas can be defined according to the conditions in which they are created.

Cold Plasma technologies provide a uniform modification of the whole surface with less material degradation than several wet chemical treatments (Karam et al. 2013). In fact, the

imparted functionalization type can be controlled by plasma gas selection like Ar, N_2, O_2, H_2O, CO_2, or NH_3, and by experimental conditions such as pressure, power, time, or gas flow rate (Kang and Neoh 2009). Plasma-Surface Modification (PSM) allows changing the chemical composition and properties such as wettability, hardness, chemical inertness, and biocompatibility of materials surfaces (Neděla et al. 2017). There are two very interesting things about cold plasma technology. Firstly, cold plasma is source of elevated temperature electrons at ambient conditions. Secondly, the cold plasma, when interacting with an atmospheric or controlled environment, elaborates many reactive components. Indeed, cold plasma is produced at low levels of power and pressures, with absence of localized thermodynamic equilibrium, it is indeed defined as non-equilibrium plasma. The provided energy breaks the gas into a several reactive species, following other reactions such as ionization, excitation and de-excitation. Moreover, the specific procedure carried out during cold plasma production determines the orientation for application alongside composition of reactive species (Taccogna and Dilecce 2016). Those reactive species can be applied for many chemical reactions in different domains of science (Gorbanev et al. 2016). Such plasma is of particular interest technically and industrially because they do not require extreme conditions that might change the material properties (Wiesemann 2014).

There are options concerning the delivery of the generated plasma species to the substrate. Firstly, the direct exposure in which the substrate is directly exposed to the plasma discharge itself. It can be the splash of a plasma jet or the field between two electrodes. The other option is the indirect or remote exposure that requires placing the surface at a distance from the plasma discharge. The long-lived components interact with the surface after recombination with several induced species (Sarangapani et al. 2018).

It is therefore important to first define the technological trajectory for cold plasma generation, which comprise those developed under reduced pressure and induced by atmospheric pressure.

Cold plasma generated under reduced pressure is known as microwave plasma directed by electromagnetic waves generated at frequencies of hundreds of MHz. In contrast to methods presenting electrodes, the microwave discharges are produced via a magnetron supplying microwaves, guided by a coaxial cable, into a process chamber. The irradiation is then absorbed and heat is produced (Fig. 3A) (Isbary et al. 2013). The inelastic collisions generate ionization reactions. The absence of electrodes in microwave plasma is considered beneficial and can be easily restarted in air. Moreover, the gas required in this technique is low comparing to the large quantities of reactive species released. In addition, this plasma is limited in space and its application to wide zones is non-workable in comparison with plasma jets. Plasma jet is a particular configuration discharge. In general, the active region is characterized by a flow of auxiliary gas, producing a burning small jet of ionization waves and active particles. High power and local practicability are profitable in those plasma types called jet, plasma torch, plasma needle or plasma pen (Fig. 3B) (Scholtz et al. 2015).

Cold plasma induced at atmospheric pressure includes dielectric barrier discharges (DBD) (Fig. 3C), corona discharge (Fig. 3D), radio frequency plasma (Fig. 3E) and gliding arc discharge (GAD) (Fig. 3F). DBD plasma is induced by an alternating current emitted when two metallic electrodes are retained apart using a dielectric material at a discharge gap ranging from 100 millimeters to a few centimeters. The dielectric impedes the generation of sparks due to charges movement. The DBD technique enclose the application of different gases, reduction of gas flow rate, a uniform discharge activation over several meters and is characterized by a good adaptability since the electrode geometries employed can be varied. However, prevention and security measures are requested since DBD needs high ignition voltages of 10 kV (Cullen et al. 2017; Fg et al. 2017).

Corona discharge plasma is elaborated surrounding sharp pointed electrodes that contains substantial electric field for developing the ionization energy of arbitrarily created electrons to the expedition for gas, molecules or atoms. High voltage is needed to generate this discharge. It is not expensive and simple to employ. Corona discharges are carried out for surface treatment and fighting microbial contamination. However, it is constrained to heterogeneous diminutive areas. Otherwise, radiofrequency plasma is generally produced when a gas is localized within an oscillating electromagnetic field, achieved by distinct electrodes maintained outside the reactor or by an induction coil. Comparable to microwaves, this class of plasma are produced at frequencies ranging Hz to MHz (Scholtz et al. 2015; Fg et al. 2017; Mandal et al. 2018).

GAD are elaborated in a reactor comprising two or more diverging metallic electrodes working at a high potential difference. In this technique, an inlet gas, composed of humid air, is pumped into the discharge gap between the electrodes. This leads to the formation of an arc in between the narrowest inter-electrode area, which is directly blown away into the diverging area by the inlet gas. Generally, GAD develops both thermal and cold plasmas, depending on the conditions. This technique is applied for both liquid and surface treatments. It is employed for chemical contaminants (e.g. organic solvents, industrial wastes) degradation and for antibacterial effect (Patil et al. 2016; Dasan et al. 2017).

Cold plasma is considered in this review since its suitable for surface coatings elaboration. Indeed, cold plasma technology applied for deposition and coating production is divided hereafter in three approaches that will be highlighted. The plasma functionalization, polymerisation and plasma-induced grafting.

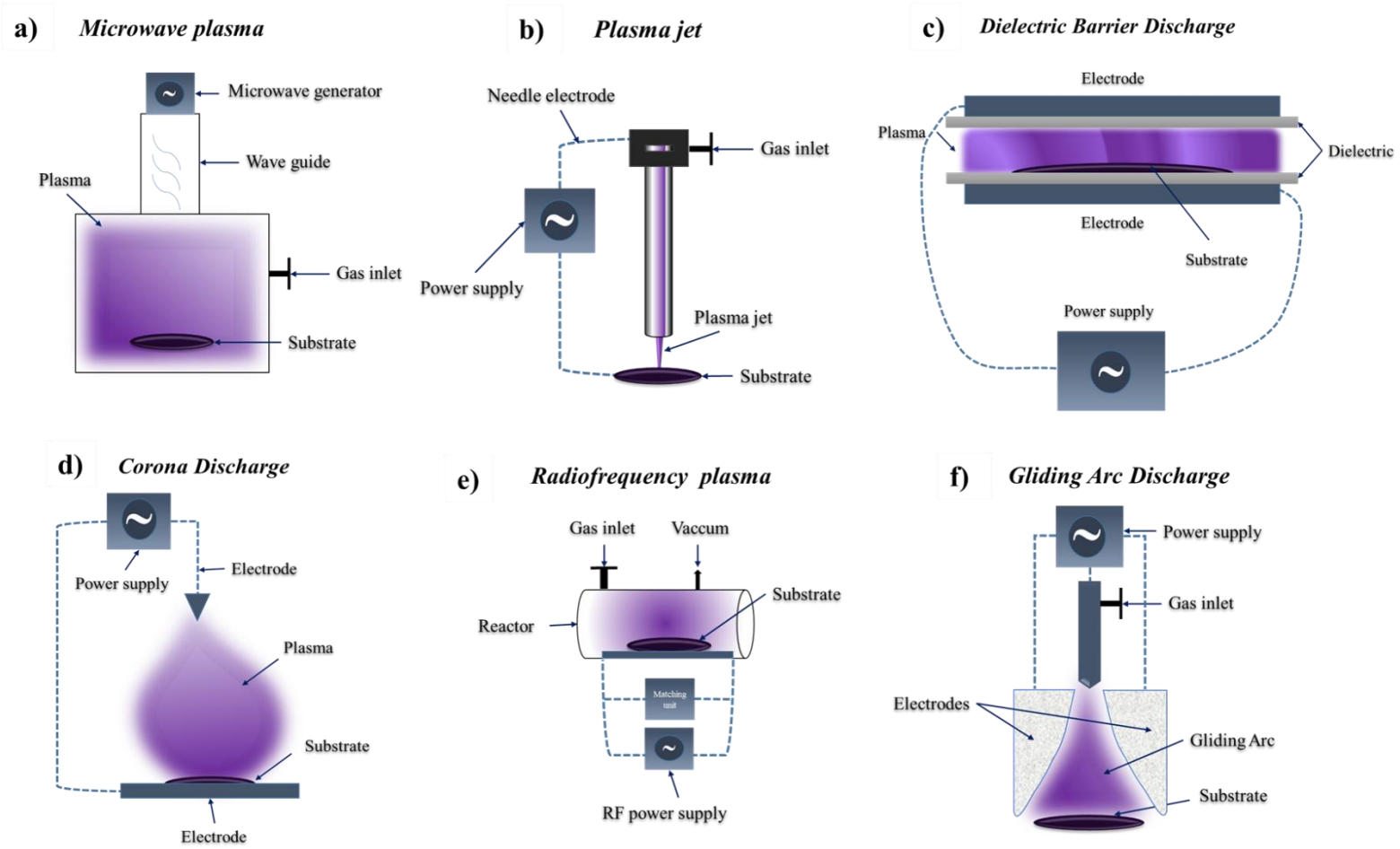

Figure 3 *Schematic setups of different low and atmospheric pressure cold plasma. a) microwave plasma, b) plasma jet needle, c) dielectric barrier discharge, d) corona discharge, e) radiofrequency plasma, f) gliding arc discharge. Adapted from (surowsky et al. 2015; scholtz et al. 2015; coutinho et al. 2018)*

Approaches for cold plasma surface modification

Plasma functionalization approach concerns a plasma treatment leading to the incorporation of new functionalities on the material surface. In fact, different reactive and inert gases are used alone or in combination in order to generate active plasma species on polymers (Karam et al. 2013). The active plasma species bombard the atoms surface and break the covalent bonds between them, conducting to hydrogen abstraction and creation of surface radicals. Radicals

have the potency to react with the gas-phase species to form several chemically active functional groups on the surface (Bogaerts et al. 2002). The type of the formed functional groups rely on the gas used for functionalization as well as the experiment conditions such as the excitation type, reactor geometry, applied power, time, temperature, flow rate and gas pressure (Chan et al. 1996; Chu et al. 2002). Oxygen plasma guides to the set-up of a variety of oxygen functional groups like carboxylic acid groups, peroxide groups, and hydroxyl groups on the polymer surfaces (Fig. 4) (Chan et al. 1996; Sanchis et al. 2006).

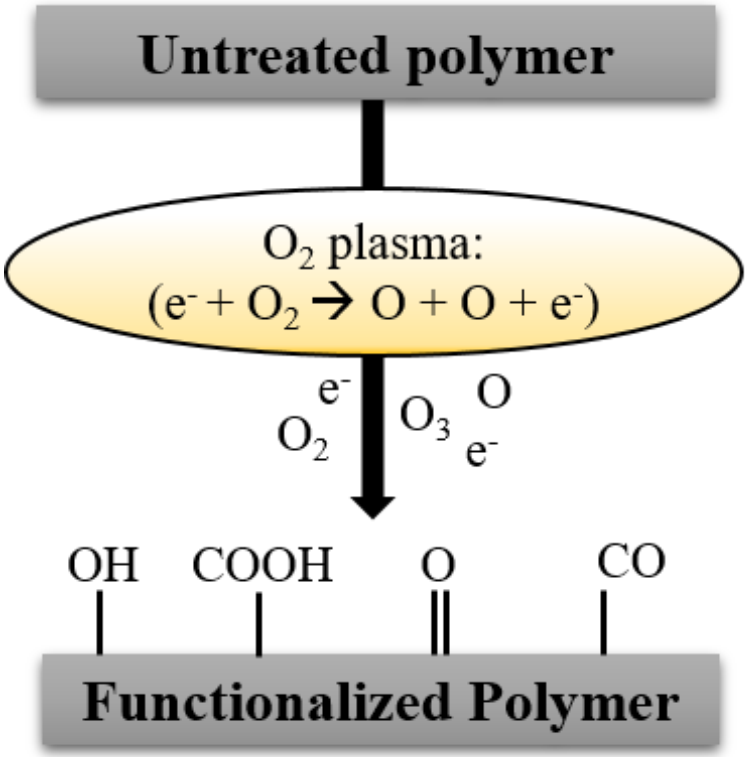

Figure 4 *Plasma functionalization process*

Otherwise, Carbon dioxide plasmas can form hydroxyls, ketones, aldehydes, esters and carboxyl groups on a selected surface (Desmet et al. 2009). Nitrogen and ammonia plasmas introduce primary, secondary, and tertiary amines, as well as amides on the material surface (Tušek et al. 2001; Kull et al. 2005). However, the plasma functionalization technique is believed to be disadvantageous regarding its inability to form a single functional group and the

instability of the changes induced to the surface. This ageing procedure is due to post-plasma oxidation, reorientation of polar groups on the surface towards the bulk, diffusion of molecules with low molar mass to the polymer, the environmental conditions like humidity, that causes the absorption of water molecules by hydrophilic coating, resulting in the disturbance of the surface properties (Schönherr et al. 2000; Upreti et al. 2006; Siow et al. 2006; Tsougeni et al. 2009) (Fig. 5).

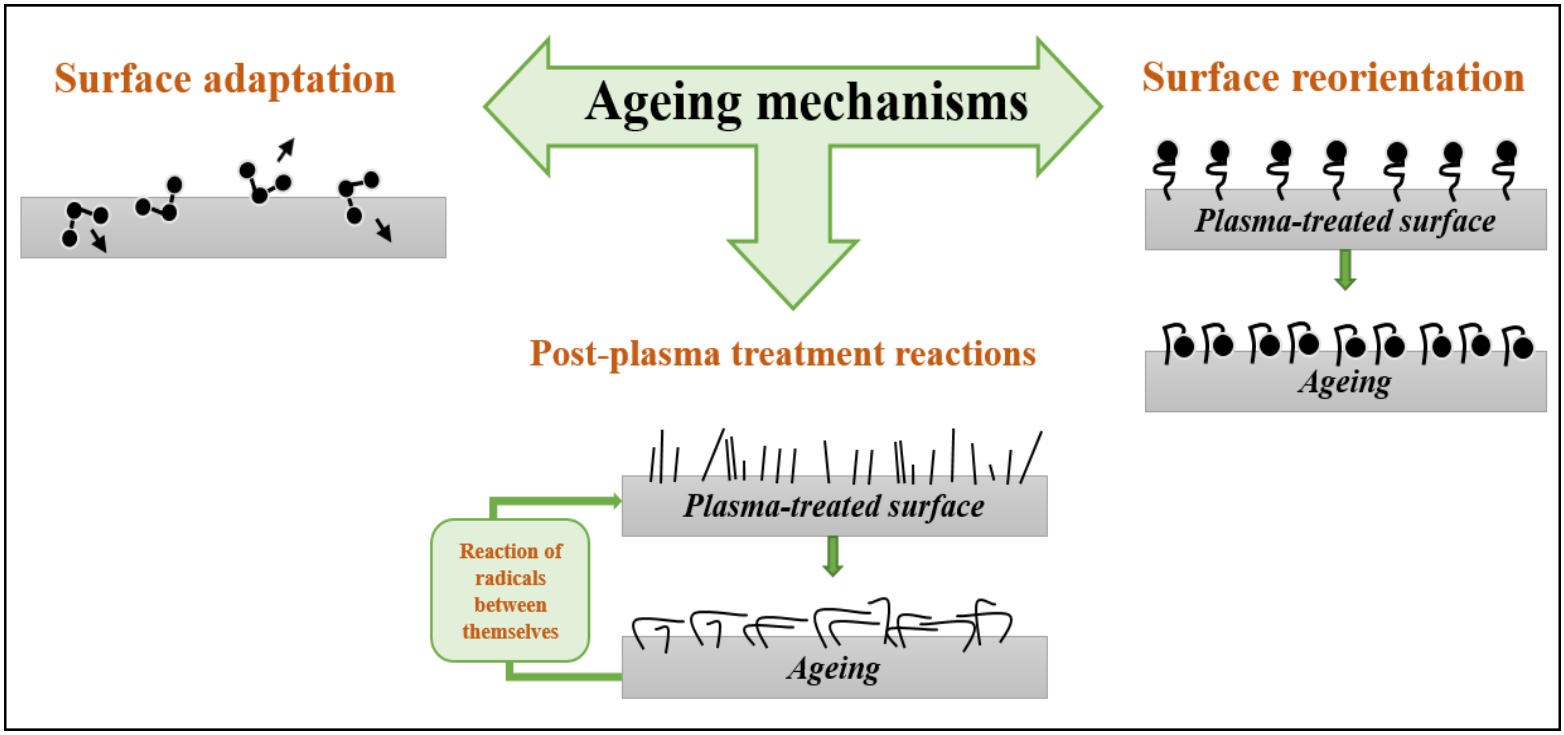

Figure 5 *Ageing mechanisms*

Another approach is the induced-plasma chemical grafting. It is a technique where there is an association of plasma functionalization and classic chemistry (Karam et al. 2013). In this technique, a polymer surface is exposed to a cold plasma of a gas such as oxygen, helium or argon to activate the surface and create free radicals (Bogaerts et al. 2002). The material is then exposed to atmospheric air that oxidizes the radicals, resulting in the formation of peroxide

functions that will allow the grafting of monomers in a further step (Legeay et al. 2006; Ma et al. 2007). The immersion in the monomer solution is carried out under heating (50°C). Indeed, the heating enhances the decomposition of peroxide and oxygen is avoided in the solution since it can stop the reactions (Gupta et al. 2001; Chu et al. 2002; Legeay et al. 2006). This method prevents the ageing effects. Since grafting chemicals onto the surface increases the stability of this treatment (Kang et al. 1996; Goddard and Hotchkiss 2007a).

In addition, the plasma polymerization (Fig. 6) is essentially a plasma-enhanced chemical vapor deposition (PE-CVD) procedure which is an effective technique to elaborate organic thin coatings on a material, and offering proper control over the film character (Hamedani et al. 2016). This approach uses electrical energy for generating a plasma that turn on the reaction by transmitting the energy of its compounds to the precursors leading to free radical creation followed by polymerization process (Vasudev et al. 2013). This polymerization is chemically and physically different from conventional polymerization involving radicals and ions even if the same monomers are used in both polymerization techniques (Chu et al. 2002).

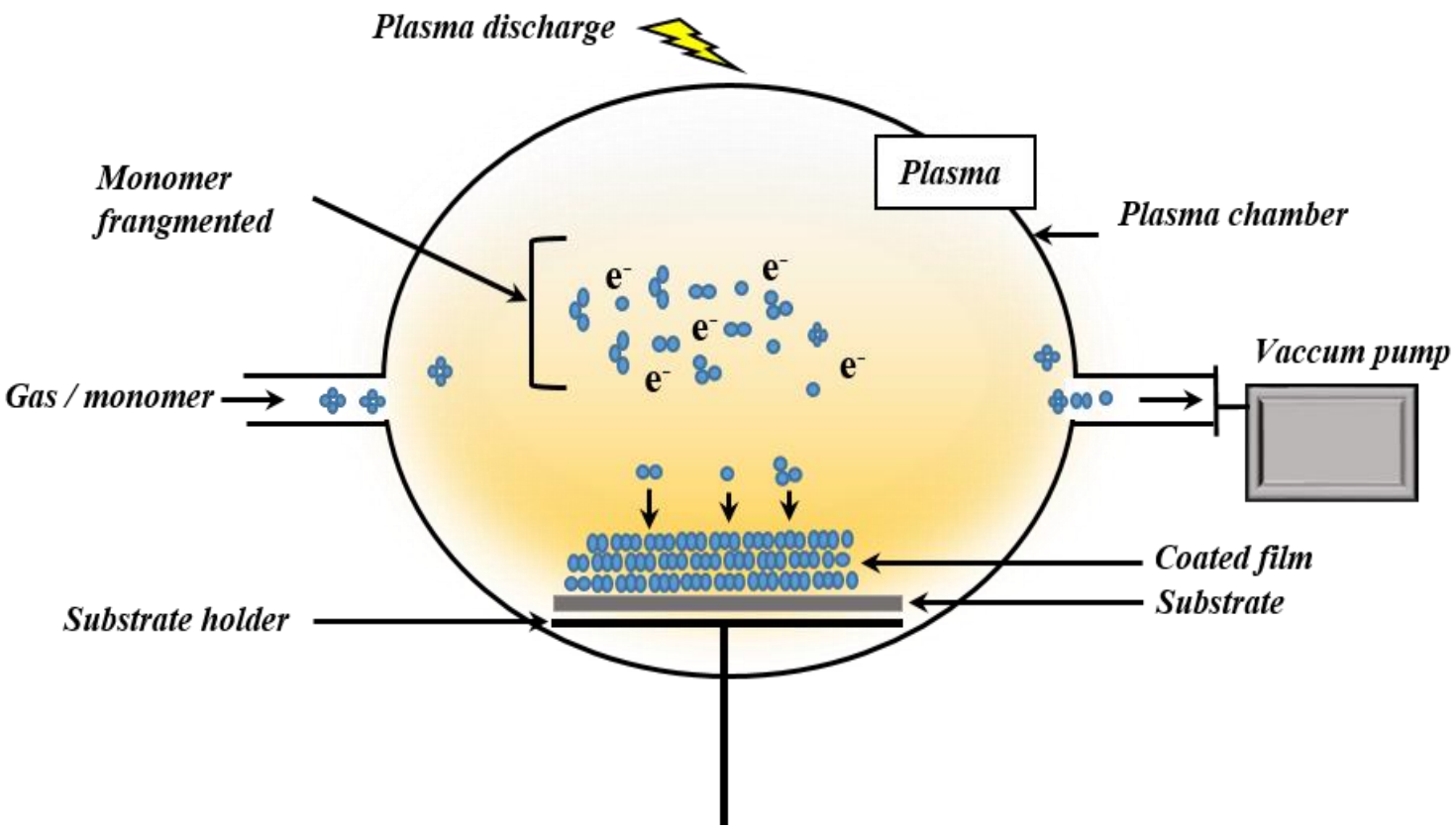

Figure 6 *Plasma polymerization process*

Plasma polymerization technique presents remarkable properties, like chemical stability because of its highly cross-linked nature, the variety of monomers and materials that can be used in this technique, the film uniform thickness. Moreover, plasma polymerization technology has diversity of potential applications that make it a spot of interest for industrials and researchers (Chu et al. 2002; Hamedani et al. 2016). With this depositing process, the selected substrates can be covered with various types of coatings starting with gaseous precursors. In general, a short-chain monomer is cross-linked, fragmented, rearranged and polymerized under the influence of the plasma to generate a long-chain polymer (Dessaux et al. 1998) (Fig. 7).

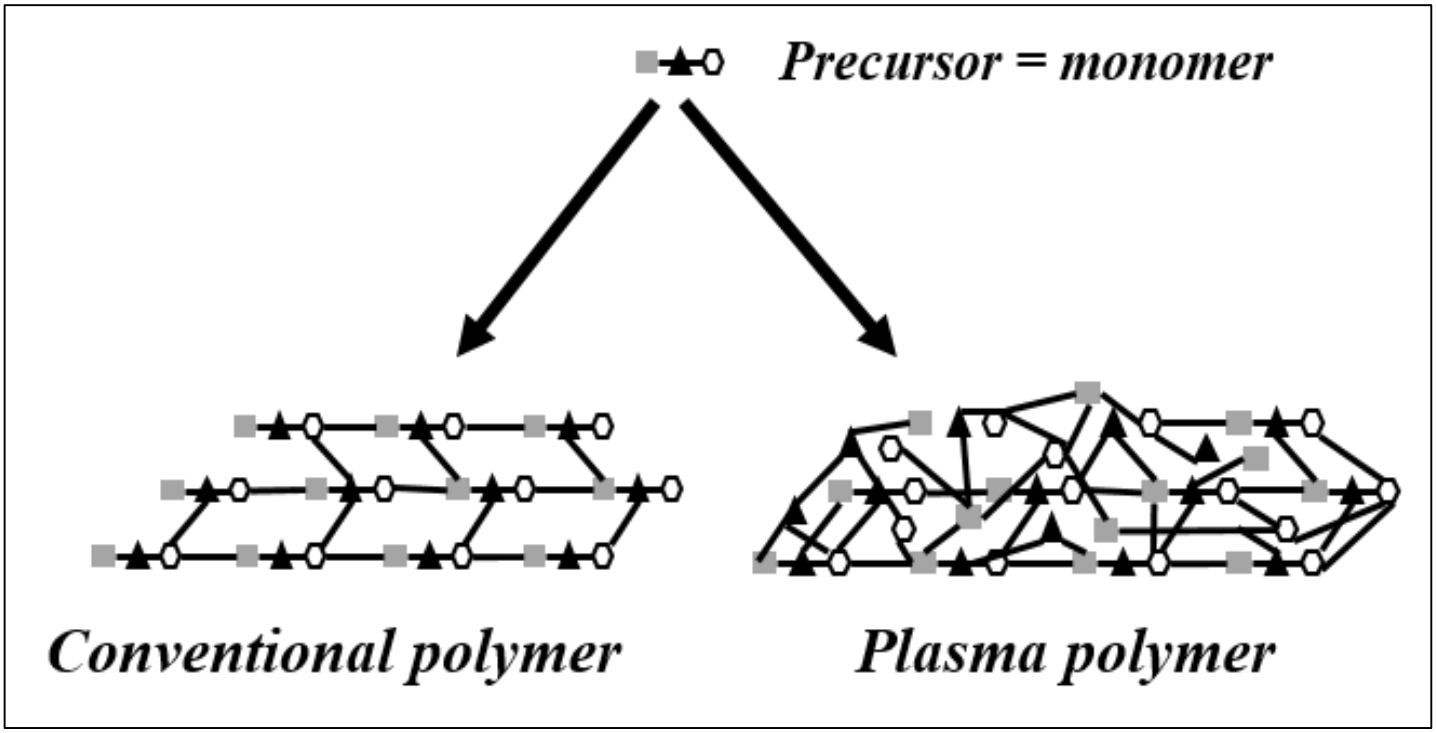

Figure 7 *Structures of polymers formed by conventional polymerization and plasma*

Plasma polymerization method permits the elaboration of thin films using organic monomers that polymerize on the surface thanks to this technique while other conventional methods do not permit their polymerization.

Plasma physico-chemical modification to surface

In this section, the main physico-chemical changes introduced to the surface after plasma surface modification (PSM) are mentioned. Biomaterial surface properties are usually described in terms of surface energy (wettability), chemistry, topography, roughness, and electrostatic charge. PSM with reactive gas leads to the introduction of active chemical function species and then, the modification of chemical properties (Károly et al. 2019).

The surface chemistry controls the charge and the hydrophobicity of a material. Thus, it has a direct effect on the cell adhesion to plasma modified surfaces. The nature of introduced chemical groups depends on the gas used to generate plasma (Amani et al. 2019).

The surface wettability is an important parameter that significantly change after PSM. Surface wettability before and after PSM is usually characterized by water contact angle (θ) measurements. θ is used to measure the surface hydrophilicity using organic (non-polar) or polar solvents by placing a droplet of liquid on a dry surface (Iqbal et al. 2019). Generally, the lower the θ, the more hydrophilic is the surface. Surface energy can be altered by PSM techniques to strongly influence cell adhesion (Rezaei et al. 2014). The free radicals generated during the plasma process react with the environmental O_2 leading to the formation of polar groups (such as -OH, -COOH). Those functions provide more hydrogen bonding, resulting in a decreased contact angle, and hence lowering of θ (Goddard and Hotchkiss 2007b). Furthermore, it was found that PSM increased the hydrophilicity and surface energy without altering the bulk properties of the materials (Sharma et al. 2002; Govindarajan and Shandas 2014; Jaganathan et al. 2015).

Surface topography and roughness are considerably modified after PSM on the micron and nanometer scale, resulting in microorganisms' behaviour modification that could interact with plasma treated materials (Jing et al. 2007a; Jing et al. 2007b). In fact, it has been reported that PSM of biomaterials have the ability to regulate cell functions such as proliferation, differentiation, and apoptosis (Ito 1999). Oxygen plasma treatment have been used by Ha et al. (1997) to modify the surface characteristics of PolyEtherEtherKetone (PEEK). They showed that PSM created spherulitic surface irregularities of PEEK characterized by an increased roughness. Moreover, it has been shown that plasma treated PolyUrethane (PU) had homogeneous surfaces after treatment, which did not lead to significant changes in PU film topography (Sanchis et al. 2007).

Furthermore, plasma treatment affects the hardness and elastic modulus of treated polymer surfaces owing to the effects of densification and cross-linking (Shi et al. 2001; Powles et al. 2005). Shi et al. (2001) study showed that the nano-hardness and elastic modulus of plasma

treated ultra-high molecular weight polyethylene UHMWPE doubled, whereas the wear resistance coefficients was significantly enhanced by a factor of three compared with the untreated samples. They concluded that improvement of wear resistance can be mainly attributed to ion bombardment induced cross-linking, and thus surface hardening. Surface charge is determined mainly by zeta potential measurements based on the quantification of electrophoretic mobility of materials in solution, depending on the polarity (charge) of the absorbed counter ions in the electric double layer, and the ionic concentration of the solvent (Khorasani and Mirzadeh 2007). Basically, PSM results in the introduction of different charged species (anionic and cationic), functional groups and free radicals, on the surfaces of materials. These created species are directly involved in the modification of the original zeta potential of initial surface. In fact, many studies have demonstrated the impact of PSM on material surface charge. Shao et al. (2017) used atmospheric-pressure dielectric barrier discharge for the modification of epoxy material surface and refine the dissipation of surface charge aiming to reduce the accumulation of surface charge. Another study demonstrated that cold plasma treatment participates in charging organic surfaces. In this investigation, the surface density of the electrical charge of lentil seeds and pepper and polymers like polystyrene, polyethylene, poly(methyl methacrylate) and polycarbonate was established experimentally (Shapira et al. 2018). Moreover, it has been reported that, the electronegativity of PVC, showed a high increase from -9 mV, to -22 mV after PSM (Khorasani and Mirzadeh 2007; Khorasani et al. 2008). In addition, in human surgery, plasma surface polymerization has been applied to set up non-thrombogenic cardiovascular implants surfaces based on the electrostatic interaction between the negatively charged plasma proteins and cationic coating due to the introduction of $-NH_2$ and -COOH groups (Lassen et al. 1992).

Cold plasma applications

Researchers are continuously fascinated by the applications offered by plasma science. Thanks to the low heat capacity of cold plasma, its production cost efficiency and the diversity of its applications, a very high interest in plasma technology is prevailing. Fig. 8 illustrates the main cold plasma applications.

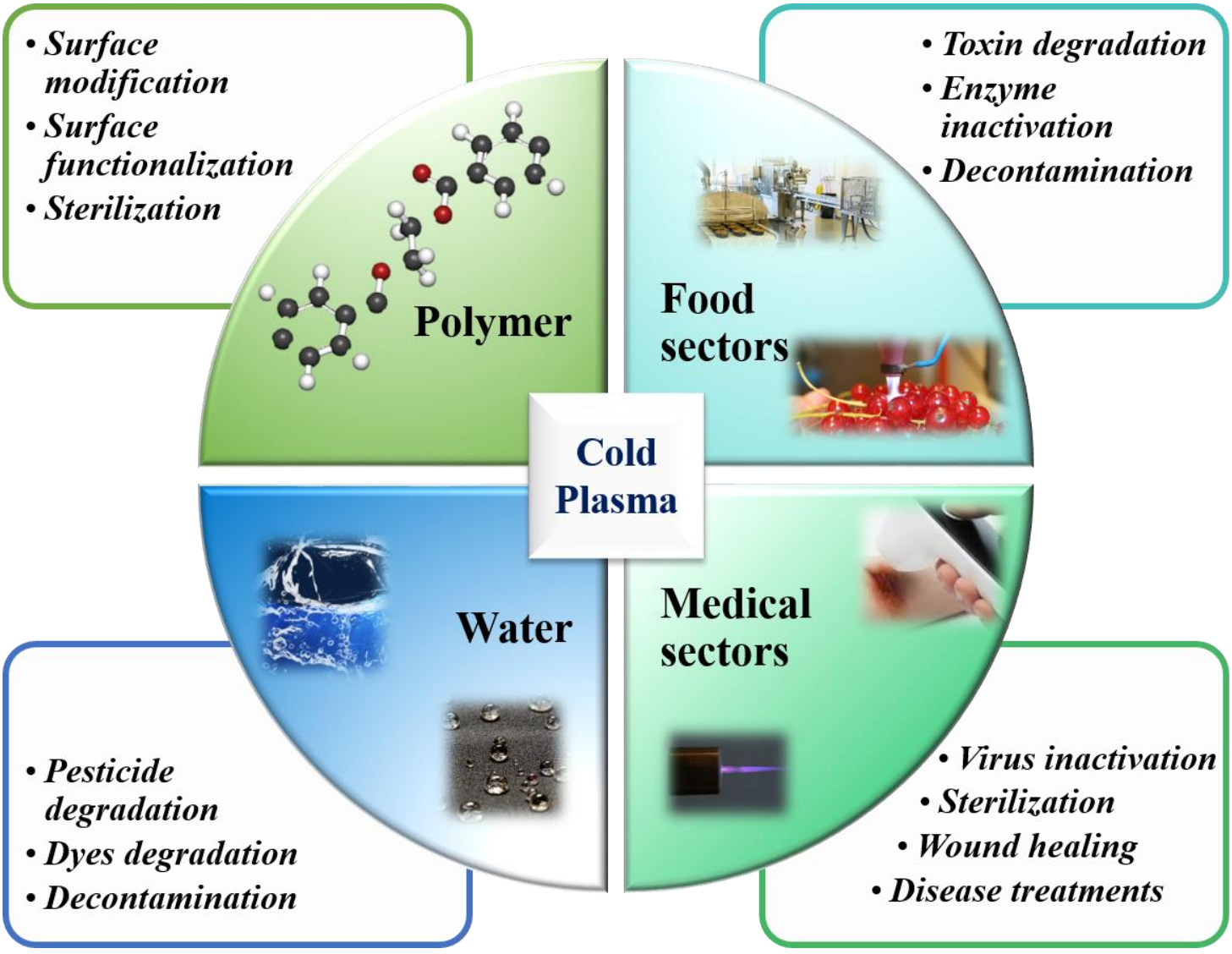

Figure 8 *The main cold plasma applications. Adapted from (pankaj and keener 2017)*

The potential employment of thermal plasma processing technology comprises a large range of activities, such as: the extraction of metals, the refining of metals, the production of fine ceramic powders, spray coatings, and the destruction and consolidation of hazardous wastes (Taylor and Pirzada 1994; Samal et al. 2010)

Otherwise, in material science, cold plasma is applied for surface properties modifications, for example in the production of computer chip (Weltmann et al. 2018). Plasma polymer films application includes anti-adhesion surfaces, humidity sensors, electrical resistors, optical filters, protective coatings, chemical barrier coatings and scratch resistance coatings that have been successfully applied on optical lenses. In environmental sciences, it finds application in air and water purification (Foster 2017), for example, it can be applied for pesticide degradation in water (Pankaj and Keener 2017).

