Bioadhesion: a review of concepts and applications

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Bioadhesion refers to the phenomenon where natural and synthetic materials adhere to biological surfaces. An understanding of the fundamental mechanisms that govern bioadhesion is of great interest for various researchers who aim to develop new biomaterials, therapies and technological applications such as biosensors. This review paper will first describe various examples of the manifestation of bioadhesion along with the underlying mechanisms. This will be followed by a discussion of some of the methods for the optimization of bioadhesion. Finally, nanoscale and macroscale characterization techniques for the efficacy of bioadhesion and the analysis of failure surfaces are described.

Keywords: bioadhesion; bioadhesives; cell adhesion; mucoadhesion

1. Introduction

The term ‘bioadhesion’ refers to widely diverse phenomena, which all involve the adherence of materials (natural or synthetic) to biological surfaces. As an interface phenomenon, bioadhesion is similar to conventional adhesion, except for the special characteristics of biological organisms and surfaces. As such, this term covers the adhesive properties of both synthetic components and the natural surfaces (such as cells). Bioadhesion could also refer to the use of bioadhesives to bond two surfaces together, which is relevant in drug delivery, dental and surgical applications. Therefore, the wide interest in bioadhesion research is due to its implications for the development of new biomaterials, therapies and technological products such as biosensors [1–6].

A few examples illustrating various manifestations of bioadhesion are shown in figure 1. Pillar-directed cell growth is an area of interest for researchers investigating the role of substrate micro-roughness on cell behaviour in the development of new biomaterials. In figure 1a, fibroblasts attach on top of the silicon pillar array substrate, and then extend to reach the surrounding pillars [7]. The height and spacing between pillars affect cell shape and thickness, which in turn, has implications for biological functions, such as cell growth and gene expression. Bioadhesion can also refer to the application of adhesives for biological interfaces in clinical use. Figure 1b is an example of the application of a medical adhesive. In this case, fibrin glue was used to adhere a surgical mesh on an

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animal peritoneum after a laparoscopy [8]. Meanwhile, figure 1c shows the use of adhesives in dental applications. In this example, a dentin adhesive was used to reattach a tooth fragment onto a fractured area [9].

Examples of naturally occurring bioadhesion are shown in figure 1d,e. The phenomenon of mucoadhesion is illustrated in figure 1d, which shows the attachment of mussels to underwater surface, such as corals or ship surfaces. The foot of the animal extends from its shell to secrete a pad of the mussel adhesive protein (MAP) on the underwater surface. It then retreats to the shell interior, leaving a thread that secures the mussel to the pad. This process is repeated multiple times, creating a strong bond [10]. The MAP has been used
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2. Mechanisms that control bioadhesion

The factors that determine biological adhesion are diverse, and can be classified into the effects of surface morphology, chemical interactions, physiological
factors and physical–mechanical interactions. These aforementioned determinants usually work in concert with one another in order to achieve adhesion between the interfaces of interest.

(a) Surface morphology effects

Cell adhesion on synthetic biomaterial surfaces is widely studied owing to its direct implications for the design and clinical performance of body implants. The adhesion of cells on biomaterials is an example that illustrates the importance of the micro/nanotopography of the substrate surface where the cells are cultured. During the implantation of a biomaterial into a living host, cells are not directly attached to the biomaterial surface. Instead, the biomaterial is rapidly coated with a protein layer. Other proteins will displace the initial layer in a process known as the ‘Vroman effect’. Various extracellular matrix (ECM) and serum proteins are involved, such as fibronectin, fibrinogen, albumin and vitronectin. The conformation of the adsorbed protein is partly determined by the morphology of the biomaterial surface, which in turn, influences the cell adhesion and proliferation process [14–18].

Model synthetic surfaces have been used to investigate the effect of surface morphology on protein conformation and cell adhesion. These surfaces have well-defined microscale or nanoscale topography, and are typically created using top-down (e.g. lithographic or dry etching methods) or bottom-up (self-assembly processes or block copolymers) approaches [19–23].

Accounts vary on the optimal feature size required for effective cell adhesion. Accounts of bone cell adhesion on patterned titanium indicate that submicrometre (greater than 100 nm) features are better than nanometre-sized (less than 100 nm) features in facilitating cell adhesion [24]. Lehnert et al. [25] performed cell adhesion and spreading studies on patterns with distances ranging from 1 to 30 μm. They found that for patterns with small areas of the adhesive ECM proteins (0.1 μm²), pattern spacings larger than 5 μm show cellular adhesion, but do not support cell spreading. However, for larger dot dimensions (9 μm²), cells can effectively connect non-adhesive regions during the spreading process as long as the distances between features do not exceed 25 μm. This variation in cell spreading as a function of the substrate geometry is shown in figure 3, where for 25 μm-spaced features, the spreading was limited and the cells were observed to appear as either triangular, ellipsoidal or round. In another study, using lithographically created patterns with deposited fibronectin, it was found that cellular adhesion increases as the spacing between features increases up to the optimal distance of 11 μm [26]. However, for cell adhesion on gold nanoparticles, it was found that for spacings less than 100 nm, closer patterns (ca 60 nm) allowed for greater cell adhesion [20]. Even though these literature accounts are not definitive, it is apparent that dimensional thresholds that enhance cell adhesion are present.

