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# Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants

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ABSTRACT

The interaction of cells and tissues with artificial materials designed for applications in biotechnologies and in medicine is governed by the physical and chemical properties of the material surface. There is optimal cell adhesion to moderately hydrophilic and positively charged substrates, due to the adsorption of cell adhesion-mediating molecules (e.g. vitronectin, fibronectin) in an advantageous geometrical conformation, which makes specific sites on these molecules (e.g. specific amino acid sequences) accessible to cell adhesion receptors (e.g. integrins). Highly hydrophilic surfaces prevent the adsorption of proteins, or these molecules are bound very weakly. On highly hydrophobic materials, however, proteins are adsorbed in rigid and denatured forms, hampering cell adhesion. The wettability of the material surface, particularly in synthetic polymers, can be effectively regulated by physical treatments, e.g. by irradiation with ions, plasma or UV light. The irradiation-activated material surface can be functionalized by various biomolecules and nanoparticles, and this further enhances its attractiveness for cells and its effectiveness in regulating cell functions. Another important factor for cell–material interaction is surface roughness and surface topography. Nanostructured substrates (i.e. substrates with irregularities smaller than 100 nm), are generally considered to be beneficial for cell adhesion and growth, while microstructured substrates behave more controversially (e.g. they can hamper cell spreading and proliferation but they enhance cell differentiation, particularly in osteogenic cells). A factor which has been relatively less investigated, but which is essential for cell–material interaction, is material deformability. Highly soft and deformable substrates cannot resist the tractional forces generated by cells during cell adhesion, and cells are not able to attach, spread and survive on such materials. Local variation in the physical and chemical properties of the material surface can be advantageously used for constructing patterned surfaces. Micropatterned surfaces enable regionally selective cell adhesion and directed growth, which can be utilized in tissue engineering, in constructing microarrays and in biosensorics. Nanopatterned surfaces are an effective tool for manipulating the type, number, spacing and distribution of ligands for cell adhesion receptors on the material surface. As a consequence, these surfaces are able to control the size, shape, distribution and maturity of focal adhesion plaques on cells, and thus cell adhesion, proliferation, differentiation and other cell functions.



**Contents**



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## 1. Introduction

In recent years, artificial materials, particularly synthetic polymers, have been of growing importance in various biomedical technologies, including tissue engineering and transplantation medicine. However, for the construction of advanced bioartificial tissues and organs, the materials should not just be passively tolerated by the cells (as often happened in the "classical" materials of earlier generations) but should actively promote specific cell responses in a controllable manner. In other words, artificial cell carriers should act as analogs of the natural extracellular matrix (ECM), i.e. they should regulate the extent and strength of cell adhesion through the binding between cell adhesion receptors (e.g. integrins) and active parts of ECM molecules (e.g. specific amino acid sequences such as Arg-Gly-Asp, Bacakova et al., 2007a).

The extent and the strength of cell adhesion then play a decisive role in regulating the subsequent cell proliferation activity and switching between the proliferation and differentiation program in the cells. Anchorage-dependent cells do not divide (and even undergo apoptosis) without previous extension on the growth substrate — cell proliferation activity is therefore usually reported to correlate positively with cell flattening. However, this is true only to a certain degree — the maximum proliferation capacity as well as maximum migration speed is achieved at the intermediate extent of cell spreading and adhesion strength. Cells with very large adhesion areas with numerous and large focal adhesion plaques, containing increased levels of specific cell adhesion molecules and associated proteins (paxillin, talin, vinculin, tensin), are usually more quiescent concerning migration and proliferation, and more active in the expression of differentiation markers (Mann and West, 2002; for a review, see Bacakova and Svorcik, 2008; Bacakova et al., 2004).

