UV-Softening Polyacrylamide Substrates for Spatio-Temporal Mechanotransduction Studies

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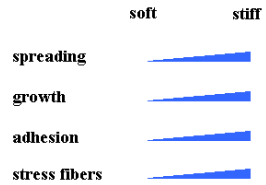
ABSTRACT

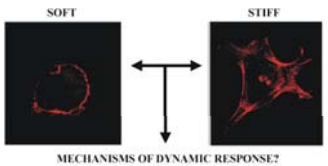
Polyacrylamide gels have been used widely for cell culture studies as substrata for their favorable optical properties and the ability to tailor their mechanical properties. Understanding cell response to substrata stiffness is important in the fields of developmental biology, regenerative medicine, wound healing, and tissue engineering. Similar to surface biochemistry, the mechanical properties of substrata provide a biological cue that affects cell behavior, such as spreading and migration speed. However, these studies are done in parallel fashion and yield no information regarding the transition dynamics of cell behavior during changes in substrata stiffness. We have created a model system for such a study by modifying polyacrylamide with a photo-cleavable crosslinking agent. Cells readily adhere to this substrate after addition of fibronectin. Based on atomic force microscopy measurements, ultraviolet light exposure reduces the substrate stiffness. Cells on the substrata before and after exposure behave in a similar fashion to cells on stiff and soft substrata respectively, while cells on glass appear to be unaffected by the same intensity of exposure. This novel substrate allows for transition dynamics of cells to be studied as a function of changing stiffness.

BACKGROUND

• Studies of cells on soft and stiff matrices show differential effects [1, 2, & 3] , but are limited to comparisons of a static nature.



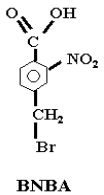
RATIONALE



[1]

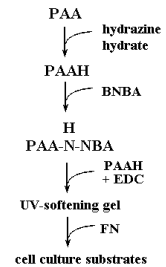
• The rationale for this project was to create and characterize a UV-softening substrate in order to study cell response to changes in substrate stiffness.

UV-SOFTENING CELL CULTURE SUBSTRATES



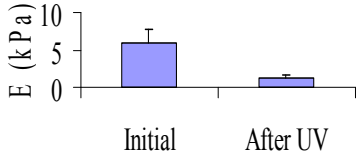
• BNBA, a caged compound, is cleavable with 365 nm UV light.

• The resultant clear gel is originally stiff, but can later be softened with minimal UV exposure.



UV EXPOSURE REDUCES STIFFNESS

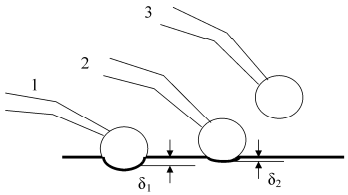
To ensure these substrates were softened with UV exposure, substrates were tested with an atomic force microscope before and after a high dose of UV exposure (n = 4) [4].



SOFTENING UPON UV-EXPOSURE IS INSTANTANEOUS

To determine the decrease in stiffness upon exposure with low dose UV and to determine the time scale of the response, a micro-sphere indenter [5] was used to detect stiffness before and after UV exposure.

Micro-sphere Indenter



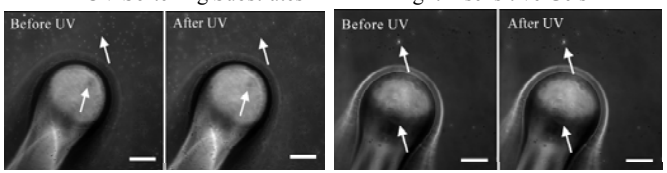
• Two indentations (δ) are made with a micro-sphere at a known force, F, to determine sample stiffness, E, from Hertz contact mechanics [6]:

$$F_{sphere} = \frac{4}{3}\frac{E}{(1-\nu^2)} R^{1/2}\delta^{3/2}$$

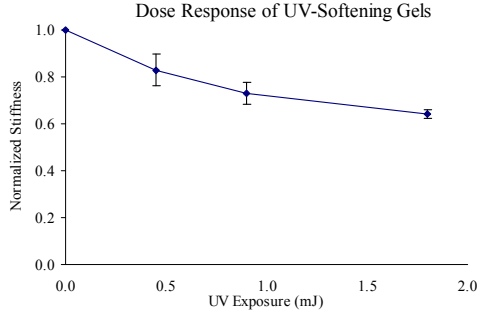
15 μm < R < 50 μm

• UV exposure increases micro-sphere indentation on UV-softening substrates (arrows indicate change in focus, scale bar = 20 μm), but not on light-insensitive gels (arrows indicate no change in focus).

UV-Softening Substrates | Light Insensitive Gels



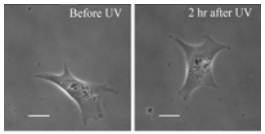
Dose Response of UV-Softening Gels



• Softening of gels is immediate and increases with UV exposure, as detected from changes in micro-sphere indentation (n = 4)

CELLS ON COVERSLIPS UNAFFECTED BY UV EXPOSURE

FN-Coated Coverslip

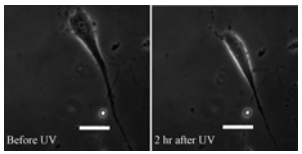


Cells on FN-coated coverslips showed no response to UV exposure (1.8 mJ).

• Phase images of migrating 3T3 cells (scale bar = 20 μm) recorded 2 hours both before and after UV exposure (λ = 365).

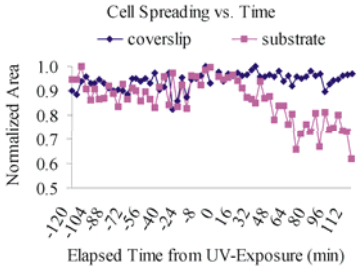
SUBSTRATE SOFTENING REDUCES CELL SPREADING

UV-Softening Substrate



Area of cell spreading is reduced on UV-softening substrates by UV exposure (0.9 mJ).

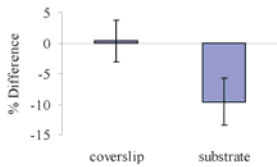
Cell Spreading vs. Time



• Normalized values of cell area show cellular response to substrate softening upon UV exposure compared to stable cell spreading on FN coated coverslips (using twice the exposure).

• Average change before and after UV exposure for cells on coverslips and UV-softening substrates (SD, n = 8 and 6, respectively).

Change in Cell Spreading After UV Exposure



CONCLUSIONS

• Crosslinking of modified polyacrylamide with BNBA and EDC and addition of supplemental FN creates a UV-softening substrate suitable for cell culture studies.
• Substrate stiffness decreases with minimal exposure to UV-light.
• Substrate softening causes reduction in cell spreading area.
• UV-softening substrates are a novel material for studying the dynamic spatial-temporal responses to changes in substrate stiffness.

REFERENCES

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