CELLULAR INTERACTIONS WITH IMPLANTED SURFACES IN THE LIVING ORGANISM AND OSSEOINTEGRATION OF IMPLANTS

ABSTRACT: The surface of a biomaterial is a platform for cellular migration and growth. Every biomaterial, when implanted in vivo, causes cellular and tissue responses. These include inflammatory and foreign body reactions, wound healing response and formation of fibrous capsule to a greater or lesser degree around the implant. Tissue growth in the interface of an implanted structure is a highly complex phenomenon that involves a variety of factors, some of them related to biomaterial and include its microarchitecture, mechanical properties of the base material, topography and roughness, both on a micrometric and macrometric scale. Regarding bone tissue specifically, a structure with interconnected pores is critical to mimic the extracellular bone matrix. The size of pores, porosity and interconnectivity between them determines the performance of the material in functions such as cell fixation and nutrient diffusion. Characteristics of the implant surface, such as roughness and porosity significantly influence cell differentiation and consequently bone growth and osseointegration. The focus of this review article is on the interactions of proteins with the surface of biomaterials implanted in the organism and the consequent activation of macrophages and development of a foreign body reaction. In addition, we described the mechanisms related to osseointegration of implants.
Key words: biomaterial; foreign body reaction; macrophage; bone regeneration

INTERAÇÕES CELULARES COM SUPERFÍCIES IMPLANTADAS NO ORGANISMO VIVO E OSTEOINTEGRAÇÃO DE IMPLANTES

RESUMO:A superfície de um biomaterial é uma plataforma para migração e crescimento celular. Todo biomaterial, quando implantado in vivo, causa respostas celulares e teciduais. Estas incluem reações inflamatórias e de corpo estranho, resposta de cicatrização de feridas e formação de cápsula fibrosa em algum grau ao redor do implante. O crescimento tecidual na interface de uma estrutura implantada é um fenômeno complexo que envolve uma variedade de fatores, alguns deles relacionados ao biomaterial e incluem sua microarquitetura, propriedades mecânicas do material de base, topografia e rugosidade, ambos em escala micrométrica e macrométrica. Em relação ao tecido ósseo especificamente, uma estrutura com poros interligados é fundamental para mimetizar a matriz óssea extracelular. O tamanho dos poros, a porosidade e a interconectividade entre eles determina o desempenho do material em funções tais como fixação celular e difusão de nutrientes. Características da superfície do implante, tais como rugosidade e porosidade influenciam significativamente a diferenciação celular e consequentemente o crescimento ósseo e a osseointegração. O foco deste artigo de revisão é a interação de proteínas com a superfície dos biomateriais implantados e a consequente ativação dos macrófagos e desenvolvimento de reação de corpo estranho. Além disso, descrevemos os mecanismos relacionados à osseointegração dos implantes.
Palavras-chave: biomaterial; reação de corpo estranho; macrófago; regeneração óssea

INTERACCIONES CELULARES CON LAS SUPERFICIES IMPLANTADAS EN EL ORGANISMO VIVO Y LA OSTEOINTEGRACIÓN DEL IMPLANTE

RESUMEN: La superficie de un biomaterial es una plataforma para la migración y el crecimiento de las células. Cada biomaterial, cuando se implanta in vivo, causa respuestas celulares y tisulares. Estas incluyen reacciones inflamatorias y de cuerpos extraños, respuesta de curación de heridas y formación de cápsulas fibrosas en algún grado alrededor del implante. El crecimiento de tejido en la interfaz de una estructura implantada es un fenómeno complejo que implica una variedad de factores, algunos de los cuales están relacionados con el biomaterial e incluyen su microarquitectura, las propiedades mecánicas del material base, la topografía y la rugosidad, tanto a escala micrométrica como macrométrica. En lo que respecta al tejido óseo específicamente, una estructura con poros interconectados es fundamental para imitar la matriz ósea extracelular. El tamaño de los poros, la porosidad y la interconectividad entre ellos determinan el rendimiento del material en funciones como la fijación celular y la difusión de nutrientes. Las características de la superficie del implante, como la rugosidad y la porosidad, influyen considerablemente en la diferenciación celular y, por consiguiente, en el crecimiento y la osteointegración del hueso. El enfoque de este artículo de revisión es la interacción de las proteínas con la superficie de los biomateriales implantados y la consiguiente activación de los macrófagos y el desarrollo de la reacción de cuerpos extraños. Además, describimos los mecanismos relacionados con la osteointegración de los implantes.
Palabras clave: biomaterial; reacción de cuerpo extraño; macrófago; regeneración ósea

