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Cell Adhesion to Biomaterials: Concept of Biocompatibility

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Additional information is available at the end of the chapter

1. Introduction

Cell adhesion is a dynamic process that results from specific interactions between cell surface molecules and their appropriate ligands. Adhesion can be found between adjacent cells (cell-cell adhesion) as well as between cells and the extracellular matrix (ECM) (cell-matrix adhesion). Adhesion is an extremely important concept in both practical and theoretical terms. Unfortunately, there is no completely satisfactory definition of the term that fulfils the needs of both the theoretical surface chemist and the practicing technologist. It is assumed as a state in which two bodies (usually, but not necessarily dissimilar) are held together by intimate interfacial contact in such a way that mechanical force or work can be applied across the interface without causing the two bodies to separate.

Cell membrane are crucial to the adhesion of the cell and therefore to its life. Indeed, plasma membrane encloses the cell, defines its boundaries, and maintains the essential differences between the cytosol and the extracellular environment. In all cells the plasma membrane also contains proteins that act as sensors of external signals, allowing the cell to change its behavior in response to environmental cues; these receptors transfer information rather than ions or molecules across the membrane. Plasma membrane has the structure of a thin film of lipid and protein molecules linked together mostly through non covalent interactions. These lipid molecules are arranged as a continuous bilayer and are responsible for the basic structure of the membrane and the protein molecules embedded into it control most of the functions of the membrane. In the plasma membrane some proteins serve as structural links that connect the membrane to the cytoskeleton and/or to either the extracellular matrix (ECM) or an adjacent cell, while others serve as receptors to detect and transducer chemical signals in the cell's environment [1].

Besides keeping a multicellular organism together, cell adhesion is also a source of specific signals to adherent cells; their phenotype can thus be regulated by their adhesive interactions. In fact, most of the cell adhesion receptors were found to be involved in signal transduction. By interacting with growth factor receptors they are able to modulate their signaling efficiency. Therefore, gene expression, cytoskeletal dynamics and growth regulation all depend, at least partially, on cell adhesive interactions [2].

In this chapter, I tried to find a possible correlation between polyelectrolyte multilayer films and human gingival fibroblasts to test these biomaterials biocompatibility. This represents a fundamental step needed to know about a possible use in a biological field (i.e. as implant). For that purpose, I characterized each solid surface used as a surface on which fibroblasts were cultured; by calculating their surface free energy and evaluating their chemical heterogeneity, roughness and wettability using contact angle measurement. Thereafter, I followed the adhesion of fibroblasts, their proliferation and their morphology.

2. Polyelectrolyte multilayer film

2.1. Biomaterials: Generality and interest

During a consensus conference in 1986, a definition was given for biomaterials. Indeed, a biomaterial is «a non-living material used and designed to be integrated with biological systems». Biomaterials are defined according to their domain of use and regroup metals and alloys, ceramics, polymers (i.e. collagen)[3].

Biomaterials were used since the pharaoh's time to replace injured and affected organs. Pharaoh had used pure natural materials but presenting integration's problems. Since that, researches had grown up rapidly in this field in order to design the "ideal" material which will be more accepted by the human body. The designed material was referred to as "biomaterial" afterwards and will recover a lot of biomedical applications for implants and tissues injuries covering.

Biomaterials' design must take into account the purpose and the place of its use. This biomaterial must have a well defined shape depending on his position within the body. Indeed, for orthopedic usage, a biomaterial must conform to some criteria and regulations such as: a good mechanical structure, a good resistance to corrosion and metal fatigue. For vascular surgery, a biomaterial must not induce thrombosis, in odontology a biomaterial must withstand changes that can occur to temperature (coffee, cool drinks), to pH (alcohol, lemon…) and to the buccal cavity [4].

Making reliable and cheap biomaterials is being a new challenge for researchers and industries. In fact, the infallibility of every biomaterial depends on the materials from which it's made of. Consequently, there's a great demand in developing new suitable biomaterials (or making the existing ones better) used in multidisciplinary fields and involving physics, chemistry and biology.

In this study, the biomaterials used for fibroblasts adhesion are made of polyelectrolytes using the layer-by-layer technique based on alternating oppositely charged polyelectrolytes on glass probes (more details are shown in paragraph III.2).

2.2. Polyelectrolytes

Polyelectrolytes are highly charged nanoscopic objects or macromolecules. Their electric charge density appears as more or less continuous, when it is seen from distances to the macromolecule equal to several times to the intercharge distance, giving them the polyelectrolytic character. Obviously, their properties will be extremely different according to their geometry. Massive spherical objects will behave like colloids, whereas linear flexible objects will keep some of the macromolecular polymeric character [5]. They are defined as materials for which the solution's properties in dissolvent presenting a high permittivity are governed by electrostatic interactions for distances superior to the molecular dimensions [6]. Polyelectrolytes are by no way a mere superposition of electrolytes and polymers properties. New and rather unexpected behaviours are observed:

• Whereas polymers exhibit only excluded volume effects, the long ranged coulomb interactions, which are present in polyelectrolytes, give rise to new critical exponents.

• The main difference with electrolytes is that one kind of ions, the counterions are stuck together along a chain, and the collective contribution of the charged monomers causes a strong field in the vicinity of the chain, even at very low dilution.

These materials are widely used in industries as dispersive substances in aqueous medium, flocculants to aggregate sludge and industrial waste. Recently, they were used to make films by alternating thin layers of polymers of medical use such as dental prosthesis, fabrication of transplantable organs etc…

Polymers differ by their structure, their surface composition and their biological properties:

2.2.1. Biological properties

The biological properties reflect the origin of polymers. Indeed, one can distinguish three different origins for polymers [7]:

• Natural polymers coming from animal, vegetal and mineral origins

• Artificial polymers with natural basic components and chemically transformed functions in their units (monomers)

• Synthetic polymers presenting synthetic basic components which are often very similar to those of natural polymers

2.2.2. Physico-chemical properties

According to Oudet [7], polymers have different physical properties. The most important are their thermal conductivity reflecting polymers' behaviour under temperature changes.

The second interesting physical property is their optical reactions towards light (refraction, reflection angle, polarization, absorption…). Moreover, polymers are characterized by their ability as electrical conductors or insulators.

From the chemical point of view, Fowkes [8] presumed the existence of different polymers surface structure: polymers with polar surfaces (polyethylene), polymers with acid (polyimide) or basic (polystyrene) sites dominance and others are regrouping both acid and basic characters (polyamide). These surfaces are governed by specific (dispersive forces attraction) and non-specific interactions (acid-base interaction).

Polymers properties are strongly influenced by molecular interactions such as Van der Waals interactions (low energy bonds), hydrogen interactions (low energy bonds having an electrostatic origin) and ionic interactions due to electrostatic attractions and repulsions between ions or ionized groups.

2.3. Polyelectrolyte multilayer film

2.3.1. Generality

In recent years, polyelectrolyte multilayer film has been widely developed in different fields and for a variety of purposes. This kind of ultrathin film can be fabricated from oppositely charged polyelectrolytes using a method called self-assembly discovered by Decher and co-workers in 1992 and allows surface modification and therefore controlling their properties at the molecular (or even the atomic) level.

These films are of a great interest for covering biomaterials used as implants [9, 10] and therefore they will be in contact with cells [11]. Layer-by-layer assembly of polyelectrolytes is a simple and suitable method for coating different substrates such as glass, silicon, thermoplastic and even curved surfaces [12, 13].

It's known that biomaterials must present two main conditions to be admitted for integration in the biological system: to be biocompatible with this system and to have definite mechanical and electrical properties depending on their use [14]. The next implants generation has a tendency to be bioactive, besides its biocompatibility, thanks to substrate coating with bioactive substances.

2.3.2. Fabrication method and application fields

Multilayer polyelectrolyte films are made by alternating oppositely charged polyelectrolytes (polyanions and polycations) on glass slides (Figure 1).

Film's formation is based on charge overcompensation of the newly adsorbed polyions. Indeed, a polyanion (negative charge) added to a polycation (positive charge), previously deposited on the substrate, will neutralise the excess of positive charges and therefore create a new negatively charged polyelectrolyte layer. This step can be repeated as many times as the needed number of layers is reached [15].
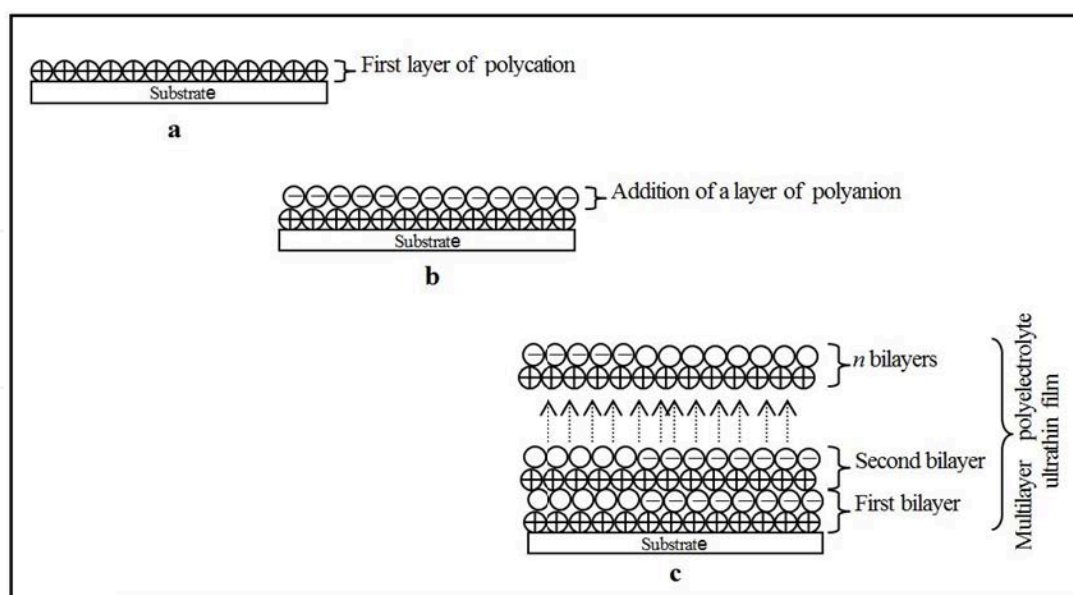
Figure 1. Layer-by-layer polyelectrolyte film's fabrication. This assembly method is based on alternating oppositely charge polyanion (positive charge) and polycations (negative charge) on a solid substrate. One bilayer consists in one polycation associated with one polyanion and the film is a set of n bilayers.

