Nanopatterned Adhesive Hydrogel to Control Ligand Spacing and Regulate human Bladder Smooth Muscle Cell (hBSMC) Spreading and Proliferation

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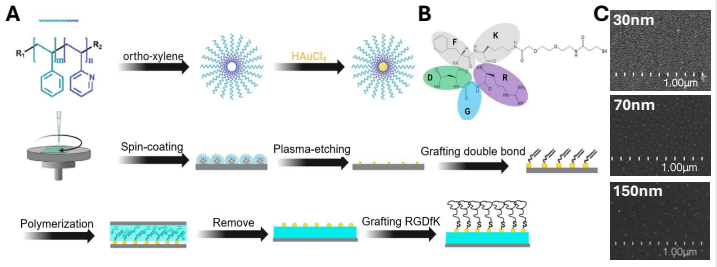
Background

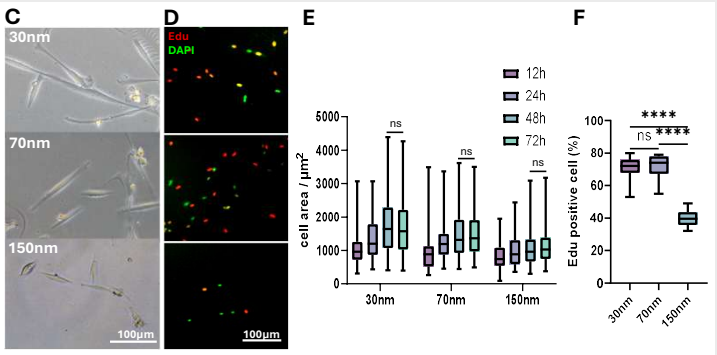
Spatial molecular patterning plays a pivotal role in regulating adhesion receptor clustering, thereby influencing critical cellular processes such as spreading and proliferation. Given the importance of human bladder smooth muscle cell (hBSMC) in bladder injury repair, this study investigates how variations in RGD ligand spacing affect spreading and proliferation of hBSMC through integrin clustering. The findings aim to provide insights into material design principles for supporting bladder tissue regeneration.

Methods

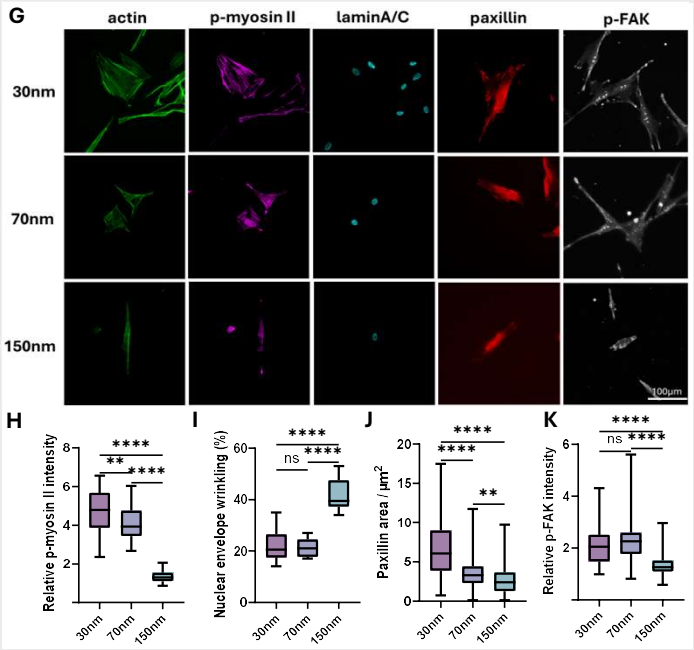


Hydrogels with defined RGD spacings were fabricated via Au-loaded micelle, polymerized with NAGA precursor, detached, and incubated with cyclic RGDfK. After UV sterilization, the hydrogels were used to culture hBSMCs. Cell adhesion, spreading, and proliferation were then quantitatively characterized.

Results and Discussions



During the first 48 hours post-seeding, cell spreading area increased progressively and stabilized by 72 hours. Cells cultured on hydrogels with 30 nm and 70 nm RGD spacings showed comparable spreading areas, which were significantly larger than those on 150 nm substrates. Based on the establishment of stable adhesion at 48 hours, this time point was chosen for subsequent proliferation analysis. EdU incorporation assays indicated significantly higher proliferation rates on RGD spacings ≤70 nm compared to 150 nm. Together, these results identify 70 nm as a critical integrin ligand spacing threshold, above which both cell spreading and proliferation are significantly impaired.



We next investigated how integrin spacing modulates cellular behavior. Immunofluorescence staining indicated that integrin clustering critically regulates focal adhesion assembly: larger spacings correlated with smaller focal adhesion sizes and significantly reduced p-FAK activation at 150 nm. Furthermore, disrupted integrin clustering led to markedly diminished intracellular tension, as indicated by decreased p-myosin II expression and increased nuclear envelope wrinkling.

Conclusion

Taken together, these results indicate that hBSMCs have difficulty spreading on adhesive interfaces where the spacing between RGD ligands exceeds 70 nm, which also significantly impairs cell proliferation. Furthermore, intracellular force transduction mediated by FA formation plays a critical role in regulating cell behavior in response to different RGD spacings. Our findings highlight the importance of controlling ligand spacing to promote hBSMCs functions, offering valuable insights for developing surface modification strategies in bladder tissue engineering.

References

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