Introduction

Tissue growth at the interface of an implanted structure is a highly complex phenomenon involving a variety of factors encompassing a cascade of cellular and extracellular biological events [1]. Blood / biomaterial interactions begin to happen simultaneously after the implantation of the biomaterial, with adsorption of proteins on its surface and the development of a transient temporary matrix sustained in the thrombus that forms at the tissue / biomaterial interface and around it [2–4]. When implanted in the tissues, the biomaterial will always incite a cellular immune response [2,3]. Among the factors related to tissue growth on an implanted material, some are related to the material itself, and include its microarchitecture (existence or not of pores, relative density), mechanical properties of the base material and topography (roughness), both at macro, micro and nanometric scales [1,5,6]. According to [7] and [8], the topographic characteristics of the biomaterial surface directly influence the behavior of the cells that are responsible for tissue repair through intracellular signal pathways mediated by their focal adhesion. Also, according to [8], the architectural orientation of the natural extracellular matrix regulates several cellular behaviors, such as cell polarity, migration capacity, adhesion and proliferation changing the cytoskeleton through the reorganization of actin filaments when the cells adhere to it. Consequently, many studies in the field of tissue engineering have been conducted in the direction of mimicking the extracellular matrix of various tissue types.

Regarding bone tissue specifically, a structure with interconnected pores is critical to mimic the extracellular bone matrix [9,10]. The size and number of the pores, besides the interconnectivity between them, determines the performance of the material in functions such as cell fixation and diffusion of nutrients, and these factors are directly related to the ingrowth of soft tissue and bone and also to the resistance of the bone-implant interface [1,10]. According to [1], for proper bone ingrowth, the porosity of the biomaterial must be greater than 50%, and the pore size between 50 and 800 μm. However, it is widely known that the increase in the porosity of the implant leads to a decrease in its mechanical strength, although it is beneficial for tissue ingrowth [5]. Adequate mechanical stability is mandatory to obtain the necessary mechanical support during the bone repair and regeneration [10]. The need for stability leads us to observe that the design of the implant and the surface characteristics of the scaffold directly influence the application or use of the biomaterial, which may be needed temporarily (absorbable materials) or permanently [11]. Therefore, the balance between porosity and mechanical resistance is the key to the success of porous biomaterials.

Also, as previously mentioned, these characteristics are important in cellular differentiation and behavior since the biological tissues will interact mainly with the implant surface [8,11]. The

interface between the surface of a porous implant and the cells determines cellular behavior, such as cell adhesion, dissemination and proliferation [10,12]. Therefore, much attention has been given to tissue engineering, which seeks to develop materials such as scaffolds made of biocompatible material, like hydroxyapatite, collagen-based materials, bioglass, among others, which bind mechanically to bone tissue [5,13].

With the expansion of research in tissue engineering and the development of new biomaterials, it is increasingly necessary to understand the interactions of tissues with implanted materials, since it is these interactions that will determine the success or failure of the implant. However, according to [14] although considerable attention is given to the development of biomaterials for clinical applications, many fail to match the functional characteristics of target tissues in vivo due to their low biocompatibility. In this way, the present work meets the need for a better understanding of biomaterial-tissue interactions, helping to clarify how they occur and how the biomaterial characteristics influence them.

Protein adsorption

The host's response to an implanted material begins immediately after its introduction and covers several overlapping phases, including injury, protein adsorption, acute inflammation, chronic inflammation, foreign body reaction, granulation tissue formation and fibrous capsule formation [6,15]. The process by which atoms, molecules or ions in a fluid are retained on the surface of solids through chemical or physical interactions is known as adsorption. In the interface region, i.e., the place of interaction between the surface of the material and the biological environment in which it is installed, physical, chemical and biological mechanisms will direct the adsorption of proteins. The nature and amount of adsorbed proteins will directly influence the adhesion, migration and subsequent cell proliferation [4,16,17]. Thus, in contact between the biomaterial and physiological fluids, the layer of adsorbed proteins will alter the implant surface to prepare it for future cell colonization [17,18]. In summary, when a material is involved in the physiological environment, the adsorption of proteins on its surface is the first stage before cell adhesion (Figure1), which plays a key role in its biocompatibility [17,19,20].