This adsorption mechanism is governed by electrostatic interactions which represent, besides other secondary interactions (hydrogen bond or dispersive force), a paramount parameter for the final structure of the formed film [16].

Polyelectrolyte multilayer films are used in different fields: orthopedic surgery (hip prosthesis...), cardiovascular (artificial heart, vascular prosthesis...), odontology (dental restoration...), ophthalmology (contact lenses...), urology (catheters, artificial kidney...), endocrinology (artificial pancreas, biosensors...), aesthetic surgery and other domains [17].

2.4. Polyelectrolyte film surface characterization

This study is possible by investigating surface wettability and calculating surface free energy. Indeed, wettability is the aptitude of a substrate to be coated by a thin liquid film while dipped in a liquid solution. This method is used to follow the substrate behaviour in relation to its environment and can be done thanks to the contact angle measurement. In this paper we are interested in the dynamic contact angle method using Wilhelmy plate method, treated later. This method, besides giving information about substrate surface hydrophilicity and hydrophobicity, allows us to evaluate the surface roughness and chemical heterogeneity. Moreover, with the results found, we measured the polyelectrolyte film's surface free energy according to Van Oss theory.

2.4.1. Contact angle measurement

There are a variety of simple and inexpensive techniques for measuring contact angles, most of which are described in detail in various texts and publications and will be mentioned only briefly here. The most common direct methods (Figure 2) include the sessile

drop (a), the captive bubble (b) and the tilting plate (c). Indirect methods include tensiometry and geometric analysis of the shape of a meniscus. For solids for which the above methods are not applicable, such as powders and porous materials, methods based on capillary pressures, sedimentation rates, wetting times, imbibition rates, and other properties, have been developed [18].
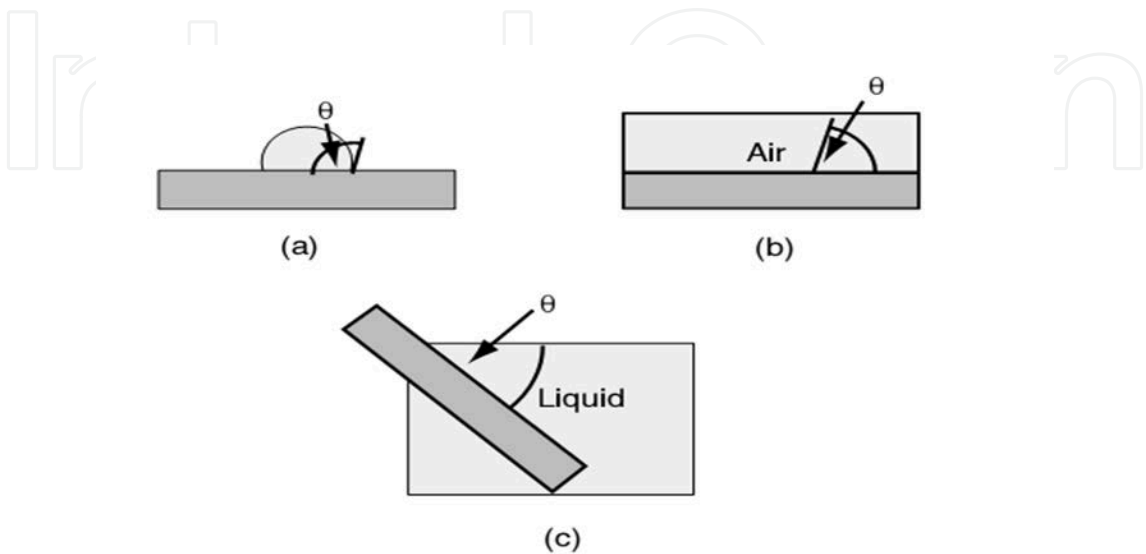

Figure 2. The more common systems of contact angle measurement showing the sessile drop (a), the captive bubble (b) and the tilting plate (c). θ is the contact angle to be measured.

2.4.1.1. The sessile drop method

It's a static contact angle measurement method which consists in putting down a liquid drop on the solid plate we want to characterize its surface by measuring the contact angle made by the drop on this surface. Indeed, when a drop of a liquid is putted down on a solid surface; three phases system occurs: solid, liquid and gas (Figure 3).
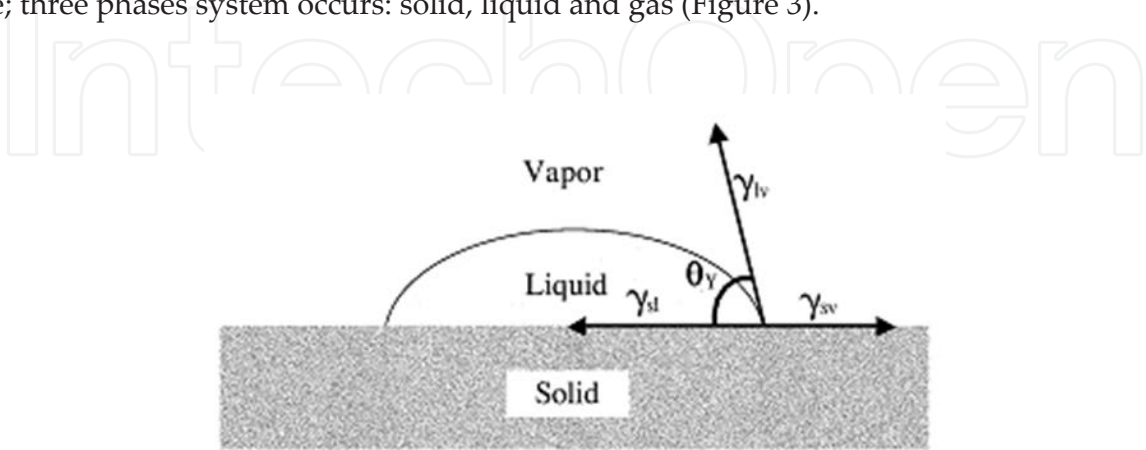

Figure 3. Static contact angle measurement with the sessile drop method

The drop's profile is being changed depending on the physico-chemical characters of the solid surface, on the adhesion forces newly created at the interface solid/liquid and on the cohesion forces of the liquid. This change will affect the contact angle value revealing the surface state (hydrophobic or hydrophilic, rough or smooth, homogeneous or heterogeneous…) and the different forces occurred are linked together according to Young's equation [19]:

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} cos\theta,$$

Where γ_{sv}, γ_{sl} and γ_{lv} represent the "surface tensions" of the interface solid/gas, solid/liquid and liquid/gas, respectively, and θ represents the contact angle.

2.4.1.2. The captive bubble method

It's a derivative of the sessile drop method and consists in making an air bubble (or a bubble from a less dense and non miscible liquid such as dodecane, octane and octadecane) on a solid surface immersed in pure water or in other liquid with a well known physico-chemical characters. So, it's possible to measure the contact angle made by this bubble with the immersed solid surface (see Figure 2).

2.4.1.3. The tilting plate method

The tilting plate method is to slowly tilt a contact angle sample until the sessile drop on it begins to move in the downhill direction. At that time, the downhill contact angle is the advancing angle and the uphill angle the receding contact angle [20].

The principal alternative to the tilting plate method is having the dispense needle remain immersed in the sessile drop and pumping in until the drop expands in base area and pumping out until the drop contracts in base area. Often the tilting plate measurement is carried out on an instrument with a mechanical platform that tilts the stage and the camera together.

It has been shown that these methods are a subject of controversy. However, the dynamic contact angle measurement using the Wilhelmy plate method has been shown to be easier for use and gives more information about the surface characterized.

2.4.1.4. The dynamic contact angle method: The tensiometer

In our study, we used the Wilhelmy plate method (Tensiometer 3S, GBX, France) which allows a dynamic measurement of the contact angle hysteresis. Indeed, the tensiometer used for the measurement will measure the force applied to the substrate while immerged in a liquid thanks to a balance where the substrate was hanged (Figure 4)

In each case, the polyelectrolyte film coated glass slide was immersed into and then drawn out of the measurement liquid. Therefore, the tensiometer will evaluate the advancing angle (θ_a) when the liquid moves forward the substrate surface and thereafter the receding angle (θ_r) when the liquid resorbs from the substrate. The difference be-

tween θ_a and θ_r is called contact angle hysteresis H (H = θ_a - θ_r) and is useful for under-standing the wettability of the film. It gives us information about the surface film mobility, its reorganization and roughness [21].
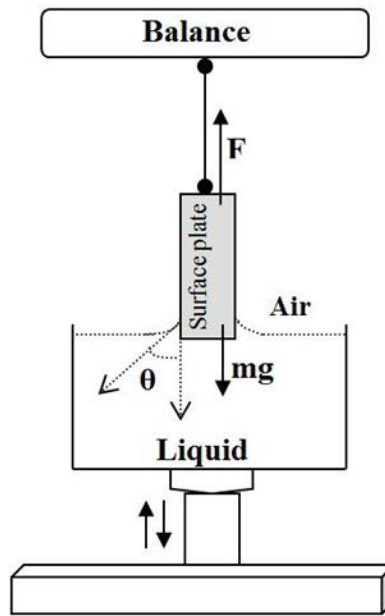

Figure 4. The Wilhelmy plate method for dynamic contact angle measurement. The surface plate is partially im-merged in the up down moving liquid container. Curves (Loops) are automatically drawn by a software associated to the Tensiometer according to F = f (Immersion depth)

When a substrate is immersed in a liquid, three forces occur (see Figure 4): the gravity force, the upthrust buoyancy and the capillary forces. Therefore, by measuring the applied force according to the immersion depth and as we previously know the dimension of the sub-strate; one can calculate the wetting forces according to the equation [22]:

$$F = mg + p * \gamma_{LV} * \cos\theta - F_b \qquad (1)$$

Where F represents the force measured (mN/m), m is the substratum mass, g is the accelera-tion constant induced by the gravity, p is the substratum perimeter (cm^2), γ_{LV} is the surface free energy (mN/m) of the liquid used for measurement (constant), θ: the contact angle be-tween the liquid and the substratum (°) and F_b is the force related to the upthrust buoyancy.