In biomedicine fields, cold plasma technology is applied for teeth and skin therapy, sterilization of medical equipment, the development of coatings for antibacterial purposes (Popelka et al. 2012; Hoffmann et al. 2013). This technology is also employed for wound healing and disease treatments (Pankaj and Keener 2017). Concerning the application of cold plasma in virus inactivation, a recent study showed that cold atmospheric plasma with argon plasma gas was efficient in the inactivation of coronavirus SARS-CoV-2 on several surfaces like metal, plastic and cardboard. These results proof the interesting potential of cold plasma in the prevention of virus transmission for different surfaces that are generally in frequent contact with individuals (Chen et al. 2020). SARS-CoV-2 infection involves recognizing and linking to the human angiotensin-converting enzyme 2 receptor on cells via the receptor binding domain (RBD) of the spike protein, and perturbation of this mechanism can effectively inhibit SARS-CoV-2 proliferation. Plasma-activated water impact on coronaviruses has been investigated by Guo et al. (2021). Indeed, in this study, pseudoviruses with SARS-CoV-2 protein S were employed as a model, and plasma-activated water effectively inactivated pseudovirus infection by inhibiting

of the protein S. RBD was employed to investigate the molecular particularities. Results showed that the binding activity of RBD was effectively knocked out by plasma-activated water via modification of RBD. These demonstrations present a new opportunity for the engineering scientific, and medical sectors.

Moreover, non-thermal plasma can be used in food industries for the development of coated surfaces with anti-adhesive and antibacterial properties aiming to fight biofilm formation (Ma et al. 2012). In this field, cold plasma is also used in the packaging process as well as in food production to reduce the risk of bacterial contamination since it can be applied for decontamination and toxins degradation. Indeed, it can help for products shelf-life extension and improve the packaging integrity (Karam et al. 2013). Plasma technology can be carried out to design functional films with different biocidal agents, including quaternary ammonium salts, silver, or antibiotics (Wang et al. 2004; Bruckert and Weidenhaupt 2010). In addition, it is applied in surface modification, functionalization, reticulation and thin films deposition of polymers surface (Pankaj and Keener 2017). The plasma-based techniques are profitable for different purposes, they can be applied for coating/depositing, cleaning/sterilization, and modification of surface chemistry of substrates. Plasma treatment can also be used as a pre-treatment to other surface modification techniques (Sabir et al. 2009; Joshy et al. 2019).

Cold plasma treatments of materials for preventing bacterial adhesion

Aiming to fight biofilm formation in medical and food fields, many studies were carried out to produce anti-bacterial and anti-adhesive modified surfaces via cold plasma treatments. Among the several materials applied in those fields, stainless steel is a predominant metal used in various application where hygiene is primordial, including food industry and medical sectors (Fouda and Ellithy 2009; Sun et al. 2015). Moreover, titanium alloy is used for dental implants, medical equipment and in food and pharmaceutical manufacturing areas (Agripa and Botef 2019). In addition, polyethylene terephthalate (PET) is a polymer commonly used in biomedical

and food applications (Perez-Roldan et al. 2014). Other polymers like polyamide, polydimethylsiloxane (PDMS), silicone and polypropylene can be applied for their physico-chemical properties in those sectors. This section highlights the strategies carried out by researchers to elaborate antiadhesive films by cold plasma treatment on the materials surfaces mentioned above. Fig. 9 summarize the main directions followed in coatings elaboration and Fig. 10 shows the general surface properties modifications after plasma treatment.

Several studies demonstrated that modifying surfaces with hydrophilic and non-charged polymers resulted in reduced cellular, protein, and bacterial attachment on different surface types (Finch 1994; Du et al. 1997; Sofia et al. 1998; Zhang et al. 2001). Indeed, it has been established that surfaces deposited with poly(ethylene glycol) (PEG) are able to reduce bacterial adhesion and biofilm formation. In effect, an investigation of coated PET and polyamide with PEG of different molecular weights using a $SiCl_4$ cold plasma treatment via the creation of C–Si–Clx functionalities permitting the covalent linkage of PEG macromolecules through a condensation reaction mechanism. Indeed, these coating showed significant inhibition of attachment and biofilm formation by *Listeria monocytogenes* and *Salmonella enterica* sv. Typhimurium compared to unmodified PET and polyamide (Dong et al. 2011). In addition, a research analyzed the coating of PEG-like compounds, 1,4,7,10-tetraoxacyclododecane ether and tri(ethylene glycol) dimethyl ether, onto stainless steel by a cold-plasma enhanced technique. The coatings were more hydrophilic and less rough than the uncoated stainless steel. Biological testing on a mixed culture of *Staphylococcus epidermidis, Salmonella* Typhimurium, and *Pseudomonas fluorescens* revealed a reduction in the bacterial adhesion and biofilm formation (Denes et al. 2001). In another study, the PEG-like compound, di(ethylene glycol) vinyl ether was deposited onto stainless steel surface via radiofrequency–plasma processes. These deposited films showed a stable chemistry and a more hydrophilic character and a decrease in roughness values in comparison with bare stainless steel. These new characteristics

leaded to an effective anti-adhesive behavior of the coatings towards *Listeria monocytogenes* strains (Wang et al. 2003). Plasma treatments can be used as preliminary preparation for surface grafting. A research investigated the antifouling characteristics of grafted plasma-modified PET surfaces. In fact, two different gases, oxygen and helium, were employed to create superhydrophilic surfaces with various surface chemistries. Oxygen reactive gas used in plasma treatment increases the oxygen groups and enhances the hydrophilic character on the surface (Krstulović et al. 2006). Researchers demonstrated an antibiofilm activity of 3D printed polylactic acid petri dishes treated surfaces. Atmospheric pressure plasma was employed for the polymerization and deposition of acrylic acid. Plasma polymerization caused an increment of oxygen polar groups (C—O and O-C=O) producing a hydrophilic character of the coatings. This hydrophilic character played an essential role in *Pseudomonas aeruginosa* and *Staphylococcus aureus biofilms reduction* (Muro-Fraguas et al. 2020)

In a study, stainless steel surfaces were treated with (3- amino propyl) triethoxysilane (APTES), tetraethyl orthosilicate (TEOS) and acrylic acid (AA) via Non-Equilibrium Atmospheric Plasma. An anti-biofilm efficient activity was detected against *Listeria monocytogenes* and *Escherichia coli* strains. *Listeria monocytogenes* registred the best results, with surfaces coated with a base of APTES and functionalized by TEOS or AA, reduced biofilm formation by 45% and 74%, respectively in comparison with uncoated SS. Surface characterization showed that the coating with the highest anti-biofilm activity had higher hydrophilicity and lower surface roughness. This results showed that the development of a hydration layer prevented the bacterial adherence, an effect that seems to be increased by low temperature conditions and when the wettability of the strains is enhanced (Fernández-Gómez et al. 2020). Indeed, the environmental conditions, physicochemical characteristics of the surface and bacterial cell envelope affects the adhesion behavior.

Moreover, surface cross-linking was carried out thanks to helium inert gas resulting in the increase in surface wettability, an important factor influencing the bacterial adhesion (Gheorghiu et al. 1997; Papakonstantinou et al. 2007). Surfaces were then grafted with PEG, Pluronic F108, Pluronic F68, mixed solutions of Pluronic and surfactant like sodium taurodeoxycholate nonaethylene glycol, monodecyl ether and hexadecyltrimethyl ammonium bromide. Those coated surfaces showed effective antifouling properties (Perez-Roldan et al. 2014). Moreover, in another study using radiofrequency plasma polymerization, stainless steel surface was deposited with ethylenediamine (EDA), a hydrophilic monomer, in different glow discharge parameters (Radiofrequency discharge power of 20-80 W with exposure time of 10 min). The modification of plasma conditions showed different efficiencies of the anti-adhesive character of the coatings tested towards *Enterobacter sakazakii*. The optimal condition showing 99.74% of attachment reduction was plasma modification by EDA at 45 W and for 10 min (Şen et al. 2012). Another coating on stainless steel by plasma technique was elaborated aiming to obtain antiadhesive properties. Effectively a silver nanoparticle component film was coated onto stainless steel to weaken the adhesion power of the model yeast *Saccharomyces cerevisiae*. The coating was done under cold-plasma parameters, mixing silver sputtering and RF glow discharge. The anti-adhesive properties of the coating were attested with shear-flow-induced detachment trial (Guillemot et al. 2008). Plasma materials treatment is a strategy to improve coating quality and affect biological response at the surfaces of biomedical devices specifically polymeric materials. A research shows the time-dependent effects of a non-thermal plasma on the surface of polypropylene polymeric implants. Findings suggest that plasma exposure enhanced resistance to *Escherichia coli* adhesion. Bacterial adhesion decreased after 1 min of plasma treatment ($p > 0.048$) whereas after 10 min and 20 min of plasma treatment, the bacterial attachment rate, in comparison with the 1 min rate, was reduced by half ($p < 0.001$). These results imply that the time exposure of a surface to plasma treatments affects its chemical

properties and behavior towards microorganisms (Gd et al. 2020). Furthermore, Lin et al. (2020) elaborated effective antiadhesive coatings towards *Escherichia coli*. In fact, PDMS polymeric surfaces were modified via an atmospheric plasma-induce polymerization with polyvinyl alcohol (PVA) and then immobilized by a zwitterionic polymer (2-methacryloyloxyethyl phosphorylcholine, MPC). Those surfaces were developed for wound dressing application in biomedical fields. The super-hydrophobic character of those modified surfaces inhibited bacterial adhesion. Titanium (Ti) alloys, often used in medical fields does not repel bacterial attachment. In an investigation, the production of radicals was carried out via non-thermal atmospheric pressure plasma jet on Ti surfaces aiming to modify its chemical properties. Bacterial adhesion of *Streptococcus sanguinis* to Ti was significantly inhibited after plasma treatment ($p < 0.05$) compared to unmodified surfaces. In this work, the anti-adhesive effect was generated by carbon cleaning, that was dependent on the gas type used on the titanium surfaces (nitrogen > ammonia and air, $p < 0.05$) (Lee et al. 2017). In a recent study, an acrylate-containing coating was elaborated on titanium surfaces through atmospheric pressure plasma treatment of 2-hydroxyethyl methacrylate, a liquid precursor. The obtained hydrophilic coatings decreased *Staphylococcus aureus* and *Escherichia coli* adhesion. These surfaces were produced for dental implants antiadhesive effectiveness (Buxadera-Palomero et al. 2021)

Moreover, in another recent study, superhydrophobic surfaces with antibacterial properties were developed. Surfaces were elaborated using trichloro(1H,1H,2H,2H-perfluorooctyl)silane (TPFOS) and titanium dioxide nanoparticles (TiO2-NPs) as chemical modifiers. The virgin PVDF membrane was pre-treated using PEG-co-PMAA, followed by plasma treatment, to increase the –COOH and –OH groups on the outer layer and enable coordinate bonds formation on the membrane surface to TiO_2. TPFOS was selected to impart a superhydrophobic character to the titanium surface. Results showed that the PVDF/PP-PT/Ti/Si (polyvinylidene difluoride/ coated PEG-co-PMAA-plasma treated/titanium nanoparticles/perfluorooctyl silane)

developed membrane registered a larger contact angle of ~152° and a better cleanability than that of the pristine PVDF membrane. Plasma treatment caused the increase in membrane porosity via the polymer ablation mechanism. Treated surfaces showed excellent antibacterial properties when tested against Staphylococcus aureus and Escherichia coli (Sinha Ray et al. 2021).

Plasma polymers elaborated using monomeric silicone-based chemicals provide excellent chemical and thermal resistance and remarkable optical, electrical, and biomedical properties (Inagaki et al. 1985; Schwarz et al. 1998; Bashir and Bashir 2015). The plasma polymerized organosilicon films can be employed as protective coatings in microelectronics (KRYSZEWSKI et al. 1979). Organosilicon are also selected for the protection of metals from corrosion (Fracassi et al. 2003). The most employed organo-silicon monomers include TetraMethylDiSilOxane (TMDSO) (Deng et al. 2015), TetraMethylSilane (TMS) (Fonseca et al. 1993), VinylTriMethylSilane (VTMS) (Bonnar et al. 1999), HexaMethylDiSilOxane (HMDSO) (Morent et al. 2009) and HexaMethylDiSilaZane (HMDSZ) (Huang et al. 2015) containing Si, H, C, O or N atoms (Gaur and Vergason 2000). Organo-silicon monomers are used in industries because they are non-toxic components and they do not generate harmful species during processing. Thus, they can be applied without any special safety considerations (European Commission, Directorate General for Health & Consumers 2014).

Among the many monomers which have been employed in plasma polymerization, the organosilicons were recognized to form coatings of special properties. Indeed, organosilanes have at least one carbon-silicon bond, which is very stable and nonpolar. In the procedure of plasma polymerization of organosilicon, the film polymerized on the substrate surface starts to grow when the long-lasting reactive particles, flowing from the microwave discharge, had enough energy to break the chemical bonds and create free radicals implied in the film formation (Callebert et al. 1994; Karam et al. 2013). The deposition zone, where CRNP appears

as a yellow afterglow, is a non-ionized zone mostly formed with reactive species like nitrogen atoms in the ground electronic state N(4S), free radicals, and electronically excited N_2 triplet states and vibrationally excited N_2 in the ground electronic state (Jama et al. 1997; Quédé et al. 2002; Esbayou et al. 2018)

Organosilanes are one of the most versatile molecules that are widely used in coatings and surface modifications cold plasma technologies. Generally, organosilane coated surfaces exhibit an increase in low surface energy and its hydrophobic characters. These types of films ensue the inhibition of bacterial growth without releasing toxic products of low molecular mass into the environment (Kregiel and Niedzielska 2014). Furthermore, an investigation showed that following plasma-assisted surface silanization, the anti-adhesive properties of coated surfaces increased due to the decrease in roughness properties (Savela et al. 2012; Kregiel et al. 2013). In another research, organosilicone-based films were developed on 316L stainless steel, by atmospheric pressure plasma spraying (APPS) of HMDSO. This plasma coating showed an antifouling character and antiadhesive properties towards *Staphylococcus aureus* (Zouaghi et al. 2018). Moreover, Kregiel & Niedzielska (2014) developed polyethylene surfaces, activated by plasma processing and modified with active organosilanes. Those coatings exhibited anti-adhesive properties towards *Aeromonas hydrophila*.

Some studies have examined the antimicrobial activity of organosilanes with active biocidal groups chemically linked to their chains. Indeed, Fortuniak et al. (2011) tested the biocidal activities of polysiloxanes linked with antibacterial quaternary ammonium salt (QAS) groups. These polysiloxanes were linear polydimethylsiloxanes with 20% siloxane units substituted at silicon by 3(dimethyl-n-octylammonio) propyl chloride or 3(dimethyl-n-hexadecylammonio) propyl chloride and terminated by silanol functions at both chain ends. Those polymers were cross-linked and added to a silicone substrate. The biocidal test resulted in thousand-fold reduction of *Staphylococcus aureus* after 15 min of contact with the substrate containing 20 wt

% of this polymer. This study permitted to conclude that polysiloxane-based surfaces can be used as pretreated substrates to link antibacterial groups and develop antimicrobial surfaces. Moreover, another research aimed to study how the modification of silicone elastomer and Polyvinyl chloride surfaces, commonly used in the water industry can reduce the attachment of *Aeromonas hydrophila*, a pathogenic bacterium that have the ability to attach to pipe materials. Silicone elastomer and Polyvinyl chloride surfaces were activated via cold plasma with reactive organo-silanes by coupling silanes with the native material. Those coated surfaces exhibited higher anti-adhesive and anti-microbial characteristics in comparison to the bare surfaces (Kregiel 2013).

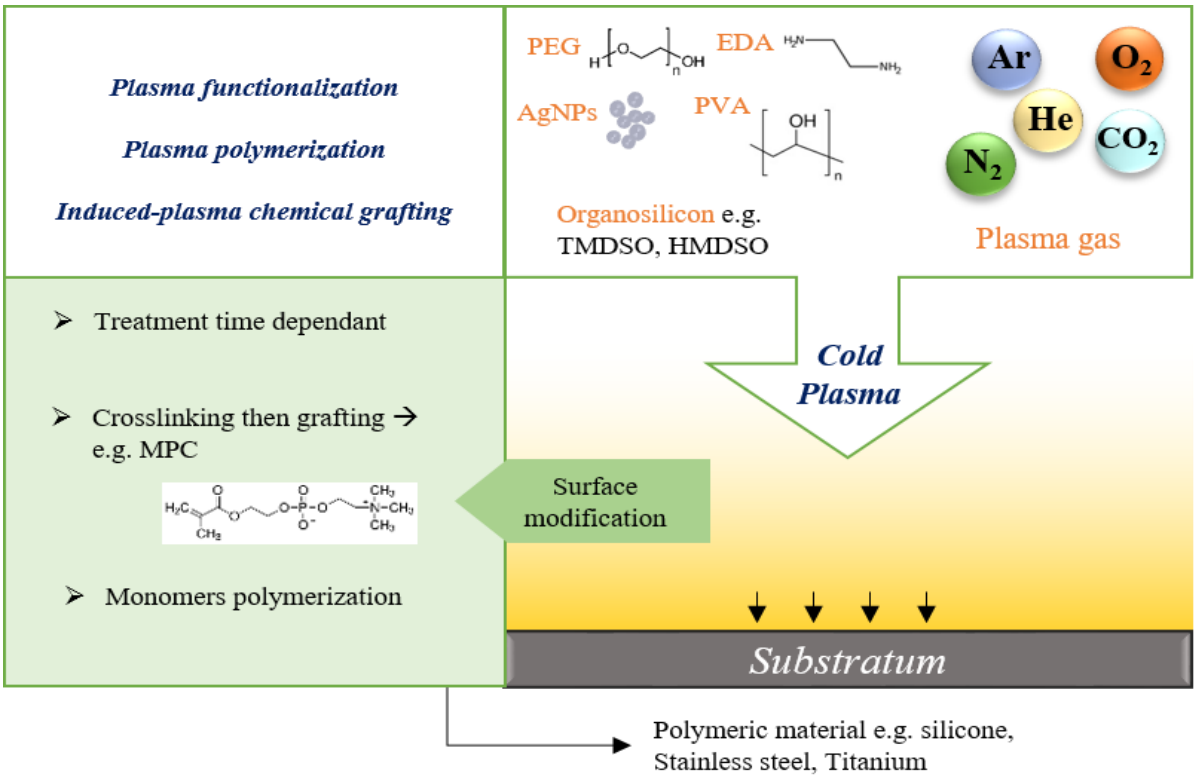

Figure 9 *Main directions followed in coatings elaboration*

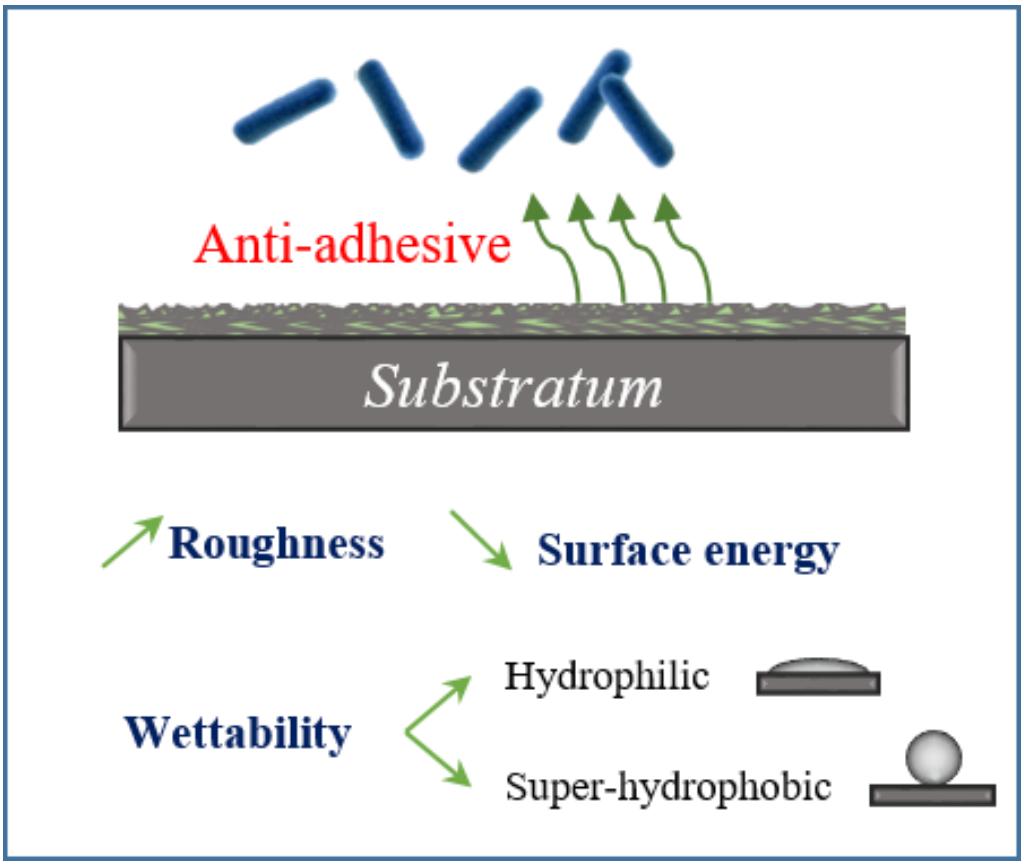

Figure 10 *General surface properties modifications after plasma treatmen*

Conclusion

Cold plasma is an innovative technology experiencing an increased popularity since it shows applications at several sectors. In food and medical sectors, pathogenic bacteria adhere on surfaces and form resistant biofilm which are responsible of many infectious diseases. This review presents general aspects of cold plasma surface modifications. It also highlights plasma coated surfaces designed to inhibit and prevent bacterial attachment on surfaces. However, cold plasma technology requires additional investigations in eco-toxicity, ageing characteristics, coatings effectiveness with time and the interactions mechanisms between the bacteria and plasma coated surface.

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CHAPTER: II

LITERATURE REVIEW – Part 2

Antimicrobial Peptides Coated Stainless Steel for Fighting Biofilms Formation for Food and Medical Fields: Review of literature

Antimicrobial peptides coated stainless steel for fighting biofilms formation in food and medical fields

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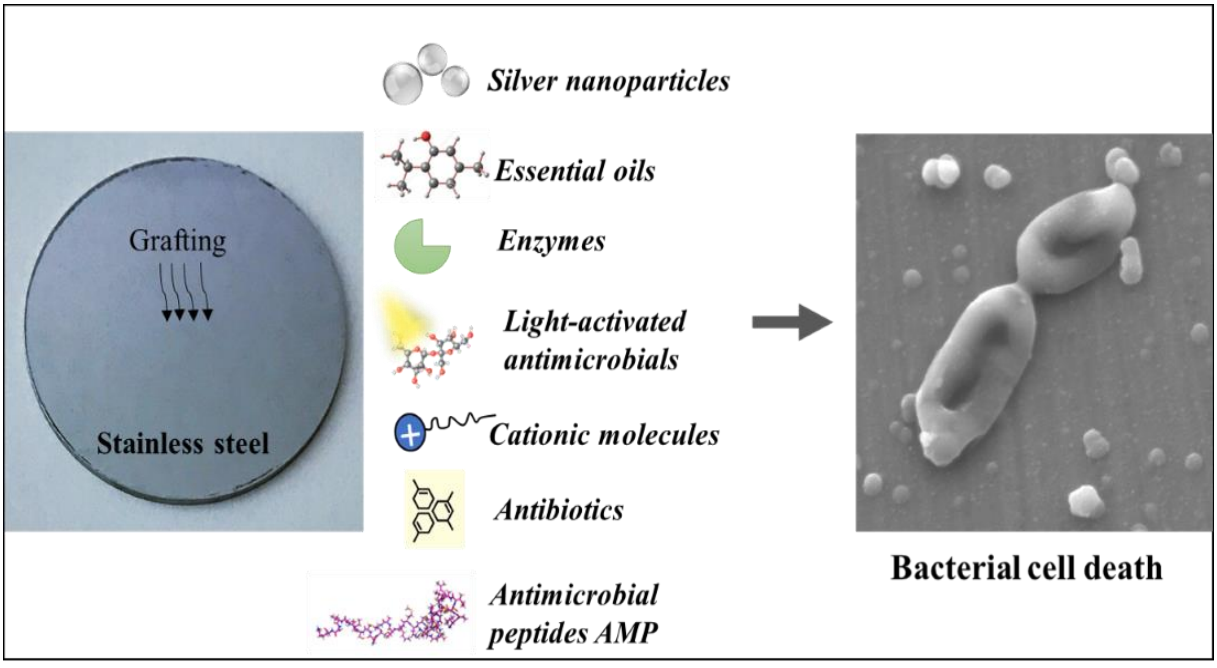
Abstract

Antimicrobial coatings emerging technologies contribute in the fight challenge of pathogenic bacterial biofilms in medical and agri-food environments. Stainless steel is a material widely used in those fields since it has satisfying mechanical properties, but it unfortunately lacks the required bio-functionality, rendering it vulnerable to bacterial adhesion and biofilm formation. Therefore, this review aims to present the coatings developed by employing biocides grafted on stainless steel. It also highlighted antimicrobial peptides (AMPs) coated stainless steel, particularly the nisin which is commonly accepted as a safe alternative to prevent pathogenic biofilms development.

Keywords: Stainless steel; Biofilms; Coatings; Antimicrobial peptides, Biocides

Graphical Abstract



Introduction

In industrial and medical environments, bacteria adhere to accessible surfaces and can grow and develop into a dense biofilm. Biofilm-associated infections in medical devices and food equipment represent a serious public health burden and negatively impact the proper functioning of the instrument resulting in huge economical loss.

The biofilm is constructed of a complex consortium of microorganisms encased in an extracellular polymeric matrix. Bacterial invasion of a substrate is a multi-stage phenomenon that includes biological and physico-chemical factors. Biofilm growth can be explained as a 5-step process comprising: the first step in the biofilm formation is the adsorption of a so-called conditional layer that consist mainly of complex exopolymeric substances as these substances are already present in the aqueous environment emitted by the microorganisms. Secondly, if the conditions are favorable, a transformation from reversible to irreversible adhesion occurs and extracellular polymers (EPS) are secreted by the bacteria; then an early development of the biofilm structure starts and formed micro-colonies develops into a mature biofilm; and finally a dispersion of the biofilm cells in the neighboring environment [1]. Many efforts have been attempted to control biofilm formation in the food industry and hospitals, especially by actively cleaning and disinfecting surfaces. Yet, this antimicrobial treatment can be compromised due to disinfectants failure in penetrating the biofilm matrix that is attached to the surface or to bacterial resistance [2,3].

This paper focuses on the problematic of pathogenic biofilm formation in the food and medical industries. Several materials can be used in these fields such as Teflon, PET, titanium or stainless steel. Each material has its specificity and the bacterial adhesion rate can be different on each material depending on its nature and surface properties. For example, the adhesion rate

of staphylococcal species on Teflon and stainless steel was compared and the results showed that the majority of strains had a moderate adhesion rate (<20%) on stainless steel while a higher adhesion rate was observed on the Teflon surface [4]. The paper will focus on stainless steel because it is widely used in medical and food applications [5,6]. This is mainly due to its relatively low cost, ease of fabrication, good corrosion resistance, mechanical characteristics and good biocompatibility. It is estimated that nearly 60% of surgical implants used in the United States [5] and about 85% of surgical instruments are made of stainless steel [7].

Different surface treatment strategies for the prevention of bacterial adhesion and biofilm formation have been developed. Coatings incorporating antimicrobials have been used under different conditions and their effectiveness depends on the nature of the surface and the surrounding environment [8]. The state of the art of the main classes of biocides grafted on stainless steel, including silver nanoparticles (AgNPs) [9], essential oils (EOs) [10], light-activated antimicrobials [11], cationic molecules [12], antibiotics [13], enzymes [14] and antimicrobial peptides (AMPs) [15], will be presented. Among these methods, surface treatment using AMPs is a very promising way to reduce bacterial contamination by killing adherent microorganisms. Special attention will be given to AMPs based coatings for their interesting properties such as low toxicity and safety [16]. Generally, the AMPs are significantly more effective than classical biocides. They present advantages over conventional antibiotics, with a large spectrum of antibacterial, antifungal and antiviral properties [17,18]. They are also powerful with a fast germ-killing capacity and low bactericidal concentration. They are even efficient on conventional antibiotic-resistant species and also have synergistic effects with classical antibiotics to neutralize endotoxin [17,19]. Moreover, these AMPs are safe, without or less toxic side effects, and not easily induce bacterial drug resistance compared to conventional biocides [20]. A special focus is carried out on nisin, a widely used bacteriocin

and a healthy option to fight biofilm formation [21]. This bacteriocin is approved by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) as a safe food additive and is currently largely applied in the biomedical fields [22]. The advantages and disadvantages of each applied biocide will also be given.

The employment of stainless steel

In food manufacturing and hospitals, cleaning and disinfection are paramount. The material and equipment selected upstream will affect the future care and disinfection methods. In those fields, metals specifically, can provide exceptional strength properties. However, strength is not the only aspect to consider. The most adequate material needs to be as inert and non-corrosive as possible.

Stainless steel is characterized by the addition of chromium at least 10.5 % of total composition. Chromium is very reactive to oxygen and immediately forms a strong barrier on its outer surface. This barrier is highly resistant and protects internal structures from additional corrosion [23]. Indeed, this stainless steel alloy is one on the most largely used material in biomedical and food fields. This metal is selected for its mechanical and chemical stability, biocompatibility, good corrosion resistance, low price and non-toxicity. It is mostly employed in medical sectors for orthopedic implants and prosthesis, cardiovascular valves and stents, and also for 3D printing of custom-made implants [24]. Moreover, in agri-food industries, stainless steel is selected since it does not affect the food's colour or taste without contaminating it. It also provides amazing performance for maintaining food safety by being effortlessly and efficiently washed up and sterilized [25].

It is of importance to note that the major issue for researchers is to provide antibacterial and antiadhesive properties to implantable stainless steel. Moreover, it is difficult to elaborate

coatings with specific mechanical properties and adequate antimicrobial/antiadhesive effect at the same time. That is why scientists tried to modify stainless steel surface via multiple strategies and elaborate coatings with the employment of several antimicrobial molecules.

Antimicrobial coatings on stainless steel incorporating biocides

Silver nanoparticles coated on stainless steel

Since ancient times, the most frequently used antibacterial heavy metal is silver. This metal species fights bacteria by disrupting enzymatic activities, disabling the membrane function, damaging the DNA and oxidative stress [26]. Moreover, silver was demonstrated to exhibit an excellent bacteriostatic and bactericidal effect towards several bacterial species [27]. Different configurations of silver were used to develop the films. Ions, metallic nanoparticles and silver halide nanoparticles were integrated into Layer by Layer (LbL) coatings on stainless steel and afterward released to kill bacteria. AgNPs has been extensively admitted to possess effective antimicrobial properties related to its oligodynamic action and multiple modes of its biocidal action [28]. Silver-based nanoparticle mixed with a cationic polymer poly(3,4-dihydroxy-L-phenylalanine)-co-poly(2-(methacryloxy)ethyl trimethylammonium chloride) (DOPA) permitted the adhesion enhancement of the LbL coating to stainless steel surface. The mixture formed an aqueous suspension with stable AgO and AgCl nanoparticles which was gathered with the polyanion poly(styrene sulfonate) (PSS) on stainless steel surface. Micelles were formed by the interaction between the positively charged DOPA and the negatively charged PSS. This LbL coating imparted an efficient antibacterial activity, related to the silver ions release from the film, against *Escherichia coli* strains [29,30]. Moreover, antimicrobial coatings were elaborated by electrodeposition of Ag on stainless steel from an $AgNO_3$ aqueous solution. These films were designed for fracture repair implant and harmless to human osteoblasts

preventing in vivo bacterial infection. The coatings were challenged with *Pseudomonas aeruginosa* and 13-fold bacterial reduction was observed after 24 h [31]. Otherwise, Cowan et al. [9] evaluated the antibacterial efficacy of stainless steel coupons coated with a zeolite matrix containing silver and zinc (AgION). These coatings showed an antimicrobial behavior against Gram-positive strains like *Listeria monocytogenes* and *Staphylococcus aureus* and on Gram-negative strains like *Pseudomonas aeruginosa* and *Escherichia coli*. In another study, antibacterial coatings based on chitosan and bioglass particles were developed on stainless steel using electrophoretic deposition (EPD). These coatings showed an efficient antibacterial activity against *Staphylococcus aureus* [32]. Moreover, stainless steel coatings based on AgNPs thin layers elaborated by reduction of Tollen's reagent were developed using a formaldehyde-radiofrequency plasma functionalization. These coatings' antimicrobial efficacy was tested and resulted in 5-log reduction in *Listeria monocytogenes* populations after 5 h of exposure [33].

