Analysis of the Adhesion and Spreading Behavior of Cellular Spheroids on Supported Lipid Layers

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Abstract:

Understanding the basic functions of cells, such as spreading and adhesion, is important to generate baseline knowledge on cellular behaviors for more targeted studies. Traditional cellular spreading studies are carried out on hard, planar surfaces which do not mimic physiological conditions. By depositing a layer of biologically relevant lipids onto both liquid-solid and liquid-liquid interfaces, cellular adhesion and spreading behaviors can be studied in multiple environments, providing a more realistic model of how cells move in the body. These cellular functions are mediated by a host of factors, including physical cues, chemical cues, and cell-cell interactions. Monocellular cultures lack these critical cell-cell interactions, making them less analogous to real cell behavior. This study aims to bridge this gap by examining both single-cell and spheroid spreading on supported lipid layers. A seemingly direct relationship was observed between cellular spreading and a cell-adhesive peptide (RGD) density after a long incubation, although a smaller proportion of 0.5% RGD seemed to have increased efficacy in the short term. Further investigation into these relationships can provide insight into cellular spreading behaviors in the body and may eventually serve as a model for cancer metastasis.

Summary of Research:

Background. Cell spreading is facilitated by both internal and external factors, including chemical cues from the cell's environment. RGD is a family of peptides responsible for cellular adhesion and spreading on the extracellular matrix and is found abundantly within the human body [4]. Supported lipid membranes (SLMs) (see Figure 1) are formed from physiologically common lipids that can be conjugated to molecules like RGD to induce cellular spreading based on the literature [2,3]. While the spreading behaviors of single cells are well documented, the behavior of cellular clusters is less well understood due to the influence of intercellular signals and forces.

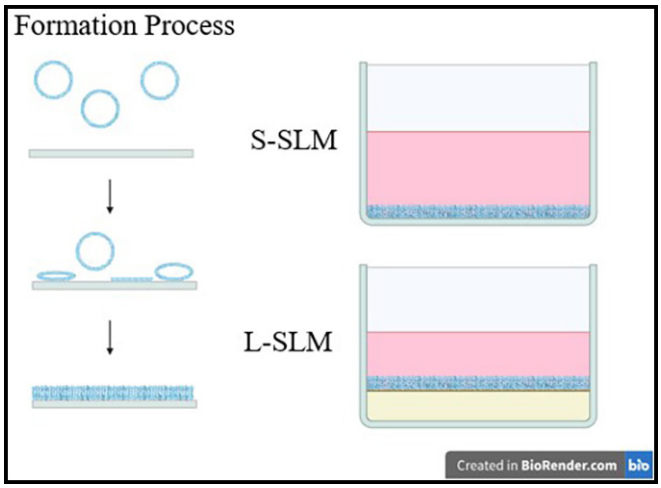

Figure 1: Process of forming SLMs. Solid-SLMs were created by depositing the bilayer at the interface between a functionalized glass slide and cell medium, while liquid-SLMs were created at the interface between perfluorocarbon and cell medium.

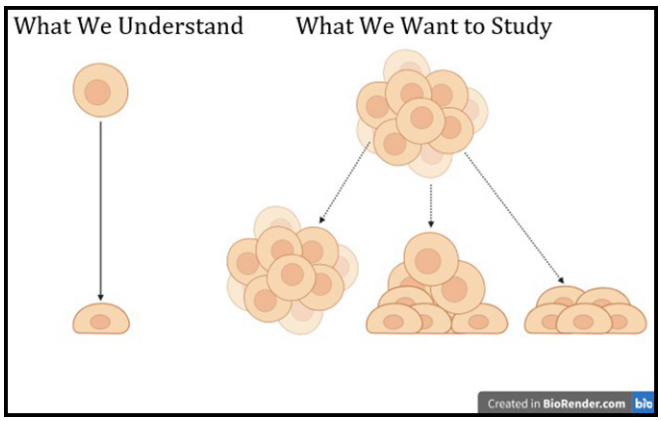

Figure 2: While single-cell spreading is well-studied, the intercellular forces of cellular spheroids make it hard to predict how they will spread.

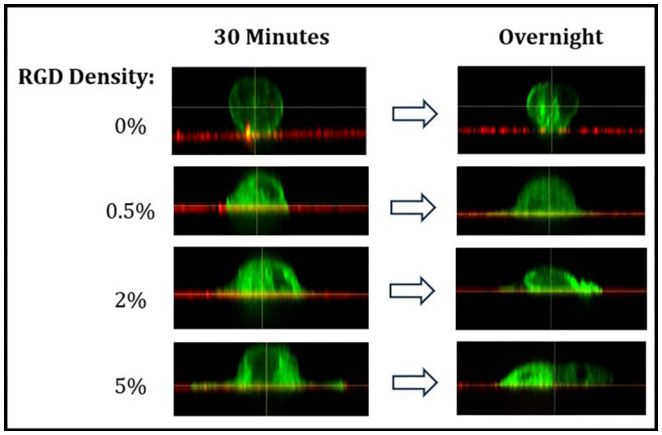

Figure 3: Comparison of confocal images (XZ plane) of cellular spreading on solid-SLMs after 30 minutes and 24 hours of incubation. Red and green fluorescence represent actin and the lipid membrane, respectively. All images are a representative sample from the images taken for that experimental condition.

