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Cell adhesive PET membranes by surface grafting of RGD peptidomimetics

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Abstract

A non-peptide mimic of the Arg-Gly Asp (RGD) active sequence of adhesive proteins (such as vitronectin) has been equipped with two different spacer-arms for surface anchorage. The covalent grafting on poly(ethylene terephthalate) (PET) membrane was realized via the activation of the hydroxyl polymer chain-ends by tosylation followed by nucleophilic substitution. The surface density of peptidomimetics was determined by X-ray photoelectron spectroscopy (XPS), on the basis of F/C atomic ratios since a fluorine tag was incorporated into the RGD-like compounds. The biological activity of soluble peptidomimetics was evaluated versus isolated human integrin $\alpha_v\beta_3$ (vitronectin receptor), and versus CaCo2 cells. Inhibition of cellular adhesion was observed after pre-incubation of CaCo2 cells with soluble peptidomimetics. On the other hand a significant promotion of cellular adhesion resulted from the surface grafting of peptidomimetics on the PET culture substrate. The best performance was obtained with the RGD-like integrin ligand bearing a triethylene glycol spacer-arm.

Keywords: RGD peptidomimetics; Poly(ethylene terephthalate) membrane (PET); In vitro cell culture; CaCo2 cells; Vitronectin; Polymer surface modification; XPS analysis; Biocompatibility

1. Introduction

Most of the mammalian cells must adhere on a substrate, the extracellular matrix (ECM) in natural tissues, in order to carry out normal metabolism, proliferation and differentiation. Proteins of ECM are ligands of membrane bound receptors, called integrins that are responsible for cell–cell and cell–matrix interactions. Integrins are heterodimeric glycoproteins, composed of non-covalently associated α and β polypeptide chains. Depending on the nature of the α and β subunits, the receptors display some selectivity for endogenous proteins [1]. The vitronectin receptor $\alpha_V\beta_3$ is expressed on endothelial, smooth muscle, bone and epithelial cells. Recently, the structure of the extracellular domain of $\alpha_V\beta_3$ has been solved by X-ray diffraction analysis, in the native state [2] and in complex with a cyclic peptide containing the RGD (Arg-Gly Asp) sequence [3]. Indeed, integrin $\alpha_V\beta_3$ interacts with vitronectin through its RGD binding domain. The RGD sequence is also present in other ECM and blood proteins, and depending on its conformation and peptide environment, is selectively recognized by different integrins [1].

Integrin $\alpha_V\beta_3$ has been implicated in the pathogenesis of a number of diseases such as osteoporosis, cancer and inflammatory conditions. Therefore, this receptor became an attractive target for the development of novel therapeutic agents. Considering the most active and selective RGD cyclic peptides as models, small peptidomimetic molecules were developed. Potent $\alpha_V\beta_3$ antagonists have been disclosed over the last 10 years and considered in (pre)clinical tests [4–10].

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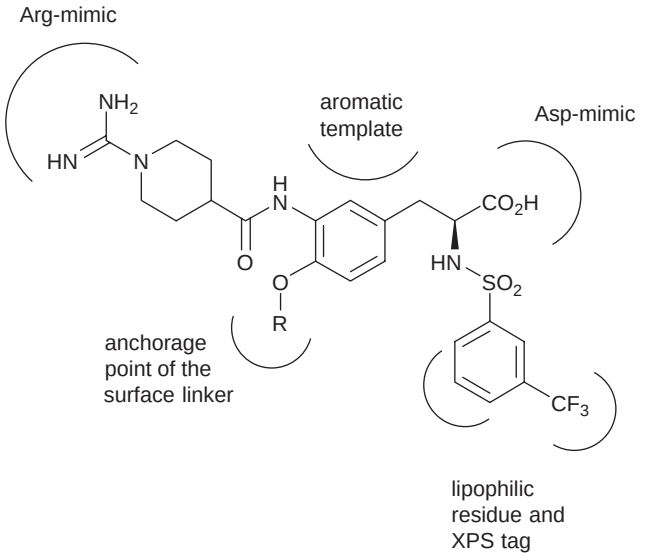

Fig. 1. Design of RGD peptidomimetic for surface grafting.

In the field of biomaterials science, RGD peptides (linear or cyclic) were also used to promote cellular adhesion, a phenomenon of crucial importance in the case of substrates for tissue engineering [11–19]. To our knowledge, except our preliminary work [20], non-peptide RGD mimics were never considered for the preparation of tailored materials with cell adhesive properties [21].

We are interested in the surface modification of poly(ethylene terephthalate) (PET) track-etched membrane by the covalent grafting of RGD peptidomimetics as $\alpha_V\beta_3$ ligands. Such a microporous support used for the in vitro cultivation of mammalian cells allows to investigate the transport of pharmacological agents through the cultured cells. For our purpose, we designed adequately functionalized molecules based on the L-tyrosine template and equipped with a spacer-arm for their anchorage on the PET membrane surface. An optimum length of this spacer is required considering the availability of the grafted ligand towards recognition by cellular receptors (Fig. 1). This article reports on the coupling to PET membranes of RGD peptidomimetics varying by the nature of the spacer-arm, and the quantification of the grafted molecules by X-ray photoelectron spectroscopy (XPS). The evaluation of the cell-attachment capacity of the resulting substrates is further described by using the CaCo2 cells which express the $\alpha_v\beta_3$ integrin; this cell line is usually considered for the development of pharmacological models.