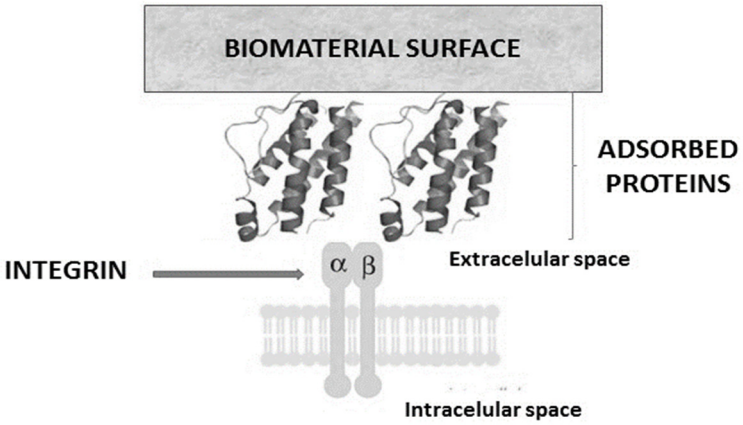

Figure 1: Schematic of the interactions between cell and proteins adsorbed on the biomaterial surface.

The events that occur at the interface between biomaterial and living tissue only a few moments after implantation are of great importance for the successful performance of the biomaterial in the organism [15,19,21]. The adsorption of non-specific proteins to a biomaterial occurs almost instantaneously after its implantation through a thermodynamically directed process to reduce the surface energy, and a monolayer of proteins can be seen within minutes [22,23]. After the formation of a protein monolayer on the surface of the material, the cells can adhere and proliferate within a period of up to 24 hours. There are numerous proteins in the living organism and their competitive adsorption, denaturation on surfaces and participation in blood clotting will critically influence the biocompatibility and consequently the performance of the biomaterial [16].

Inflammatory cells are able to recognize implanted materials as foreign by adsorbed proteins, thus initiating the cascade of events that lead to foreign body reaction [22], a process similar to opsonization. Therefore, plasma proteins play an important role in cell recruitment and tissue response to biomaterial implantation [18–20]. It is important to consider that this process of protein adsorption by the surface of the biomaterial is inevitable and practically irreversible except under certain conditions, as in the Vroman effect, for example [24]. In this phenomenon, observed by Vroman and Adams in 1969, a competitive protein exchange occurs on surfaces of the material, in which proteins already adsorbed from a protein mixture solution, are displaced by subsequently arriving proteins [23,25].

It is considered that the adsorption of proteins is a dynamic process and influenced by several factors such as the type of non-covalent interaction that will occur (hydrophobic interactions, hydrogen bonds, electrostatic forces and Van der Waals forces). It is also influenced by the characteristics of the proteins (size and structure, which in turn, influence the type of interaction), besides the biomaterial itself (roughness, chemistry and surface energy) [17,23,26]. According to [23] there

are three major forces that determine the rate of protein adsorption on the surface of a biomaterial. They are, the difference in protein concentration between the liquid phase and the surface, the inherent affinity of the protein for the surface and the size of the protein (that is, its molecular weight).

An example of how the surface of the biomaterial influences protein adsorption is the so-called wettability. It is known that hydrophobic interaction plays a major role in protein adsorption phenomena. Protein adsorption is greater on a hydrophobic surface than on a hydrophilic one [2,26]. Although protein adsorption is generally low on hydrophilic materials due to their low surface energy in aqueous environments, proteins, as has been reported, may still adsorb to hydrophilic materials [22]. Examples of this phenomenon are observed with fibronectin, a protein that promotes cell adhesion, and fibrinogen, important for blood coagulation. Both are adsorbed in greater quantities on hydrophilic and hydrophobic surfaces [17,20,22]. In the case of bone tissue, wettability can influence the first stage of osteoblast adhesion and the quality of osseointegration [17,20,27], since it is known that osteoblasts recognize and adhere to the adsorbed fibronectin on the surface of the implanted material [17,20].

Another characteristic of biomaterials that have an important influence on protein adsorption and consequent cellular adherence, especially in bone tissue, is the surface roughness [8,19,27]. The nanoscale morphology of the implant surface shows evident advantages in inducing cell proliferation and differentiation, as well as good bone formation. [27]. Many studies have shown that the increase in surface roughness in micro and nanoscale, exhibiting size characteristics comparable to those of the resorption pits and cellular dimensions, leads to a greater differentiation of osteoblasts and local production of factors in vitro. In this way, there is an increase in bone implant contact in vivo leading to better clinical wound healing rates. The superficial roughness in nanometric scale, which corresponds directly to the sizes of proteins and cell membrane receptors, can also play an important role in the differentiation of osteoblasts and in the regeneration of injured tissues [19].