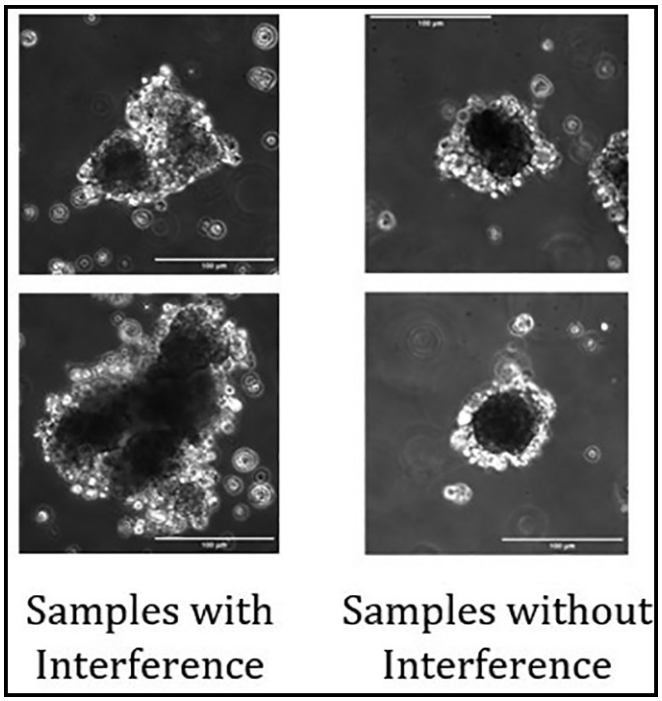

Figure 4: Brightfield images of cellular spheroids showing the adverse effects of shaking during spheroid growth.

Spheroids, or globular clusters of cells, are an important tool as they more closely mimic physiological conditions through the inclusion of these cell-cell interactions [1]. While integrins, which are proteins that control cellular adhesion, bind to the RGD peptide, this force lies in opposition to the proteins binding individual cells in spheroids together. This conflict makes it unclear which forces will win out in spreading experiments (see Figure 2) but can allow for a more accurate picture of how cells migrate within the body.

Case Study: Single Cell Spreading. To first validate the experimental setup and to establish a baseline of spreading behaviors, initial experiments were performed using single Madin-Darby Canine Kidney (MDCK) cells. Cells were incubated overnight on the supported lipid layers and imaged using a confocal microscope after 30 minutes and 24 hours of incubation (see Figure 3), drawing comparisons at different RGD densities. These experiments found that increased cellular spreading appeared directly related to increased levels of RGD in the lipid membrane after overnight incubation. However, cells incubated on 0.5% RGD membranes for 30 minutes seemed to display more spreading than expected, suggesting that perhaps a low proportion of RGD can initially stimulate increased spreading, but that this phenomenon is taken over by more linear trends after a longer incubation period.

Spheroid Formation and Spreading. Cellular clusters were generated from MDCK cells using EZSphere plates. Unfortunately, the non-adhesive coating on the microwells of these plates makes them susceptible to adverse shaking effects. Spheroidal morphology was highly dependent on movement of the plates during medium changes and imaging, and increased shaking would lead to spheroids migrating from one microwell to the next and fusing, creating non-circular amalgamations (see Figure 4). While truly spherical clusters were not formed during the course of this experimentation, "disk-shaped" clusters with a three-dimensional aspect served as an initial approximate to study overall spreading patterns.

Conclusions and Future Steps:

Initial results from these experiments are promising, indicating a potentially linear trend between RGD density and cellular spreading, especially over longer time periods. More investigation is required to understand the potentially increased spreading seen in early culture on 0.5% RGD, but our findings suggest that this RGD density can induce quick early spreading behaviors that slow drastically in the long-term.

Future work will focus on improving spheroid formation techniques to create more uniform and rounded cell clusters. Once a finalized spheroid formation protocol has been established, further study of these cellular clusters on both solid- and liquid-SLMs will be performed, specifically studying the role that intercellular forces play in modifying cell spreading.

In the future, this understanding of spheroid migration and spreading could be used as a rudimentary model for tumor metastasis.

References:

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