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Short communication

Static adhesion of cancer cells to glass surfaces coated with glycosaminoglycans

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ABSTRACT

Using a previously described method for the functionalization of glass substrates with glycosaminoglycans (GAGs), *in vitro* experimental comparison of adhesion levels of cancer cells to glycosaminoglycan-modified substrates was performed with non-treated and heparin-treated human cancer cells of different metastatic activity. For both non-treated and heparin-treated cells, our results indicate that heparan sulfate is the preferred substrate for adhesion while keratan sulfate shows anti-adhesive properties. The observed net effect of heparin is a cell-dependent reduction in the adhesion figures. Overall, our results suggest that tissues with higher composition of heparan sulfate chains may be preferential metastatic targets and indicate that the effective use of heparin as anti-metastatic or anti-inflammatory agent may also depend on glycosaminoglycan composition of the affected organs.

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1. Introduction

Biomolecules deposited onto solid substrates provide interesting models that can be used in biological applications, including controlled adhesion and growth of cells, biosensors or immunoassays [1]. In particular, the study of the processes relating biointerfaces and cancer biology will have increased interest in coming years, as is clear from recent events with modified heparins [2]. To contribute to the effort of polymer and surface scientists in biopolymers applications [3], here we present the results of experiments of the adhesion on cancer cells to glycosaminoglycan (GAG)-modified substrates.

GAGs are linear polysaccharides that are found in animal tissues, normally in covalent association with proteins, known as proteoglycans. These GAGs are primarily composed of the disaccharide repeats galactose and glucosamine – keratan sulfate (KS); glucuronic acid and glucosamine – heparan sulfate (HS); or glucuronic acid and galactosamine – chondroitin sulfate A (CSA) and chondroitin sulfate C (CSC). On average, the number of charges per dimer in these polysaccharides can range from around 0.5 for KS to about 4 for heparin, taking into account sulfate and carboxylic groups. The interactions of the GAG chains with their environment provide proteoglycans with their biological properties – hence, the importance of developing techniques that could permit analysis of their interactions with whole cells.

Initially, we reported a novel method describing the controlled attachment of glycosaminoglycans on glass substrates [4]. In our experimental procedure the cover slips simply expose a layer of isolated GAG chains, which permit the analysis of their interaction with the cells. Previous methods for the deposition of isolated biomolecules on substrates frequently have consisted of culturing cells on the surfaces and then eliminating part of the biological material via lysis [5]. However, these methods cannot study individualized interactions between cells and single molecular species of choice, because the lysis procedure cannot eliminate all but one single molecule.

The GAGs used in our experiments have different biological functions and, of particular interest for our study, they mediate cell adhesion [6]. Sulfated polysaccharide and GAG involvement in tumor biology appears to be related to the effect of the charge density of the biopolymer [7,8], among other factors. Mainly the degree of sulfation and position of anionic groups within the polysaccharide chains affect the strength of the interaction between carbohydrate and polypeptides. Some indications suggest that the degree of sulfation appears to have been the cause of the medical complications in the recent heparin case [9]. With this application as motivation, we have presented elsewhere an analysis of the adhesion focused on the electronic charge per dimer of glycosaminoglycan and its chain length [10]. Furthermore, seeking to understand how physical properties influence the adhesion of the

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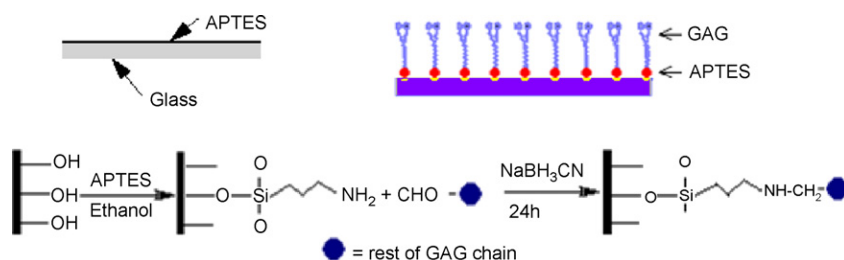

Fig. 1. Conceptual representation of the procedure for the functionalization of glass substrates with GAGs. These polysaccharide substrates were then used in cancer cell adhesion experiments.

cells on the substrates, we also have elaborated the hypothesis that the adhesion of whole cancer cells to glycosaminoglycan substrates maybe a function of polysaccharide radius of gyration, and applied the WLC polymer model for this analysis [11].