(b) Chemical interactions

Various interactions between two chemically active surfaces (i.e. not inert) facilitate the bioadhesion process. Strong adhesion can occur if the two surfaces are capable of forming either covalent, ionic or metallic bonds. At the same time, weaker forces, such as polar (dipole–dipole), hydrogen bonding or van
Figure 3. Fluorescence microscopy images showing the effect of substrate geometry on cell adhesion and spreading: (a) homogeneous substrate (hs), (b) 0.1 µm² dots, approximately 1 µm apart, (c) 1 µm² dots, 2 µm apart, (d) 9 µm² dots, 10 µm apart, (e) 9 µm² dots, 15 µm apart, (f) 9 µm² dots, 20 µm apart, (g–i) 9 µm² dots, 25 µm apart (adapted from Lehnert et al. [25]). (Online version in colour.)

Der Waals interactions (induced dipoles) also aid in bonding the two surfaces [27–29]. A schematic of possible protein–solid surface interactions is shown in figure 4. Here, it is assumed that the surface is uniform and that there is only one main type of interaction between the protein and the surface. The strength of protein adsorption depends on the net charge and polarity of the side of the protein available for adsorption and the composition of the substrate surface. The protein and substrate could either be predominantly hydrophobic, positively charged, negatively charged or neutral hydrophilic. The interaction of a neutral hydrophilic side of the protein with a surface having a similar polarity tends to lead to weak adsorption. Ionic interactions between proteins and substrate surfaces will lead to relatively moderate adsorption. Meanwhile,
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Figure 4. Schematic of a protein adsorbing on surfaces with different net charge and polarity characteristics. The strength of adsorption depends on the net charge and polarity of the side of the protein (whether it is mainly hydrophobic, positively charged, negatively charged or neutral hydrophilic) available for adsorption and the charge and polarity of the substrate surface [30].

the interactions between hydrophobic protein moieties and the substrate lead to strong adsorption [30]. This illustrates schematically the role of surface chemistry in protein adsorption. However, proteins contain complex arrangements of hydrophobic, charged and neutral hydrophilic groups, such that the resulting interactions will be combinations of the cases presented. A discussion of protein composition is provided in appendix A for completeness.

Specific examples, illustrating chemical interactions in the formation of bioadhesive bonds, are discussed below.

(i) Mussel adhesion

The case of mussel adhesion on underwater surfaces is an example of how the chemical composition of the contacting surfaces affects the adhesion mechanism. The initial interaction between the mussel and the underwater surface involves the removal of weak boundary layers (mostly water). If the underwater surface is non-polar, then the water boundary layer interacts through weak dispersive forces. Because the MAP is larger than a water molecule, the protein experiences an entropic gain and greater dispersive interactions with the non-polar surface relative to water, leading to the displacement of the water boundary layer, and adhesion of the protein. In the case of polar underwater surfaces, the water boundary layer cannot be easily displaced. In this case, the MAP uses its hydrophilic amino acid side chains, which contain aminoalkyl, hydroxyalkyl and phenolic groups (such as 3,4-dihydroxyphenylalanine or DOPA), all of which are capable of forming strong hydrogen bonds. Hence, the MAP is able to displace water and the mussel is able to adhere to polar underwater surfaces as
well [31,32]. The unique chemical composition and properties of the MAPs have led to the development of synthetic analogues for potential use as mucoadhesives for drug-delivery systems.

(ii) Cell adhesion to biomaterials

Cell adhesion to biomaterial surfaces is a complex phenomenon. As discussed in §2a, the micro/nanoscale surface topography has a direct impact on cell adhesion and proliferation. But aside from morphology, the chemical composition of the biomaterial surface has also been established to play a significant role in the cell adhesion and proliferation process. For instance, cell adhesion as mediated by the integrin group of cell surface receptors has been shown to depend on the conformation of the ECM protein fibronectin, which in turn is sensitive to the chemical composition of the synthetic surface. To demonstrate this concept, Keselowsky et al. [33] used various self-assembled monolayers (SAMs) with OH, COOH, NH2 and CH3 termini in order to create surfaces that are hydrophilic and neutrally charged, hydrophilic and acidic, hydrophilic and basic, and hydrophobic, respectively. They found that the adhesion strength of cell binding (as determined by a centrifugation assay) followed the trend: OH > COOH > NH2 > CH3. This trend was found to correlate with the integrin-binding profile on the SAMs. In another study, these researchers found that other events related to cell differentiation, such as osteoblast-specific gene expression, alkaline phosphatase enzyme activity and matrix mineralization, were also surface chemistry dependent, such that OH- and NH2-terminated surfaces were more advantageous compared with COOH and CH3 SAMs [34].

