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Contributions of Surface and Interface Physical Chemistry to Biology

Ahmed Hamraoui

Université Paris Cité, CNRS UMR 8003, Saints-Pères Paris Institute for the Neurosciences, 45 rue des Saints-Pères, 75006 Paris, France; ahmed.hamraoui@u-paris.fr

Abstract: Understanding and controlling cell-implant interactions is crucial for enhancing biomaterial performance and reducing post-implantation complications. Among the diverse surface properties that influence these interactions, surface wettability has emerged as a critical factor affecting cell adhesion, migration, proliferation, and differentiation. This review emphasizes the strong correlation between wettability and cell behavior on adhesion surfaces, highlighting its potential as an accessible and effective parameter for surface characterization. Wettability experiments are relatively simple to conduct, offering practical entry points for investigating surface properties. However, misinterpretations of these measurements can lead to erroneous conclusions, especially when directly linking cell responses to surface energy without accounting for other influencing factors. This review aims to bridge the gap between physical chemistry and biological sciences by providing a comprehensive yet accessible resource for biologists and researchers unfamiliar with surface and interface science. By elucidating the principles of wettability, surface energy, and their role in governing cell behavior, we offer insights that help ensure accurate interpretation of experimental results. This understanding prevents oversimplified correlations and fosters more nuanced experimental designs. Additionally, the review outlines key experimental techniques and their relevance to biological responses, enabling researchers to optimize surface properties for biomedical applications. By integrating interdisciplinary knowledge, we not only clarify the molecular mechanisms underlying cell-surface interactions but also empower biologists to leverage these insights in designing advanced biomaterials and implants. This approach ensures meaningful advancements in biomedical science while avoiding pitfalls from incomplete or incorrect interpretations.

Keywords: wettability; cell adhesion; surface properties; biomaterials; surface energy; cell-surface interactions

1. Introduction

Defining and controlling the interface between cells, tissues, and implants is a cornerstone challenge in modern biomedical and bioengineering research. Since the pioneering studies on cell-surface interactions[1–5], it has been firmly established that the physical and chemical properties of a substrate can profoundly influence cellular behaviors, including adhesion, survival, proliferation, differentiation, cytoskeletal organization, and migration. These interactions not only govern fundamental biological processes but also underpin a wide array of applications in medicine, biomaterials science, and surgery.

For example, surface roughness and wettability play a pivotal role in implant integration. In orthopedic research, nanoscale surface modifications of titanium implants have been shown to enhance osteoblast attachment and differentiation, significantly improving osseointegration. Similarly, hydrophilic coatings on cardiovascular stents reduce platelet aggregation and thrombus formation, minimizing the risk of post-implant complications. Such findings exemplify how the careful design of surface properties can address clinical challenges.

Beyond implantology, cell-surface interactions are critical in tissue engineering and regenerative medicine. Recent studies have demonstrated that hydrophilic polymeric scaffolds can support epithelial cell adhesion and proliferation, making them promising candidates for wound healing applications. In my research, I have investigated how functionalized polymer films can guide neural stem cell differentiation, offering potential pathways for designing advanced neural interfaces and treatments for neurodegenerative diseases.

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In our research, we demonstrated the potential of functionalized surfaces in guiding the differentiation of PC12 cells, a neuronal model. For instance, chemically modified polystyrene substrates promoting specific ligand presentation have successfully enhanced the differentiation and neurite outgrowth of PC12 cells. This observation underscores how surface engineering enables precise manipulation of cellular responses, facilitating applications in neural tissue repair and neuroprosthetics.

Moreover, the study of cell-surface interactions is indispensable in understanding disease mechanisms. For instance, the role of substrate stiffness and topography in influencing metastatic cancer cell migration provides insights into tumor progression and invasion. Such work has implications for developing accurate in vitro models and testing anti-cancer therapies.

The interdisciplinary nature of this field bridges fundamental biology, physical chemistry, and applied science. Advances in surface engineering have enabled the creation of "smart" biomaterials, such as coatings that release growth factors upon cell adhesion or antimicrobial agents in response to bacterial colonization. These innovations not only improve the performance of biomedical devices but also expand their functional versatility.

This article aims to synthesize current knowledge on cell-surface interactions, offering a resource that bridges physical chemistry and biology. By highlighting both the fundamental principles and practical applications, this work underscores the transformative potential of surface engineering in implant design, tissue engineering, and therapeutic development.

Table 1. Definitions of adhesion phases from different authors, specifying cell lines and phases of the adhesion process.

Author	Cell Line	Phases of Adhesion Process
P. Clark, P. Connolly, A. S. Curtis *et al.*	Murine macrophages	Topographical control of macrophage activation through interaction with grooved substrata.
B. Wójciak-Stothard, A. Curtis *et al.*	Murine macrophages and fibroblasts	Activation by surface topography; role of cytoskeleton in cellular response to grooves.
L. Chou, J. D. Firth *et al.*	Human fibroblasts	Regulation of fibronectin mRNA stability, secretion, and assembly due to surface topography.
G. Dunn, A. Brown	Fibroblasts	Alignment of fibroblasts on grooved surfaces using geometric transformations.
A. Webb, P. Clark *et al.*	Oligodendrocytes and progenitor cells	Guidance of cells by substratum topography, influencing shape and differentiation.
A. Cooper, H. Munden, G. Brown	Mouse neuroblastoma cells	Growth orientation control using thin films of silicon monoxide.
J. Meyle, K. Gultig *et al.*	Human fibroblasts	Variation in contact guidance on microstructured surfaces.
C. Oakley, D. Brunette	Epithelial cells	Responses to substratum topography, affecting cluster formation and alignment.
D. Brunette, G. S. Kenner *et al.*	Human gingival explants	Orientation and migration of cells on grooved titanium surfaces.
E. T. D. Braber, J. E. de Ruijter *et al.*	Fibroblasts	Effects of microgrooved surfaces and surface energy on cellular growth and orientation.

2. Surface Energy and Its Role in Cell Behavior

The physical-chemistry of surfaces is defined as the study of phenomena that occur at the interface of two phases [6]. The notion of surface tension was introduced by Thomas Young[7] and Pierre-Simon Laplace, while the idea of free surface energy was suggested by Josiah W. Gibbs [6,8].

2.1. Relevance to PC12 Cell Differentiation

Surface energy directly affects cellular behavior by modulating the adsorption of proteins and other biomolecules, which in turn dictate cell adhesion and signal transduction. In our research, we explored how engineered surfaces influence PC12 cells, a model system for studying neuronal differentiation. Functionalized polystyrene surfaces with controlled surface energy demonstrated significant impacts on neurite outgrowth and differentiation efficiency. By fine-tuning the energy

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and chemical composition of these surfaces, we achieved enhanced neuronal responses critical for applications in neural tissue repair and neuroprosthetics.

Specifically, surfaces with hydrophilic functional groups facilitated the adsorption of differentiation-promoting extracellular matrix proteins. These proteins mediated integrin signaling pathways that triggered cytoskeletal rearrangements essential for neurite elongation. Examples from our publications include:

2.1.1. Recent Findings on Surface Energy Gradients

Our work[9] demonstrates that PC12 cells not only respond to the surface energy landscape but also show remarkable sensitivity to nanoscale chemical heterogeneities. Self-assembled monolayers of alkylsiloxanes on glass were used to modify the culture substrate. By altering the structure, ordering, and chemical composition of these monolayers, we engineered varying surface energy spatial distributions. While both well-ordered CH_3-terminated substrates and bare glass (OH-terminated) substrates did not support PC12 adhesion, highly disordered CH_3/OH substrates promoted strong adhesion and rapid neuritogenesis within 48 hours, even in the absence of nerve growth factor. These findings reveal that surface free-energy gradients, derived from nanoscale chemical heterogeneities, are pivotal in biological processes such as nerve regeneration and neuronal differentiation on biomaterials.

By systematically studying these interfaces, we highlighted the broader implications of surface energy in directing cell behavior. These insights form the basis for designing advanced biomaterials capable of eliciting specific biological responses in neural regeneration and beyond.

2.1.2. Chiral Surfaces and Neuronal Behavior

Chiral molecular functionalization of surfaces has emerged as a compelling strategy to direct cellular behaviors, particularly in neural tissue engineering. Recent studies highlight how chiral configurations influence protein adsorption, cellular adhesion, and downstream signaling. For instance, surfaces functionalized with D-glutamic acid were shown to enhance neurite outgrowth in PC12 cells significantly more than their L-glutamic acid counterparts. This chirality-dependent effect is hypothesized to arise from selective interactions with adhesion molecules and receptors, which trigger signaling cascades critical for neuronal differentiation and growth cone extension[10].

