Mechano-sensing of laterally mobile viscoelastic films induced biphasic cell spreading response

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Statement of Purpose:

Mechanobiology of cell adhesion has primarily been studied using purely elastic and immobile substrates.**[1]** However, natural extracellular matrix (ECM) is viscoelastic and contains mobile components.**[2]** In this work, we combined chemistry and cell biology tools to design laterally mobile viscoelastic polymer films that induced previously unreported cell spreading response. We showed that biphasic spreading is related with the mechano-sensing of lateral mobility that controls the formation of more than one sub-cellular structures.

Methods:

Integrin specific films that display lateral mobility were fabricated by Langmuir-Blodgett and Langmuir-Schaefer deposition of poly(butylene)-b-poly(ethylene oxide) and poly(butylene)-b-poly(ethylene oxide)-Arg-Gly-Asp-Ser-OH.**[3]** Film mobility was tuned by addition of polyisobutylene chains and measured by fluorescence recovery after photobleaching (FRAP). We imposed Arg-Gly-Asp (RGD) spacing of *50 nm* **[4,5]** to focus solely on mechano-responses to lateral mobility. Focal adhesions (FAs) of 3T3 NIH fibroblasts were monitored by immunostaining of vinculin proteins. Contact (integrin-RGD complex) clustering was estimated by centrifugation-adhesion assay**[6]** after treatment with Y27632 to remove FA effects.**[7]** The relative change in cell projection area ($\delta A/A$) after treatment with Y27632 gave the contribution of FAs on ***A*.** The contribution of contact clustering on ***A*** is given by ***1-*** $\delta A/A$***.***

Results:

Cell adhesion on mobile films exhibited biphasic cell projection area (***A***) **(Fig.1.A)** and FA density responses **(Fig.1.B)**. In order to explain this biphasic behavior, we focused on FA size and contact clustering whose growth depends on the physical characteristics of the substrate.**[8]** FA size is proportional to cell generated lateral forces,**[9]** and therefore the decreasing FA size with mobility **(Fig.1.C)** is due to small lateral forces sustained by the more mobile films. Contact clustering effects are manifested by the relative adhesion strength ($\delta W/W$).**[6]** Herein, $\delta W/W$ was increased on films with higher mobility (Fig.2.D), therefore contact clustering is facilitated on more mobile films due to higher fluidity. FA size and contact clustering dependence on film mobility had direct effects on cell spreading. The relative change in ***A*** **(Fig.1.A)** due to FAs ($\delta A/A$) was lowered with mobility **(Fig.1.E).** In contrast, the relative change in ***A*** due to contact clustering ($1-\delta A/A$) was increased **(Fig.1.F).** Based on the above, the summative (due to FAs and contact clustering together) cell spreading is dominated by contributions of FAs and contact clustering at low and high mobility films, respectively.

Conclusions:

We created viscoelastic polymer films with tunable lateral mobility. Cells responded to lateral mobility by the adjusted formation of focal adhesions and contact clustering. The combined effects of focal adhesions and contact clustering are responsible for a previously unreported biphasic dependence of the cell projection area to the lateral mobility of the films. Hence, lateral mobility is a biomaterial feature intimately related with the mechano-sensing of viscoelastic substrates. This study introduced lateral mobility as a controllable cue with well defined impact on cell adhesion and created a new perspective for the design of novel ECM biomaterials.

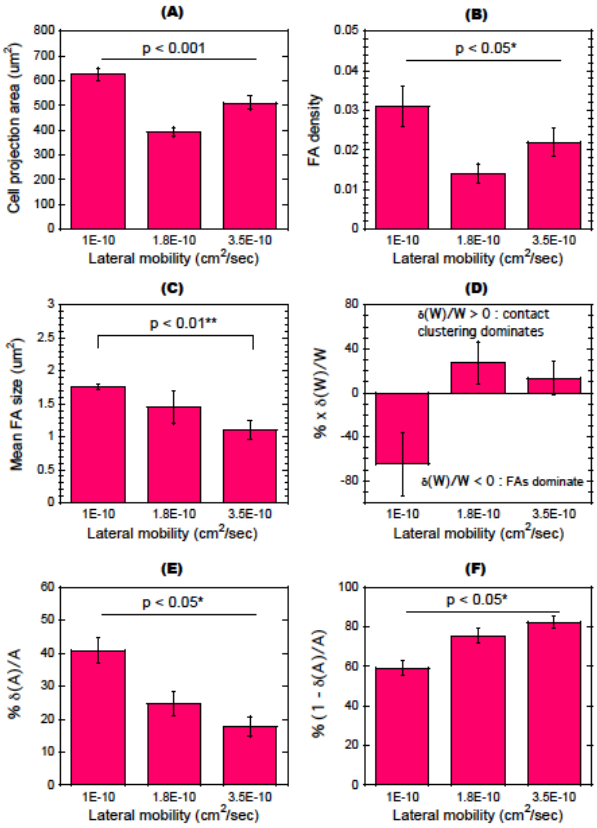

Figure 1: (A) Cell projection area : *A* **(B)** FA density (# of FAs per um² of ***A***), **(C)** FA size, **(D)** Relative adhesion strength due to contact clustering ($\delta W/W$), **(E)** % of ***A*** due to FAs, **(F)** % of ***A*** due to contact clustering. One-way ANOVA was used for statistical comparison of the data. Error bars correspond to the standard error of the mean.

References:

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