Biological activity of a recombinant fragment of fibronectin ($FNIII_{7-10}$) on poly(ethyl acrylate)

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Statement of Purpose: Integrin-Fibronectin (FN) interactions, governed mainly by the $\alpha_5\beta_1$ dimer, lead to the formation of extracellular matrix fibrils from the newly secreted FN (Mao Y. Matrix Biol 2005;24:389). FN binding to integrins induces reorganization of the actin cytoskeleton that facilitates the unfolding of the native globular FN structure, thus exposing cryptic domains that were not available in the compact form of soluble FN. We have recently shown that FN is able to self-assemble into a network on hydrophobic poly(ethyl acrylate), PEA, in the absence of cells; that is to say, as a consequence of the protein-material interaction, leading to a so-called substrate-induced fibronectin fibrillogenesis (Gugutkov D. Langmuir 2009;25:10893). Since cell-FN-material interaction involves the RGD sequence, located on the 10th subunit of the III domain, we have investigated the conformation and distribution of a recombinant fragment of FN ($FNIII_{7-10}$) that incorporates both the PHSRN synergy sequence and the RGD binding motifs on this particular chemistry by atomic force microscopy (AFM). The biological activity of the fragment on PEA as compared with model methyl (CH_3) and hydroxyl (OH) terminated SAMs was investigated via MC3T3 initial adhesion. The effect of incorpoting –OH groups in the PEA chemistry on focal adhesions formation and actin cytoskeleton development was addressed.

Methods: $FNIII_{7-10}$ was produced and purified as described previously from *E. coli* transformed with cDNA coding for human $FNIII_{7-10}$ (Cutler SM, Biomaterials 2003;24:1759). Polymer sheets were obtained by polymerization of a solution of ethyl acrylate using 0.1wt% of benzoin as a photoinitiator. Copolymer sheets were obtained by polymerization of a solution of both monomers ethyl acrylate and hydroxyethyl acrylate in different ratios (x_{OH}). Self assembled monolayers (SAMs) were prepared from alkanethiols (1-dodecanethiol and 11-mercapto- 1-undecanol) on Au-coated glass coverslips. Au surfaces were immersed in alkanethiol solutions and SAMs were allowed to assemble overnight. AFM was performed in a NanoScope III from Digital Instruments (Santa Barbara, CA) operating in the tapping mode. ELISA with monoclonal antibodies HFN7.1 and mAb1937 were used to assess the conformation of the adsorbed fragment. Focal adhesion formation (vinculin) and actin cytoskeleton development were followed by inmunofluorescence for MC3T3 osteoblast-like cells on the different surfaces.

Results: AFM images of $FNIII_{7-10}$ adsorbed on PEA after immersion for 10 min at protein solutions of different concentrations: 2, 5, 10, 20, and 50 μg/mL were obtained. The lowest concentration results in isolated globular molecules homogeneously distributed on the material. For a concentration of 5 μg/mL, $FNIII_{7-10}$ molecules are observed in higher density. The incipient formation of a protein network on the material occurs when the fragment was adsorbed from a solution with a concentration of 20 μg/mL. Protein adsorption from solutions of higher concentration (50 μg/mL) gives rise to the formation of FN networks on the material.

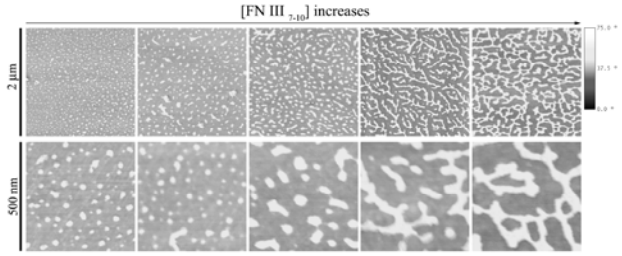

Figure 1. AFM images of $FNIII_{7-10}$ on PEA from solutions of increasing concentration (2, 5, 10, 20, 50 μg/ ml).

The availability of cell adhesion domains after protein adsorption was evaluated by ELISA with two monoclonal antibodies. HFN7.1 binds to the flexible linker between the 9th and 10th type III repeats; mAb1937 is directed against the 8th type III repeat. The affinity of both antibodies for the fragment is higher on the PEA surface than on the glass, especially when protein adsorption is done from solutions of high concentrations (20 μg/ml). The development of focal adhesions and the formation of F-actin cytoskeleton were investigated after 3h on the protein-coated surfaces. Cells presented prominent actin fibers inserting into well-developed focal adhesion complexes on PEA. CH_3 and OH SAMs were used for comparison. Also, the effect of incorporating increasing amounts of hydroxyl groups in the material was investigated. F-actin cytoskeleton was well developed in cells cultured on PEA and the copolymer x_{OH}=0.3. However, the degree of formation of the actin cytoskeleton decreased as the fraction of hydroxyl groups in the sample increases. For the more hydrophilic samples (x_{OH}= 0.7, 1), only globular-like cells are observed with no trace of actin fibers but only some peripheral actin. Accordingly, focal adhesions were only visible on PEA and x_{OH} =0.3.

Conclusions: Upon adsorption on PEA, $FNIII_{7-10}$ undergoes unfolding, leading to an extended conformation which enhances inter-molecular contacts so that, from a certain concentration of the adsorbing solution (50 μg/ml), an interconnected network is obtained on the surface. More importantly is that the unfolding of the FN fragment on the PEA substrate is biologically active as obtained by actin cytoskeleton development and focal adhesion formation. When the number of hydroxyl groups in the sample increases, the conformation of the fragment remains globular and the cell-material interaction worsens.