So, how does the surface charge and hydrophilicity/hydrophobicity influence cell adhesion? The answer lies in the effect of the surface chemistry on the adsorption of the ECM proteins such as fibronectin, vitronectin, collagen and laminin. In the context of the four model surfaces (OH-, COOH-, NH2- and CH3-terminated SAMs), it has been found that fibronectin undergoes the largest extent of denaturation on the CH3-terminated SAM, which corresponds to poor cell adhesion characteristics. The denaturation of fibronectin prevents the surface exposure of the arginine–glycine–aspartic acid (RGD) groups on its cell-binding domain, which are known to mediate cell adhesion. In contrast, the hydrophilic, neutrally charged SAM (OH terminus) has been found to induce the least extent of unfolding or denaturation, leading to a good cell adhesion on the fibronectin/SAM surface [35]. The extent of denaturation of fibronectin on the OH and CH3 surfaces is attributed to functional group dehydration and water-restructuring effects brought about by the substrate surface [36,37].

It has also been established that the surface chemistry of the substrate plays a role in the formation of focal adhesions, which are adhesive complexes with signalling molecules responsible for cell migration, survival and differentiation. The focal adhesion complexes (or plaques) have been found to contain integrin receptors and cytoplasmic proteins, such as talin, vinculin and α-actinin. An example is shown in figure 5 of the localization of talin on fibronectin-coated SAMs [38]. In these SAMs, the termini were also modified to possess OH, COOH, NH2 and CH3 termini. Talin formed large clusters on the OH and COOH SAMs, fewer structures on NH2 SAMs, and the least amount on the
CH$_3$ surface. The extent of cluster formation is directly related to the amount of focal adhesions formed, which is indicative of the success of cell adhesion on the substrate surface.

The studies on integrin binding, cell adhesion, fibronectin conformation and focal adhesion formation are all consistent in demonstrating that modulating the surface chemistry of the biomaterial has a large impact on optimizing the cell adhesion process. A schematic illustrating the effect of surface chemistry on the cell adhesion process is shown in figure 6 [39]. Figure 6a illustrates the case for cell adhesion to a hydrophobic surface. Because the ECM protein (such as fibronectin) is denatured, specific amino acid sequences (such as the RGD groups of fibronectin) are inaccessible for the integrin receptors. The receptors cannot cluster into focal adhesion complexes and bind to cytoplasmic proteins (such as talin), which is required for cell adhesion. In contrast, when the biomaterial surface is moderately hydrophilic (figure 6b), the adsorbed ECM proteins are more flexible and not denatured, such that the receptors can form focal adhesions and link the integrins to the actin cytoskeleton of the cells [39].

(iii) Cyanoacrylate bioadhesives

Bioadhesives for surgical applications is another area where chemical interactions are important. There are two main types of surgical tissue adhesives,
namely cyanoacrylates and fibrin tissue adhesives (the latter will be discussed in a separate section later). The main difference between the two is that while cyanoacrylates are synthetic compounds (the same type of materials found in ‘super glue’), fibrin glues are composed of endogenous biomolecules. Cyanoacrylates are used as surgical adhesives owing to their wound-sealing properties and antimicrobial activity [4].

Cyanoacrylate monomers polymerize with the aid of water molecules available on the surface or present as relative humidity in the air, leading to rapid polymerization. The first cyanoacrylate compound to be considered as a surgical adhesive was methyl-2-cyanoacrylate. However, this compound has been found to cause histotoxic (toxicity to biological tissues) reactions, and other cyanoacrylate compounds have been investigated since. The latest generation of cyanoacrylate adhesives used in surgery is based on octyl-2-cyanoacrylate, and is sold under the brand name Dermabond Topical Skin Adhesive (Ethicon, Somerville, NJ, USA). This adhesive is deemed non-toxic and has been approved by the US Food and Drug Administration for topical wound closure applications [4].

(iv) Solubility parameters and dental restoratives

The solubility parameter concept, which is used as a guide for the miscibility of polymer and solvent systems, can be used to predict the bonding of systems such as resin composites to dentin. Initially introduced by Hildebrand, the solubility parameter ($\delta$) of a liquid is the square root of the energy of vaporization per unit volume, expressed as [40,41]

$$\delta = \left[ \frac{\Delta H_v - RT}{V} \right]^{1/2},$$  

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where $\Delta H_v$ is the molar enthalpy of vaporization, $R$ is the universal gas constant, $T$ is the temperature and $V$ is the molar volume. In this concept, liquids with comparable solubility parameter values are predicted to be miscible with each other. As applied to dental biomaterials, it has been found that there is a correlation between the solubility parameters of adhesive monomers and the dentin and the ability of the monomers to permeate into the substrate [42–44].

