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Biomaterials 27 (2006) 1187–1199
Biomaterials
www.elsevier.com/locate/biomaterials

Leading Opinion

Modelling approach in cell/material interactions studies ☆

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Received 1 July 2005; accepted 9 October 2005
Available online 27 October 2005

Abstract

Based on our experiments, we propose a statistical modeling approach of the in vitro interactions between biological objects and materials. The objective of this paper is to provide basic principles for developing more ambitious experiments comparing the simultaneous influence of more than one or two parameters on various observations, taking advantage of convenient statistical and mathematical techniques for the treatment of measured data. Analyzing some examples of our own experiments, the essential features needed for modeling cell/material interaction studies are presented. Firstly, we describe the initial process of designing appropriate experiments that allow for comprehensive modeling. In the second part, we illustrate the different applications of a specific statistical modeling technique, the bootstrap protocol, on either the amplification of data, the elimination of correlation existing between measured parameters or, out of a set of parameters, identification of the most relevant parameter for further statistical analysis. Finally, based on recent statistical analysis tools such as the bootstrap, we illustrate the relative influence of biological and physical parameters in phenomenological studies of cell/material interactions.

Keywords: Cell adhesion; Modeling; Surface roughness; Surface analysis; Cell proliferation

1. Introduction

Recently, many efforts have been undertaken to improve bone regeneration by the use of cellular or none-cellular implants. In vitro studies of bone cell responses to artificial materials are the basic tools to determine the material surface/tissue interactions on a cellular level [1,2]. Classically, the majority of these in vitro investigations focus either on cell attachment after some hours or on proliferation during some days of culture. The effects of materials composition, as well as the effects of their surface chemistry or surface topography on cell adhesion and proliferation have been largely studied on bone-derived cells [3–11]. The material composition always influences cell attachment [4,6,7], whereas variations of surface chemistry of titanium-based substrates following surface treatments like anodization have generally little influence on osteoblasts attachment capacity [10,11]. Likewise, Ahmad et al. [12] described a not significant difference of osteoblastic cell attachment between Grade 1 and Grade 4 pure titanium. On the contrary, the surface roughness of titanium substrates is known to have a considerable effect on osteoblastic cell attachment as well as on cell adhesion, proliferation and differentiation [4,5,13–19]. Attachment is generally increased on rough surfaces ($Ra > 1\,\mu m$) produced for example by sandblasting compared to smooth ones [13,20–24] but sometimes no effects are described [9,25].

Different groups perform complementary and sometimes controversial studies to elucidate the interactions between cells and various materials (Fig. 1). One group prefers to

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0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.10.009

analyze the interaction of one isolated cell with a substrate. Then, specific methods like atomic force microscopy, magnetic twisting cytometry or micropipette aspiration [2,26] are used to quantify the cell adhesion strength. Another group focuses on the interactions of a population of cells with the material. Other specific methods like fluid shearing in flow cells, centrifugation, ultrasounds, enzymatic detachment are used to quantify adhesion strength [27–33]. However, these interactions are analyzed directly after the first minutes or first hours of cell–material contact [34,35]. The objective in this second approach is to exclusively consider short-term adhesive events occurring between cells and a given surface before cell proliferation begins and before cell/cell interactions are established. Finally, in a third approach, authors consider all together, short-term adhesive events or long-term adhesion, proliferation and differentiation phases [3,10,11,15–19,21,33,36]. We esteem this third approach as more valuable since it allows to approximate the in vivo situation. One of its major inconveniences is, however, the simultaneous involvement of many parameters of which a large number cannot be fully controlled.

This is one reason why in most typical studies of cell/material interactions, only one cellular parameter (attachment, proliferation) and one surface parameter are considered (surface roughness, surface composition). In most cases, the surface parameter is poorly defined and its influence not entirely analyzed. Moreover, it has never been proven if this parameter is not correlated also with other surface parameters. For example, the surface topography is often defined only by an average roughness amplitude parameter (Ra) although several other pertinent parameters exist, which describe the surface topography, for example frequency parameters [18,37–39]. Additionally, our team has shown that, analogous to mean roughness amplitude, cell adhesion is also correlated to frequency parameters describing the organization of the surface topography [18,33].

