Surface Display using Self-assembly for Screening Cell-adhesive Peptides

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Statement of Purpose: Biomaterials used in applications such as implants, tissue engineering scaffolds, and sensors are often functionalized with proteins or peptides to promote biological activity based on specific recognition. The surfaces of engineered tissue, in particular, may have to be decorated with multiple peptides in order to achieve cell adhesion, growth and differentiation. This paper describes a self-assembly based approach to displaying multiple peptides on surfaces. Supported bilayers consisting of lipids and 'peptide amphiphiles' – peptides covalently linked to lipid-like hydrocarbon tails – were constructed via fusion of vesicles on glass surfaces. Multiple peptide displays were created simply by mixing vesicles incorporating different peptide amphiphiles. Adhesion of NIH 3T3 fibroblasts to surfaces displaying RGD peptides was studied, and peptide conformation and density required for optimal adhesion were determined.

Methods:
Peptide amphiphiles were synthesized by an extension of standard solid-phase peptide synthesis, as previously described (Berndt P *et al.*, JACS 1995; 117: 9515-9522) and purified by reverse-phase HPLC. Lipids were purchased from Avanti Lipids (Alabaster, AL).

Supported bilayers were characterized by epi-fluorescence microscopy and photobleaching. In addition, QCM-D measurements on a D300 instrument from Q-Sense (Glen Burnie, MD) and AFM images from a Digital Instruments Multimode AFM (Santa Barbara, CA) were used to verify bilayer formation and topography.

NIH 3T3 cells were obtained from ATCC (Manassas, VA) and cultured in DMEM supplemented with 10% bovine calf serum. Cell attachment to peptide surfaces was evaluated at 1h after incubation in serum-free media by formaldehyde fixation followed by nuclear staining using the Hoechst dye.

Results:
Supported bilayers incorporating peptide amphiphiles were formed on glass surfaces via fusion of vesicles. This was evidenced by smooth fluorescence from bilayers incorporating 1 %mol. fluorescent lipid DiD. Photo-bleaching experiments showed that the bilayers were fluid with diffusion coefficients in the range expected (~ 1-5 $\mu m^2/s$). Further, QCM-D measurements showed the formation of an elastic surface layer after initial adsorption of vesicles. The mass increase observed by QCM-D was found to correspond to the mass of a single bilayer. AFM images of fully hydrated samples in tapping mode showed bilayer islands with some defects.

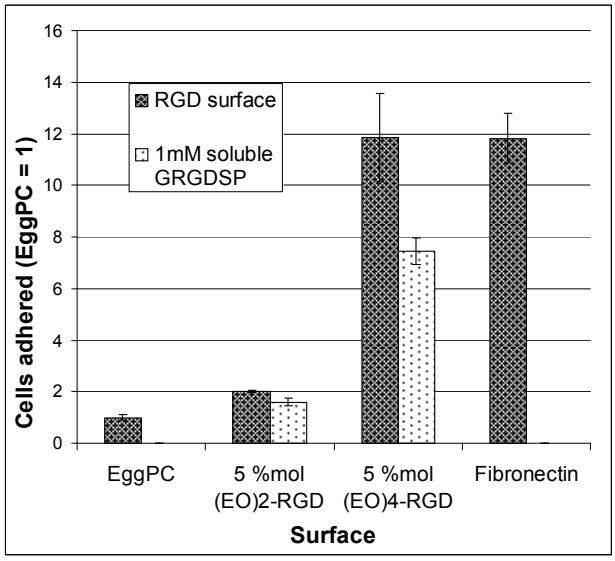

Fig. 1: Cell adhesion to PC bilayers displaying GRGDSP peptide. Significantly higher number of 3T3 cells adhere when a longer spacer ($(EO)_4$ instead of $(EO)_2$) is used; further, this adhesion is due to specific RGD recognition.

GRGDSP peptides constructed with Ethylene Oxide (EO) spacers of various lengths were displayed on EggPC bilayers. 3T3 cells were incubated on these surfaces in serum-free medium. The results are shown in Fig. 1. Cell adhesion was significantly greater when the peptide was presented using a longer spacer. Further, incubation in the presence of soluble GRGDSP peptide inhibited cell adhesion, proving that the adhesion was due to specific RGD recognition.

Vesicles displaying GRGDSP peptides were mixed with pure PC vesicles to generate surfaces with varying densities of GRGDSP peptide in a facile way. GRGDSP peptides modified with a fluorescent Rhodamine moiety were used to verify the modulation of peptide surface density. Cell adhesion to these surfaces increased with increasing RGD density. Finally, mixed-peptide surfaces presenting GRGDSP peptide as well as the synergy sequence PHSRN were created by mixing vesicles. Cell adhesion to the resultant surfaces depended on both peptide densities.

Conclusions:
Self-assembly of peptide amphiphiles is shown to be an easy and powerful way to create peptide displays on surfaces. The cell-binding ability of such surfaces was found to be a function of both the peptide density as well as its accessibility. Surfaces displaying a range of peptide concentrations, as well as multiple peptides, could be created simply by mixing. This technique provides an efficient way to screen peptides that can be used to trigger cell adhesion and growth on biomaterial surfaces.