(c) Physiological factors

Biological adhesion can also take place through specific physiologically related mechanisms. Two examples are discussed, namely fibrin glues and the plant lectin adhesive.

(i) Fibrin tissue adhesive

The fibrin tissue adhesive is one of the two major categories of adhesives for surgical applications (the other type being cyanoacrylates). Fibrin tissue adhesives can be applied below the dermis as a sealant (or haemostatic clamp) for skin grafts and flaps, and for laparoscopic surgeries [4,8]. These adhesives are typically packaged as two components, which are then mixed together during surgery. The first component contains fibrinogen, plasma glutaminase (also called Factor XIII) and CaCl$_2$. The second component contains thrombin and antifibrinolytic agent. Fibrin tissue adhesives work based on the physiology of the blood coagulation process. Thrombin cleaves the protein fibrinogen during clotting into smaller fibrin subunits, which then go through end-to-end and side-to-side polymerization. Factor XIII is responsible for the cross-linking of the subunits into a stable fibrin clot in the presence of calcium [4].

(ii) Lectins

Lectins are carbohydrate-binding proteins found in both animals and plants. One of the common animal lectins is the C-type or calcium-dependent binding lectins. One of these C-type lectins, the asialoglycoprotein lectins, is specific to liver cells and is involved in animal biological function. Meanwhile, plant lectins are abundant and can be found in common plants such as tomatoes and in the seeds of legumes. Some plant lectins are regarded as toxins when they bind to animal cells [27,45]. However, owing to their ability to bind specifically to glycosylated cell membrane components, lectins are being investigated for their ability to transport macromolecules, with implications for drug delivery to the gastrointestinal tract [46,47]. Aside from lectins, other molecules that are being considered for bioadhesive-based drug delivery are bacterial fimbrins and invasins [3].

(d) Physical and mechanical effects

The physical and mechanical factors that influence bioadhesion were originally developed for polymer–polymer adhesion, but are directly applicable to biological adhesion as well [1]. The mechanisms of wetting and interpenetration will be discussed as follows.
Figure 7. Schematic of the relevant interfacial energies ($\gamma$) that determine the formation of a bioadhesive bond, as illustrated for the adhesion between a polymer (P) and the mucous layer (M) on a biological membrane in the presence of a liquid medium (L) (adapted from Buckton [2]).

(i) Wetting phenomenon

The interfacial energy is an important determinant of successful bioadhesion. In systems exhibiting bioadhesion, the liquid environment influences the spreading of one material phase over another. Consider the case of a polymer surface adhering to the mucous gel layer on a biological membrane immersed in liquid medium (e.g. in drug-delivery applications). Figure 7 is a schematic illustrating the interfacial energy components that should be considered in evaluating the thermodynamic work of adhesion of this system. During adhesion, a unit interface between the polymer and the liquid and between the mucus and the liquid vanishes, whereas an interface between the polymer and mucus forms. The thermodynamic work of adhesion ($W_{\text{PM}}^{\text{adh}}$) or the energy of adhesion per unit area between the polymer and mucus is then defined by the Dupre equation as follows [2,28]:

$$W_{\text{PM}}^{\text{adh}} = \gamma_{\text{PM}} - (\gamma_{\text{PL}} + \gamma_{\text{ML}}), \quad (2.2)$$

where $\gamma$ represent surface energies and the subscripts P, M and L are for polymer, mucus and liquid, respectively. A positive value for $W_{\text{PM}}^{\text{adh}}$ is regarded to be a necessary condition to achieve successful bonding between the two surfaces.

(ii) Interpenetration

While interfacial contact and chemical bonding interactions are needed for the initial stages of bioadhesion, the interpenetration or interdiffusion between the molecules of the two contacting surfaces will maintain the adhesive bond. If a polymer is one of the contacting systems involved in the bioadhesion process, then the interpenetration process involves the mobility of the individual chains and their entanglement in the opposing biological membrane. A related concept is the swelling capacity of the polymer, which is the ratio of the wet to dry weights. A high swelling capacity for a given polymer implies that it has greater chain mobility, and a higher tendency towards interpenetration [2].
For bioadhesion in dental prostheses, mechanical interlocking or interpenetration is the primary adhesive mechanism. When bonding an acrylic resin restorative to tooth enamel, surface etching is performed to create micropores on the surface that will increase the penetration of the restorative material. This method was pioneered by Buonocore [48], who discovered that conditioning human enamel with 85 per cent phosphoric acid improves the adhesion of acrylic resin to the enamel surface. This is an illustration of how a surface chemical treatment roughens the surface and facilitates adhesion via mechanical interlocking effects.

