Applicability of polymeric substrates for subcellular live cell micropatterning experiments

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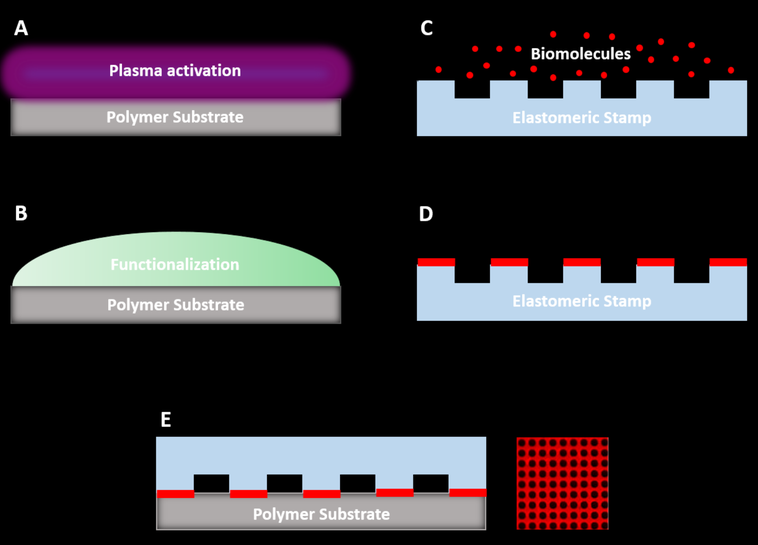
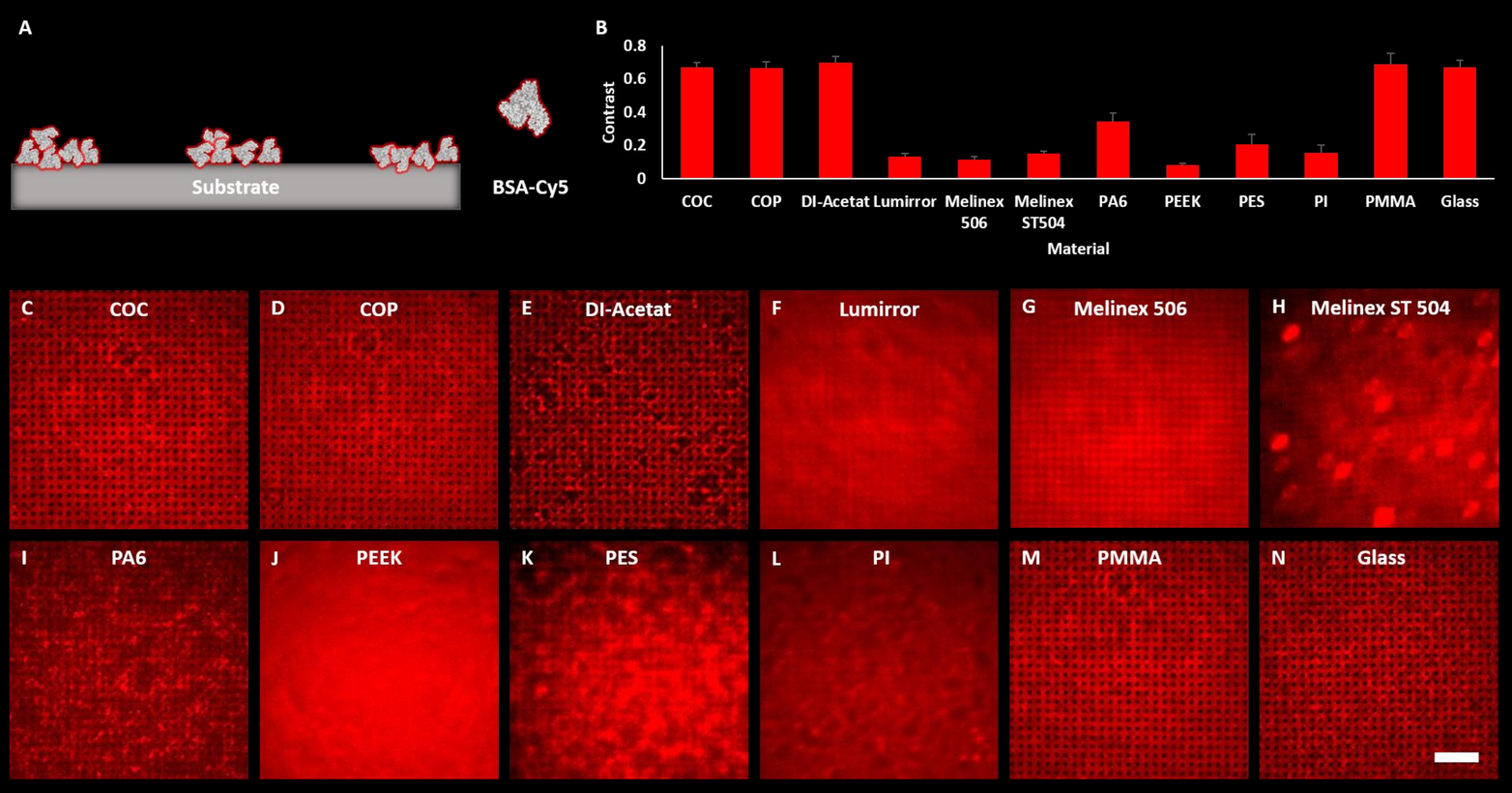


