Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment

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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 in-dwelling medical devices. As such, the fundamental principles of bacterial cell attachment and biofilm formation are also discussed. Functional organic plasma polymerised coatings are also discussed for their potential as biosensitive interfaces, connecting inorganic/metallic electronic devices with their physiological environments.

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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 in-dwelling 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 responses. 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 in-dwelling 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 tailoring of 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 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 adherence 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 thermal equilibrium of its entire range of species. The temperatures required to generate near-equilibrium plasmas generally range between 4000 and 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 bioglass–ceramic composites [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 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 Fig. 1. The nature of the interactions between the excited species and the solid surface will determine the type and degree of the chemical and physical modifications 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 vapor 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, hydrolysis, 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 to a specific application. Despite the many advantages associated with the 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 [24–29]. 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 of adhesion. Furthermore, the development of biomaterial-associated infections can arise in several ways, the most common being the introduction of etiological 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 late hematogenous infection of the implant, particularly when medical devices are directly exposed to the blood, 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 physico–chemical interactions being followed by intricate irreversible molecular and cellular interactions. Bacterial cells move, or are moved, by 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 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 number of adhesion ligands on the substratum, and the net direction of cell movement to the gradient of adhesion [42]. It has also been suggested that haptotaxis influences the attachment preferences of 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 non-specific 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 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 provide a protected habitat, with reduced shear forces [44]. It has also been demonstrated that bacterial cells attach more favorably 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 more stable adhesion of the microorganism to the surface [46]. Various polymeric structures, such as capsules, fimbriae, pili, and slime, that may be present on the surface of the bacterial cell and engage in specific irreversible molecular reactions with 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 colonisation 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 with 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 bacterial cells can precondition the target surface by being adsorbed onto 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 colonisation the hyaluronic acid capsule of S. pyogenes attaches to a 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 colonisation of pharynx keratinocytes in vivo [60,61]. Furthermore, the presence of bound (capsular) and free (slime) extracellular material may significantly increase the chances of survival of the attached microorganism 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 in a mouse model involving capsular mutant strains of S. pyogenes showed 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 revertable mutant strains, 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 behavior of the pathogen [70]. A carbohydrate structure comprising a core oligosaccharide and a polysaccharide known as O-antigen is anchored to the bacterial
membrane by lipid A [71]. Although the O-antigen is flexible and can extend outwards depending on the ambient environmental conditions surrounding the microorganism, the preferred conformation is thought to position the O-antigen lying flat on top of the cell surface, covering the saturated fats and phospholipids of lipid A and possibly non-polar sites on 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].

Adhesive interactions between microorganisms and their 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 Si₃N₄, TiO₂, SiO₂, and Al₂O₃ [71].

### 3.3. Coloniser proliferation and biofilm formation

Biofilm formation can be initiated by multiplication of the primary coloniser without the release of progeny cells and/or the recruitment of co-aggregate members of the same or different species, boosting their individual potential for colonisation of various ecological niches [46,74,75] Fig. 2. 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 interspecies co-aggregation within the biofilms by providing a matrix for the formation and stabilization of the film 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 and magnesium) to increase the binding forces within the 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 the biofilm matrix [77,78]. Extracellular DNA has also been demonstrated to be an important component of the biofilm matrix, via the introduction of favorable 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 initial attachment of the coloniser to the surface, and these forces also contribute to subsequent biofilm formation and structural development.