An adhesive protein of particular interest in this field is fibrinogen, an important component of plasma, which is adsorbed to the surfaces of the biomaterial and thus participates in the acute inflammatory response to the implanted material [28,29]. Their function in relation to biomaterials is due to the fact that proteins are surfactants, which means that they adsorb very quickly to foreign surfaces. Therefore, almost any biomaterial that comes in contact with biological fluids such as blood plasma or peritoneal fluids will cause a rapid adsorption of fibrinogen [29]. Besides fibrinogen, other proteins can adsorb to the surface of biomaterials, such as vitronectin and fibronectin. These proteins and others involved in cellular adhesion are rich with the tripeptide sequence, Ar-Gly-Asp (RGD), which has been shown to bind to a number of integrins [28]. In the bone tissue, the presence of RGD-related peptides on the implant surface accelerates the osseointegration process, as they serve as a signal and

transmit instructions to the cells, such as adhering to the surface, spreading, differentiating and inducing bone tissue formation [28,30,31].

Plasma proteins, and also those present at the interstitial site of the implant, that are adsorbed on biomaterial, undergo changes in its structure, including denaturation on the implant surface, supporting subsequent cellular adhesion [3,16]. In addition, platelets release chemotactic signals that stimulate cell migration. With respect to platelets, their adhesion on the implant surface is also influenced by the characteristics cited in the previous paragraphs [32–34]. Authors [32], demonstrated that platelet adhesion on the surface of a biomaterial is directly related to its complexity, with more irregular surfaces favoring the adhesion of the same. Specifically, platelets activated by contact with a material surface may release cytokines that activate other cells, including other platelets, in the implant surface, modulating the degree of platelet activation [33].

Macrophage Activation and Foreign Body Reaction (FBR)

The implantation of a biomaterial within the body initiates in the host a series of immune reactions collectively called foreign body response (FBR), which try to eliminate and / or isolate the implanted material [14,35–37]. Inflammatory cells are thought to recognize implanted materials as foreign through the adsorbed proteins, thus initiating a cascade of events that lead to the FBR [22]. The most prominent cells in the FBR are macrophages, which attempt to phagocyte the material, though complete engulfment and degradation are often difficult. The macrophages, activated in the process of interacting with a biomaterial, may elaborate cytokines which stimulate inflammation or fibrosis [35].

As described above, after the implantation of a biomaterial, an influx of blood and interstitial fluid proteins creates a random protein coating on the surface of the biomaterial (blood-based provisional matrix) [15,38,39]. This matrix is formed by fibrinogen, vitronectin, complement, and fibronectin. In response for these formation, platelet activation occurs with the constitution and proliferation of thrombus and activation of cells of the inflammatory response [39]. Protein adsorption and chemotaxis of neutrophils and mast cells to the implant site direct the acute inflammatory phase. The formation of these provisional matrix can take from a few hours to days [14]. The inflammatory cells interact with the adsorbed proteins and are activated. This process induces recruited macrophages to the implant site, as well as resident macrophages. In fact, although neutrophils arrive early, they are quickly replaced by macrophages, the orchestrators of FBR [40]. Then, they begin to secrete chemotactic cytokines and other signaling molecules, which contribute to the development of FBR [15,28,38].

Adsorbed fibrinogen promotes adhesion of platelets and monocytes/macrophages, playing a key role in the foreign body reaction associated with biomaterial implants [29]. It is important to mention the importance of Mac-1, a leukocyte integrin present in macrophages that binds to fibrinogen when it is adsorbed. This integrin is responsible for the direct adhesion of macrophages and their activation on the implant surface [28,29]. Integrins are transmembrane receptors that act as bridges in cell-cell and matrix-cell interactions. This allows quick and flexible responses to events on the cell surface. Integrins contain two sub-units, α and β (Figure 1). During the process of attachment to any surface these subunits group together and recruit other cytoplasmic proteins [18]. Then occurs the formation of a complex called "focal contact" or “focal adhesion” which measures cell adhesion and migration [11,18].

