Dielectric Nano-biopatterning for the Analysis of Cell-ligand Interactions by Super-resolution Fluorescence Microscopy

Ashish Pandey[1], Kashish Jain[2], Taerin Chung[1], Arash Nemati[1], Hao Wang[1], Pakorn Kanchanawong[2], Michael Sheetz[2], Rishita Changede[2], Haogang Cai[1]

1. Tech4Health Institute and Department of Radiology, NYU Langone Health, New York, NY, USA.

2. Mechanobiology Institute, National University of Singapore, Singapore, Singapore.

E-mail: haogang.cai@nyu.edu

Oral contribution

Used by cells to sense the physicochemical nature of microenvironment, cell-extracellular matrix (ECM) adhesions are imperative for critical cell functions that regulate growth, differentiation and disease, forming the foundation for the organization of tissues and organs.[1] Biomolecular nanopatterning has become a powerful tool for probing cell-matrix interactions at the single-molecule level, which is typically based on metallic nanopatterns such as gold nanoparticle (AuNP) arrays. A major finding was the spatial effect of integrin ligand positioning on cell spreading and adhesion formation, with a threshold of ~60 nm spacing.[2] However, AuNP arrays limited ligand manipulation to single sites in hexagonal arrangement, while the plasmonic quenching[3] limited further investigation by fluorescence-based super-resolution imaging. In order to eliminate the plasmonic quenching effect, we have developed a novel TiO_2 nano-biopatterning technology (Fig. 1a).[4] Using e-beam lithography, TiO_2 nanopatterns with arbitrary geometries can be created on glass surfaces. A new functionalization scheme was developed[5] to immobilize ECM binding peptides Arg-Gly-Asp (RGD) on nanopatterns and cover the glass background by supported lipid bilayers (SLBs) to prevent non-specific adsorption.

Both optical simulations and photobleaching experiments show drastic reduction of fluorescence intensity on Au nanopatterns and minimal interference on TiO_2 nanopatterns (e.g., 100 nm-diameter nanodiscs, Fig. 1b). By enabling dual-color super-resolution imaging, high precision and consistency among nanopatterns, bioligands (RGD ligands with neutravidin linkers), and integrin nanoclusters in fibroblast cell adhesion was observed (Fig. 1c,d). It validates the high quality and integrity of nanopattern functionalization and SLB passivation. While the selective formation of integrin nanoclusters on nanopatterns was always a basic assumption, it was only demonstrated on our new platform with simultaneous molecular-level patterning and imaging (Fig. 1d). The fibroblast cell spreading area, paxillin recruitment, and YAP nuclear localization are highly dependent on the nanodisc diameter (Fig. 1h). There is an optimal threshold at ~100 nm, matching the intrinsic integrin cluster size formed on SLBs with mobile RGD ligands.

Our work presents a high-fidelity nano-biopatterning technology compatible with super-resolution imaging. The ligand geometric requirement for cell-ECM adhesion formation is generalized to a basic unit with a diameter of ~100 nm. In the specific case of hexagonal single-ligand arrays, the minimal threshold of 60 nm spacing corresponds to four ligands in a quadrilateral with a diagonal dimension of ~100 nm, which is also in agreement with our new finding. Super-resolution imaging revealed that unliganded integrins co-cluster with liganded ones, which implies more importance of liganded sites surrounding the cluster edge (spanning a characteristic distance ~100 nm) than the central region.[6] Therefore, the 100 nm threshold could be related to the intrinsic integrin nanocluster dimension determined by factors like the cell membrane curvature, which shed light on the molecular mechanism of cell-matrix adhesion. It not only builds a solid foundation for precise quantitative cell biology studies to address molecular-scale signaling events, but also expands the scope of using nanopatterns for cellular and molecular bioengineering.

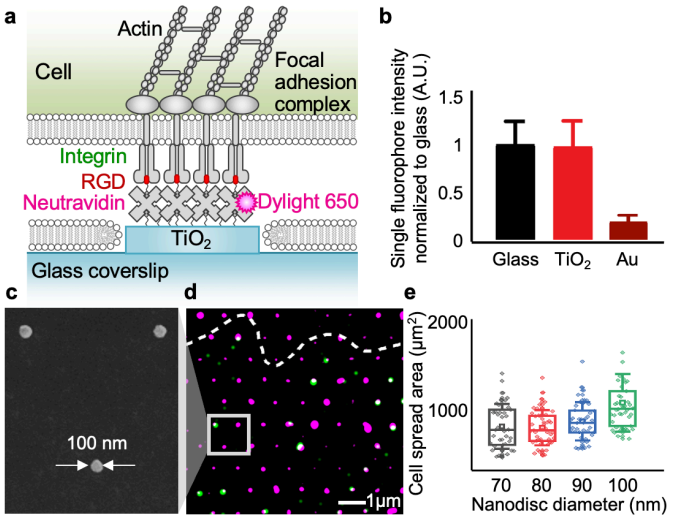

Fig. 1. (a) Schematic diagram of cell adhesion cluster formation on TiO_2 nanopatterns. (b) Single fluorophore intensity on TiO_2 and Au nanodiscs (diameter 100 nm) normalized by that on glass. (c) SEM images of TiO_2 nanodisc arrays. (d) Super-resolution images of Integrin β3-mEos2 and neutravidin Dylight 650 imaged using PALM and dSTORM respectively. (e) Cell spread area on nanodisc arrays with various diameters.

References

[1] Hynes, R. O., Cell. **110**, 673 (2002).
[2] Arnold, M. et al. Chemphyschem. **5**, 383 (2004).
[3] Cai, H. et al. ACS Nano. **10**, 4173 (2016).
[4] Jain, K. et al. Advanced Materials. 36, 2309284 (2024).
[5] Jain, K. et al. Nature Protocol. **17**, 2275 (2022).
[6] Changede, R., Cai, H., et al. Nature Materials. **18**, 1366 (2019).