Biomaterial-associated infections remain a major concern in the use of most implanted or intravascular devices, including orthopedic 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, 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 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 glucons 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 a biofilm S. aureus cells have been shown to synthetize and secrete an auto-inducing peptide signal that accumulates in the extracellular environment. This is then used for cell–cell communication, otherwise known as quorum sensing, an ubiquitous regulatory mechanism that controls the extent of S. aureus pathogenicity and biofilm development [86]. Upon reaching its critical concentration, the auto-inducing peptide signal binds to a surface receptor, activating an agr regulatory cascade, which results in 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, i.e. a 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 orthopedic 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 colonisation 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 the bacterial cell, the presence of flagellae, 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 protruding EPS chains to reconfigure in terms of their spatial arrangement upon approaching a solid surface will determine whether 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 attractive interactions of 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 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 mechanism activation, and the chemical composition and fluid flow in the 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 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 of 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, having an impact on 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 1–2 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, in which microorganisms race against 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 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 favorable 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 integration of the biomaterial into the host systems without generating an excessive immune response. In addition, 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 a 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 –CH₃, is inert in terms of protein and cell adsorption, whereas charged groups such as –COOH and –NH₂ encourage cell and protein attachment [113].

Plasma polymerisation is frequently employed for the functionalisation of surfaces where hydrophobic behavior is required. It allows the introduction of a wide range of functional moieties and/or combination of moieties, including carboxyl (–COOH), amino (–NH₂), 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 in in-dwelling 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 polymerisation 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 has a 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 the 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 major component of tea tree oil responsible for the oil’s broad spectrum antimicrobial and anti-inflammatory properties, using radio frequency plasma polymerisation under varied input power conditions [119]. When produced at 10 W the surfaces inhibited the adhesion and growth of both S. epidermidis and S. aureus (Fig. 4), however, when fabricated at higher power the coatings promoted attachment, adhesion, and metabolic activity of the pathogens and encouraged biofilm formation.

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

Fig. 3. Hydrophobicity and chemical composition of polystyrene (PS), polyethylene (PE), polyetherether ketone (PEEK), polyethylene terephthalate (PET), polyethylene naphthalate (PEN), polycarbonate (PC), polymethyl methacrylate (PMMA) and fluorinated (PTFE) polymers amino functionalised using ammonia plasma treatment: (1) water contact angle and (3) respective N/C and –NH₂/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 the low pressure plasma system [114].
surfaces are more attractive to hydrophilic S. aureus bacterial cells [120]. A real time investigation of S. epidermidis adhesion dynamics to hydrophilic glass and hydrophobic dimethyl dichlorosilane-coated glass highlighted that a number of adsorption and desorption events occurred, with a twofold 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 a 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 with their negatively charged counterparts, yet 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 gram-positive Bacillus brevis exhibited a decreased surface pH when attached to a negatively charged glass surface. This resulted in an enhanced proton motive force and increased 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 opposite effect was apparent, resulting in a drop in metabolic activity and, possibly, cell death, which may explain the antibacterial effect frequently reported for such surfaces [98].

