Plasma assisted surface modification of organic biopolymers

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Abstract: Despite many synthetic biomaterials having physical properties that are comparable or even superior to those of natural body tissues, they frequently fail due to the adverse physiological reactions they cause within the human body, such as infection and inflammation. The surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness. Amongst the numerous surface modification techniques available, plasma surface modification affords device manufacturers a flexible and environmentally friendly process that enables tailoring of the surface morphology, structure, composition, and properties of the material to a specific need. There are a vast range of possible applications of plasma modification in biomaterial applications, however the focus of this review paper is on processes that can be used to develop surface morphologies and chemical structures for the prevention of adhesion and proliferation of pathogenic bacteria on the surfaces of indwelling medical devices. As such, the fundamental principles of bacterial cell attachment and biofilm formation are also discussed. Functional organic plasma polymerized coatings are also discussed for their potential as biosensitive interfaces, connecting inorganic/metallic electronic devices with their physiological environments.

Keywords: bacterial adhesion; biofunctional coatings; plasma modification.

1. Introduction

For decades, biomaterials have played an important role in disease management and the advancement of health care. Their applications range from coatings for tablets or capsules in pharmaceutical preparations to being essential components of extracorporeal devices such as contact lenses or kidney dialyzers, and indwelling devices and implants. Many of these materials were not originally designed for medical applications, and while they addressed many important medical issues, their use frequently led to complications, such as poor biocompatibility, time-dependent material degradation and subsequent mechanical failure, infection, inflammation and blood clot formation. The biomaterials were often selected only for their bulk properties, such as mechanical strength and

inertness, and as a result, many widely used biomaterials exhibited significant drawbacks. Many possessed sub-optimal surface biological properties such as high hydrophobicity and high friction, resulting in deleterious effects such as inflammation and irregular tissue response. Recently, advanced surface characterisation techniques have allowed a better understanding of the reactions occurring at the interface between the biomaterial surface and host tissues. This has allowed an insight into the important role that the surface properties of biomaterials play with regard to the response of the biological environment to the indwelling medical devices [1]. As a consequence, novel techniques have been developed that can impart desirable chemical, physical, and biological properties to the biomaterials. This can occur through the synthesis of a new material with desirable properties built directly into its matrix, or by the modification of materials already being used by the medical industry [2]. The surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness. One such modification technique is plasma surface modification. This provides device manufacturers with a flexible and environmentally friendly process that allows for tailoring the surface properties of the material to suit a specific need [3-8]. In addition, exposure to plasma has been shown to irreversibly damage bacterial cells, allowing for *in situ* sterilisation of the biomaterial during the surface modification process. For example, plasma sterilisation has been demonstrated to be effective against *Escherichia coli* [9-10], *Staphylococcus aureus* [9, 11], *Pseudomonas aeruginosa* [10], *Bacillus cereus* [10], *Bacillus subtilis* [12] and *Geobacillus stearothermophilus* [13]. The resultant plasma coatings have been shown to possess spatial uniformity and strong adhesion to the substrate. They result in a smooth, defect-free surface with sound chemical and physical stability [14-16]. Furthermore, coatings manufactured using plasma technologies display interesting optical and electrical properties, making them suitable candidates for integration into a range of electronic devices that can interface between organic/inorganic electronics and physiological environments [17]. This paper discusses the processes used to develop plasma-modified surfaces with morphologies and chemical structures that prevent the adhesion and proliferation of pathogenic bacteria.

2. Plasma modification

A plasma is defined as a partially or wholly ionised gas, with approximately equal amount of positively and negatively charged particles. Near-equilibrium plasmas are formed under high temperature conditions and are characterised by a thermal equilibrium of its entire range of species. Temperatures required to generate near-equilibrium plasmas generally range between 4000 K to 20 000 K, depending on the ionisation potential of the element. These extreme conditions are not likely to be appropriate for the surface modification of biomaterials constructed from polymers [1], although they can be used for the evaporation and deposition of bioactive metals and ceramics, such as natural hydroxyapatite-based bio-glass–ceramics [18-19] and zirconia coatings [20-21] for artificial bones and hard tissues. Non-equilibrium plasmas, on the other hand, can be initiated at substantially lower temperatures, enabling their application for the surface cleaning and functionalisation of polymer surfaces. The ion mobility in a low-temperature plasma is significantly lower than that of the electrons

that transport the energy through the electric field [22]. The plasma can also be classified according to the pressure at which it is initiated, or according to the energy source used to energise the gas [23].

During plasma surface treatment, the substrate is exposed to a reactive environment of a partially ionised gas comprising large concentrations of excited atomic, molecular, ionic, and free-radical species. The nature of the interactions between the excited species and the solid surface will determine the type and the degree of the chemical and physical modification that will take place. The processing conditions, such as power, pressure, gas etc., and the nature of the substrate will determine whether the surface modification is one of film deposition, substitution, or ablation. Plasma polymerisation can take place when a monomer, either in vapour phase or at the surface, is fragmented into reactive species that can then recombine and be deposited onto the surface of the substrate. Monomers that do not necessarily contain functionalities associated with conventional thermo-chemical polymerisation, such as unsaturation or ring structures, can be deposited in this way.

In plasma treatment, gases that do not fragment into polymerisable intermediates upon excitation are used. These include air, nitrogen, argon, oxygen, nitrous oxide, helium, tetrafluoromethane, water vapor, carbon dioxide, methane, and ammonia. Exposure to such plasmas can lead to the introduction of chemical functionalities, with the nature of the functionalities being highly dependent on the chemical composition of the biomaterial and the process gas. For instance, plasma oxidation, nitration, hydrolyzation, or amination will increase the surface energy and hydrophilicity of the biomaterial, therefore changing the way in which the biomaterial interacts with its immediate physiological environment. Free radicals are also created on the surface, since the surface is being bombarded by energetic particles and high energy UV radiation.This can lead to surface ablation, cross-linking or surface activation. Ablation is a process by which lower molecular weight species, such as volatile oligomers and monomers, are desorbed. Cross-linking occurs when radicals from one chain on the surface of the polymer combine with radicals from another polymer chain to form a bond. Surface activation, however, involves the recombination of surface radicals with atoms or chemical groups that are different from those that were originally present at the surface of the biomaterial.

The surface functionalities that arise as a result of plasma treatment can serve as a platform for further surface modification processes, such as the grafting of biomolecules and other functional structures. Further surface modification can be performed in order to tailor the properties of the biomaterial for a specific application.

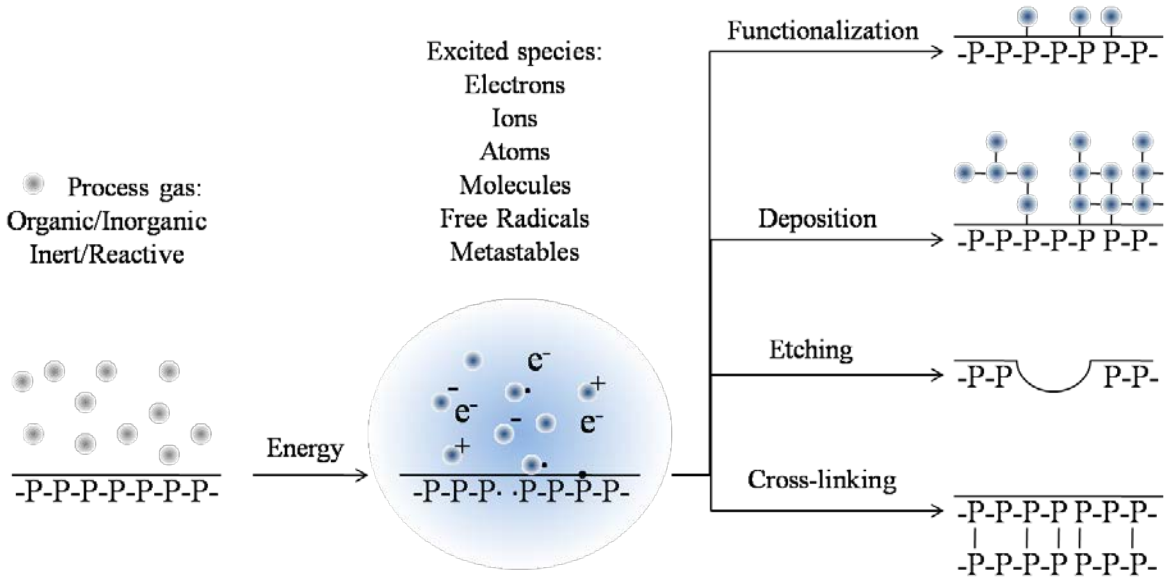

Figure 1. Surface modification processes that can be achieved using the plasma technique

Despite the many advantages associated with use of conventional plasma techniques for surface functionalisation, polymer thin films fabricated using this method are typically characterised as highly cross-linked and amorphous. Furthermore, these films retain only a limited amount of the original monomer functionality due to the high degree of fragmentation and recombination that takes place during the plasma polymerisation process. If low input power deposition and low levels of substrate heating are used, the original chemical structure of the monomer can be retained to a large extent, however a relatively low degree of cross-linking results, rendering these coatings inferior in terms of their mechanical properties and dynamic stability, hence limiting their *in vivo* applicability [24]. A number of papers have been published that detail the use of a pulsed plasma technique. This technique allows the precise control of chemical functionality and surface morphology and results in a coating with good stability [24-28] [29]. The plasma duty cycle was found to be an important determinant in controlling the degree of retained surface functionality [30], and hence a greater degree of compatibility with biomolecules, bacterial and host cells, and liquid media [31]. Moreover, the surface properties of the coating could be varied using this technique by changing the duty cycle between the 'pulse on' (ion implantation) and 'pulse off' (plasma exposure) periods during treatment, with a high ion implantation/plasma exposure time ratio being achieved by increasing the pulsing frequency and elongating the duration of the pulse [32].

3. Principles of bacterial attachment and biofilm formation

Designing a coating that will be effective in controlling bacterial adhesion and proliferation requires an in-depth understanding of the forces that govern these processes, the attachment and colony formation dynamics, and the consequences for both the coloniser and the abiotic target as a result of adhesion. Furthermore, the development of biomaterial-associated infections can arise in several ways, the most common being the introduction of aetiological agents from direct contamination of the implant during surgery [33] or post-operative care [34]. In addition, microorganisms that originate from an infection site elsewhere in the body can spread through the blood, causing the late

haematogenous infection of the implant, particularly when medical devices are directly exposed to the blood stream, such as in the case of artificial valves [35-36].

3.1. Mechanism of initial attachment of planktonic bacteria to surfaces

The process of bacterial adhesion is complex, with initial reversible physicochemical interactions being followed by intricate irreversible molecular and cellular interactions. Bacterial cells move, or are moved, by the flow towards the surface of the material through and by the effects of physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge and hydrophobic interactions [37]. Bacterial motility mechanisms, including swimming, swarming, and twitching, are known to play important roles in bacterial attachment and biofilm formation, with directed motility being influenced by chemotaxis functions [38]. Chemotactic sensing is prevalent in almost all bacterial species and can influence bacterial colonisation of surfaces via regulation of expression of certain cellular adhesion components and bacterium-bacterium and bacterium-surface interactions [39-40]. Approaching a so-called chemoattractant, such as an amino acid, sugar, or an oligopeptide, encourages a bacterium to move in frequent runs, whilst a decreasing concentration of attractant and/or increasing concentrations of repellent such as an extreme pH, certain metal ions, or a hydrophobic amino acid instigates increased tumbling by the microorganism [41]. Haptotaxis is a mechanism that relates the cell speed and/or random turning behaviour to the magnitude of the adhesion ligands in the substratum, and the net direction of the cell movement to the gradient of adhesion [42]. It has also been suggested that haptotaxis also influences the attachment preferences of the microorganisms [43]. When the distance between the bacterial cell and other cells or abiotic surfaces is larger than 50 nm, the interactions between these two entities are nonspecific and are directly related to the distance and free energy characteristics pertinent to these two surfaces [37]. The nature of these forces, i.e. whether they are attractive or repulsive, will either facilitate the bacterial attachment or prevent the cell from moving into the molecular or cellular phase of adhesion. Studies have shown that bacterial adhesion and settlement increases with increasing surface roughness, due to the presence of a greater surface area for colonisation. In addition, the so-called ‘valleys’ on rough surfaces that provide a protected habitat, with reduced shear forces [44]. It has also been demonstrated that bacterial cells attach more favourably and rapidly to hydrophobic and non-polar surfaces rather than those with more hydrophilic properties [45]. When the distance separating these surfaces becomes less than 5 nm, chemical interactions such as hydrogen bonding, ionic and dipole interactions, hydration and/or hydrophobic interactions become significant, resulting in a more stable adhesion of the microorganism to the surface [46]. Various polymeric structures such as capsules, fimbriae, pili, and slime that can be present on the surface of the bacterial cell engage in the molecular specific irreversible reactions with the chemical features of the tissue or abiotic surface.