Usually, we make several immersion/emersion cycles for the substratum we are investi-gating and the different loops (one loop corresponds to one immersion/emersion cycle) are drawn by a software associated to the Tensiometer according to Force = f (immer-sion depth). Moreover, the substratum weight is assumed to be nil by a direct correction fixing the pre-immersion force to the value of zero. Therefore, the previous equation ([Eq. 1]) becomes:

$F_{(zero\ immersion)} = p * \gamma_{LV} * \cos\theta$

As the surface energy of the liquid of measurement is previously known, therefore the contact angle could be deduced.

It has been shown that the contact angle changes depending on the nature of the film and on its charges and thickness. The nature of liquid of measurement, the speed and temperature of measurement are also involved in this change [23]. Indeed, the thickness of the film can affect its elasticity which will induce a difference in the liquid diffusion into this film and therefore the film's swelling level changes affecting the contact angle. A previous study made by Elbert et al.[24] has shown a clear effect of the film layers' number on the wettability of the film.

The liquid used for measurement can affect the surface wettability by the mean of its pH which varies from a liquid to another and controls the acid or base character as well as the liquid polarity. These parameters are responsible for the rearrangement of the biomaterial's groups at its contact. This reorganization is also depending on the liquid diffusion into the polymer and on the effect of solubilization induced by the liquid to this polymer. This phenomenon represents an interesting mechanism for explaining contact angle hysteresis especially when the liquid concerned is water. Indeed, water has small molecules which allow it to diffuse easily. Therefore, after diffusion into a polymer, water will confer its hydrophilic character to this polymer which is being to have some kind of elasticity responsible for the reorganization of its polar groups as a reaction to the high surface energy level of water which is responsible for the high energy level at the interface [25]. Concerning the dynamic contact angle measurement speed, it affects the contact period between the biomaterial and the liquid and therefore it will change the period of time needed for the rearrangement of the surface polar groups during contact with the liquid. As each film has its own defined reorganization time, therefore different contact angles were found for the same surface at different measurement speeds. Moreover, every polymer has a defined glass transition temperature (T_g) able to induce a change on the surface wettability depending on the temperature of measurement [26].

2.4.2. Surface free energy calculation

It's interesting to know the value of surface free energy of a biomaterial because it has an effect on wettability as shown by Van Oss [27]. While the contact between the biomaterial and the liquid generates an interface solid/liquid which will consume, during its formation, a defined energy called the interface energy. The reversible adhesion force represents, therefore, the difference in the energy level between the initial state characterized by two surfaces [28, 29]: solid surface with the energy (γ_s) and liquid surface with the energy (γ_l); and the final state (γ_{sl}).

The surface free energy is a kind of an attraction force of the surface which cannot be measure directly but calculated after contact angle measurement in different measurement liquids (with different surface free energies) according to Owends et Wendt or Van Oss' approaches. Their theories are complementary but Van Oss' approach has been shown to give more information. It consists in the following equation [27]:

$$\gamma_S = \gamma_S{}^{LW} + 2 \, (\gamma_S{}^+ \cdot \gamma_S{}^-)^{1/2}$$

where γ_S represents the surface free energy of the biomaterial surface, $\gamma_S{}^{WL}$: the dispersive component and $\gamma_S{}^+$, $\gamma_S{}^-$ represent the polar components (acid- base).

The different components of the solid and the liquid surface free energies as well as the contact angle are related by this equation:

$$\gamma_L \, (1 + \cos\theta) = 2 \, ((\gamma_S{}^{LW} \cdot \gamma_L{}^{LW})^{1/2} + (\gamma_S{}^+ \cdot \gamma_L{}^-)^{1/2} + (\gamma_L{}^+ \cdot \gamma_S{}^-)^{1/2}),$$

This equation contains three unknown parameters: γ^{LW}, γ^+ and γ^-; the contact angle measurement must be done with three different measurement liquids in order to solve this equation and calculate the surface free energy of our polyelectrolyte film. For this purpose, we used three different liquids: water, diiodomethane and formamide.

2.4.3. Evaluation of the surface roughness and heterogeneity

Theses parameters are deduced from the shapes of the curves drawn (loops). Indeed, the more the surface is rough; the more the curve is deformed (non linear curve). However, the more the surface is smooth; the more the curve presents a linear shape (no deformations observed). Otherwise, a roughness of about 100 nm has been shown to induce contact angle hysteresis. As for surface heterogeneity, it can be concluded from the different contact angle hysteresis values measured in the case of a negligible roughness.

Concerning the different polyelectrolyte films used in this study, a previous investigation was made by Picart and coworkers [30]. They measured the roughness by the AFM technique, refractive index and thickness are estimated by optical waveguide light mode spectroscopy, and zeta potential is measured by streaming potential measurements. Indeed, these parameters give us information about the chemical heterogeneity of the polyelectrolyte used.

Many studies had observed an important dependence of the contact angle hysteresis on the surface composition and topography (roughness) [31, 32]. Therefore, the more the surface is rough; the more it's hydrophilic and vise versa and the more this surface is composed of small molecules, the less the liquid diffusion in the biomaterial surface is disturbed leading to a low contact angle value. According to Morra et al.[33], this is may be due to existence of two different effects while studying the wettability of rough and homogeneous biomaterials: the barrier effect, where hysteresis increases with increasing the surface roughness due to an important rigidity of the substrate, and the capillary attraction at the surface which can affect Young's concept. Indeed, the capillary effect induces an increase of both the advancing and receding contact angles in the case of a surface presenting a contact angle superior to 90° at the equilibrium state. In the opposite case (contact angle inferior to 90° at the equilibrium state), the inverse situation will happen and the contact angle variations will be less important than those corresponding to the barrier effect. Only in the case of a contact angle equals to 90°, the capillary effect is negligible.

2.5. Conclusion

When a drop of liquid is placed on a solid surface, the liquid will either spread across the surface to form a thin, approximately uniform film or spread to a limited extent but remain as a discrete drop on the surface. The final condition of the applied liquid to the surface is taken as an indication of the wettability of the surface by the liquid or the wetting ability of the liquid on the surface. The quantitative measure of the wetting process is taken to be the contact angle, which the drop makes with the solid as measured through the liquid in question.

The wetting of a surface by a liquid and the ultimate extent of spreading of that liquid are very important aspects of practical surface chemistry. Many of the phenomenological aspects of the wetting processes have been recognized and quantified since early in the history of observation of such processes. However, the microscopic details of what is occurring at the various interfaces and lines of contact among phases has been more a subject of conjecture and theory than of known facts until the latter part of this century when quantum electrodynamics and elegant analytical procedures began to provide a great deal of new insight into events at the molecular level. Even with all the new information of the last 20 years, however, there still remains a great deal to learn about the mechanisms of movement of a liquid across a surface.

3. Fibroblast cells

3.1. Human gingival fibroblasts

3.1.1. Generality

Fibroblasts are spindle-shaped connective-tissue cells of mesenchymal origin that secretes proteins and especially molecular collagen from which the extracellular fibrillar matrix of connective tissue forms. They have oval or circular nucleus and a little developed cytoplasm giving rise to long prolongation forms [34]. These cells do not have a basal lamina and their surfaces are often in contact with the fibers of the collagen. Their cytoplasm contains a rough endoplasmic reticulum, an important Golgi apparatus, few mitochondria and a little bit quantity of cytoplasmic filaments. Fibroblasts synthesize enormous quantities of the extracellular matrix constituents. Indeed, the majority part of the extracellular matrix components consists of collagen made in the intracellular space where fibroblasts sustain structural modifications.

3.1.2. Gingival tissue

It's the tissue that surrounds the necks of teeth and covers the alveolar parts of the jaws; broadly: the alveolar portion of a jaw with its enveloping soft tissues [35]. It consists in a pink connective tissue with fibrous collagen surrounded by an epithelial tissue. Its pink color changes from one person to another, depending on pigmentation, epithelium thickness, its keratinization level and on the underlying vascularization [36]. Fibroblasts are the basic

component of the gingival chorion whose intercellular matrix is essentially formed by colla‐ gen and elastin.

3.2. Cell-Biomaterial: Interface and interactions

3.2.1. Biocompatibility concept

While a cell is in contact with a biomaterial, many reactions can occur and a sensing phe‐ nomenon will launch between this cell and the biomaterial [37]. Indeed, the cell has a signal network reached as a result of the surface exploration and sensing made in order to verify whether the new environment (biomaterial) is in accordance with its expected physiological conditions necessary for a normal biological activity [38]. Thus, before putting a new materi‐ al in contact with a cell it's of a great importance to choose the corresponding material in such a way that this material obey the cell's norm by not being toxic or injurious and not causing immunological rejection. In one word, this material must be biocompatible.

The biological tolerance of a biomaterial led scientists to regroup the different parameters and mechanisms controlling the interface biomaterial/cell (or tissue) so that they can deduce a concrete and a common definition for biocompatibility concept. Indeed, biocompatibility includes the understanding of the interactive mechanisms relating the biomaterial with its biological environment. Generally, biocompatibility represents the ability of a material to be accepted by a living organism.