Additionally, chiral surface designs have been applied to guide the differentiation of PC12 cells into neuron-like phenotypes under controlled conditions. These surfaces influence growth cone navigation and axonal elongation, critical for reconstructing neural circuits. For example, functionalized carbon nanotube hydrogels with chiral polymer coatings improved neurite elongation and neuronal marker expression compared to non-chiral surfaces[11,12].

Another recent study found that PC12 cells exhibited enhanced differentiation on nanostructured hydrogels integrating topographic and chiral chemical cues. These surfaces induced a two-fold increase in neurite length and elevated expression of neuronal differentiation markers, such as GAP)-43 and synapsin-1[12].

Together, these findings underline the profound impact of molecular chirality in modulating cellular behavior. By leveraging the stereochemical preferences of neural cells, chiral surfaces open pathways for innovative approaches in neural repair and regeneration.

2.2. Surface Tension and Its Fundamentals

Surface energy is a critical property that governs the behavior of materials at interfaces, influencing how surfaces interact with their environment. For biomaterials, this property plays a significant role in determining their compatibility and effectiveness in biological applications. Molecules at a material's surface experience unbalanced forces due to fewer cohesive interactions compared to those in the bulk. This imbalance manifests as surface free energy J/m^2, a thermodynamic property that influences interactions at the molecular and cellular levels.

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In biological systems, this property underpins cell-material interactions, including adhesion, proliferation, and differentiation. Creating new interfaces requires energy, proportional to the area (A) and surface tension (γ), as expressed in the equation:

$$dW = \gamma dA$$

Here we start by presenting a brief review of the concept of surface energy (or surface tension), define the liquid or solid surface tension and how they vary with the state of the surface and the relationship between them. Any liquid or solid consisting of molecules, atoms or ions are linked to each other by a more or less intense cohesive force ensuring the cohesion of the material. On the surface there are a number of free (unsaturated) bonds, and this confers on the constituent particles of the surfaces a state of energy and cohesion stresses different from that of the elements constituting the bulk. The elements of the surface constitute a superficial phase, the state of the molecules or atoms on the material surfaces is radically different from that of the molecules or atoms in the bulk, material on a surface is in a tense state, that is to say subjected to a stress. At the surface, molecules has less nearest neighbors and excess unsaturated bonding compared to those in the bulk phase. Unbounded molecules have higher potential energy than those that form bonds. This thermodynamic quantity which describe the state of equilibrium of atoms or molecules in the surface layer of materials, is called surface free energy, in other words the surface tension describes the unequal distribution of forces between the bulck of the material and its surface. Because of this unbalance between attractive forces, liquids get spherical shape that corresponds to the minimum surface area. The surface free energy is an intrinsic characteristics for each substance or material and is measured in the energy per area units, J/m^2. To create an interface, energy should be applied which is equal to the product of the area of the new interface by the interface free energy. The free energy of the formation of stable interface it is required to be positive. The work necessary for increasing isothermally the surface area by dA is:

$$dW = \gamma dA \tag{1}$$

We can consider γ as the amount of energy when the surface area is increased per unit area or for separating two phases in equilibrium for creating a new surface unit. The excess free energies of interfaces can also be considered as surface tension forces. These forces are applied to the triple line (three-phase contact line) and oriented toward the corresponding interface. In the original work of Young [7], his equation was formulated in early 1800s from equilibrium of forces acting at the three-phase contact line, after the thermodynamic concept of free surface energy was introduced in the second half of the 19th century by principally Helmholtz and Gibbs [6,8]

2.3. Liquid-Fluid Interface

The interface, which is also a superficial phase, is a frontier between two thermodynamically stable phases. A liquid surface can be described as having an elastic envelope which renders the liquid capable of obtaining a shape with a minimum surface area. Each element, atom or molecule of the interface has an excess free energy, and then the expansion of the contact zone (the interface) between two phases is strongly unfavorable and, in the absence of external influences, the shape of a drop of liquid is spherical (see subsection 2.2). For a curved interface (bubble) of radius R, varying the radius of the bubble for a small variation dR, this would result in both a surface change of $8\pi R dR$ and the volume of $4\pi R^2 dR$. If pressure inside the bubble extends the outside pressure by ΔP, the work of pressure is given by $4\pi R^2 dR\,\Delta P$, whereas the change of the surface energy is given by $8\pi R dR\,\gamma_{\ell v}$. The surface is at mechanical equilibrium if these energy changes are equal, that is, $\Delta P = \dfrac{2\gamma_{\ell v}}{R}$. In the general case, the surface is not necessarily spherical, and the pressure change along the curved interface is given by the Laplace equation [6,8].

$$\Delta P = \gamma_{\ell v}\left(\frac{1}{R_1} - \frac{1}{R_2}\right) = \gamma_{\ell v}\, C \tag{2}$$

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with C is the curvature of the interface.

2.4. Solid-Fluid Interface

The interface between a solid and its vapour or a gas is characterized by its surface free energy. Classically the Zisman method [13–15] is used to estimate the solid surface energy. We will discuss below, about this method. The Zisman plot [13–15] is an empirical technique, generally used by engineers in industry laboratories, or researchers to estimate largely the surface energy of solids. This is a default estimate of the surface energy of solids. Then, from Zisman plot, which consist of a plot of the cosine of contact angle against liquid-gas surface tension, we define the critical surface tension, γ_c. Fox and Zisman observes that $\cos\theta = f(\gamma_\ell)$ is often linear and complete wetting is achieved if $\gamma_\ell \leq \gamma_{critic}$. The critical surface tension is the surface tension of the liquid that switch exactly to zero contact angle (or $\cos\theta = 1$) with the solid. It is important to note that the linearity observed by Fox and Zisman is obtained using a homologous series of alkanes.

Now let us discuss about the linearity of the function $\cos\theta = f(\gamma_\ell)$. The origin of this linearity is coming from the approximation of the Good-Girafalco equation [16] :

$$\cos\theta = -1 + 2\sqrt{\frac{\gamma_c}{\gamma}}$$

and the first-order approximation around γ_c gives:

$$\cos\theta \approx 1 - \frac{\gamma - \gamma_c}{\gamma_c}$$

On the one hand, the "critical surface tension" exists for any solid, brought into contact with a series of liquids, on the other hand it is not related to the free surface energy of the solid often in a simple manner. The critical surface tension γ_c was calculated using the Fox-Zisman approximation. In this work, it can be understood as a first-order approximation of the Good-Girifalco equation[17], for a surface tension of the liquid $\gamma_L(\gamma_L \geq \gamma_c)$ close to the γ_c of the solid. Using a linear regression analysis, Zisman plots, $\cos\theta = f(\gamma)$, were traced for each substrate by fitting the data obtained with the test liquids. γ_c values were read where the line fits intersect $\cos\theta = 1$, as described by Zisman[15].

2.5. Contact Angle

At the solid-liquid interface, the contact angle is an macroscopic angle at the triple-phase line, between the tangents at the two surfaces (Fig.1a). concretely when a drop of liquid is deposited on a solid surface, if the liquid does not wet the solid, the liquid drop takes on a lens shape and a finite contact angle is observed then $\gamma_{sv} < \gamma_{s\ell} + \gamma_{\ell v}$. Or it may that the liquid spread indefinitely over the solid surface, when the liquid completely wet the solid with a macroscopic uniform liquid layer covering the whole solid surface. From the Young relation (Eq (4)) the condition for equilibrium requires that, in the absence of gravity effects the contact angle between the liquid and the solid surface is given by :

$$\cos(\theta_e) = \frac{\gamma_{sv} - \gamma_{s\ell}}{\gamma_{\ell v}} \tag{3}$$

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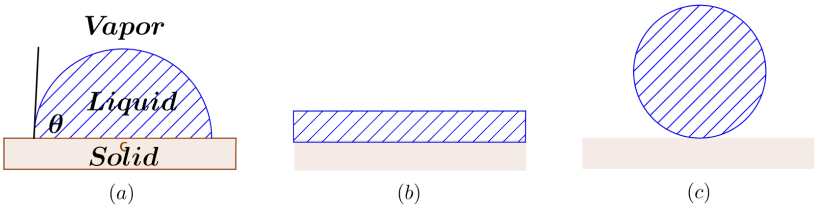

Figure 1. Three wetting situations which can a liquid drop placed on a solid surface. In (a) $\theta = 0$: complete wetting situation, the vapor is excluded from the contact to the solid. The situation (b) is called partial wetting, where $0 < \theta < \pi$. The other limite situation is (c) it called non wetting situation, or one can say the vapor wett completely the liquid-solid interface.