Several studies concerning AgNPs toxicity reported that a safe range can be established for the use of AgNPs in designing antimicrobial coatings [28]. However, despite silver's effective antibacterial activity, the toxic effect of AgNPs against the mammalian cells limited their use [34]. Moreover, Sung et al. [35] outlined that a constant exposure to AgNPs beyond 90 days caused inflammatory lesions that considerably weakened lung function of rats.

Essential oils coated on stainless steel

Essential oils (EO) are natural compounds that are produced by plants with an antimicrobial effect for their protection against Gram-negative and Gram-positive bacteria, fungi, viruses and yeasts [36].

EOs have a variety of structures, their mechanism of action against microorganisms is not clearly understood. They can be bacteriostatic in low concentration but bactericidal at higher

concentrations [37]. It is known to include hydrophilic and hydrophobic interactions with cell membranes of targeted microorganism. These interactions cause the increase membrane permeability leading to the loss of the cell's vital components like ions and molecules and ATP production and finally to cell death [38].

The application of essential oils in the development of antimicrobial coatings on stainless steel aimed precisely for their edible particularity. A study demonstrated the effective antimicrobial activity of isoeugenol coated stainless steel against *Staphylococcus aureus*, *Listeria monocytogenes* and *Pseudomonas fluorescens*. Indeed, the surface eliminated all viable cells from the coated stainless steel surface [10]. Moreover, another research evaluated the bactericidal power of Mentha piperita essential oil coated stainless steel. The coating highlighted an anti-adhesive behavior against *Escherichia coli* [39].

However, the native odor of EOs might be problematic to their applications by industrials since a consequent concentration is needed of EOs to achieve their antimicrobial efficiency. Furthermore, the presence of EO on equipment used to process food could adversely impact its sensory perception [40]. EOs are effective after migration from the coating. Indeed, EO-coated material will lose its activity with time [41]. Moreover, EOs activity can be considerably reduced when exposed to high pH levels and to high and low temperatures. It is also diminished in the presence of lipids, proteins, oxygen and polysaccharides [42]. Furthermore, their non-stability, volatility and immiscibility in water may cause problems for their applications.

Light-Activated Antimicrobials coated on stainless steel

Antimicrobial coatings can also be developed using light activated antimicrobial that are photo-activated compounds. Those species might be organic, aromatic like porphyrin, chlorophyll derivatives or inorganic oxides like titanium dioxide (TiO_2). The antimicrobial

activity become efficient when those compounds are exposed to light with a specific wavelength. The reaction gives reactive oxygen species that interact with the bacterial protective membranes and DNA and results in bacterial death [43,44]. These species are regarded as bacteriostatic rather than bactericidal [45]. Regarding stainless steel coated substrate with those photo-activated compounds, a study involved grafting a thin film of TiO2. This modified stainless steel showed an efficient antibacterial activity against *Bacillus pumilus* [11]. Moreover, the frequently touched surfaces play an important role in the spread of bacterial infections in hospitals. In a research, nanostructured TiO_2 coatings on stainless steel surface were elaborated. They were made via chlorine chemistry and elaborated to kill bacteria under visible light, via an enhanced photocatalytic activity. These coatings showed greater than 3-log reduction in viable *Escherichia coli* after 4 hours visible light exposure [46]. However, some of these antimicrobial coatings can only be activated by UV light or by very high intensity light sources which may be dangerous to human health [47].

Cationic molecules coated on stainless steel

Cationic molecules have the ability to impart a bactericidal activity while released from an LbL film or when are immobilized on the surface. They can be bacteriostatic when employed at a lower rate than the minimum inhibitory concentration (MIC) and bactericidal when used at higher concentration than the MIC [48]. Bacteria will be killed once in contact with these positively charged molecules. The antimicrobial properties of the cationic functional groups stabilized on a surface persist more than the released ones [30]. In a research, a type of coating using a polycation has been generated to impart antifouling and antimicrobial properties to stainless steel surface. Chitosan, a versatile hydrophilic polysaccharide was used for its large antimicrobial spectrum. The stainless steel surface was grafted with chitosan via polymer brushes based on poly (2-hydroxyethyl methacrylate) (PHEMA). The coating was developed

using LbL process, firstly, as an initiator, a layer of barnacle cement (BC) holding the alkyl bromide, an atom transfers radical polymerization (ATRP) of 2-hydroxyethyl methacrylate, was fixed to the surface. The hydroxyl groups of PHEMA were then converted to carboxyl groups for linking to chitosan. The coated surface reduced bacterial adhesion and presented an antibacterial efficacy against *Escherichia coli* [12].

The main positively charged structure used for its bactericide power are quaternary ammonium compounds (QACs) [49]. In a study, QACs were coated on silanized stainless steel substrates via alkylation of immobilized ethylene diamine using cold plasma techniques. The coated films showed bactericidal efficacy against *Staphylococcus aureus* and *Klebsiella pneumonia* [50].

In order to fight biofilm formation by preventing the bacterial adhesion, highly hydrophobic coatings were elaborated by LbL assembly technology using fluorinated polyelectrolytes. Stainless steel coated with 1% Nafion, a sulfonated tetrafluoroethylene based fluoropolymer-copolymer characterized with ionic properties. This ionomer has a large amount of sulfonate groups providing a negative surface charge. These coatings significantly reduced *Escherichia coli* adhesion thanks to electrostatic repulsion between the negatively charged bacterial cells and film surface [51].

However, the large employment of these biocides in food and healthcare sectors constitutes a risk to patients and consumer's health. Moreover, the extended use of cationic antimicrobials in these fields caused the development of bacterial resistance [52].

Antibiotics coated on stainless steel

In multiple studies concerning fighting bacterial contamination, antibiotics were incorporated in coatings and released from the films to exert its bactericidal effect. Those therapeutics were used especially in favor of multifunctional biomedical coatings, they can be bacteriostatic or bactericidal according to the drug type [53]. However, bacteria may develop resistance to antibiotics over time. Also, antibiotic grafted surfaces are not suitable for food industry employment [13,54,55]. However, in medical fields, the antibiotic resistance develops naturally via mutation, new bacterial resistance mechanisms are growing and disseminating worldwide, essentially owing to the overuse and/or misuse of antibiotics, as well as inconvenient infection prevention [56]. Antibiotic-resistant bacteria are regarded as an arising global disease and constitute a major public health problem [57]. Analyses done by European Centre for Disease Prevention and Control showed that antimicrobial resistance remains an important threat to public health in Europe [58]. This is why researchers need to find another solution to fight against pathogens.

Enzymes coated on stainless steel

Antimicrobial enzymes are ubiquitous in nature, participating meaningfully in the defense mechanisms of organisms against bacterial infection and fungi. They are wall-degrading enzymes that result in cell wall breakage and cell death [59]. They can be bacteriostatic and bactericidal depending on the concentration employed [60]. Antimicrobial enzymes are also employed for bactericidal film development. For example, stainless steel, pretreated with poly(ethylene imine) (PEI), was grafted with lysozyme and/or poly(ethylene glycol). It showed an antimicrobial activity against *Listeria ivanovii* and *Micrococcus luteus* [14]. Moreover, the efficiency of serine protease trypsin in preventing biofilm formation was

reported [61]. The trypsin-grafted bioactive coating was developed on stainless steel showed an efficient antibacterial activity against *Staphylococcus epidermidis* biofilm [61]. Despite their effectiveness, the economic cost for enzymes extraction and purification before use is very expensive [62].

Generalities on antimicrobial peptides

Multiple studies aimed to develop stainless steel coated with biocides like antibiotics, heavy metals and other new synthesized antimicrobial compounds. In fact, the bacterial resistance and the complexity of biocides validation process and clinical phase analyses compromise their application in food industry and medical fields. In this context, there is a pressing need to find alternative strategies for preventing microbial contamination on surfaces. AMPs were highlighted as promising candidates for antimicrobial surfaces elaboration due to their biocompatibility, low toxicity and effectiveness [16]. AMPs are amino acid sequences that constitute a key component of the innate immune system of many types of species like animals, plants, insects and microorganisms. The AMPs defense role grant them protection from invading microorganisms. Indeed, those protein-like antibacterial agents are obtained by extraction from the living organisms [63]. They have an effective antimicrobial effect against a broad spectrum of microorganisms including the antibiotic-resistant planktonic bacteria and biofilms [15]. Those agents are mostly cationic amphipathic peptides that connect with specific constituents of the negatively charged bacterial envelope via electrostatic interaction. Their commonly low molecular weight makes them capable of getting entrapped within the cell wall. This reaction results in disruption, destabilization and depolarization of the bacterial plasma membrane conducting to bacterial deadly permeabilization, after the leakage of the essential biomolecules [19,52]. In order to produce biocidal surfaces, AMPs can be linked covalently via

their amine, hydroxyls, carboxylic acid and thiols groups, to materials currently used in medical and food fields. There are several types of surface used for antimicrobial coating procedure, like stainless steel [6], titanium [65], polystyrene [66], glass [67], magnetic nanoparticles [68] and silicon [69]. More particularly, bacteriocins are AMPs ribosomally synthesized by bacteria of certain type of species, as a bacterial defense system. Bacteriocins target cells are the bacteria closely related to their producer strain which stay unharmed thanks to their specific immunity proteins [70]. Those proteinaceous toxins are either bactericidal with or without cell lysis, or bacteriostatic inhibiting the growth of bacteria [71]. In many food industries, bacteriocin are employed to avoid food spoilage and bacterial contamination [72]. Easily degraded by proteolytic enzymes of the mammalian gastrointestinal tract, bacteriocins are believed to be safe [73]. Indeed, they have a high prevalence in nature and established without associated public health risk. Lactic acid bacteria (LAB), generating bacteriocins, and bacteriocins are used as natural preservatives in food industries [74]. Another efficient alternative for medical and food sectors are the immobilization of bacteriocins onto surfaces, which improve their antimicrobial activity and stability in the equipment [75]. The very popular and most studied bacteriocin is nisin, a valuable compound for food and healthcare fields [76].

Stainless steel coatings incorporating antimicrobial peptides

In a previous study, covalent grafting of Antimicrobial Peptides (AMPs) such as magainin I and nisin was reported on a stainless steel surface pre-coated with a chitosan polymeric layer. The results showed that the modified stainless steel decreased the *Listeria ivanovii* adhesion and underlined an efficient antibiofilm activity [6]. Another work using LbL technique showed that nisin can be incorporated into a cross-linked coating with durable and strong antimicrobial power against *Bacillus subtilis*. The properties of such coatings were optimized using

successive immersions of the substrate. Firstly, in a solution of a polycationic copolymer to strongly anchor to the surface, and secondly, a successive dipping of the surface was carried out into a solution of a poly(methacrylamide) bearing oxidized poly(3,4-dihydroxyphenylalanine) moieties and then into a solution of a polymer bearing primary amines [77]. Moreover, a study investigated the covalent fixation of AMPs nisin, tritrpticin (Trp11) and 4K-C16 a tetrapeptide of lysine conjugated with a C16 aliphatic acid. The immobilization reaction was performed via a reaction between amine groups on the peptides and surface epoxy groups obtained by organic–polymeric interlayer deposited by RF-glow discharge plasma on stainless steel. The modified stainless steel showed an effective bactericidal power against *Escherichia coli* and *Bacillus subtilis* [78]. In another study, aiming to develop an easy cleaning stainless steel surface, C. Vreuls et al. [79] elaborated multilayer coatings that included peptides with antibacterial properties like nisin, Trp11, 4K-C16 and anti-adhesive properties like heparin and mucin. The antimicrobial behavior of the films was attested against Gram-positive and Gram-negative strains. Moreover, coated surfaces with antiadhesive molecules showed 95% effective reduction of *S. epidermidis* in comparison with uncoated stainless steel. The multilayered coatings obtained by combining both types of compounds, antibacterial and antiadhesive demonstrated a better cleanability. Indeed, the antiadhesive effect inhibited the formation of a dead bacterial layer that could have decreased the antibacterial efficacy by reducing the film exposure to cells. Furthermore, Cao et al. [80] carried out a research where polished stainless steel 304 was treated with dopamine as a coupling agent permitting a strong binding ability with two types of synthetic peptides. The peptide-modified stainless steel surfaces showed an antibacterial capacity and anti-biofilm power against *Staphylococcus aureus* correlating with topography characteristics. A group of researchers developed a two-step covalent bonding method, for the fixation of 1-Ethyl-3-(3-

dimethylaminopropyl)carbodiimide/N-Hydroxysuccinimide (EDC/NHS) functionalized nisin onto stainless steel, already coated with a stable polymerized aminosilica interlayer by organosilicon-based cold plasma coatings system. It highlighted that the modified stainless steel acquired an effective antibacterial property that was demonstrated against *Bacillus subtilis* 168. Indeed, almost 4 log10 reduction of Gram-positive cells were reached in comparison with bare stainless steel [81]. The same researchers inverted a wet chemical strategy, by functionalizing stainless steel surface with vinyltrimethoxysilane, maleic anhydride and EDC/NHS. Nisin was then linked to the surface via an amide bond formed between its NH_2 functional group and the EDC/NHS functionalized surface. The surfaces showed an effective antimicrobial power. Indeed, each of these immobilization approaches would result in a different orientation of nisin, that would affect the antibacterial efficacy [82,83].

Friedlander et al. [84] carried out a study aiming to find a solution for biofilm contamination on surfaces in dairy industries. A peptide-coated stainless steel was developed with antiadhesive effect towards Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Bacillus licheniformis* preventing biofilm formation. In addition, the elaborated surfaces did not impact the technological properties of dairy products. Another approach was developed for stainless steel 304L and titanium Ti-6Al-4V surfaces using an oxide coating deposited using nanosecond pulsed laser technology. Different surface morphologies were observed, a mudflat cracked surface on titanium, but no cracks on the steel. The two surfaces were nisin-infused and showed an active anti-microbial performance against *Listeria monocytogenes*. Moreover, the nisin fixed on those surfaces reacted differently to the release tests. Indeed, it was slowly liberated from the non-cracked SS surface and showed no liberation from the Ti-6Al-4V surface due to its immobilization in the micro-cracks created on the surface [85].

Recently, Cao et al, reported an operative pathway to manage marine biofouling on the surface of SS that relies on the interactions between a derived AMP (M2-DA) and the surface of SS. M2-DA was formulated by conjugating magainin II, a cationic amphipathic antimicrobial peptide, and dopamine, which was then used to modify the surface of SS. AFM, XPS and contact angle measurement showed that M2-DA was successfully linked to the substrate surface, while the morphology and wettability significantly changed after the surface modification. The thickness and robustness of M2-DA on the coating were also determined. *Vibrio natriegens* and *Citrobacter farmeri* adhesion test results demonstrated that M2-DA treated surfaces exhibit a strong antibacterial behavior, and the bacterial adhesion rate decreased by 99.79% and 99.33%, respectively [86].

Another valuable consideration is to characterize the adhesion and durability of each type of surface coating prior to its use, regardless of the biocide used. Several storage and sterilization procedures were tested for preservation of the integrity and the efficiency of coated materials. Saini et al. (2016) showed that cellulose nanofibers treated using non covalently bonded Nisin exhibited very low bactericidal activity against strong and resistant bacteria such as *S. aureus* after washing [87]. This means that non-covalent linkage of AMP to the surface weakens the antibacterial efficiency and the life service of the coating such findings corroborates those of Arakha et al. [88]. On the same wave, a research showed that if the AMP employed is linked covalently to the surface, the coating durability will be affected positively [77]. For example, Espejo et al. (2019), Cao et al (2020) and Qi et al (2011) adopted the covalent bonding of Nisin on the surface material. The elaborated coatings were demonstrated to be effective and to have a good life service [85,86,89]. Dutz et al. (2017) demonstrate that the storage period of protein coated materials might be extended by using optimized storing conditions (e.g., lower temperature, oxygen exclusion, reduced humidity) and that damaging

effects of the coated materials could be suppressed using UV radiation for sterilization. Such studies suggest that the storage conditions and post-treatments of coated materials must be adapted and optimized for long-term use applications [90]

Nisin qualification

Nisin is an AMP applied for its bactericidal properties in about 50 countries for the past 40 years [91]. It was approved by the United States FDA (Food and drug administration) as "generally recognized as safe" GRAS, for its application as a safe additive and antimicrobial agent limiting pathogenic contamination during the manufacturing chain in food industries [92]. Nisin was also confirmed by the EU as a safe preservative and added to the list of food additives under the European number E 234 [93].

Nisin applications has been extended to biomedical fields since it can prevent the growth of antibiotic-resistant bacterial strains [94] . There are six variants of nisin, the nisin A is the originally isolated form of nisin, which is a cyclic polypeptide that consists of 34 amino acids, and there are another five natural variants, Z, F, Q, U, and U2 differing by up to 10 amino acids in comparison with nisin A [95]. Nisin is a lantibiotic that belongs to class I bacteriocins [96]. It is a ribosomally elaborated and a post-translationally modified lantibiotic, produced by *Lactococcus* species for the formation of nisin A, Z, F and Q and *Streptococcus* species for the formation of nisin U and U2 [94,95].

In fact, the post-translational shift is responsible for providing the lantibiotics, including nisin, their biological activity [97]. Nisin is a positively charged polypeptide with a 3500 Da molar mass presenting 34 amino acid allocated in hydrophilic residues at the COOH-terminus and hydrophobic residues at the NH_2-terminus [98,99].

It is distinguished by intramolecular rings established by the unusual thioether-bridged amino acids called lanthionine, by 3-methyllanthionine, and it also holds other uncommon dehydrated amino acids like dehydrobutyrine (Dhb) and dehydroalanine (Dha) [100]

Moreover, according to De Vuyst & Vandamme [101] the rare amino acids present in its structure might be the reason for its thermo-stability and solubility in an acidic solution and its particular activity against bacteria. Nisin stability is directly linked to its solubility since nisin solutions are boilable in diluted hydrochloric acid at pH 2.5 or less without losing its activity. However, the stability of nisin remains constant after autoclaving at an acidic pH close to 2, but it gradually loses its activity as it increases to a basic pH.

Nisin antimicrobial properties and mechanism of action

Bacteriocins exert their antimicrobial activity mainly through interactions with the cell wall of its targeted microorganism [102]. Nisin bactericidal powers are efficient at pico to nanomolar concentrations relying on various mechanisms of action [103]. It interacts with the cell membrane precursor lipid II, implicating the inhibition of bacterial cell wall biosynthesis and pores formation in the membrane [104]. Nisin antibacterial mode of action involves firstly the binding of its cationic COOH-terminus with the anionic lipids of the bacterial cell wall via electrostatic interactions [105]. After nisin binding to the membrane, its amphiphilic character allows its hydrophobic NH_2-terminus to slide, parallely to the membrane surface, into the lipidic membrane via hydrophobic interactions [106,107]. The increased concentration of anionic lipids is crucial for an effective and deeper insertion of the peptide into the lipidic part of the bacterial cell wall. Indeed, several studies indicated that the ring structures and NH_2-terminus of nisin are relevant for its interaction with Lipid II. The different susceptibility of bacterial strains to nisin and their minimum inhibitory concentration (MIC) ranges could be due to the

difference of lipid II amounts between several strains [105,108,109]. Thereafter, a trans-membrane pore is created by nisin-lipid II complexes resulting in cytoplasmic membrane disruption and rapid efflux of essential cellular components like amino acids, ATP and ions leading to a cellular death [93,107].

Nisin antimicrobial spectrum and applications

Nisin is a well-studied compound of the lantibiotic family, and it has high antimicrobial activity against a broad range of Gram-positive bacteria while it is almost nil against Gram-negative strains. Nisin is bioactive against many foodborne pathogens like *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus* [106]. Moreover, several investigations have revealed that nisin can inhibit the growth of antibiotic-resistant bacterial strains, such as *Clostridium difficile*, *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* and *Enterococci* [94]. However, some studies showed that nisin, combined with other antimicrobials or bioengineered, have developed a bactericidal potency against both Gram-positive and Gram-negative species [100,110]. Indeed, there is an important interest in employing nisin with another molecule to extend its target spectrum. A study showed that nisin exposed to chelating compounds, freezing or sub-lethal heat developed an activity against Gram-negative bacteria [111] . The chelator EDTA damaged the outer cell wall, exposing the Gram-negative bacteria to bacteriocins [112]. Otherwise, bacterial strains can develop resistance to nisin by producing a nisin destroying enzyme, nisinase. Also, physiological changes can occur in the bacterial membrane composition [113,114]. That’s why it’s important to optimize the use of bacteriocins rather than exaggerate its use for avoiding bacterial adaptation [107].

The International Unit (IU) of nisin activity has been fixed as the activity encased in 1 µg of this International Reference Preparation, which is equivalent to the nisaplin, the unique commercial nisin. Nisaplin is elaborated with an activity standardized at the same level of 1 x 106 IU / g. Thus, 1 g of nisaplin has an activity of 106 IU, when 1 g of pure nisin contains 40 X 106 IU. A bioactivity of 40 IU consequently, is equal to 1 µg of pure nisin [101]. Nisin has found applications in cosmetics products as a natural antibacterial agent. A study showed that nisin, when combined with other preservatives usually causing allergies to consumers enabled a use of smaller quantities of those chemicals [115]. It is also applied in veterinary and biomedicine fields for certain infections treatments [116]. Also, in food sectors, nisin was used as a preservative and for elongating products shelf life [111,114]. Moreover, for advanced technologies, it was employed in antimicrobial food packaging development [107]. Bacteriocins were largely employed in food and medical sectors for antimicrobial coatings preparation. Several studies were carried out to set up innovative nisin-coated surfaces.

Conclusion

Antibacterial treated stainless steel surfaces are of everlasting relevance, but the requirements for such coatings are more challenging in biomedical and food applications. Preventing the formation of bacterial biofilms is a goal that researchers have attempted to achieve. From an overall perspective, it is difficult to predict which type of coating and which molecule are most suitable for employment. It will be determined by the surrounding environment, the conditions, and whether their applicability is intended to be short-term or long-term. It is also important to characterize the adhesion and life service of each coating type before its employment, regardless the biocide used. Such tests can give an idea of the coated surface application, for disposable or long-term employment. The AgNPs, EOs, light activated

antimicrobials, antibiotics, cationic molecules and enzymes were grafted on SS surfaces, in several studies, to be tested against pathogenic bacteria. However, the application and use of AMPs is of increasing importance. Indeed, AMPs are a diverse class of natural compounds that are generated as the first line of defense by all multicellular living entities. These peptides can have a broad range of activity to immediately destroy bacteria, yeasts, fungi and viruses. Grafting these natural molecules onto stainless steel is a promising approach. However, it is important to try multi-strategies to control bacterial contamination and eradicate microbiological risk in the food and medical sectors.

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CHAPTER: III

Cold plasma assisted deposition of organosilicon coatings on stainless steel for *Salmonella enterica* serovar Enteritidis adhesion prevention

Cold plasma assisted deposition of organosilicon coatings on stainless steel for *Salmonella enterica* serovar Enteritidis adhesion prevention

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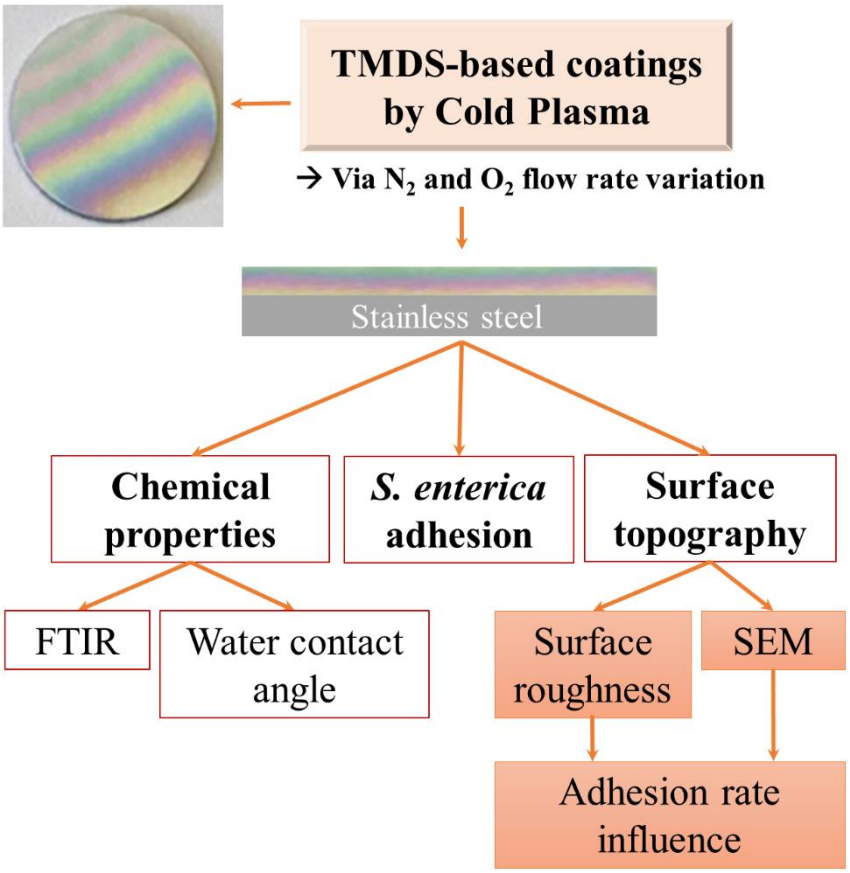
Abstract

Salmonella enterica persistence on abiotic surfaces in hospitals and agri-food industries leads to several infections worldwide. In this context, this work aimed to study the adhesion of *S.* Enteritidis on plasma-modified stainless steel to prevent biofilm-associated-infections. Surface modification was achieved by the elaboration of organosilicon coatings from the monomer 1,1,3,3-tetramethyldisiloxane, mixed with oxygen, using a flowing nitrogen microwave post-discharge plasma polymerization technique. The effect of cold plasma parameters on the coatings properties, coated surface topography and *S.* Enteritidis cells adhesion was studied. Results showed that the surface topography influenced the bacterial adhesion rate. Indeed, rough surfaces did not repel *S.* Enteritidis since the number of attached cells on these coatings was between 30 ± 4 to 65 ± 4 bacteria per microscopic field. Otherwise, smoother surfaces demonstrated an anti-adhesive character since the number of attached cells was almost nil on these coatings.

Keywords: *Salmonella* Enteritidis*;* biofilm; 1,1,3,3-tetramethyldisiloxane; coating; cold plasma; adhesion

Graphical abstract



Introduction

Preventing microbiological contamination of surfaces in food industries and medical fields is crucial since it impacts directly and negatively on population health. Indeed, bacterial contamination is responsible for foodborne illness and nosocomial infections around the world (Bhatta et al. 2018). *Salmonella* species are involved in 93.8 million food poisoning and 155,000 deaths per year and they are reported to be one of the most common foodborne pathogens (Eng et al. 2015). This pathogen is the cause of many hospital disease outbreaks, and it can be deadly for susceptible patients (Lee & Greig 2013). Moreover, according to the numbers released by the World Health Organization (WHO), *S.* Enteritidis is one of the 15 serovars of *Salmonella* that most often occurs in food and humans or on environmental surfaces (WHO 2018). In such environments, when conditions are suitable, adherent bacteria can grow and form a complex ecosystem, called a biofilm.

Bacteria within biofilms are known to be up to 1000-fold more resistant to disinfecting agents than free-floating planktonic ones (Costerton et al. 1987; Stewart & William Costerton 2001; Abdallah et al. 2014; Khelissa et al. 2017). Therefore, it is important to find a solution to get rid of bacterial adhesion and biofilm formation on surfaces. It has previously been reported that *S.* Enteritidis can adhere and form strong biofilms on materials such as stainless steel, polyurethane and polyethylene (Manijeh et al. 2008). Indeed, the adhesion capacity of *S.* Enteritidis is recognized as a virulence factor (Oliveira et al. 2007). In the current work, coatings were elaborated in an attempt to reduce bacterial adhesion, which is an important step in biofilm formation by *Salmonella* Enteritidis. The technology of surface characteristic modifications is a very promising approach to further prevent surface contamination (Feng et al. 2015). Hence, organosilicon-based coatings were deposited by a Cold Remote Nitrogen Plasma (CRNP) which is a part of Plasma-enhanced Chemical Vapour Deposition (PE-CVD) technique. With this coating process, substrates can be covered with various types of coatings

using gaseous precursors. In general, a gas monomer is fragmented, rearranged and polymerized under the influence of the plasma to generate a cross-linked coating (Dessaux et al. 1998). The deposition occurs in a downstream zone far from the discharge zone. Therefore, electrons or ions are no longer available, resulting in a higher deposition rate in comparison to the one obtained in the discharge zone. In previous works, remote plasma was used with 1,1,3,3-Tetramethyldisiloxane (TMDS) to obtain plasma polymerized films showing a polysiloxane-like structure (Callebert et al. 1994; Jama et al. 1996; Quédé et al. 2002). The Cold Remote Nitrogen Plasma (CRNP) is a non-ionized zone containing reactive species like nitrogen atoms in the ground electronic state N (4S), electronically excited N_2 triplet states and vibrationally excited N_2 in the ground electronic state. The monomer TMDS was employed for its ability to impart a hydrophobic character to coatings (Aoyagi et al. 2012; Dimitrakellis & Gogolides 2018). These coatings are used in a wide variety of applications. Their fire-retardant properties facilitate the production of protecting polymers such as polyamide-6 (Quédé et al. 2004; Bras et al. 2005). An efficient barrier effect, using these coatings, has been used to hinder Zn^{2+} diffusion from rubber cups to pharmaceutical liquid (Jama et al. 1996).

Several studies have demonstrated the effect of organosilanes on anti-adhesive, anti-bacterial and surface properties. Organosilane coated surfaces exhibit an increase in hydrophobic character and a decrease in free surface energy. These types of films ensure the inhibition of bacterial growth (Kregiel & Niedzielska 2014). Savela et al. (2012) and Kregiel et al. (2013) showed that following plasma-assisted surface silanization, the roughness of native surfaces decreases considerably, leading to an increase in anti-adhesive properties of coated surfaces. Furthermore, organosilicon-based films developed on 316L stainless steel, by atmospheric pressure plasma spraying (APPS) of hexamethydisiloxane, showed an antifouling character and anti-adhesive properties towards *Staphylococcus aureus* (Zouaghi et al. 2018).

In the current study, the effect of CRNP assisted organosilicon coatings on bacterial attachment was assessed. The effect of CRNP parameters such as nitrogen and oxygen gas flow rates during the coating elaboration on the surface chemistry and morphology was studied. Fourier transform infrared and water contact angle measurements were used to chemically characterize the coatings. Bacterial attachment tests were also performed. Then and in order to understand the results, the surface topography of the deposits was analyzed by measuring the surface roughness and evaluating contrasts by scanning electron microscopy.

Materials and Methods

Materials, bacterial strains and culture condition

Circular SS 314L coupons of 40 mm diameter and 1 mm thickness were used for coating elaboration (Equinox, Willems, France). Nitrogen and oxygen gases (grade 99.998 %) used for CRNP deposition process were provided by Air Liquide (Paris, France). The monomer 1,1,3,3-tetramethyldisiloxane 97% was acquired from Sigma Aldrich (Lyon, France). Distilled water was used for contact angle measurements. The bacterial strain selected for this study was *Salmonella enterica* serovar Enteritidis (Collection Institut Pasteur CIP 8297 - ATCC 13076 - NCTC 12694). The strain was stored at -20°C in Tryptic Soy broth (TSB; Biokar Diagnostics, France) containing 40% (v/v) of glycerol. The pre-culture was prepared by inoculating 100 µl of the stock culture into 5 ml of sterile TSB which was incubated for 24 h at 37°C. The working culture was started in 50 ml of sterile TSB inoculated with 10^4 CFU ml^{-1} of *S.* Enteritidis from the pre-culture and incubated under stirring (160 rpm) at 37°C for 16 hours to be harvested in the late exponential phase.

Plasma coatings Elaboration

The stainless steel (SS) coupons were standardized before plasma coating. Briefly, coupons were soaked for 10 minutes in absolute ethanol (Brabant, France), then air-dried before being autoclaved at 120°C for 20 min. Cold remote nitrogen plasma (CRNP) was used to elaborate coatings from TMDS mixed to dioxygen. The reactor used was described elsewhere by Esbayou et al. (2019). The TMDS monomer was selected in order to provide antifouling properties to SS surface. A stainless steel disc was placed on the substrate-holder already fixed in the center of the reaction chamber (30 cm diameter, 60 cm height). The air in the plasma reactor chamber was pumped out and the pressure in the reactor was reduced to 3 Pa then the pre-treatment step, also called substrate activation step, was started. A nitrogen gaseous flow (purity = 99.995%)

was injected by a continuous pumping (Primary Pfeiffer pump with roots Pfeiffer vacuum). The nitrogen flow rate used for the different coating types was fixed to 4.3 or 3 slpm by a MKS mass-flow controller. Once the nitrogen flow rate stabilized, a plasma discharge was created by exciting the nitrogen, using an electrodeless discharge through a coaxial device in a quartz tube of 19 mm diameter and operating with a microwave generator at 2450 MHz with a transmitted power of 1000 W. The long-term life excited species traversed a 1.1 m distance from the discharge to reach the deposition zone in the chamber where the CRNP appeared as a yellow afterglow. This activation step lasted for 5 minutes. After this step, the TMDS at a flow fixed at 3 sccm mixed to dioxygen set at 0.01, 0.025, or 0.05 slpm was injected through a coaxial Pyrex tube at a distance of 15 cm from the substrate. The deposition time for each condition 5 was minutes. Table 1 shows the different TMDS, oxygen and nitrogen flow rates used for coating elaboration. Sample references were divided into two categories: TMDS-N_2-3 and TMDS-N_2-4.3 where TMDS flow rate always fixed at 3 sccm and nitrogen (N_2) flow rates were fixed at 3 and 4.3 slpm respectively. For each category oxygen (O_2) flow rates were varied from 0.01 to 0.025 to 0.05 slpm. For each test, the uncoated stainless steel was taken as a control.