Macrophages are activated while they are participating in local tissue responses including the FBR, wound healing, and certain diseases [41]. The two best studied macrophage phenotypes are M1 or classically activated and M2, or alternatively activated. Regarding the role played by each phenotipe, M1 macrophages are known to secrete pro-inflammatory cytokines such as IL-12, IL-23 and tumour necrosis factor alpha (TNF-α) [38,42,43]. M1 macrophages are critical for the initiation of angiogenesis and osteogenesis [44]. M2 macrophages promote extracellular matrix synthesis and cell proliferation, being considered as an anti-inflammatory phenotype and pro-wound healing [14,38,42,43]. M2 macrophages secrete large amounts of anti-inflammatory and pro-fibrotic cytokines such as IL-10 and transforming growth factor (TGF-β) [38]. In addition, the transition (balance) between the phenotypes M1 and M2 plays an important role in the formation and thickness of the fibrous capsule around implanted biomaterials [15]. In normal tissue repair, macrophages exhibit a pro-inflammatory phenotype (M1) at early stages and a pro-healing phenotype (M2) at later stages [41–43]. Macrophage polarization can be influenced by both wettability of the implant surface [38] and pore size in the case of porous implants [41]. Many studies have linked the macrophage polarization with FBR outcome. It is well known that the FBR is a dynamic series of events, and macrophage polarization earlier and later, may play an important role on it [41,42].

Macrophages can be found during the first weeks after the implantation of a biomaterial and in the final phase of the resorption process. This final step depends on the degradation time of the one in question [2,36,40]. Macrophages are considered the main cellular mediators of FBR in biodegradable materials. They coordinate a complexity of cellular reactions, which integrate inflammatory cytokines, growth factors and complement components with various cell types, including other macrophages, neutrophils, lymphocytes, endothelial cells and fibroblasts [36–38]. Macrophages are sensitive to minimal changes in the biological environment and set up a rapid response to implanted materials. They can also fuse under the influence of cytokines IL-4 and IL-13, released by mast cells (Figure 2), forming foreign body giant cells (FBGCs). Macrophages and FBGCs stimulate immune

(e.g., lymphocytes) and stromal cells (e.g., fibroblasts), leading to inflammation and fibrosis involving the implant [38,39,45]. The macrophages activation is directly related to the development of FBR, since macrophages alternatively activated (M2) are responsible for the formation of the fibrous capsule and also of the BFGC [40]. The intensity of this inflammatory reaction and fibrosis is related to many factors linked to the implant, as previously described [5,8,10]. Changes in the roughness of the implant surface, in micrometric and nanometric scales, influence the orientation, adhesion, spreading and formation of the macrophage cytoskeleton and also the thickness of the fibrous capsule formed around the implant [15]. Small particles are phagocyted by macrophages, large particles stimulate the formation of FBGC [38,45].

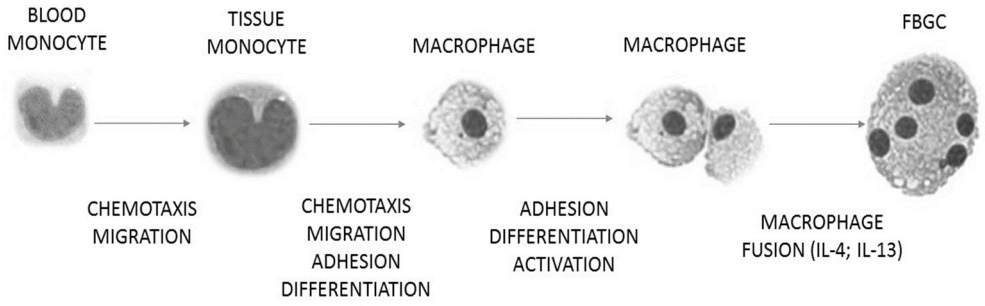

Figure 2: Activation and migration of monocytes and the subsequent fusion of macrophages forming a giant foreign body cell

FBGCs are considered a hallmark of FBR and can cause degradation of the implanted biomaterial, leading to its poor performance at the implantation site. Being more specific, FBGCs secrete reactive oxygen species, degradation enzymes, and create an acidic environment at the interface of the biomaterial. After these events, the formation of a collagenous and largely avascular capsule occurs, involving the biomaterial within 2 to 4 weeks after being implanted. Pro-fibrotic signals at the implant site are responsible for this phenomenon. The confinement of the material within this capsule prevents the actual integration of the implant with the surrounding tissue [3,37]. The formation of this capsule is directly linked to the action of macrophages [2,3,39] and FBGCs that secrete pro-migratory molecules and TGF-β, which leads to the recruitment of fibroblasts that deposit extracellular matrix and encapsulate the implant. These foreign body capsules can reach a thickness of 50 to 200 μm and completely envelop the implant in a largely avascular space, which consists of dense and highly organized collagen fibers [3]. The fibrous capsule formation is also related to several factors associated to the implant, such as its size, shape and texture, as well as its chemical properties, porosity, implant location, and chemical and physical stimuli caused by the implant [13,37,46].