In 1987, Williams D.F suggested the following definition «biocompatibility is the ability of a material to be used with an appropriate and suitable reaction of the host for a spe‐ cific application».

According to Exbrayat [39] « biocompatibility is a set of the different interrelations between a biomaterial and its environment, and their biological local or general consequences, imme‐ diate or delayed, reversible or definitive».

Indeed, biocompatibility is a group of networks that liaises between the biomaterial and its environment and takes into account the possible effect of this biomaterial on its environ‐ ment and vice versa. Interactions existing in the interface biomaterial/biological environ‐ ment differ by their intensity and their duration period depending both on the biomaterial and on the tissue in contact.

Characterizing the surface properties of a biomaterial before putting it in contact with a cell seems to be an obligation. This step allows us to know about different parameters and char‐ acters of this biomaterial (topography, roughness, surface energy etc.) in order to find a cor‐ relation with the cell behavior and therefore we can adjust these physico-chemical properties, when making the biomaterial, so that we have a normal and physiological cell behavior in contact with that biomaterial.

3.2.2. Cell adhesion

It is well known that during the contact between a cell and a material, information will be transferred from the material surface to the cell and this contact will induce, in return, an alteration to the material. This situation may cause material remodelling [40,22].

Cells adhere to surfaces through adhesion proteins (i.e. fibronectin, collagen, laminin, vitronectin) using specific cell receptors, called integrins, attached to the cell membrane. Indeed, when fibroblasts grow on a substrate, most of their cell surface is separated from the substratum by a gap of more than 50 nm; but at focal contacts, this gap is reduced to 10 to 15 nm. The main transmembrane linker proteins of focal contacts belong to the integrin family and the cytoplasmic domain of the integrin binds to the protein talin, which in turn binds to vinculin, a protein found also in other actin-containing cell junction. Vinculin associates with α-actinin and is thereby linked to an actin filament [1].

Besides their role as anchors, focal contacts can also relay signals from the extracellular matrix (ECM) to the cytoskeleton. Several protein kinases are localized to focal contacts and seems to change their activity with the type of the substratum on which the rest. These kinases can regulate the survival, growth, morphology, movement, and differentiation of cells in response to new environment. Figure 5 shows a possible arrangement of these different proteins during a focal contact.
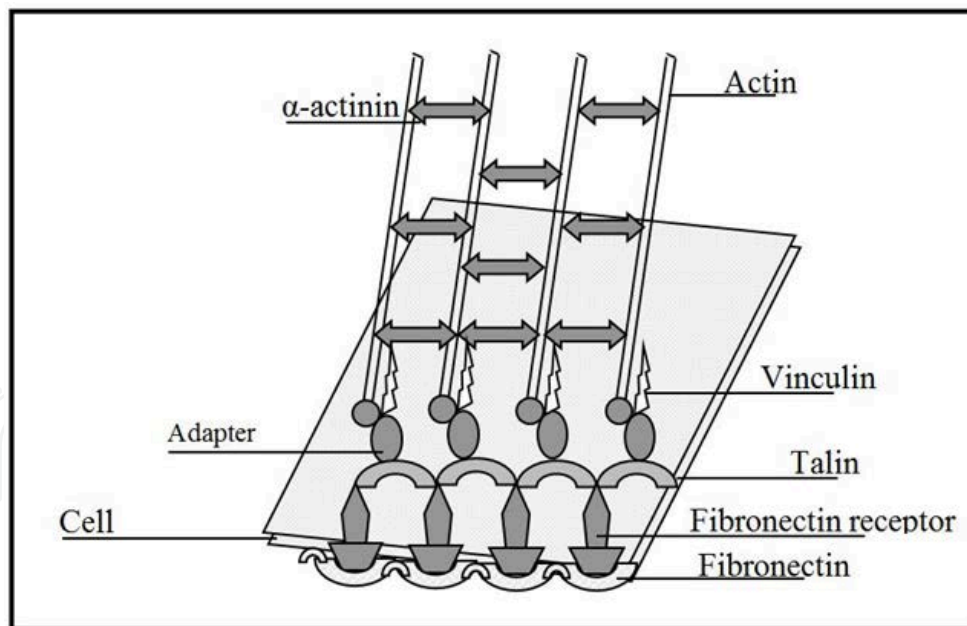

Figure 5. Adhesion proteins involved in focal contacts

The formation of focal contacts occurs when the binding of matrix glycoprotein, such as fibronectin, on the outside of the cell causes the integrin molecules to cluster at the contact site. Fibronectins are associated together by proteoglycans and constitute thins fibers of the extracellular matrix (ECM).

3.2.2.1. Extracellular matrix

The extracellular matrix (ECM) represents an important element in the processes of cell ad‐hesion. Indeed, at this level, cell adhesion is under the control of a well defined zone in the cytoplasmic membrane called focal contact. At this zone, filaments of actin are linked to fi‐bronectin through an intracellular complex of proteins, the adherence complex. The extracel‐lular matrix (ECM) is made of different proteins such as fibronectins, collagen, laminin, vitronectin [41] and represents the mediator of cell adhesion thanks to its integrins.

Although the extracellular matrix generally provides mechanical support to tissues, it serves several other functions as well. Different combinations of ECM components tailor the extrac‐ellular matrix for specific purposes: strength in a tendon, tooth, or bone; cushioning in carti‐lage; and adhesion in most tissues. In addition, the composition of the matrix, which can vary, depending on the anatomical site and physiological status of a tissue, can let a cell know where it is and what it should do (environmental cues). Changes in ECM components, which are constantly being remodeled, degraded, and resynthesized locally, can modulate the interactions of a cell with its environment. The matrix also serves as a reservoir for many extracellular signalling molecules that control cell growth and differentiation. In addition, the matrix provides a lattice through or on which cells can move, particularly in the early stages of tissue assembly [42].

Many functions of the matrix require transmembrane adhesion receptors that bind directly to ECM components and that also interact, through adapter proteins, with the cytoskeleton. The principal class of adhesion receptors that mediate cell–matrix adhesion are integrins, a large family of $\alpha\beta$ heterodimeric cell surface proteins that mediate both cell–cell and cell–matrix adhesions and inside-out and outside-in signalling in numerous tissues.

3.2.2.2. Adhesion proteins and receptors in fibroblast cells

Different proteins and their receptors are involved in fibroblast cells adhesion process. The most important and known are fibronectins and their receptors; integrins:

- Fibronectins

  Fibronectins are dimers of two similar polypeptides linked at their C-termini by two di‐sulfide bonds; each chain is about 60–70 nm long and 2–3 nm thick. The combination of different repeats composing the regions, another example of combinatorial diversity, con‐fers on fibronectin its ability to bind multiple ligands [40].

  Fibronectins help attach cells to the extracellular matrix by binding to other ECM compo‐nents, particularly fibrous collagens and heparan sulfate proteoglycans, and to cell sur‐face adhesion receptors such as integrins. Through their interactions with adhesion receptors (e.g., $\alpha5\beta1$ integrin), fibronectins influence the shape and movement of cells and the organization of the cytoskeleton. Conversely, by regulating their receptor-mediated attachments to fibronectin and other ECM components, cells can sculpt the immediate ECM environment to suit their needs.

- Integrins

Integrins are the principle adhesion receptors; a large family of $\alpha\beta$ heterodimeric cell sur‐ face proteins that mediate both cell–cell and cell–matrix. They are transmembrane pro‐ teins that mediate interactions between adhesion molecules on adjacent cells and/or the extracellular matrix (ECM). They have diverse roles in several biological processes includ‐ ing cell migration during development and wound healing, cell differentiation, and apop‐ tosis. Their activities can also regulate the metastatic and invasive potential of tumor cells. They exist as heterodimers consisting of alpha and beta subunits. Some alpha and beta subunits exhibit specificity for one another, and heterodimers often preferentially bind certain cell adhesion molecules, or constituents of the ECM.

Although they themselves have no catalytic activity, integrins can be part of multimolecu‐ lar signalling complexes known focal adhesions. The two subunits, designated as alpha and beta, both participate in binding.
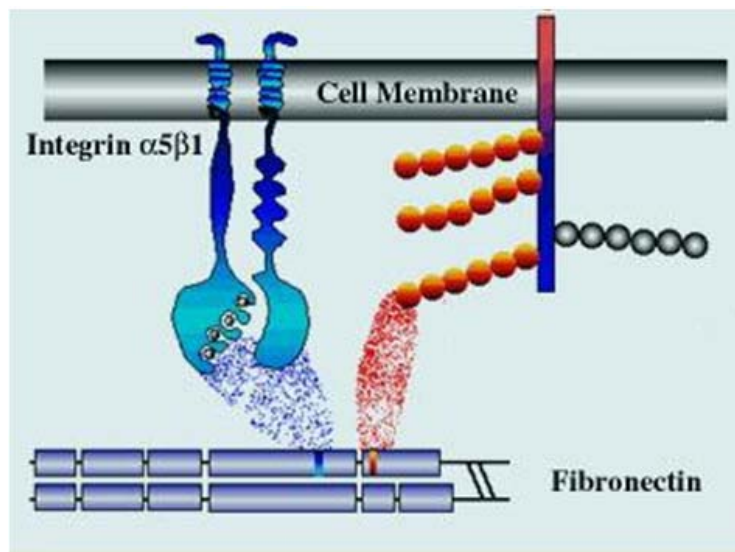

Figure 6. Fibronectin binding to its Integrin receptor (adapted from internet)

Integrins participate in cell-cell adhesion and are of great importance in binding and interac‐ tions of cells with components of the extracellular matrix such as fibronectin. Importantly, integrins facilitate "communication" between the cytoskeleton and extracellular matrix; al‐ low each to influence the orientation and structure of the other. It is clear that interactions of integrins with the extracellular matrix can have profound effects on cell function, and events such as clustering of integrins activates a number of intracellular signally pathways.