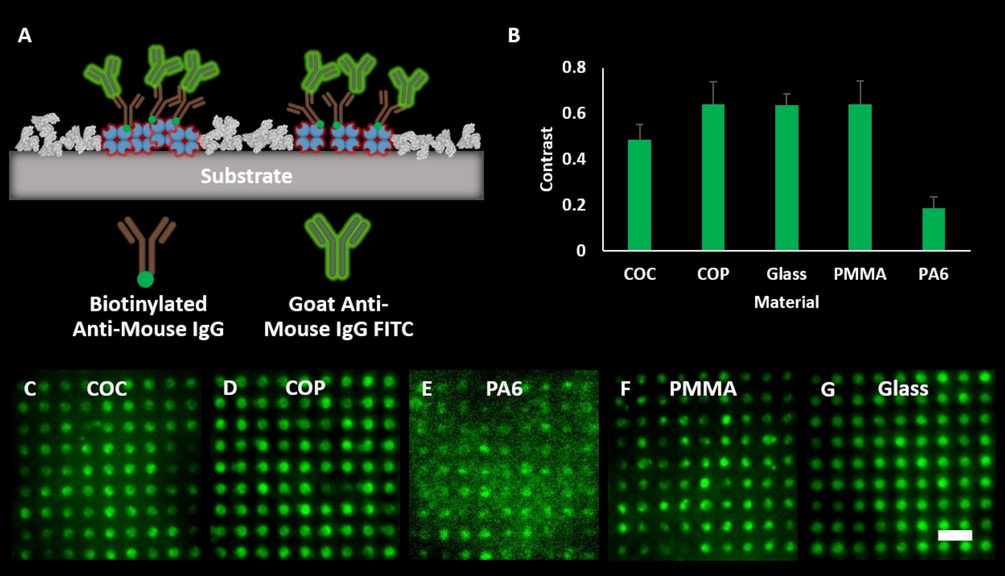
Fabrication of micropatterns via µCP: (A) Plasma activation, (B) introduction of functional groups, (C) stamp incubation with biomolecules, (D) generation of a biomolecule layer and (E) µCP on polymer substrate.