Implant Osseointegration

As described above, the biocompatibility of a material is closely related to the behavior of cells in contact with its surface, especially cell adhesion. Immediately after the insertion of the implant into the bone tissue, the cellular mechanisms that culminate in osseointegration are initiated. In this phase the characteristics of the implant surface have a great influence. The material's surface properties affect the adsorption of proteins and modulate cell proliferation and differentiation, influencing not only the osteoblast adhesion process but also the tissue response. Surface characteristics such as roughness, porosity, chemistry and energy significantly influence cell differentiation and, consequently, bone growth and osseointegration of the implant [1,8,11] because they play in important role in osteoblast adhesion on biomaterials [31].

Osteoblasts adhere to implanted surfaces and to ECM (extracellular matrix) through adhesion molecules, as previously described [21,47]. The cell-matrix adhesions mechanically connect the internal actin filaments to the matrix. The complex is known as a focal adhesion, focal plaque or focal contact [47]. Focal adhesions mediate intracellular signaling pathways as they are directly linked to the cytoskeleton, thus influencing cellular behavior (Figure 3) [8,31]. Therefore, fixation, adhesion and spreading are part of the first phase of cell/material interactions and the quality of this first phase will direct the cell's ability to proliferate and to differentiate itself in contact with the implant [31]. This means that stable cell adhesion is essential for further proliferation and differentiation [48].

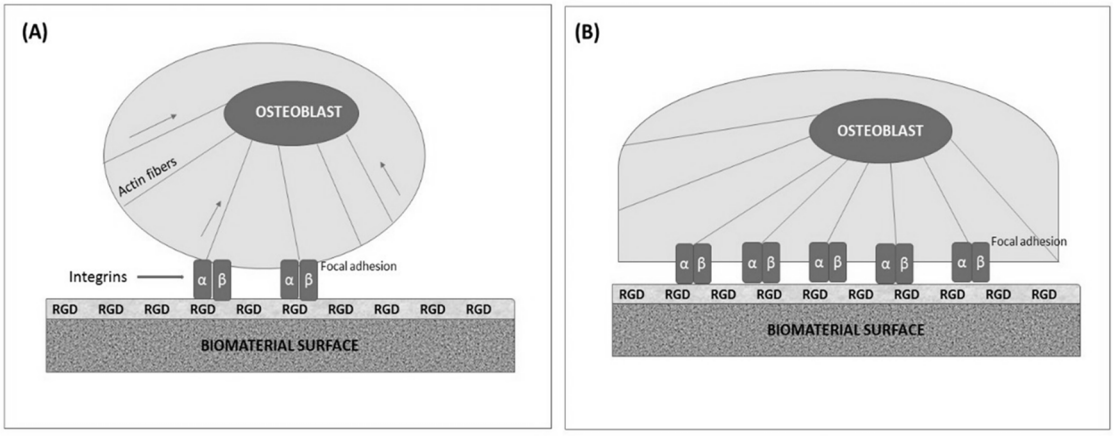

Figure 3: Schematic diagram of osteoblastic cell adhesion process. (A) Integrins recognize RGD sequences and mediate the cell-biomaterial anchorage. Then, signs initiate the recruitment of cytoskeletal proteins resulting in the formation of focal adhesions. (B) Spreading: actin microfilaments and the cytoskeletal network rearrange and reshape cell morphology.

The recruitment and migration of a population of potentially osteogenic cells is the first step to promote bone formation on the implant surface, then it is necessary to differentiate this population into mature secretory cells, i.e., osteoblasts. Osteogenic cells migrate through the blood clot formed at the site of the fracture or bone defect and reach the surface of the implant or the bone where it was

implanted. They then differentiate and form the new bone [17]. In order to favor the mechanisms involved in bone formation around an implanted material, it is necessary that the implant has micro and nanotopographical characteristics that allow the connections of the extracellular matrix proteins with its surface. Studies show that both micrometric and nanometric characteristics influence cell adhesion, proliferation and osseointegration [7,18,27]. Depending on the implant surface morphology, on the first day of implantation, mesenchymal cells, preosteoblasts and osteoblasts adhere to the implant surface [17].

