Synthetic cells with ordered protein patches

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Anisotropy is a hallmark of functional efficiency in many natural systems[1] (e.g. reactive sites on molecules, active sites of enzymes, immune cell synapse etc.). At the macro scale, it is possible to produce a similar form of anisotropy using synthetic microspheres with protein or polymer patches. Such may represent a new way to produce unique functional efficiency in applications such as sensors, biomedicine and photonics. However, fabrication methods that are capable of producing uniform surface patterning of microspheres are not currently available. Ideally, a viable technique would include mild conditions during processing while still permitting fine control over size and order of the patterns. Recently, we have developed a contact-induced mask lithography technique to accomplish this task on a microsphere surface. To demonstrate proof-in-principle of this new technique, we have employed a lateral self-assembly process to control the ordering of polystyrene (PS) microspheres. Subsequently, we use a Poly Dimethyl Siloxane (PDMS) scaffold-based protection/ deprotection and labeling procedure that produces polymer or protein patterns reproducibly under mild conditions. Scanning Electron Microscopy Energy Dispersive X-ray Analysis (SEM-EDAX) studies indicate that the pattern of PDMS patch formation on the surface is robust. Confocal Laser Scanning Microscopy (CLSM) studies reveal regular and ordered dual protein patterns. We are currently exploring several applications for our dual-labeled particles including drug delivery, immunotherapeutic, catalysis, and photonic applications.

Methods:

Commercially available carboxylated PS (mean diameter 6.37µm) microspheres were purchased from Bangs Laboratories Inc, USA. The microspheres were washed and re-suspended in water. Microbiology-grade glass cover slips were washed under bath sonication for 15min each. "Microwells" of microspheres were developed by spotting 3µl of microsphere suspension (10%w/v in water) onto a cover slip and dried sequentially. The wells were further filled with a concentrated microsphere suspension (30%w/v). The PDMS ((PDMS/ catalyst) (10:1) (w/w)) (Sylgard 184 silicone elastomer kit, Dowcorning Corp, MI, USA) solution was added and allowed to saturate and stabilize the interstitial spaces for 10min. This mixture was immediately heated to 90°C for 15min. After setting of the PDMS scaffold (16hrs at 40°C), the cover slip was carefully removed to prepare the PDMS scaffold / colloidal crystal for protein patterning.

As a result of these processing steps, the particle-based colloidal crystal was embedded inside a PDMS scaffold where masks had nucleated at the contact points between microspheres. Subsequently, two different chemistries were explored for functionalisation of proteins at contact (direct covalent immobilization) and non-contact (avidin-biotin bioconjugate) regions. The non-contact region was first labeled with Biotin-PEG. Subsequently, the PDMS mask was removed rhodamine albumin was labeled directly onto this newly exposed region. Flourescien-Avidin was then added to bind with the immobilized biotin to produce dual-labeled microspheres (Figure 1C).

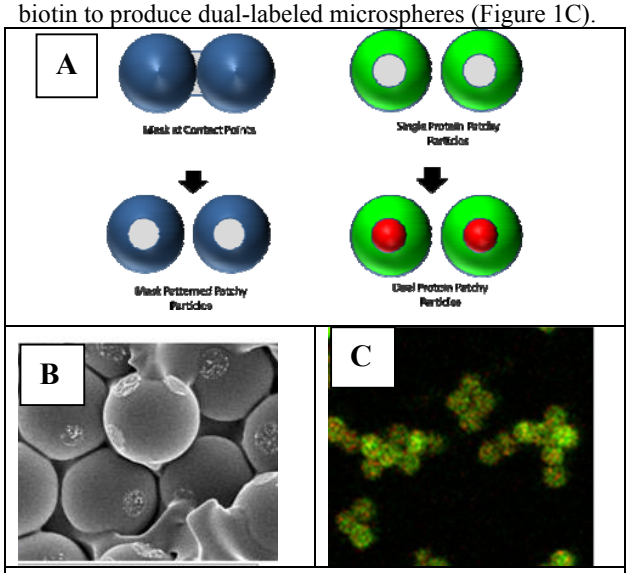

Figure-1: Protein patterned patchy microspheres, (A) Fabrication process of patchy microspheres (B) Mask Patterned Microspheres, (C) Protein patterned microspheres

Results: Laterally self-assembled microspheres form a colloidal crystal, which reduced the movement of the particles and created masks at the contact points between particles (Figure 1B). The polymer incompatibility between the PDMS and the PS leads to relaxation of the PDMS from MS surface in all other regions besides the contact points. Dual labeling (prior to, and then subsequent to, "deprotection" of the PDMS mask) leads to dual protein-labeled, anisotropic, patchy microspheres (Figure-1C).

Conclusion

This new technique employs condensation of a protective material only at contact points between microspheres in a given packing. Using this template, an iterative, sequential protein labeling strategy produces uniformly-labeled, and anisotropic microspheres with protein patches, which (to our knowledge) is the first demonstration of such in the literature.

References

[1] S. C. Glotzer, M. J. Solomon, *Nat. mat.,* **2007**, *6*, 557.