3.2. Influence of physiological status and substrate specific biological response on bacterial attachment

Bacteria secrete an elaborate variety of extracellular polymeric substances, including polysaccharides, proteins, and nucleic acids, that perform a wide range of biological functions, including shielding the cell surface, affording the cell protection from major bacterial pathogens [47],

providing resistance to desiccation [48], and impeding antibody opsonization and phagocytosis [49]. Importantly, these substances play a significant role in mediating the bacterial colonization of surfaces by facilitating cell adhesion to biotic (i.e., epithelial and endothelial cells) and abiotic surfaces (i.e. mineral surfaces or medical implants) and cohesion to each other via dipole interactions, covalent or ionic bonding, steric interactions, and hydrophobic association [50-55].

For example, components of free extracellular polymeric substances released onto the surfaces that may otherwise be regarded as unfriendly for settlement by the bacterial cells will pre-condition the target surface by adsorbing to it hence making it more appropriate for bacterial attachment. The temperature, solution pH, electrolyte and macromolecule concentration, and adsorbent surface chemistry will directly affect the chemical composition and structure of the polymeric substances produced by the bacteria [56-57]. Cell adhesion to biotic targets such as host tissues has also been shown to be strongly associated with the presence of extracellular polymeric substances. *Streptococcus pyogenes*, for example, colonises the pharynx and is associated with infections such as necrotizing fasciitis and pharyngitis [58-59]. During the colonisation, the hyaluronic acid capsule of *S. pyogenes* attaches to CD44 receptor on human cells. CD44 is a hyaluronic acid-binding protein that mediates human cell-cell and cell-extracellular matrix–binding interactions, hence facilitating the colonisation of the pharynx keratinocytes *in vivo* [60-61]. Furthermore, the presence of the bound (capsular) and free (slime) extracellular material may significantly increase the chances for survival of the attached microorganism in the environment by acting as a permeability barrier that facilitates selective transportation of nutrients, whilst at the same time providing a protective barrier that excludes harmful substances, including systemic antimicrobial agents [62-67].

Factors such as the solution chemistry, abundance of nutrients, and the cell growth phase will exert a significant influence over the nature and distribution of the extracellular polymeric substances produced in these conditions[68]. *In vivo* studies on mice model involving acapsular mutant stains of *S. pyogenes* showed a spontaneous excision of the transposon from the capsule-synthesis region of the bacterial chromosome upon injection into a host, producing a high number of encapsulated revertants in subjects inoculated with the revertible mutant stains, resulting in mortality levels similar to those caused by parental encapsulated *S. pyogenes* [69]. In addition to secreted polymeric substances, lipopolysaccharides present on the outer leaflet of the outer membrane of gram-negative bacteria also affect the adhesive behaviour of the pathogen [70]. A carbohydrate structure comprised of a core oligosaccharide and a polysaccharide known as O-antigen is anchored to the bacterial membrane with the lipid A [71]. Although the O-antigen is flexible and can extend outwards depending on the ambient environmental conditions that surround the microorganism, the preferred conformation is thought to position the O-antigen to lie flat on top of the cell surface, covering the saturated fats and phospholipids of the lipid A and possibly non-polar sites of the surface of the pathogen [70]. As such, absence or attenuation of the O-antigen has been demonstrated to enhance the extent of bacterial attachment to hydrophobic surfaces [70].

The adhesive interactions between the microorganism and its environment have also been shown to depend on the length and heterogeneity of the O-antigen [71-72]. In *E. coli*, for example, the lipopolysaccharide core and O-antigen have been identified as the key components that mediate bacterial binding with inorganic surfaces and facilitate aggregation with other cells [73], with

hydrogen bonding having been shown to be an important factor in controlling O-antigen adhesion to inorganic molecules such as Si_3N_4, TiO_2, SiO_2, and Al_2O_3 [71].

3.3. Coloniser proliferation and biofilm formation

Biofilm formation can be initiated by the multiplication of the primary coloniser without release of progeny cells and/or the recruitment of co-aggregate members of the same or different species, boosting their individual potentials for colonisation of various ecological niches [46, 74-75]. A biofilm can comprise bacteria, algae, fungi and protozoa enfolded in a dynamic aggregation of polymeric compounds that are predominantly polysaccharides, but also contain proteins, nucleic acids, lipids, and humic substances. These extracellular polymeric substances (EPS) mediate the interspecies co-aggregation within the biofilms by providing a matrix for formation and stabilization of the film's architecture. The composition and quantity of the extracellular polymeric substances that form the matrix of the biofilm will change according to the type of microorganism, the age of the aggregation and the environmental circumstances in which the formation exists, including oxygen and nitrogen levels, the extent of desiccation, temperature, pH, and availability of nutrients [46]. Charged non-carbohydrate components such as uronic acids or ketal-linked pyruvates present in the EPS further enhance the anionic nature of the surface polysaccharides of gram-negative bacteria, thus allowing the association of divalent cations (i.e., calcium, magnesium) to increase the binding forces within biofilm [76]. These non-carbohydrate components also strongly influence the tertiary structure and the physical properties of the EPS. Certain polysaccharide–surface combinations result in irreversible attachment. In these instances, the binding forces between the individual cell and the abiotic surface improve the overall stability of biofilm matrix [77-78]. Extracellular DNA has also been demonstrated to be an important component of the biofilm matrix via the introduction of favourable acid-base interactions. The removal of extracellular DNA from Gram-positive bacteria has been shown to reduce the initial adhesion and aggregation of bacteria on surfaces [79]. A combination of electrostatic interactions, hydrogen bonds and London dispersion forces are responsible for the initial attachment of the coloniser to the surface, and these forces also contribute to the subsequent biofilm formation and structural development.

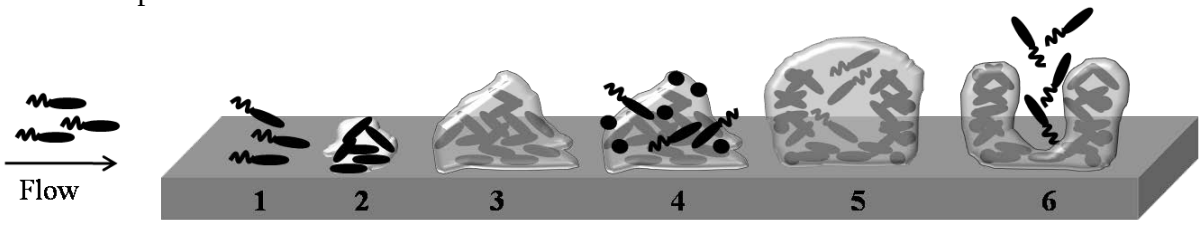

Figure 2. A model of *P. aeruginosa* biofilm development in stages: (1) reversible attachment of the bacterial cells to the surface governed by electrostatic forces, Brownian motion and flagella-mediated locomotion; (2) irreversible attachment mediated by extracellular polymeric substances, loss of flagella-driven motility; (3) development of early biofilm architecture by bacterial reproduction, EPS secretion and twitching motility; (4) attachment of other organisms to the biofilm; (5) formation of a complex biofilm architecture and biofilm maturation; (6) dispersion of single motile cells from biofilm microcolonies into the medium.

Biomaterial-associated infections remain a major concern in the use of most implanted or intravascular devices, including orthopaedic prostheses, artificial valves, urinary tract and cardiovascular catheters, intraocular lenses and dentures [35-36]. Bacterial attachment and subsequent biofilm formation frequently results in indwelling device related infections, often resulting in device failure [80-81]. The state of the biofilm acts as a defence mechanism against predation by phagocytes, and that serves as a permeability barrier against harmful agents [82]. In the biofilm state, pathogenic bacteria are less susceptible to host defence mechanisms and systemic antibiotics. They are also more resistant to detachment under flow conditions, and as a result, the surgical removal of the infected device is often required [33]. For instance, the extracellular substances produced by Gram-negative *P. aeruginosa* cells limit the oxygen available to the microorganism, resulting in a reduction in the metabolic activity of the pathogen. Furthermore, recent studies on a mutant strain of *P. aeruginosa* showed that while they were still capable of forming biofilms with the characteristic *P. aeruginosa* architecture, they did not develop any high-level biofilm-specific resistance to three different classes of antibiotics. It was shown that periplasmic glucans synthesized by the bacteria interacted physically with antimicrobial agents, hence preventing the latter from reaching their sites of action [83-84]. In other words, in addition to the biofilm acting as a diffusion barrier for antibiotics, the bacteria within these films employed distinct mechanisms to resist the action of antimicrobial agents. The presence of a biofilm can also offer certain nutritional advantages to the bacteria over their planktonic state, in that the film acts as a 'sorptive sponge' which binds and concentrates organic molecules and ions close to cells [85]. While growing within the biofilm, *S. aureus* cells have been shown to synthesize and secrete an autoinducing peptide signal that accumulates in the extracellular environment. This is then used for cell-to-cell communication, otherwise known as quorum-sensing, a ubiquitous regulatory mechanism that controls the extent of *S. aureus* pathogenicity and biofilm development [86]. Upon reaching its critical concentration, the autoinducing peptide signal binds to a surface receptor, activating an agr regulatory cascade, which results in the increased expression of invasive factors, including toxins, hemolysins, proteases, and other tissue-degrading enzymes. Furthermore, the agr system also decreases the expression of surface adhesions, triggering a dispersal pathway and detaching cells from a surface-bound biofilm. Reverted to their planktonic state, these cells are then able to establish new colonisation sites elsewhere in the host, thus spreading the infection.

Nutrient depletion can also trigger cell detachment and drift. When a bacterial inoculum reaches a critical size and overcomes the local host defence, chronic infections can establish [75]. For instance, prolonged subclinical infections, that is bacterial presence without any signs of infection, have been linked to *Staphylococcus epidermidis* related capsular contracture formation around a silicone implant, the most common complication of augmentation mammaplasty and other procedures involving breast implants [87]. The biofilm formed on the outer surface of an implant triggers irritation and chronic inflammation, leading to accelerated capsular contracture. Another study involving *S. epidermidis* reported that in the presence of small colony variants on the surface of orthopaedic implants, osteoblasts initially adhered and spread on the surface of the implant, but were killed within 2 days [88].

4. Factors that influence bacterial adhesion

The stability with which a cell can attach to a solid surface and the degree of subsequent colonization have been shown to vary with the surface properties of the abiotic target. These surface properties include surface architecture and energy, the nature of the medium, and the surface characteristics of the microorganism itself. Microorganism-specific factors influencing the rate and degree of attachment to the surface include the hydrophobicity and surface energy of bacterial cell, the presence of fimbriae and flagella, the extent of EPS production and the type of polymeric materials being produced by the cell [44]. The hydrophobicity of bacteria is commonly inferred from water contact angle measurements on bacterial lawns deposited on membrane filters or from bacterial adhesion to hydrocarbons, whereas the electrical properties are determined by the zeta potential, which is related to the electrophoretic mobility of the microorganism [89]. In addition to these surface properties, the ability of the protruding EPS chains to reconfigure in terms of their spatial arrangement upon approaching the solid surface will determine if the subsequent interactions between the cell and the surface are attractive or repulsive [90]. The irreversible adhesion of Gram-negative *Stenotrophomonas maltophilia* to glass was shown to be facilitated by the attractive interactions of the long chain polysaccharides within the surface of the substrate. This interaction, known as bridging, resulted in a higher affinity between these surface structures and the surface of the solid. Lipopolysaccharide polymers, on the other hand, displayed a higher affinity for the medium than the substrate, and hence lower levels of attachment were observed as a result of the steric repulsion between the surface and the microorganisms [91]. Bridging is generally observed in instances where both the surface of the bacterium and the surface of the substrate are hydrophobic [92-93].

