THE ROLE OF CELL ADHESION TO BIOMATERIAL

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Abstract

The efficacy of biomaterials-either as replacements for damaged tissues, or as a temporary scaffolds for the manufacture of engineered tissues and organs- relies on the ability of the material surface to regulate cell adhesion. Adhesion plays a critical role to regulate proliferation, differentiation and phenotypic behaviour, therefore consequently impact tissue development and function. Cell adhesion to biomaterials may be characterized in terms of specific and nonspecific interactions. Specific interaction entail cell receptor recognition, whereas nonspecific interaction encompass noncovalent attractive forces. This paper gives a review of the phenomena occuring in cell/material interaction and particularly the role of cell adhesion.

***Keywords**: cell adhesion; biomaterial; specific interaction; nonspesific forces*

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In the last decades, a novel multidisciplinary field of science called tissue engineering has been developed as a new therapeutic approaches for variety tissues. Its main purposes are production of tissues and organs substitute that can replace or restore the natural features and physiological functions of natural tissues in vivo. The underlying principle of tissue engineering involves the utilization of biocompatible and mechanically suitable scaffolds, combined with an appropriate source of cells and bioactive molecules to promote the differentiation and maturation of the cell type of interest. These components, when combined, form a tissue-engineered construct, which can function as the tissue replacement material and, in principle, facilitate a faster rate of tissue repair [1,2].

Cell adhesion is fundamental process involved in tissue integration of biomaterial. The biocompatibility of biomaterials is very closely related to cell behaviour on contact with them and particularly to cell adhesion to their surface. The term 'adhesion' in the biomaterial domain covers two different phenomena: the attachment phase and the adhesion phase. The attachment phase occurs rapidly and involves short-term events like physicochemical linkages/non specific forces between cells and materials, involving ionic forces, van der Walls forces, etc. while the adhesion phase occurring in the longer term and involving various biological molecules with specific interactions [3,4].

The adhesion of anchorage-dependent mammalian cells to a substratum surface occurrs in four major steps: protein adsorption, cell-substratum contact, cell-substratum attachment, and cell adhesion/spreading These first phases will influence the cell's capacity to proliferate and to diferentiate itself on contact with the biomaterial and profoundly influence integration with tissue and eventual success or failure of a broad range of implanted biomaterials [3,4,5]. Thus, a complete understanding of cell behaviour on biomaterial and particularly the role of cell adhesion is needed for successful outcomes of tissue engineering.

This paper gives a review of the phenomena occuring in cell adhesion to biomaterial. The aim of the author is to highlight useful information for the understanding of cell/biomaterial interaction to improve present biomaterials and the future development of new biomaterials.

Cell adhesion

Initial attachment phase

Cell contact, attachment, and subsequent adhesion of anchorage-dependent cells are among the first phases of cell-material interactions [5]. Protein adsorption at the biomaterial surface is the early step of cell-material interactions, because cells interact with adsorbed protein on a biomaterial surface rather than the surface itself [5,6]. Cells do not see a naked material, in vivo or in culture. The material is conditioned by components of the fluid in which the material is immersed, whether it be serum, saliva, crevicular fluid or cell culture media. When an artificial material is exposed to cells suspended in a culture medium supplemented with fetal bovine serum, proteins in the serum are rapidly adsorbed onto its surface before the cells adhere. The adsorbed proteins determine the subsequent cell adhesion [7].

Cell contact and attachment involves gravitation/sedimentation to within 50 nm or so of a surface whereupon physical and biochemical forces conspire to close the cell-surface distance gap. Initial cell contact with the substratum presumably occurs by extension of filopodia that penetrate an

electrostatic barrier between cell-and-substratum surfaces that usually bear similar net-negative charges (fig 1.a). Filopodia attach firmly to the substrate and play an important role in orienting cells on the surface and begin the process of customizing the substratum for improved cell adhesion (fig 1.b). The early-attachment phase does not include significant extracellular matrix (ECM) production but rather is dominated by physical forces [5].

Cells attached to a surface then slowly (typically within hours) spread over the surface, depending on compatibility with the surface, expressing a strong 'biological component of adhesion' that includes secretion of ECM and results in the flattening of cells on the substratum (fig.1.c). Time required to complete contact-and-attachment steps in a simple, stagnant culture-dish arrangement is usually of the order of 30 minutes for typical soft-tissue cells, but clearly depends on a complex interplay between cell, surface and suspending fluid-phase composition [5].