Abstract

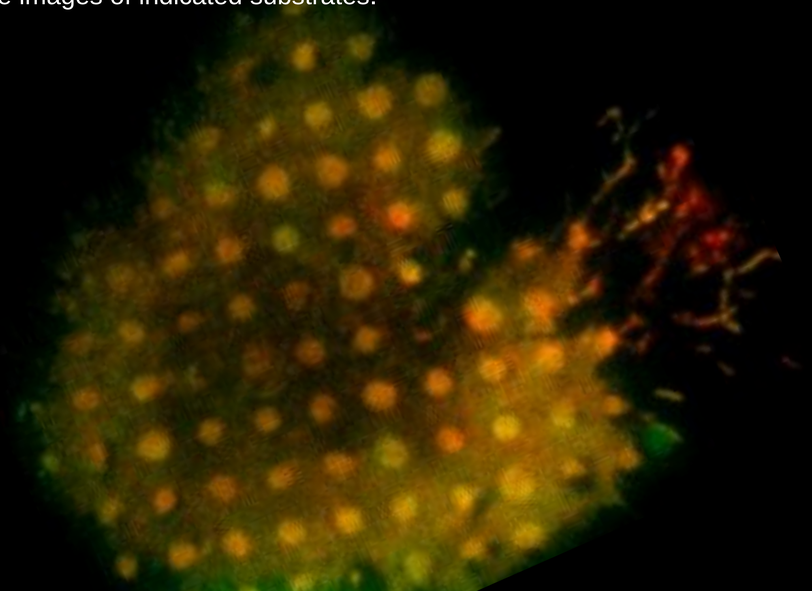
Polymeric materials play an emerging role in the development of new biomedical and biosensing interfaces. Within this regard, polymer substrates can serve as a superior surface for binding and patterning of biomolecules. However, detailed information about the applicability of different polymers for surface functionalization and quantitative fluorescence microscopy is missing. Therefore, we characterized eleven different polymer foils and glass as a reference: cyclic olefin polymer (COP), cyclic olefin copolymer (COC), polymethylmethacrylate (PMMA), di-acetate, lumirror, melinex 506, melinex ST504, polyamide 6 (PA6), polyethersulfone (PES), polyether ether ketone (PEEK) and Polyimide (PI). We have recently introduced two different approaches (microcontact printing (µCP) and photolithography) for the fabrication of biomolecule micropatterns on various functionalized polymer substrates. [1, 2]. However, the implementation of photolithographic approaches for the fabrication of microstructured surfaces is expensive and labor-intensive compared to µCP. Hence, we focused on µCP for the fabrication of biomolecule micropatterns. The absence of functional groups in many polymeric materials does not allow for the immobilization of biomolecules onto these substrates by means of common surface chemistry. Therefore, we used plasma activation and wet chemistry for the introduction of functional groups on these surfaces and evaluated the coating performance via contact angle measurement and scanning electron microscopy (SEM). We gathered information about transmission and absorption properties of the different polymers via UV-VIS spectroscopy. Furthermore, we give an overview about their suitability for epifluorescence and total internal reflection fluorescence (TIRF) microscopy and evaluated these methods via contrast measurement. In addition, we tested these micropatterned polymers concerning their applicability in cell-based protein-protein interaction assays. Overall, we tested eleven different polymer substrates to evaluate their suitability for fluorescence microscopy and subcellular live cell micropatterning assays. COC, COP and PMMA turned out to be cheap and flexible alternatives to glass substrates with comparable chemical and optical properties.

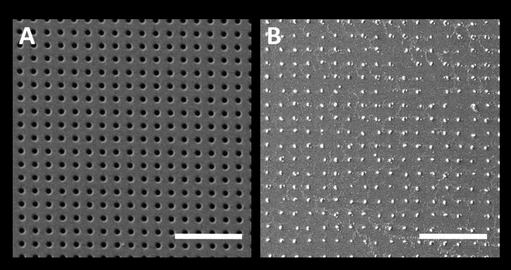


Characterization of pattern contrast on various substrates using fluorescence microscopy. (A) Schematic overview of fluorescently labeled BSA bound to the substrate. (B) Quantitation of fluorescence contrast and (C-N) representative fluorescence images of indicated substrates.