The ambient environment can promote or hinder colonisation by exerting selective pressure on the coloniser. This can be done by regulating its size, shape, growth rate and the substances the bacterium secretes, and also by directly affecting the surface properties of the abiotic target. Medium characteristics such as temperature, time of exposure, bacterial and antibiotic concentration, the degree of host immunity defence mechanisms activation, and the chemical composition and fluid flow in proximity of the surface also directly influence the dynamics of bacterial adhesion and biofilm development, with the latter often regarded as the most prominent factor. The pH and ionic strength of the medium can alter the surface hydrophobicity of the bacterium, and therefore the strength of the electrostatic interactions within the forming biofilm, hence affecting the stability and development of the biofilm architecture [75].

Cations such as magnesium and calcium actively contribute to biofilm cohesion and matrix development. They act as cross-linkers, contributing to the integrity of the outer membrane of the cell and the lipopolysaccharides. They also facilitate a physiology-dependent attachment process by acting as essential cellular cations and enzyme cofactors [94]. The presence of iron has also been shown to be a crucial factor for bacterial growth and biofilm formation; hence, targeting iron uptake systems may present an effective way by which the extent of biofilm formation can be restricted [95]. A study on the urinary tract biofilm-forming *E. coli* cells showed that biofilm formation can be impaired by the addition of divalent metal ions, such as Zn(II) and Co(II), which inhibit iron uptake by virtue of their higher-than-iron affinity for the master controller protein of iron uptake [95]. Biofilm formation can also be initiated in order to protect the bacteria from the presence of toxic compounds. *E. coli* bacterial cells, for example, have been shown to change from a planktonic to biofilm state in order to mitigate the harmful effects of a sub-inhibitory concentration of nickel [96]. In this case, the nickel-induced

biofilm formation in *E. coli* was an adaptation process, occurring through a transcriptional effect on genes coding for adherence structures. Silver is also known to suppress bacterial growth and biofilm formation in a wide range of pathogenic bacteria, including *E. coli, Serratia proteamaculans, Serratia liquefaciens, P. aeruginosa,* and *P. chlororaphis* [97].

The pH of the medium has been shown to directly influence the surface hydrophobicity of the bacteria. Recent studies of the electrostatic potential and pH of bacteria upon adhesion to a solid surface indicated that the proton concentration at the surface of an adhered bacterium can vary greatly from that of one existing in the bulk medium, impacting cellular bioenergetics [98].

Mass transport conditions are also important factors that determine the efficiency of bacterial deposition and irreversible microbial adhesion, controlling the rate that the organisms arrive at the solid surface during adhesion [99]. Furthermore, time resolved studies of adsorption, desorption and transmission within biological systems have shown that desorption probabilities of microorganisms decrease by several orders of magnitude within 1 to 2 min after contact with a substratum surface, with microbial adhesion forces strengthening exponentially over time by progressively invoking acid–base interaction forces [99-100].

5. Effect of material properties of the substrate

As mentioned previously, bacterial attachment to a solid surface is highly dependent on the surface properties of the material, such as its chemical composition and reactivity, surface energy and hydrophobicity [101], surface roughness [102-103], and porosity. Furthermore, bacterial attachment is a competitive process where microorganisms race alongside the host proteins and cells for the colonisation surface [104]. A study using a microfluidic device for real time imaging of osteoblasts in response to the presence of very limited numbers of *S. epidermidis* showed that during the early stages of culture osteoblast adhesion, spreading and proliferation were not adversely affected. Towards the end of the culture, however, the osteoblasts became damaged because the *S. epidermidis* actively proliferated in the co-culture channels and formed small clusters on the alloy surface. This changed the microenvironment so that it was no longer favourable for the sustainance of osteoblasts [105]. Therefore, the ideal surface configuration of the biomaterial would be one that actively promoted the binding and attachment of host cells, while promoting tissue healing. This would encourage the mediation of host biomolecule attachment only to a level that facilitates the integration of the biomaterial into the host systems without generating an excessive immune response. In addtion, this would concomitantly prevent bacterial attachment and biofilm formation, the latter being the foremost cause of device related infections and device failure [80-81, 106].

5.1. Chemical composition, hydrophobicity and surface free energy

Functional groups presented on the surface of the biomaterial will determine the hydrophobicity and surface charge of the abiotic target. Surface free energy is an important indicator of the type of interactions that occur at the solid-liquid interface, such as surface wettability [101]. It has been shown that the surface events that take place immediately after the insertion of a material into biological fluids will predetermine the subsequent response to the material. Surface events include the wetting of the material by physiological liquids, and the adsorption of proteins and cells to the surface [107].

There is a correlative relationship between surface wettability and blood-, cell-, or tissue-compatibility [108-110], with higher degrees of wettability corresponding to higher levels of cell attachment and subsequent spreading rates [111]. In addition, the friction behavior of an implantable tribological system is greatly affected by the extent of surface wettability, with higher wettability levels generally resulting in better tolerance of the biomaterial by the body [112]. A functional group, such as $–CH_3$, is inert in terms of protein and cell adsorption, whereas charged groups such as –COOH and $–NH_2$ encourage cell and protein attachment [113].

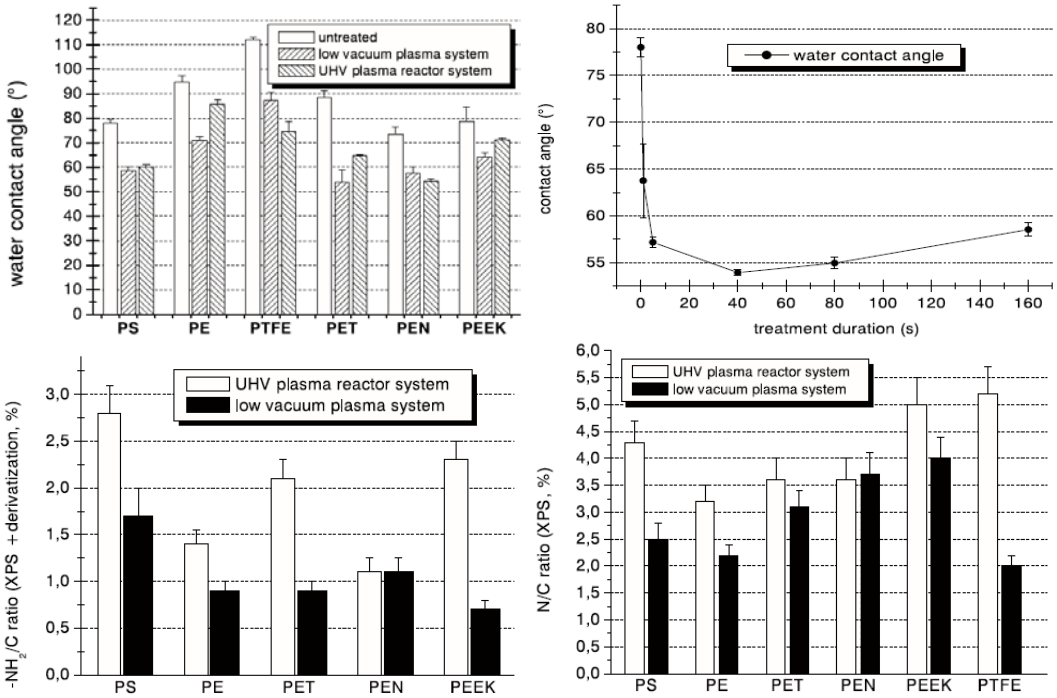

Figure 3. Hydrophobicity and chemical composition of polystyrene (PS), polyethylene (PE), polyetheretherketone (PEEK), polyethyleneterephthalate (PET), polyethylenenaphthalate (PEN), polycarbonate (PC), polymethylmethacrylate (PMMA) and fluorinated (PTFE) polymers amino functionalised using ammonia plasma treatment: (1) water contact angle and (3) respective N/C and $–NH_2$/C ratios after treatment of polymers in the low vacuum and the ultra high vacuum plasma reactors; (2) time dependence of the functionalisation effects in low pressure plasma system [114].

Plasma polymerization is frequently employed for the functionalisation of surfaces where hydrophobic behaviour is required. It allows for an introduction of a wide range of functional moieties and/or combination of moieties, including carboxyl (−COOH), amino ($−NH_2$), and alkene (−C=C) groups to name but a few, over a broad assortment of substrates [26]. Plasma polymer coatings derived from allylamine interact favorably via their amine functionality with both DNA and mammalian cells, making this material an attractive option for applications requiring biomolecule manipulation, such as on indwelling devices, whereas poly(ethylene glycol) films are being investigated for their ability to reduce protein fouling and limit cell attachment [115]. Films fabricated from maleic anhydride retain anhydride group functionality that can be then used for further modification via attachment of amino-functionalized alkyl chains, the polymerization of styrene, and protein attachment [30-31]. Nitrogen plasma treated bacterial cellulose improves the adhesion and proliferation of microvascular and

neuroblast cells by increasing the porosity and changing the surface chemistry of the material, without affecting its wettability [116]. Argon plasma treatment has been demonstrated to reduce bacterial attachment, resulting in reduced levels of *S. epidermidis* adhesion to Ar-treated polyethylene [117]. According to Kumar et al., a surface engineering approach to the prevention of biofilm formation on surfaces of biomaterials involves designing a surface that is hydrophilic and with high surface energy; hydrophobic and inert with a low surface energy; or decorated with tethered antimicrobial self sterilizing agents which are attached directly to the surfaces of the devices [118].

Our recent studies on fabrication of thin film coatings from essential oils and their individual constituents showed their potential in limiting bacterial attachment and proliferation. Coatings were deposited from terpinen-4-ol, a majour component of tea tree oil responsible for the oil's broad spectrum antimictobial and anti-inflammatory properties, using RF plasma polymerisation under varied input power conditions [119]. When produced at 10 W, the surfaces inhibited adhesion and growth of both *S. epidermidis* and *S. aureus* (Figure 4), however, when fabricated at higher power, the coatings promoted attachment, adhesion and metabolic activity of the pathogens, and encouraged biofilm formation.