The tooth dentin, on the other hand, contains tubules that radiate from the pulp. These tubular structures facilitate the penetration of resin monomers into the dentin and their retention once the resin has been polymerized [42]. As discussed earlier, chemical effects (i.e. solubility) play a role in the adhesion between a restorative and dentin. However, the other dimension in this adhesive event is the permeation of the adhesive into the collagen fibrillar network found in the tooth dentin [5]. A list of hydrophilic monomers that have been investigated for this purpose is given in table 1. It is thought that the entanglement of the polymer network formed during the polymerization of the monomer on the collagen network of dentin is responsible for the observed adhesion.

3. Optimization of bioadhesion

The bioadhesion between two surfaces in contact may be enhanced using various physical, chemical and mechanical processing techniques.

(a) Physical processes

Various methods are used to modify surfaces, such as exposure to plasma, corona discharge, ions and ultraviolet (UV)-ozone. Plasma processing is a widely used technique for surface cleaning and modification of the surface chemical composition. The plasma, which is generated by applying an electric field to a low-pressure gas in vacuum, produces reactive species such as ions and free radicals. For surface cleaning purposes, the plasma breaks most organic bonds, such as C−H, C−C, C=C, C−O and C−N. In addition, if oxygen plasma is used, another cleaning mode is possible. The activated oxygen species (O•, O+, O−, O2+, O2−, O3, among others) can combine with organic compounds to form H2O, CO, CO2 and low molecular weight hydrocarbons, thereby removing organic contaminants on the surface [49]. Surface modification through plasma processing can render the surface more hydrophilic or hydrophobic. If the gas used is oxygen or carbon dioxide, polar groups are introduced to the surface (such as C−OH, C=O and COOH), enhancing surface hydrophilicity. The presence of more polar surface groups is valuable in enhancing adhesion, e.g. between proteins and polymeric biomaterials used in tissue engineering applications. The application of this concept is shown schematically in figure 8[39]. Two examples are shown to illustrate how plasma treatment leads to an activated surface with free radicals. After the plasma processing, molecules can be grafted onto the activated surface, such as polyethylene glycol in figure 8a or dithiol and
Table 1. Monomers used as priming agents for tooth dentin adhesives [5].

<table>
<thead>
<tr>
<th>common abbreviation</th>
<th>full name</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMA</td>
<td>2-hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>GLUMA</td>
<td>adduct of glutaraldehyde and HEMA</td>
</tr>
<tr>
<td>NPG-GMA</td>
<td>N-phenyl glycine/glycidyl methacrylate (adduct)</td>
</tr>
<tr>
<td>NTG-GMA</td>
<td>N-((p-tolyl)glycine and glycidyl methacrylate</td>
</tr>
<tr>
<td>GDMA</td>
<td>glyceryl dimethacrylate</td>
</tr>
<tr>
<td>GPDM</td>
<td>glycerophosphoric acid dimethacrylate</td>
</tr>
<tr>
<td>NMBu</td>
<td>N-methacryloyl butyric acid</td>
</tr>
<tr>
<td>NMGlu</td>
<td>N-methacryloyl glutamic acid</td>
</tr>
<tr>
<td>NAasp</td>
<td>N-acryloyl aspartic acid</td>
</tr>
<tr>
<td>NMHyph</td>
<td>N-methacryloyl hydroxyproline</td>
</tr>
<tr>
<td>NMGly</td>
<td>N-methacryloyl glycine</td>
</tr>
<tr>
<td>DIPENTA</td>
<td>pentaacryloyldipentaerythritol phosphoric acid</td>
</tr>
<tr>
<td>PMDM</td>
<td>diadduct of pyromellitic anhydride with 2-hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>MEM</td>
<td>2-methacryloyloxyethyl hydrogen maleate</td>
</tr>
<tr>
<td>MMEM</td>
<td>mono-2-(methacryloyloxy)ethyl maleate</td>
</tr>
<tr>
<td>MMPM</td>
<td>mono-2-(methacryloyloxy)propyl maleate</td>
</tr>
<tr>
<td>MDPM</td>
<td>3-methacryloxypropyl phosphate</td>
</tr>
<tr>
<td>MBP</td>
<td>4-methacryloyloxybutyl phosphoric acid</td>
</tr>
<tr>
<td>MOP</td>
<td>8-methacryloyloxyoctyl phosphoric acid</td>
</tr>
<tr>
<td>ACE</td>
<td>acryloxyloxyethyl citraconate</td>
</tr>
<tr>
<td>BMEP</td>
<td>bis[2-(methacryloyloxy)-ethyl]phosphate</td>
</tr>
<tr>
<td>EGMP</td>
<td>ethylene glycol methacrylate phosphate</td>
</tr>
<tr>
<td>MDP</td>
<td>10-methacryloyldeacmethylene phosphoric acid</td>
</tr>
<tr>
<td>PhenylP</td>
<td>2-methacryloyloxy phenyl phosphate</td>
</tr>
<tr>
<td>4-META</td>
<td>4-methacryloyxyethyl trimellitate anhydride</td>
</tr>
<tr>
<td>PA</td>
<td>2-acryloyloxyethyl phosphate</td>
</tr>
<tr>
<td>PM</td>
<td>2-methacryloyloxy ethyl phosphate</td>
</tr>
<tr>
<td>SBMA</td>
<td>3-sulpho-2-butyl methacrylate</td>
</tr>
<tr>
<td>SEMA</td>
<td>2-sulphoethyl methacrylate</td>
</tr>
<tr>
<td>MSPMA</td>
<td>3-methoxy-1-sulpho-2-propyl methacrylate</td>
</tr>
</tbody>
</table>