Nonspecific physical forces

Several types of nonspecific forces that involves in the initial approach and binding of a cell to a substratum are: coulombic (or electrostatic) forces, which exist between any charged bodies, van der Waals (or electrodynamic) forces, which may arise between electrically neutral bodies, and hydrogen bonds [3,8].

Electrostatic force are repulsive between bodies of same charge and are attractive between bodies of opposite charge. In biological systems, electric charges arise from mobile ions (principally Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, H^+, and OH^-) and from fixed chemical moieties that covalently bound to macromolecules (such as PO_4^{3-}, COO^-, and NH_3^+) and undergo acidic or basic dissociation or association in aqueous solution. Under most condition, intact cells have an overall net negative charge, therefore cells would be attracted to positively charged substrata [8].

The van der Waals force arises as a consequence of the spontaneous transient fluctuations of charge distribution which occur in a body. The charge fluctuations arise from thermal motion and changes in the positions and momenta of electrons and atomic nuclei in the bodies [8]. Hydrogen bonds represents a special form of polar interaction in which an electropositive hydrogen atom is partially shared by two electronegative atoms [9].

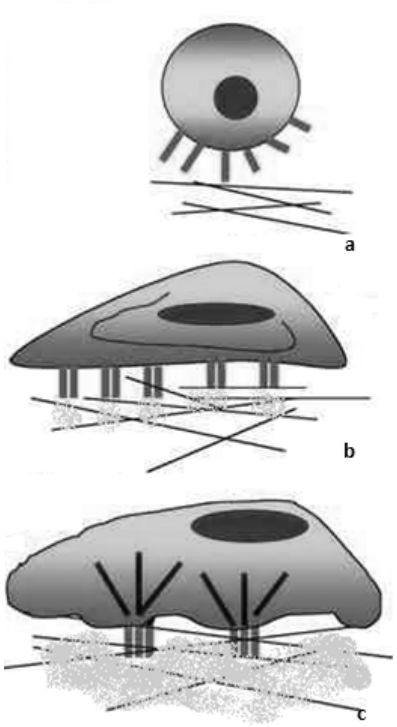

Figure 1. Ilustration of cell attachment with initial cell contact (a), filopodia attach to the substrate (b), expression of ECM (c)

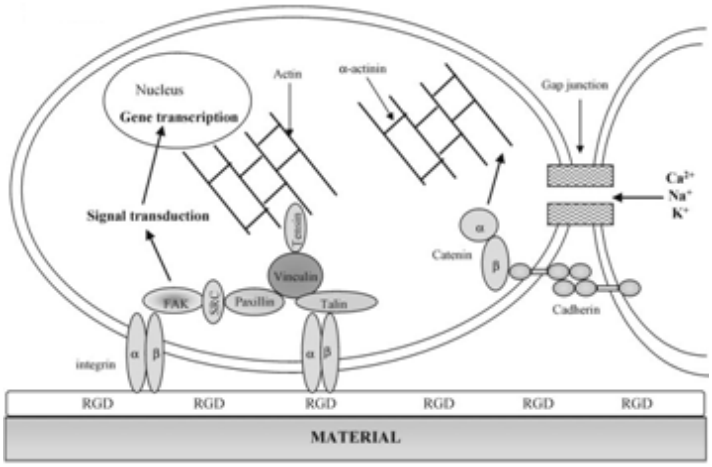

Figure 2. Representation of the cell proteins involved in cell adhesion on biomaterial [4]

Adhesion phase

The adhesion phase involves ECM proteins, cell membrane proteins, and cytoskeleton proteins which interact together to induce signal transduction, promoting the action of transcription factors and consequently regulating gene expression [4]. The interaction between cell and the substratum is mediated by trans-membrane receptor proteins, such as integrin, connected to the cytoskeleton of the cell. Strong adhesion are seen within 24 hours, though may occur within minutes [10]. C*ytoskeleton protein*

The site of adhesion is called focal contacts or adhesion plaque, where the extracellular domain presents integrin and on the cytoplasmic domain, some proteins are mediating interaction between actin filaments and integrin (figure 2). Focal contacts are closed junctions where the distance between the substrate surface and the cell membrane is between 10-15 nm. This type of junction is rare in vivo except for endothelial cells in vessels with high hydrodynamic stress [4].