Here, we present the results of adhesion experiments by cell line and type of GAG substrate using these continuously GAG-coated surfaces. Although the experiments described in this paper were not designed to elucidate the molecular mechanisms of the adhesion of the cells to GAGs, an hypothetical model for the observed adhesion is given. The main objective was to observe the differential response of the cells to these mucopolysaccharides functionalized surfaces and determine what substrates are preferred substrates for cancer cell adhesion. Additionally, the overall effect of heparin on adhesion was evaluated, by comparing the adhesion of non-treated tumor cells and tumor cells that had been resuspended in heparin-containing medium, in order to determine the effect of heparin on cell adhesion to the modified GAGs substrates. The use of heparin was mainly motivated by the reported reduction in the incidence of metastasis, up to 90% in some cases [12] by non-anticoagulant species of heparin and polysulfated polysaccharides of low molecular weights.

2. Materials and methods

2.1. Silanization and GAG deposition

Glass cover slips were modified in a two-step procedure, as previously reported by Peramo et al. [4], using the silane agent APTES (3-aminopropyltriethoxy-silane) and conceptually reproduced in Fig. 1. This modification initially produces an NH_2 terminated sub-monolayer or monolayer on the glass surface that is later incubated for 24 h at room temperature in solutions of either 0.1 μg/ml of HS, KS, CSC (all from Seikagaku America) and CSA (Sigma–Aldrich) in PBS 1X with $NaBH_3CN$ (Acros Organics) at a concentration of 3 μg/ml.

2.2. Tumor cell lines and cell adhesion assays

Human BT20 (moderately metastatic) breast tumor cells and A431 (highly metastatic) human epidermoid carcinoma cells were purchased from ATCC, while MCF7 (non-metastatic) breast tumor cells were kindly provided by Dr. William Dalton, Moffitt Cancer Center. The only factor considered in the selection of the cell lines were their different metastatic potentials. All cell lines are adherent and were maintained in tissue culture using the specifications recommended by ATCC. For adhesion assays, tumor cells were grown to confluence in two different groups: non-treated cells and cells resuspended in heparin-containing media. Cells were trypsinized and resuspended in the corresponding complete media (for non-treated cells) or in a solution of complete media containing 10 μg/ml of heparin for 30 min previous to the adhesion assays with heparin [13]. Cells then were transferred to GAGs-coated cover slips placed on the bottom of Petri dishes. Deposited cells (2.5×10^5) were allowed to incubate at 37 °C for 2 h for A431 and BT20 cells and 8 h for MCF7 cells. This seeding time was determined by comparing the number of cells adhered at 30 min, 1 h, 2 h and 4 h (for A431 and BT20) and between 4 h, 6 h, 8 h and 10 h (for MCF7), and selecting between the times that showed a stable trend in adhesion, determined by 15% or less difference in the number of cells adhered. Bound cells were carefully washed with PBS kept at room temperature to remove non-adherent cells. The remaining cells were detached using 250 μl of a solution of PBS-EDTA and then counted. In order to compare the results, the density of GAG molecules present on the substrates have been normalized to equivalent surface density, determined as previously described [14]. For each experimental group shown in Figs. 2 and 3, the results are expressed as the mean number (±S.D.) of adhered tumor cells in eight cover slips. Paired Student t-tests were performed to estimate the significance of the results, with $p < 0.05$ considered significant.

3. Results and discussion

Results are presented independently for each cell line and later for all cell lines together. For each cell line, results also are presented for the two conditions tested during the adhesion experiments. An additional analysis is performed presenting GAG adhesion information for cells treated with heparin relative to non-treated cells.

3.1. Analysis by cell type

Fig. 2 represents absolute number of cells attached (in cells per ml) to the four different GAG substrates. As seen in panel (A), non-treated MCF7 cells adhere in higher numbers to HS and CSA but not to CSC and KS substrates. Globally, HS appears to be a preferred substrate for adhesion. For all substrates, the number of adherent cells is higher for non-treated cells and lower for heparin suspended cells. Compared to non-treated cells, MCF7 heparin treated cells show a 65% decrease in adhesion to CSA ($p < 0.0001$), 60% to KS ($p < 0.0001$), 56% to HS ($p < 0.0001$) and 21% to CSC ($p < 0.005$).

In general, the effect of heparin is the most intense for this cell line, providing strong indication that the mechanism used by MCF7 cells in their binding to glycosaminoglycans is partially disrupted by heparin. Adherence of cells in heparin-containing media does not vary substantially among substrates, indicating that cells are probably using more than one mechanism for adhesion, one that is blocked by heparin and another that is not blocked by heparin and which is not affected by changes in the substrate. In panel (B), BT20 cells show strong preference for HS substrates and low preference for KS, as with the other two cell lines, for both conditions. A reduction in adhesion of heparin suspended cells with respect to untreated cells is also observed. BT20 heparin treated cells show a 14% reduction adhering to HS ($p < 0.05$), 19% reduction to CSC ($p < 0.03$) and 29% reduction to KS substrates ($p < 0.01$), while for

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