3.2.3. Cell adhesion: The physical process

Biological systems exhibit electromagnetic activity in a wide frequency range from the static or quasistatic electric field to optical bands. Fröhlich [43] presumed that biological matter has anomalous polarization properties (e.g. induction of great electric dipole after electric field application). Static charge distribution of dipole and/or multipole nature exists (e.g. in protein molecules). Vibrations in biological molecules, therefore, generate an electromagnet‐

ic field [44]. Pokorny et al.[45], assume that the Fröhlich electromagnetic field can be a fundamental factor of cell adherence.

Surface topography is of an important interest in cell adhesion as well as its chemical composition. Indeed, it has been shown that cells adhere and proliferate depending on the surface roughness and the more the surface is rough the more cell adhesion and proliferation is better [46]. This effect depends on the cell type. For fibroblasts, they line up along the biomaterial surface microstructures and may adapt their shape with uneven surfaces.

Moreover, recent studies had shown that a weak change in the surface roughness may induce different cell reactions such as change in their shape and their way of adhesion [47, 48].

3.2.3.1. Forces involved in cell adhesion

According to Richards [49], cell adhesion to biomaterials is done thanks to focal adhesion sites which represent strict contact sites with the substrate in a so limited space. For fibroblasts, it has been shown the existence of a force called cohesion force responsible for keeping contact between cells themselves. However, this force is weaker than the adhesion force involved while a cell adheres to a biomaterial. This difference in force level depends on the cell type and on the nature of the biomaterial used for adhesion, and may explain the different ways of cell adhesion and spreading on different surface structures.

3.2.3.2. Surface free energy

Surface free energy is a thermodynamic measurement which contributes to the interpretation of the phenomena occurring in interfaces. It has an important effect on cell adhesion in the way that every change in its value induces the modification of the surface wettability, and therefore cell behaviour will be affected too [50, 51, 52].

Cell-biomaterial interface depends on the physico-chemical properties of the biomaterial and every change in the chemical composition or in the electric charge of the surface will affect its surface free energy.

3.2.4. Parameters involved in cell adhesion

3.2.4.1. Surface roughness

Surface roughness has been the subject of many studies as a deciding factor in the process of cell adhesion to biomaterials. Ponsonnet et al.[53] had studied the behaviour of fibroblast cells while adhering to titanium surface with different roughness; they found that cells had adhered to the surface using thin cytoplasmic structures. Indeed, these cells presented a flattened shape spreading practically over the substrate surface after adhesion to smooth surfaces. However, on rough surfaces, cell morphology was affected by the surface grooves and they were reoriented by the surface structure.

According to Richards [48], smooth titanium surfaces always increase fibroblasts adhesion and proliferation better than rough surfaces. They suggested that this kind of surfaces

should be a better candidate for biological implant thanks to their ability to resist to bacterial infections. Indeed, their weak roughness is unfavourable to the adhesion of bacteria.

3.2.4.2. The electric charge effect

In the majority of the studies carried out about biomaterials made from polyelectrolyte film, as in our case, the electric charge effect is in proportion with the thickness of the film built and depends on the charged functional group of the polyelectrolyte used [54].

For Andrade [25], the notion of the nature of an electric charge is important to be mentioned but its effect is not significant and doesn't induce an efficient change on surface wettability. However, it has been shown that a better adhesion of cells was observed on negatively charged polyelectrolyte [55]. In reality, most of the existed cells and their corresponding adhesion proteins are negatively charged. Nevertheless, this charge can be without any effect in the case when functional groups become able to control cell adhesion mechanism by their hydrophilic or hydrophobic character as it will be shown later in this text. Dubois [56] presumed that an electric charge trapped within an insulating biomaterial, none associated to a particular chemical group, is able to affect its biological environment. Moreover, Maroudas [57] revealed the dependence of cell adhesion and spreading on a solid surface on the surface charge of the substrate.

3.2.4.3. Chemical composition

The different chemical components of a biomaterial must be studied and known before to start investigating cell adhesion to that biomaterial. Therefore, this step is fundamental for concluding about the biocompatibility of a given biomaterial and its effect on cell adhesion [58].

The wettability of a surface depends on the chemical composition of the material and each change than can occur at this level will disturb cell adhesion process [59]. Besides the effect of the biomaterial, the adhered cell type plays an important role in adhesion. Indeed, for the same biomaterial surface, different cell reactions were observed for two types of cells [60]; this kind of biomaterial seems to be biocompatible with one cell type but not tolerated by the other cell type.

According to Marmur [61], most of the materials in the nature are rough and heterogeneous and contact angle may change along the contact line with a value depending on the roughness and heterogeneity level.

3.2.4.4. Surface hydrophilicity and hydrophobicity

Contact angle measurement allows us to calculate surface free energy [62]. It also allows knowing about the polar or non polar nature of the interactions at the interface liquid/ solid. Moreover, one can deduce from it the hydrophilic or the hydrophobic character of a surface [63].

A study about polyelectrolyte films found that hydrophobic interactions on a surface induce the adsorption of proteins and stabilise the complex formed [64]. Indeed, it has been proved that myoglobin or lysozymes are able to adhere to polystyrene sulfonate (PSS) and form many layers. However, this adhesion was not possible when using another surface having the same electric charge as PSS but with a hydrophilic character. The electrostatic interactions between the protein complex and this hydrophilic surface were easily destructed after water rinsing. Thus, surface hydrophilicity and hydrophobicity are a determinant parameter for substrate wettability on account of the rearrangement of the functional groups at the surface of a biomaterial in contact with a cell [65, 66, 67]. Indeed, it has been shown that fibroblast cells adhere and proliferate better on biomaterials with a moderate hydrophilicity [68, 69].

Andrade [66] presumed that, in the case of deformable materials, an elasticity model of 3.5 10^5 dyn/cm^2 is necessary for avoiding contact angle change. A roughness below 0.1 μm has a negligible effect on contact angle. Most of the materials holding over than 20 to 30 % of water present a receding contact angle (θ_r), in water, near zero because of the hydrophilic character which dominates the interface in these conditions. The same author estimated that the majority of polymers have a changeable volume which can be the reason for contact angle change: this change is depending on the duration of the contact with water, on the nature of the liquid and on the temperature of measurement. Non existent contact angle hysteresis may be due to the duration of contact between the material and the liquid which is shorter or longer than the measurement time needed for recording contact angle change. Therefore, surface hydrophilicity and hydrophobicity depends on the volume blowing of the material, on the diffusion phenomenon and on the mobility and reorientation of the molecules on the material surface.

Some materials are able to go out of shape in contact with a liquid depending on their mechanical properties and on their relaxation time and temperature. So, what characterizes a polymer is its chemical composition, roughness, mobility, wettability, surface free energy and its electric charge [70].

3.2.4.5. Surface wettability: Contact angle hysteresis

Contact angle hysteresis is the result of contact angle change between the surface we are characterizing and another ideal surface physico-chemically homogeneous. It's the direct result of a different sensitivity to the wettability process of heterogeneous surfaces. According to Rupp et al.[71], the receding contact angle value (θ_r) is under the control of the small hydrophilic particles of the surface which are able to disturb or to delay the non wettability process. Indeed, when the hysteresis remains constant after many immersion and emersion cycles it's called thermodynamic (or true) hysteresis. However, in the opposite case, it's called kinetic hysteresis (see Figure 7).

Thermodynamic hysteresis is due to the surface roughness and heterogeneity. Nevertheless, kinetic hysteresis is caused by the adsorption mechanisms (due to the liquid phase), surface polar group's reorientation and surface deformation [24].
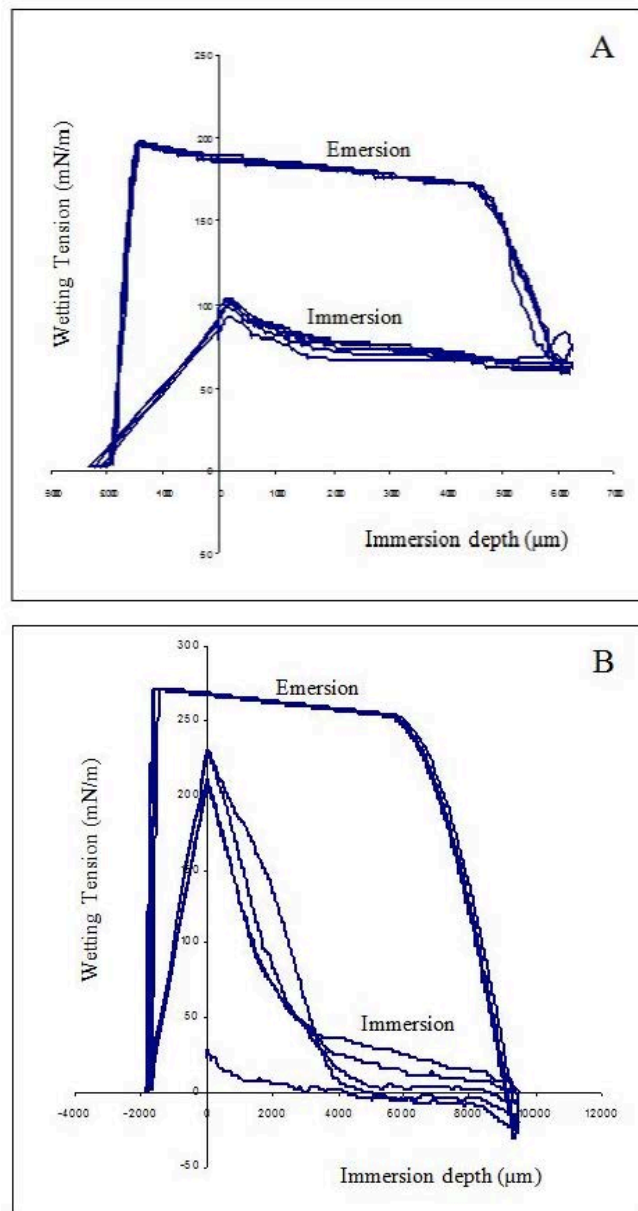
Figure 7. Immersion and emersion loops showing the two types of hysteresis: (A): thermodynamic hysteresis and (B): kinetic hysteresis. The sample is repeatedly immersed in the liquid leading to typical hysteresis loops. From each loop, wettability parameters (advancing and receding contact angle or wetting tension) can be calculated

Contact angle hysteresis is often assigned to the surface roughness and heterogeneity. Actually, a study made by Lam et al. [26], have shown that hysteresis is related to the molecules' mobility, the liquid diffusion and the surface swelling. These authors had observed a close dependence between the liquid molecules size and the liquid/material contact duration. Liquid resorption and retention are the direct causes of hysteresis. However, as the liquid surface free energy is higher that that of the material; therefore the liquid retention into the material will increase the material surface free energy and thus reduces the receding contact angle (θ_r). Indeed, liquids having smaller molecular

chains (or smaller molecular weight) diffuse faster into the polymer surface leading to an important decrease in contact angle.