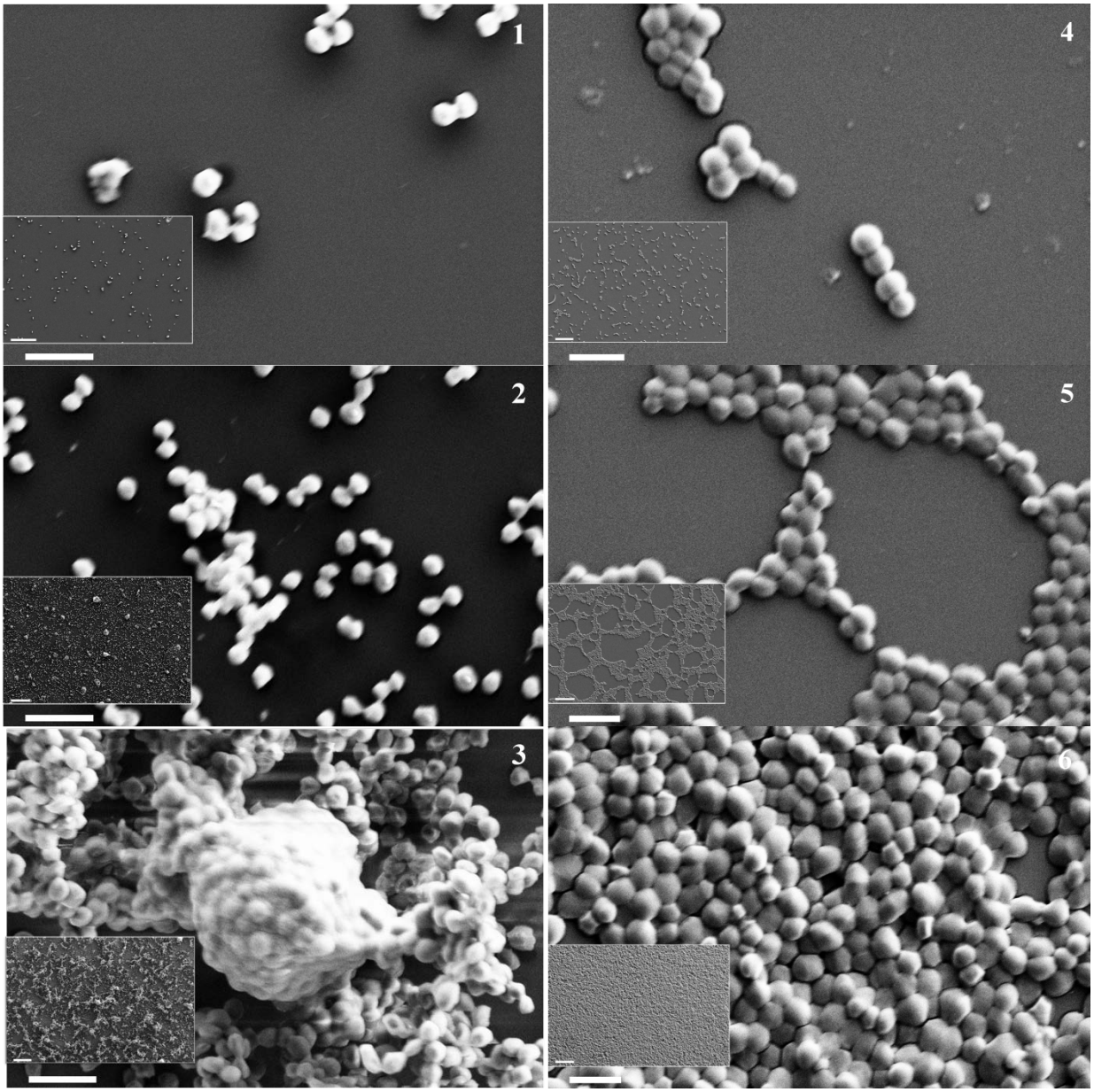

Figure 4. Scanning electron microscopy images of attachment and proliferation of *Streptococcus epidermidis* (left panel) and *Staphylococcus aureus* (right panel) after 18 h of incubation on surfaces treated with monoterpene alcohol plasma deposition under varied input power conditions: (1,4) 10 W; (2,5) 25 W; (3,6) 50 W. Scale bar 2 µm; scale bar 20 µm for inset

Hydrophobic material surfaces, such as those possessed by many polymers, are thought to be more attractive in terms of colonisation to hydrophobic microorganisms such as *S. epidermidis*, with the hydrophobicity of the material surface being identified as the more detrimental factor in the bacterial adhesion process than the hydrophobicity of the bacteria [75]. Similarly, metal surfaces are frequently hydrophilic due to the presence of surface hydroxyl groups on the surface oxide layer of the material. These surfaces are more attractive to the hydrophilic *S. aureus* bacerial cells [120]. A real time investigation of *S. epidermidis* adhesion dynamics to hydrophilic glass and hydrophobic dimethyldichlorosilane-coated glass highlighted that the number of adsorption and desorption events occurred, with a two-fold higher number of bacteria attaching to the hydrophilic surfaces [121]. The modes of adhesion were also found to differ, with approximately 20% of cells sliding over the surface of glass prior to either the fixed adhesion or desorption event, whereas a comparable mobile adhesion mechanism was virtually absent (1%) on the hydrophobic substrate, with only 2% of all *Staphylococci* desorbing from their adhesion site. The presence of high affinity sites on the surface of the hydrophobic coating was shown to initiate an attractive acid–base interaction with the surface of the cell, thus facilitating a closer approach and enhanced extent of immobile adhesion. Low affinity sites were associated with desorption and sliding of the bacterial cells.

A number of studies have reported that positively charged surfaces exhibit increased levels of bacterial attachment compared to their negatively charged counterparts, yet the subsequent growth was found to be more prominent on the latter surfaces [120]. Previous reports of antimicrobial activity increasing in the presence of positively charged surface sites [122] have been explained by a recent study that linked adhesion onto differently charged surfaces to changes in the charge-regulation process and cellular bioenergetics of the coloniser [98]. This study proposed that changes in the proton concentration at the cell surface can affect the periplasmic space, altering the levels of metabolic activity of the adhered bacteria. It has been shown that Gram-negative *E. coli* and the Gram-positive *Bacillus brevis*, when attached to a negatively charged glass surface, exhibited a decreased surface pH. This resulted in an enhanced proton motive force and an increased extent of ATP production, which may have assisted the cells to colonise the surface [123]. When these bacteria were attached to a positively charged surface, however, the effect was the opposite, resulting in a drop in metabolic activity, and possibly cell death, which may explain the antibacterial effect frequently reported for such surfaces [98].

Ultra hydrophobic hydrocarbons exhibit extremely low water solubility, are poorly bioavailable for bacterial colonisation, and can be toxic to bacterial cells due to their permeabilizing effect on the cytoplasmic membranes, leading also to a loss of ATP and a decrease in the proton gradient. In order to colonise such surfaces, bacteria may modify their cellular energetics through activation of their electron transport phosphorylation systems, allowing homeostasis of the ATP level and energy, which also results in a reduced growth yield [124].

Since most pathogens are hydrophilic under physiological conditions, decreasing the water contact angle of the material may improve its antibacterial properties. Indeed, a d.c. oxygen treatment of medical-grade poly(vinyl chloride) yielded a 70% reduction in bacterial adhesion for the four strains of *P. aeruginosa* [125]. However, this reduction was unlikely to be sufficient to prevent the *P. aeruginosa* colonization of endotracheal intubation devices [126]. Oxygen plasma-treatment of plasma deposited diamond-like carbon coatings resulted in the formation of superhydrophilic surfaces, but the presence of this surface did not increase the bactericidal properties of the material [127]. In addition to oxidation, the surface polarity of a substrate can also be increased by the plasma polymerisation of a coating that is not subject to hydrophobic recovery using an appropriately chosen monomer and carrier gas [128]. Hydrophobic recovery is a process of reorientation of the surface functionalities after oxidation with time attributed to the tendency to minimize the surface energy of the oxidized polymer and facilitated by the flexibility of polymer chains that allows for such a movement. Treatment of polyethylene terephthalate with helium and 20% of oxygen in helium (He/O_2) plasma were demonstrated to significantly reduce *S. epidermidis* bacterial adhesion compared to the untreated material, however, the ageing effect and the subsequent decrease in the surface free energy of the substratum surfaces with time, particularly in the case of He treated surfaces, were found to favour bacterial adhesion and aggregation [129]. The surface energy and hydrophobicity of the substrate are greatly influenced by the chemical composition and pH of contact medium, consequently affecting the free energy of solvent-mediated interaction between the cell and the substrate. For instance, attachment of *S. aureus* to glass was predicted to be at its maximum at pH 3 and pH 11, whereas the higherst adehsion to Teflon should be observed at pH 5 [130]. The same study found that adhesion of *S. aureus* to glass was mediated by both short range forces (Lewis acid–bases forces) and by long range forces (van der Waals forces), whereas the attachment of the bacteria to Teflon was likely governed by short range forces only. Plasma polymerized functional coatings are particularly susceptible to changes induced by the chemical composition of the liquid medium, such as the aqueous solution and body fluid, which can pose limitations on the potential applications of these structures as biomaterial or biocompatible surfaces [131]. Even before implantation, plasma polymers are vulnerable to degradation under ambient conditions, which may affect the storage and shelf life of the plasma deposited coating and undermine their usefulness. Upon immersion into a liquid, the swelling behaviour commonly observed in plasma polymers can cause the coating to increase in thickness and in volume. For instance, plasma polymerised maleic anhydride films have been shown to swell in water to form what is probably a polyelectrolyte film [31]. Interactions between ionisable functional groups of plasma polymers, such as acids and amines, and the ions of the liquid medium as a function of pH and ionic concentration of the solution will affect the swelling and degradation dynamics, and ultimately will influence the stability and bioactivity of these coatings. Leaching of small molecular weight compounds from the coating can also take place, a phenomenon that can be successfully utilised when designing a biodegradable or the diffusion-controlled release of a biocidal ingredient from the polymer system. Pulsed plasma polymerised allylamine films deposited onto silicon showed a pronounced pH dependence of the magnitude of the average pull-off forces which was attributed to protonation of the amino groups, with the pull-off forces decreasing significantly for pH values below 5.5 [132]. The same study demonstrated that by varying the duty cycle of the deposition, coatings with controlled content of amino and nitrile groups can be achieved, creating a heterogeneous local

environment in terms of chemical functionality and hydrophobicity on the nanometer scale. The adhesion behaviour of a product of pulsed plasma modification of polydimethylsiloxane substrates with maleic anhydride, with subsequent hydrolysation to promote the formation of dicarboxylic acid groups showed clear dependence on pH and electrolyte nature and concentration. The adhesion force was demonstrated to almost vanish under high pH in the presence of monovalent cation K^+ (due to condensation of counterions on the carboxylate groups), whilst it was oserved to increase slightly under high pH in the presence of divalent cation Ca^{2+}, due to ions bridging between two carboxylate groups [26]. The patterns of substrate-liquid medium interactions will therefore impact the substrate-biomolecule interactions.

Since proteins are regarded as the primary and the most significant player in mediating biomaterial–host interactions, the status of the proteins which adhere to the material surface will determine the ultimate biocompatibility of the given material, and the extent of bacterial cell attachment to such a surface [133]. For instance, globular proteins, such as fibronectin, adsorbed onto polymer films of various hydrophobicity, charge density and swelling characteristics have been shown to differ in terms of their adsorption and displacement patterns, which in turn affected their functional characteristics due to an altered availability for molecular interactions attributed to the conformational changes, orientation and/or the anchorage of the surface-confined proteins [134]. Fibronectin is a key protein of the extracellular matrix that enables cell adhesion and an important prerequisite for the differentiation of the cells, with the latter being dependent on the binding strength of the protein. In order to achieve specific cell responses, the coatings should be designed so as to reduce the non-specific protein adsorption that may lead to undesirable side effects, such as surface-induced thrombosis, while induce specific protein adsorption and anticipated cell responses by decorating the material surface with specific chemical functionalities [133].

The adhesion of the coating to the biomaterial substrate is also greatly affected by the properties of the ambient fluid, with partial loss of adhesion or full delamination of the coating being a serious hindrance to *in vitro* plasma polymer application. The adhesion can be significantly improved by pre-treatment of the substrate prior to film deposition, with the specific treatment dependent on the properties of the substrate and the coating. Exposing polymer substrates to oxygen or nitrogen plasma for short time facilitates energetic species mediated hydrogen abstraction and polymer bond breakage, and hence allows for activation of the substrate surface. Adhesion promoting layers, such self-assembled monolayers and silicon oxide film, are an effective solution for adhesion improvement between plasma polymerised coating and an inorganic or metallic substrate. In addition to improving stability of plasma films in aqueous environment, such an interlayer may enable more precise and more reproducible chemical reactivity of plasma deposited coatings for biomaterial applications [135]. A radio frequency oxygen glow discharge was used to pre-functionalise medical-grade poly(vinyl chloride) prior to sodium hydroxide and silver nitrate wet treatment and monovalent silver incorporation in order to reduce *P. aeruginosa* adhesion and colonisation [136]. Oxygen plasma pre-functionalisation step was demonstrated to be a necessary step to ensure reproducible biomaterial surfaces amongst production lots, as well as to increase the amount of ether/alcohol, esters and carboxyl functional groups. The resultant modification completely inhibited bacterial adhesion of four strains of *P. aeruginosa* and efficiently prevented colonization over longer periods. Plasma-

modification was also used to successfully enhance the adhesion and uniformity of an electroless silver coating to polyurethane catheter surfaces [137].

Certain types of plasma polymers, such as fluorocarbon-based coatings, have been demonstrated to be stable and impermeable in a medium reproducing the physiological conditions, and can therefore be successfully applied as protective encapsulating coatings for biomaterials used for long-term implantation, such as intravascular stents and other metallic devices. Upon prolonged exposure to blood and other body fluids, these biomaterials can undergo degradation in terms of their mechanical properties, with a high potential for the release of toxic metallic compounds, such as nickel-based oxides and metal ions [138]. The application of a strongly adherent plasma-polymerised fluorocarbon coating can serve as a barrier against ion release, while being biocompatible with demonstrated thromboresistance properties and protein retention capability [139-140]. Furthermore, the *in vivo* stent implantation to support the narrowed lumen of atherosclerotic stenosed arteries requires *in situ* stent expansion, a step that generates local plastic deformation of up to 25% and may cause coating failures, including cracking and delamination [141]. Fluorocarbon coatings with a thickness below 100 nm exhibited the required cohesion and interfacial adhesion to resist the stent expansion without cracking or delaminating [142].