In recent decades, several studies have suggested that distinct micro and/or nanoscale structural characteristics that result from different surface modification techniques can play a significant role in influencing the behavior of target cells and thus define the quality of bone integration. Despite many pre-clinical in vitro and in vivo studies on the evaluation of new implant biomaterials, there is still scarce knowledge about the link between the specific characteristics of an implant surface and the cellular response that occurs at its interface with the surrounding tissue [8,21,48]. Many authors stated that osteoblastic cells adhere more rapidly on rough surfaces where there is more pronounced proliferation of extracellular matrix synthesis than on smooth surfaces [11,49,50]. However, there is no consensus on this issue since cell behavior depends on the degree of roughness. These might explain differences in studies, as roughness parameters vary from one study to another [51]. In addition, there is no uniformity in the cell lines used in the experiments and cells with different phenotypes may show different responses to the surface of the materials [19,21,51]. Studies show that changes in cell shape, which occur after its attachment to the surface of the biomaterial, are directly linked to its phenotypic characteristics. And these shape changes can alter cell metabolism, leading therefore to different results depending on the cell type used [47].

Regarding the surface wettability of the implants, it was observed by [51] that hydrophilic surfaces were more likely to favor the adhesion of osteoblasts than fibroblasts. And according to [21], cells adhere more easily to hydrophilic surfaces. Authors [27] observed greater cellular adhesion in titanium implants as their surface wettability increased. As described above, wettability affects not only protein adsorption, but also platelet adhesion, thus influencing the subsequent phase, that is, the colonization of the implanted surface by osteogenitor cells [34].

After adhesion and migration, cells divide and proliferate across the surface. They further differentiate which means that they synthesize the molecules they normally synthesize in their tissue of origin. Osteoblasts will synthesize collagen and the other proteins (non-collagenous proteins) normally found in bone tissue [21]. Most of these (especially fibronectins, osteopontines, bone sialoproteins, type I collagen, vitronectins) are involved in the process of cell adhesion and chemotaxis [52,53] due to the RGD sequence present in its molecules [28,30,52]. Fibronectin and vitronectin adsorption are important in

osseointegration since they induce the reorganization of actin microfilaments promoting adhesion and scattering, which in turn affects cell morphology and migration [30,52]. In addition, osteoblasts recognize and adhere to the adsorbed fibronectin on the surface of the biomaterial [30,53].

Adhesion molecules are characterized by the ability to interact with specific binders, which may be located in the membrane of neighboring cells or be proteins in the extracellular matrix. These adhesion molecules belong to different families. However, in relation to osteoblasts, the most important are integrins [11,18,30]. After binding to specific sites within the extracellular matrix proteins, especially to the sequence of RGD trypeptides, integrins quickly associate with the cytoskeleton modifying it to form focal adhesions (Figure 3). This interaction of integrins with the extracellular matrix proteins is necessary for the gene expression of osteoblasts. Together with growth factor receptors, focal adhesions signal pathways for cell proliferation [30,53]. Both the chemical composition of the material and its topography influence the interaction of integrins with the substrate and integrins with cellular behavior [11,18,49]. Cell migration requires an integration between cell, substrate and cytoskeleton. First, the cells develop a protrusion forming the lamellipod and then use the adhesive interactions to generate traction and energy for movement. Finally, the release of the adhesion points occurs, followed by the detachment and retraction [18].

Not only osteoblasts, but also osteoclasts play a role in bone healing, development and remodeling and their complementary activities are important in bone-biomaterial interaction [11,54]. For many applications, it would be beneficial to identify the surface characteristics that would be ideal to promote bone formation by osteoblasts, suppressing the resorptive activity of osteoclasts. If the biomaterial is to be used as a synthetic bone substitute, then the degradation rate should be comparable to bone. However, if the intended application is a permanent implant, then the resorption rate should be minimized [11]. An ideal, bioactive material for bone substitution should be able to be resorbed and replaced by new bone, before having its stability compromised. In other words, the ideal bone substitution material should be osteoinductive, osteoconductive and only stay in the body as long as necessary to replace the defect by newly formed bone [55]. Therefore, considering the importance of osteoclasts for bone remodeling and for responses triggered by trauma or functional demands, the understanding of the mutual interactions of osteoblasts, osteocytes, osteoclasts and biomaterials is of great interest [54].