When the liquid droplets adhere to surface their macroscopic shape are determined by the surface and interracial energies. The contact angle, is the primary parameter which characterizes wetting of any immiscible phases at least one of which is a liquid, it depends on several parameters other than thermodynamic factors, such as surface state, preparation its cleanliness, roughness and electrostatic charges[6,18]. The contact angle gives the ratio of the energy gained in forming unit area of the solid-liquid interface to that required to form unit area of the liquid-vapor interface. One can also see it as the ratio between the adhesion and cohesion forces. The adhesive forces tend to spread the liquid over the solid surface while the cohesive forces tend to *curled up* the liquid on itself.

In order to avoid any misinterpretation and any mislead of its significance properly, contact angle measurements and their interpretation depend heavily on the procedure, the methodology and the conditions under which the measurements were made [19].

2.6. Wetting

Surface wettability plays a fundamental role in regulating the cell behavior on surface of biomaterial, and several studies in the literature have shown that moderately wettable surfaces favor the adhesion and growth of cells [20–24]. The term wetting covers all phenomena that occur when a liquid is brought into contact with a solid or with an immiscible liquid. We will focus here only on the solid-fluid interface. The first theoretical works are attributed to Young [7] and Laplace [25] and dates back to the late 19th and early 20th century, and many studies are still being undertaken in this area of static and dynamic wetting.

When a liquid is in contact with a rigid solid (flat surface) in presence of vapor (gas phase) , the intersection of the solid, liquid and the gas (vapor) phases is called three-phase contact line. When this line move for a small distance $d\ell$ results in a net energy changes of

$$dE = d\ell(\gamma_{s\ell} - \gamma_{sv} + \gamma_{\ell v}\cos\theta)$$

Therefore if the drop is at equilibrium then $dE = 0$ and we obtain: the Young equation [7]:

$$\gamma_{sv} - \gamma_{s\ell} - \gamma_{\ell v}\cos(\theta_e) = 0 \tag{4}$$

Where γ_{ij} denotes the surface tension between phases i and j. The equilibrium contact angle can be measured by different methods.

In Young relation the first hypothesis is that the solid is rigid (non deformable), so that only the horizontal projection of the tensions contribute to the equilibrium of the liquid drop, therefore, the vertical component of the force, it is balanced by the elastic response of the solid surface. Two limit cases can occurs, complete wetting and non-wetting of the solid by the liquid corresponding to $\theta_e = 0$ (Antonov's relation) and $\theta = \pi$ respectively which leads to the relationships:

$$\frac{\gamma_{sv} - \gamma_{s\ell}}{\gamma_{\ell v}} = \begin{cases} 1 \text{ for } \theta = 0 \\ -1 \text{ for } \theta = \pi \end{cases} \tag{5}$$

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For $theta = 0$ complete wetting takes place and the liquid solid spread on the solid surface, In other words, there is a complete exclusion of the vapor from contact with the solid. Conversely, for $theta = \pi$, there is a complete rejection of the liquid from contact with the solid. In this wetting situation, one can say that the vapor completely wett the solid-liquid interface. The intermediate situation which is the partial wetting is the most common situation and which correspond to

$$\frac{\gamma_{sv} - \gamma_{s\ell}}{\gamma_{\ell v}} < 1$$

3. Roughness

We discuss here the heterogeneous interface resulting from the roughness and introduce the equations that govern the contact angle for the heterogeneous interface. The real surfaces are neither physically nor chemically homogeneous, the majority of materials have a surface with a wavy landscape, steps or pores / holes. It is well known that the roughness of a solid surface has an important effect on the spreading of a liquid on that surface. Thus, the presence of roughness affects the apparent contact angle (Equation 6) which varies depending on whether the surface is smooth or rough[26]. The presence of the roughness of the solid surface must increase the contact surface between the solid and the liquid, then it is possible to increase or decrease the wettability of the surface by modifying its roughness. The wettability induced by the roughness makes it a subject of research of intensive activities. When the air is trapped in the asperities of a rough surface, the interface between the liquid and the solid becomes textit composite alternating liquid-solid and liquid-air contacts in the same contact surface area Cite CassieBaxter . The contact angle of a liquid droplet on rough solid surface is governed by Young [7], Wenzel[26] and Cassie equations [27], these laws relate contact angle, surface and interface energies and roughness.

In Wenzel's description, the roughness[26] is defined as the ratio between the real area of the solid and the geometric area (projection) described by its spatial limit.

Among other things, the topography of a surface considerably affects the macroscopic behavior of a material [28]. On real surface (rough) the measured contact angle θ^* is called the apparent contact angle of a liquid on a surface composed of two different fractions, with a fractional area φ_i and the contact angle θ_i $(i = 1, 2)$. The apparent contact angle is given by the Cassie equation :

$$\cos\theta^* = \varphi_1 \cos\theta_1 + \varphi_2 \cos\theta_2 \tag{6}$$

The background hypothesis of the Cassie (6) equation is that the heterogeneities at the surface are distinct and quasi-periodically distributed. The assumption of an average surface energy is not so erroneous. But in the case where the heterogeneities are compared to molecular dimensions the average should be on the dipole moment and instead of the Cassie (6) equation one should be use [29]:

$$(1 + \cos\theta^*)^2 = \varphi_1(1 + \cos\theta_1)^2 + \varphi_2(1 + \cos\theta_2)^2 \tag{7}$$

For the case where the interface is composite, consisting of solid-liquid and liquid-air fractions such as $\varphi_1 = \varphi_{s\ell}$, $\varphi_2 = \varphi_{\ell v} = 1 - \varphi_{s\ell}$ and $\cos\theta_2 = -1$ if we replace in the Cassie equation (6) that yields to the Cassie-Baxter equation :

$$\cos\theta = -1 + \varphi_{s\ell}(1 + \cos\theta_0) \tag{8}$$

When the liquid is inside cavities the contact angle $\theta_2 = 0$, this situation yield to the relation :

$$\cos\theta = 1 + \varphi_{s\ell}(\cos\theta_0 - 1) \tag{9}$$

It is important to highlight that, in many cases, surface roughness can overshadow the influence of interfacial energies [30]. Biological interactions with surfaces have also been shown to depend significantly on surface topography. Curtis and Wilkinson [31] provided a comprehensive review on

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how topographical features control cell adhesion and activity, while a broader review of the role of polymer biomaterials can be found in Griffith's work [32].

3.1. Contact Angle Hysteresis

The phenomenon of contact angle hysteresis, that appears in many practical applications, remain a complex phenomenon although it has been recognized and studied for numerous decades. Several experimental and theoretical research have been carried out to study its mechanisms [33–36]. The general observation is that the contact angle measured for a liquid advancing across a surface exceeds that of one receding from the surface. An everyday example is found in the appearance of a raindrop moving down a windowpane or an inclined surface [37,38]. This difference, known as contact angle hysteresis, can be quite large, as much as 50° for water on mineral surfaces. This can be quite

Although not fully understood, contact line hysteresis is generally attributed to surface roughness, surface heterogeneity, solution impurities adsorbing on the surface, or swelling, rearrangement or alteration of the surface by the solvent. The local tilting of a rough surface or the local variation in interfacial energies on a heterogeneous surface can cause the contact angle to vary. It is not yet clear whether, like other hysteretic phenomena (such as found in magnetism), contact angle hysteresis can be described by irreversible transitions or "jumps" between domains of equilibrium states [41 admason p355]. Here we review some of the main features of heterogeneous or rough surfaces and their effect on contact angle measurements.

Solid surface (or, more exactly, solid–liquid, solid–gas, or solid–vacuum interface) has complex structure and properties depending upon the nature of the material and the method of surface preparation. All solid surfaces, both natural and artificial, irrespective of the method of their formation, contain irregularities.Numerous researchers have studied its origin experimentally and theoretically [39,40]. The general observation is that the contact angle measured for a liquid advancing across a surface exceeds that of one receding from the surface. Contact angle hysteresis is the difference between the advancing and receding contact angles, which are two stable values.

4. Adsorption

First of all, it is very important to note that the cells never see a bare surface (modified or not) but a surface previously coated with adsorbed proteins and water from blood, biological interstitial fluids or culture media. Then, cells sense foreign surfaces through this adsorbed layer, which means that cell interactions with an exogenous material surface are conditioned by these the early steps surface activities leading to that initially cells respond to the adsorbed proteins, rather to the surface itself [41] Thus, starting of cell-surface interactions/adhesion are mediated by non-specific receptor interactions.