Table 1: TMDS, N_2 and O_2 flow rates used for coating elaboration.

Samples reference		**TMDS (sccm)**	**N_2 (slpm)**	**O_2 (slpm)**
TMDS-N_2-3	TMDS-N_2-3-O_2-0.01	3	3	0.01
	TMDS-N_2-3-O_2-0.025	3	3	0.025
	TMDS-N_2-3-O_2-0.05	3	3	0.05
TMDS-N_2-4.3	TMDS-N_2-4.3-O_2-0.01	3	4.3	0.01
	TMDS-N_2-4.3-O_2-0.025	3	4.3	0.025
	TMDS-N_2-4.3-O_2-0.05	3	4.3	0.05

Fourier transform infrared characterization

The spectra of deposited films and TMDS monomer were recorded at room temperature by Fourier Transform Infrared (FTIR) spectrometry using a Nicolet iS50 FT-IR spectrometer (Thermo Scientific Waltham, USA). Light source of transmittance was in the middle range infrared 500–4000 cm^{-1} with 64 scans and a resolution of 4 cm^{-1}. All the spectra were analyzed using the OMNIC software. A spectrum of the TMDS monomer was taken as a control.

Water contact angle measurements

The wettability of TMDS-coated surfaces was determined by water contact angle (WCA) measurements using a Digidrop ASE (GBX, France) goniometer. Measurement values were calculated by Young-Dupré's law using Windrop ++ software. Briefly, five droplets (5 µl) of demineralized water were deposited on the coupon's surface in distinct zones. Average and standard deviation were calculated and WCA greater than 90° were considered hydrophobic.

MATS: Microbial affinity to solvents

The hydrophobic / hydrophilic nature of *S.* Enteritidis surface was determined by the MATS test (Bellon-Fontaine et al. 1996). It involves the evaluation of the affinity of the bacteria's surface towards polar and apolar solvents. The solvents employed were, hexadecane (apolar), decane (apolar), chloroform (monopolar and electron-acceptor), ethyl acetate (monopolar and electron-donor). *S.* Enteritidis cells were grown in TSB overnight at 37°C, and were washed with saline (0.85% NaCl) two times by centrifugation at 5000 *g* for 10 min at 20°C. The test was carried out using 2.4 ml of bacterial suspension at 10^8 CFU ml^{-1} (corresponding to an optical density of OD_{620} nm = 0.110 ± 0.005 (Jenway spectrophotometer 6320D, Brumath, France) and 0.4 ml of the solvent. The mixture was vortexed at maximal speed (2400 rpm) for 2 min to form an emulsion and left for 30 min to settle allowing separation of the two phases. The optical density (OD) of the aqueous phase was measured at 400 nm and the percentage of

affinity to solvent was established using the equation: $\% \, Affinity = \left[1 - \left(\frac{A}{A_0}\right)\right] . 100$

➔ where A is the OD of the bacterial suspension before mixing and A_0 is the OD after mixing.

The low (<30%) / high (>70%) affinity for apolar solvents (decane, hexadecane) translates to a hydrophilic / hydrophobic character. In addition, a higher affinity for ethyl acetate than for decane reflects a basic character (electron donor) and a higher affinity for chloroform than for hexadecane indicates an acidic character (electron acceptor).

Bacterial suspension and adhesion test

S. Enteritidis cells were collected by centrifugation (5000 *g*, 10 min, 20°C) and washed twice with 20 ml of sterile physiological saline solution (NaCl 0.85%). The cells were then re-suspended in 20 ml of saline solution. The *S.* Enteritidis suspension (1×10^8 CFU ml^{-1}) was sonicated at 37 kHz for 5 minutes at 20°C (Elma S40 Elmasonic, Germany) to disperse cells before the spectrophotometry reading. This suspension was then diluted 10 times for bacterial adhesion tests (10^7 CFU ml^{-1}). Thereafter, to proceed to the adhesion test, both coated and uncoated-SS slides, were placed individually in a sterile NEC-biofilm system as described by Abdallah et al. (2015). Five ml of the prepared bacterial suspension were incubated on either coated or uncoated slides at 20°C for 60 min, under static conditions, to allow bacterial to attach. After removal of the suspension, the slides were gently rinsed with 20 ml of sterile saline solution to remove weakly attached cells. Then, adherent cells were stained with 2 ml of Acridine Orange (AO) for 10 minutes in the dark. Furthermore, substrates were rinsed twice with distilled water to clear off the excess of AO and left to air-dry in the dark. Cells stained with AO were observed under an epifluorescence microscope (Olympus BX53) with 100 x objective. A total of 25 fields per coupon was captured with a digital camera and stained cells

were enumerated. Mean value of adhered *S.* Enteritidis per microscopic field and its relative standard deviation were presented. This test was repeated three times. Three slides for each coating type were studied in each test.

Surface roughness and thickness analyses

The surface roughness and the thickness of plasma deposited coatings was determined using a surface profilometer Alpha-step IQ (Kla Tencor, Milpitas, California). The topography of the coating was characterized by scanning the surface with a diamond stylus supported by a sensor that registers the movement. The stylus measures the vertical position (*Z*) when it moves horizontally (*X*) on the surface, allowing to find the surface profile $Z = f(X)$. Bare SS was the control sample. Substrates were scanned over a length of 1 mm with a scan speed of 20 μm s^{-1}, a sampling rate of 50 Hz and a resolution of 400 nm. Each sample was scanned in three locations. The average roughness (R_a) and the root-mean-squared roughness (R_q) were calculated according to the following equations $R_a = \frac{1}{n}\sum_{i=1}^{n}|Y_i|$ and $R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} Y_i^2}$.

The coatings thickness was also measured. For this, tape was stuck on a part of the stainless steel sample, and then the organosilicon coating is elaborated over the entire surface of the sample including the covered part. Immediately after the plasma treatment, five different measurements between these two zones were carried out. The average values for coatings thickness were then calculated.

Evaluation of coating adhesion to stainless steel

The adhesion of coatings on a metal substrate was evaluated by the cross-cut method according to the standard test ASTM D3359-17 (D01 Committee 2017). In this method, a scratch is made on the coating surface, tape is applied then it is peeled off and the percentage of chipped areas in the coating is calculated. The adhesion test results are classified from 5B to 0B. The 5B class shows no peeling of the coating and none of the squares of the lattice are detached. The other

levels 4B, 3B, 2B, 1B to 0B show the presence of chipping at the corners and along the edges from 5 %, 5-15 %, 15-35 %, 35-65 % and 65-100% respectively. Three repetitions for each type of coating were carried out.

Scanning electron microscopy analyses

Scanning electron microscopy (SEM) was performed to visualize the topography of bare and plasma coated SS surfaces. SEM analyses were carried out using a Hitachi S-4700 SEM equipped with a field emission gun (FEG). Beforehand the observations, samples were sputter coated with carbon to become conductive using a BAL-TEC SCD 005 Sputter Coater.

Statistical analysis

All experiments were carried out at least three times. Statistical analysis was performed with IBM SPSS 19 statistics software using one-way ANOVA. Results were considered significantly different when $P < 0.05$.

Results

Chemical properties of TMDS coatings

The TMDS monomer's chemical structure is presented in Figure 1.

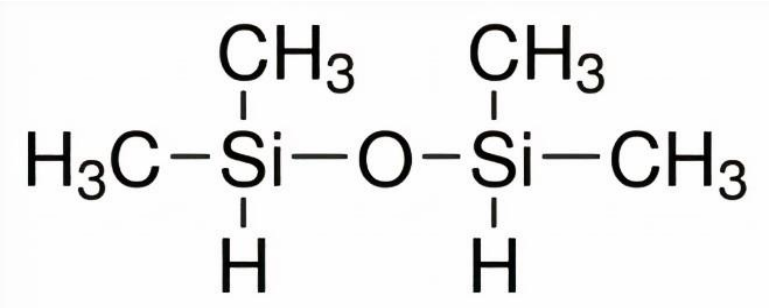


Figure 1. Chemical structure of 1,1,3,3-Tetramethyldisiloxane

This monomer and the TMDS coated surfaces were analyzed by Fourier transform infrared spectroscopy (FTIR). A comparison of TMDS spectrum (spectrum A: control) and the coated surfaces spectra (spectra B) was investigated (Figure 2). The FTIR spectrum of the TMDS

monomer showed peaks related to the chemical bonds present in the TMDS monomer. Spectrum B showed peaks related to chemicals bonds present in the polymerized TMDS (Table 2). The absorbance spectra corresponding to each type of coating, elaborated at different nitrogen and oxygen flow rates, were broadly similar. Indeed, peaks occurred at the same wavenumber and their absorbance became greater with an increase in the oxygen flow rate. In all spectra of coated films and TMDS spectrum, different peaks indicated the presence of methyl (CH_3) groups. The asymmetric $CH_3(\nu(CH_3))$ stretch was detected at 2960 cm^{-1}. The Si-$(CH_3)_2$ group was identified by its strong, sharp band at 1260 cm^{-1} and blocks of dimethyl units were represented by a relatively weak band at 860 cm^{-1}. The peaks at 850-830 cm^{-1} indicated the presence of Si-CH_3 groups. Moreover, the peak at 784 cm^{-1} represented the elongation band (ν(Si-C)). The broad band at 3300 cm^{-1}, appeared distinctly in the case of an oxygen flow rate equal to 0.05 slpm, and were associated to the formation of Si-OH (silanols) groups while this peak was not observed in the TMDS spectrum. Otherwise, the adsorption band located at 2122 cm^{-1} on the TMDS spectrum, was attributed to ν(Si-H) which disappeared from the spectra of coated films. Siloxane products showed very strong infrared peaks in the wavenumber region of 1130-1000 cm^{-1} representing the Si—O—Si band. As the siloxane chains became longer or branched, the Si—O—Si absorption became broader and more complex, showing two or more overlapping bands. Indeed, the appearance of a shoulder at 1080 cm^{-1} overlapping the main band at 1030 cm^{-1} was related to the symmetric stretching of Si-O groups. The absorbance

intensity of these peaks increased with oxygen flow rate for both TMDS-N_2-3 and TMDS-N_2-4.3 coatings (spectra B) (Launer & Arkles 2013).

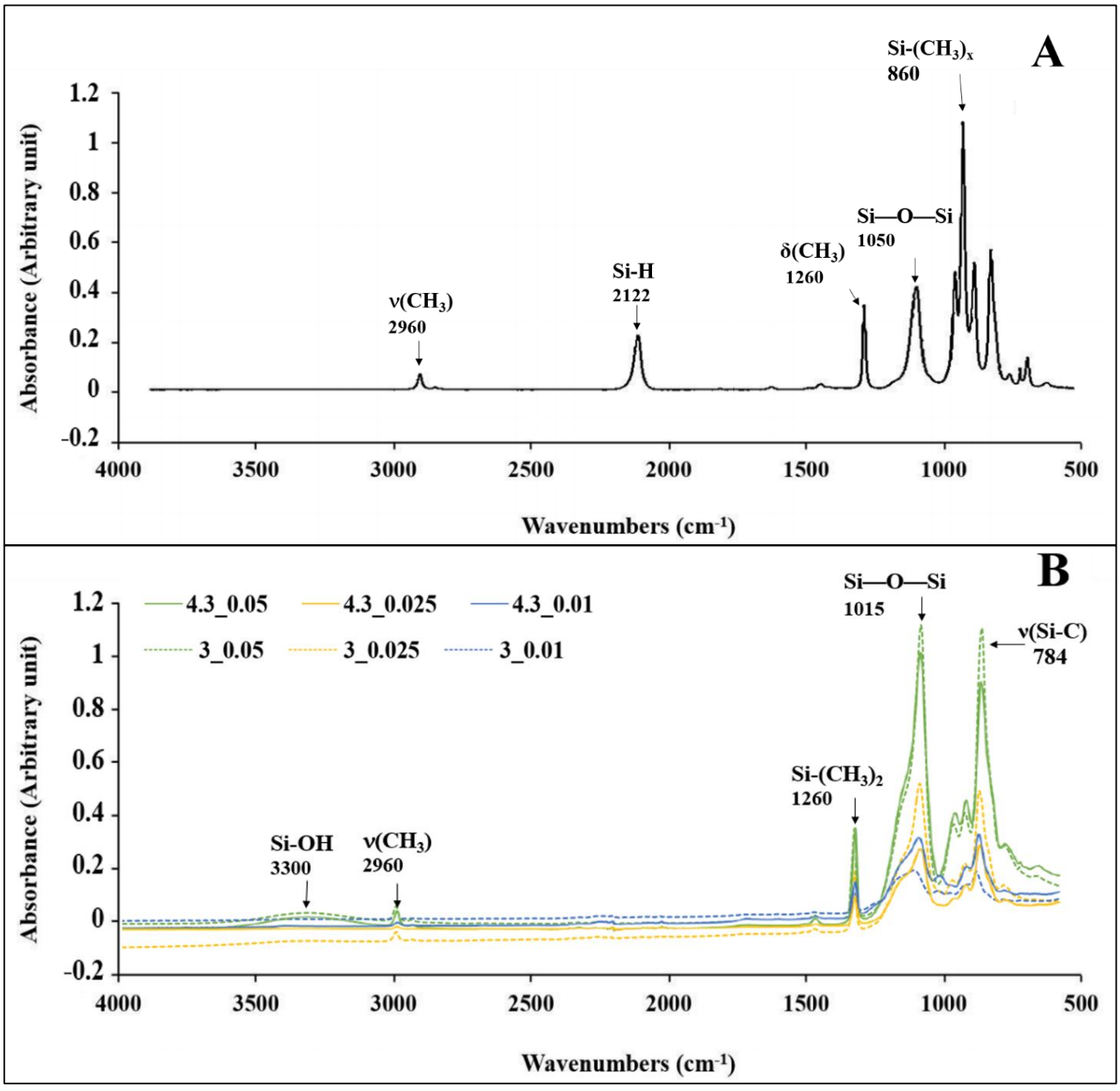


Figure 2. (A) FTIR Spectrum of TMDS monomer as a control. (B) FTIR Spectrum of TMDS coated surfaces; N_2 flow rate = 4.3 and 3 slpm varying O_2 flow rate from 0.01 to 0.025 to 0.05 slpm.

Table 2: FTIR peak positions and assignments.

Peak position (cm^{-1})	Assignments
3300	ν(OH), Si-OH
2960	$\nu(CH_3)_{as}$
2910	$\nu(CH_3)_s$
2122	ν(Si-H)
1260	$\delta(CH_3)_s$, Si-$(CH_3)_2$
1050	ν(Si-O-Si)$_{as}$
860	$\delta(CH_3)$, ν(Si-C), Si-$(CH_3)_2$
846	$\delta(CH_3)$, ν(Si-C), Si-$(CH_3)_2$
784	ν(Si-C), Si-$(CH_3)_2$

Water contact angles (WCA) of TMDS-coated or uncoated surfaces were measured in order to evaluate surface wettability in each coating condition. The WCA of bare SS coupons, employed as a control, was of 61° ± 3, showing the relatively hydrophilic character of an uncoated SS surface. The TMDS-N_2-3 coatings had a hydrophobic character, as their WCA measurements were around 95° regardless of oxygen flow values conditions. Moreover, the WCA measurements of TMDS-N_2-4.3 coatings showed a more hydrophobic character than the TMDS-N_2-3 coatings. The WCA of these coatings increased respectively from 109° ± 1 to 128° ± 1 to 138° ± 1 when the oxygen flow rate decreased from 0.05 to 0.025 to 0.01 slpm (Figure 3).

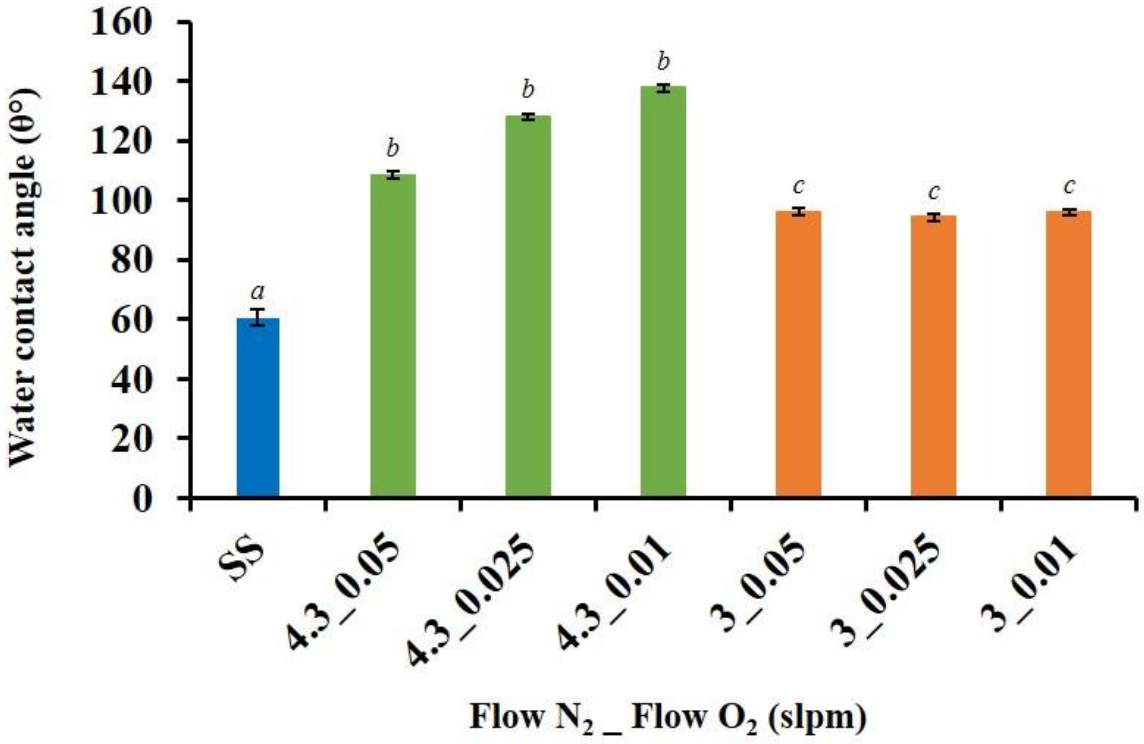


Figure 3. Water contact angle measurements for uncoated and coated SS; (Blue) uncoated SS; (Green) TMDS coatings with N_2 flow rate = 4.3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm (Orange) TMDS coatings with N_2 flow rate = 3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm. (*) each represented value is a mean of 6 measures for each type of coupon. Different letters on error bars (a, b, and c) indicate significant differences ($P < 0.05$); same letters on error bars indicate non-significant differences ($P > 0.05$).

The results of the MATS test showed that the low percentage affinity for apolar solvents (hexadecane and decane) translated to a hydrophilic character of *S.* Enteritidis. Indeed, a percentage of 1.0 ± 0.6 and 1.5 ± 0.7 was obtained for hexadecane and decane respectively. Moreover, *S.* Enteritidis displayed maximal affinity of 22 ± 6 percent for the acidic solvent (chloroform) and a low affinity of 2 ± 1 for the basic solvent (ethyl acetate). The adhesion of *S.* Enteritidis was higher to chloroform than to hexadecane, two solvents with similar van der Waals properties. These results, demonstrated that cell surface of this strain was strongly electron donor (i.e. basic) and very weakly electron acceptor (i.e. acidic) (Table 3).

Table 3: Affinity of *S.* Enteritidis for solvents used in MATS test

	Hexadecane	Decane	Chloroform	Ethylacetate	Electron donor	Electron acceptor
% Affinity	1.0 ± 0.6	1.5 ± 0.7	22 ± 6	2 ± 1	21 ± 6	1 ± 2

Anti-adhesive character of TMDS coatings

A bacterial attachment assay was performed on uncoated-SS as a control and TMDS-coated samples in order to give evidence of the link between the bacterial adhesion and the chemical character of the coating. The number of attached *S.* Enteritidis cells was estimated after AO staining using epifluorescence microscopy. The average number of adherent *S.* Enteritidis per microscopic field on uncoated-SS was 20 ± 3 (Figure 4). Figure 5 shows one of the 25 fields captured of the uncoated SS, presenting 23 adhered *S.* Enteritidis cells. The rate of bacterial attachment on the TMDS-N_2-4.3 and TMDS-N_2-3 coatings was significantly different ($P < 0.05$). The figures representing the fields showing the enumerated *S.* Enteritidis of each type of coating were shown in Figure 5. Indeed, TMDS-N_2-4.3 coatings contained more attached cells than uncoated-SS and attachment increased with a decrease in O_2 flow rate. The number of cells attached varied from 30 ± 4 cells per microscopic field for TMDS-N_2-4.3-O_2-0.05, to 63 ± 4 and 65 ± 4 for TMDS-N_2-4.3-O_2-0.025 and TMDS-N_2-4.3-O_2-0.01 respectively (Figure 4). The same tendency of bacterial attachment presented in Figure 4 is presented in Figure 5, for samples (4.3_0.05), (4.3_0.025) and (4.3_0.01). However, TMDS-N_2-3 coatings showed a very low number of adhered *S.* Enteritidis regardless of the oxygen flow value employed. Indeed, the registered values of adhered cells on TMDS-N_2-3-O_2-0.05 was 1 ± 1 cells and TMDS-N_2-3-O_2-0.025 was 2 ± 1 cells and TMDS-N_2-3-O_2-0.01 was 1 ± 1 cells. Figure 5 shows, for samples (3_0.05), (3_0.025) and (3_0.01), that the number of adhered bacteria was almost nil. The difference in the number of cells attached between TMDS-N_2-4.3 and TMDS-N_2-3 was surprising since FTIR analyses results showed a similar chemical structure for both coatings.

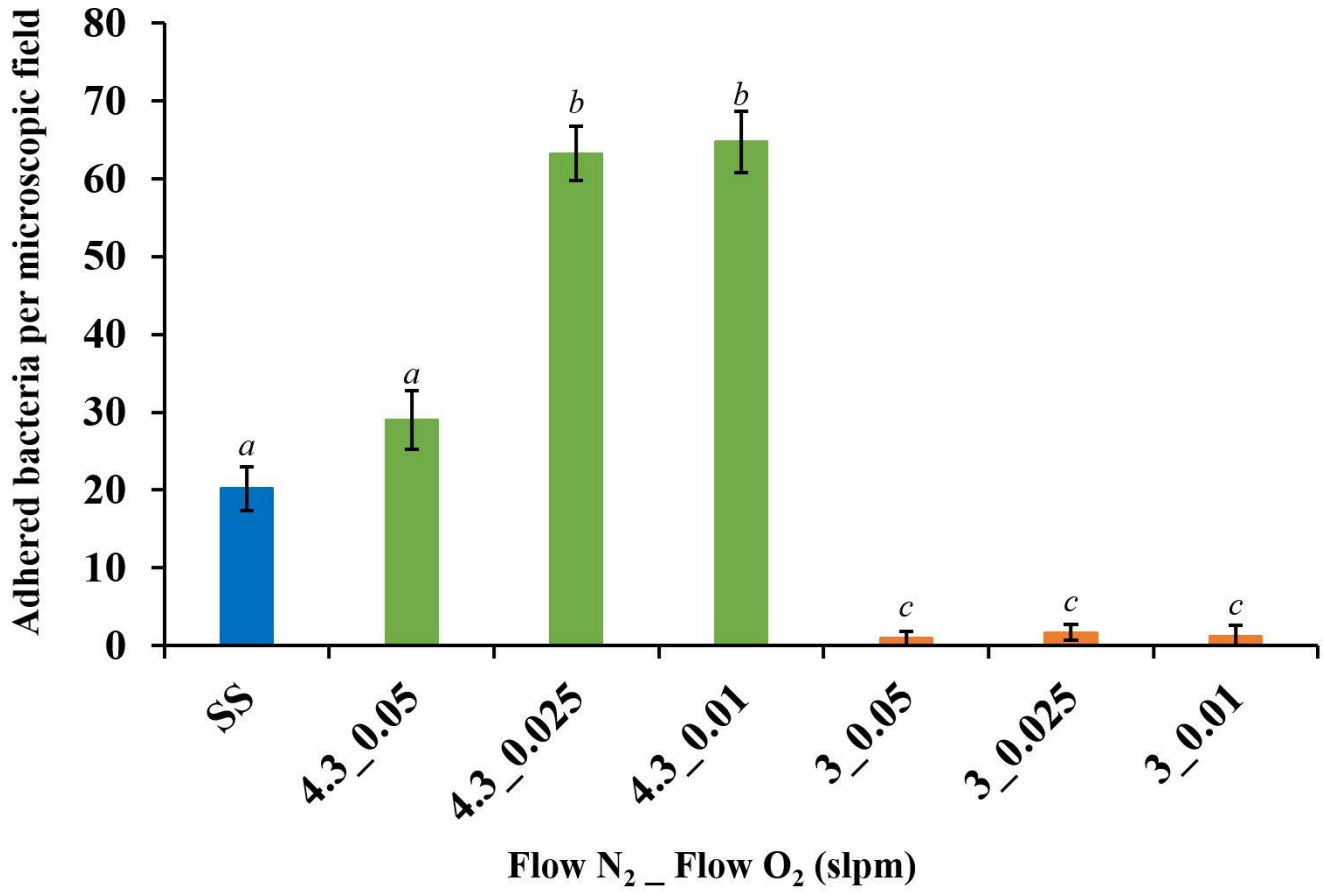


Figure 4. Adhered *Salmonella* Enteritidis per microscopic field on the coupons according to the variation of N_2_O_2 flow rate. (Blue) uncoated SS; (Green) TMDS coatings with N_2 flow rate = 4.3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm (Orange) for TMDS coatings with N_2 flow rate = 3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm. Different letters on error bars (a, b, and c) indicate significant differences ($P < 0.05$); same letters on error bars indicate non-significant differences ($P > 0.05$).

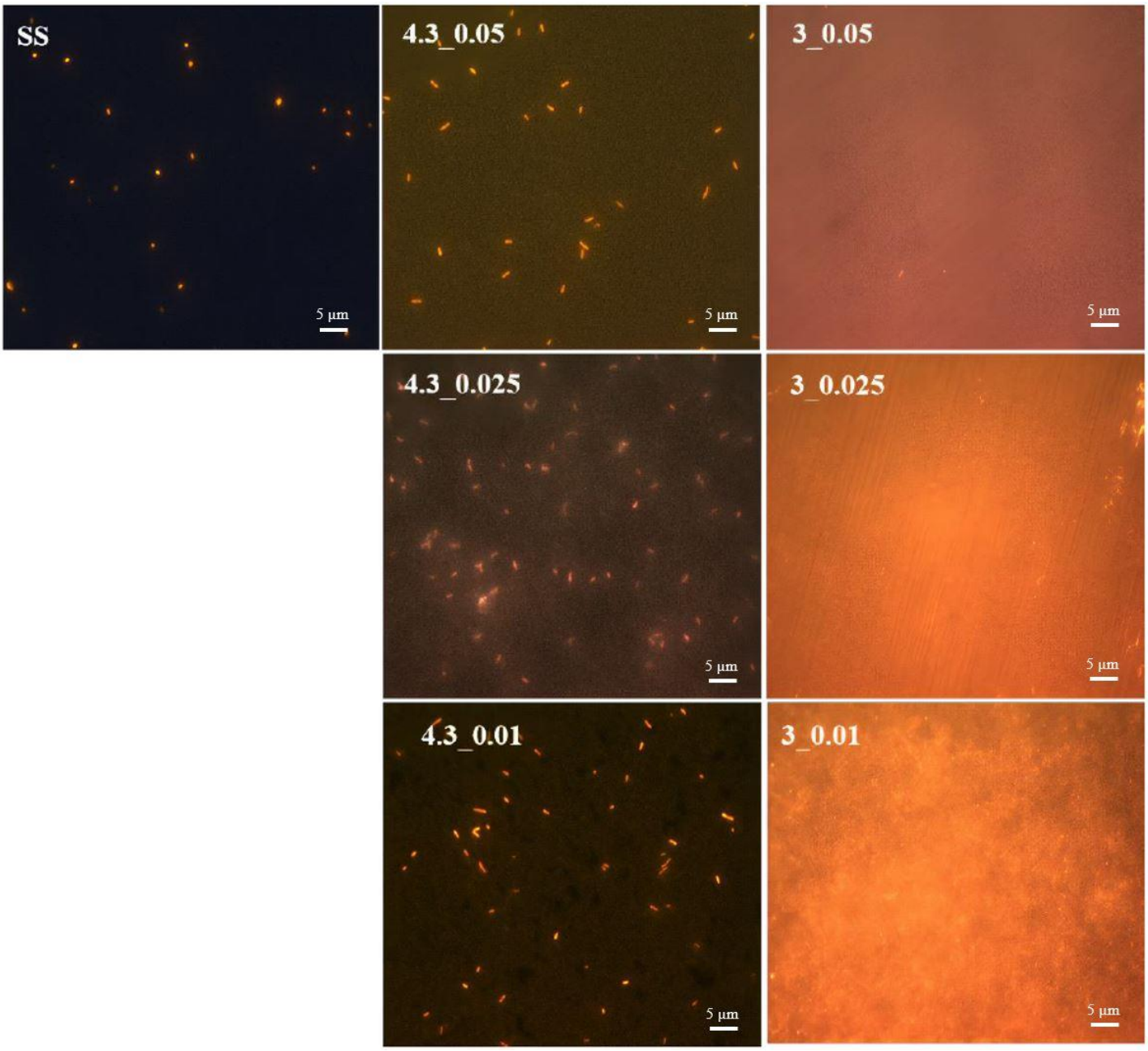


Figure 5. Epifluorescence microscopy of *Salmonella* Enteritidis adhesion; (SS) Adhered *S.* Enteritidis on uncoated SS; (4.3_0.05, 4.3_0.025; 4.3_0.01) Adhered *S.* Enteritidis on TMDS coatings with N_2 flow rate = 4.3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm; (3_0.05, 3_0.025; 3_0.01) Adhered *S.* Enteritidis on TMDS coatings with N_2 flow rate = 3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm.

Topography of TMDS coatings

The surface roughness results showed that uncoated-SS had a smooth surface, where the average roughness R_a was equal to 3.0 ± 0.1 nm and R_q equal to 4.0 ± 0.5 nm. Coated surfaces showed different roughness values where TMDS-N_2-4.3 surfaces were rougher than TMDS-N_2-3. For TMDS-N_2-4.3 surfaces, the roughness values increased with decreasing oxygen flows, although it did not affect the roughness of TMDS-N_2-3 surfaces. Indeed, TMDS-N_2-4.3-O_2-0.05 surface roughness increased to a value R_a equal to 20.5 ± 0.1 nm and R_q equal to 33 ± 1 nm. As the oxygen flow rates decreased, the surfaces roughness increased. The TMDS-N_2-4.3-O_2-0.025 surfaces R_a was equal to 54.5 ± 0.6 nm and R_q equal to 70 ± 1 nm and the TMDS-N_2-4.3-O_2-0.01 surfaces R_a was equal to 70 ± 9 nm and R_q equal to 83 ± 7 nm. Otherwise, TMDS-N_2-3 coatings were characterized by a smooth surface where their Ra and Rq did not exceed 5 ± 1 nm and 9 ± 1 nm respectively (Figure 6). The results of thickness test showed that thickness increased with an increase in oxygen flow rate and that the nitrogen flow rate did not modify the thickness values. Indeed, TMDS-N_2-4.3-O_2-0.05 and TMDS-N_2-3-O_2-0.05 coatings registered the highest thickness equal to 0.9 ± 0.2 µm. In addition, both TMDS-N_2-4.3-O_2-0.025 and TMDS-N_2-3-O_2-0.025 coatings registered 0.7 ± 0.2 µm thickness. This value decreased for TMDS-N_2-4.3-O_2-0.01 and TMDS-N_2-3-O_2-0.01 which registered 0.6 ± 0.2 µm and 0.6 ± 0.1 µm respectively. The adhesion of the coatings to SS substrate showed for all the coatings a high adhesion of 5B classification (Table 4).

