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Brief communication

Cell spreading correlates with calculated log*P* of amino acid-modified surfaces

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Abstract

The interactions of cells with synthetic surfaces are a critical factor in biomaterials design and it would be invaluable if these interactions could be precisely controlled and predicted. Hydrophobicity or lipophilicity of the surface is commonly used to rationalize cell attachment to materials. In the pharmaceutical sciences it is common practice to use log*P*, the partitioning coefficient between water and octanol, as a reliable indicator of the hydrophobicity or lipophilicity of (drug) molecules. A number of methods are available to reliably predict log*P* values directly from molecular structure. In this paper we demonstrate that log*P* values calculated on the basis of the molecular structure of a range of surface-tethered groups correlate well with cell spreading. To our knowledge this is the first method to predict cell spreading on chemically modified surfaces via nonspecific interactions.

Keywords: Amino acid; Log*P*; Surface; Osteoblast; Surface functionalization

1. Introduction

It is widely recognized that for a given substrate compliance, the interactions of cells with synthetic surfaces are dictated by the chemical and topographical properties of the material surface [1–4]. In protein-containing environments, e.g. serum-containing media, these interactions take place via an adsorbed surface protein layer. The identities, orientations and molecular conformations of the proteins in this layer ultimately determine the cellular response through specific interactions. Since Leo Vroman first described the effect in the late 1960s, the molecular and structural composition of the protein layer has been known to be dynamic, undergoing competitive adsorption, rearrangement and desorption over time [5]. It is possible to regulate cell spreading to surfaces by choosing a suitable surface chemistry [6–10], although the precise relationship between surface chemistry on protein adsorption and cell adhesion are complex and consequently not fully understood [1–4].

In the pharmaceutical sciences it is common practice to use log*P*, the partition coefficient between water and octanol, as a reliable indicator of the hydrophobicity or lipophilicity of (drug) molecules [11,12]. At least 40 different methods are currently available that predict log*P* from molecular structure, with the most reliable methods generally considered the fragmental ones, with Hansch and Leo's Clog*P* (computed log*P*) probably the most widely known and used [11,12]. This method simply adds assigned values for structural parts of a molecule together and

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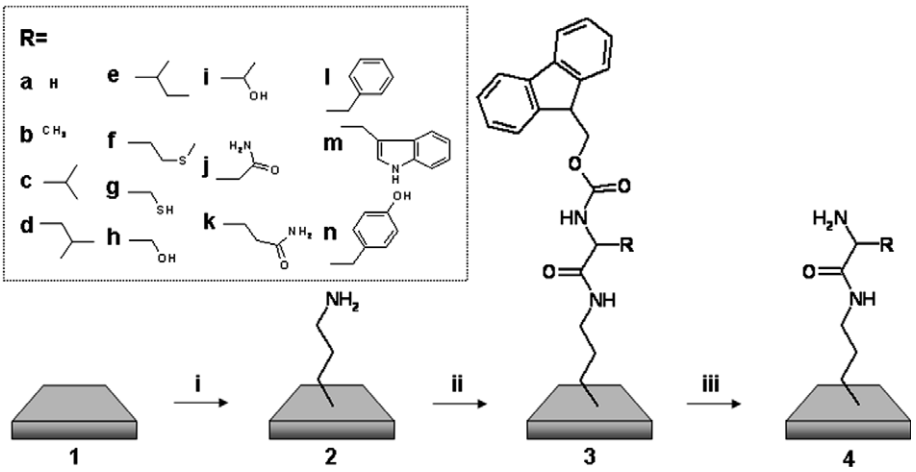

Fig. 1. Procedure to make homogeneous chemically functionalized surfaces, where 1 = glass coverslip; (i) = silanization step using APS; 2 = surface presenting free amines; (ii) = standard DIC/HOBt coupling steps; 3 = surface modified with Fmoc-amino acids with side groups R = a–n; (iii) = chemical removal of Fmoc and other protecting groups to reveal the underlying amino acid; 4.

applies extra factors depending on the way the parts of the molecule interact.

In this paper we used a subset of the naturally occurring amino acids as a source of chemical functionalities – we exclusively studied amino acids with uncharged side chains (at physiological conditions), thereby separating the effects of hydrophobic interactions and electrostatic interactions. This left us with a library of 14 amino acids, providing a range of different properties (see Fig. 1). These comprise Gly a, amino acids with aliphatic groups (Ala b, Val c, Leu d and Ile e), sulphur-containing groups (Met f, Cys g), alcohols (Ser h, Thr i), amides (Asn j, Gln k) and aromatic groups (Phe l, Trp m, Tyr n). We calculated their Clog*P*, using the Clog*P* calculator from Daylight Chemical Information Systems, Inc. (www.daylight.com), characterized them using water contact angles, time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS), and established the varying degree of osteoblast spreading onto these surfaces.

2. Materials and methods

2.1. Sample preparation

All chemicals and reagents, unless stated otherwise, were purchased from Sigma Aldrich Company Ltd. and used as received. All cell culture reagents, media and buffers were purchased from Invitrogen Ltd.

Borosilicate glass coverslips (Chance Glass Ltd, 12 mm diameter, No. 2 thickness) and all other glassware used in the experiments were cleaned by immersion in "Piranha" solution – a 3:7 mixture of 30% hydrogen peroxide solution and concentrated sulphuric acid – for 30 min, followed by rinsing in copious amounts of deionized water and drying in an oven at 100 °C overnight.

Amino acid surfaces were prepared by a well-established method used in our laboratory; first, glass coverslips were functionalized [13] (Fig. 1, surface 1) by immersion for 1 h in a solution of 1% (3-aminopropyl)triethoxysilane (APS), 4% deionized water and 94% ethanol (Fig. 1, step i). The glass slides were rinsed in ethanol and dried in a stream of nitrogen both prior to and following immersion in the APS solution. The slides were then dried further in an oven at 50 °C for 10 min. This produced amine-functionalized surfaces (Fig. 1, surface 2). 9-Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (Bachem Ltd) were then coupled to the amine-functionalized glass surfaces in the presence of 1-hydroxybenzotriazole (HOBt) and *N*,*N*′-diisopropylcarbodiimide (DIC) in *N*,*N*-dimethylformamide (DMF) (Fig. 1, step ii). All samples were rinsed with 1% trifluoroacetic acid (TFA) in acetonitrile (ACN), DMF, 1% TFA in ACN, ethanol, 25:75 ethanol:deionized water, deionized water and finally ethanol, and were then dried in a stream of nitrogen. Fmoc–amino acid coupling was carried out twice, by immersion in solution for 2.5 h in the first instance followed by rinsing as described above, then immersion in fresh solution for 18 h, again followed by rinsing as described above, to produce surfaces 3a–n (see Fig. 1). To expose the attached amino acids (Fig. 1, surfaces 4a–n), the Fmoc-protecting groups were removed by immersion in 10% piperidine in DMF for 30 min and other side-protecting groups (*t*-Butyl (*t*Bu) on Ser h, Thr i, Cys g and Tyr n, and *t*-butyloxycarbonyl (Boc) on Trp m) were removed by immersion in aqueous TFA (90%) for 20 min (Fig. 1, step iii). The aforementioned rinsing scheme was employed following both protecting group removal stages.

2.2. ToF-SIMS analysis

Secondary ion mass spectrometric analysis was carried out using a SIMS IV time-of-flight (ToF-SIMS) instrument (ION-TOF GmbH, Münster, Germany) equipped with a gallium liquid metal ion gun and a single-stage reflectron