The phenomenon of adsorption of atoms and molecules at various interfaces is of particular importance in the surface and colloid sciences since the beginning of this century and in many industrial processes, such as biology, enviromental protection, in many catalytic processes and purification of water, sewages, air and soil are concerned too. The adsorption still the main way by which one can alter interfaces energies. While theoretical aspects of the phenomenon can be complex that is why most approaches are empirical. The adsorption studies represent intrinsically an interdisciplinary area between chemistry, physics, biology and engineering.

The adsorption can be interpreted as the change in concentration of a given substance at the interface as compared with the bulk phases is denoted to as adsorption. Adsorption processes concern mainly at the industrial scale, the solid-gas and solid-liquid interfaces, in some laboratory separation techniques all types of interfaces are concerned [6,42]

Increased use of synthetic materials from polymers and ceramics, where it is necessary to replace organs, vessels and tissues with manufactured products. What makes the most important domain in which adsorption processes are largely involved is the biocompatibility of implants or the prosthesis where protein adsorption at the solid-liquid interface plays a major role in the integration of Biomaterial or not [43].

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Many authors have reported that moderately wettable surfaces promote cell adhesion and growth. Hydrophobic surfaces promote the adsorption of proteins while hydrophilic surfaces are less favorable for protein adsorption and, more generally, the wettability properties of biomaterial surfaces play an important role in cell behavior. [20–24,44]. Cell growth is directly related to that expected from protein adsorption on the surface of biomaterilas. When a material surface resists protein adsorption, cell proliferation is mediocre at the surface because the interaction of cellular material is mainly controlled by the adsorbed proteins.Thus, surface wettability is one of the important parameters that influence the biocompatibility of biomaterials. Modification of the conformation of the adsorbed proteins could also result in poor growth of cells on the surface of biomaterials.

5. Metal Surface

Metals and their oxides are solids with high surface energies, generally of the order of several hundred $mJ \cdot m^{-2}$. Consequently, all liquid (organic or not) should completely wet a clean metal surfaces, which means also that such surfaces are easily contaminated.

Materials used to manufacture implants must be with specific properties that meet requirements of biocompatibility, *ad hoc* mechanical properties and resistance to the corrosion. The known problems of metal-based biomaterials related to strength and corrosion properties are circumvented by the use of titanium materials. Thus, the research activities can focus on the implant - tissue interface in order to improve for best controlling biocompatibility. This objective will be achieved if the topography of the surface can be modified in several ways and at several levels. Chemical surface modification using covalently or noncovalently attached (bio)molecules contribute to enhance the biocompatibility of surface. The presence of surface oxide layer with its beneficial physical and chemical properties, provides the corrosion resistance, biocompatibility and low dissolution in body fluids[45,46].

6. Adhesion

6.1. Work of Adhesion and Cohesion

What does adhesion mean? From an energetic point of view, it is the energy necessary to bring two surfaces closer from infinite until the real contact. When two free surfaces of two phases A and B come in close proximity to one another, an adhesive interaction results, often analogous to the internal cohesion of the atoms or molecules in the bulk. In general, the work of adhesion between two immiscible phases A and B, e.g. *liquid-liquid*, *solid-liquid* and *solid-solid*. The work of adhesion can also be defined as the work needed to separate an interface into two separate surfaces and is given by the *Dupré* equation :

$$W_{AB} = \gamma_A + \gamma_B - \gamma_{AB} \tag{10}$$

In the case of solid-liquid interface, we obtain, when combined the Young (4) and Dupré equations (10):

$$W_{s\ell} = \gamma_{\ell v}(1 + \cos\theta_e) \tag{11}$$

The total wetting thus corresponds to the maximum of the adhesion energy. From a phase perspective, adhesion occurs between two distinct phases, as illustrated in Figure 2, whereas cohesion takes place within the same phase. However, this does not imply that the types of interactions in cohesion and adhesion are fundamentally different.

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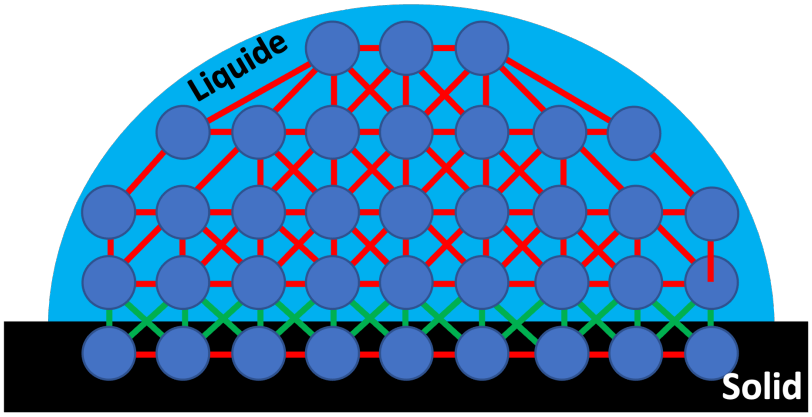

Figure 2. The Green lines represent the Work of adhesion between two different phases, here: Solid-Liquid. The red lines represent the cohesion energy between molecules within the same phase, here: Liquid-Liquid or solid-solid.

6.2. Spreading Coefficient

The liquid's spreading power is determined by comparing surface cohesion energy W_c of the liquid to the work of adhesion : if $W_c < W_a$ the liquid spread when adhesive forces dominates and will not spread if cohesive forces dominates i.e. $W_c > W_a$ with respect to the surface of the liquid or solid upon which the spreading is to occur.

We define also :

$$W_c = 2\gamma_c \tag{12}$$

is the work of cohesion for a single surface, which is the work needed to break the surface and is equal to two times the value of its surface tension.

The spreading tendency or power of spreading of one drop of liquid placed upon the surface of another liquid or on a solid surface is defined by the Harkins [47,48] spreading coefficient, S, which quantifies the wetting or spreading property of a *solid-liquid-gas* system:

$S = \gamma_A - (\gamma_B + \gamma_{AB})$ using equations (10) and (12) we obtain :

$$S = W_a - W_c \tag{13}$$

In the case of the liquid-liquid interface (water-oil or water hydrocarbons interfaces) we get the following situations

$$\begin{cases} W_c < W_a \Rightarrow S > 0 & \text{the liquid spread over the substrate i.e. adhesive forces dominate} \\ W_c > W_a \Rightarrow S < 0 & \text{the liquid not wet the substrate i.e. cohesive forces dominate} \end{cases}$$

In the case of solid-liquid interface and combining equations (13) and (4) we obtain:

$$S = \gamma_{\ell v}(\cos\theta_e - 1) \tag{14}$$

then, and as showed in figure 1, we obtain:

$$\begin{cases} S < 0 & \text{the liquid not spread upon the solid surface and form a lense} \\ S = 0 & \theta_e = 0 \text{ then the liquid spread on the solid-vapor interface} \\ S = -2\gamma_{\ell v} & \theta_e = \pi \text{ then the vapor spread on the solid-liquid interface} \end{cases}$$

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7. Decomposition of the Work of Adhesion for Determining the Solid Surface Energy

The work of adhesion W_{AB} between two materials A and B can be decomposed into several contributions based on different types of interactions at the interfaces. According to the Owens model, these contributions are:

Dispersion Forces: Dispersion forces, also known as van der Waals forces, arise from transient dipole interactions between molecules. These forces contribute significantly to the adhesion energy and are represented as W_{AB}^{D}. The dispersion forces are related to the surface energies of the materials and depend on the electron density and polarizability of the surfaces. This corresponds to the part of the surface tension due to non-polar interactions like Van der Waals forces.

non-dispersive forces : In the context of surface energy, particularly when discussing the contributions to surface energy of a material, the term "non-dispersive contribution" can be used to describe the part of the surface energy that does not depend on the frequency of electromagnetic radiation or wave interactions.This corresponds to the polar component, which includes dipole-dipole, hydrogen bonding, ...

Non-dispersive Contribution to Surface Energy:
The non-dispersive contribution to surface energy refers to the component of the surface energy of a material that is independent of the frequency of interactions, such as electromagnetic waves or acoustic waves. This includes contributions from static or low-frequency interactions that do not change with frequency. In contrast, dispersive contributions to surface energy arise from frequency-dependent interactions and phenomena, such as van der Waals forces or specific types of electromagnetic interactions, which can vary with the wavelength or frequency of the applied energy.

In summary, while dispersive contributions involve frequency-dependent effects, the non-dispersive contribution reflects the intrinsic surface characteristics that remain constant regardless of the frequency of interaction.

Polar Forces Polar forces result from permanent dipole interactions between molecules. In the Owens model, this contribution is denoted as W_{AB}^{P}. These interactions are particularly important for polar materials and can be influenced by factors such as surface charge and molecular orientation.