Fig. 4. Scanning electron microscopy images of attachment and proliferation of Streptococcus epidermidis (left panel) and Staphylococcus aureus (right panel) after 18 h incubation on surfaces subjected to monoterpene alcohol plasma deposition under varied input power conditions: (1, 4) 10 W; (2, 5) 25 W; (3, 6) 50 W. Scale bars: 2 μm; 20 μm (inset).
Ultra-hydrophobic hydrocarbons exhibit extremely low water solubilities, are poorly bioavailable for bacterial colonisation, and can be toxic to bacterial cells due to their permeabilizing effect on the cytoplasmic membrane, leading 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 the activation of electron transport phosphorylation systems, resulting in ATP level and energy homeostasis, 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) resulted in a 70% reduction in bacterial adhesion for the four strains of *P. aeruginosa* [125]. However, this reduction was unlikely to be sufficient to prevent *P. aeruginosa* colonisation 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 bacterialic properties of the material [127]. In addition to oxidation, the surface porosity of a substrate can also be increased by 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 with time after oxidation, attributed to a tendency to minimise the surface energy of the oxidised polymer and facilitated by the flexibility of polymer chains that allow for such movement. Treatment of polyethylene terephthalate with helium and 20% oxygen in helium (He/O2) plasma were demonstrated to significantly reduce *S. epidermidis* bacterial adhesion compared with the untreated material, however, the aging effect and subsequent decrease in the surface free energy of the substratum surfaces with time, particularly in the case of He-treated surfaces, were found to favor bacterial adhesion and aggregation [129]. The surface energy and hydrophobicity of the substrate are greatly influenced by the chemical composition and pH of the contact medium, consequently affecting the free energy of solvent-mediated interactions 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 highest adhesion 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 (Lewis acid–base forces) and long-range (van der Waals forces) forces, whereas the attachment of bacteria to Teflon was likely governed by short-range forces only. Plasma polymerised 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 limit 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 behavior 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 diffusion-controlled release biocide carrier polymer system. Pulsed plasma polymerised allylamine films deposited onto silicon showed a pronounced pH dependence of the magnitude of the average pull-off force, which was attributed to protonation of amino groups, with the pull-off force decreasing significantly for pH values below 5.5 [132]. The same study demonstrated that coatings with controlled contents of amino and nitrile groups can be achieved by varying the duty cycle of the deposition, creating a heterogeneous local environment in terms of chemical functionality and hydrophobicity on the nanometer scale. The adhesion behavior of a product of pulsed plasma modification of polydimethylsiloxane substrates with maleic anhydride, with subsequent hydrolization to promote the formation of dicarboxylic acid groups showed a clear dependence on pH and electrolyte nature and concentration. The adhesion force was demonstrated to almost vanish at high pH in the presence of the monovalent cation K+ (due to condensation of counterions on the carboxylate groups), whilst it was observed to increase slightly at high pH in the presence of the divalent cation Ca2+, due to ions bridging between two carboxylate groups [26]. The patterns of substrate–liquid medium interactions will therefore have an impact on 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 affect their functional characteristics due to altered availability for molecular interactions attributed to the conformational changes, orientation, and/or 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 non-specific protein adsorption, which may lead to undesirable side-effects, such as surface-induced thrombosis, while inducing specific protein adsorption and the anticipated cell responses by decorating the material surface with specific chemical functionalities [133].

Adhesion of the coating to the biomaterial substrate is also greatly affected by the properties of the ambient fluid, with a partial loss of adhesion or full delamination of the coating being a serious hindrance to in vitro plasma polymer application. Adhesion can be significantly improved by pretreatment 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 an oxygen or nitrogen plasma for short times facilitates energetic species-mediated hydrogen abstraction and polymer bond breakage and, hence, allows activation of the substrate surface. Adhesion-promoting layers, such as self-assembled monolayers and silicon oxide films, are an effective solution to adhesion improvement between a plasma polymerised coating and an inorganic or metallic substrate. In addition to improving the stability of plasma films in an aqueous environment, such an interlayer may enable more precise and more reproducible chemical reactivity of plasma-deposited coatings for biomaterials applications [135]. Radio frequency oxygen glow discharge was used to prefunctionalise 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]. The oxygen plasma prefunctionalisation step was demonstrated to be necessary to ensure reproducible biomaterial surfaces amongst production lots, as well as to increase the number of ether/alcohol, ester and carboxyl functional groups.
The resultant modification completely inhibited bacterial adhesion of four strains of \textit{P. aeruginosa} and efficiently prevented colonisa-
tion over longer periods. Plasma modification was also used to suc-
cessfully enhance the adhesion to and uniformity of an electroless
deposited silver coating on 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 physiological conditions and can, there-
fore, be successfully applied as protective encapsulating coatings
for biomaterials used for long-term implantation, such as intravas-
cular stents and other metallic devices. Upon prolonged exposure
to blood and other body fluids these biomaterials can undergo deg-
radation of their mechanical properties, with a high potential for
the release of toxic metallic compounds, such as nickel-based oxy-
des 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
thrombo–resistance properties and protein retention capabilities
[102,103]. Furthermore, in vivo stent implantation to support the
narrowed lumen of atherosclerotic stenosed arteries requires
in situ stent expansion, a step that generates local plastic deforma-
tion of up to 25% and may cause coating failure, including cracking
and delamination [141]. Fluorocarbon coatings with a thickness of
less than 100 nm exhibited the required cohesion and interfacial
adhesion to resist stent expansion without cracking or delaminat-
ing [142].