2. Experimental

2.1. Synthesis of peptidomimetics

The biologically active molecules depicted in Fig. 1 (Table 1) were prepared according to Refs. [22,23]. The compounds were characterized by NMR analysis and high resolution mass spectrometry (HRMS, see Table 1).

Reference ***4*** (R = Me): ^{1}H (D_2O, 500 MHz) δ: 7.87 (s, 1 H), 7.85 (d, $J = 8.2$ Hz, 1H), 7.76 (d, $J = 8.2$ Hz, 1 H), 7.55 (dd, $J = 8.2$, 8.2 Hz, 1 H), 7.26 (d, $J = 2.1$ Hz, 1 H), 6.90 (dd, $J = 2.1$, 8.5 Hz, 1 H), 6.76 (d, $J = 8.5$ Hz, 1 H), 4.12 (dd, $J = 4.1$, 11.1 Hz, 1 H), 3.95 (m, 2 H), 3.89 (s, 3 H), 3.21 (m, 2 H), 3.12 (dd, $J = 4.1$, 13.7 Hz, 1 H), 2.80 (m, 1 H), 2.69 (dd, $J = 11.1$, 13.7 Hz, 1 H), 2.02 (m, 2 H), 1.77 (m, 2 H); ^{13}C (D_2O, 125 MHz): δ 178.3, 178.0, 158.6, 151.8, 142.7, 133.2, 132.9, 132.7, 132.5, 132.0, 131.1, 129.5, 127.8, 127.0, 126.0 (CF_3), 114.0, 61.0, 58.3, 47.6, 44.7, 39.5, 30.1.

Peptido ***2*** (R = $(CH_2)_3$-NH_2): ^{1}H (D_2O, 500 MHz) δ: 7.87 (s, 1 H), 7.85 (d, $J = 8.2$ Hz, 1 H), 7.76 (d, $J = 8.2$ Hz, 1 H), 7.55 (dd, $J = 8.2$, 8.2 Hz, 1 H), 7.26 (d, $J = 2.1$ Hz, 1 H), 6.90 (dd, $J = 2.1$, 8.5 Hz, 1 H), 6.76 (d, $J = 8.5$ Hz, 1 H), 4.12 (dd, $J = 4.1$, 11.1 Hz, 1 H), 4.09 (t, $J = 7.5$ Hz, 2 H), 3.95 (m, 2 H), 3.21 (m, 2 H), 3.19 (m, 2 H), 3.12 (dd, $J = 4.1$, 13.7 Hz, 1 H), 2.83 (m, 1 H), 2.69 (dd, $J = 11.1$, 13.7 Hz, 1H), 2.17 (m, 2 H), 2.02 (m, 2 H), 1.77 (m, 2 H); ^{13}C (D_2O, 125 MHz) δ : 178.3, 177.7, 158.6, 151.8, 142.6, 133.2, 132.7, 132.6, 132.1, 131.4, 130.2, 127.6, 127.4, 125.9 (CF_3), 126.0, 115.1, 68.1, 60.4, 47.7, 44.6, 39.7, 39.3, 30.1, 29.2.

Peptido ***3*** (R = $(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$-NH_2): ^{1}H (D_2O, 500 MHz) δ: 7.86 (s, 1H), 7.83 (d, $J = 8.4$ Hz, 1H), 7.76 (d, $J = 8.4$ Hz, 1H), 7.54 (d, $J = 1.8$ Hz, 1H), 7.54 (dd, $J = 8.4$, 8.4 Hz, 1H), 6.85 (dd, $J = 8.5$, 1.8 Hz, 1H), 6.73 (d, $J = 8.5$ Hz, 1H), 4.17 (dd, $J = 10.8$, 4.2 Hz, 1H), 3.98 (m, 2 H), 3.96 (m, 2 H), 3.85 (m, 4 H), 3.83 (m, 2 H), 3.28 (m, 2 H), 3.26 (m, 2 H,), 3.14 (dd, $J = 4.2$, 14.0 Hz, 1H), 2.92 (m, 1H), 2.72 (dd, $J = 10.8$, 14.0 Hz, 1H), 2.07 (m, 2 H), 1.83 (m, 2 H); ^{13}C (D_2O, 125 MHz) δ : 175.3, 175.0, 156.1, 148.1, 140.3, 130.5, 130.2, 130.0, 129.4, 128.6, 126.6, 125.6, 123.4, 123.3, 112.2, 69.9, 69.7, 69.2, 67.4, 66.6, 57.9, 45.2, 42.1, 39.2, 36.8, 27.6.

2.2. Polymer surface chemistry

We used PET track-etched microporous membrane from Whatman SA (Louvain-la-Neuve, Belgium). This membrane was characterized by a thickness of 12 μm, a pore density of 1.45×10^6 pores/cm^2, and a mean pore diameter of 0.49 μm [24,25]. Reagents and solvents were of analytical grade.

2.2.1. Coupling of peptidomimetics *2*, *3* and RGDS

Samples of 4×4 cm^2 were cut from the PET membrane. The samples were individually activated by immersion into a solution of tosyl chloride (3.5 g) and pyridine (1.5 mL) in dry acetone (20 mL) and heating for 1H at 50 °C under argon atmosphere, and stirring. Samples were washed by successive immersion, under shaking (Edmund Bühler strirrer KL-2), into acetone