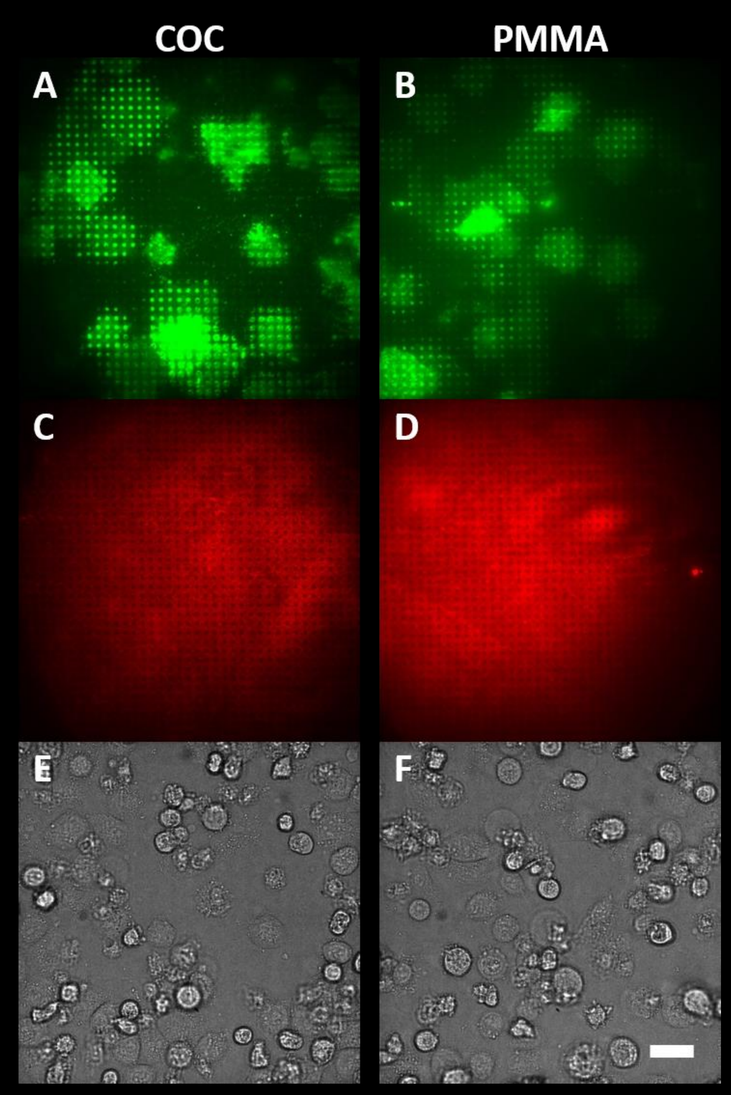


Characterization of pattern contrast on various substrates using TIRF microscopy. (A) Schematic overview of micropatterned anti-mouse IgG detected with goat anti-mouse IgG FITC. (B) Quantitation of fluorescence contrast and (C-G) representative fluorescence images of indicated substrates.

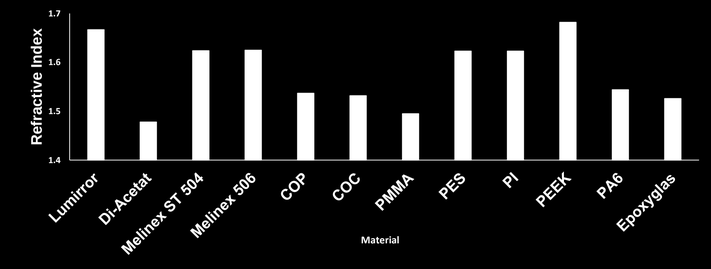




Scanning electron micrograph of (A) PDMS stamp and (B) micropatterned BSA molecules on functionalized COP foil. Scale bar: 30 µM.



Live cell assay for the detection of PPIs on selected polymer substrates (COC and PMMA). Patterning of bait-presenting artificial receptors (bait-PARs) for coimmunoprecipitation of cytosolic protein complexes. (A, B) Specific bait-prey interactions in living cells, (C, D) BSA-Cy5 grid for surface passivation and (E, F) bright-field images of selected area.



References

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2. Hager, R.; Müller, U.; Ollinger, N.; Weghuber, J.; Lanzerstorfer, P. Subcellular micropatterning for visual immunoprecipitation reveals differences in cytosolic protein complexes downstream the EGFR. bioRxiv 2021.05.25.445547; doi: https://doi.org/10.1101/2021.05.25.445547

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