Recently, surface grafted stimuli-responsive polymers, such as
poly(N-isopropylacrylamide) have attracted significant attention
due to their ability to change their physico-chemical characteris-
tics upon induction of environmentally triggered phase changes
[143]. Of particular interest is the possibility to control biomolecu-
lar adsorption, bacterial cell attachment and release, and cell func-
tion, such as the production of extracellular substances by the
adsorbed microorganisms, using these materials. For instance, at-
tached bacterial cells can be released from the surface due to
changes in the anchorage strength of cells brought about by phys-
ico-chemical changes in the surface upon induction of environ-
mentally triggered phase changes [144]. Plasma immobilised
thermo-responsive poly(N-isopropylacrylamide)-co-N-(1-phenyl-
ethyl)acrylamide films were demonstrated to successfully modu-
late initial attachment and adhesion strength of the diatom \textit{N. perminuta} [145].

5.2. Surface architecture and porosity

There is much debate about 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 mech-
anism of bacteria, with cells demonstrating no preference for adhe-
sion 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 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 a situation increased the binding potential of the bacteria
by maximising bacteria–surface contact area [147], whereas fea-
tures appreciably smaller than the bacterial size led to a reduction
in binding as a result of the decrease in contact area [148]. Exam-
ination of the adhesion preferences of \textit{P. aeruginosa} to poly(methyl
methacrylate) contact lenses indicated that surfaces with a root
mean square roughness of 14 nm or above increased the extent of
microorganism attachment [149]. Studies on the attachment
behavior of the human pathogens \textit{Pseudomonas fluorescens} and
\textit{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 attach-
ment of these bacteria to smooth surfaces did not follow a distinct
pattern [146,151]. Furthermore, the surface architecture of the abi-
otic 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 \textit{P. fluorescens} localized
attachment in the trenches of the surfaces compared with native
gold surfaces, with cells showing limited EPS synthesis and re-
duced cell size compared with those attached to non-nano-
patterned surfaces [151]. Our recent investigation on pathogenic
strains of \textit{S. aureus} and \textit{P. aeruginosa} have shown evidence of
increased adhesion to “nanosmooth” glass, polymer, and titanium
surfaces, with a 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 anisotropically topographies such as ridges and grooves affect individual
cell behavior (cells align along the anisotropic direction),
isotropic topographies, such as evenly or randomly distributed
peaks and valleys, influence collective cell behavior [113].

In general, porous materials are associated with higher infection
rates compared with dense and smooth materials. A recent study of
biofilm formation on bone grafts and bone graft substitutes re-
ported a shorter biofilm detection time and a 10-fold (\textit{S. epidermi-
dis}) or 100-fold (\textit{S. aureus}) higher bacterial count on porous
samples (β-tricalcium phosphate, processed human spongiosa)
compared to smooth samples (PMMA and PE) [156]. It is assumed
that the shear forces are significantly lower inside pores, even at
high bulk fluid velocities, resulting in a protected environment
for bacteria to attach and grow [157]. The dynamics of microbial
attachment and biofilm formation within the pores of the sub-
strates will be affected by the degree of porosity, pore size, and
permeability distribution of the porous network [158]. For in-
stance, recent studies of osteoconductive hydroxyapatite and bi-
phasic 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 internalisation 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 strongly depend on the bacterial
species and the prevailing hydrodynamic and nutritional condi-
tions, ranging from continuous, smooth films to discontinuous,
highly irregular colonies [160]. For porous substrates biofilm
development involves the initial formation of smooth biofilms on
the pore walls, inducing changes in the geometry and topology of
the porous medium and, hence, having an impact on the macro-
scopic properties of the porous medium, including its porosity
and permeability, drastically changing fluid flow and mass trans-
port through the porous medium. Gradually the smooth biofilm
morphs into more irregular biofilm forms, creating biofilm strands
spanning the pores, separated by water channels (web-like struc-
ture) [160]. Plasma polymerisation can be used to decrease the size of
the accessible pores, making them unavailable for colonisation.
Furthermore, surface roughness and porosity are also known to af-
flect the friction behavior of the material, an important property
for surfaces that undergo insertion into body conduits such as blood
vessels or the urethra or for high wear applications, such as a
replacement for articular cartilage in joints [161]. Plasma treat-
ment with inert gases 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 pacemakers, 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 the 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 the assembly of complex functional structures. For instance, covalent attachment (i.e. “tethering”) of antimicrobial and anti fouling agents to a component of the coating system can be used to significantly extend the service lifetime of the device, resulting in 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 cross-linked polysiloxane matrix produced a hybrid anti fouling/foul release coating with biocidal activity against marine Cellulophaga lytica, with 4 wt.% QAS moieties resulting in an 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 ethylene diamine plasma functionalisation generated films rich in secondary and tertiary amines [167]. Pretreatment 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 Klebsiella 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 plasma-treated polyethylene methylacrylate which was further modified with transparent TiO₂ films. These surfaces exhibited excellent photo-induced antibacterial effects against S. aureus and E. coli under indoor natural light, with approximately 100% of bacteria being inactivated within 2 h of illumination [168].

