European Cells and Materials Vol. 7. Suppl. 1, 2004 (page 17) ISSN 1473-2262

Interactions of cells and proteins with molecularly designed surfaces

Mário A. Barbosa[1,2] (mbarbosa@ineb.up.pt), Mª Cristina L. Martins[1] (cmartins@ineb.up.pt) , & Judite N. Barbosa [1,,2] (judite@ineb.up.pt)

[1] *INEB – Instituto de Engenharia Biomédica, Laboratório de Biomateriais, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal (www.ineb.up.pt)*

[2] *Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e Materiais, Porto, Portugal*

INTRODUCTION: Protein adsorption to implant materials influences the events leading to cell-material interactions, including thrombogenesis, which is induced by a series of events, including platelet adhesion and activation processes. One of the strategies defended to improve blood compatibility is the deposition of a thin layer of albumin on the material surface in order to minimise adsorption of other proteins. The problem is the non-specific nature of such adsorption, denaturation of the protein and exchange with other proteins over time, which renders the strategy limited to short-term applications. The possibility of using highly ordered structures constituted by self-assembled monolayers (SAMs) of long alkanethiols on gold substrates offers the possibility of modelling the substrate at the molecular level. The interaction of single and multi-functional surfaces with proteins and cells is thus possible, enabling a more precise control of the role of surface properties over such interactions.
In this paper we describe the influence of the terminal functional group over adsorption of albumin and the inflammatory response. For this purpose alkanethiols with three terminal functional groups (OH, COOH and CH_3) were tested. While CH_3 produces a hydrophobic surface, the other two have a hydrophylic character. Mixed monolayers, with hydroxyl- and methyl-terminated monolayers, were also investigated to elucidate in which manner the predominance of one type over the other influences the hydrophilicity of the surface and its tendency to induce selective albumin adsorption over fibrinogen adsorption.

METHODS: The SAMs were prepared by a method described previously [1]. Esssentially, the method consists in coating gold substrates with monolayers of 11-mercapto-1-undecanol ($SH-(CH_2)_{11}OH$), 1-decanethiol ($SH-(CH_2)_9CH_3$) and 11-mercaptoundecanoic acid ($SH-(CH_2)_{10}COOH$). The monolayers we produced by immersing gold surfaces in an ethanol solution of the alkanethiol with a final concentration of 1 mM over 24h. For protein adsorption studies human serum albumin (HSA) was dissolved in PBS in a concentration normally of 0.1 mg/ml. The adhesion of human leukocytes to the SAMs was studied by seeding the samples with two subpopulations of leukocytes: polymorphonuclear (PMN) and mononuclear leukocytes, non-activated and activated with phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA). The techniques are described in detail in reference [2]. The acute inflammatory response and the adhesion of cells to these surfaces was studied *in vivo* using a rodent air-pouch model of inflammation [3].

RESULTS: The coverage of SAMs was found to be linearly dependent on the amount of HSA on the surface, which increased in the order $OH>COOH>CH_3$. The coverage took place preferentially at active sites on the surface. Blocking of these sites was very effective in terms of electron transfer processes with the solution. When mixed monolayers ($C11OH + C15CH_3$) were investigated the surface remained hydrophobic until the percentage of C11OH was ca. 70% of the total amount of alkanethiols in solution. However, the hydrophylicity was linearly related with the surface concentration of C11OH. HSA adsorption was stronger on the more hydrophobic surfaces. Moderately hydrophylic surfaces (65% C11OH) show considerable HSA adsorption, which can be easily exchanged by HSA in solution but not by fibrinogen. Therefore, this surface may possess selectivity towards HSA adsorption.
Mononuclear and PMN leukocytes have a higher affinity towards methyl-terminated SAMs than for the more hydrophylic OH- and COOH-terminated alkanethiols. *In vitro* activation of both leukocytes further increased cell adhesion. In the animal model experiments the inflammatory response was rather intense with the OH and CH_3 groups. However, the number of cells adhering to the CH_3-terminated SAMs was rather low. This high tendency for CH_3 groups to recruit a large number of inflammatory cells but to originate few attached cells is not understood.

REFERENCES: [1]M.C.L. Martins, C. Fonseca, M.A. Barbosa, B.D. Ratner (2003) *Biomaterials* 24:3697-3706. [2]Judite N. Barbosa, Mário A. Barbosa, Artur P. Águas (2003) *J Biomed Mater Res* 65[A] :429-434. [3]Judite N. Barbosa, Mário A. Barbosa, Artur P. Águas (2004) *Biomaterials*, 25:2557-2563.