Recently, surface-grafted stimuli-responsive polymers, such as poly (*N*-isopropylacrylamide) have attracted notable attention due to their ability to change their physicochemical characteristics upon induction of environmentally-triggered phase changes [143]. Of particular interest is the possibility to control biomolecular adsorption, bacterial cell attachment and release, and cell function, such as production of extracellular substances by the adsorbed microorganisms, using these materials. For instance, attached bacterial cells can be released from the surface due to changes in the anchorage strength of cells brought about by the physico-chemical changes of the surface upon induction of environmentally-triggered phase change [144]. Plasma immobilised thermo-responsive poly(*N*-isopropylacrylamide)-*co*–*N*-(1-phenylethyl) acrylamide films were demonstrated to successfully modulate initial attachment and adhesion strength of the diatom, *N. perminuta* [145].

5.2. Surface architecture and porosity

There is much debate as to the extent to which the surface topography of a solid substrate influences bacterial attachment and their subsequent proliferation to form biofilms, particularly on a nano-scale level [102-103]. Several early studies concluded surface roughness to be a ‘minor factor’ in the attachment mechanism of bacteria, with cells demonstrating no preference for adhesion to surface features such as scratches or grooves [102-103]. Subsequently, Scheuerman et al. described preferential adherence of bacteria to grooved and braided surfaces, with the increased adhesion effect being attributed to the increase in contact surface area [146]. It was reported that, where the size of the surface features were comparable with the size of the individual microorganism, such situation increased the binding potential of the bacteria by maximising bacteria-surface contact area [147], whereas features appreciably smaller than bacterial size led to reduction in the binding as a result of the decrease in the contact area [148]. Examination of the adhesion preferences of *P. aeruginosa* to poly(methyl methacrylate) contact lenses indicated that surfaces with root-mean-square roughness parameter of 14 nm or above increased the extent of micro-organism attachment [149]. Studies on the

attachment behaviour of human pathogens, *Pseudomonas fluorescens* and *S. aureus*, concluded that the topography of micro-rough titanium surfaces affected the extent of cell attachment and preferential growth along the trenches in long rows [150], whilst the attachment response of these bacteria towards smooth surfaces did not follow a distinct pattern [146, 151]. Furthermore, the surface architecture of the abiotic target has also been demonstrated to affect the metabolism and morphology of the coloniser [152-153]. Nano-patterning of gold surfaces has been shown to enhance *P. fluorescens* localized attachment in the trenches of the surfaces compared to native gold surfaces, with cells showing limited EPS synthesis and reduced cell size compared to those attached to non-nano-patterned surfaces [151]. Our recent investigation on pathogenic strains of *S. aureus* and *P. aeruginosa* have shown evidence of increased adhesion to "nanosmooth" glass, polymer and titanium surfaces, with concurrent elevation in cellular metabolic activity, augmented production of EPS, and increased number of bacterial cells undergoing attachment [153-155]. It has been proposed that as anisotropic topographies such as ridges and grooves affect the individual cell behavior (cells align along the anisotropic direction), isotropic topographies, such as evenly or randomly distributed peaks and valleys influence collective cell behaviors [113].

In general, porous materials are associated with higher infection rates compared to dense and smooth materials. A recent study of biofilm formation on bone grafts and bone graft substitutes reported a shorter biofilm detection time and a 10-fold (*S. epidermidis*) or 100-fold (*S. aureus*) higher bacterial counts on porous samples (β-TCP, processed human spongiosa) compared to smooth samples (PMMA and PE) [156]. It is assumed that the shear forces are significantly lower inside pores even under high bulk fluid velocity allowing for a protected environment for bacteria to attach and grow [157]. The dynamics of microbial attachment and biofilm formation within the pores of the substrates will be affected by the degree of the porosity, pore size and permeability distribution of the porous network [158]. For instance, recent studies of osteoconductive hydroxyapatite and biphasic calcium phosphate ceramic materials with pores ranging in size from 50 to 300 nm, with a mean pore diameter of 200 nm, demonstrated that this pore size is not sufficiently large to allow the internalization of *Staphylococci* due to the rigid structure of the cell wall of Gram-positive bacteria [159]. The morphology of biofilms in porous media will also depend strongly on the bacterial species and the prevailing hydrodynamic and nutritional conditions, ranging from continuous, smooth films to discontinuous, highly irregular colonies [160]. For porous substrates, the biofilm development involves initial formation of smooth biofilms on the pore walls, inducing changes in the geometry and topology of the porous medium, hence impacting the macroscopic properties of the porous medium, including its porosity and the permeability, drastically changing the fluid flow and mass transport through the porous medium. Gradually, the smooth biofilm would morph into more irregular biofilm forms, creating biofilm strands spanning the pores and separated by water channels (web-like structure) [160]. Plasma polymerisation can be used to decrease the size of the accessible pores, making those unavailable for colonisation. Furthermore, surface roughness and porosity are also known to affect friction behaviour of the material, an important property for surfaces that undergo insertion into body conduits such as blood vessels or urethra or for high wear applications, such as a replacement for articular cartilage in joints [161]. Plasma treatment with inert gasses such as argon or helium can facilitate the formation of a highly cross-linked and smooth surface layer, hence improving the friction and wear properties of the biomaterial, as is the case with radio frequency glow discharge surface

treatment of the silicone rubber covering of electrical heart pacemaker which leads to a significant improvement in their slip properties [162]. Argon plasma sputtering of rough and smooth surfaces with amorphous carbon and titanium films to improve their biocompatibility showed an increased number of colony forming units on rough surfaces, especially on the a-C surfaces, with the degree of adhesion also dependent on bacterial taxa and surface chemistry of the coatings [163].

5.3. Plasma mediated grafting of surfaces

Plasma activation, film synthesis, ion implantation and grafting are tools frequently utilised for assembly of complex functional structures. For instance, covalent attachment (*i.e.* "tethering") of antimicrobials and antifouling agents to a component of the coating system can be used to significantly extend service lifetime of the device, forcing compatibility and uniform dispersion of the active ingredient throughout the polymer matrix even in cases where some preferred drugs and polymer carriers may be incompatible [164]. Polymer cushions prepared using plasma polymerisation have also been used to assemble various types of polymer-supported lipid bilayer membranes by tethering of a lipid monolayer containing reactive anchor lipids onto the surface of the plasma polymer [165]. Tethering quaternary ammonium salts (QASs) to a crosslinked polysiloxane matrix produced a hybrid antifouling/fouling-release coating with biocidal activity toward marine *Cellulophaga lytica*, with 4 wt% QAS moieties resulted in approximately 50% reduction in *C. lytica* biofilm retention without any leachate toxicity [166]. Bottom-up chemical synthesis of quaternary ammonium groups on stainless steel and filter paper surfaces using low-pressure ethylenediamine plasma functionalisation generated films rich in secondary and tertiary amines [167]. The pre-treatment of the surfaces with oxygen and hexamethyldisiloxane plasma ensured covalent attachment of quaternary ammonium structures. Modified steel surfaces exhibited greater than a 99.9% and 98% decrease in *S. aureus* and *K. pneumoniae* counts, respectively, whereas porous filter paper surfaces with immobilized QAS groups inactivated 98.7% and 96.8% of *S. aureus* and *K. pneumoniae*, respectively. The antibacterial properties of plasma treated surfaces can be further improved, such as in the case of the plasma-treated polymethyl methacrylate which was further modified with transparent TiO_2 films. These surface exhibited excellent photoinduced antibacterial effect against *S. aureus* and *E. coli* for the sterilisation of pathogen under indoor natural light, with approximately 100% of bacteria being inactivated within 2 h of illumination [168].

Plasma modification was used to activate poly(dimethyl siloxane) elastomer commonly used as a biomaterial, and to sequentially promote the attachment of Pluronic® F-68 synthetic surfactant or poly(ethylene glycol) methyl methacrylate to improve material hydrophilicity and bacterial cell repulsion properties [169]. The modification resulted in an increase of the oxygen content at the surface, with all materials found to be non-haemolytic and displaying no cytotoxicity. Asadinezhad et al. used surface activation by diffuse coplanar surface barrier discharge plasma followed by radical graft copolymerization of acrylic acid through surface-initiated pathway to render a structured high density brush on the surface of medical-grade polyvinyl chloride [170]. The brush modification was found to be remarkably effective to diminish the adherence of *E. coli*. Subsequent coatings with antibacterial agents, including bronopol, benzalkonium chloride, and chlorhexidine, were demonstrated to induce up to 85% reduction in adherence of *E. coli*, however only chlorhexidine coating was

capable of retarding the adhesion of *S. aureus*, with a reduction of 50%. Active screen plasma alloying treatment of medical grade stainless steel has been demonstrated to produce highly durable antimicrobial surfaces with concomitant increase in surface hardness and sliding wear resistance; the nanocrystalline silver alloyed S-phase steel surfaces achieved 93% reduction in *E. coli* after 6 h contact time compared to untreated steel samples [171]. Silver ions introduced into plasma sprayed 57% SiO_2/3% Al_2O_3/ 34% CaO/6% Na_2O glass coating on titanium alloy and stainless steel substrates demonstrated *in vivo* antimicrobial action against *S. aureus*, while maintaining its biocompatibility, and has been suggested as a suitable coating for bone healing and prosthetic devices [172].

Plasma immersion ion implantation has been used by several teams to modify medical-grade poly(vinyl chloride) to enhance its antibacterial properties. Zhang et al coated triclosan (2, 4, 4P-trichloro-2P-hydroxydiphenylether) and bronopol (2-bromo-2-nitropropane-1,3-diol) on oxygen plasma activated poly(vinyl chloride) surfaces, followed by an argon plasma treatment to improve the antibacterial properties of the triclosan and bronopol-coated poly(vinyl chloride) samples [173]. The modification resulted in enhanced antibacterial properties against *S. aureus* and *E. coli*, with triclosan treated surfaces being more effective against *E. coli* compared to those modified with bronopol. The antibacterial efficacy of both coatings, however, was demonstrated to decrease with time. Kwok et al. reported plasma immersion ion implantation of polycarbonate and polytetrafluoroethylene using argon and oxygen, respectively, under varied pulse and frequency conditions [32]. High energy oxygen treatment resulted in super-hydrophobic polytetrafluoroethylene surface that was characterised by higher affinity for human cell and *S. aureus* attachment. Acetylene (C_2H_2) plasma immersion ion implantation used to treat polyethylene terephthalate increased the hemocompatibility and antibacterial properties of the biomaterials, with a significant decrease of bacteria adhesion and growth reported for *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa* [174]. The plasma immersion ion implantation technique can also be successfully used for modification of orthopedic nickel-titanium shape memory alloys and cardiovascular materials with diamond-like carbon containing nitrogen and phosphorus doping agents [175]. The coating was found to possess adequate surface mechanical properties and host tissue compatibility, enhancing the biocompatibility of the materials, effectively mitigating nickel out-diffusion, whilst allowing the NiTi rods to retain their shape recovery properties. Biocompatibility of polyurethane undergone acetylene plasma immersion ion implantation was also reported to improve, while argon plasma was used to pre-treat surface for subsequent grafting with heparin, albumin or polyethylene oxide bindings [174, 176].

Concluding remarks

The utilisation of implantable materials and devices to replace missing tissues or restore a function has progressed rapidly over the past several decades. Continuous research efforts in the field of surface technology are directed toward enhancing tissue/surface interactions and advancing long-term performance of these materials. Furthermore, the ability to subtly modify surface properties can be potentially utilised to enrich our knowledge regarding the immune response, particularly the highly complex processes that govern the covalent binding of biomolecules, such antibodies and enzymes. Equally so, the intricate interactions between an abiotic surface and different types of living cell,

including that of bacteria and fungi, can be investigated in greater detail in order to improve our ability to predict the biological responses to changes in surface properties of these biomaterials.