Plasma modification was used to activate poly(dimethylsiloxane) 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 the material hydrophilicity and bacterial cell repulsion properties [169]. The modification resulted in an increase in the oxygen content at the surface, with all materials found to be non-hemolytic and displaying no cytotoxicity. Asadinezhad et al. used surface activation with a diffuse co-planar surface barrier discharge plasma followed by radical graft co-polymerisation of acrylic acid via a surface-initiated pathway to produce a structured high density brush on the surface of medical grade polyvinyl chloride [170]. The brush modification was found to be remarkably effective in diminishing the adherence of E. coli. Subsequent coatings with antibacterial agents, including bronopol, benzalkonium chloride, and chlorhexidine, were demonstrated to induce up to an 85% reduction in adherence of E. coli, however, only the 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 a concomitant increase in surface hardness and sliding wear resistance. The nanocrystalline silver alloyed S-phase steel surfaces showed a 93% reduction in E. coli after 6 h contact time compared with untreated steel samples [171]. Silver ions introduced into a 57% SiO₂/3% Al₂O₃/34% CaO/6% Na₂O glass coating plasma sprayed onto titanium alloy and stainless steel substrates demonstrated in vivo antimicrobial action against S. aureus while maintaining 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 the triclosan-treated surfaces being more effective against E. coli compared with those modified with bronopol. The antibacterial efficacy of both coatings, however, decreased 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 a super-hydrophobic polytetrafluoroethylene surface that was characterised by a higher affinity for human cell and S. aureus attachment. Acetylene (C₂H₄) plasma immersion ion implantation used to treat polyethylene terephthalate increased the hemocompatibility and antibacterial properties of the biomaterials, with a significant decrease in 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–titianium 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, and effectively mitigating nickel out diffusion, whilst allowing the NiTi rods to retain their shape recovery properties. The biocompatibility of polyurethane that had undergone acetylene plasma immersion ion implantation was also reported to be improved, while argon plasma was used to pretreat surfaces for subsequent grafting with heparin, albumin or polyethylene oxide [174,176].

6. 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 effort in the field of surface technology has been directed towards enhancing tissue–surface interactions and advancing the 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, the intricate interactions between an abiotic surface and different types of living cell, including 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 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 tailoring of the surface properties of the material to suit a specific need.
exposure to plasma has been shown to irreversibly damage bacterial cells, allowing in situ sterilization of the biomaterial during the surface modification process. Despite numerous auspicious results reported in the literature, real life applications are frequently hindered by a limited understanding of the influence of the process parameters, including, among others, the geometry of the reactor, the input energy, and the pressure. The combination of these parameters determines the nature of the reactive species and, ultimately, the surface modifications produced. Further advancement in the areas of immunology, biology, and analytical techniques are necessary for the successful design and implementation of biomaterials.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Fig. 1, is difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.12.024.

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