According to Shananan et al.[72], contact angle hysteresis is related to the polymer polarity. Indeed, when a polymer gets in touch with a polar liquid (water), it orients its mobile polar groups on the surface in order to increase the interfacial water/polymer energy and therefore decreasing the system surface free energy. In the other hand, when the polymer is contact with a non polar liquid, its functional groups conserve their state and will not reorient. These authors assumed the existence of two parameters behind hysteresis: the intrinsic polarity of the material and the mobility of its polar groups on the surface. Nishioka et al.[73], had observed that the advancing contact angle hysteresis is under the control of surface sites more hydrophobic than those controlling the receding contact angle hysteresis.

The contact angle hysteresis observed on hydrophilic and hydrated polymers is due to the polar groups' orientation on the interfaces polymer/liquid and polymer/air. This reorientation represents the polymer reaction to every environmental change (air, liquid). The receding contact angle (θ_r) depends on the contact duration with water, the environment temperature and on the glass transition temperature (T_g) of the material itself. Each material has its own glass transition temperature (T_g) allowing a defined molecular mobility sufficient for an important rearrangement [74].

3.3. Conclusion

The concepts of solid surfaces assumed that the surfaces in question were effectively rigid and immobile. Such assumptions allow one to develop certain models and mathematical relationships useful for estimating and understanding surface energies, surface stresses, and specific interactions, such as adsorption, wetting, and contact angles. It is assumed that the surfaces themselves do not change or respond in any specific way to the presence of a contacting liquid phase, thereby altering their specific surface energy [75]. Although such assumptions are (or may be) valid for truly rigid crystalline or amorphous solids, they more often than not do not apply strictly to polymeric surfaces.

In contact with condensed phases, especially liquids, surface relaxations and transitions can become quite important leading to a possible dramatically change in the interfacial characteristics of a polymer with possibly important consequences in a particular application. And since the processes are time-dependent, the changes may not be evident over the short span of a normal experiment. For critical applications in which a polymer surface will be in contact with a liquid phase, such as implant device for biomedical application, it is not only important to know the surface characteristics (e.g., coefficient of friction, adhesion, adsorption)under normal experimental conditions but also to determine the effects of prolonged (equilibrium) exposure to the liquid medium of interest. It is therefore important for biomedical as well as many other applications that the surface characteristics of a material of interest be determined under conditions that mimic as closely as possible the conditions of use and over extended periods of exposure to those conditions, in addition to the usual characterizations.

4. Experimental and results

4.1. Polyelectrolyte multilayer film preparation

Before use, glass slides were cleaned in 0.01 M SDS and then in 0.1 N HCl, both for 10 min in a boiling water bath, followed by a pure water rinse. Polyelectrolyte solutions were prepared by dissolution of the polyelectrolyte powders in 0.15 M NaCl (using ultrapure water filtered with a MilliQ system, Millipore) at a concentration of 1mg/l for PLL, PGA and HA and 5 mg/l for PEI, PSS and PAH. For all the films, the precursor layer was always PEI (polycation), followed by the alternate adsorption of polyanions/polycations for 12 min adsorption times and two rinses in the 0.15 M NaCl solution [76]. The glass slides held in a slide holder were dipped into the different polyelectrolyte baths for the preparation of three different types of film, ending either by the polycation or polyanion: $(PSS/PAH)_{10}$, $(PSS/PAH)_{10}$–PSS; $(PGA/PLL)_5$, $(PGA/PLL)_5$–PGA; and $(HA/PLL)_5$, $(HA/PLL)_5$–HA. Cleaning was made before film characterization. The films were all prepared at the same pH before being in contact with culture medium. Poly(styrene-4-sulfonate) (PSS,MW=70 kDa), Poly(allylamine hydrochloride) (PAH,MW=70 kDa) and Poly(ethyleneimine) (PEI,MW=70 kDa) are purchased from Aldrich. Poly(l-lysine) (PLL, MW=32 KDa) Poly(l-glutamic acid) (PGA, MW=72 KDa) were obtained from Sigma and Hyaluronan (HA,MW=400 kDa) from Bioiberica. Sodium dodecyl sulfate (SDS) was purchased from Sigma and sodium chloride (NaCl, purity ~ 99%) from Aldrich, glass slides (18x18 cm^2 square and 14x14 cm^2 disk), respectively, were obtained from CML, France.

4.2. Contact angle measurement and Surface Free Energy (SFE) calculation

The measurements were performed with a Wilhelmy balance for the characterization of solids using the 3S tensiometer and the corresponding software (GBX, France). For these experiments, the glass slides were coated with polyelectrolyte multilayer films on both sides. Before beginning the measurements, the films were washed in 18.2 MΩ Millipore water for 30–45 min in order to eliminate the NaCl traces that could modify the results. Samples were then dried at 30 °C for 2 h. The dynamic contact angle hysteresis was determined at 20°C for each film and five wetting/dewetting cycles were carried out at a 50 μm/s speed.

Three liquids were used as a probe for surface free energy calculations: diiodomethane, formamide (Sigma Chemical CO, St Louis, MO, USA) and distilled water. The final contact angle used for this calculation was the average of the 2nd to 5th cycle advancing contact angle (θ_a) and the surface free energies of the different films were calculated using the Van Oss (VO) approach, as usual with sessile drop method contact angles:

$$\gamma_S = \gamma_S{}^d + 2\,(\gamma_S{}^+ . \gamma_S{}^-)^{\frac{1}{2}}$$

This method produces the dispersive ($\gamma_S{}^d$) and the polar acid–base ($\gamma_S{}^+$, $\gamma_S{}^-$) components. Solid and liquid SFE components and contact angle are related according to the equation below:

$$\gamma_L\,(1 + cos\theta) = 2\,((\gamma_S{}^d . \gamma_L{}^d)^{\frac{1}{2}} + (\gamma_S{}^+ . \gamma_L{}^-)^{\frac{1}{2}} + (\gamma_L{}^+ . \gamma_S{}^-)^{\frac{1}{2}})$$

Were γ_L is the SFE of the liquid and γ_s the SFE of the surface.

4.3. Cell adhesion, viability and morphology study

For adhered cell counting, image analysis was performed on a Quantimet 570 (Leica, UK) fitted to an epifluorescence microscope (Axioplan, Zeiss, DE) and a black-and-white charge-coupled device (CCD) camera (LH51XX-SPU, Lhesa Electronique, FR). The scanning was carried out using a ten times lens (NA=0.3) and a filter set adapted for propidium iodide fluorescence observation (BP 546/12 nm, DM 580 nm, LP 590 nm). Microscope focus and stage were motorized and software controlled.

The cell viability was determined with the MTT colorimetric assay. It was measured at 570 nm with a 96-well microplate reader (Becton Dinkinson, Lincoln Park, USA) on a spectrophotometer (Bio-Tek Instruments, Winooski, USA). The blank reference was taken for wells containing only the MTT solution.

The morphology of the cells was analyzed after 120 min (day 0), 2 and 7 days of culture using a scanning electron microscopy (Philips, EDAX XL-20) and phase contrast microscopy.

4.4. Results

4.4.1. Contact angle measurement

The different contact angle values found are shown in Table 1. Experiments were performed at 20 °C at a speed of 50 μm/s. One can observe that contact angle depends on the film's nature (physico-chemical composition) which differs from a polymer to another.

	Water	Formamide	Diiodomethane
Glass	43 ± 2	23 ± 3	43 ± 3.1
(HA/PLL)$_{10}$	12 ± 2	00	00
(HA/PLL)$_5$	81.9 ± 1.8	49.6 ± 2	43.5 ± 3
(HA/PLL)$_5$-HA	87.8 ± 1.2	00	45 ± 2.9
(PGA/PLL)$_5$	55.2 ± 3	14.7 ± 2.5	39.1 ± 1.2
(PGA/PLL)$_5$-PGA	44.1 ± 3.1	00	40.7 ± 1.6
(PSS/PAH)$_{10}$	49.2 ± 1.8	23.6 ± 3	00
(PSS/PAH)$_{10}$-PSS	53 ± 1.9	12.1 ± 3.3	00

Table 1. Dynamic contact angle

4.4.2. SFE values

SFE and its component's values are summarized in Table 2. (HA/PLL) films have the lowest SFE value and (PSS/PAH) films have the highest value. The outermost layer of the film does not have a great influence.