In this paper, we have reviewed the advantages of the family of plasma-assisted techniques for the production and modification of biomaterials. The plasma surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness. This provides device manufacturers with a flexible and environmentally friendly process that allows for tailoring the surface properties of the material to suit a specific need. In addition, exposure to plasma has been shown to irreversibly damage bacterial cells, allowing for in situ sterilisation of the biomaterial during the surface modification process. Despite numerous auspicious results reported in literature, the real life applications are frequently hindered by limited understanding of the influence of process parameters, including among others geometry of the reactor, input energy, and pressure. The combination of these parameters determines the nature of the reactive species and ultimately the surface modifications produced. To the same extent, further advancements in the areas of immunology, biology and analytical techniques are necessary for the successful design and implementation of biomaterials.

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References and Notes

[1] Desmet T, Morent R, Geyter ND, Leys C, Schacht E, Dubruel P. Nonthermal plasma technology as a versatile strategy for polymeric biomaterials surface modification: a review. Biomacromolecules 2009;10:2351-78.

[2] Raynor JE, Capadona JR, Collard DM, Petrie TA, Garcia AJ. Polymer brushes and self-assembled monolayers: versatile platforms to control cell adhesion to biomaterials (Review). Biointerphases 2009;4:FA3-FA16.

[3] Biederman H, Slavínská D. Plasma polymer films and their future prospects. Surf Coat Technol 2000;125:371-6.

[4] Denes FS, Manolache S. Macromolecular plasma-chemistry: an emerging field of polymer science. Prog Polym Sci 2004;29:815-85.

[5] Johnston EE, Ratner BD. Surface characterization of plasma deposited organic thin films. J Electron Spectrosc Relat Phenom 1996;81:303-17.

[6] Muccini M. A bright future for organic field-effect transistors. Nat Mater 2006;5:605-13.

[7] Shi FF. Recent advances in polymer thin films prepared by plasma polymerization: Synthesis, structural characterization, properties and applications. Surf Coat Technol 1996;82:1-15.

[8] Yasuda H. Glow discharge polymerization. J Pol Sci: Macromol R 1981;16:199-293.

[9] Korachi M, Gurol C, Aslan N. Atmospheric plasma discharge sterilization effects on whole cell fatty acid profiles of *Escherichia coli* and *Staphylococcus aureus*. J Electrostat;In Press, Corrected Proof.

[10] Sohbatzadeh F, Hosseinzadeh Colagar A, Mirzanejhad S, Mahmodi S. *E. coli*, *P. aeruginosa*, and *B. cereus* bacteria sterilization using afterglow of non-thermal plasma at atmospheric pressure. Appl Biochem Biotechnol 2010;160:1978-84.

[11] Sureshkumar A, Sankar R, Mandal M, Neogi S. Effective bacterial inactivation using low temperature radio frequency plasma. Int J Pharm 2010;396:17-22.

[12] Roth S, Feichtinger J, Hertel C. Characterization of *Bacillus subtilis* spore inactivation in low-pressure, low-temperature gas plasma sterilization processes. J Appl Microbiol 2010;108:521-31.

[13] Zaaba SK, Akitsu T, Ohkawa H, Katayama-Hirayama K, Tsuji M, Shimizu N, et al. Plasma disinfection and the deterioration of surgical tools at atmospheric pressure plasma. IEEJ Transactions on Fundamentals and Materials 2010;130:355-61.

[14] Kim MC, Cho SH, Han JG, Hong BY, Kim YJ, Yang SH, et al. High-rate deposition of plasma polymerized thin films using PECVD method and characterization of their optical properties. Surf Coat Technol 2003;169-170:595-9.

[15] Lahann J. Vapor-based polymer coatings for potential biomedical applications. Polym Int 2006;55:1361-70.

[16] Sajeev U, Mathai C, Saravanan S, Ashokan R, Venkatachalam S, Anantharaman M. On the optical and electrical properties of rf and a.c. plasma polymerized aniline thin films. Bull Mater Sci 2006;29:159-63.

[17] Bettinger CJ, Bao Z. Biomaterials-based organic electronic devices. Polym Int 2010;59:563-7.

[18] Yoganand CP, Selvarajan V, Cannillo V, Sola A, Roumeli E, Goudouri OM, et al. Characterization and in vitro-bioactivity of natural hydroxyapatite based bio-glass-ceramics synthesized by thermal plasma processing. Ceram Int 2010;36:1757-66.

[19] Yoganand CP, et al. Bioactivity of thermal plasma synthesized bovine hydroxyapatite/glass ceramic composites. J Phys Conf Ser 2010;208:012099.

[20] Wang G, Meng F, Ding C, Chu PK, Liu X. Microstructure, bioactivity and osteoblast behavior of monoclinic zirconia coating with nanostructured surface. Acta Biomater 2009;6:990-1000.

[21] Nawale AB, Kulkarni N, Karmakar S, Das AK, Bhoraskar SV, Mathe VL. Phase controlled structure formation of the nanocrystalline zirconia using thermal plasma technique. J Phys Conf Ser 2010;208:012121.

[22] Dautov GY. Generators of nonequilibrium low-temperature plasma. J Eng Phys Thermophys 1987;53:1434-41.

[23] Inbakumar S, Morent R, De Geyter N, Desmet T, Anukaliani A, Dubruel P, et al. Chemical and physical analysis of cotton fabrics plasma-treated with a low pressure DC glow discharge. Cellulose 2010;17:417-26.

[24] Yang Z, Wang X, Wang J, Yao Y, Sun H, Huang N. Pulsed-plasma polymeric allylamine thin films. Plasma Processes Polym 2009;6:498-505.

[25] Denis L, Marsal P, Olivier Y, Godfroid T, Lazzaroni R, Hecq M, et al. Deposition of functional organic thin films by pulsed plasma polymerization: a joint theoretical and experimental study. Plasma Processes Polym 2010;7:172-81.

[26] Geissler A, Vallat M-F, Vidal L, Voegel J-C, Hemmerle J, Schaaf P, et al. Chemical force titration of plasma polymer-modified PDMS substrates by using plasma polymer-modified AFM tips. Langmuir 2008;24:4874-80.

[27] Nelea V, et al. Deposition of fluorocarbon thin films on outer and inner surfaces of stainless steel mini-tubes by pulsed plasma polymerization for stents. J Phys D: Appl Phys 2009;42:225208.

[28] Swaraj S, Oran U, Lippitz A, Friedrich JE, Unger WES. Surface analysis of plasma-deposited polymer films: analysis of plasma deposited allyl alcohol films before and after aging in air. Plasma Processes Polym 2005;2:572-80.

[29] Gristina R, D'Aloia E, Senesi GS, Milella A, Nardulli M, Sardella E, et al. Increasing cell adhesion on plasma deposited fluorocarbon coatings by changing the surface topography. J Biomed Mater Res B Appl Biomater 2009;88B:139-49.

[30] Liu S, Vareiro MMLM, Fraser S, Jenkins ATA. Control of attachment of bovine serum albumin to pulse plasma-polymerized maleic anhydride by variation of pulse conditions. Langmuir 2005;21:8572-5.

[31] Chifen AN, Forch R, Knoll W, Cameron PJ, Khor HL, Williams TL, et al. Attachment and phospholipase A2-Induced lysis of phospholipid bilayer vesicles to plasma-polymerized maleic anhydride/SiO2 multilayers. Langmuir 2007;23:6294-8.

[32] Kwok DTK, Tong L, Yeung CY, Remedios CGd, Chu PK. Hybrid plasma surface modification and ion implantation of biopolymers. Surf Coat Technol 2010;204:2892-7.

[33] Subbiahdoss G, Kuijer R, Grijpma DW, van der Mei HC, Busscher HJ. Microbial biofilm growth vs. tissue integration: "The race for the surface" experimentally studied. Acta Biomater 2009;5:1399-404.

[34] Busscher HJ, Ploeg RJ, van der Mei HC. Snapshot: biofilms and biomaterials: mechanisms of medical device related infections. Biomaterials 2009;30:4247-8.

[35] Hanssen AD. Managing the infected knee: As good as it gets. J Arthropl 2002;17:98-101.

[36] Montanaro L, Campoccia D, Arciola CR. Advancements in molecular epidemiology of implant infections and future perspectives. Biomaterials 2007;28:5155-68.

[37] Gottenbos B, van der Mei HC, Klatter F, Nieuwenhuis P, Busscher HJ. In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials 2002;23:1417-23.

[38] Merritt PM, Danhorn T, Fuqua C. Motility and Chemotaxis in Agrobacterium tumefaciens Surface Attachment and Biofilm Formation. J Bacteriol 2007;189:8005-14.

[39] Velasco-Casal P, Wick LY, Ortega-Calvo J-J. Chemoeffectors decrease the deposition of chemotactic bacteria during transport in porous media. Environ Sci Technol 2008;42:1131-7.

[40] Jenal U, Silversmith RE, Sogaard-Andersen L, Sockett L. Sense and sensibility in bacteria. EMBO Rep 2005;6:615-9.

[41] Kirov SM. Bacteria that express lateral flagella enable dissection of the multifunctional roles of flagella in pathogenesis. FEMS Microbiol Lett 2003;224:151-9.

[42] Dickinson RB, Tranquillo RT. A stochastic model for adhesion-mediated cell random motility and haptotaxis. J Math Biol 1993;31:563-600.

[43] Pavithra D, Mukesh D. Biofilm formation, bacterial adhesion and host response on polymeric implants issues and prevention. Biomedical Materials 2008;3:034003.

[44] Donlan RM. Biofilms: microbial life on surfaces. Emerging Infect Dis 2002;8:881-90.

[45] Flemming HC, Wingender J. Relevance of microbial extracellular polymeric substances (EPSs) – Part I: Structural and ecological aspects. Water Sci Technol 2001;43:1-8.

[46] Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, et al. The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int J Biol Macromol 1999;26:3-16.

[47] Dong C, Beis K, Nesper J, Brunkan-LaMontagne AL, Clarke BR, Whitfield C, et al. Wza the translocon for *E. coli* capsular polysaccharides defines a new class of membrane protein. Nature 2006;444:226-9.

[48] Roberson EB, Firestone MK. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas sp*. Appl Environ Microbiol 1992;58:1284-91.

[49] Jayaratne P, Keenleyside WJ, MacLachlan PR, Dodgson C, Whitfield C. Characterization of rcsB and rcsC from *Escherichia coli* O9:K30:H12 and examination of the role of the rcs regulatory system in expression of group I capsular polysaccharides. J Bacteriol 1993;175:5384-94.

[50] Beveridge TJ, Graham LL. Surface layers of bacteria. Microbiol Mol Biol Rev 1991;55:684-705.

[51] Bruno C, Yves F. In situ characterization of bacterial extracellular polymeric substances by AFM Colloids Surf B: Biointerfaces 2002;23:173-82.

[52] Decho AW. Microbial cxopolymcr secretions in ocean environments: Their role(s) in food webs and marine processes. Oceanogr Mar Biol 1990;28:73-153.

[53] Flemming HC. Biofouling und biokorrosion - die folgen unerwünschter biofilme. Chem Ingnieur Technik 1995;67:1425-30.

[54] Absoiom DR, Lamberti FV, Policova Z, Zingg W, Van Oss DJ, Neumann AW. Appl Environ Microbiol 1983;46:90.

[55] Beveridge TJ, Fyfe WS. Metal fixation by bacterial cell walls. Can J Earth Sci 1985;22:1893-8.

[56] Cheng SS, Chittur KK, Sukenik CN, Culp LA, Lawrence K. The conformation of fibronectin on self-assembled monolayers with different surface composition: an FTIR/ATR study J Colloid Interface Sci 1994;162:135-43.

[57] Ong JL, Chittur KK, Lucas LC. Dissolution/reprecipitation and protein adsorption studies of calcium phosphate coatings by FT-IR/ATR techniques. J Biomed Mater Res 1994;28:1337-46.

[58] Hoge CW, Schwartz B, Talkington DF, Breiman RF, Macneill EM, Englender SJ. The changing epidemiology of invasive group-A streptococcal infections and the emergence of Streptococcal toxic shock-like syndrome - a retrospective population-based study JAMA 1993;269:1638-.

[59] Kaul R, McGeer A, Low DE, Green K, Schwartz B, Simor AE. Population-based surveillance for group A streptococcal necrotizing fascitis: Clinical features, prognostic indicators, and microbiologic analysis of seventy-seven cases. Am J Med 1997;103:18-24.

[60] Cywes C, Stamenkovic I, Wessels MR. CD44 as a receptor for colonization of the pharynx by group A Streptococcus. J Clin Invest 2000;106:995-1002.