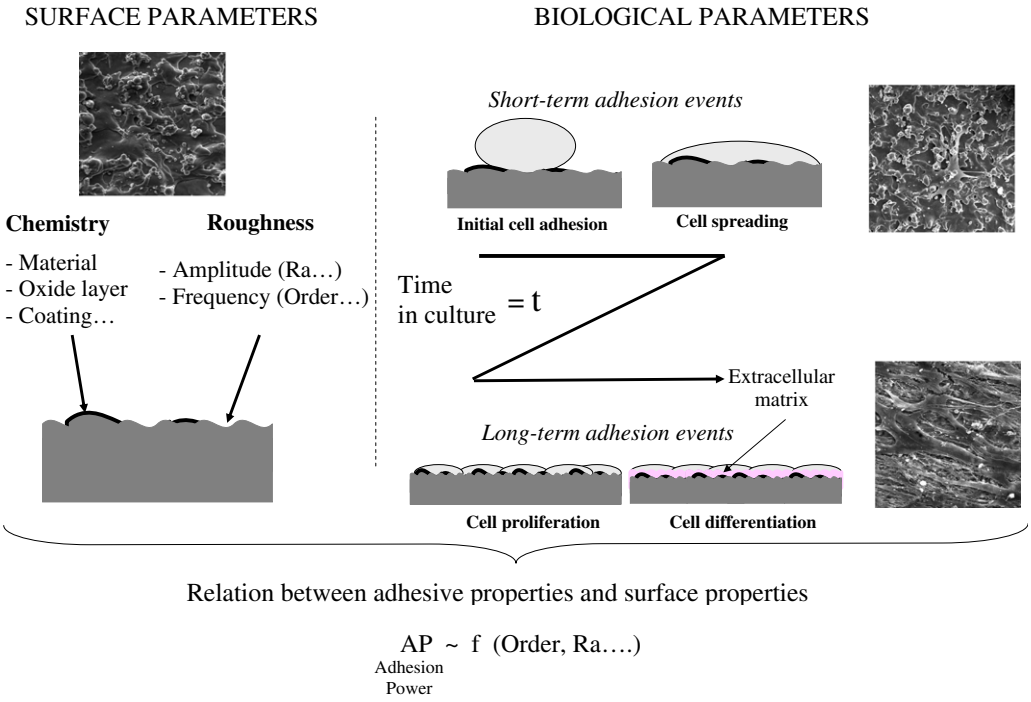

Fig. 1. Various surface and biological parameters are involved in cell/material interactions. On the material side, surface chemistry and surface roughness have together a main influence on cell behavior. The surface topography has to be characterized by more than only amplitude parameters. Frequency parameters need also to be measured in order to characterize also the organization of the topography (Table 2). In the same way, the deep analysis of surface chemistry is also essential. Indeed it has been shown that process used for increasing surface roughness can also modify surface chemistry [15] and it is necessary to take this into account when analysing the cell response to a surface. As for surface topography, we advise to measure several significant parameters describing the surface chemical state of the substrate. This could be achieved using only one technique but it must be preferable to use several techniques to fully characterize the surface chemistry. We cannot specify the techniques to use since they depend on the material studied but we want to insist on the final objective i.e. to be able, at the end, to do a quantitative measurement of one or several parameters describing the surface chemical state of the substrate for correlation with biological parameters. On the biological side, different phases can be distinguished with time in culture in the cell response to material. The first one is the initial cell adhesion phase involving non-specific electrostatic forces (e.g. Van Der Waals) and passive formation of ligand–receptor bonds, followed during the further hours by the cell spreading phase involving receptor recruitment, clustering to anchoring sites and interactions with cytoskeletal elements. The second phase concerns the proliferation and differentiation phases involving the extracellular matrix formation. In our cell culture model, this last phase belongs to what we call the long-term adhesion events. The quantification of the short-term adhesion, long-term adhesion and proliferation of cells allowed to establish relations between adhesive properties of cells (e.g. adhesion power) and surface properties (order, Ra) thanks to a specific statistical modeling approach.