Biphasic Cell Responses on Laterally Mobile Films

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Statement of Purpose:
Biomaterials have been widely explored for studying anoikis, metastasis, and other cancer-relevant cell functions. In nearly all of these works, the biochemical cues/ligands are chemically attached to the underlying substrate and therefore these systems are inherently static. By contrast, the cellular micro-environment is dynamic and remodeled by biochemical actions and biophysical forces. Borrowing this concept from Nature, we created polymer films by an interfacial self-assembly process. These polymer films can be reorganized by cells via receptor-ligand interactions. Here we show that the substrate mobility is an important and previously unreported aspect of mechano-sensing and can give a new insight on the extracellular physical environment.

Methods:
Poly(butylene)-b-poly(ethylene oxide) (PB-PEO, Mw = 10.2 kg/mol, PDI = 1.14 and w_{EO} = 0.39), Poly(butylene)-b-poly(ethylene oxide)-fluorescein isothiocyanate (PB-PEG-FITC) and Poly(butylene)-b-poly(ethylene oxide)-Arg-Gly-Asp-Ser-OH (PB-PEG-RGDS) were produced by modification of the 1,2-PBd-PEO amphiphilic block copolymer. 1,2-PBd-PEO and Poly(isobutylene) (PIB, Mw = 0.9 kg/mol) were purchased by Polymer Source. Assembly of the block copolymer chains with different amounts of PIB was performed by Langmuir-Blodgett and Langmuir-Schaefer techniques [1]. To probe the lateral mobility of the films, we incorporated 10mol% PB-PEO-FITC at the topmost layer through the Langmuir-Schaefer deposition and performed fluorescence recovery after photobleaching (FRAP) for all the different concentrations of PIB. The normalized diffusion coefficient of the films D* ($D^* = D/D_0$, D_0 = 6.88 x 10^{-10} cm^2/sec: diffusion coefficient of the neat film) was found to depend on the amount of PIB. The values of D* = 1, 2, 5 and 8 correspond to neat, 10^{-6}, 10^{-3}, and 1 mol% PIB.

For cell studies, we tune the RGD spacing on the films by controlling the mixing fraction of PB-PEO and PB-PEO-RGDS polymer chains and assuming ideal conditions. NIH 3T3 fibroblasts were cultured over films with different mobility at an initial density of 3 x10^3 cells/cm^2 under complete DMEM (10% bovine calf serum, 0.5% penicillin – streptomycin). After seeding for 24h (T=37°C and 5% CO_2), we use phase contrast microscopy to capture cell spreading. Films were always maintained under aqueous conditions.

Results:
The mean cell projection area on films with average ligand spacing of 50 and 150nm and at different values of lateral mobility D* is presented in Figure 1. Cells display higher projection area on films with RGD spacing of 50nm than on the corresponding of 150nm. This behavior is consistent with the need for a minimum ligand spacing for sufficient cell spreading [2].

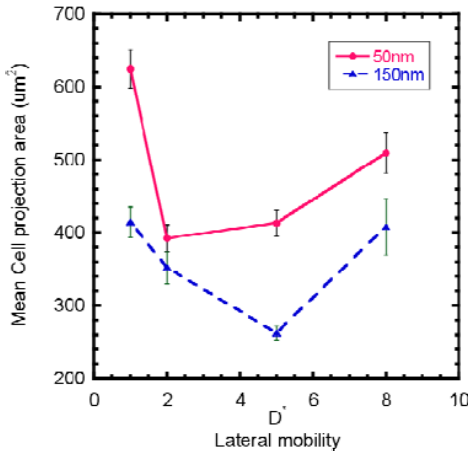

Figure 1: Mean cell projection area against the lateral mobility of the films (D*).

At the same RGD spacing, lateral mobility dramatically affects cell spreading. Cells on films with extreme D* values (e.g., 1 and 8) exhibit higher projection area than those with intermediate D* values (e.g., 2 and 5). We suggest that our films enable two mechanisms that facilitate cell spreading. On films with low D*, the films allow for strong resistance to the cell-generated force load. On films with high D*, the films facilitate cell-driven ligand remodeling. Based on our results (Figure 1), when either of these mechanisms is dominant, cell spreading is promoted. On the other hand, when both mechanisms contribute to similar extents, the resulting competition gives attenuated cell spreading.

Conclusions:
Herein, we fabricated a novel biomaterial with independent control over substrate lateral mobility and ligand density. Fibroblast cells show a biphasic spreading behavior versus the lateral mobility of these films. These results create a new perspective on the role of cell-ECM interactions; for example, the mobility of biochemical cues could depend on the pathophysiology of the ECM or vice versa. We envision decorating our films with synergy peptides (PHSRN) and other protein components, to better mimic the biophysical character of the ECM. These platforms could eventually be used to study the response of other cell types and their cancerous counterparts.

References:
[1] Lerum RV et al., ChemPhysChem 2010; 11: 665-669.
[2]Arnold M et al., ChemPhysChem 2004; 5: 383-388.