Hydrogen Bonding Hydrogen bonding is a specific type of polar interaction where hydrogen atoms are attracted to electronegative atoms, such as oxygen or nitrogen. This contribution to the adhesion energy is represented as W_{AB}^{H}. Hydrogen bonding plays a significant role in adhesion for materials where this interaction is prominent.

The total work of adhesion can be expressed as:

$$W_{AB} = W_{AB}^{d} + W_{AB}^{p} + W_{ab}^{H} + \dots \tag{15}$$

Each component reflects different aspects of the molecular interactions at the interface. Analyzing these contributions separately provides insights into the specific interactions that dominate the adhesion process and enables the optimization of material properties and surface treatments to enhance adhesion performance. This detailed analysis is essential for the development of effective adhesive systems and the improvement of material performance in various applications.

7.1. Determination of Surface Free Energy

The surface free energies (SFE) of samples were calculated using the Owens Wendt theoretical model [32]. This model gives the long-range dispersion (LifshitzVan der Waals; γ^{d}) and the short-range non-dispersive (example : hydrogen bonding; γ^{p}) components of SFE according to the following equation:

$$W_{s\ell} = (1 + \cos\theta)\gamma_{\ell} = 2\left(\sqrt{\gamma_s^d \gamma_\ell^d} + \sqrt{\gamma_s^{nd} \gamma_\ell^{nd}}\right) \tag{16}$$

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where γ_s is the SFE of the surface, γ_ℓ is the SFE of the liquid and $W_{s\ell}$ is the solid-liquid interface energy. Two liquids were can be used as probes for SFE calculation. For a liquid, the overall surface tension (γ_ℓ) is a combination of dispersive and non-dispersive components, whose values are indicated in tables[49]. The measured contact angles of liquids will be reported in Eq. 16 for each solid substrate, then sample SFE components were calculated.

When choosing two liquids, one can build a linear system of two equations and solve it exacltly:

$$\begin{cases} W_{s\ell_1} = (1+\cos\theta_1)\gamma_{\ell_1} = 2\left(\sqrt{\gamma_s^d\gamma_{\ell_1}^d} + \sqrt{\gamma_s^{nd}\gamma_{\ell_1}^{nd}}\right) \\ W_{s\ell_2} = (1+\cos\theta_2)\gamma_{\ell_2} = 2\left(\sqrt{\gamma_s^d\gamma_{\ell_2}^d} + \sqrt{\gamma_s^{nd}\gamma_{\ell_2}^{nd}}\right) \end{cases}$$

Given that these equations might be complex depending on the specific values, you can solve them numerically or use computational tools if needed (see section Annexe 1 p. 12). For diffrent values of liquids see the Annexe 2 p 12

Finally, the solid SFE is :

$$\gamma_s = \gamma_s^d + \gamma_s^{nd} \tag{17}$$

8. Cell-surface Interaction

Understanding and directing the interactions between cells and the natural or artificial extracellular matrix is of major importance for discovering the mechanisms that control cellular behaviors, such as migration and differentiation, and for the development of new biomaterials. Identifying the main interactions between cells and their complex micro and nano environment, which affect cellular functions, will contribute to our understanding of cells behaviors for developing artificial materials for medical applications. Both biologists and material scientists are interested in understanding how local interactions between cells and their surrounding micro and nano environment can regulate cellular behavior [50–57]. The cells are integrated in a micro and nano complex and dynamic environment containing, in addition to the extracellular matrix (ECM), surrounding cells, growth factors and cytokines. Cell adhesion to the ECM substrate involves a physical connection to the ECM proteins *via* specific cell surface receptors. Cell adhesion uses protein binding on the ECM surface *via* specific receptors on the cell surface. Integrins are one of the most important transmembrane proteins. They are responsible for the connection of the intracellular cytoskeleton to the ECM. Once binding of integrins to ECM ligands is established, aggregates of integrins result in focal adhesions. The adhesion process triggers a cascade of intracellular signaling events that produce changes in cell behavior, primarily in the form of growth and/or differentiation [58,59].

Rahmati *et al.* emphasized the significance of surface wettability and functional group properties in biomaterials, demonstrating that hydrophilic and zwitterionic groups enhance protein adsorption and cell adhesion[60]. Imsirovic *et al.* explored biofilm formation in silicone mammary implants, identifying persistent inflammation as a major contributor to fibrosis, and suggested antimicrobial-impregnated materials and surface modifications to mitigate this[61]. Haugen *et al.* reviewed the relationship between physicochemical surface properties and biochemical signaling, highlighting how surface chemistry optimization improves implant integration[60]. Together, these studies provide valuable insights for advancing biomaterial design in therapeutic applications.

The surfaces of biomaterials are rarely planar at the molecular level, such surfaces appear only after cleavage of monocrystalline materials in particular planes. Many studies has explore the effects that roughness has on cells. Hallab et al. studied which surface energy or surface roughness had the most influence on the adhesion and colonization of biomaterials by cells [62]. They used cell proliferation and extracellular matrix secretion as a parameter to characterize the colonization of 3T3MC fibroblasts on different polymers and metal-based implants. It is well known that metals have a higher surface energy than polymeric materials (see section 5). As expected, they confirm that polymeric materials exhibit a significant increase in adhesion strength associated with increased surface roughness (see section 3) and a linear correlation between the surface energy of metals and cell adhesion. Finally, they conclude that surface energy plays a much larger role in cell adhesion and proliferation than surface roughness [63] in directing cell adhesion and colonization of cells

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on artificial tissue scaffolds. Ponsonnet *et al.* [64] studied the spread of human fibroblasts on the surface of the substrate as a function of hydrophobicity, surface free energy, interfacial free energy and surface roughness to determine which of these parameters is predominant. Their experiments were carried out on used various engineered titanium surfaces and on titanium-aluminium-vanadium alloy $Ti-6Al-4V$, and titanium–nickel $NiTi$. A relationship between cell spreading and the polar component of surface free energy was found. Free interfacial energy values were low for all surfaces of the alloys studied and good biocompatibility for these surfaces. It should also be noted that fibroblast cells migrate on a metal-deposited surface along the gradient and in the direction of the hight deposit which correspond to the high region with strong energy of adhesion[65,66]. Tan *et al.*[67] discloses a simple method of micropanning cells on glass, silicone rubber and polystyrene. Various cell types including fibroblasts, smooth muscle cells, adipocytes, stem cells, cardiomyocytes, and endothelial cells were used. In addition, they used self-assembled alkanethiol monolayers on gold as model surfaces to adjust the wettability of the substrate surface. Adjusting the wettability of common tissue culture substrates can be a tool to study the role of spatial organization in cell behavior [9,63,68,69]. Rodriguez-Valverde *et al.* [70] studied the effects physico-chemical properties of the Ti surface, such as roughness and wettability on osteoblasts-like cells adhesion. Different roughness was carried out by polishing, etching and blasting the surfaces. A serious analysis of the roughness characteristics are given in this paper. As expected their results shown a direct effects of the surface topography and the surface energy (wettability) on cell adhesion. The good cell response at long times is obtained on the etched Ti surface by hydrofluoric acid. Explanations of the relationship between cell behavior and roughness were provided by Curtis *et al.*[31,71] . These interpretations are based on observations of cell movements on rough surfaces. The proposed interpretations highlight only the effects of the geometry *i.e.* discontinuities of the surface and not the energy of the surface on the behaviors of the cells. They based this on the observation of the positions of cells reacting to topography and also on the phenomenon that cells align and move along the sharp intersections not descending to flatter areas even though the ridges are themselves concave as they stretch from one 'peak' to another. In this analysis, the discontinuities are considered as a physical heterogeneity in the sense of peak and valley with respect to a plane surface, which has consequences on the spatial distribution of the interaction energies. From the point of view of biologists, discontinuities are a certain difference in curvature between two positions at the surface, which leads to a simplistic interpretation of how cells detect changes in surface topography. When the cells are in contact with a rough surface, deformation and / or stretching of the cell occurs and induces activation of the receptors *via* their deformations [31,71], Which implies also an organization of cytoskeletal elements, F-actin followed by microtubules, along edges of discontinuities [72–74]. In the particular case of the grooved surfaces, which is the most feature type used for investigating effects of surface structure on cells[72,75–82,82–102]. The cells are aligned along the axis of the grooves more affected by the depth of the grooves than the the width [71,89,91]. Roughness has also been used to guide cells and neurons onto substrates structured by grooves or pillars [103]. Ge *et al.* Ge et al. have shown that changes in the surface morphology of the titanium alloy induce an increase in the dispersive surface tension and significantly improve the wettability of the titanium alloy[104].