Au nanoparticles in figure 8b. In these modified polymer surfaces, successful adhesion of fibroblast cells was achieved, indicating the cytocompatibility of the plasma-processed polymer [39, 50].

It should be noted that a similar effect on enhancing surface hydrophilicity can be obtained by using UV-ozone surface treatment on polydimethylsiloxane (PDMS). Compared with the untreated polymer surface, the surface-treated PDMS tends to be rougher and more hydrophilic. An increase in the cell attachment as well as an enhancement in cell spreading was observed with the use of the UV-ozone-treated PDMS [51].

On the other hand, the use of fluorinated gases such as CF$_4$ in the plasma results in the introduction of a low surface energy polytetrafluoroethylene-like structure, enhancing surface hydrophobicity. This approach has been used to prevent the undesirable protein adhesion and the unwanted generation of inflammatory cells on poly(methyl methacrylate) (PMMA) contact lenses [52].
(b) Chemical processes

Specific functional groups can be covalently attached to substrate surfaces using chemical synthesis techniques to attach biomolecules and construct model systems for use in adhesion studies. Covalent bonding will lead to enhanced retention of the biomolecule relative to simply relying on its innate adsorption to the substrate. An example is shown in figure 9, illustrating how a silica surface is chemically functionalized with the protein streptavidin [53]. In this illustration, the functionalization process consists of multiple steps, starting with the addition
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3-aminopropyltriethoxysilane (3-APTES)

\[ \text{CH}_2\text{CH}_2\text{O} \quad \text{SiO}_2 \quad \text{SiO}_2 \quad \text{O} \quad \text{O} \]

streptavidin has four biotin-binding pockets. Two or one may be attached to the biotin on the surface, with the remaining two or three available to bind the biotin analyte.

Figure 9. Schematic showing the steps in the functionalization of a silica surface with the protein streptavidin. Adapted from Bhushan et al. [53].

of the aminosilane 3-aminopropyltriethoxysilane (APTES) in order to introduce amine groups to the silica surface. This is then followed by reacting the surface with sulpho-N-hydroxysuccin-imido-biotin (abbreviated as biotin), which forms a covalent bond with the amine group of the APTES. In the final step, the protein streptavidin will bind to biotin. Streptavidin has a high affinity to biotin, and this interaction is regarded as one of the strongest non-covalent bonds known. This technique can be implemented for the functionalization of the silica surface with other proteins as well [22,23].

(c) Mechanical interactions

Surface modification through mechanical texturing is a simple and cost-effective method to roughen biomaterials and to create anchor points for enhanced adhesion. In this method, abrasive particles (such as silicon carbide or alumina) impinge on the biomaterial, and the resulting impact increases its surface roughness [28,54]. This method can be used on orthopaedic implant materials such as titanium or cobalt/chrome.

In the design of artificial cartilage scaffold, the surface modification of a non-degradable polymer could be undertaken to enhance cell adhesion. This involves the addition of a non-woven mesh composed of polymer fibres.
bonded onto a non-porous surface. The presence of the mesh on the surface creates a porous surface on which cells can proliferate. As the ECM develops, the fibres remain interpenetrated, and ensuring the bonding of the scaffold onto the cartilage. An image is shown in figure 10 illustrating this concept [55]. The short arrows point to the fibres (made of poly(l-lactic acid)), whereas the long arrows indicate the presence of cartilage growth on the fibres.

4. Characterization techniques

In this section, nanoscale and macroscale experimental techniques for quantifying bioadhesion are discussed.

(a) Nanoscale

Fundamental studies of bioadhesion at the nanoscale are conducted with atomic force microscopy (AFM). Aside from its high-resolution surface imaging capability, AFM can measure forces in the pico- to nanonewton range, allowing for the detection of single-molecule interactions [6,56]. In AFM, force–distance curves for the contact between the probe and the sample surface can be obtained. An example is shown in figure 11. The force–distance curve consists of two segments, namely the advancing curve (corresponding to AFM piezo extension), which shows how the probe approaches the surface, and the retracting curve, which illustrates how the probe detaches from the surface. In the retracting force curve, a distinct snap-off point is observed, which corresponds to the force necessary to separate the tip from the sample surface. This is the measured adhesive force.
Figure 11. Atomic force microscopy force–distance curve between a tip functionalized with a fibronectin antibody and fibronectin deposited on a polymer surface obtained in phosphate-buffered saline medium.