	$(HA/PLL)_5$	$(HA/PLL)_5$-HA	$(PGA/PLL)_5$	$(PGA/PLL)_5$-PGA	$(PSS/PAH)_{10}$	$(PSS/PAH)_{10}$-PSS	Glass	Thermanox
Surface Free Energy (mN/m)	42.4	44.95	53.3	48.26	57.1	58.9	50	35
Dispersive component (mN/m)	38.8	37.1	40.23	39.5	50.8	50.8	35	36
Acid component (mN/m)	1.2	11.85	3.56	9.46	0.46	1.7	3	3
Basic component (mN/m)	2.86	1.3	14.6	2.4	23.76	7	25	0
Acid-basic component (mN/m)	3.56	7.8	13.9	8.86	6.3	8.1	17	1

Table 2. Surface Free Energy (SFE) and its components for the different films used. The SFE of PSS/PAH is higher compared to the other films.

4.4.3. Cell adhesion

Figure 8 shows the percentage of fibroblasts that have adhered after 2 h in culture. The highest adhesion is found with $(PGA/PLL)_5$ film (95%) and the lowest on $(HA/PLL)_5$ film (49%).
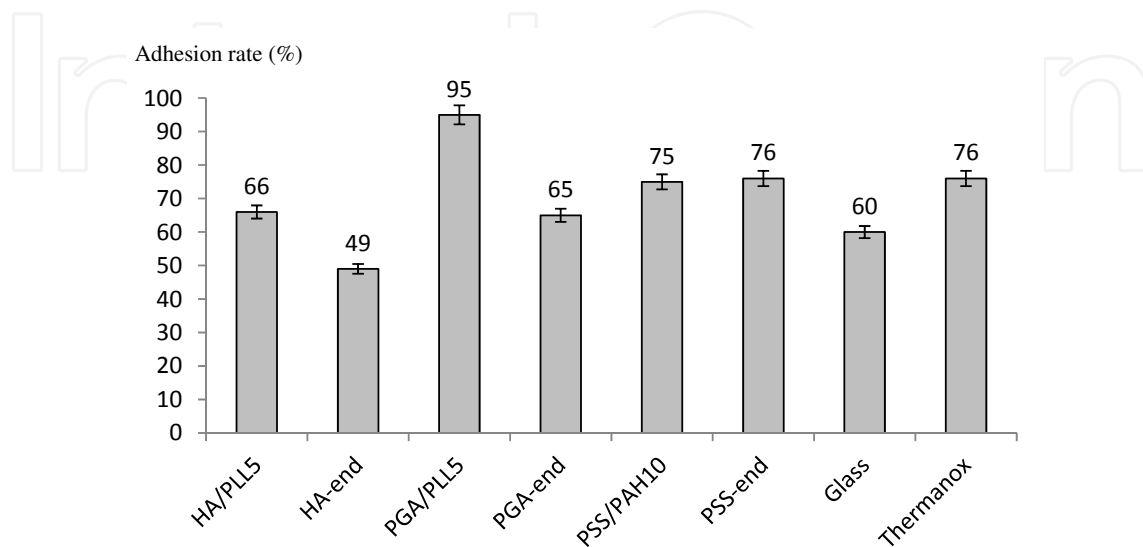
Figure 8. Fibroblast adhesion rate after 2 h in culture onto different films. The percentage represents the number of the adhered cells compared to the initial number of seeded cells.

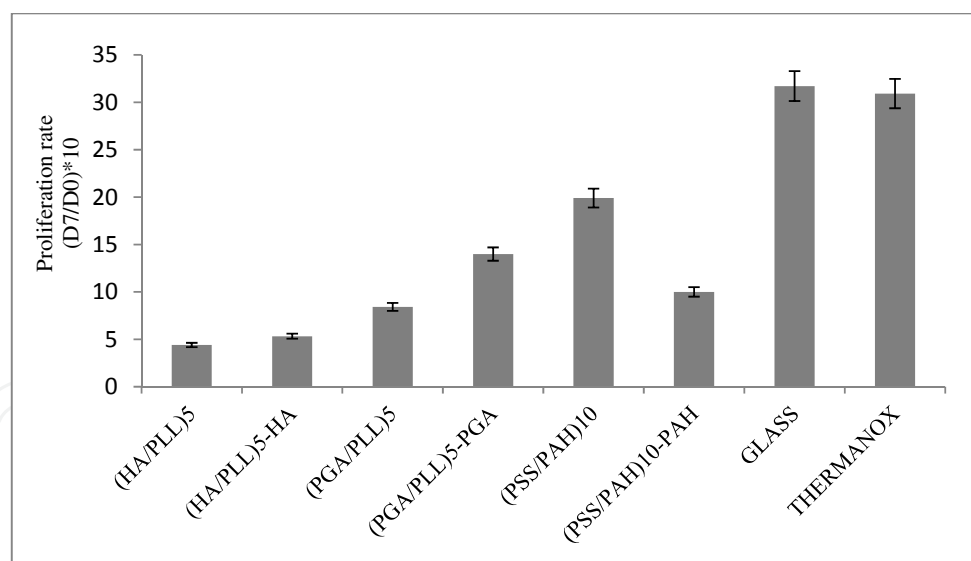### 4.4.4. Cell viability and proliferation rate

Cell viability was evaluated on the different types of film at different time intervals (0, 2 and 7 days) with the MTT assay (Figure 9A). The $(PGA/PLL)_5$–PGA films exhibited a good proliferation rate (Figure 9B) and the $(PSS/PAH)_{10}$ films were the most favorable to cell proliferation.

4.4.5. Cell morphology

Good adhesion is observed on $(PGA/PLL)_5$ film (Figure 10A) whereas bad adhesion was found on $(HA/PLL)_5$-HA film (Figure 10B). Typical morphology at day 2 on a $(PGA/PLL)_5$–PGA film is presented in Figure 10C. After seven days in culture, the difference in morphology for the cells that had adhered to the different films was even more striking. Cells in contact with $(HA/PLL)_5$–HA exhibit necroses (Figure 11A) whereas the cells exhibit elongated and spread morphologies on the highly proliferative $(PSS/PAH)_{10}$ films (Figure 11B).
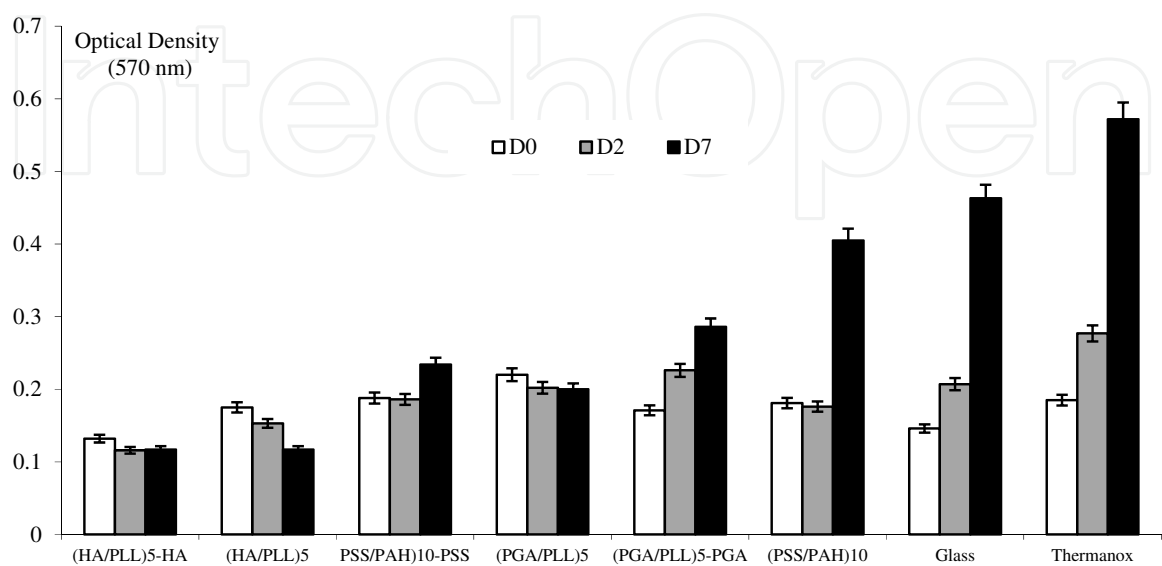
A