The extent and strength of cell adhesion, and subsequent cell proliferation and differentiation, depend strongly on the physical and chemical properties of the biomaterial surface. Therefore, in this review, based mainly on our results obtained in the course of more than 10 years of research on biomaterials, the effects of the following factors on the adhesion, proliferation and phenotypic maturation of cells colonizing artificial materials are discussed:

- material surface chemistry, surface energy and wettability, and changes in these parameters, e.g. on synthetic polymers modified by ion-, ultraviolet light and plasma irradiation,
- the electrical charge and conductivity of the material surface,
- surface roughness and morphology,
- rigidity and deformability of the cell adhesion substrate,
- micropatterning the material surface with cell-adhesive and non-adhesive domains,
- nanopatterning and grafting the material surface (creation of adhesive nanodomains manipulating the assembly of focal adhesion plaques on cells).

It can be supposed that in most materials currently used for constructing tissue replacements, these factors have a common (universal) influence on the cell behavior, irrespective of material type. In various cell types, some common reactions to the basic physical and chemical properties of the adhesion substrate can also be expected. This review therefore focuses on common relations between the physico-chemical properties of material surface and cell behavior. Specific materials are only used as examples to demonstrate these relations. Specific materials (e.g. natural and synthetic polymers, metals, ceramics, carbon-based materials, composite materials and their specific organic and inorganic coatings) and specific types of tissue replacements (e.g. vascular prostheses, bone implants) have been discussed in several review articles and book chapters that we have published earlier (Bacakova and Svorcik, 2008; Bacakova et al., 2004, 2008, 2011; Parizek et al., 2011; Vagaska et al., 2010; Vandrovcova and Bacakova, 2011).

## 2. Chemistry, energy, polarity, wettability and zeta potential of the material surface

It has been shown repeatedly that cell adhesion to an artificial material depends strongly on the physico-chemical properties of the material surface, e.g. its chemical composition, energy, polarity and wettability. The chemical composition of the material surface is an important factor determining the surface energy, polarity, wettability and zeta potential, and consequently the character of the cell–material interaction. For example, the presence of oxygen-containing chemical functional groups increases the energy, polarity and wettability of the material surface, and supports the adhesion and growth of cells on this surface (Bacakova et al., 1996; Detrait et al., 1998; Feng et al., 2003; Kubova et al., 2007; Svorcik et al., 1995a,b).

*The surface energy of a material* is defined as the amount of energy per area required to reversibly create an infinitesimally small unit surface (Zhuang and Hansen, 2009). Surface energy quantifies the disruption of the intermolecular bonds that occur when a surface is created. In the physics of solids, surfaces must be intrinsically less energetically favorable than the bulk of a material, otherwise there would be a driving force for surfaces to be created, removing the bulk of the material, e.g. by a sublimation process. Surface energy may therefore be defined as the excess energy at the surface of a material compared to the bulk. The surface energy which can be converted into work has been referred to as "free surface energy" (also known as "free enthalpy", "available energy" or "affinity" in chemical systems). Free surface energy can be analyzed into polar and nonpolar (i.e. dispersive) components using the Owens–Wendt–Kaeble equation:

$$W_a = \gamma_L(\cos\theta + 1) = 2\left(\gamma L^D \gamma S^D\right)^{1/2} + 2\left(\gamma L^P \gamma S^P\right)^{1/2},$$

where $\gamma L^D$ and $\gamma L^P$ are the dispersive and polar components of the liquid surface tension, and $\gamma S^D$ and $\gamma S^P$ are the dispersive and polar components of the solid surface free energy, respectively (Youssef et al., 2001).

*The dispersive component of surface energy* results from molecular interaction due to London forces. These forces are part of the van der Waals forces and represent the weak intermolecular forces arising from quantum-induced instantaneous polarization multipoles in molecules. These forces can therefore act between molecules without permanent multipole moments. London forces are exhibited by nonpolar molecules because of the correlated movements of the electrons in interacting molecules. *The polar component of surface energy* comprises all other interactions due to non-London forces. Polar molecules interact through dipole–dipole intermolecular forces and hydrogen bonds. Molecular polarity is dependent on the difference in electronegativity between the atoms in a compound and the asymmetry of the structure of the compound. For example, a molecule of water is polar because of the unequal sharing of its electrons in a "bent" structure, whereas methane is considered non-