Spreading of Cells on Supported Peptide Amphiphile Bilayer Membranes

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Abstract:

Peptide amphiphile bilayer membranes may lead to the design of better biomaterials, which will have a variety of uses in medical research. A couple of these areas include in vitro tissue formation and cell specific drug delivery. Currently, work is being done to determine a composition of lipid and peptide amphiphile that will best facilitate cell adhesion and spreading. Vesicle solutions of naturally derived lipids and peptide amphiphiles are prepared. These vesicles are allowed to form a bilayer on a hydrophilic glass substrate. Mouse fibroblast cells are introduced to the bilayer environment and allowed to interact for three hours. Fluorescence and optical microscopy were used to visualize the bilayer and the cells. To quantify cell spreading, imaging software is used to determine the shape factor of the cells.

Introduction:

Cell adhesion to synthetic surfaces is a topic that has undergone much research in the past fifteen years. In an effort to contribute to the understanding of this topic, our primary goal is to identify important parameters for cell adhesion. The synthetic surfaces that are used in this study are supported bilayer membranes. A bilayer is the result of the self-assembly of amphiphiles, molecules that have a hydrophilic head group and a hydrophobic tail group. In solution, the amphiphiles arrange with the tails inward and the heads exposed to the water/bilayer interface. It is imperative that these membranes are not exposed to air; if that occurs, the membrane would be irreparably damaged. Figure 1 shows a simple bilayer membrane.

A class of amphiphiles contains a hydrophilic peptide head group, a spacer, and hydrophobic tails. These molecules are called peptide amphiphiles (PA). In this study, the peptide head group contains the peptide sequence known as RGD, which has been shown to promote adhesion with many types of cell integrins, molecules on the cell surface that are integral to adhesion [1].

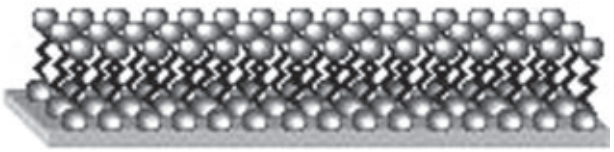

Figure 1: Phospholipid bilayer membrane.

This study focuses on how two different peptide amphiphiles facilitate cell adhesion. The only difference between the two is the size of the spacer group present. For naming purposes, the peptide amphiphiles will be called by their spacer group name, C2 and PEO (polyethylene oxide).

Procedures:

Vesicle solution of ten percent PA, eighty-five percent egg phosphatidylcholine (egg PC), and five percent Texas Red (fluorescence marker) was prepared and dried under nitrogen to a thin film. The film was then hydrated for an hour and extruded to form vesicles with a diameter of roughly 100 nm. An additional solution of Texas Red and egg PC was prepared using the same techniques.

Bilayers were formed on hydrophilic glass substrates that were cleaned via plasma oxidation. The substrates were affixed to the bottom of a sterile petri dish and a drop of sodium chloride solution was pipetted onto the substrate followed by the vesicle solution. Sodium chloride acted as an agent to increase the pressure on the vesicles and thus, rupture them. As the vesicles ruptured, a continuous, supported bilayer was formed on the substrate. The petri dish was filled with deionized water to insure that the bilayer would not be exposed to air.

To prepare the environment for the addition of cells, some of the water was removed and replaced with Dulbecco's cell medium. Mouse fibroblast cells were introduced to the bilayer environment and incubated for three hours. A similar, more detailed procedure can be seen in a study done by Kam and Boxer [2].

After the cells were allowed to adhere to the surface, optical microscopy was used to visualize their morphologies. Digital pictures of the bilayers were taken and imported into Scion Imaging to determine the shape factor. The shape factor is represented by the equation:

$$4\pi(a)(p)^{-2}$$

Shape factor values range between 0 and 1, with 1 representing a perfect circle. It should be noted that shape factor alone does not indicate adhesion; rather, one sample's shape factor in relation to another sample gives a quantitative idea of how much spreading actually occurred.

Results:

The bilayers composed of egg PC and 10% C2 PA showed no cell adhesion, with average shape factors of 0.85 and 0.84, respectively. Bilayers composed of 10% PEO PA showed evidence of cell adhesion with an average shape factor of 0.76, or 9.5% cell spreading from the original round morphology.

Discussion:

The results obtained in this study could be explained by the different lengths of the spacer groups. It is believed that the longer spacer makes the RGD peptide more accessible to integrins; however, this hypothesis must undergo further experimental work.

Future Work:

The current way of preparing bilayers allows for only one composition of PA to be present on the substrate. It is desired to have one substrate with many corrals of bilayers, each containing a different composition. As shown in Figure 2, there would be an array of bilayers separated by protein barriers.

This would give researchers a versatile tool that allows individual corral study or observation of how the entire concentration gradient affects cell adhesion. To achieve such a gradient, two solutions, one of pure lipid and one of lipid/PA would be exposed to the substrate via a microfluidic device. Much work is being done to design such a device; however, there are many obstacles to overcome, for example; the fluid mechanics and fabrication of such a small apparatus.

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References:

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[2] Kam L, Boxer SG. Cell adhesion to protein-micropatterned-supported lipid bilayer membranes. Journal of Biomedical Materials Research 2001; 55:487-495.

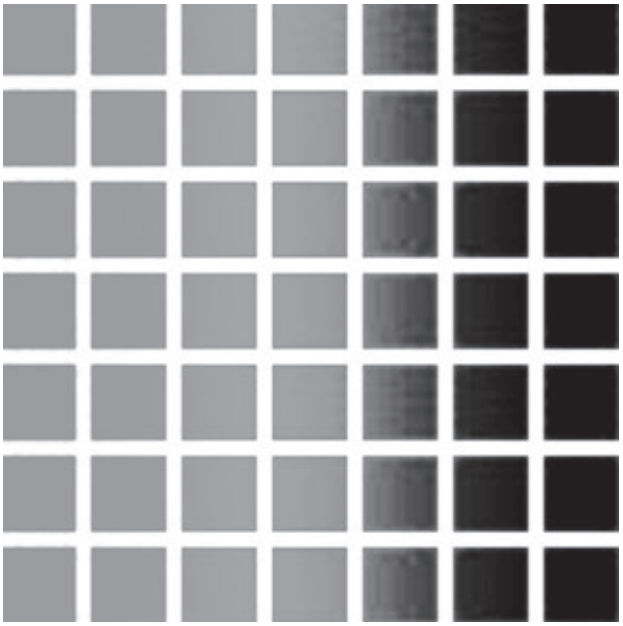

Figure 2: Sample concentration gradient.