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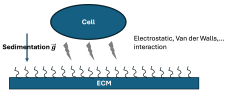
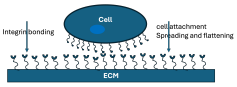
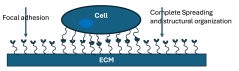
Table 2. Combined Evaluation and Definitions of In Vitro Cell Adhesion Phases

Cell Adhesion Phases	**Phase I** (Initial Attachment)	**Phase II** (Flattening)	**Phase III** (Full Spreading and Structural Organization)
Schematic Diagram of Cell Adhesion	 Cell attaches to ECM via electrostatic interaction	 Cell flattens as integrin bonding occurs	 Cell spreads and forms focal adhesions
Schematic Diagram of Transformation of Cell Shape	Initial attachment	Flattening	Full spreading and stable adhesion
Cell Adhesion Intervention	Electrostatic interaction	Integrin bonding	Focal adhesion
Adhesion Stages	Sedimentation	Cell attachment	Cell spreading and stable adhesion
Adhesion Definitions by Authors	**A. J. Garcia** Stick (Van der Waals and ionic forces)	Grip (Integrin-ligand binding, receptor clustering)	Extra-cellular matrix synthesis
	J. E. Murphy-Ullrich Attachment	Spreading (Increase in contact area)	Focal adhesion and stress fiber formation
	A. Pierres Flattening	Spreading	
	K. Anselme Short-term adhesion	Alignment (Cytoskeletal interactions, mechanical prestress)	Long-term adhesion

8.1. Materials and Types of Topography That Affect Cells

The physical and chemical properties of material surfaces, as well as their topographical features, significantly influence cellular behavior such as adhesion, migration, proliferation, and differentiation. Cells interact with various biomaterials through surface receptors, responding differently based on the material's properties, including its roughness, wettability, and surface energy.

Metals and Metal Oxides: Metals such as titanium and its alloys are widely used in biomedical implants due to their biocompatibility and mechanical strength. Surface modifications, such as roughening or creating micro- and nano-scale features, have been shown to enhance osteoblast adhesion and promote osseointegration [45**?**]. For instance, titanium's high surface energy improves cell attachment, while surface topography can direct cell alignment and migration.

Because titanium is the most widely used material primarily in dental care as an implant material in the human body. We begin this section by examining the use of processes to control the physicochemical properties of surfaces, namely the topography of the surface and gradients in the energy of adhesion, to reveal the pathways of surface influence, as an extracellular artificial matrix, in cell behaviors, especially in adhesion [31].

Polymers: Polymers are another class of materials frequently used in biomedical applications. The topography of polymeric surfaces can influence cell behavior, especially in terms of adhesion and proliferation. Griffith [32] reviewed the role of polymers in biomaterials, emphasizing how variations in surface roughness and chemistry can affect cell responses. Moreover, studies have shown that polymer surfaces with nano-patterned features can guide stem cell differentiation by mimicking the natural extracellular matrix [31].

Topographical Features: Surface topographies, such as grooves, pits, and ridges, play a crucial role in determining cell behavior. Curtis and Wilkinson [31] highlighted that micro- and nano-grooved surfaces can guide the orientation and movement of cells. Similarly, biomaterials with specific topographical patterns have been found to influence the differentiation of stem cells into specific lineages

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without the need for biochemical stimuli. Such features are essential in developing biomaterials for tissue engineering applications, where controlled cell behavior is necessary.

In conclusion, the interaction between cells and biomaterials is highly dependent on the material's surface characteristics, including topography. By designing materials with specific surface features, it is possible to control cellular responses for various biomedical applications, including implants, tissue engineering, and regenerative medicine.

Table 3. Materials, Topography, and Effects on Cell Behavior

Material	Topography	Effect on Cells	Ref.
Titanium (Ti)	Micro/nano grooves	Enhances osteoblast adhesion, promotes osseointegration	[45,46]
Polymers	Nano-patterned surfaces	Guides stem cell differentiation, mimics extracellular matrix	[32]
Silicon	Nano-pillars	Enhances neuronal cell growth, guides axonal alignment	[31]
Stainless Steel	Micro-textured surfaces	Improves endothelial cell adhesion, reduces bacterial adhesion	[32]
Hydrogels	Soft, deformable textures	Promotes cell migration and proliferation in wound healing applications	[31]
Biodegradable Polymers	Porous scaffold structures	Enhances tissue regeneration, supports 3D cell growth and nutrient diffusion	[32]
Human Fibroblasts	Micro-grooved surfaces	Alters fibronectin mRNA stability, secretion, and assembly	[93]
Murine Macrophages	Grooved substrata	Topographical control of macrophage activation	[89]
Fibroblasts	Aligned grooves	Orientation and migration of fibroblasts	[94]
Oligodendrocytes / Progenitor Cells	Grooved substrata	Guidance of cells, influencing shape and differentiation	[91]

The Table 3 combines the materials, types of topography, and their effects on different cell types with the appropriate references. It covers both the materials commonly used in biomaterials (like titanium and polymers) and the cell-specific interactions with various topographies (like grooves or nano-pillars).

9. Neurons and Model of Neuronal Cells Cultured on Surfaces

During the development of the nervous system, neuronal growth is significantly influenced by contact interactions with surrounding surfaces. Curtis and colleagues explored various methods of nerve repair using conduits, also known as tubes, to aid nerve regeneration after injury [31]. These approaches, thoroughly reviewed by Doolabh et *al.* and Aebischer *et al.* [105,106], introduced microfabricated topography to control cell orientation. The declared intention was to develop materials made from biodegradable polymers. Additionally, Brunette et al. fabricated microgrooves on the surface of tooth crowns, aiming to prevent fibroblasts from invading the gingiva [75,79,87].

Neuronal growth cones are steered by a variety of molecules, which may either diffuse or be bound to a substrate or membrane. Diffusible factors, such as Netrins, Semaphorins, Neurotrophins, and Slit proteins, can act as either repellents or attractants for growth cones, depending on the context [107]. Recent studies have shown that the response of a growth cone to a diffusible factor is determined by transmembrane receptors, particularly their cytoplasmic domains, which dictate whether a cue is perceived as attractive or repulsive. Cytoplasmic signal transduction pathways that mediate repulsion and attraction in neurons converge onto the same signaling pathways, often modulated by cyclic nucleotides [108]. The study of Holt *et al.* [109] explores how cyclic nucleotides (cAMP and cGMP) modulate the response of neuronal growth cones, switching between attraction and repulsion depending on their levels.

Understanding how axonal and dendritic growth is regulated is essential for comprehending the development of the nervous system, axonal regeneration, and functional adaptations. Physicochemical properties of adhesion substrates or extracellular matrices (ECM), whether natural or artificial, have a

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fundamental impact on cellular processes in both physiological and pathological conditions [41,110]. Early studies on the nervous systems of certain invertebrates revealed a consistent architecture, suggesting that embryonic axonal growth is not random [111]. This foundational work explores the architecture and functional modality of invertebrate nervous systems, emphasizing how their development follows organized patterns rather than random growth. This discovery led to further investigations into the mechanisms underlying ordered axonal growth.

In 1975, Letourneau *et al.* [112] studied the behavior of axonal growth cones on patterned surfaces using dorsal root ganglia dissected from eight-day-old chicken embryos [112]. They concluded that interactions between microtips and the substrate adjacent to the growth cone play critical roles in determining the direction and pathways of axonal elongation. This work confirmed that contact guidance is the key force in axonal growth, with axon orientation controlled by the topographic features of the substrate surface, such as model ECM, cell surfaces, or interactions with other axons and growth cones [109,113–115].

Marcus et al. [116] analyzed the adhesion of neurons on different substrates to understand the role of contact guidance in neurite extension. Their study highlights the importance of engineered biomaterial scaffolds, which provide structural support for neuronal growth and serve as guidance systems for nerve regeneration, particularly when natural reconstruction is not possible. By examining neuron-surface interactions, they emphasized how these scaffolds can be optimized not only for nerve repair but also for the development of advanced neuronal interfaces, which are critical for improving the stability and precision of brain-machine communication."

Murnane *et al.* studied the influence of large-scale surface energy gradients on neurite initiation in neuron-like cells [117]. They found that when PC12 cells were grown on substrates showing nonspecific adhesion gradients, under nerve growth factor (NGF) treatment, neurites preferentially initiated in directions of changing adhesivity, regardless of whether that change was in the direction of increasing or decreasing adhesivity. This pattern indicated that substrate adhesivity interacts with the cell's motility mechanisms through intracellular signaling involving the inhibitory monomeric GTPase Rho.