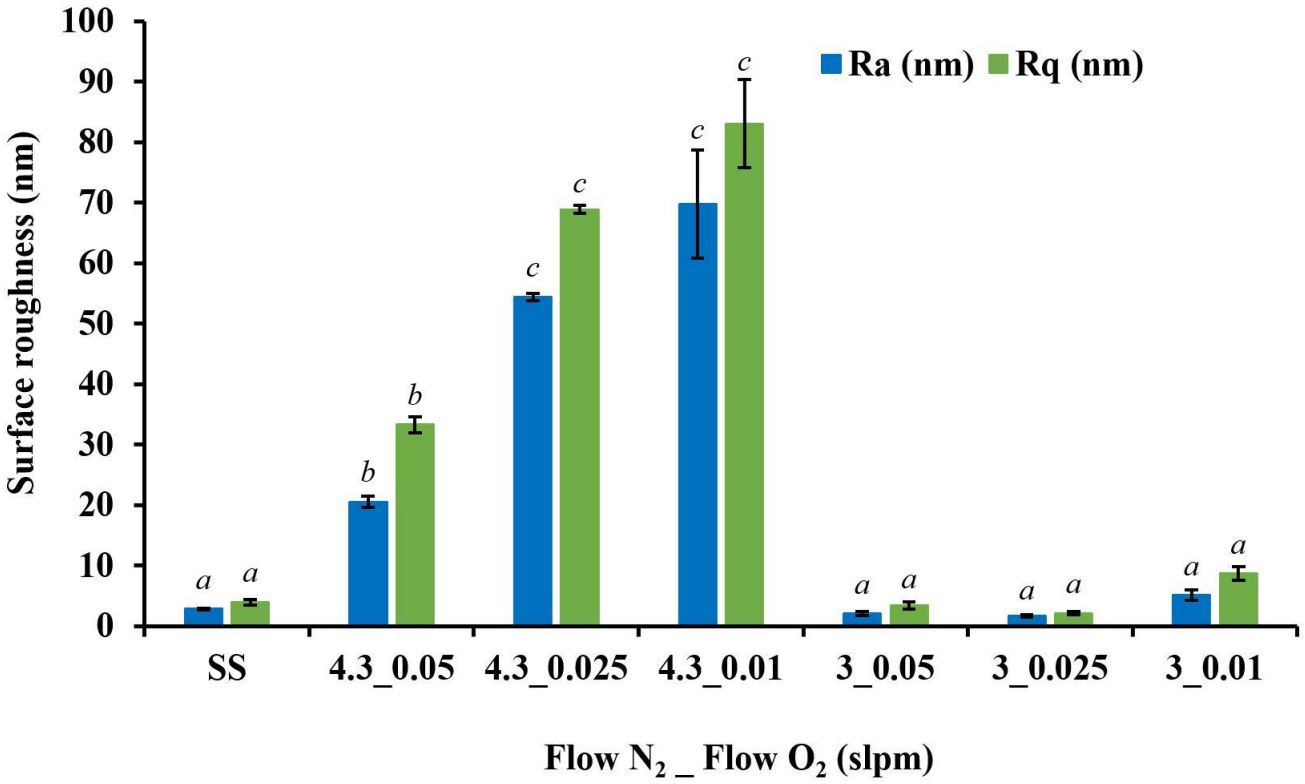


Figure 6. Surface roughness variation of bare SS and TMDS-based coatings. (Blue) representing the Ra (nm) and (Green) representing the Rq (nm). (*) each represented value is a mean of 6 measures for each type of coupon. Different letters on error bars (a, b, and c) indicate significant differences ($P < 0.05$); same letters on error bars indicate non-significant differences ($P > 0.05$).

Table 4: Surface characterization: thickness (µm) and adhesion to SS.

Coating Type	Surface characterization	
	Thickness (µm)	Adhesion to stainless steel
TMDS-N_2-3-O_2-0.01	0.8 ± 0.1	5B
TMDS-N_2-3-O_2-0.025	0.7 ± 0.2	5B
TMDS-N_2-3-O_2-0.05	0.9 ± 0.2	5B
TMDS-N_2-4.3-O_2-0.01	0.6 ± 0.2	5B
TMDS-N_2-4.3-O_2-0.025	0.7 ± 0.2	5B
TMDS-N_2-4.3-O_2-0.05	0.9 ± 0.2	5B

After surface roughness, thickness and adhesion analyses, SEM was used to qualitatively characterize the topography of the coatings. Uncoated-SS showed a smooth surface with small

holes and crevices. This was probably due to its mechanical polishing. The SEM micrographs showed that the TMDS coatings covered all the SS surface whatever the studied condition. The TMDS-N_2-4.3-O_2-0.05 coatings were characterized by a relatively smooth surface with the presence of spherical microstructures distributed homogeneously on the coating. However, when the oxygen flow rate was diminished to 0.025 and 0.01 slpm, TMDS-N_2-4.3-O_2-0.025 and TMDS-N_2-4.3-O_2-0.01 coatings, were distinguished by a topography presenting large structures with some asperities. However, TMDS-N_2-3 coatings showed a smooth homogenous and regular topography. The concentration and dimension of

these coated granular structures increased with a decrease in the oxygen flow rate (Figure 7).

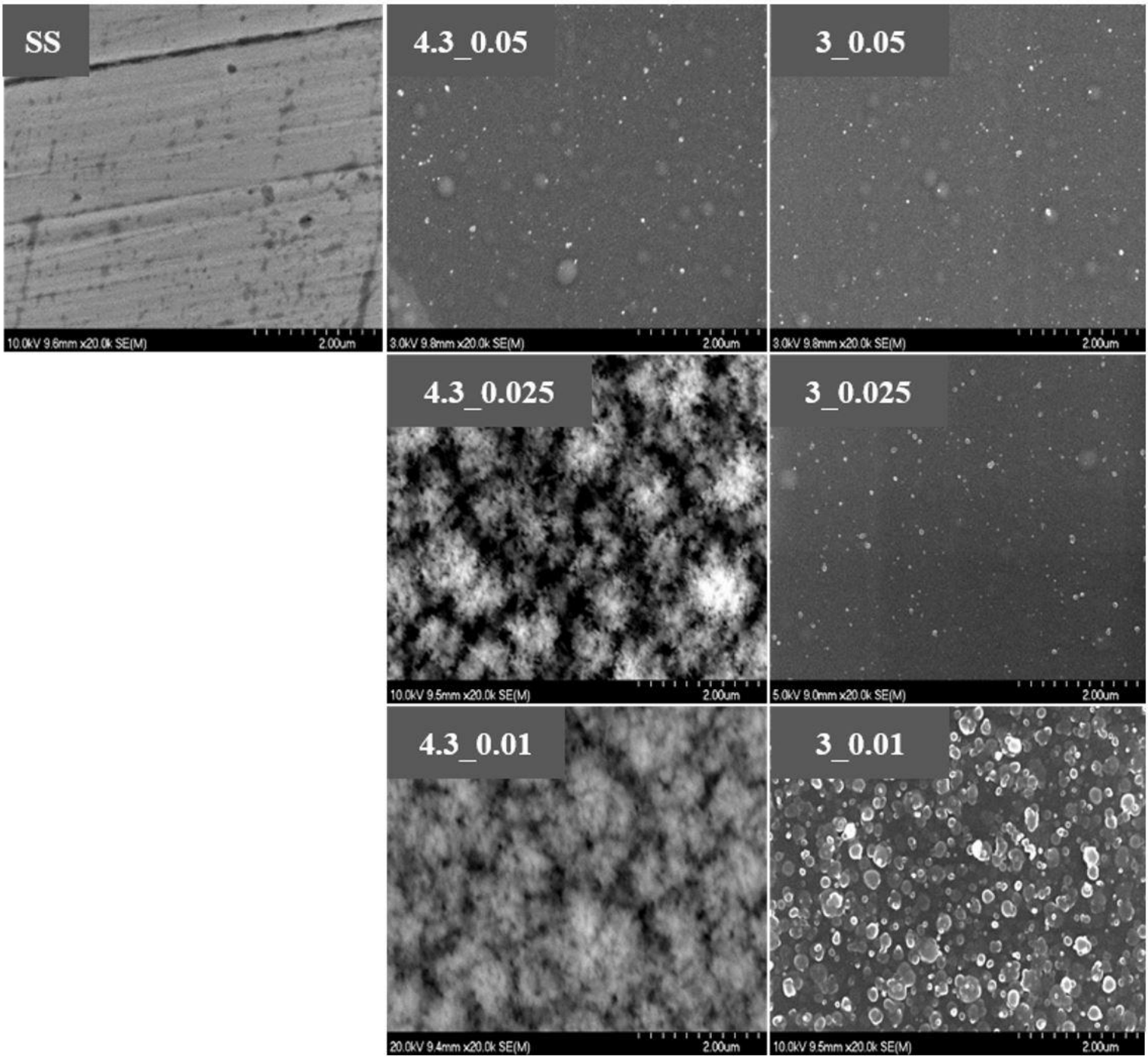


Figure 7. Scanning electron microscopy (SEM) micrographs; (SS) Micrograph of uncoated SS; (4.3_0.05, 4.3_0.025; 4.3_0.01) Micrographs of TMDS coatings with N_2 flow rate = 4.3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm; (3_0.05, 3_0.025; 3_0.01) Micrographs TMDS coatings with N_2 flow rate = 3 slpm varying O_2 flow rate from 0.05 to 0.025 to 0.01 slpm

Discussion

The adhesion of pathogenic bacteria and biofilm formation on abiotic surfaces is one of the biggest problems that industrial and medical fields are facing. This study aimed to find a solution regarding *S.* Enteritidis adhesion onto SS surfaces. In this context, an organosilicon monomer TMDS was polymerized on SS surfaces using microwave cold nitrogen plasma post-discharge, in order to confer to SS an antifouling character. Coatings were elaborated using different oxygen and nitrogen flow rates and a constant TMDS flow rate. The effect of these variations on the chemical, anti-adhesive and morphological coating properties were investigated.

The chemical composition of coated samples was studied using Fourrier transform infrared spectroscopy analyses. The TMDS-N_2-4.3 and TMDS-N_2-3 coatings showed the same infrared bands. These results imply a similar chemical composition on each elaborated coating. However, the infrared spectra showed some differences in the band absorbance intensity with the increase of oxygen flow rates. Hence, the polymerization of the monomer and the chemical structure of the coating are affected by the amount of O_2 added to TMDS during the plasma deposition. The chemical groups related to peaks identified in the spectra, showed that TMDS was fragmented by the CRNP reactive species, forming radicals that have polymerized on the stainless steel surface. The structure of the deposited film was a polysiloxane-like structure as already shown by Quédé et al. (2004). These results are consistent with those reported for hexamethyldisiloxane plasma deposited films by Agres et al. (1996). They suggest that the variation of O_2 flow rates permitted the formation of different functional groups inducing some changes in the polymer structure. Moreover, since all the coatings have a similar chemical structure, the difference in the number of cells attaching to each one was surprising. These results suggested that the chemical structure of the coatings was not the factor affecting bacterial attachment and permitted to redirect the research towards the analysis of surface topography.

The coatings thickness measurements supported the FTIR analyses since the increase of the oxygen flow rate increased the thickness while the nitrogen flow rate did not affect the thickness.

A TMDS monomer was used for its capacity to confer a hydrophobic character to SS surfaces. Some previous research has demonstrated that bacteria adhere more rapidly onto non-polar and hydrophobic surfaces than hydrophilic materials (Fletcher & Loeb 1979; Pringle & Fletcher 1983; Bendinger et al. 1993). In effect, some sort of hydrophobic interactions happens between the cell membrane and the material surface. Those interactions lead to the irreversible adherence of the bacteria since they enable the cell to overcome repulsive forces that are active within a specific distance from the surface (Donlan 2002). However, many studies have reported that increasing the surface hydrophobicity reduces the bacterial adhesion by a repelling action when the bacterial membrane is hydrophilic (Park et al. 2008; Pagedar et al. 2010). The findings in the current study were in contrast with these results since the MATS test showed that the *S.* Enteritidis cell surface has a hydrophilic character and electron donor properties. The coated surfaces are neutral since they mostly present Si-CH_3 which is neutral. The electron donor character of the bacterial strain used did not interfere in the adhesion process. The TMDS-N_2-4.3 coatings registered high WCA values (around 108°) and high hydrophobic character but remarkably high bacterial adhesion rates. Furthermore, the TMDS-N_2-3 coatings recorded lower WCA around 95° and allowed significant reduction of bacterial adhesion values. These results showed that the hydrophilic surface of *S.* Enteritidis did not interact with the hydrophobic surfaces via repulsive forces to inhibit the bacterial adhesion.

It is important to note that the increase in the nitrogen flow rate from 3 to 4.3 rendered the surface more hydrophobic without conferring any significant anti-adhesive property. In addition, the bacterial attachment test results showed that gas flow rate was an effective

parameter to modify coating structure and control antifouling properties. Indeed, the N_2 to O_2 ratio could be an effective parameter influencing the bacterial adhesion to plasma treated SS.

According to the results, TMDS-N_2-4.3 are recognized as non-repelling films and TMDS-N_2-3 coatings are considered as anti-adhesive for *S.* Enteritidis in all O_2 flow rate conditions. Therefore, to understand the reason of distinct adhesion behavior of *S.* Enteritidis towards both coatings types, and the factors related to the efficient anti-adhesive character of TMDS-N_2-3 coatings, the topography of the coatings have been studied. The topography results highlighted the effect of N_2 and O_2 flow rate variation on the morphology of coated surfaces. In fact, TMDS-N_2-4.3 coatings were more hydrophobic and rougher than TMDS-N_2-3 coatings. However, the TMDS-N_2-4.3 coatings presented an important difference in the number of attached cells in comparison with TMDS-N_2-3 coatings. These results suggest that the bacterial attachment was guided by the morphological character of the coating surface. Moreover, the TMDS-N_2-4.3 surface became increasingly rougher with an increase in O_2 flow rate, leading to a favorable substrate for the *S.* Enteritidis adhesion. The smooth surface of TMDS-N_2-3 coatings inhibited bacterial attachment. The increase in the nitrogen flow rate leaded to a more complex polymerized and rougher surface topography. The irregularities and cavities on these surfaces help bacteria to stick, adhere to the surface and form biofilms. These results suggest that the topography and roughness of the surface are very important parameters which significantly influence the rate of bacterial adhesion. The current results correlate with findings by Giraldez et al. (2010), who studied the effect of hydrogel contact lenses surface roughness and hydrophobicity on *Staphylococcus epidermidis* adhesion. The hydrophobic surfaces with the highest surface roughness had the highest number of attached cells. In this case the surface roughness modulated the bacterial adhesion. Moreover, Fernandes et al. (2014) studied the influence of the surface roughness and wettability of two different surfaces on the adhesion of *Salmonella enterica* serovar Typhimurium. Adhesion tests were carried out on the two,

hydrophobic and hydrophilic surfaces. Results showed that *S.* Typhimurium adhesion on both surfaces was similar. According to the literature and the current findings, *S.* Enteritidis has a surface which is hydrophilic (Lima et al. 2013). Thus, in this study, it was expected that *S.* Typhimurium would show a lower number of attached cells on the hydrophilic surface. However, this surface was rougher than the hydrophobic substrate, which permitted the bacteria to attach in similar numbers to both surfaces. Furthermore, Giraldez et al. (2010) investigated the impact of wettability and surface texture on bacterial adhesion of *Streptococcus sanguinis* and *Staphylococcus epidermidis* on different titanium and ceramics implant surfaces. This study showed that the differences in surface roughness did not affect *S. epidermidis* adhesion, but the higher roughness increased *S. sanguinis* adhesion. In contrast, a high bacterial adhesion was detected on hydrophobic surfaces for *S. epidermidis* but not for *S. sanguinis*. In this context, surface wettability and roughness orientated the bacterial adhesion on materials and the factor influencing the adhesion depends here on the bacterial species. The bacterial adhesion mechanism and behavior depends on the bacterial species and the morphological characteristics. Indeed, the adhesion of micro-organisms to a surface is a complex process involving physico-chemical interactions like electrostatic, Lifshitz–Van der Waals, acid–base and hydrophobic forces (Giraldez et al. 2010).

This investigation highlights the impact of surface morphology on bacterial adhesion. At the same time, the previously cited studies showed that the adhesion behavior may vary depending on the type of bacterial strain. These findings emphasize that the surfaces used in the food and medical sector must be smooth and the worn ones must be replaced by new surfaces in order to avoid issues associated with the formation of biofilms.

Conclusion

In this work, coatings were elaborated from the polymerization of TMDS doped with O_2 with a cold remote nitrogen plasma technique. Study on the effect of oxygen and nitrogen variation on the coating's nature were carried out. The chemistry and topography was analyzed by the FTIR, water contact angle, surface roughness and SEM. The effect of each type of coatings on *S.* Enteritidis adhesion was highlighted. The results underlined that bacterial adhesion was guided by the surface topography parameter. In fact, the rougher surfaces exhibited the higher number of attached *S.* Enteritidis cells. Overall, this study clearly showed that coating SS using TMDS under controlled conditions makes it repulsive for *S.* Enteritidis. A good perspective would be the grafting of antimicrobial groups on TMDS-coated surfaces by plasma treatment. The SS surfaces obtained would provide an antia-dhesive and anti-microbial property to SS. This strategy would help to prevent biofilm formation passively and actively.

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CHAPTER: IV

Nisin-based coatings for the prevention of biofilm formation: Surface characterization and antimicrobial assessments

Nisin-based coatings for the prevention of biofilm formation: Surface characterization and antimicrobial assessments

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ABSTRACT

Pathogenic bacterial biofilms invading surfaces in food and medical fields are a challenge to overcome. Despite all the strategies applied to fight their formation, the microbiological risk associated to bacterial biofilms remains an important threat for at risk population and for food and healthcare sectors. The prevention of biofilm formation might be an effective approach to confront this problem. In this study, stainless steel surfaces were functionalized by nisin, a natural antimicrobial peptide. The mechanism of action of immobilized nisin against sensitive bacteria is not fully understood. Therefore, nisin was grafted onto the surface by either its carboxylic group or its amino group. The generated coating's chemical, topographical and antibacterial properties were studied to understand the nisin mode of action, when immobilized, and identify the section of the bacteriocin responsible for the antimicrobial activity. The antimicrobial activity of the elaborated coatings was tested against *Listeria monocytogenes*. Indeed, the surfaces coated with nisin linked by its amino group showed an efficient antibacterial activity while the surface with nisin linked by its carboxylic group showed less antimicrobial effect. The antimicrobial results showed almost 2 log reduction of colony forming units for efficient antibacterial coatings while the other showed no bacterial reduction. The surface properties analysis permitted to understand the chemical and topographical characteristics of treated surfaces including nisin conformation and quantification. A tight relation was concluded between the surface topography, the nisin conformation, and the antibacterial activity of the bacteriocin-coated surfaces.

Keywords: Nisin*;* Stainless Steel; *Listeria monocytogenes*; Biofilms; Surface; Antimicrobial properties.

Abreviations

- stainless steel (SS)
- stainless steel/polydopamine (SD)
- stainless steel/polydopamine/nisin (SDN)
- stainless steel/polydopamine/glutaraldehyde (SDG)
- stainless steel/polydopamine/glutaraldehyde/nisin (SDGN)
- stainless steel/polydopamine/succinic acid (SDA)
- stainless steel/polydopamine/succinic acid/nisin (SDAN)
- Scanning Electron Microscopy (SEM)
- Fourier transform infrared analyses (FTIR)
- Time-of-flight secondary ion mass spectrometry (ToF-SIMS)
- X-ray photoelectron spectroscopy (XPS)
- N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)
- N-Hydroxysuccinimide (NHS)
- *Listeria monocytogenes (L. monocytogenes)*
- Colony-forming unit (CFU)
- Tryptic soy broth (TSB)
- Tryptone soy agar (TSA)
- average roughness (Ra)
- root-mean-squared roughness (Rq)
- antimicrobial peptides (AMPs)
- acridine orange (AO)

Introduction

Contamination of facilities with bacterial pathogenic biofilms in food and medical sectors is an evolving problem and will be for many years if an efficient remedy is not found. Indeed, these pathogenic structures, formed on equipment, are involved in a variety of foodborne illness and nosocomial infections [1,2]. A study carried out by the Foodborne Disease Burden Epidemiology Reference Group (FERG), established by the World Health Organization (WHO) in 2007, estimated that 31 foodborne hazards caused 600 million foodborne illnesses and 420,000 deaths in 2010 [3]. On the other hand, health-care-associated infections, known as nosocomial infections are considered as the most common unfortunate events threatening patient safety worldwide [4-6].

Stainless steel (SS) is a widely employed material in agri-food and healthcare domains for its appropriate properties. However, bacterial biofilms can develop and persist on SS-based equipment. The disinfections strategies applied by industrials and hospitals do not eliminate biofilms perfectly, especially the resistant ones. The plans aiming to control biofilm formation, negatively affect the environment and have a consequent impact on the economy of these fields. Indeed, it is of great importance to find solutions to get rid of biofilm contamination.

The objective of this work is to design an effective tool to fight against biofilm formation. The elaboration of surfaces with antimicrobial bacteriocin-based coatings seems to be a powerful remedy in killing pathogenic bacteria. Moreover, the use of antimicrobial peptides (AMPs) adsorbed onto surfaces is one of the possible innovative and proactive approaches to prevent contaminations and infections [7]. Nisin is a member of these antimicrobial peptides which can be grafted on materials. It is a positively charged peptide with a 3352 Da molar mass presenting 34 amino acids allocated in hydrophilic residues at the COOH-terminus and hydrophobic residues at the NH_2-terminus (Fig. 1). It is known as a safe food additive that has been accepted and applied for food preservation in over 50 countries for a period for almost 70 years [11]. The

bactericidal potency of nisin is effective at pico to nanomolar concentrations and is based on various mechanisms of action [9]. It interacts with the cell membrane precursor, lipid II, which involves the inhibition of bacterial cell wall biosynthesis and contributes to the pore formation in the membrane [10]. At first, the antibacterial mode of action of nisin involves the binding of its cationic COOH terminus with the anionic lipids of the bacterial cell wall, through electrostatic interactions [11]. It also has been reported that the covalent grafting of antimicrobial peptides (AMPs) such as magainin I and nisin on a stainless steel surface pre-coated with a chitosan polymer layer lead to a decreased in the adhesion of *Listeria ivanovii* and underlined an efficient antibiofilm activity [12].

Nisin is produced by *Lactococcus lactis* subsp. *lactis* and have an interesting inhibitory effect on a large number of Gram-positive bacteria, including *Listeria monocytogenes* and *Staphylococcus aureus* [12]. *L. monocytogenes* is a Gram-positive bacteria involved in Listeriosis a foodborne illness. Its fatality rate is around 30%, possibly higher for pregnant women and people with weakened immune system [14].

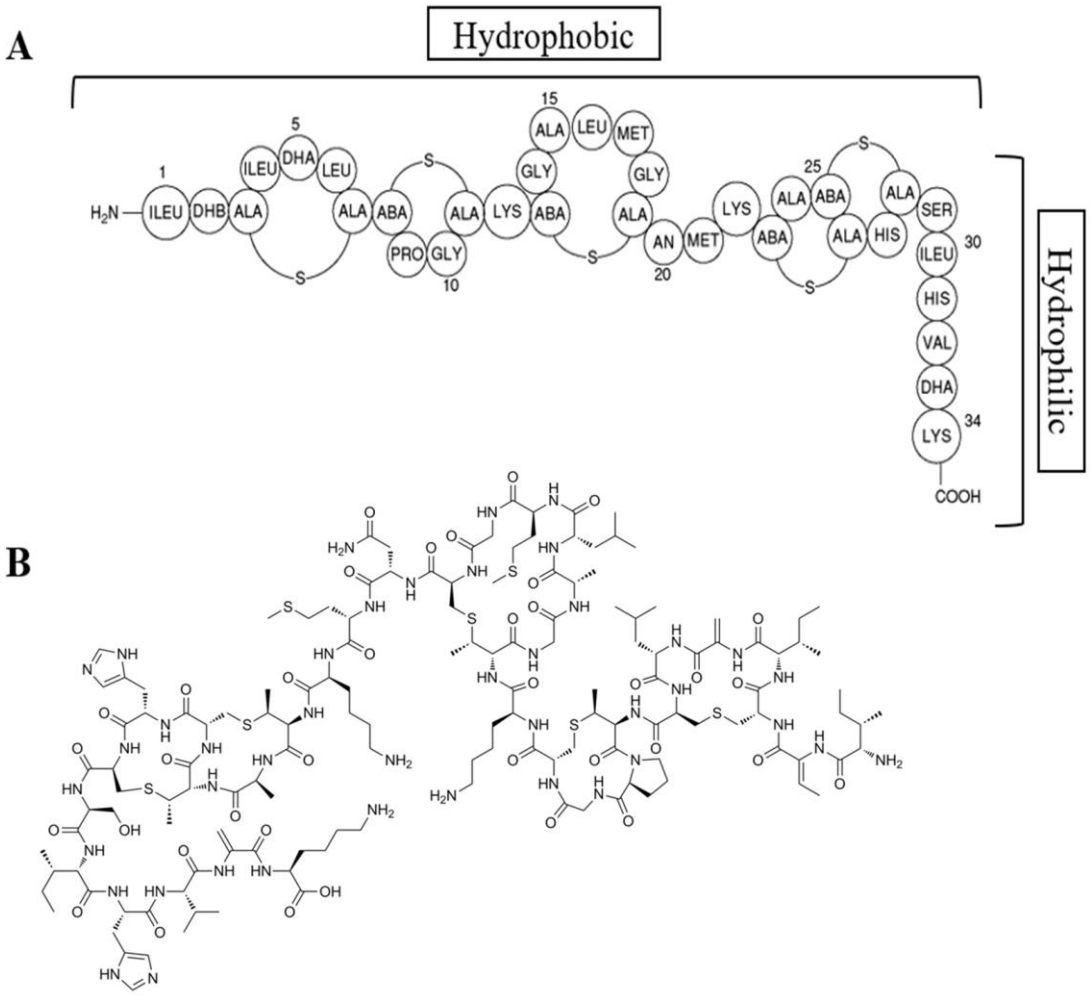

Fig. 1. (A) Primary structure of nisin A presenting the 34 amino acids distribution in the hydrophobic and hydrophilic parts (adapted from [13]) (B) Chemical structure of nisin showing NH_2-terminus and COOH-terminus (adapted from [14]).

In a recent study, a glass surface was modified via electron-transfer and chemical reactions employing dopamine to attach nisin directly. The results of XPS and FTIR showed that nisin was successfully grafted. Moreover, the modified surfaces inhibited the adhesion of both algae *Phaeodactylum tricornutum* and bacteria *Bacillus* spp. [14]. Otherwise, according to several studies, the most common cross-linker used is glutaraldehyde. But, unfortunately it is toxic [15]. In the search for a potential harmless analogue, succinic acid, a dicarboxylic acid, was selected. Succinate is a food additive acidulant/pH modifier, used as a flavoring agent in the food markets. It is also used in the markets of production of health-related agents, including

pharmaceuticals, antibiotics, amino acids, and vitamins [16]. Indeed, succinic acid is generally recognized as safe by the U.S. Food and Drug Administration [17,18].

To characterize these coatings before and after the nisin immobilization, in term of antimicrobial and chemical properties, several analyses were carried out. The antimicrobial efficiency of the treated surfaces was carried out towards *L. monocytogenes.* The adhesive power test of treated surfaces was performed to assess the comparability of bacterial adhesion rates on each treated surface. An antibacterial challenge test was used to assess the antibacterial activity of the treated surfaces. In addition, a direct antibacterial assessment was carried out using the LIVE/DEAD Kit, qualitatively testing the surfaces on a bacterial lawn, and Scanning Electron Microscopy (SEM) analysis of the bacterial state. The activity time selected for the nisin-coated surfaces was 3, 5 and 24 hours. Indeed, the optimal time for nisin activity is 3 h according to several studies [8,19]. The contact time of 5 h was selected to compare it with the 3 h contact time and to characterize the activity evolution. Finally, the 24 h was selected since it is the time for a biofilm to become mature. The chemical analysis and the molecules conformation and presence was demonstrated via water contact angle (WCA) measurements, Fourier transform infrared analyses (FTIR), surface roughness and thickness, SEM analyses, Time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS).

Material and methods

Approaches for coatings elaboration

In this work, two approaches of nisin conformation linkage were applied in the coating elaboration. During the different coatings elaboration, at each step, coatings were kept apart to be tested as controls during all the experiments (SD, SDG and SDA). The first approach was the linkage of nisin by its amino group while its only carboxylic group is free. The second one was to link the nisin by its only carboxylic group while its amino groups are free. In the process, firstly, dopamine was polymerized on SS to acquire amino groups on the surface (SD). After that, according to the first approach of nisin linkage, glutaraldehyde was fixed (SDG) between polydopamine and nisin, which was added finally, resulting in a model surface called stainless steel/polydopamine/glutaraldehyde/nisin (SDGN). Moreover, according to the same approach, succinic acid was linked to polydopamine (SDA), and nisin was grafted in the end, resulting in a model surface called stainless steel/polydopamine/succinic acid/nisin (SDAN). In the second linkage approach, nisin was directly attached by its carboxylic group to polydopamine without a linking agent resulting in a model surface called Stainless steel/polydopamine/nisin (SDN). Fig. 2 explains the linkage approaches.

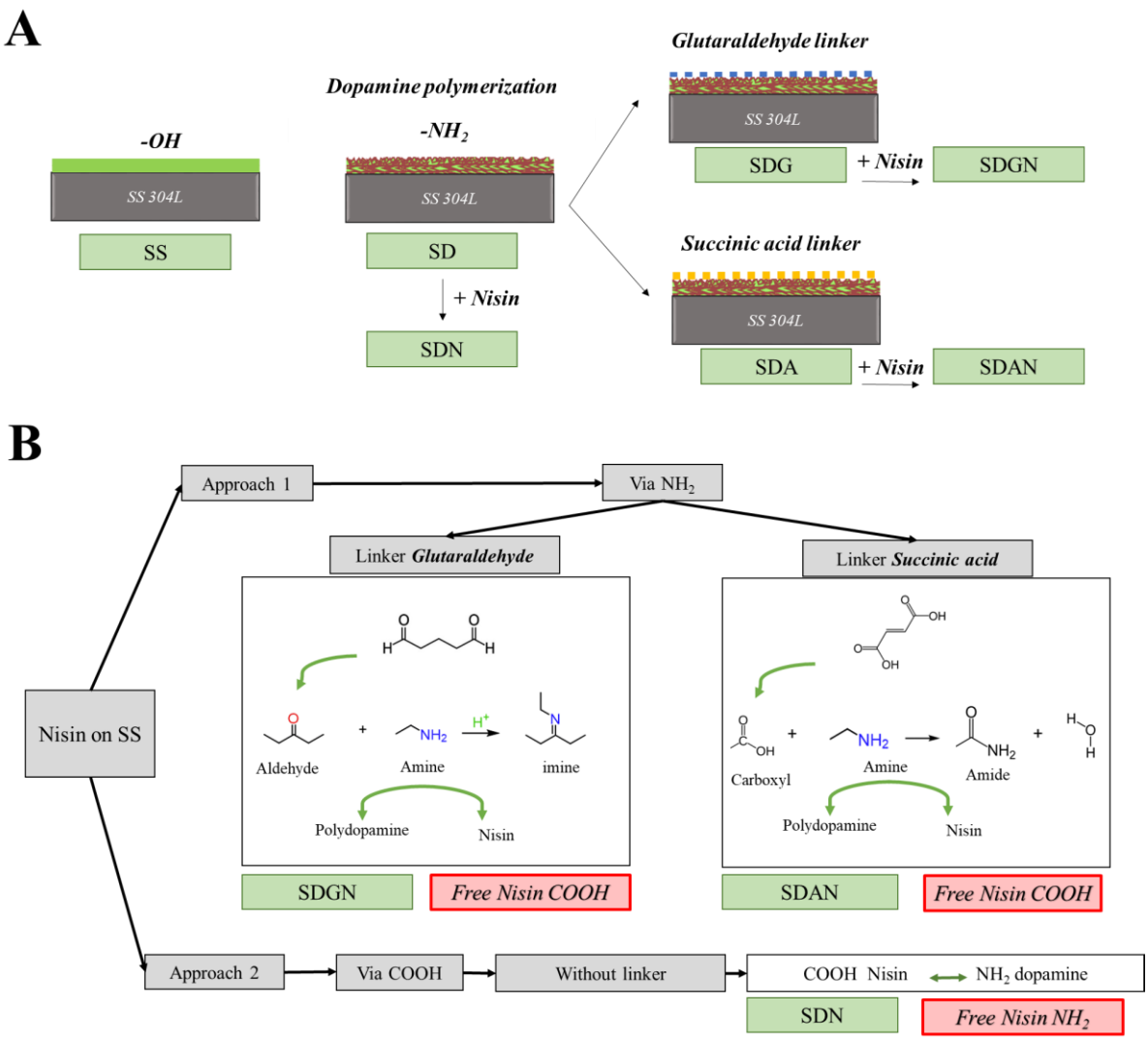

Fig. 2. (A) Schematic representation of coatings elaboration. (B) Schematic representation of nisin grafting approaches.

Standardized SS slides preparation

The SS circular slides, purchased from Acciai Speciali Terni (Terni, Italy) and polished by Equinox (Willems, France), are 40 mm of diameter and 1 mm of thickness. After removing the SS protection film, the slides were soaked for 10 min in absolute ethanol (Brabant, France), then rubbed with a paper towel dipped with ethanol to remove the sticky residues of the protection films. They were then air-dried and autoclaved at 120°C for 20 min for sterilization. SS slides were then collected in sterile Petri dishes

Bacterial strain, culture conditions and suspension preparation

Listeria monocytogenes ATCC 35152 (LM/NCTC, United Kingdom) is the bacterial strain selected for this research. The cryogenic vials containing this strain with Tryptic Soy Broth (TSB; Biokar Diagnostics, France) and $^{-1}$ glycerol (40%) were stored at – 20°C. For pre-culture preparation, 100 µl of the frozen tubes was inoculated into 5 ml of TSB and then incubated at 37°C. After 24 h of incubation, and to prepare the culture of *L. monocytogenes,* 100 µl of this pre-culture that contains 10^4 CFU ml^{-1} were inoculated into 50 ml of TSB culture medium in 500 ml sterile Erlenmeyer. The culture was incubated at 37 °C under stirring condition at 160 rpm, and bacterial cells were collected in the late exponential condition after 15 h. *L. monocytogenes* cells were collected by culture centrifugation (5000 *g*, 10 min, 20 °C) and washed twice with 20 ml of sterile physiological saline solution (8.5 g l^{-1} NaCl) to purify and eliminate the suspension from TSB and bacterial debris. Finally, cells were re-suspended in 20 ml of saline. The *L. monocytogenes* suspension was prepared to a concentration of (1×10^8 CFU ml^{-1}) via spectrometric reading (Jenway 6320D spectrophotometer). It was then sonicated at 37 kHz for 5 min at 20 °C (Elma S40 Elmasonic, Germany) for cell dispersion. This bacterial suspension was then diluted 10-fold and 1000 fold to obtain the concentrations of 1×10^7 CFU ml^{-1} and 1×10^5 CFU ml^{-1} respectively, needed for the different anti-bacterial tests to be performed.