The external faces of focal contacts present specific receptor proteins such as integrins. On the internal face, some proteins like talin, paxillin, vinculin, tensin are known mediating interactions between actin filaments and membrane receptor proteins (integrins). Many proteins colocalize with vinculin and talin in the adhesion plaque: integrin, cytoskeletal proteins, proteases, protein kinases and phosphatases, signalling molecules, etc. These proteins are involved in signal transduction [3,4].

The formation of focal contacts occurs essentially in cells with low motility and is promoted in vitro by extracellular matrix proteins like fibronectin or vitronectin. The architecture of the actin cytoskeleton is essential to the maintenance of cell shape and cell adhesion. If assembled in long bundles, F-actin supports finger-like protrusions of the plasma membrane known as filopodia; if assembled in the form of a mesh, it supports sheet-like protrusions known as lamellipodia. If present in bundles coupled with adhesion plaques, actin 'stress fibers' may transmit forces to the substrate [4].

Adhesion molecules

Adhesion molecules are characterized by their capacity to interact with a specific ligand. These ligands may be situated on the membrane of neighbouring cells or may be extracellular matrix proteins. Adhesion molecules belong to diferent families. The four main classes are selectins, immunoglobulin superfamily, cadherins and integrins [3,4].

Integrins: cell-substrate adhesion. The integrin family is composed of 22 heterodimers of two types of sub-units α and β. Integrins are transmembrane heterodimers consisting of noncovalently associated α and β sub-units. Each sub-unit is made up of a large extracellular domain, a transmembrane domain and a short cytoplasmic domain. The integrin spanning the cell membrane acts as an interfacer between the intra- and extra-cellular compartments and can translate the attachment of external ligands to internal information which induces adhesion, spreading, or cell migration and consequently regulates cell growth and diferentiation [3,4].

Cadherin: cell-cell adhesion. As cell-substrate adhesions are based on integrin-type receptors, adherens junctions containing cadherins mediate cell-cell adhesion (CAM). Cadherins are transmembrane glycoproteins acting with intracellular partners: catenins which interact with intracellular proteins. Association with α, β or γ-catenin is a

prerequisite for the adhesive function of cadherins. The cadherin family is composed of numerous types of calcium-dependent molecules (E, P, N, L, R, 6B, 7, 11, 4). They associate in a zipper homophilic model of interactions between cadherin molecules exposed on the plasma membrane of adjacent cells [3,4].

Gap junctions: cell-cell communications. Cell recognition and adhesion precede and control cell-cell communication via gap junctions. Intercellular communications occur through direct exchange of ions via gap junctions or through signals produced by the action of CAM [4].

Factors influencing cell adhesion on biomaterial

The process of cell interactions on materials is highly dynamic and depends on various parameters influencing the cell responses [11]. Cell adhesion and proliferation on materials are influenced by its surface characteristic such as wettability (hydrophilicity/hydrophobicity or surface free energy), chemistry, charge, topography and rigidity [11,12]. These surface characteristics determine how biological moleculs will adsorb to the surface and more particularly determine the orientation of adsorbed molecules [4].

Material surface with amino-, hydroxyl-, carboxyl-, sulfonic-, acylamino-groups favor cell adhesion and growth. Polymer surface grafted with amine groups was best for cell adhesion, spreading and growth in aqueous cell culture medium than hydroxyl groups and amide groups due to its positively charged properties. Positively charged surfaces also gives better support for cell adhesion, spreading and growth than negatively charged or negatively charged or neutral surfaces. [12,13].

Hydrophilicity of a material surface could affect the degree of cell adhesion and proliferation. Hydrophilicity of a material was believed to be a factor affecting the surface energy (surface tension), which might influence serum proteins that adhered to the material, and in turn governed the biological response, such as cell adhesion and proliferation [5]. Although hydrophobic surfaces tend to bind more protein, many cell studies have been reported that cells attached and spread more effectively on surface with proper hydrophilicity than on hydrophobic surfaces [6,12].

Conclusion

The phenomena occuring in cell/material interaction and particularly the role of cell adhesion are very complex. A complete understanding of cell behaviour in contact with the material is becoming more essential in developing advance materials for tissue engineering. The current challenge is to develop bioactive materials that can act through different types of cell receptors to regulate cell and tissue behaviour.

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