The substrates used in Murnane's study were based on a polycationic material, polyethyleinimine, with gradients elaborated by evaporating a metal layer (gold-palladium alloy or gold alone) onto the surface. Neurite outgrowth occurred in a pattern that suggested substrate adhesivity interacts with intracellular signaling, although no surface energy characterization was performed to confirm the presence of an adhesion gradient. The addition of NGF minimized the effect of the hypothetical gradient in adherence. Nonetheless, the key observation was that neurites followed the adhesion gradients (see figure 2 in reference [117]). Regardless of whether the variation in adhesion energy is increasing or decreasing, because in this experiment, the emergence of an increasing gradient due to gold evaporation also leads to the appearance of a decreasing gradient.

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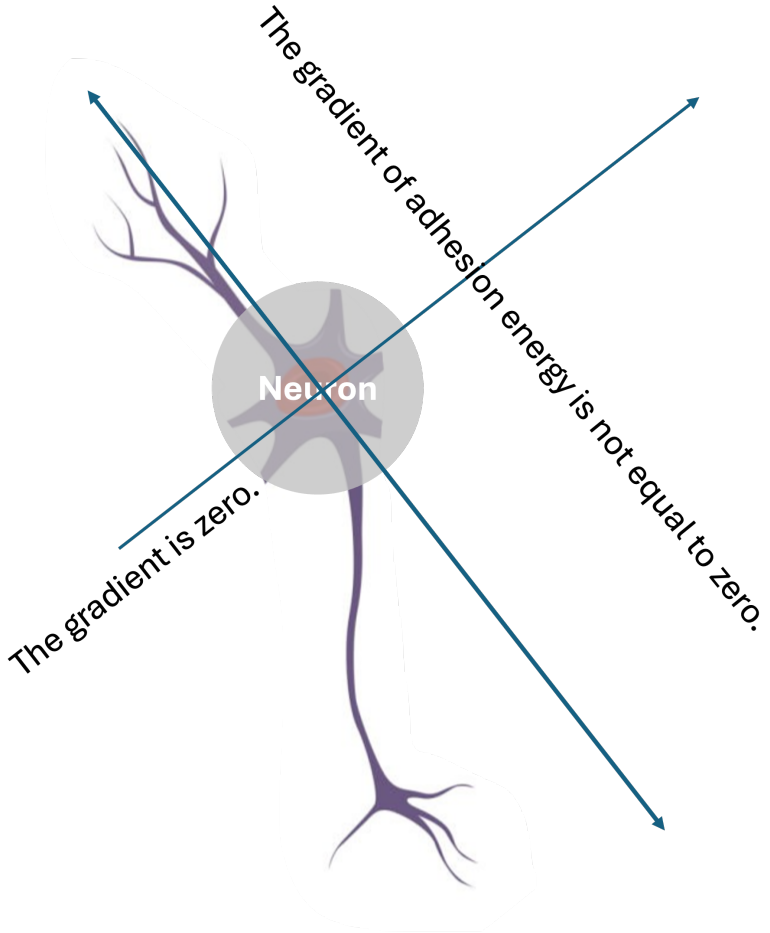

Figure 3. Neurite outgrowth patterns suggest an interaction between substrate adhesivity and intracellular signaling, despite the lack of surface energy characterization to confirm an adhesion gradient. The addition of NGF reduced the effect of the hypothetical gradient in adherence. Notably, neurites aligned with adhesion gradients, which in this experiment, exhibited both increasing and decreasing gradients due to gold evaporation. Adapted from Figure 2 of ref [117].

She *et al.*[118] demonstrated that hiPSC-RGCs cultured on PEDOT, with controlled gradients of adhesion, exhibited marked improvements in neurite outgrowth, axon guidance, and electrophysiological performance. Their work suggests that gradient PEDOT may provide a valuable scaffold for hiPSC-based therapies aimed at restoring retinal ganglion cell function in degenerative retinal diseases and optic neuropathies.

This observation is consistent with more recent research, which shows that the strength of adhesion to the surface is not the critical factor for axonal growth and elongation but gradients in energy of adhesion are very important.

In summary, the study of neurons and their interaction with model surfaces has provided valuable insights into how surface topography and energy guide neuronal growth. Understanding these interactions is crucial for developing materials and techniques to control nerve regeneration and repair.

10. Stem Cell Differentiation Depending on Different Surfaces

Stem cells are highly sensitive to their microenvironment, and this environment plays a crucial role in dictating their fate. They are exposed to a multi-scale topographic environment that significantly influences their behavior and differentiation potential. This environment ranges from macroscopic structures, such as bones and ligaments, to micrometer-sized cells and nanoscale proteins and ligands.

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The role of surface topography has gained considerable attention, as studies have shown that it can produce effects comparable to chemical stimulation, including the activation of growth factors [119].

Topographical features of a surface can direct stem cell behavior, including adhesion, proliferation, and differentiation. For instance, micro- and nano-topographies can induce specific lineage commitments in mesenchymal stem cells (MSCs) without the need for external chemical stimuli. This phenomenon is primarily due to the activation of mechanical signaling pathways like RhoA, which respond to surface shape and stiffness [120,121].

Chen *et al.* conducted pioneering research demonstrating that human MSCs cultured on surfaces patterned with fibronectin could be induced to differentiate based on surface shape. MSCs on round islands typically underwent adipogenesis, whereas those on larger, spread-out islands tended toward osteogenesis [55]. This differentiation was linked to mechanical cues, particularly through the activation of RhoA, which subsequently influences the ROCK kinase pathway.

Furthermore, another study by McBeath *et al.* [122] The study explores how human mesenchymal stem cells (hMSCs) commit to adipocyte or osteoblast lineages, emphasizing the role of cell shape, cytoskeletal tension, and RhoA signaling. It demonstrates that hMSCs undergo osteogenesis when spread and flatten, while rounded, unspread cells differentiate into adipocytes. The research identifies RhoA activity as a crucial mediator in this process, where dominant-negative RhoA induces adipogenesis and constitutively active RhoA promotes osteogenesis. ROCK (a downstream effector of RhoA) was shown to drive osteogenesis regardless of cell shape, but its function is dependent on actin-myosin-generated cytoskeletal tension. This study underscores the integral role of mechanical cues, like cell shape and cytoskeletal forces, in stem cell fate decisions, alongside biochemical factors. Conversely, cells on smaller nanotubes $(30nm)$ exhibited no significant differentiation [123]. This shift was also attributed to the activation of RhoA, reinforcing the notion that topographical cues can guide stem cell fate *via* mechanical signaling (see Figure 1 Ref. [124]).

In their article McNamara *et al.* [125] focuses on how nanotopography influences stem cell behavior and differentiation, with a primary focus on skeletal stem cells. Nanotopographical cues, such as nanoscale surface features, guide cell differentiation by altering cell adhesion, morphology, and mechanotransduction processes. The article emphasizes that nanotopography can induce differentiation without the use of chemical factors, providing a durable and non-invasive means for controlling cell fate. The mechanisms by which stem cells respond to these topographical cues involve both biochemical signaling and physical force transduction, ultimately affecting gene expression and protein production. Various methods of nanotopographical fabrication, like electron beam lithography and colloidal lithography, are highlighted for their ability to produce controlled surface features. The findings present promising applications in regenerative medicine, especially in tissue engineering and implantable devices.

This section advances the understanding of how nanoscale physical cues can guide stem cell differentiation and emphasizes their relevance for clinical applications in developing advanced biomaterials. By exploring the interplay between surface topography and stem cell behavior, we can harness these mechanical signals to improve the design of biomaterials and scaffolds for tissue engineering. Stem cell-based therapies and regenerative medicine stand to benefit significantly from insights into how factors such as surface shape, chemistry, and stiffness influence cell fate decisions. Ultimately, the manipulation of topographical features presents a powerful tool for directing stem cell differentiation in a controlled, non-invasive manner, offering promising avenues for enhancing tissue regeneration and therapeutic outcomes.

10.1. Influence of Surface Chemistry and Energy on Stem Cell Differentiation

The chemistry and surface energy of materials also have a profound impact on stem cell behavior. Hydrophobic and hydrophilic surfaces can affect protein adsorption, which in turn influences cell adhesion and differentiation. Hydrophobic surfaces tend to promote the adsorption of proteins,

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enhancing cell adhesion, while hydrophilic surfaces generally reduce protein adsorption and may inhibit certain cellular functions [21].

Van Wachem *et al.* studied the interaction between human endothelial cells and polymeric surfaces with varying wettability. They found that moderately wettable surfaces promote better cell adhesion and growth compared to highly hydrophilic or highly hydrophobic surfaces [23,24]. Similarly, Arima and Iwata demonstrated that surface functional groups, combined with controlled wettability, could influence MSC differentiation, suggesting that surface chemistry plays a critical role in modulating stem cell fate [20].