Protocols for coatings elaboration

- *Stainless steel/polydopamine (SD) coatings*

Dopamine hydrochloride H8502-25G (Sigma-Aldrich, France) was dissolved in Tris-Base buffer solution (10 mM) to obtain a solution of 2 mg ml^{-1} and pH was rectified to 8.5 using sodium hydroxide solution (2 M). Then 20 ml of the solution were spilled in a Petri dish of 8.5 cm of diameter containing two standardized SS slides. The Petri dish was incubated at 20°C for 24 h under stirring condition at 160 rpm after wrapping it in parafilm. This continuous agitation

allows homogeneous polymerization of dopamine hydrochloride to polydopamine and prevents the deposition of undesirable microparticle on the samples. After incubation, the slides were gently rinsed four times with 20 ml of autoclaved ultrapure water (Milli-Q IX Pure Water System). These coated surfaces are called SD. All the treated surfaces hereafter were incubated and rinsed in the same conditions as SD.

- *Stainless steel/polydopamine/nisin (SDN) coatings*

The antimicrobial peptide nisin (Pure grade of nisin A - Danisco Beaminster Dorset, United Kingdom), being insoluble in water was dissolved in hydrochloric acid solution 0.01 M 1 mg ml^{-1}). The dissolution was carried out gently avoiding foam formation. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) 98% (Both purchased from Sigma-Aldrich, France), were dissolved in phosphate buffer pH = 6.2 and added to the prepared nisin solution. The final concentration of EDC and NHS was 0.02 M and 0.01 M, respectively. To immobilize nisin, 20 ml of the solution was directly poured into a Petri dish containing two SD samples. The system was incubated then rinsed in the standardized condition. These coated surfaces are called SDN.

- *Stainless steel/polydopamine/glutaraldehyde/nisin (SDGN) coatings*

Glutaraldehyde (Sigma-Aldrich, France), employed as a spacer arm, was diluted in sterile ultrapure water to 3 ml 100 ml^{-1}. Then, 20 ml of this solution were poured into a Petri dish containing two samples of SD. The system was incubated then rinsed in the standardized condition. These coated surfaces are called SDG. Nisin was dissolved gently in hydrochloric acid solution 0.01 M (Using the rate Nisin: HCl = 10mg: 1ml). Then, the solution was diluted in sterile ultrapure water to obtain a concentration of 1 mg ml^{-1}. The pH is then neutralized to 7 by adding sodium hydroxide solution (2 M). Then 20 ml of the solution were spilled into a Petri dish containing two rinsed SDG. The system was incubated then rinsed in the standardized condition. These coated surfaces are called SDGN.

- *Stainless steel/polydopamine/succinic acid /nisin (SDAN) coatings*

A solution containing 3 g 100 ml^{-1} of succinic acid (Janssen-Beerse, Belgium), 0.02 M EDC and 0.01 M NHS was prepared in phosphate buffer (pH = 6.2, 10 mM). The pH of the solution was adjusted to 4.3 by adding 2 M of sodium hydroxide solution. Then 20 ml of this solution were poured into a Petri dish containing two SD samples. The system is incubated then rinsed in the standardized condition. These coated surfaces are called SDA. For the elaboration of nisin-coated SDA, the same steps of SDA preparation were followed, changing the incubation time of the solution before adding nisin. Indeed, 19.5 ml of the solution were poured in a Petri dish that was closed with parafilm and incubated at 20°C under stirring at 160 rpm for 3 h. Then, 0.5 ml of the dissolved nisin (1 mg ml^{-1}) in hydrochloric acid solution of 0.01 M were added into the Petri dish and incubated again on the stirring plate for 21 h at 20 °C. After that, the standardized rinsing protocol was followed. These coated surfaces are called SDAN.

Bacterial adhesion tests in NEC biofilm system

Visualization of adhered bacteria on coated surfaces was carried out with epifluorescence microscopy 100 × magnification (Nikon Optiphot-2 EFD3). Bacterial adhesion assessment allows to quantify the number of cells adhered on a surface that has been put in contact with a bacterial suspension. This analyze aimed to prove a comparability of the number of adhered bacteria between all the surfaces. The bacterial adhesion test was carried out on the prepared coated surfaces and non-coated SS by exposing them to a *L. monocytogenes* suspension in *NEC biofilm system* [18]. Bare SS was taken as control. Briefly, 5 ml of 10^{7} CFU ml^{-1} *L. monocytogenes* suspension was statically incubated on each sample at 20°C for 1 h to allow bacterial adhesion. The solution was then removed, and coupons were rinsed with 20 ml of sterile physiological water to eliminate all loosely attached cells. The adhered cells were then stained with 2 ml of acridine orange (AO; 0.01 g 100 ml^{-1}) for 10 min in darkness. After that, the intercalating agent was withdrawn, and the coupons were rinsed twice with 2 ml of

physiological water to clear out excess of AO. The samples were then dried and observed under the microscope. A total of 25 fields per coupon were captured with a digital camera and stained cells were enumerated. Mean value of adhered bacteria per microscopic field and its relative standard deviation were presented. Three repetitions were carried out for each coating type.

Antibacterial challenge test

After the adhesion rate test, the challenge test was carried out by treated surfaces, towards *L. monocytogenes* to assess the antimicrobial activity of these surfaces, specifically the ones grafted with nisin. Uncoated SS was taken as a control. The protocol for the challenge test was carried out according to the procedure proposed in the norm ISO 22196 [19]. The concentration of *L. monocytogenes* solution tested was 10^5 CFU ml^{-1} prepared in a diluted TSB to 1/100 volume. Each coating was collected in a sterile pot and 1 ml of the test inoculum was deposited on the entire surface for 3, 5 and 24 h under sterile condition, at 20°C. After incubation, 1 ml of deposited bacterial solution and the sample were recovered and placed upside down in a sterile container containing 9 ml of sterile tryptone salt (Biokar, France) solution (9.5 g l^{-1}). To unhook the cells, the container was vortexed for 15 seconds, sonicated in an ultrasonic bath for 5 min at 37 kHz at 20°C (Elmasonic S60H, Elma, Germany) and again vortexed for 15 seconds. Then 10-fold dilution was performed for each condition. The bacteria were enumerated in Tryptone Soy Agar (TSA; Biokar Diagnostics, France) after incubation at 37°C for 24 h. The mortality rate of bacteria on a coated SS was assessed by comparing the relative colony-forming unit (CFU) to the one associated with the uncoated SS. Log reduction of CFU was then calculated. Three repetitions were carried out for each coating type.

Assessment of the bacterial viability with the Live/Dead backlight viability kit

The viability assessment of bacteria exposed to treated surfaces was carried out. Bare SS was taken as a control. Briefly, after 3, 5 and 24 h of exposure to coupon's surface, 1 ml of

10^5 CFU ml^{-1} of bacterial solution on each coupon was mixed with 4 ml of sterile tryptone salt solution. For each coupon condition, bacterial solutions were stained with LIVE/DEAD BacLight Bacterial Viability kit (Invitrogen Molecular Probes, USA), according to the manufacturer instruction for 15 min in the dark. After this time, 1 ml of the solution is vacuum filtered through a 0.2 µm pore-size polycarbonate membrane filters (Millipore, France). Stained cells were washed once with 1 ml of saline solution (8.5 g l^{-1} NaCl) and filters were placed on microscopic slides for the epifluorescence microscopic enumeration. The viable and dead cells were counted in 25 microscopic fields. Results are expressed as mean (± Standard deviation) of three repetitions for each coating type.

Qualitative antibacterial assessment of treated surfaces

The qualitative testing was carried out on all the treated surfaces to assess the antibacterial activity of efficient nisin coated surfaces and prove the nisin linkage to the films without its diffusion. Mueller Hinton agar medium (Biokar Diagnostics, France) was seeded with *L. monocytogenes*. The face-up of the treated surfaces were placed on the agar surface. The plate was incubated at 20°C for 3 h for nisin activity. The coupons were then removed and the plate was incubated at 37 °C for 24 h. Nisin activity was assessed as an inhibition of *L. monocytogenes* growth on the zone where the film was placed.

Water contact angle measurements

Water contact angle (WCA) measurements were obtained by a DSA100 drop shape analyser (Krüss, Germany). Droplets of 2 µl deionized water were deposited onto the surface of samples at room temperature. Measurements were taken on five different areas of each coupon. Contact angle was calculated with Advance software. Data are representative of three different measurements on five droplets deposed randomly on the coating surfaces.

Surface roughness and thickness analyses

The surface roughness of the coatings was determined using a surface profiler Alpha-step IQ (Kla Tencor, Milpitas, California). Samples were scanned over a length of 1 mm with a scan speed of 20 μm s^{-1} and sampling rate of 50 Hz. Each sample was scanned in three locations. The resolution was 400 nm. SS was always taken as control. Each measurement was repeated three times. The average roughness (R_a) and the root-mean-squared roughness (R_q) were presented and calculated using the following equations:

$$R_a = \frac{1}{n}\sum_{i=1}^{n}|Y_i| \quad (1)$$

$$R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} Y_i^2} \quad (2)$$

The thickness of the treated surfaces was also measured. A tape was stuck on a part of the SS slide before its treatment. After coatings elaboration, the tape was removed and the thickness was measured on 5 different zones. The average values for coatings thickness were then calculated with its standard deviation. Each measurement was repeated three times.

Scanning Electron Microscopy *analysis*

Scanning Electron Microscopy was carried out to visualize the morphology of bare SS, coated surfaces, and to observe the state of bacteria in contact with coatings. SEM micrographs were taken at 20,000× magnifications. Standard procedures for fixing and embedding sensitive biological samples were carried out on coatings with adhered bacteria. SEM analyses were done using a Hitachi S-4700 SEM equipped with a field emission gun (FEG). Beforehand the observations, samples were sputter coated with carbon to become conductive with BAL-TEC SCD 005 Sputter Coater.

Ion polishing of coatings

Prior to SEM imaging, sample cross sections were first polished using SiC polishing sheets up to a grade 1200 and then using a Fischione Instruments 1061 SEM Mill ionic polishing

system at 4 kV for 2 h to obtain a smooth surface. Prepared samples were carbon coated with a Bal-Tec SCD005 sputter coater and SEM images were taken using a JEOL JSM 7800F LV scanning electron microscope at 5 kV.

Fourier Transform Infrared analysis

The FTIR spectra of treated surfaces were recorded by a Fourier Transform Infrared (FTIR) spectrometry (Nicolet iS50 FT-IR spectrometer - Thermo Scientific Waltham, USA) at room temperature. 64 spectral scans were recorded, which was sufficient to achieve good resolution. All the spectra were analyzed using OMNIC software.

ToF-SIMS analysis

ToF-SIMS is a surface analytical technique. In this method, a pulsed beam of primary ions already passed over the surface of the sample, produces secondary ions in a sputtering process. Analyzing the secondary ions provides information about the molecular and elemental species present on the surface. ToF-SIMS measures were performed on a ToF.SIMS 5 instrument by ION-ToF GmbH (Germany). It was equipped with a Bi liquid metal ion gun (LMIG). The measurements were carried out with pulsed primary Bi^{3+} ions. (25 keV and 0.4 pA). A low-energy (20 eV) electron flood source was employed for charge compensation. For each coating, positive and negative mass spectra were picked from a surface of 500 x 500 mm corresponding to 30 scans. Data were analyzed using SurfaceLab 6.2 software.

XPS analysis

XPS measures were performed using XPS KRATOS, analytical AXIS UltraDLD spectrometer Thermo Scientific KAlpha XPS system (UK). The monochromatized Aluminium-Kα X-ray source ($h\upsilon$ = 1486.6 eV) was carried out via an electromagnetic lens mode and in a constant analyzer energy mode (CAE = 150 eV for survey spectra and CAE = 30 eV for high resolution spectra). The binding energy scale was initially calibrated using the Ag $3d_{5/2}$ (368.2

eV), Cu $2p_{3/2}$ (932.7 eV) and Au $4f_{7/2}$ (84 eV) peak positions. In addition, the C 1s hydrocarbon (285.0 eV) binding energy (BE) was used as internal reference for calibration. Simulation and quantification of the experimental peaks were performed using the CasaXPS software. Moreover, quantification took into account a nonlinear Shirley background subtraction [20].

Statistical analysis

All quantitative measurements were reproduced in triplicate. Statistical analysis was carried out with IBM SPSS 19 statistics software using one-way ANOVA. Results were considered significantly different when $p < 0.05$.

Results

Antibacterial effect of nisin coated SS

Bacterial adhesion test was carried out to ensure that coatings had comparable adhesion rates before performing antibacterial activity tests. Bacterial adhesion test on bare SS showed an average of 26 ± 5 adhered *L. monocytogenes* per microscopic field. The average of adhered cells per microscopic field obtained for each coating type was comparable for all coating types (Fig. 3). Indeed, the adhesion rate was 18 ± 2, 20 ± 3, 20 ± 3, 27 ± 4, 23 ± 2 and 25 ± 3 for SD, SDN, SDG, SDGN, SDA and SDAN, respectively. Fig. 4 shows one microscopic field representing the tendency of adhered bacteria on each sample.

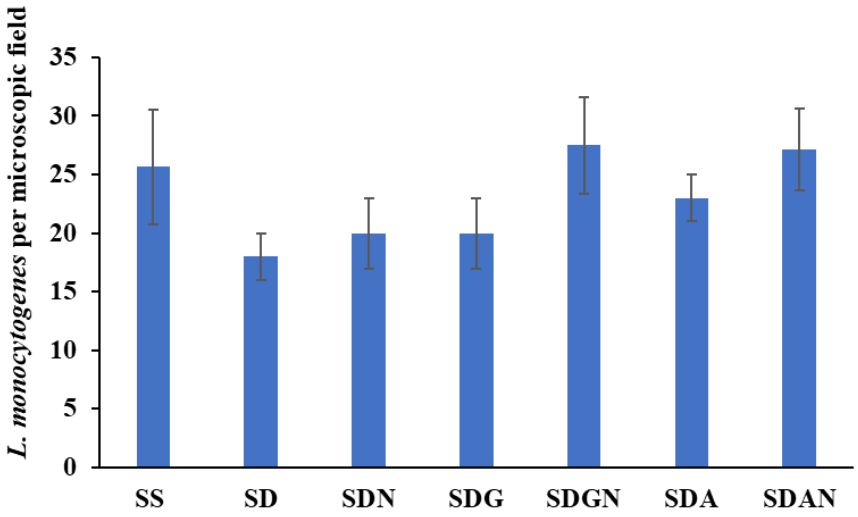

Fig. 3. Assessment of *Listeria monocytogenes* adhesion on SS, SD SDN SDG SDGN SDA and SDAN. (*) *The statistical analysis for these results indicates non-significant differences (p > 0.05).*

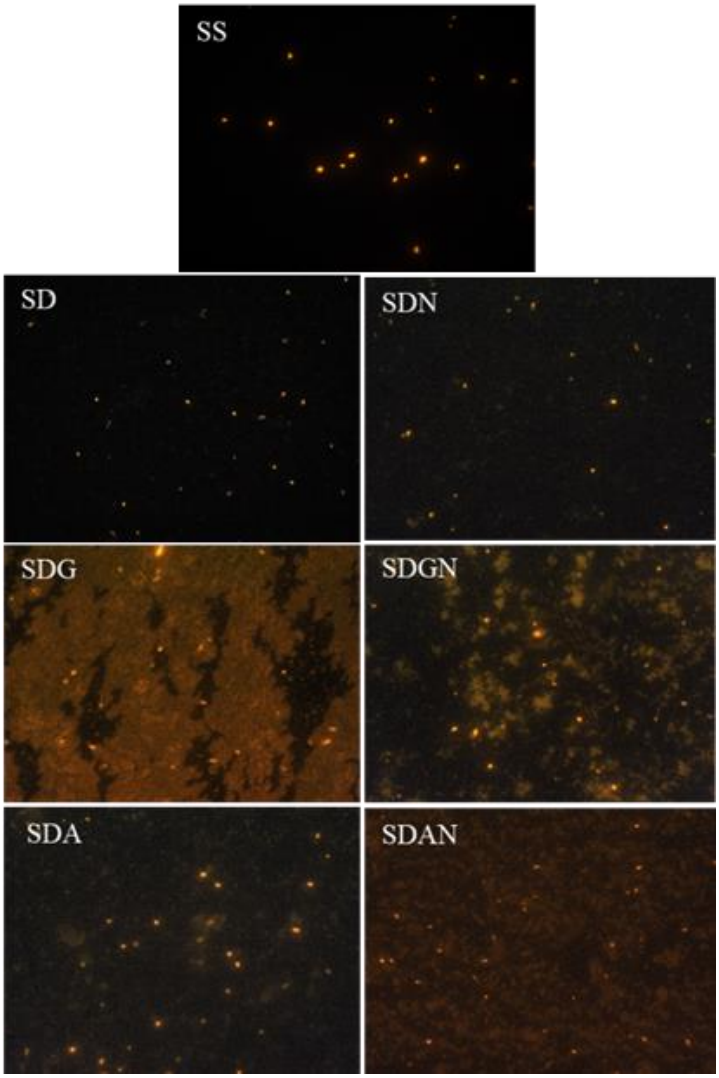

Fig. 4. *Listeria monocytogenes* adherent cells on different coated SS. Acridine orange staining and epifluorescence microscopy were used to assess *L. monocytogenes* adhesion capabilities.

To characterize the antibacterial activity of coated surfaces, a challenge test adapted from the antibacterial ISO 22196 test against Gram positive bacteria, was carried out. After 3 h of *L. monocytogenes* contact with SS, SD, SDG and SDA, the bacterial load was log 5.3 ± 0.1, log 5.2 ± 0.1, log 5.2 ± 0.1 and log 5.2 ± 0.1 CFU cm^{-2} respectively. After 5 h, SS, SD, SDG and SDA similar results were observed as for 3 h. The bacterial load was 5.4 ± 0.1, 5.2 ± 0.1, 5.2 ± 0.1 and 5.2 ± 0.1 CFU cm^{-2} respectively. Nisin coated SDN registered after 3 and 5 h a bacterial log of 5.2 ± 0.1 and 5.2 ± 0.1 CFU cm^{-2} respectively.

Otherwise, for SDGN and SDAN, the bacterial load decreased of almost 2 and 1.5 CFU cm^{-2} respectively. Indeed, SDGN registered after 3 and 5 h a bacterial load of 2.2 ± 0.2 and 2.6 ± 0.1 CFU cm^{-2} respectively, and SDAN registered after 3 and 5 h a bacterial load of 3.5 ± 0.1 and 3.6 ± 0.1 respectively CFU cm^{-2}. After 24 h of bacterial contact, the bacterial load increased for all coated surfaces. In fact, SS, SD, SDN, SDG, SDGN, SDA and SDAN registered respectively log values of 7.3 ± 0.1, 7.2 ± 0.1, 7.1 ± 0.1, 7.2 ± 0.1, 7.1 ± 0.1 and 7.2 ± 0.1 CFU cm^{-2} (Fig. 5A).

Bacterial suspension incubated for 3, 5 and 24 h upon control and nisin-coated substrates, was stained by LIVE/DEAD Kit and observed using the epifluorescence microscopy. The enumeration of viable cells underlined that *L. monocytogenes* on SS, SD, SDG and SDA showed predominantly viable cells after 3 and 5 h. Similar results of bacterial viability were observed after 24 h for SS and all coated surfaces with or without the bacteriocin. Our results also showed that, bacterial viability decreased of 60 % and 50% after 3 and 5 h of contact with SDGN and SDAN respectively while SDN did not decrease the bacterial viability (Fig. 5B).

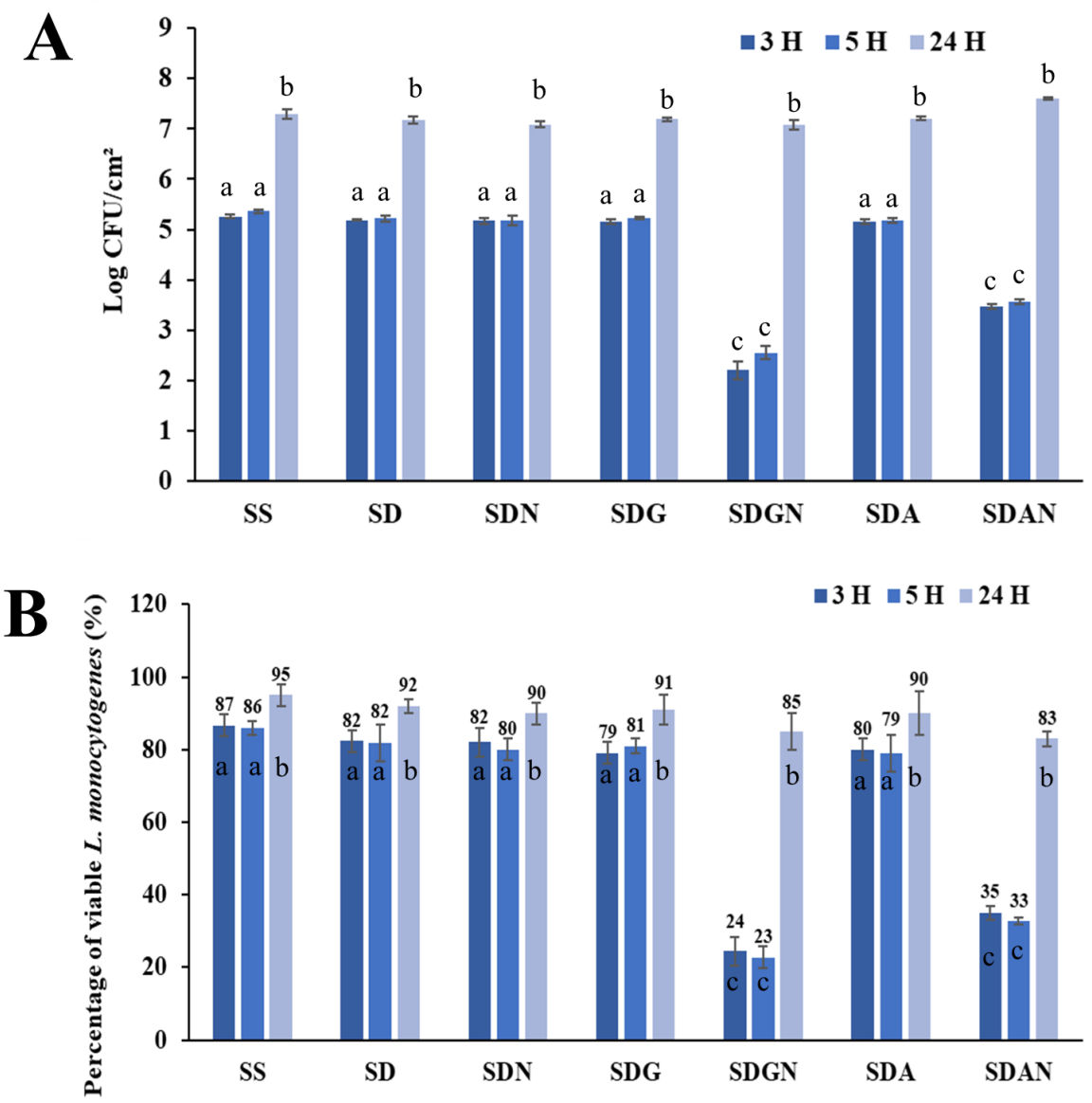

Fig. 5. (A) Antimicrobial assessment of the different treated SS surfaces on *Listeria monocytogenes*. (B) Viability percentage of *L. monocytogenes* with LIVE/DEAD kit after 3, 5 and 24 h of contact with SS and coated SS surfaces (± SD). (*) Different letters on top or below error bars (a, b, and c) indicate significant differences ($p < 0.05$); the same letters on error bars indicate non-significant differences ($p > 0.05$).

SEM analyses were carried out to observe the bacterial state around effective antimicrobial coatings, SDGN and SDAN. SS was taken as a control. *L. monocytogenes* state was intact and the cell wall presented no degradation after 3 and 5 h of contact with SS (Fig. 6A and 6D).Moreover, SEM micrographs in Fig. 6B, 6C, 6E, and 6F showed the damaged cell wall of *L. monocytogenes* on SDGN and SDAN after 3 and 5 h of contact. Indeed, the cell walls of the bacteria are sunken proving that the material is out of the cells and that the membrane is destroyed. Nisin granulations were clearly detected near the bacteria (Fig. 6).

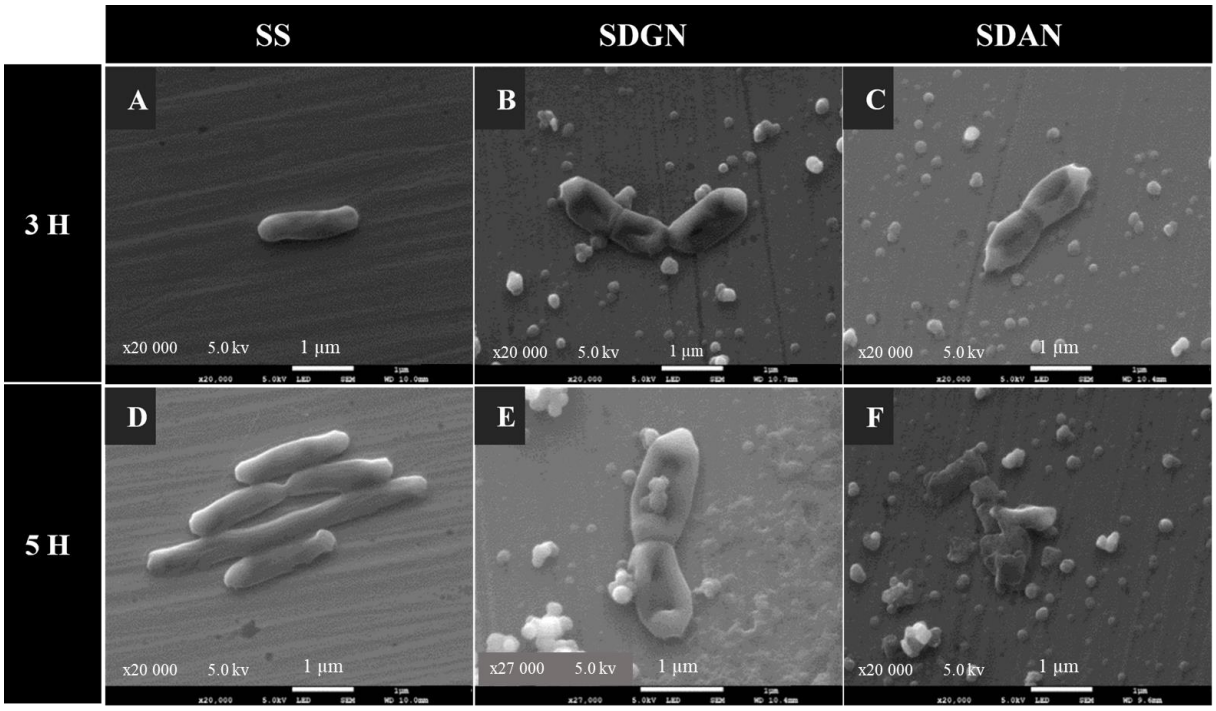

Fig. 6. Scanning Electron Microscopy micrographs showing the state of *Listeria monocytogenes* adhered to non-coated SS surface (A-D) and to coated SS surfaces (B-C-E-F) after 3 and 5 hours of contact with the surfaces.

The results presented in figure 6 showed a significant antibacterial activity linked to SDGN and SDAN while SDN did not show an inhibition zone. SS, SD, SDG and SDA, taken as control did not show any antimicrobial activity. Cellulose disc soaked in pure nisin showed a diffusion circle while no diffusion was observed for SDGN and SDAN as shown in Fig. 7.

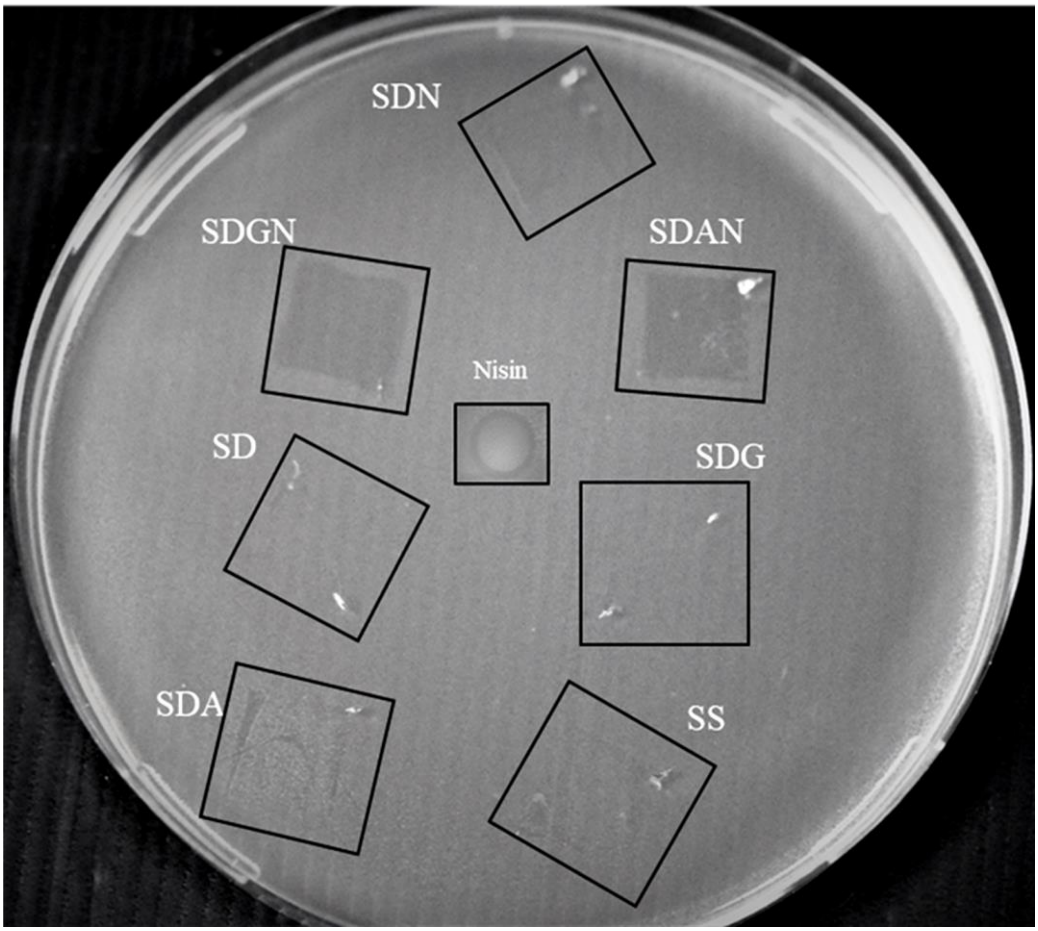

Fig. 7. Antibacterial assessment toward *Listeria monocytogenes* of different coated SS surfaces and nisin (1 mg ml^{-1}). The experiment was performed on Mueller Hinton agar medium seeded with *L. monocytogenes*.

Surface characterization of SS coated films

To understand the properties of treated surfaces, the presence of molecular compounds and their relative quantities and nisin conformation on coatings, different tests for analyzing the chemical properties of the treated surfaces were carried out.

WCA was measured on the different treated surfaces to make sure that nisin is fixed onto SDGN, SDAN and SDN samples. WCA of bare SS was 61 ± 2°. Treated surfaces SD, SDG and SDA generated a decrease in the WCA, to 54 ± 1°, 42 ± 3° and 42 ± 2° respectively. These coatings provided the steel surface a more hydrophilic character. However, after nisin addition to pretreated surfaces (SD, SDG and SDA), SDN, SDGN and SDAN were

characterized by higher WCA measurements that went from 81 ± 3°, 83 ± 3° and 73 ± 2° respectively (Table 1).

Table 1

WCA measurements on bare and different treated surfaces.

Surface	WCA (°)	Standard deviation (°)
SS	61	± 2
SD	54	± 1
SDG	42	± 3
SDA	42	± 2
SDN	81	± 3
SDGN	83	± 3
SDAN	73	± 2

The surface roughness of coatings was analyzed by *Alpha-Step IQ, stylus-based surface profiler*. Bare SS was characterized by a roughness R_a equal to 2.8 ± 0.1 nm and R_q equal to 3.9 ± 0.4 nm. After polydopamine immobilization on the SS surface (SD) the roughness values decreased to R_a equal to 1.9 ± 0.3 nm and R_q equal to 2.5 ± 0.4 nm. For SDN, after nisin grafting on SD, roughness increased significantly to 4.8 ± 0.8 and 6.0 ± 0.3 nm for R_a and R_q respectively. SDG showed a similar roughness to bare SS and SDA presented a higher roughness values R_a equal to 3.4 ± 0.5 nm and R_q equal to 4.4 ± 0.5 nm.Moreover, the nisin fixation on SDG and SDA, barely increased the roughness of almost 0.5 nm for SDGN and 1 nm for SDAN. Indeed, SDGN registered 3.1 ± 0.2 nm for R_a and 4.0 ± 0.2 nm for R_q and SDAN registered 4 ± 0.4 nm for Ra and 5.4 ± 0.4 nm for R_q (Fig. 8A).

On the same wave, the thickness results showed for SD a value of 310 ± 7 nm. After nisin addition to SD, the SDN thickness value increased significantly to 356 ± 10 nm. Otherwise, the thickness of SDG and SDA was 321 ± 4 nm and 327 ± 3 nm respectively. After nisin addition to SDG and SDA, the thickness values of SDGN and SGAN barely increased to 326 ± 5 nm and 337 ± 2 nm respectively (Fig. 8B).