Figure 12. Effect of pH on the adhesion between streptavidin and biotin as measured by atomic force microscopy. Adapted from Bhushan et al. [57].

The force resolution of AFM enables it to detect differences in the net charge of biomolecules brought about by a change in the surrounding ionic environment. In another AFM study, Bhushan et al. [57] were able to demonstrate that the adhesion between streptavidin and biotin increases with the pH of the buffer solution, as shown in figure 12. This is related to the charge interactions between the two biomolecules. Streptavidin has an isoelectric point of pH 5.5, such that it has a net positive charge at pH 4.4 and net negative charge at pH 7.4 and 9.1. The net negative charge in streptavidin facilitates adhesive interactions with the biotin molecule.

Force–distance curves can also be taken over a predefined area divided into an array of points, where the average adhesive force can be determined. This method is useful when analysing the adhesive properties of next-generation biomaterials such as block copolymers, which have heterogeneous surface properties. Their morphology is a function of the composition and molecular weight of the individual blocks, as well as the spatial relationship of the blocks; for instance,
Figure 13. Atomic force microscopy (AFM) data on the average adhesive force for the interaction between block copolymers containing poly(methyl methacrylate) (PMMA)/poly(acrylic acid) (PAA) with the proteins fibronectin, BSA and collagen (attached to functionalized AFM tips) in pH 7.4 (phosphate-buffered saline) and pH 6.2 buffer media, with data for PMMA as a reference [22].

A–B block copolymers (or diblock) will have a different morphology from A–B–A block copolymers (or triblock), and so on. This variation in the morphology translates to observable differences in the adhesive force. Figure 13 is a summary of the average adhesive force for the interactions between three proteins, namely fibronectin, bovine serum albumin (BSA) and collagen, on block copolymers composed of PMMA and poly(acrylic acid) (PAA), but with different block arrangements (random, diblock and triblock) [22].

The data in figure 13 show the variation of the adhesive force at two ionic environments, pH 7.4 (phosphate-buffered saline, PBS) and pH 6.2, where it is seen that the liquid environment affects the adhesive force. For both buffer media, the highest average adhesive force was observed in the triblock copolymer (PAA-b-PMMA-b-PAA, where ‘b’ denotes block copolymer), and the lowest force was obtained from the PMMA reference. The higher average adhesive force in the triblock copolymer surface can be due to greater ordering on its surface, which would expose more PAA-rich areas than it would for the diblock or random copolymer.

The average adhesive forces measured at pH 6.2 are higher throughout the entire series, relative to the PBS medium (pH 7.4) data. This is attributed to higher repulsion at pH 7.4 (or conversely, increased attractive forces at the lower pH). Chemical interactions between the protein and the polymer surface are influenced by the surface charges of the protein and the polymer itself. The amine groups of the proteins are protonated at both pH 7.4 and 6.2. But these proteins carry a net negative charge when immersed in either the pH 7.4 or 6.2 buffer media, because the isoelectric points of fibronectin, BSA and collagen are all lower than 6.2 [22]. There are more negative charges on the protein surface at pH 7.4 than at pH 6.2. Also, the acrylic acid chains are ionized (to acrylates) during the adhesive force mapping experiment as they are immersed in aqueous
medium. The presence of negative charges on both the tip and sample surface leads to repulsive interactions. As illustrated in figure 14, the reduced repulsion between the acrylate groups in the acrylic acid and the smaller amount of surface negative charges in fibronectin, BSA and collagen at the lower pH increase the measured adhesion between the two surfaces [22]. At the higher pH (7.4), the proteins carry a greater net negative charge. Because the acrylic acid in the block copolymer is present in its ionized form, the repulsion from the negative charges present on both the protein and the polymer surface is more significant at pH 7.4 than at pH 6.2. This accounts for the higher adhesive force measured at pH 6.2. The results demonstrate how AFM is able to measure the effect of the ionic environment on bioadhesion.

(b) Macroscale

Traditional adhesion characterization techniques, such as crack growth, peel and shear tests, could be applied to evaluate bioadhesion. A few examples of the use of these methods are provided below.

For dental restorations, the Griffith energy balance model could be used to characterize the crack growth using a bending test, where the cracks originate from the shrinking of the implanted dental composite material upon polymerization [58]. The energy balance concept, as applied to restorations, deals with the balance between the elastic energy in the tooth (which is actually composed of the individual elastic energies of the tooth and the restorative material) and the surface energy associated with the crack. Experimentally, either the stress intensity factor ($K$, also referred to as the fracture toughness) or the strain energy release rate ($G$, also referred to as the fracture energy) is evaluated from the measurement of the crack growth. Fracture toughness is obtained from [58]

$$K = \sigma (a\pi)^{1/2},$$

where $\sigma$ is the applied stress and $a$ is half the length of the crack. A tabulation of the fracture toughness of adhesive joints, tooth components, and restorative materials is presented in table 2. The fracture energy can be obtained as long as the modulus and Poisson’s ratio of the material are known.
Table 2. Fracture toughness of selected dental materials [58].