Another approach to controlling stem cell differentiation is through the regulation of chirality. Manipulating this parameter raises several questions, the most important being: do chiral molecules in 3D maintain their chirality once adsorbed or fixed to a surface? an exemple of work is that of Yao *et al.*[126] demonstrate that molecular chirality on material surfaces serves as an indirect regulator of stem cell differentiation, with d-cysteine surfaces promoting osteogenesis and l-cysteine surfaces favoring adipogenesis. They also found that mesenchymal stem cells (MSCs) exhibited greater adhesion and reduced spreading on l-cysteine surfaces compared to d-cysteine surfaces at confluence

10.2. Applications in Regenerative Medicine

The ability to control stem cell behavior through surface modifications offers exciting possibilities for regenerative medicine and tissue engineering. By manipulating surface topography and chemistry, researchers can guide stem cell differentiation in desired directions without the need for external chemical inducers. This approach reduces the risk of side effects from chemical treatments and enhances the precision of tissue engineering applications.

Moreover, surface engineering of biomaterials is increasingly being integrated into the design of scaffolds for tissue regeneration. These biomaterials are tailored to provide specific mechanical and biochemical cues to stem cells, promoting the formation of desired tissue types, such as bone, cartilage, or neural tissues. The field is moving towards creating dynamic, responsive surfaces that can adapt to the changing needs of cells during the regeneration process [127].

Surface topography and chemistry are key regulators of stem cell behavior and differentiation. Advances in the understanding of how mechanical and biochemical cues from surfaces influence stem cells will continue to shape the development of novel biomaterials and scaffolds for regenerative medicine. Future research will likely focus on creating more sophisticated surface designs that mimic the complex microenvironments found in vivo, paving the way for more effective stem cell-based therapies.

11. Conclusion

The interactions between cells and biomaterial surfaces are crucial for advancements in biomedical applications, particularly in tissue engineering, regenerative medicine, and implant design. This article has explored how the physical chemistry of surfaces, including factors like surface energy, wettability, and topography, influence cell behavior, ranging from adhesion to differentiation.

Understanding the mechanisms behind cell-surface interactions provides valuable insights into controlling cell adhesion, proliferation, and differentiation. For example, surface modifications, whether at the nanoscale or microscale, have been shown to significantly impact cellular responses. Surface roughness, chemical composition, and energy all play a role in determining how cells interact with materials, which is critical for optimizing the performance of implants and scaffolds in vivo.

For neuronal cells, surface topography and energy gradients have been shown to guide growth and repair, providing a promising avenue for the development of nerve regeneration technologies. Meanwhile, stem cell differentiation is highly dependent on both surface topography and stiffness, as mechanical signals from the substrate can activate pathways like RhoA, directing cells towards specific lineages such as osteoblasts or adipocytes.

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Moreover, surface chemistry plays a significant role in biomaterial design. Surface wettability and functional groups determine protein adsorption, which in turn affects cell adhesion and growth. Moderately wettable surfaces have proven to be more favorable for cell behavior, providing a balance between hydrophobic and hydrophilic properties.

In conclusion, the physical chemistry of biomaterial surfaces offers powerful tools for controlling and optimizing cell responses. By fine-tuning surface properties, researchers can enhance biocompatibility, improve implant integration, and direct tissue regeneration processes. Future research will continue to explore more dynamic and responsive surfaces, paving the way for advanced biomaterials that mimic the complex environments cells encounter in the body. The interdisciplinary approach combining physics, chemistry, biology, and material science holds great potential for future breakthroughs in the biomedical field.

12. Acknowledgments

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Annexe 1

To isolate a solutions for γ_s^{nd} and γ_s^d, we'll use the given equations and isolate these parameters. Here's the step-by-step process:

1. Rewrite the given equations for $W_{s\ell_1}$ and $W_{s\ell_2}$:
From the first set:

$$W_{s\ell_1} = 2\left(\sqrt{\gamma_s^d \gamma_{\ell_1}^d} + \sqrt{\gamma_s^{nd} \gamma_{\ell_1}^{nd}}\right)$$

$$W_{s\ell_2} = 2\left(\sqrt{\gamma_s^d \gamma_{\ell_2}^d} + \sqrt{\gamma_s^{nd} \gamma_{\ell_2}^{nd}}\right)$$

From the second set:

$$W_{s\ell_1} = (1 + \cos\theta_1)\gamma_{\ell_1}$$

$$W_{s\ell_2} = (1 + \cos\theta_2)\gamma_{\ell_2}$$

2. Solve for γ_{ℓ_1} and γ_{ℓ_2}:
Rearrange the equations from the second set to express γ_{ℓ_1} and γ_{ℓ_2}:

$$\gamma_{\ell_1} = \frac{W_{s\ell_1}}{1 + \cos\theta_1}$$

$$\gamma_{\ell_2} = \frac{W_{s\ell_2}}{1 + \cos\theta_2}$$

3. Substitute γ_{ℓ_1} and γ_{ℓ_2} into the equations for $W_{s\ell_1}$ and $W_{s\ell_2}$:
Substitute γ_{ℓ_1} into the first equation:

$$W_{s\ell_1} = 2\left(\sqrt{\gamma_s^d \cdot \frac{W_{s\ell_1}^d}{(1 + \cos\theta_1)^d}} + \sqrt{\gamma_s^{nd} \cdot \frac{W_{s\ell_1}^{nd}}{(1 + \cos\theta_1)^{nd}}}\right)$$

Simplify this equation:

$$W_{s\ell_1} = 2\left(\sqrt{\frac{\gamma_s^d W_{s\ell_1}^d}{(1 + \cos\theta_1)^d}} + \sqrt{\frac{\gamma_s^{nd} W_{s\ell_1}^{nd}}{(1 + \cos\theta_1)^{nd}}}\right)$$

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Similarly, for γ_{ℓ_2}:

$$W_{s\ell_2} = 2\left(\sqrt{\gamma_s^d \cdot \frac{W_{s\ell_2}^d}{(1+\cos\theta_2)^d}} + \sqrt{\gamma_s^{nd} \cdot \frac{W_{s\ell_2}^{nd}}{(1+\cos\theta_2)^{nd}}}\right)$$

4. Isolate γ_s^d and γ_s^{nd}:
Let's focus on isolating γ_s^d and γ_s^{nd} from the above equations.
For γ_s^d:

$$\sqrt{\gamma_s^d} = \frac{W_{s\ell_1}}{2 \cdot \left(\sqrt{\frac{W_{s\ell_1}^d}{(1+\cos\theta_1)^d}} - \sqrt{\frac{\gamma_s^{nd} \cdot W_{s\ell_1}^{nd}}{(1+\cos\theta_1)^{nd}}}\right)}$$

Squaring both sides gives:

$$\gamma_s^d = \left(\frac{W_{s\ell_1}}{2 \cdot \left(\sqrt{\frac{W_{s\ell_1}^d}{(1+\cos\theta_1)^d}} - \sqrt{\frac{\gamma_s^{nd} \cdot W_{s\ell_1}^{nd}}{(1+\cos\theta_1)^{nd}}}\right)}\right)^2$$

$$\gamma_s^d = \left(\frac{W_{s\ell_1}}{2 \cdot \left(\sqrt{\frac{W_{s\ell_1}^d}{(1+\cos\theta_1)^d}} - \sqrt{\frac{\gamma_s^{nd} \cdot W_{s\ell_1}^{nd}}{(1+\cos\theta_1)^{nd}}}\right)}\right)^2$$

Squaring both sides gives:

$$\gamma_s^{nd} = \left(\frac{W_{s\ell_2}}{2 \cdot \left(\sqrt{\frac{W_{s\ell_2}^{nd}}{(1+\cos\theta_2)^{nd}}} - \sqrt{\frac{\gamma_s^{d} \cdot W_{s\ell_2}^{d}}{(1+\cos\theta_2)^{d}}}\right)}\right)^2$$

Annexe 2

Table 4. Dispersive and Non-Dispersive Surface Tensions of Various Liquids

Liquid	**Dispersive Surface Tension (γ^d, mN/m)**	**Non-Dispersive Surface Tension (γ^{nd}, mN/m)**
Water	21.8	51.0
Glycerol	34.0	30.0
Ethanol	18.0	8.0
Acetone	15.5	10.4
Hexane	18.4	0.0
Dimethyl Sulfoxide (DMSO)	36.0	8.0
Methanol	22.1	2.3
Benzene	28.9	0.0
Toluene	28.5	0.4
Chloroform	27.1	3.8
Formamide	39.0	19.0
Diiodomethane	50.8	0.0
1-Bromo-Naphthalene	44.4	0.0

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