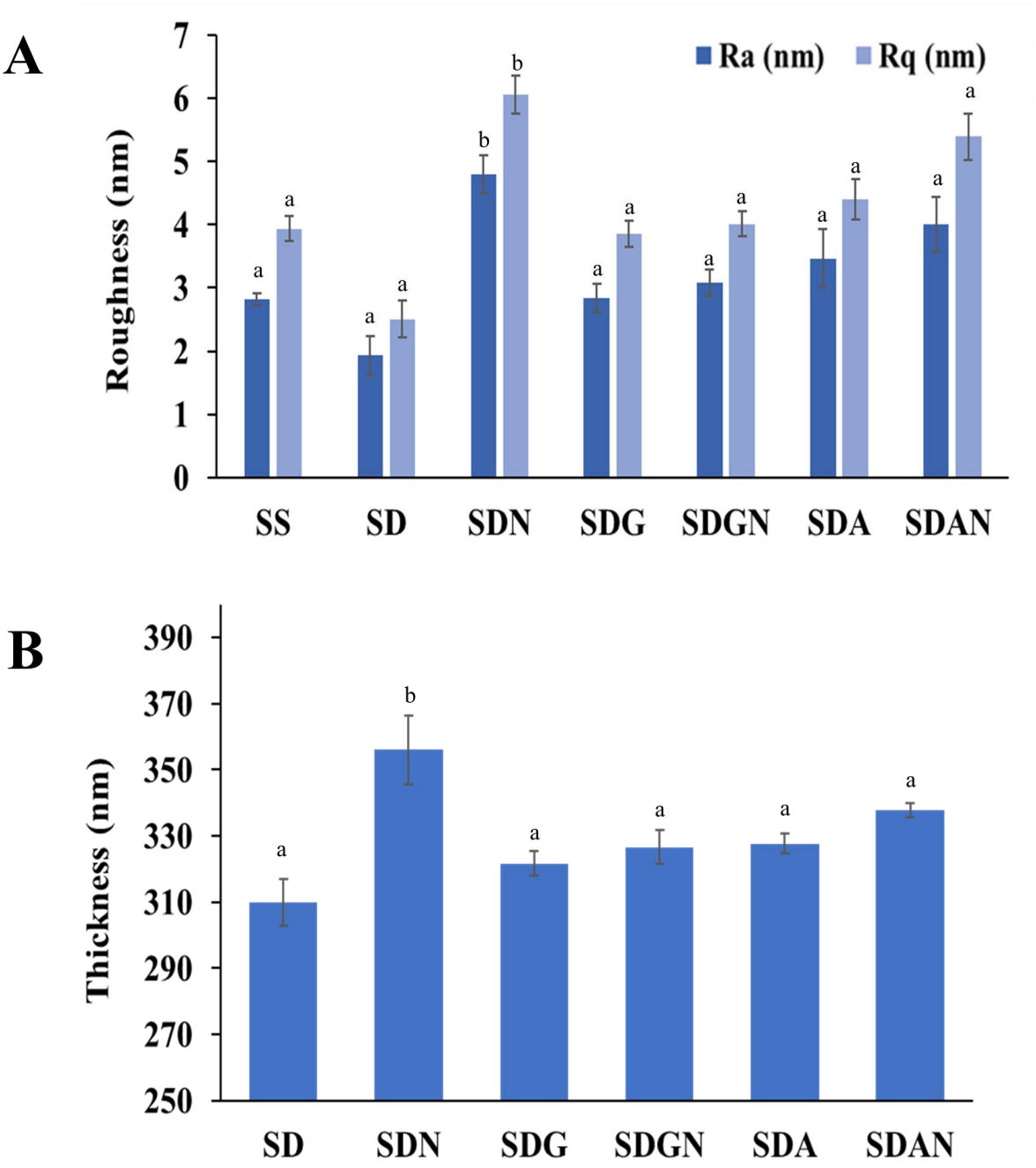

Fig. 8. (A) Surface roughness parameters R_a and R_q for bare SS and coated SS surfaces. (B) Thickness of treated surfaces defined by averaged values in nm ± SD. (*) Different letters on top of error bars (a, b) indicate significant differences ($p < 0.05$); the same letters on error bars indicate non-significant differences ($p > 0.05$).

Aiming to visualize the morphological modifications after molecular grafting on SS, SEM analyze was carried out. Micrographs, shows that bare SS surface is smooth with small

holes and crevices. Moreover, SD is characterized by granular spherical structures that result from the 3D polymerization of dopamine. Moreover, the two-layers coatings, SDG, SDA and SDN shows a shallower topography without permitting to see in detail the rougher structure, while the three-layers coatings SDGN and SDAN are characterized by bigger granular structures (Fig. 9).

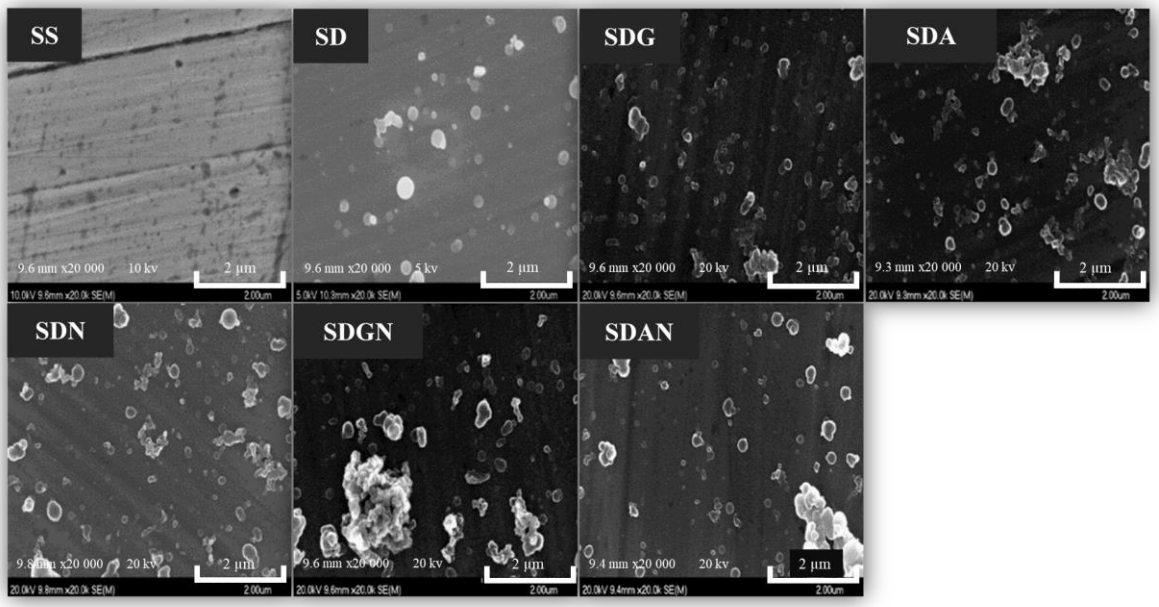

Fig. 9. Scanning electron microscopy micrographs of bare SS coated surfaces.

After SEM visualization, ion polishing was carried out to detect variations in the different coatings structure of SD, SDGN, SDAN and SDN, as given in Fig. 10. The cross-section showed variable specifications of each coating type. The micrographs A and B representing SD sample showed a full coverage of SS and polymerized dopamine mass respectively.

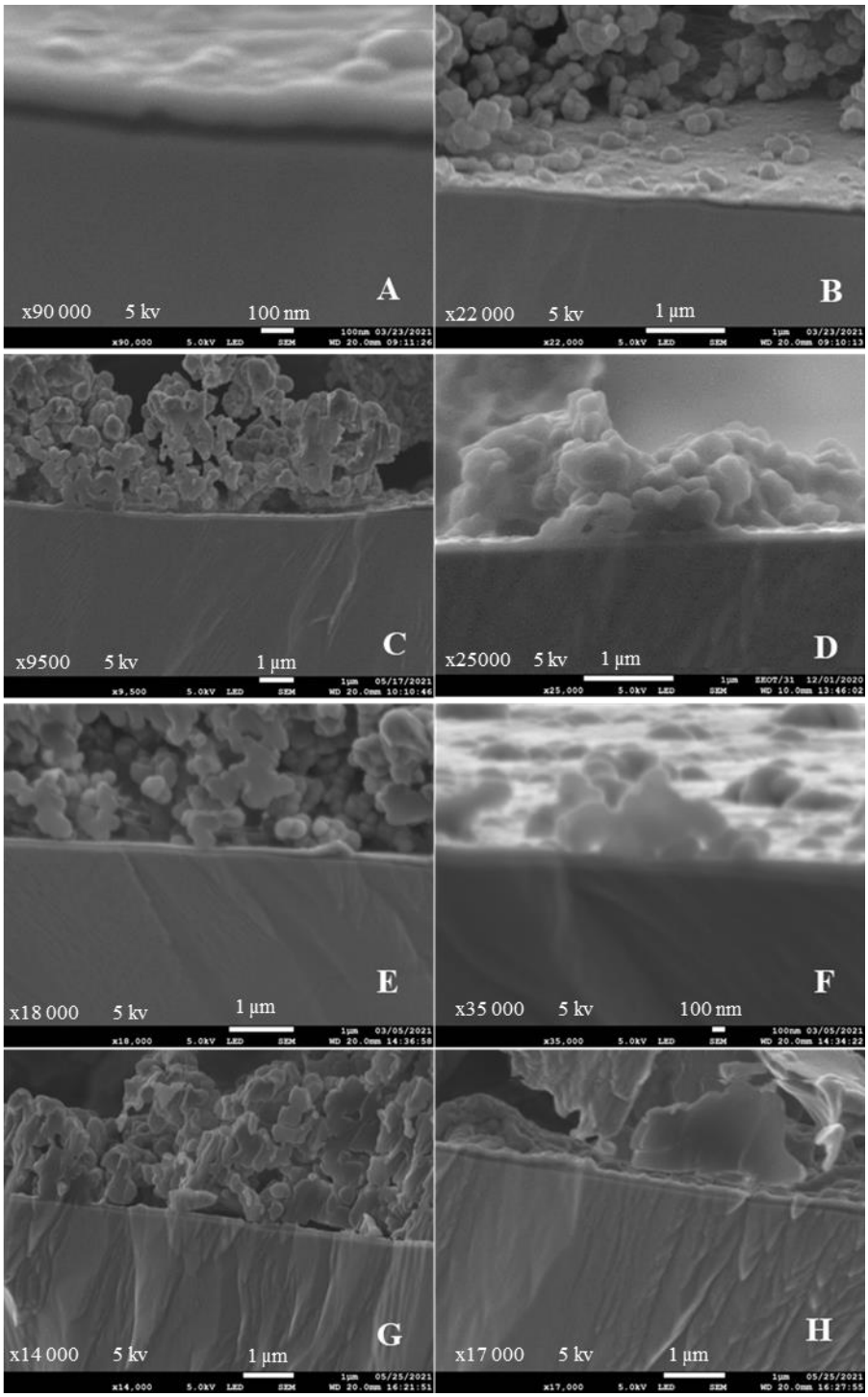

Fig. 10. SEM micrographs of cross section of coated surfaces after ion beam polishing. SD (A-B), SDGN (C-D), SDAN (E-F), SDN (G-H).

SDGN samples were represented by C and D micrographs where a mass of polydopamine/Glutaraldehyde/Nisin was detected on the sample with a full coverage preservation. E and F shows SDAN polished samples where the mass of

polydopamine/Succinic acid/Nisin was detected. However, holes were detected on the sample (Micrograph F). The micrographs G and H presents the SDN coating samples. A low coverage of polydopamine and nisin accumulation was detected on G and H respectively.

ATR-FTIR analysis was performed to characterize and determine changes in the infrared bands related to the nisin immobilization on the polydopamine coated SS surfaces. Normalized FTIR spectra of nisin and grafted nisin on the polydopamine coated surface are presented in Fig. 11. Free nisin exhibited characteristic absorption bands at 3296 cm^{-1} (axial O–H and N–H stretching vibrations), 2962 cm^{-1} (C–H stretching vibrations), 1640 cm^{-1} (the absorption peak of amide band), 1514 cm^{-1} (bending of primary amines), 1441 cm^{-1} (COO^- symmetric stretching vibrations), and 1234 cm^{-1} (C–N stretching vibrations) [21]

FTIR spectra of different treated SS surfaces showed a broadening and shift in N–H stretching peak of nisin to higher wavenumber, which indicated that a new intermolecular H-bonding between N–H groups of nisin with O–H stretch of polydopamine in the case of SDN, succinic acid in the case of SDAN, and glutaraldehyde in the case of SDGN [22]. Amide groups of nisin most likely caused a shifting in the wavenumbers when grafted onto polydopamine coatings, without linker in the case of SDN (1600 cm^{-1}) or with linker in the case of SDAN (1607 cm^{-1}) and SDGN (1606 cm^{-1}). These subtle alterations may suggest that the intermolecular interactions exist between amino groups of the nisin and polydopamine without or with addition of linkers [23]. Besides, the nisin immobilization on the polydopamine coated SS surface caused an increase in the wavenumber of C-N from 1234 cm^{-1} in free nisin to 1290 cm^{-1} approximatively in SDN, SDAN and SDGN. This change is probably attributed to the existence of intermolecular interactions between nisin and polydopamine in both cases, without and with addition of linkers (succinic acid or glutaraldehyde).

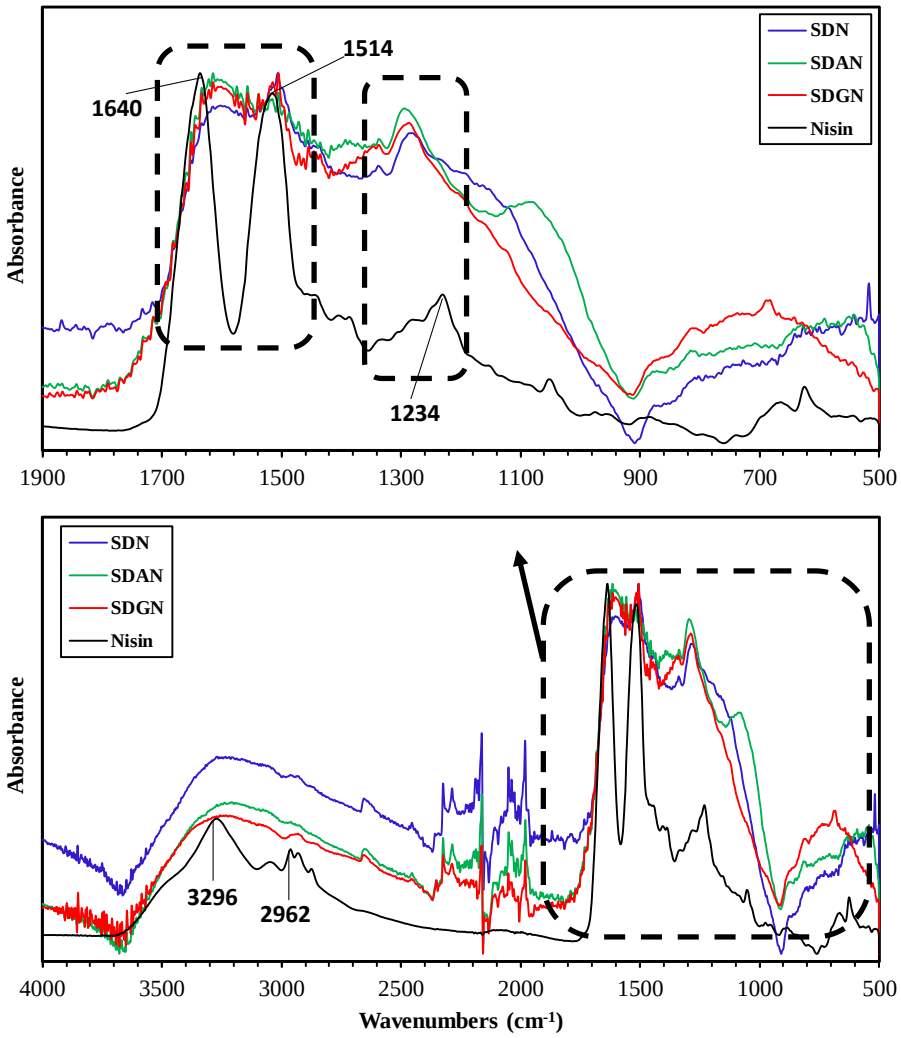

Fig. 11. FTIR spectra of different Nisin based coated SS surfaces.

ToF-SIMS spectra showed the relative intensity counts of the secondary ions corresponding to a molecular compound present on the grafted samples. Table 2 summarized the secondary ions detected by ToF-SIMS analyses of all coated surfaces SD, SDG, SDA, SDN, SDGN and SDAN and the main characterizations concerning the quantifications and the differences between surfaces. The ToF-SIMS spectra showed three detected secondary ions $C_8H_7NO_2^+$, $C_{12}H_{28}N^+$ and $C_{23}H_{20}N_3O_4^+$ at *m/z* 149.04, 186.24 and 402 respectively as shown in Fig. S1. These ions are relative of dopamine. The highest intensity was detected for SD treated surfaces, this intensity decreased with the addition of molecular layers on SD coating.

Table 2

ToF-SIMS main characterizations.

Positive and negative secondary ions	*m/z*	Related compound	Coating type with high spectra intensity count	References
$C_8H_7NO_2^+$	149.04	Dopamine hypochloride: $C_8H_{11}NO_2$	SD	[24–27]
$C_{12}H_{28}N^+$	186.24	Dopamine hypochloride: $C_8H_{11}NO_2$	SD	[24–27]
$C_{23}H_{20}N_3O_4^+$	402	Dopamine hypochloride: $C_8H_{11}NO_2$	SD	[28]
$C_3H_5O^+$	57.03	Glutaraldehyde: $C_5H_8O_2$	SDGN > SDG	[29]
$C_{12}H_{26}^+$	170.2	Succinic acid: $C_4H_6O_4$	SDA > SDAN	[30]
$C_4H_9^+$	57.06	Succinic acid: $C_4H_6O_4$	SDA > SDAN	[30]
NH_4^+	18.03	$C_8H_{11}NO_2$ and all nisin amino acids	SDGN > SDN > SDAN	[31-33]
$C_2H_5S^+$	61.01	Methionine (Met) of nisin	SDGN > SDAN > SDN	[31-33]
S_2^{2-}	31.9	^{32}S (95,02 %) of nisin	SDGN > SDAN > SDN	—
Fe^+	55.95	SS containing Fe	SDN > SDA > SDG	—

The spectrum of the secondary ion $C_3H_5O^+$ was also detected at m/z 57.03 as given in Fig. S2. This ion corresponds to glutaraldehyde molecule. The intensity was the highest for SDG and SDGN containing glutaraldehyde. The succinic acid was detected via two secondary ions $C_4H_9^+$ and $C_{12}H_{26}^+$ spectra represented at *m/z* values are 57.06 and 170.2, respectively. The intensity count was the most important for coatings containing succinic acid SDA and SDAN. The highest intensity was for SDA then for SDAN in which the nisin covered succinic acid compound (Fig. S3). Nisin was detected by three different secondary ions. NH_4^+ spectrum was detected at *m/z* = 18.03. This ion corresponds to dopamine containing amino groups or for nisin containing 34 amino acids. The intensity was the highest for SDGN then SDN then SDAN. Methionin amino acid of nisin was detected on the spectrum of the secondary ion $C_2H_5S^+$ at *m/z*

= 61.01. Moreover, the negative secondary ion S_2^{2-} was detected at *m/z* = 31.9. For both spectra, the intensity was the highest for SDGN then SDAN then SDN (Fig. S4). To understand the homogeneity of the treated surfaces, the iron spectrum was analyzed. Iron secondary ion spectrum was detected on *m/z* = 55.95. The intensity counts from the highest to the lower was in the following order: SDN, SDA, SDG, SDAN, SDGN and SD (Fig. S5).

Supplementary figures

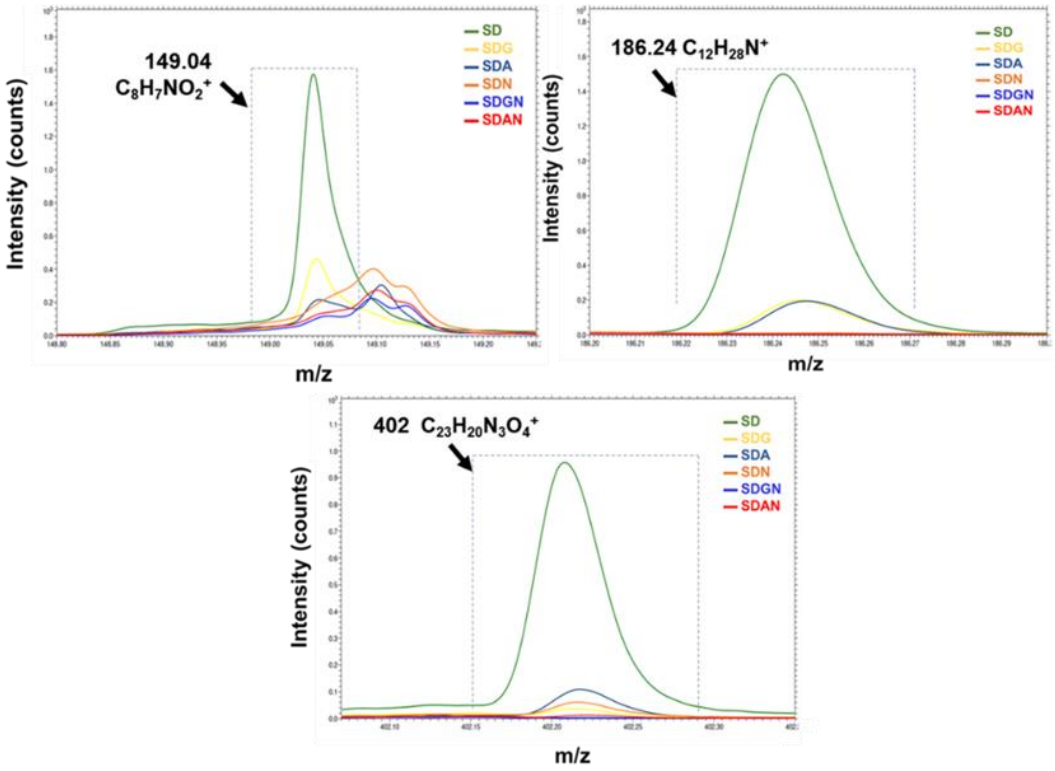

Fig. S1. ToF-SIMS spectra representing dopamine secondary ions.

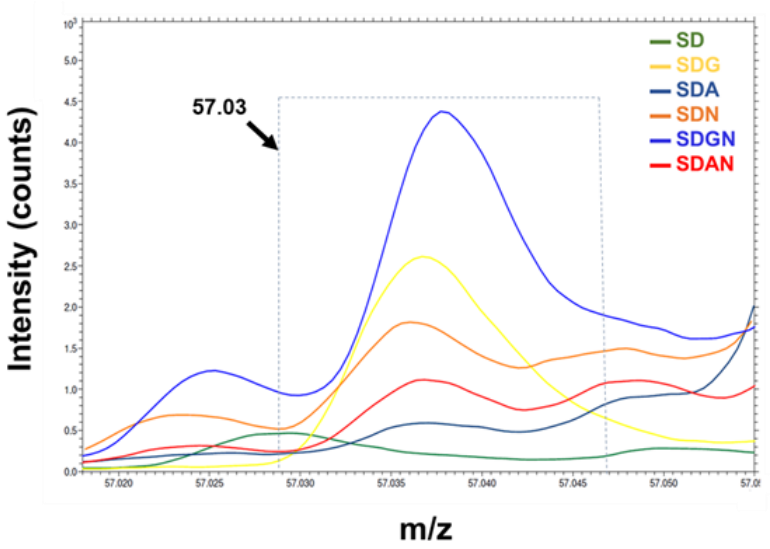

Fig. S2. ToF-SIMS spectra representing glutaraldehyde secondary ions.

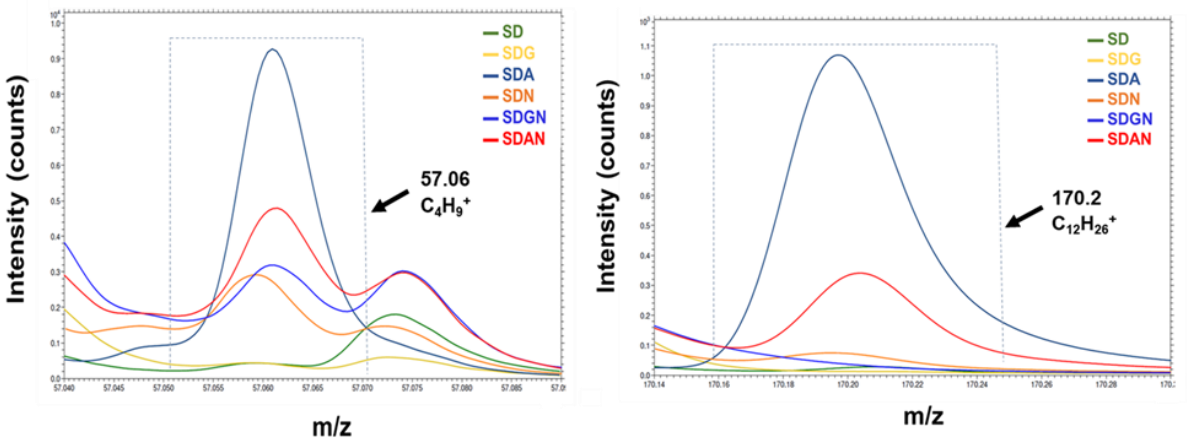

Fig. S3. ToF-SIMS spectra representing succinic acid secondary ions.

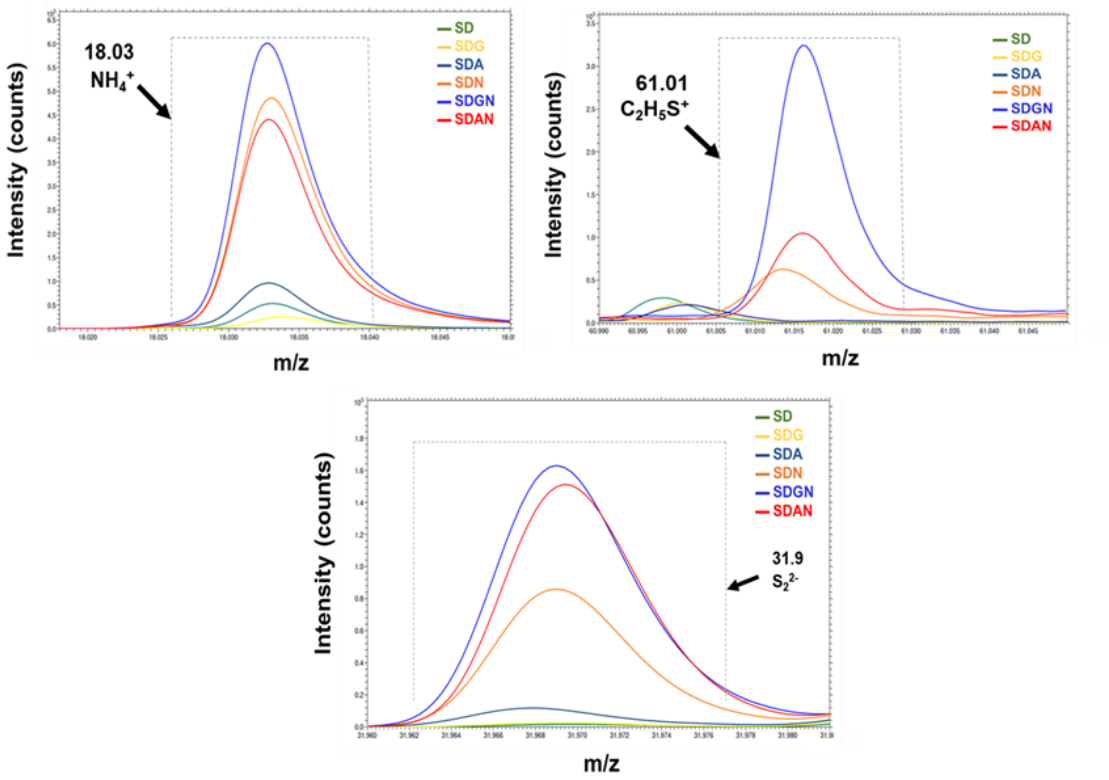

Fig. S4. ToF-SIMS spectra representing nisin secondary ions.

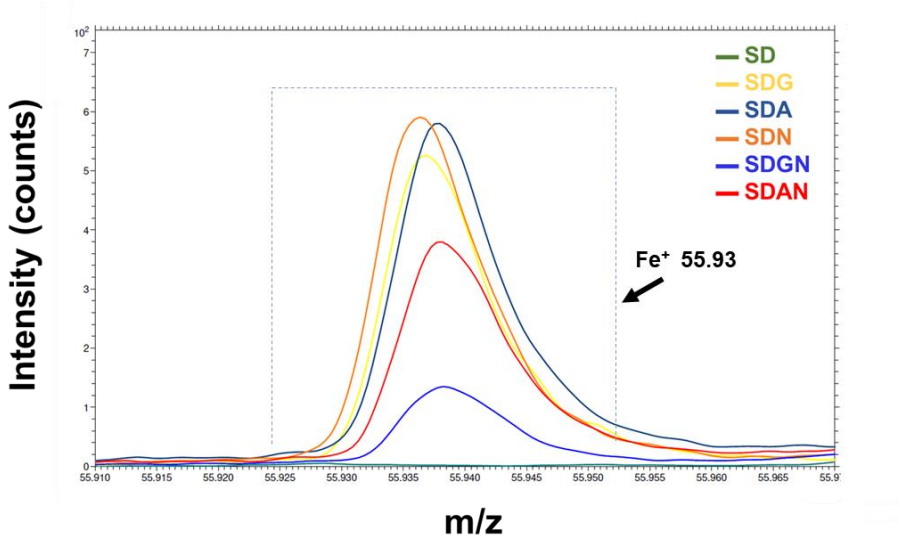

Fig. S5. ToF-SIMS spectra representing iron present in stainless steel.

XPS analyses were performed to provide the elemental composition and the distribution of functional groups of the formed layer on the different polydopamine coated SS surfaces

without and with addition of nisin. The obtained XPS wide-scan (survey) spectra of different coated SS surfaces (SD, SDN, SDAN and SDGN) are shown in Fig. 12. The low intensity of the Fe2p signals in all cases is probably due to the higher coatings thickness on the SS. The obtained high-resolution peaks for C 1s, O 1s and N 1s core levels, through a deconvoluted fitting procedure using the CASA XPS software, were given in Fig. 13. The obtained values of binding energy (BE, eV), the corresponding quantification (%) as well as their assignment for each component were given in Table 3. XPS surface elemental analyses of different coated SS surfaces (SD, SDN, SDAN and SDGN) were performed and given in Table 4. In all cases, the sum of the atom concentrations was normalized to 100% to easily compare surface concentrations for the different elements. Carbon and oxygen atomic percentages showed no significant difference between the different coated SS surfaces. However, nitrogen atomic percentage increased significantly after nisin addition for all treated surfaces. The maximum N content value was found in the case of SDGN. Moreover, the sulfur element was absent for SD and the highest value was observed for SDGN followed by SDN and SDAN. The percentage of iron was nil on SD and SDGN while it was detected (very low) on SDN and SDAN. Hence it is clear that glutaraldehyde linker has a significant contribution in the covalently incorporation nisin on the coating.

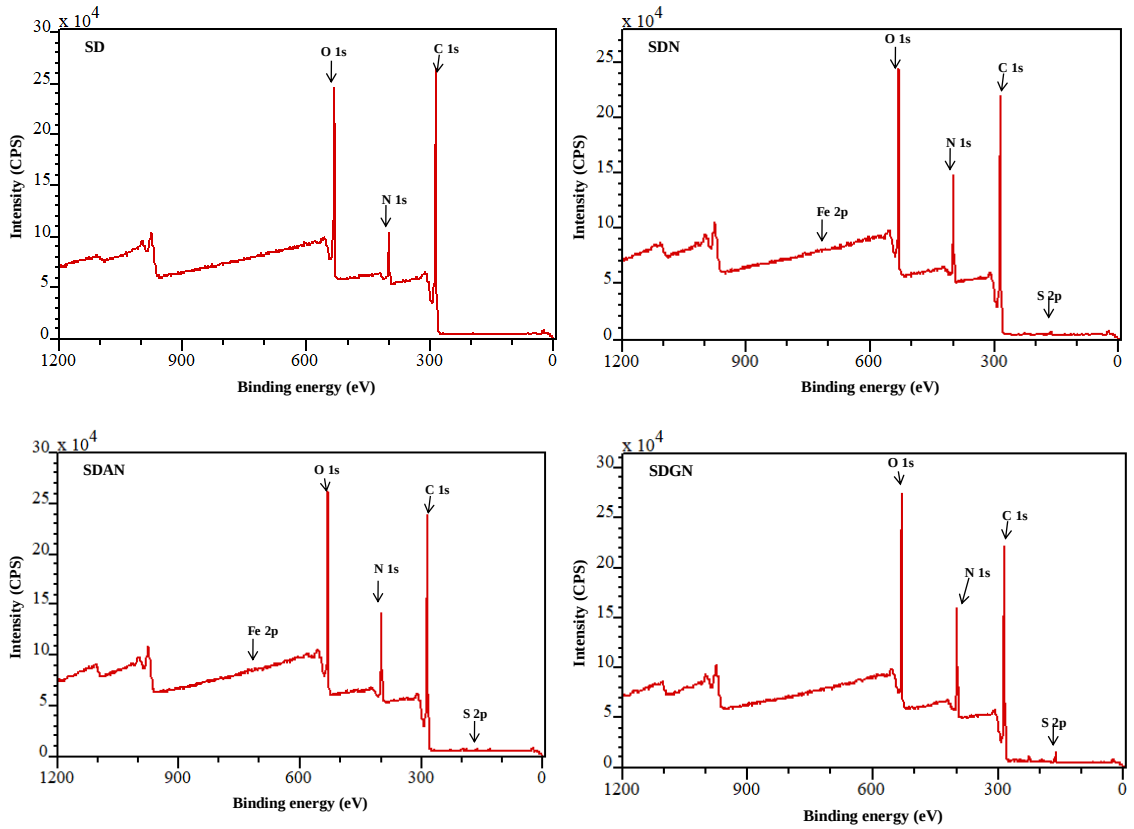

Fig. 12. XPS survey spectra of different coated SS surfaces.