<table>
<thead>
<tr>
<th>material</th>
<th>fracture toughness (MPa m$^{1/2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>adhesives bonded to dentin</td>
<td>0.1–1.2</td>
</tr>
<tr>
<td>adhesives bonded to enamel</td>
<td>1.1</td>
</tr>
<tr>
<td>enamel</td>
<td>0.65–2.5</td>
</tr>
<tr>
<td>dentin</td>
<td>1.0–4.0</td>
</tr>
<tr>
<td>dentin–enamel junction</td>
<td>0.8–3.4</td>
</tr>
<tr>
<td>composite resins</td>
<td>0.7–1.9</td>
</tr>
<tr>
<td>amalgams</td>
<td>1.4–2.4</td>
</tr>
</tbody>
</table>

As a qualitative tool, fractographic analysis through either scanning electron microscopy (SEM) or transmission electron microscopy is usually conducted on dental restoration–dentin interfaces analysed in vitro using tensile testing. Examples of fracture surface SEM images are shown in figure 15, where it is seen that either adhesive or cohesive failure can take place. The determination of the failure mode is useful when comparing between different adhesive resin formulations [59].

Standard American Society for Testing and Materials (ASTM) characterization procedures, such as the tensile test (ASTM D368), peel test (ASTM D903), shear strength (ASTM D3654) and shear adhesion failure temperature (SAFT, ASTM D4498), are conducted on interfaces where an adhesive is applied to the biomaterial. Schematics for the peel and shear tests are shown in figure 16. These tests are applicable to systems such as fibrin glues used in surgical operations, PAA hydrogels and pressure-sensitive adhesives (PSAs) intended for transdermal drug-delivery applications [8,54,60,61]. Figure 17 shows examples of macroscale adhesion data on PSAs. The adhesive system investigated was PDMS. It was loaded with an organo-clay based on montmorillonite (MMT) to form a composite material. The addition of the organo-clay was shown to improve the shear strength and the SAFT, with a minimal reduction in the peel strength [61].
Figure 16. Schematics of macroscale adhesion tests. (a, b) The scotch tape test, (c, d) examples of the peel test and (e) the lap shear test [54].

The flow-through method, which measures the flow rate needed to remove a bioadhesive-coated sphere, is a common macroscale experiment suited for the characterization of mucoadhesion of potential drug-delivery systems. A widely used biophysical assay involves the monitoring of molecular weight change through the variation in the sedimentation coefficient as measured by an analytical ultracentrifuge [62]. The Wilhelmy plate experiment is another technique for evaluating bioadhesion at the macroscale. Instead of having a liquid
medium such as water (as used in surface tension measurements), it is replaced with either natural or synthetic mucus and the plate is coated with the polymer of interest [2,54]. This method is useful for determining interfacial forces between mucus and polymer, as well as for the investigation of changes in bioadhesion properties over time.

5. Conclusion

The subject of bioadhesion covers many different concepts, mechanisms and applications. To illustrate the diversity of bioadhesion processes, relevant
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Figure 18. Chemical structures of the 20 amino acids found in proteins. Adapted from [63].

examples, such as cell adhesion to biomaterials, dental restorative adhesives and mucoadhesives, were discussed in this paper. Bioadhesion can be beneficial, as it can facilitate the desired adhesion of cells and biomolecules on various natural and synthetic substrates, which then leads to the development of novel biomaterials,
therapies and technologies such as biosensors. Various processing techniques that could enhance bioadhesion are available. The characterization of bioadhesion must be performed at the length scale appropriate to the phenomenon (either at the nanoscale or macroscale), as it relates to the application where the adherence between two interfaces is desired.

Appendix A. The composition of proteins

Proteins are known as the most abundant class of macromolecules in cells. Each protein molecule can be envisaged as a polymer composed of amino acids, which are molecules that contain an amine group, a carboxylic acid group and a variable side chain. The chemical structures of the 20 common amino acids found in proteins, along with their three-letter and one-letter abbreviations, are shown in figure 18 [63]. On the basis of their chemical composition, the amino acids alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine are classified as non-polar. Glycine, serine, threonine, cysteine, asparagine and glutamine are considered as uncharged polar amino acids. Aspartic acid, glutamic acid, lysine, arginine and histidine have charged side groups [64].

The amino acid sequence of human serum albumin is shown in figure 19 to illustrate the side chain composition variation found on a typical protein. This protein contains 585 amino acids in the form observed in the blood [65]. A close examination of this sequence reveals that charged, uncharged polar and non-polar amino acids are present in varying arrangements throughout this protein.

References


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