[61] Ashbaugh CD, Moser TJ, Shearer MH, White GL, Kennedy RC, Wessels MR. Bacterial determinants of persistent throat colonization and the associated immune response in a primate model of human group A streptococcal pharyngeal infection. Cell Microbiol 2000;2:283-92.

[62] Sutherland IW. CRC Crit Rev Microbiol 1984;60:434.

[63] Veiga MC, Jain MK, Wu WW, Hollingsworth RI, Zeikus JG. Composition and role of extracellular polymers in methanogenic granules. Appl Environ Microbiol 1997;63:403-7.

[64] Camesano TA, Logan BE. Probing bacterial electrosteric interactions using atomic force microscopy. Environ Sci Technol 2000;34:3354-62.

[65] van Loosdrecht MCM, Norde W, Lyklema J, Zehnder AJB. Hydrophobic and electrostatic parameters in bacterial adhesion. Aquatic Sci 1990;53:103.

[66] Williams V, Fletcher M. Appl Environ Microbiol 1996;62:100.

[67] Cescutti P, Otto H, Patrick JB, Mark von I. Bacterial capsular polysaccharides and exopolysaccharides. Microbial Glycobiology. San Diego: Academic Press; 2010. p. 93-108.

[68] Omoike A, Chorover J. Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: Aqueous chemistry and adsorption effects. Biomacromol 2004;5:1219-30.

[69] Wessels MR, Bronze MS. Critical role of the group A streptococcal capsule in pharyngeal colonization and infection in mice. Proc Natl Acad Sci U S A 1994;91:12238-42.

[70] Fletcher M. Bacterial attachment in aquatic environments: a diversity of surfaces and adhesion strategies In: Fletcher M, editor. Bacterial adhesion: Molecular and ecological diversity. New York: Wiley-Liss; 1996. p. 1-24.

[71] Strauss J, Burnham NA, Camesano TA. Atomic force microscopy study of the role of LPS O-antigen on adhesion of E. coli. J Mol Recognit 2009;22:347-55.

[72] Abu-Lail NI, Camesano TA. Role of lipopolysaccharides in the adhesion, retention, and transport of *Escherichia coli* JM109. Environ Sci Technol 2003;37:2173-83.

[73] Sheng H, Lim JY, Watkins MK, Minnich SA, Hovde CJ. Characterization of an *Escherichia coli* O157:H7 O-antigen deletion mutant and effect of the deletion on bacterial persistence in the mouse intestine and colonization at the bovine terminal rectal mucosa. Appl Environ Microbiol 2008;74:5015-22.

[74] Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, et al. Bacterial biofilms in nature and disease. Annu Rev Microbiol 1987;41:435-64.

[75] Pavithra D, Mukesh D. Biofilm formation, bacterial adhesion and host response on polymeric implants' issues and prevention. Biomed Mater 2008;3:034003.

[76] Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 2001;147:3-9.

[77] van Hullebusch E, Zandvoort M, Lens P. Metal immobilisation by biofilms: Mechanisms and analytical tools. R Environ Sci Biotech 2003;2:9-33.

[78] Romaní A, Fund K, Artigas J, Schwartz T, Sabater S, Obst U. Relevance of polymeric matrix enzymes during biofilm formation. Microb Ecol 2008;56:427-36.

[79] Das T, Sharma PK, Busscher HJ, van der Mei HC, Krom BP. Role of extracellular DNA in initial bacterial adhesion and surface aggregation. Appl Environ Microbiol 2010:AEM.03119-09.

[80] Liu X, Chu PK, Ding C. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Mater Sci Eng R 2004;47:49-121.

[81] Stobie N, Duffy B, McCormack DE, Colreavy J, Hidalgo M, McHale P, et al. Prevention of *Staphylococcus epidermidis* biofilm formation using a low-temperature processed silver-doped phenyltriethoxysilane sol-gel coating. Biomaterials 2008;29:963-9.

[82] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science 1999;284:1318-22.

[83] Mah T-F, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 2003;426:306-10.

[84] Sadovskaya I, Vinogradov E, Li J, Hachani A, Kowalska K, Filloux A. High-level antibiotic resistance in *Pseudomonas aeruginosa* biofilm: the ndvB gene is involved in the production of highly glycerol-phosphorylated β-(1→3)-glucans, which bind aminoglycosides. Glycobiology 2010;20:895-904.

[85] Decho AW. Microbial biofilms in intertidal systems: an overview. Continent Shelf Res 2000;20:1257-73.

[86] Lauderdale KJ, Malone CL, Boles BR, Morcuende J, Horswill AR. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. J Orthop Res 2010;28:55-61.

[87] van Heerden J, Turner M, Hoffmann D, Moolman J. Antimicrobial coating agents: can biofilm formation on a breast implant be prevented? J Plast Reconstr Aesthet Surg 2009;62:610-7.

[88] Lee J-H, Wang H, Kaplan JB, Lee WY. Microfluidic Approach to Create 3D Tissue Models for Biofilm-Related Infection of Orthopaedic Implants. Tissue Eng C: Methods.

[89] Dorobantu LS, Bhattacharjee S, Foght JM, Gray MR. Analysis of force interactions between AFM tips and hydrophobic bacteria using DLVO theory. Langmuir 2009;25:6968-76.

[90] Cappella B, Dietler G. Force-distance curves by atomic force microscopy. Surf Sci Rep 1999;34:5-104.

[91] Jucker BA, Harms H, Zehnder AJB. Polymer interactions between five gram-negative bacteria and glass investigated using LPS micelles and vesicles as model systems. Colloids Surf B: Biointerfaces 1998;11:33-45.

[92] Rijnaarts HHM, Norde W, Bouwer EJ, Lyklema J, Zehnder AJB. Bacterial adhesion under static and dynamic conditions. Appl Environ Microbiol 1993;59:3255–65.

[93] Davies KG, Afolabi P, O'Shea P. Adhesion of Pasteuria penetrans to the cuticle of root-knot nematodes (*Meloidogyne* spp.) inhibited by fibronectin: a study of electrostatic and hydrophobic interactions. Parasitology 1996;112:553-9.

[94] Song B, Leff LG. Influence of magnesium ions on biofilm formation by *Pseudomonas fluorescens*. Microbiol Res 2006;161:355-61.

[95] Hancock V, Dahl M, Klemm P. Abolition of Biofilm Formation in Urinary Tract *Escherichia coli* and *Klebsiella* Isolates by Metal Interference through Competition for Fur. Appl Environ Microbiol 2010;76:3836-41.

[96] Perrin C, Briandet R, Jubelin G, Lejeune P, Mandrand-Berthelot M-A, Rodrigue A, et al. Nickel promotes biofilm formation by *Escherichia coli* K-12 strains that produce curli. Appl Environ Microbiol 2009;75:1723-33.

[97] Radzig M, Koksharova O, Khmel' I. Antibacterial effects of silver ions on growth of gram-negative bacteria and biofilm formation. Molec Genetics Microb Virol 2009;24:194-9.

[98] Hong Y, Brown DG. Alteration of bacterial surface electrostatic potential and pH upon adhesion to a solid surface and impacts to cellular bioenergetics. Biotechnol Bioeng 2009;105:965-72.

[99] Busscher HJ, Norde W, Sharma PK, van der Mei HC. Interfacial re-arrangement in initial microbial adhesion to surfaces. Curr Opin Colloid Interface Sci 2010;In Press, Corrected Proof.

[100] Andrews SS. Accurate particle-based simulation of adsorption, desorption and partial transmission. Phys Biol 2009;6:046015.

[101] Piglowski J, Gancarz I, Staniszewska-Kus J, Paluch D, Szymonowicz M, Konieczny A. Influence of plasma modification on biological properties of poly(ethylene terephthalate). Biomaterials 1994;15:909-16.

[102] Bos R, Van Der Mei HC, Busscher HJ. Physico-chemistry of initial microbial adhesive interactions - Its mechanisms and methods for study. FEMS Microbiol Rev 1999;23:179-229.

[103] An YH, Friedman RJ, Draughn RA, Smith EA, Nicholson JH, John JF. Rapid quantification of *staphylococci* adhered to titanium surfaces using image analyzed epifluorescence microscopy. J Microbiol Methods 1995;24:29-40.

[104] Subbiahdoss G, Pidhatika B, Coullerez G, Charnley M, Kuijer R, van der Mei HC, et al. Bacterial biofilm formation versus mammalian cell growth on titanium-based mono- and bi-functional coating. Eur Cell Mater 2010;19:205-13.

[105] Lee J-H, Wang H, Kaplan JB, Lee WY. Effects of Staphylococcus epidermidis on osteoblast cell adhesion and viability on a Ti alloy surface in a microfluidic co-culture environment. Acta Biomater;In Press, Corrected Proof.

[106] Donlan RodneyÂ M. Biofilm formation: a clinically relevant microbiological process. Clin Infect Dis 2001;33:1387-92.

[107] Hao L, Lawrence J, Li L. The wettability modification of bio-grade stainless steel in contact with simulated physiological liquids by the means of laser irradiation. Appl Surf Sci 2005;247:453-7.

[108] Schakenraad JM, Busscher HJ, Wildevuur CRH, Arends J. The influence of substratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. J Biomed Mater Res 1986;20:773-84.

[109] van Wachem PB, Beugeling T, Feijen J, Bantjes A, Detmers JP, van Aken WG. Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. Biomaterials 1985;6:403-8.

[110] Tamada Y, Ikada Y. Cell adhesion to plasma-treated polymer surfaces. Polymer 1993;34:2208-12.

[111] Ponsonnet L, Reybier K, Jaffrezic N, Comte V, Lagneau C, Lissac M, et al. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour. Materials Science and Engineering: C 2003;23:551-60.

[112] Annarelli CC, Fornazero J, Cohen R, Bert J, Besse JL. Colloidal protein solutions as a new standard sensor for adhesive wettability measurements. J Colloid Interface Sci 1999;213:386-94.

[113] Pashkuleva I, Marques A, Vaz F, Reis R. Surface modification of starch based biomaterials by oxygen plasma or UV-irradiation. J Mater Sci: Mater Med 2010;21:21-32.

[114] Schröder K, Meyer-Plath A, Keller D, Besch W, Babucke G, Ohl A. Plasma-induced surface functionalization of polymeric biomaterials in ammonia plasma. Contrib Plasma Physics 2001;41:562-72.

[115] Hook AL, Thissen H, Quinton J, Voelcker NH. Comparison of the binding mode of plasmid DNA to allylamine plasma polymer and poly(ethylene glycol) surfaces. Surf Sci 2008;602:1883-91.

[116] Pertile RAN, Andrade FK, Alves Jr C, Gama M. Surface modification of bacterial cellulose by nitrogen-containing plasma for improved interaction with cells. Carbohydr Polym 2010;82:692-8.

[117] Wang IW, Anderson JM, Jacobs MR, Marchant RE. Adhesion of *Staphylococcus epidermidis* to biomedical polymers: Contributions of surface thermodynamics and hemodynamic shear conditions. J Biomed Mater Res 1995;29:485-93.

[118] Kumar V, Rauscher H, Bretagnol F, Arefi-Khonsari F, Pulputel J, Colpo P, et al. Preventing biofilm formation on biomedical surfaces. In: Rauscher H, Perucca M, Buyle G, editors. Plasma Technology for Hyperfunctional Surfaces: Food, Biomedical, and Textile Applications2010.

[119] Bazaka K, Jacob MV, Truong VK, Wang F, Pushpamali WA, Wang J, et al. Effect of plasma-enhaced chemical vapour deposition on the retention of antibacterial activity of terpinen-4-ol. Biomacromolecules 2010;11:2016-26.

[120] Gottenbos B, Grijpma DW, van der Mei HC, Feijen J, Busscher HJ. Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. J Antimicrob Chemother 2001;48:7-13.

[121] Boks NP, Kaper HJ, Norde W, van der Mei HC, Busscher HJ. Mobile and immobile adhesion of staphylococcal strains to hydrophilic and hydrophobic surfaces. J Colloid Interface Sci 2009;331:60-4.

[122] Kugler R, Bouloussa O, Rondelez F. Evidence of a charge-density threshold for optimum efficiency of biocidal cationic surfaces. Microbiology 2005;151:1341-8.