The C 1s peak deconvolution for polydopamine coated SS surfaces without incorporation of nisin (SD), and with its incorporation without linker addition (SDN) and with linker addition (SDAN, SDGN) may be fitted into three components as shown in Fig. 13 and Table 3. SD shows two dominant components attributed to the C–C, C=C and C–H bonds at 284.8 eV and C–N/C–O at 286.2 eV of dopamine polymerized structure and one small component attributed to C=O at 287.8 eV that corresponds to possible tautomers of polydopamine [34]. The incorporation of nisin onto polydopamine coated SS surfaces without or with linker addition reveals a significant increase in the contribution of the component at high binding energy side, probably due to the apparition of –COOH and –CONH groups. Indeed, all nisin characteristic functions were observed on the coatings and C 1s results permitted to confirm the nisin covalent incorporation into the different investigated coatings (SDN, SDAN, SDGN).

The O 1s region of SD, given in Fig. 13, is fitted in two peaks at 531.2 and 533.0 eV assigned to O=C and O−C species, respectively. The XPS deconvoluted O 1s spectra of SDN, SDAN and SDGN support that the incorporation of nisin without and with linker addition increase the percentage of C=O in the coatings, which prove that the nisin molecules were successfully linked into the different coated SS surfaces.

The N 1s region of SD surface is fitted into two components at 400.2 and 402.3 eV (Fig. 13). The first one represents the most contribution (91 %) and can be assigned to secondary amine (R−NH−R), while the second component can be attributed to the primary amine ($R-NH_2$) functionality [35]. Indeed, the secondary amine component dominates the N 1s region, as it is associated to polydopamine. In the case of SDN surface, the N 1s spectrum is fitted into two components, the small one (4%) at 402.2 eV attributed to the primary ($R-NH_2$) and the dominant one (96 %) assigned to the secondary (R−NH−R), and tertiary/aromatic (=N−R) amine functionalities [35]. The secondary amine is associated to both polydopamine and nisin, and the tertiary amine is associated to nisin only. The same behaviour was observed in the case of SDAN coating. However, the main component at 399.9 eV can be also attributed to the formation of amid functional group as result of the reaction of NH_2 group of nisin with the succinic acid used as linker in the SDAN coating. On the other hand, the N 1s peak was shifted to lower binding energy side for the SDGN coating, compared to other coatings (SD, SDN and SDAN) as shown in Fig. 13. The high resolution deconvoluted XPS spectra of N 1s peaks for SDGN surface show two types of nitrogen at 399.4 eV for tertiary/aromatic (=N−R) amine, 399.9 eV for the secondary (R−NH−R) amine type. It is worth noting that the primary ($R-NH_2$) amine has disappeared, and the quantification result, given in Table 3, shows that the percentage of =N−R was the highest (88 %). This can be explained by the possible formation of imine functional group, resulting of the reaction of NH_2 group of nisin with the glutaraldehyde used as a linker in the SDGN coating.

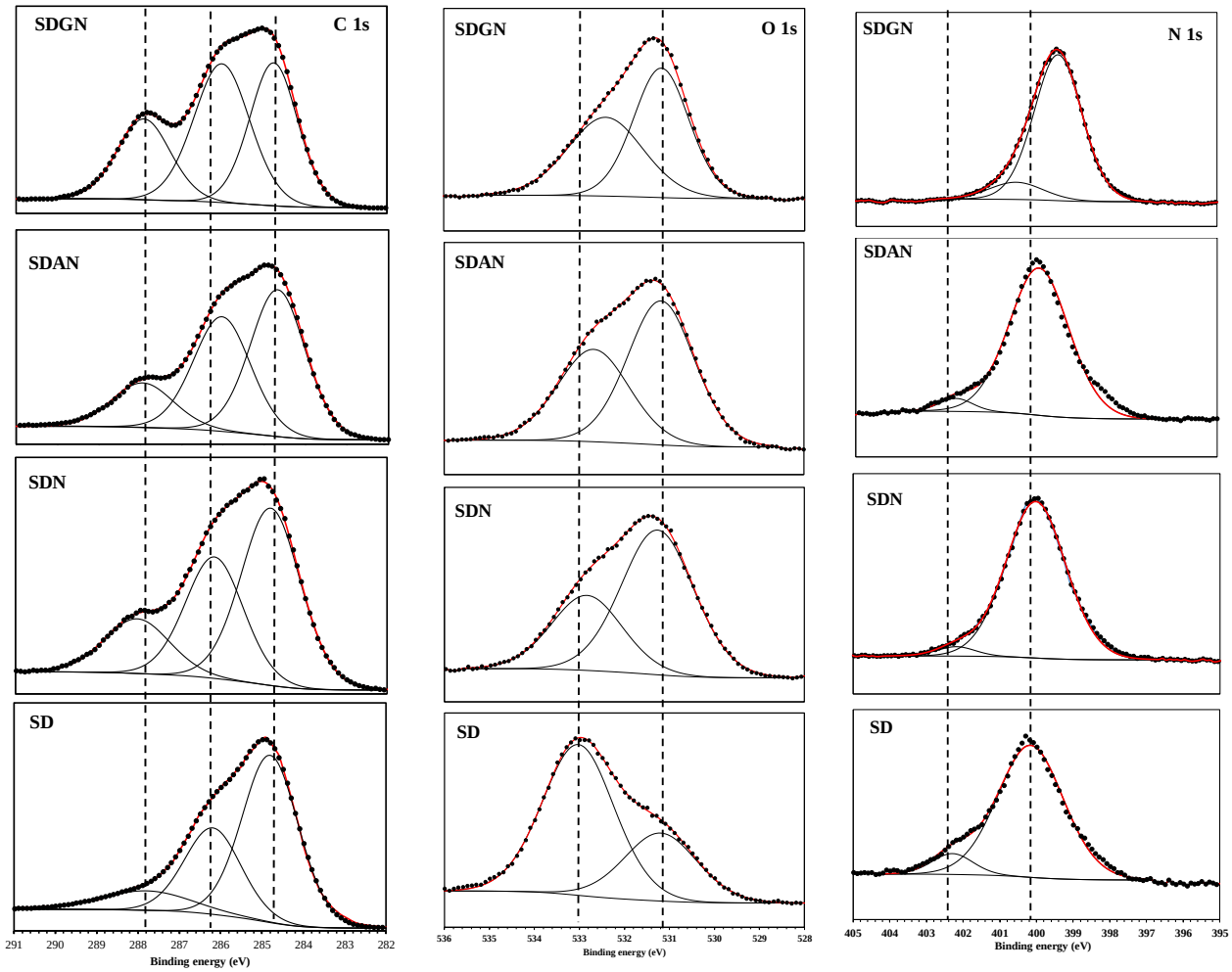

Fig. 13. High-resolution X-ray photoelectron deconvoluted profiles of C 1s, O 1s and N 1s for different coated SS surfaces.

Table 3
Binding energies (eV), relative intensity and their assignment for the major core lines observed different coated SS surfaces.

Element	Position (eV)	Assignment
	SD	
C 1s	284.8 (59 %)	C–H / C–C / C=C
	286.2 (30 %)	C–N / C–O
	287.8 (11 %)	C=O
O 1s	531.2 (32 %)	O=C
	533.0 (68 %)	O–C
N 1s	400.2 (91 %)	–NH
	402.3 (9 %)	$–NH_2$
	SDN	
C 1s	284.8 (50 %)	C–H / C–C / C=C

	286.2 (33 %)	C–O / C–N / C=N / C–S
	288.1 (17 %)	–COOH / –CONH
O 1s	531.3 (66 %)	O=C
	532.8 (34 %)	O–C
N 1s	399.9 (96 %)	=N– structure / –NH
	402.2 (4 %)	$–NH_2$
	SDAN	
C 1s	284.7 (47 %)	C–H / C–C / C=C
	286.0 (38 %)	C–O / C–N / C=N / C–S
	287.9 (15 %)	–COOH / –CONH
O 1s	531.2 (59 %)	O=C
	532.7 (41 %)	O–C
N 1s	399.9 (95 %)	=N– structure / –NH
	402.2 (5 %)	$–NH_2$
	SDGN	
C 1s	284.4 (37 %)	C–H / C–C / C=C
	285.6 (40 %)	C–O / C–N / C=N / C–S
	287.5 (23 %)	–COOH / –CONH
O 1s	530.8 (51 %)	O=C
	532.0 (49 %)	O–C
N 1s	399.4 (88 %)	=N– structure
	400.6 (12 %)	–NH

Table 4

Atomic percentage of atoms (%) on coated SS surfaces by XPS.

Atom	SD	SDN	SDGN	SDAN
C	76.62	70.14	67.87	70.38
O	16.17	16.60	16.66	17.35
N	7.16	12.83	14.40	11.91
S	0.00	0.34	1.07	0.20
Fe	0.00	0.10	0.00	0.16

Discussion

The antimicrobial grafting on materials used in medical and food sectors, like SS, constitutes a promising way forward. In this research, bacteriocin nisin produced by *Lactococcus lactis* subsp *lactis*, was successfully grafted on the surface of SS. The aim of this study was to investigate the antimicrobial effect and chemical properties of elaborated coatings. After ensuring that the bacterial adhesion rate was similar and comparable on all coated surfaces. Nisin was grafted onto the steel according to two approaches using its $–NH_2$ and –COOH groups (Fig. 2). Moreover, studies on nisin mechanism suggested that its effective action

requires the presence of its carboxylic and amino groups in a non-linked state [10,36,37]. However, our results showed an effective antimicrobial activity for SDGN followed by SDAN when nisin is linked by its amino group keeping the carboxylic terminal group free. The antimicrobial effect was the most efficient after 3 and 5 h of contact while the bacterial population increased after 24 h. Otherwise, for SDN samples, nisin's carboxylic group was linked and its amino group was free, no antimicrobial effect was observed. This demonstrates the involvement of hydrophilic side of nisin, containing the non-linked –COOH, in the antimicrobial activity. On the same wave, the viability percentages of *L. monocytogenes* decreased of almost 70 % for SDGN and SDAN while no decrease was detected for SDN. However, the loss of the antimicrobial efficiency after 24 h of contact with bacteria suggest a one-use antimicrobial surfaces application. Additional studies on the possible alternatives to improve those coating's antibacterial efficiency within time could be an interesting perspective.

The antibacterial qualitative assessment demonstrated bacterial efficiency only for SDGN and SDAN and that nisin linkage to these coatings inhibited its diffusion while free nisin soaked in a cellulose disc showed an inhibition zone due to its diffusion. Nisin mode of action was highlighted by the MEB analysis of the effective antibacterial SDGN and SDAN. Indeed, the micrographs presented the damaged bacterial membrane of dead *L. monocytogenes*. To understand these difference of antimicrobial efficacy between SDGN, SDAN and SDN, surfaces properties were characterized chemically and morphologically.

WCA is a technique that is sensitive to the extreme surface of a coating [38]. It was carried out for all elaborated surfaces to detect hydrophobicity modification after molecules grafting. Indeed, the hydrophobicity increased on SDN, SDAN and SDGN proving nisin attachment to samples. Furthermore, studies have shown that the hydrophilicity of a surface may contribute in the prevention of bacterial contamination of coated surfaces [39,40]. However, the antibacterial results showed that nisin-coated surfaces were not all bactericidal

while WCA measurements were of the same order. Indeed, the hydrophilic character of nisin-coated surfaces was not involved in the antimicrobial efficiency.

FTIR analyses permitted the detection of functional groups linked to nisin molecule in the free and grafted state. The results gave the evidence that nisin was attached to the polydopamine coating. In all coated surfaces SDN, SDGN and SDAN, intramolecular interactions between polydopamine and nisin were detected regardless the presence of linkers or not between the two molecules.

ToF-SIMS and XPS analyses were carried out to understand nisin conformation and quantitative aspects of elements on the coatings. Tof-SIMS spectra showed the relative intensity counts of the secondary ions corresponding to each grafted compound present on the samples. The depth profiling limit of Tof-SIMS was around few nanometers. Each compound grafted in the external layer of each developed coating was identified via the secondary ions and analyzed quantitatively and qualitatively. Polydopamine, glutaraldehyde and succinic acid were identified on the coatings already containing each compound. For dopamine, when layers were added, the intensity count of the ion corresponding to the internal layer decreased (SD > SDG-SDA-SDN > SDGN-SDAN). Concerning nisin specific secondary ions, the intensity counts of each ion was compared between SDN, SDAN and SDN containing nisin. NH_4^+ spectra showed the highest intensity for SDGN then for SDN then for SDAN. Otherwise, $C_2H_5S^+$ and S_2^{2-} spectra intensity followed the level of antibacterial efficiency, from efficient to less efficient to inefficient. Indeed, the intensity for both ions was the highest for SDGN then SDAN then SDN. The intensity of NH_4^+ spectra was higher for SDN than SDAN due to the presence of polydopamine directly linked to nisin in SDN. Indeed, NH_4^+ is a secondary ion representing dopamine and nisin at the same time. The SDN peak intensity of NH_4^+ represented both nisin and polydopamine. Therefore, nisin quantity was the highest for SDGN then SDN then SDAN. Moreover, $C_2H_5S^+$ secondary ion representing the methionine amino acid located at sites 17 and

21 of nisin that are closer to the side containing the carboxylic terminal group. This ion was more pronounced for SDGN and SDAN where nisin –COOH side is free than for SDN where nisin –COOH side is engaged. This result permitted to confirm the nisin orientation. Moreover, to investigate the coatings homogeneity, Fe^{+} intensity peaks showed that SD was fully covered by polydopamine and the coating was homogeneous. For SDN, SDG and SDA, the nisin, glutaraldehyde and succinic acid addition on SD respectively, guided the polydopamine with it and SS bare surface was detected via Fe^{+} ions. However, For SDGN and SDAN, the nisin addition on SDG and SDA decreased the intensity peak of $Fe^{+\cdot}$ The decrease was more important for SDGN than SDAN. Indeed, SDGN was considered homogeneous while SDAN presented some heterogeneities.

XPS results were in concordance with ToF-SIMS. The sampling depth of XPS analysis is approximatively of 70 nm. The atomic percentage of nitrogen was the most important for SDGN followed by SDN then SDAN. The nitrogen percentage of SDN was higher than SDAN because of the presence of nitrogen in polydopamine, detected for SDN. The percentage of sulfur was nil on SD due to the absence of nisin containing disulfide bridges. SDGN had the highest atomic percentage followed by SDN and SDAN. The atomic percentage of iron, on the same wave of ToF-SIMS results, was nil for SD and SDGN and detected for SDN and SDAN. The XPS survey spectra certified the interpretation of ToF-SIMS and XPS atomic percentages. Indeed, the peak analyses confirmed that nisin quantity on SDGN was the highest followed by SDN then SDAN. Moreover, the detection on iron peaks on SDN and SDAN confirmed the heterogeneity of these two coatings while SD and SDGN were homogenous and SS was fully covered. For the C 1s signal, C=N, C–S and C=O assignments detected on SDN, SDAN and SDGN represented nisin adsorption especially the carbon-sulfur bond conferring to disulphide bonds in nisin. Peaks representing the functional groups –COOH and –CONH were also detected on those three coatings with different percentages. Indeed, for SDGN, it was the

highest (23%) then SDN (17%) then SDAN (15%). This percentages distribution indicated the difference in nisin quantity grafted on each surface. For the O 1s signal, on SD, H_2O adsorption was linked to its hydrophilic character while –O=C bond appeared for nisin grafted coatings. The highest percentages were detected for SDGN (49 %) and SDAN (41 %) while it was less pronounced for SDN (34 %). This difference is linked to the conformation of nisin on those surfaces. The acidic function is free and more exposed for SDGN and SDAN while nisin is linked by its carboxylic function to polydopamine on SDN (Fig. 13, Table 3).

Several studies demonstrated that the increase in a surface roughness induces a decrease in the antimicrobial effect [41–44]. Moreover, a rough surface provides an adequate environment for the bacteria to adhere on a surface and generates a biofilm. These results are consistent with the literature, SDN coating was inactive against *L. monocytogenes* while its surface roughness was the highest. However, SDGN and SDAN registered the lowest surface roughness and showed an effective antimicrobial activity. Indeed, the use of several approaches in grafting nisin may lead to its conformation modification on the surface that can be the cause of the variation of surface roughness and antimicrobial activity.

Roughness and thickness results illustrated the morphology of each coating type. Roughness and thickness of SDN after nisin addition increased significantly while it barely increased after nisin addition to SDA and SDG. Indeed, nisin tended to accumulate on SDN while it is distributed evenly without increasing the roughness and thickness of SDGN and SDAN. Moreover, the presence of iron detected on SDAN and SDN proved their heterogeneity. Nisin accumulation and iron presence on SDN demonstrate a columnar coating. This accumulation and chemical bonding might be the cause of nisin inactivity towards *L. monocytogenes*. Otherwise, SDGN and SDAN seems to present a similar structure despite the presence of coverage heterogeneity on SDAN (Fe detection). The antimicrobial highest effectiveness of SDGN was linked to nisin highest quantity adsorption, to nisin free carboxylic

group playing a role in antibacterial mechanism and to homogeneity of the coating. Moreover, SDAN antimicrobial effectiveness was linked to nisin chemical linkage with a free carboxylic group like SDGN and nisin distribution on succinic acid without its accumulation. The diminution of its activity in comparison with SDGN is linked to nisin lower quantity and heterogeneity in the coverage. SEM of coatings permitted to show the global morphology and detect different morphologies after molecules grafting. It showed the surface from the top but did not allow to see some other differences in coating microstructure such as columnar aspect, porosity and stainless steel/dopamine interface. Therefore, ion polishing was carried out to analyse the cross section of each coating. The observed microstructural variations of the different coatings were in concordance with FTIR, XPS, ToF SIMS, roughness and thickness analysis. Indeed, SD was homogeneous and polymerized dopamine was detected and the surface was fully covered. Moreover, SDGN was also homogeneous and the surface was fully covered. The presence of porosity in SDAN micrographs outlined the Fe detection by ToF SIMS. This explains why the antimicrobial effectiveness of SDGN was higher than SDAN. Otherwise, SDN micrographs showed a non-homogenous and non-uniform covering coating presenting nisin accumulation in a tubular aspect. This structure in blocs correlates with the results of the surface roughness and thickness where it was the highest for SDN coatings. The accumulation and chemical conformation of nisin initially linked via its carboxylic group lead to the inactivation of antimicrobial efficacy. Indeed, nisin mode of action concern the binding of its cationic COOH terminus with bacterial cell wall leading to its damage. The unavailability of this COOH group leads to the loss of antibacterial effect. Moreover, these results explain the high roughness and thickness of SDN after nisin addition in comparison with SDGN and SDAN.

Conclusion

This study permitted to reach the main goal concerning the prevention of biofilm formation on SS surface to protect population health. The functionalized surfaces were analyzed chemically and biologically. Challenge tests, antibacterial qualitative testing, SEM and LIVE/DEAD techniques towards *L. monocytogenes* demonstrated the antimicrobial effectiveness of SDGN followed by SDAN and the non-efficient activity of SDN. The chemical grafting of molecules on the different prepared surfaces provided information of nisin linkage conformation on each surface. The results showed that nisin was linked in SDGN and SDAN by its amino group to glutaraldehyde and succinic acid respectively, and in SDN by its carboxylic group to polydopamine. Moreover, ToF-SIMS, XPS, surface roughness and thickness analysis, ionic polishing and WCA enabled the understanding of surfaces homogeneities, nisin presence and quantification, and coatings qualifications. Indeed, those analyses showed that the nisin carboxylic group participate in the antibacterial effect.

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CONCLUSIONS AND PERSPECTIVES

Unlike free-floating bacteria, biofilms have a very organized structure. In some situations, they are more resistant to sanitization procedures and antimicrobial display, revealing up to 1,000-fold greater MIC value than their planktonic homologues (Araújo et al. 2011). They are composed of bacteria that attach to surfaces and begin to grow into large clusters surrounded by a protective matrix constituted of proteins, DNA and polysaccharides. The extracellular matrix serves as a protection against environmental factors. The destruction of biofilms will be therefore difficult and complicated. In the food industry, different microorganisms can contaminate surfaces according to the food handled or processed. If not eradicated, they can grow and form resistant biofilms. However, their persistence is a challenging concern. The contamination of the distributed food is a danger for public health and results in a huge economic loss to healthcare and industrial sectors. Moreover, biofilms are the main cause of hospital-acquired infections. They develop on medical devices such as cardiac pacemakers, heart valves and catheters. They take root inside wounds, rippling and pulsating as they expend (Stoodley et al. 1999). Many are impervious to antibiotics. They also cost the health care system billions each year, as patients often require surgery to remove and replace contaminated implants (Bryers 2008).

The prevention of biofilm formation is an interesting goal that researchers are trying to reach. In our studies, we succeeded to elaborate coated surfaces, via different techniques that prevent biofilms formation. Each study was introduced by a literature review describing the details of the techniques and the different studies following similar strategies.

In this manuscript, two main approaches were considered to prevent biofilm formation. The passive approach presented in the first (review) and third chapter (Article), and the active approach presented in the second (review) and the fourth (Article) chapter.

In the first chapter of this manuscript, the cold plasma innovative technology was detailed, presenting the overall features of cold plasma surface modifications. It also focuses on plasma-

coated surfaces designed to inhibit and prevent the attachment of bacteria to surfaces. However, cold plasma technology requires further research on eco-toxicity, ageing characteristics, coating efficiency with time, and the mechanisms of interaction between bacteria and the plasma-coated surface. The study that was introduced with this review was detailed in the third chapter of the manuscript. In this study involving stainless steel plasma-treated surfaces, we succeeded to reach the objective of the passive prevention of biofilm formation. In this work, coatings were developed from the polymerization of TMDS supplemented with O_2 with a cold remote nitrogen plasma technique. Several analyses were carried out to determine the effect of oxygen and nitrogen variation on the coating's nature. The chemistry and topography was analyzed by water contact angle, FTIR, surface roughness and SEM imaging. The effect of each type of coatings on *S.* Enteritidis adhesion was highlighted. The results showed that bacterial adhesion was guided by the surface topography parameter. In fact, the rougher surfaces exhibited the higher number of attached *S.* Enteritidis cells. Overall, this study clearly showed that coating stainless steel using TMDS under controlled conditions makes it repulsive for *S.* Enteritidis.

The second approach considered as the active one was detailed in the second chapter. It highlighted the strategies carried out to develop active antimicrobial stainless steel coatings to prevent biofilms formation. It focused on the antimicrobial peptides, especially the nisin, grafted on stainless steel. However, the application and use of AMPs is of growing relevance. In fact, AMPs are a diverse category of natural molecules that are produced as the first-line of resistance by all multicellular living entities. Such peptides can have an extensive activity to instantly defeat bacteria, yeast, fungi and viruses. A promising approach is the grafting of these natural molecules onto stainless steel. Indeed, in our study considering the active approach, we reached the main goal concerning the prevention of biofilm formation on stainless steel surface. The functionalized surfaces were tested chemically and biologically. Challenge tests, qualitative antibacterial tests, SEM and LIVE/DEAD analysis towards *L. monocytogenes*

proved the antimicrobial efficacy of SDGN followed by SDAN and the non-effective activity of SDN. The chemical grafting of the compounds onto the surfaces provided information on the binding configuration of nisin on each specific surface. The findings revealed that nisin was linked in SDGN and SDAN via its amino group to glutaraldehyde and succinic acid, respectively, and in SDN via its carboxyl group to polydopamine. In addition, ToF SIMS, XPS, surface roughness and thickness analysis, ion polishing, and WCA provided insight into the homogeneity of the surfaces, the availability and quantification of nisin, and the characterization of the coatings. Indeed, these analyses demonstrated that the carboxylic group of nisin participates in the antibacterial effect and that the accumulation of nisin on the surface conducts to the inhibition of its antimicrobial effect.

In this research, the prevention of biofilm formation is considered, passively and actively. The development of biofilms on the generated surfaces, and the analyse of its properties is a good perspective. It can permit the analyse of the EPS matrix properties, the density of the biofilm formed and its vulnerability to disinfectants.

In conclusion, a good perspective for the passive strategy would be grafting of antimicrobial groups onto TMDS-coated surfaces by plasma treatment. The obtained stainless steel surfaces would provide an anti-adhesive and antimicrobial property at the same time. This strategy would passively and actively prevent biofilm formation. Moreover, testing the effect of generated surfaces against several bacterial types also, can be a good perspective.

Concerning the active strategy, on the same wave, the addition of a repulsive compound on effective nisin coated surface could optimize the anti-biofilm result. Moreover, the nisin is efficient against gram-positive bacteria, it would be a good perspective to add a compound to permit the surface to work against both types of bacteria, gram-positive and gram-negative. Indeed, the external membrane of gram-negative bacteria functions as a permeable shield for the cell and inhibits nisin from accessing its cytoplasmic membrane. Combining nisin with

chelating agents such as sodium salts of ethylenediaminetetraacetate (disodium EDTA) is an efficient strategy in improving the antimicrobial activity of nisin against gram-negative bacteria (Vaara 1992; Gill and Holley 2003; Khare et al. 2014). Several factors, such as compounds grafted types, pH in the surrounding environment, bacterial species and surface characteristics can affect the antimicrobial and antiadhesive efficiency. The optimization of all the factors can be a promising strategy to get rid of biofilm formation. Nevertheless, it is important to try multiple strategies to control bacterial contamination and eradicate microbiological risk in the food and medical sectors.

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ABSTRACT

The ambient operating environments in food and medical fields allows bacteria to adhere and develop on substrates, which results in the growth of resistant pathogenic bacterial biofilms. Indeed, the first stage of biofilm formation is the non-reversible adhesion of bacteria. Preventing and suppressing such adhesion is a passive strategy to inhibit the development of biofilms. These pathogenic structures are responsible for several foodborne diseases and nosocomial infections. Consequently, to combat this public health burden, one possible approach is the use of cold plasma technologies in coatings formulation. This work presents different factors influencing bacterial adhesion to a substrate. In addition, strategies for the development of passive coatings to prevent biofilm formation by cold plasma surface treatments are described as well as the anti-adhesive properties of the developed surfaces. General features of surface treatment, including the surface physicochemical changes and the use of cold plasma technologies, are also presented. In this context, a study was conducted to control, via cold plasma treatment of stainless steel, the persistent bacterium *Salmonella enterica*. Indeed, *Salmonella enterica* is responsible for several infections worldwide due to its persistence on abiotic surfaces in hospitals and food processing industries. Aiming to avoid the formation of *Salmonella enterica* biofilm, a surface modification process was carried out by the elaboration of a hydrophobic organosilicon coating from the monomer 1,1,3,3-tetramethyldisiloxane, mixed to oxygen, using a nitrogen flow microwave post-discharge plasma polymerization technique. The effects of cold plasma parameters on the coating properties, on the surface topography and on the adhesion of *Salmonella enterica* cells were investigated. The results revealed that surface topography influenced the rate of bacterial adhesion. Indeed, rough surfaces did not repel *Salmonella enterica* since the number of cells adhering to these surfaces varied from 30 ± 4 to 65 ± 4 bacteria per microscopic field. In contrast, smoother surfaces exhibited anti-adhesive behavior since the number of attached cells was close to zero on these coatings. A complementary approach to this passive strategy of anti-adhesive surface elaboration is the development of active surfaces. Emerging technologies for active and effective antimicrobial coatings are helping to address the challenge of eliminating pathogenic biofilms formed on materials used in medical and food processing environments. Stainless steel is a commonly employed material in these fields but it regrettably has insufficient bio-functional properties, which makes it susceptible to bacterial adhesion and biofilm generation. Therefore, in this thesis, a review of coatings developed by employing biocides and antimicrobial peptides (AMPs) grafted on stainless steel is presented. Moreover, a new active approach based on stainless steel coated with nisin, a common AMP accepted as a safe alternative to prevent pathogenic biofilms development, is developped. In this active strategy, stainless steel surfaces were functionalized by nisin which was grafted to the surface by either its carboxylic group or its amino group. The antimicrobial activity of the elaborated coatings was tested against *Listeria monocytogenes*, a dangerous pathogenic bacterium, with a high fatality rate. Indeed, the surfaces coated with nisin linked via its amino group exhibited a powerful antibacterial activity while the surface with nisin linked via its carboxyl group showed no antimicrobial effect. Surface property analyses provided a better understanding of the antibacterial effects, chemical and topographical characteristics of the treated surfaces, as well as the configuration and quantification of nisin.

Keywords: Biofilms; bacterial adhesion; cold plasma; 1,1,3,3-tetramethyldisiloxane; *Salmonella enterica*; Stainless steel; Coatings; Antimicrobial peptides, Biocides; Nisin*; Listeria monocytogenes*.

RESUMÉ

L'environnement opératoire dans les domaines alimentaire et médical permet aux bactéries de se fixer et de se développer sur les surfaces, ce qui entraîne la formation de biofilms bactériens pathogènes et résistants. En effet, la première étape de la formation des biofilms est l'adhésion irréversible des bactéries. Prévenir et supprimer cette adhésion est une stratégie passive pour inhiber le développement des biofilms. Ces structures pathogènes sont responsables de plusieurs maladies d'origine alimentaire et d'infections nosocomiales. Par conséquent, pour lutter contre ce fléau de santé publique, une approche possible est l'utilisation des technologies plasma froid pour l'élaboration de revêtements sur différents matériaux. Ce travail présente les différents facteurs influençant l'adhésion bactérienne à un substrat. En outre, les stratégies d'élaboration de revêtements passifs visant à prévenir la formation de biofilms par des traitements de surface par plasma froid sont décrites ainsi que les propriétés antiadhésives des surfaces élaborées. Les aspects généraux du revêtement, y compris les modifications physicochimiques de la surface et l'utilisation des technologies par plasma froid, sont également présentés. Dans ce contexte, une étude a été menée dans le but d'inhiber l'adhésion de la bactérie pathogène *Salmonella enterica* à la surface de l'acier inoxydable, via son traitement par plasma froid. En effet, *Salmonella enterica* est responsable de plusieurs infections dans le monde en raison de sa persistance sur les surfaces abiotiques dans les hôpitaux et les industries agroalimentaires. Dans le but de limiter la formation du biofilm de *Salmonella enterica*, des revêtements organosiliciés à partir du monomère 1,1,3,3-tétraméthyldisiloxane, mélangé ou non à l'oxygène, ont été élaborés par polymérisation par plasma post-décharge micro-ondes d'azote. L'effet des paramètres du plasma froid sur les propriétés du revêtement, sur la topographie de la surface et sur l'adhésion des cellules *Salmonella enterica* a été étudié. Les résultats ont révélé que la topographie de la surface influençait de façon significative le taux d'adhésion des bactéries. En effet, les surfaces rugueuses n'ont pas inhibé l'adhésion de *Salmonella enterica* puisque le nombre de cellules adhérant à ces surfaces variait de 30 ± 4 à 65 ± 4 bactéries par champ microscopique. En revanche, un comportement anti-adhésif vis-à-vis de *Salmonella enterica* a été mis en évidence pour les surfaces plus lisses. En effet, le nombre de cellules attachées était proche de zéro sur ces revêtements. Une approche complémentaire à cette stratégie passive d'élaboration de surfaces anti-adhésives est le développement de surfaces actives. Les technologies émergentes de revêtements antimicrobiens actifs et efficaces permettent de relever le défi de l'élimination des biofilms pathogènes formés sur les matériaux utilisés dans les milieux hospitaliers et agroalimentaires. L'acier inoxydable est un matériau couramment utilisé dans ces domaines, mais il possède malheureusement des propriétés bio-fonctionnelles insuffisantes, ce qui le rend susceptible à l'adhésion bactérienne et au développement de biofilms. Dans ce contexte, cette thèse présente une revue des revêtements développés en employant des biocides et des peptides antimicrobiens (AMPs) greffés sur l'acier inoxydable. De plus, une nouvelle approche active basée sur l'acier inoxydable revêtu de nisine, un AMP commun accepté comme une alternative sûre pour prévenir le développement de biofilms pathogènes, est développée. Dans cette etude, des surfaces en acier inoxydable ont été fonctionnalisées par la nisine qui a été greffée à la surface soit via son groupe carboxylique ou via son groupe amino. L'activité antimicrobienne des revêtements élaborés a montré une grande éfficacité contre *Listeria monocytogenes*, une bactérie pathogène menaçante, avec un taux de mortalité élevé. En effet, les surfaces revêtues de nisine greffée via son groupe aminé ont montré une puissante activité antibactérienne tandis que la surface greffée avec la nisine liée par son groupe carboxyle n'a montré aucun effet antimicrobien. Les analyses des propriétés de surface ont permis de mieux comprendre les effets antibactériens, les caractéristiques chimiques et topographiques des surfaces traitées ainsi que la configuration et la quantification de la nisine.

Mots clés : Biofilms ; adhésion bactérienne ; plasma froid ; 1,1,3,3-tétraméthyldisiloxane ; *Salmonella enterica* ; acier inoxydable ; revêtements ; peptides antimicrobiens, biocides ; Nisine ; *Listeria monocytogenes.*