B

Figure 9. A. Cell viability (MTT test) on each film type followed over a seven day period at: day 0 (D0), day 2 (D2), and day 7 (D7), B. Proliferation rate on the different films as estimated by the ratio (D7/D0)
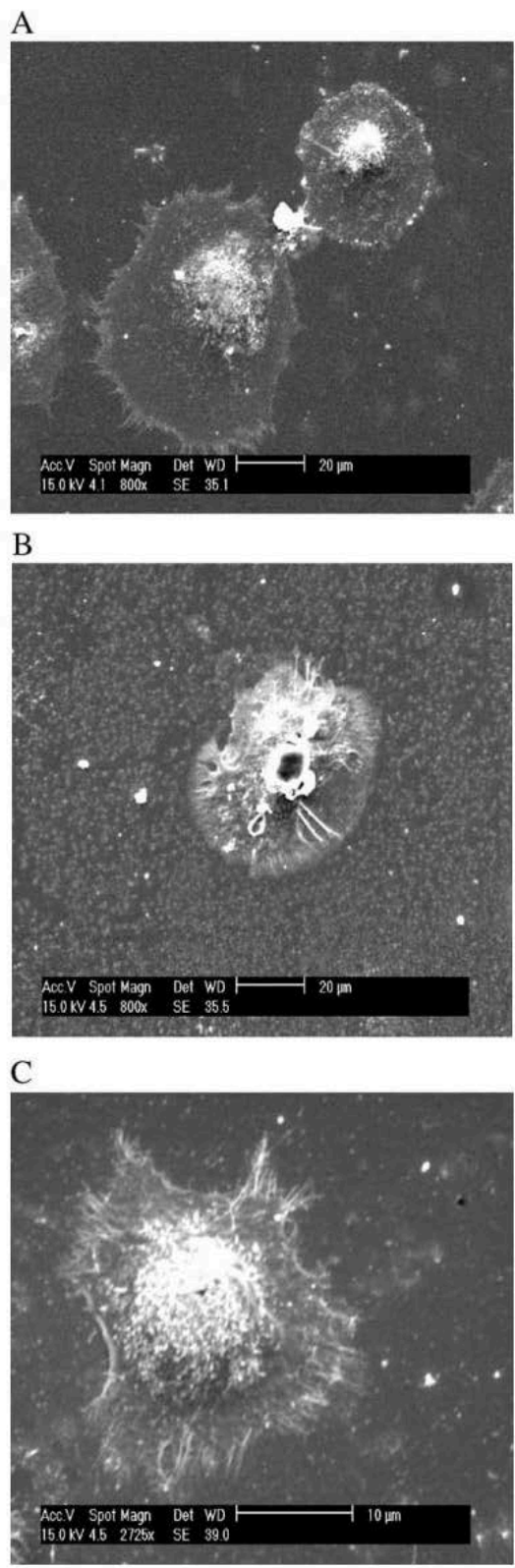
Figure 10. SEM images of cells adhering to different polyelectrolyte multilayer films. (A) (PGA/PLL)5(x800) on the first day, (B) HA/PLL$_5$–HA (x800) on the first day, (C) (PGA/PLL)$_5$–PGA film observed on the second day (x2725).
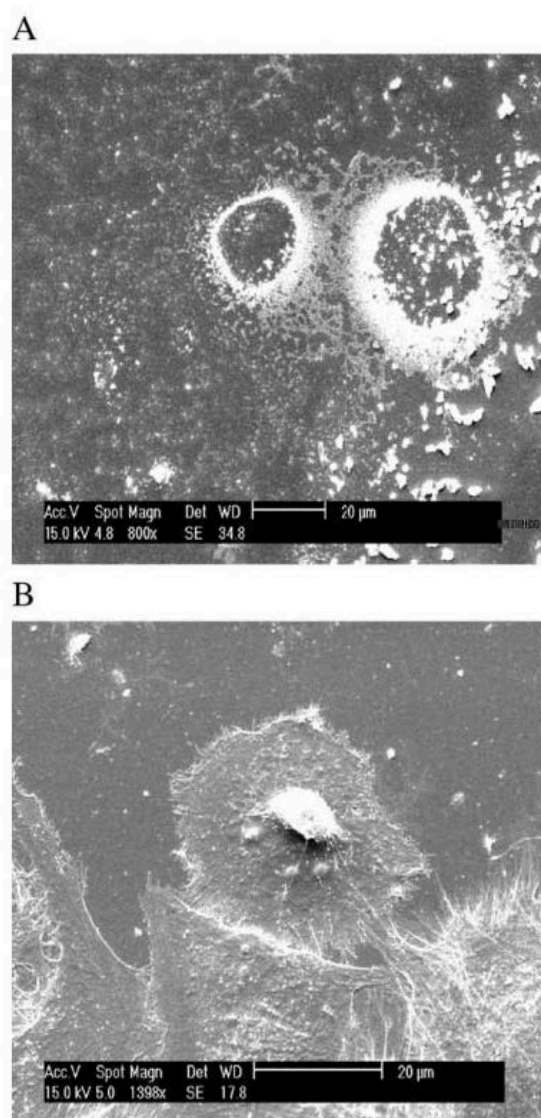
Figure 11. SEM images of cell morphology after seven days of culture. (A) (HA/PLL)$_5$–HA (x800) film, a (PSS/PAH)$_{10}$ film observed at different magnifications (B) (x1398): some areas are at confluency.

4.4.6. Correlation between cell adhesion and films SFE

No correlation was found between the wettability parameters or the SFE parameters and the fibroblast proliferation ratio. However, the adhesion rate at 2 h was correlated to both SFE basic component and the SFE acid component (Figure 12). For the adhesion rate, the SFE ba-

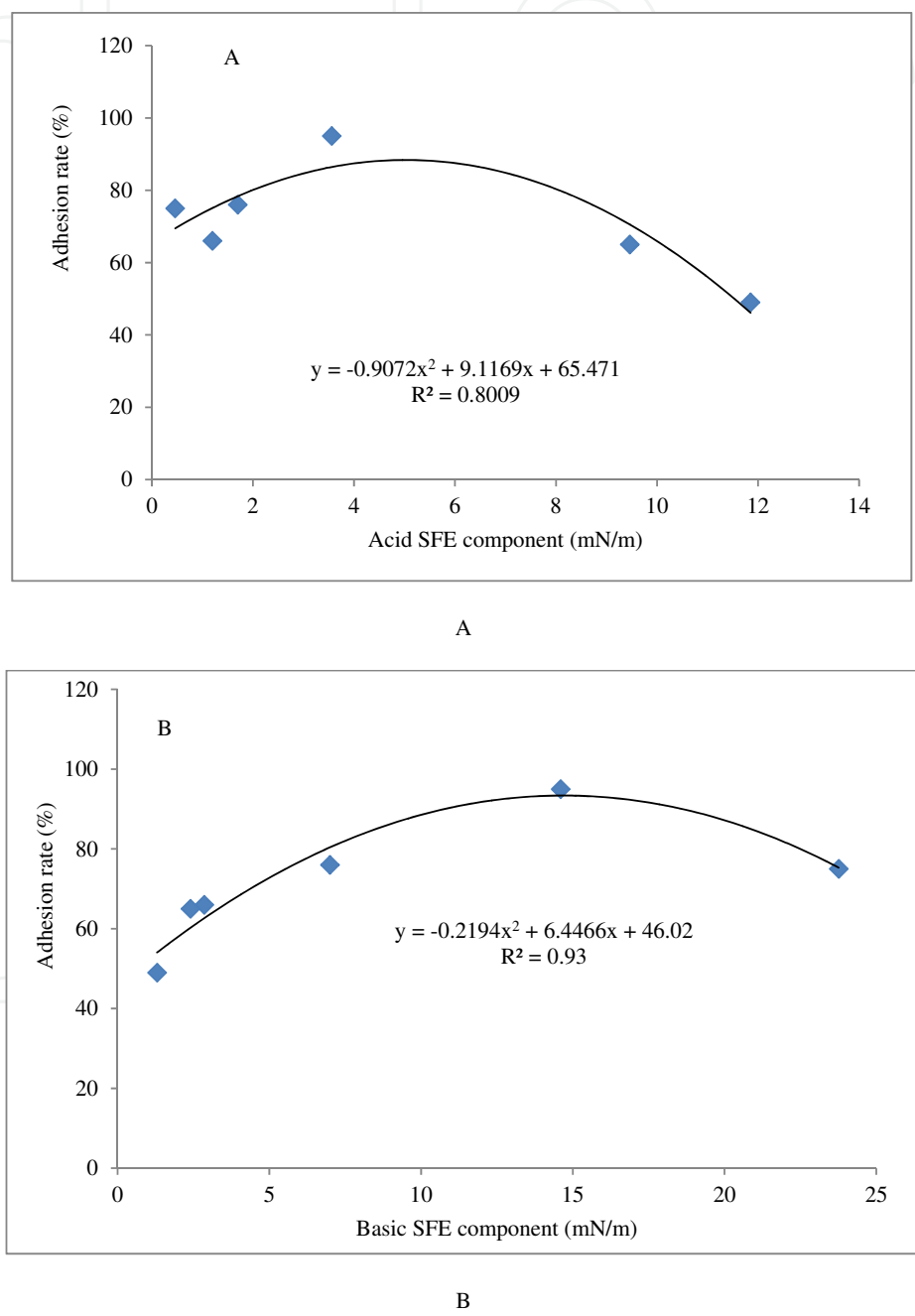sic component is optimum at 15 mN/m (Figure 12A) whereas the acid one is optimum at about 5 mN/m (Figure 12B).

A

B

Figure 12. SEM images of cell morphology after seven days of culture. (A) (HA/PLL)$_5$–HA (x800) film, a (PSS/PAH)$_{10}$ film observed at different magnifications (B) (x1398): some areas are at confluency. Figure 12B. Correlation between cell adhesion rate and Basic SFE component. An optimum is found for 15 mN/m with good polynomial correlation (R^2=0.93)

4.5. General conclusion

Cell adhesion is a paramount parameter for the biomaterial tissue. These biomaterials, by their surface properties (chemical composition, topography, roughness, surface energy) hold the key of the control of the cell adhesion, proliferation and orientation. Thus, the concept of biocompatibility is seen imposed, it is primarily focused on the interface, sites of the interactions between cells and biomaterials.

The influence of different polyelectrolyte multilayer films (PEM) on gingival fibroblast cell response was studied. Roughness and hydrophobicity/hydrophilicity of the PEM were characterized by contact angle measurement. Polar (acid-basic) components of the surface free energy (SFE) were determined. Surface advancing and receding angles were measured and hysteresis was determined. Cell adhesion, viability and morphology were analyzed.

This work pointed out that cell adherence is a complex process modulated by numerous parameters. Usually, in cell adherence studies and particularly in biomaterial approaches, surface physico-chemical properties are analysed (chemistry, roughness, motility, wettability…).

In our work we tackled the subject of the cellular behavior in contact with a biomaterial by the characterization of the surface of this material. We were interested in physical (topography) and chemical (composition) properties of various polyelectrolyte multilayer films deposited on glass slides, with different charge densities scale and thickness. We have evaluated the wettability of theses biomaterials by measuring the contact angle hysteresis using the Wilhelmy balance tensiometry to study their physico-chemical characteristics in order to understand the effects of surface roughness and chemistry on the fibroblasts behavior. Epifluorescence microscopy, SEM, phase contrast microscopy and MTT test were used to study cell adhesion, proliferation and morphology in order to correlate the film's properties and the cultivated cells response.

Surface hydrophobicity and roughness were found to be unfavourable for both adhesion and proliferation. Adhesion and proliferation were found not to be correlated.

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