The reabsorption of bone and mineral substrates depends on the formation of a transient resorption complex, which is composed of actin rings and a ruffled plasma membrane [56]. This ring is associated with a “sealing zone”, that constitutes the place where the osteoclasts firmly adhere to the bone forming a diffusion barrier, which in turn establishes a resorption compartment in which proteolytic enzymes are secreted [54,56]. In the case of synthetic apatite biomaterials, acidification of this compartment

causes mineral dissolution and formation of a resorption pit. Therefore, the resorption of ceramic materials by osteoclastic activity occurs similarly to bone resorption [11,54]. Authors [11] observed that surface topography also affects the activity of osteoclasts, and more activity is observed on smoother surfaces. In their study they also observed that the topography affects the capacity of the osteoclasts to resorb hydroxyapatite, since they did not observe osteoclastic resorption pits in the rougher material. Apparently, the smoother surface is a facilitator of the connection of these cells with the biomaterial.

Authors [57] observed osteoclasts and FBGCs in close proximity during foreign body reactions. According to the authors [58] FBGCs may resorb bone substitutes in collaboration with osteoclasts during the process of involvement into bone. In accordance to the authors, when biodegradable implants are implanted in bone defects, their degradation and osteogenesis occur simultaneously, and FBGCs and / or osteoclasts can contribute to the replacement of implants by bone. Unlike FBGCs, osteoclasts are physiologically essential for bone metabolism and even if there is no pathological stimulation of osteoclastic activity, which is usually induced by inflammation, osteoclasts can contribute to the regeneration of bone defects that received biodegradable implants.

A relevant factor in the osseointegration of an implant is the ability of the material to provide a sufficient supply of nutrients and oxygen to the implant site [42,59]. This means that ideally, the implant should be able to promote blood vessel ingrowth from the surrounding host tissue, connecting the scaffold to the host vasculature [42]. Vascularization is crucial for the development and the repair of most tissues, and is a precondition for the healing of bone defects. A functional vascularization is necessary for bone formation and the substitution of biomaterials with osseous tissue [59]. The growth of new blood vessels is critical not only to stimulate osteogenesis, but also to support cell viability [42,60]. In developing bone, differentiation of mesenchymal stromal cells (MSCs) into osteoblasts is coupled with the invasion of capillaries, and the capillary network serves as a template for the bone ingrowth [42,61]. The natural inflammatory response to injury or to an implanted biomaterial determines the course of angiogenesis, healing, and repair [2,42].

It is well known that porous bone substitutes permit vessel ingrowth and thus facilitate osteogenesis [1,60,61]. Several studies have been performed in order to evaluate the pore size influence on bone and blood vessels ingrowth through porous biomaterials. Authors [59] observed, in their study using a porous, biphasic calcium phosphate ceramics, that the onset of blood-vessel formation occurred after a shorter time period, and the functional capillary density (FCD) was higher in association with ceramics whose pore sizes exceeded 140 µm than with either those whose pore sizes were smaller than 140 µm or dense particles. They conclude that the observed differences in angiogenesis and vascularization were a function of pore size. The minimum pore size that is required

to generate mineralized bone is considered to be 50 µm [1,59]. As previously exposed, larger pores are better for vascular growth and bone formation, however they compromise mechanical stability [1,5,59].

Conclusion:

The ideal biomaterial should provide a biomimetic environment to ensure cell survival and also direct cell migration in order to that relevant cells migrate and adhere to the implant, thus ensuring its effectiveness. The type of cellular and tissue response to implanted biomaterials is dependent on their features. The main characteristics that influence the success of a biomaterial after its implantation are related to the surface that comes into contact with the tissues, such as topography (roughness), wettability and surface chemistry. These characteristics will guide the protein adsorption and the inflammatory response related to the implant. For biomaterials implanted in bone, the most important features influencing implant osseointegration and osteoconduction are surface roughness, wettability and porosity. Thus, the manipulation of the surface characteristics of implants has been increasingly the target of research, with the aim of improving to the maximum the performance of biomaterials in the various tissues in which they are used.

Acknowledgements:

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel), CNPQ (National Council for Scientific and Technological Development) and FAPEMIG (Research Support Foundation of the State of Minas Gerais) for financial support.

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