[123] Hong Y, Brown DG. Variation in bacterial ATP level and proton motive force due to adhesion to a solid surface. Appl Environ Microbiol 2009;75:2346-53.

[124] Timmis KN, Heipieper HJ, Cornelissen S, Pepi M. Surface properties and cellular energetics of bacteria in response to thepresence of hydrocarbons. Handbook of Hydrocarbon and Lipid Microbiology: Springer Berlin Heidelberg; 2010. p. 1615-24.

[125] Balazs DJ, Triandafillu K, Chevolot Y, Aronsson BO, Harms H, Descouts P, et al. Surface modification of PVC endotracheal tubes by oxygen glow discharge to reduce bacterial adhesion. Surf Interface Anal 2003;35:301-9.

[126] Triandafillu K, Balazs DJ, Aronsson BO, Descouts P, Tu Quoc P, van Delden C, et al. Adhesion of *Pseudomonas aeruginosa* strains to untreated and oxygen-plasma treated poly(vinyl chloride) (PVC) from endotracheal intubation devices. Biomaterials 2003;24:1507-18.

[127] Marciano FR, Bonetti LF, Da-Silva NS, Corat EJ, Trava-Airoldi VJ. Wettability and antibacterial activity of modified diamond-like carbon films. Appl Surf Sci 2009;255:8377-82.

[128] Kaufmann T, Ravoo BJ. Stamps, inks and substrates: polymers in microcontact printing. Polymer Chemistry 2010;1:371-87.

[129] Katsikogianni M, Amanatides E, Mataras D, Missirlis YF. *Staphylococcus epidermidis* adhesion to He, He/O2 plasma treated PET films and aged materials: Contributions of surface free energy and shear rate. Colloids Surf B: Biointerfaces 2008;65:257-68.

[130] Hamadi F, Latrache H, Zekraoui M, Ellouali M, Bengourram J. Effect of pH on surface energy of glass and Teflon and theoretical prediction of *Staphylococcus aureus* adhesion. Materials Science and Engineering: C 2009;29:1302-5.

[131] Atkin R, Craig VSJ, Hartley PG, Wanless EJ, Biggs S. Adsorption of ionic surfactants to a plasma polymer substrate. Langmuir 2003;19:4222-7.

[132] Schonherr H, van Os MT, Forch R, Timmons RB, Knoll W, Vancso GJ. Distributions of functional groups in plasma polymerized allylamine films by scanning force microscopy using functionalized probe tips. Chem Mater 2000;12:3689-94.

[133] Chen H, Yuan L, Song W, Wu Z, Li D. Biocompatible polymer materials: Role of protein-surface interactions. Prog Polym Sci 2008;33:1059-87.

[134] Déjardin P, Pompe T, Renner L, Werner C. Fibronectin at polymer surfaces with graduated characteristics. Proteins at Solid-Liquid Interfaces: Springer Berlin Heidelberg; 2006. p. 175-98.

[135] Förch R, Chifen AN, Bousquet A, Khor HL, Jungblut M, Chu LQ, et al. Recent and expected roles of plasma-polymerized films for biomedical applications. Chem Vap Deposition 2007;13:280-94.

[136] Gray JE, Norton PR, Alnouno R, Marolda CL, Valvano MA, Griffiths K. Biological efficacy of electroless-deposited silver on plasma activated polyurethane. Biomaterials 2003;24:2759-65.

[137] Balazs DJ, Triandafillu K, Wood P, Chevolot Y, van Delden C, Harms H, et al. Inhibition of bacterial adhesion on PVC endotracheal tubes by RF-oxygen glow discharge, sodium hydroxide and silver nitrate treatments. Biomaterials 2004;25:2139-51.

[138] Haïdopoulos M, Turgeon S, Laroche G, Mantovani D. Chemical and morphological characterization of ultra-thin fluorocarbon plasma-polymer deposition on 316 stainless steel substrates: a first step toward the improvement of the long-term safety of coated-stents. Plasma Processes Polym 2005;2:424-40.

[139] Sevast'yanov V, Vasilets V. Plasmochemical modification of fluorocarbon polymers for creation of new hemocompatible materials. Russ J Gen Chem 2009;79:596-605.

[140] Choudhury NR, Kannan AG, Dutta NK, Klaus F, Alois KS. Chapter 21. Novel nanocomposites and hybrids for lubricating coating applications. Tribol Interf EngSer Elsevier; 2008. p. 501-42.

[141] Hale P, Turgeon Sp, Horny P, Lewis Fo, Brack N, Van Riessen G, et al. X-ray photoelectron emission microscopy and time-of-flight secondary ion mass spectrometry analysis of ultrathin fluoropolymer coatings for stent applications. Langmuir 2008;24:7897-905.

[142] Lewis F, et al. Study of the adhesion of thin plasma fluorocarbon coatings resisting plastic deformation for stent applications. J Phys D: Appl Phys 2008;41:045310.

[143] Cordeiro AL, Zimmermann R, Gramm S, Nitschke M, Janke A, Schafer N, et al. Temperature dependent physicochemical properties of poly(N-isopropylacrylamide-co-N-(1-phenylethyl) acrylamide) thin films. Soft Matter 2009;5:1367-77.

[144] Ista LK, Mendez S, Lopez GP. Attachment and detachment of bacteria on surfaces with tunable and switchable wettability. Biofouling J Bioadh Biofilm Res 2010;26:111 - 8.

[145] Cordeiro A, Pettit M, Callow M, Callow J, Werner C. Controlling the adhesion of the diatom *Navicula perminuta* using poly(*N*-isopropylacrylamide-*co*–*N*-(1-phenylethyl) acrylamide) films. Biotechnol Lett 2010;32:489-95.

[146] Scheuerman TR, Camper AK, Hamilton MA. Effects of substratum topography on bacterial adhesion. J Colloid Interface Sci 1998;208:23-33.

[147] Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater 2004:37-57.

[148] Edwards KJ, Rutenberg AD. Microbial response to surface microtopography: the role of metabolism in localized mineral dissolution. Chem Geol 2001;180:19-32.

[149] Whitehead KA, Colligon J, Verran J. Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. Col Surf B: Biointerfaces 2005;41:129-38.

[150] Harris LG, Tosatti S, Wieland M, Textor M, Richards RG. *Staphylococcus aureus* adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(-lysine)-grafted-poly(ethylene glycol) copolymers. Biomaterials 2004;25:4135-48.

[151] Diaz C, Cortizo MC, Schilardi PL, de Saravia SGG, de Mele MAFL. Influence of the nano-micro structure of the surface on bacterial adhesion. Mater Res 2007;10:11-4.

[152] Truong VK, Rundell S, Lapovok R, Estrin Y, Wang JY, Berndt CC, et al. Effect of ultrafine-grained titanium surfaces on adhesion of bacteria. Appl Microbiol Biotech 2009;83:925-37.

[153] Mitik-Dineva N, Wang J, Truong VK, Stoddart P, Malherbe F, Crawford RJ, et al. *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* attachment patterns on glass surfaces with nanoscale roughness. Curr Microbiol 2009;58:268-73.

[154] Mitik-Dineva N, Wang J, Mocanasu RC, Stoddart PR, Crawford RJ, Ivanova EP. Impact of nano-topography on bacterial attachment. Biotech J 2008;3:536-44.

[155] Ivanova EP, Mitik-Dineva N, Wang J, Pham DK, Wright JP, Nicolau DV, et al. *Staleya guttiformis* attachment on poly(tert-butylmethacrylate) polymeric surfaces. Micron 2008;39:1197-204.

[156] Clauss M, Trampuz A, Borens O, Bohner M, Ilchmann T. Biofilm formation on bone grafts and bone graft substitutes: Comparison of different materials by a standard in vitro test and microcalorimetry. Acta Biomater 2010;6:3791-7.

[157] Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microb Cell Fact 2005;4:24.

[158] Shafahi M. Biofilm Growth Within Porous Media. AIP Conf Proc 2010;1254:193-7.

[159] Kinnari TJ, Esteban J, Martin-de-Hijas NZ, Sanchez-Munoz O, Sanchez-Salcedo S, Colilla M, et al. Influence of surface porosity and pH on bacterial adherence to hydroxyapatite and biphasic calcium phosphate bioceramics. J Med Microbiol 2009;58:132-7.

[160] Kapellos GE, Alexiou TS, Payatakes AC. Hierarchical simulator of biofilm growth and dynamics in granular porous materials. Adv Water Resour 2007;30:1648-67.

[161] Covert RJ, Ott RD, Ku DN. Friction characteristics of a potential articular cartilage biomaterial. Wear 2003;255:1064-8.

[162] Coury AJSP, MN), Cahalan, Patrick T. (Stein, NL), Di Domenico Jr., Edward D. (Anoka, MN), Keeney, Kenneth W. (Forest Lake, MN), Swoyer, John M. (Coon Rapids, MN). Radio frequency glow discharge surface treatment of silicone tubing used as covering for electrical leads to improve slip properties thereof. United States: Medtronic, Inc. (Minneapolis, MN); 1992.

[163] Almaguer-Flores A, Ximénez-Fyvie LA, Rodil SE. Oral bacterial adhesion on amorphous carbon and titanium films: Effect of surface roughness and culture media. J Biomed Mater Res B Appl Biomater 2009;92B:196-204.

[164] Kugel A, Chisholm B, Ebert S, Jepperson M, Jarabek L, Stafslien S. Antimicrobial polysiloxane polymers and coatings containing pendant levofloxacin. Polymer Chemistry 2010;1:442-52.

[165] Meier WP, Knoll W, Bender K, Förch R, Frank C, Götz H, et al. Polymer-tethered bimolecular lipid membranes. Polymer Membranes/Biomembranes: Springer Berlin / Heidelberg. p. 87-111.

[166] Majumdar P, Lee E, Patel N, Stafslien S, Daniels J, Chisholm B. Development of environmentally friendly, antifouling coatings based on tethered quaternary ammonium salts in a crosslinked polydimethylsiloxane matrix. J Coat Technol Res 2008;5:405-17.

[167] Jampala SN, Sarmadi M, Somers EB, Wong ACL, Denes FS. Plasma-enhanced synthesis of bactericidal quaternary ammonium thin layers on stainless steel and cellulose surfaces. Langmuir 2008;24:8583-91.

[168] Su W, Wang S, Wang X, Fu X, Weng J. Plasma pre-treatment and TiO2 coating of PMMA for the improvement of antibacterial properties. Surf Coat Technol;In Press, Corrected Proof.

[169] Pinto S, Alves P, Matos CM, Santos AC, Rodrigues LR, Teixeira JA, et al. Poly(dimethyl siloxane) surface modification by low pressure plasma to improve its characteristics towards biomedical applications. Colloids Surf B: Biointerfaces 2010;81:20-6.

[170] Asadinezhad A, Novák I, Lehocký M, Sedlarík V, Vesel A, Junkar I, et al. An in vitro bacterial adhesion assessment of surface-modified medical-grade PVC. Colloids Surf B: Biointerfaces 2010;77:246-56.

[171] Dong Y, Li X, Sammons R, Dong H. The generation of wear-resistant antimicrobial stainless steel surfaces by active screen plasma alloying with N and nanocrystalline Ag. J Biomed Mater Res B Appl Biomater 2010;93B:185-93.

[172] Miola M, Ferraris S, Di Nunzio S, Robotti P, Bianchi G, Fucale G, et al. Surface silver-doping of biocompatible glasses to induce antibacterial properties. Part II: plasma sprayed glass-coatings. J Mater Sci: Mater Med 2009;20:741-9.

[173] Zhang W, Chu PK, Ji J, Zhang Y, Liu X, Fu RKY, et al. Plasma surface modification of poly vinyl chloride for improvement of antibacterial properties. Biomaterials 2006;27:44-51.

[174] Huang N, Yang P, Leng YX, Wang J, Sun H, Chen JY, et al. Surface modification of biomaterials by plasma immersion ion implantation. Surf Coat Technol 2004;186:218-26.

[175] Chu PK. Enhancement of surface properties of biomaterials using plasma-based technologies. Surf Coat Technol 2007;201:8076-82.

[176] Huang N, Leng YX, Yang P, Chen JY, Sun H, Wan GJ, et al. Plasma surface modification of biomaterials applied for cardiovascular devices. Plasma Science, 2003 ICOPS 2003 IEEE Conference Record - Abstracts The 30th International Conference on2003. p. 439.