Patchy Particles

DOI: 10.1002/ange.201101217

Patchy, Anisotropic Microspheres with Soft Protein Islets**

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Surface anisotropy (i.e. uniform patterns of discrete functional moieties) is a common feature of many natural systems and a hallmark of functional efficiency.[1] For example, atoms in the "functional" groups of a molecule,[2] endow Ångstrom-level variability in electronic properties, leading to specific relative orientations that impart micro- and macroscale bulk properties. A similar example could be cited on the nanoscale for proteins such as enzymes. On the microscale, biological signatures of "patchiness" on cell surfaces are observed during tissue organization[3] and immune responses.[4] Mimicking this anisotropy with synthetic systems could represent a new way of encoding information to produce new functionality[5] in the areas of catalysis,[6] sensors,[7] optoelectronic devices,[8] modulators,[9] and delivery systems for medicine.[10]

Synthetic microspheres are attractive candidates to represent these basic building blocks on the macroscale as they are easily tunable with respect to size and surface functionality. Indeed, other forms of anisotropy are already being explored through elegant techniques that produce hemisphere-based (Janus-like)[11] as well as shape-based[12] anisotropy with microparticles. However, a method to produce microspheres with more complex forms of anisotropy[1] such as multiple regular and ordered patches on the entire surface has thus far been elusive.[13]

Here, we report a new technique to produce ordered patches on the surface of microspheres using interfacial condensation of a liquid mask and the proximity of the particles to its neighbors to determine a mask pattern. The organization of the particles with respect to one another may be as simple as particle doublets (one contact point between particles) to lines of particles (two contact points per particle 180° on opposite poles), to more complex formations that lead to a controllable number of contact points between solid microspheres. The liquid-phase component of the processing technique takes advantage of surface tension and dewetting such that solutions of masking material may be localized only to the contact points between microspheres.

As one possible manifestation of this procedure, we first created a self-assembled colloidal crystal arrangement of microspheres in order to generate a predictable number of soft-material patches over the entire surface. Specifically, carboxylated polystyrene (PS) microspheres were self-assembled into a colloidal crystal on a glass cover slip (Figure 1 a, b) using a "well-filling" strategy (see Supporting Information, Figure S1). In this step, suspended microspheres are packed towards the edge of an evaporating drop.[14] Iterative filling of this well with subsequent drops of microsphere suspensions

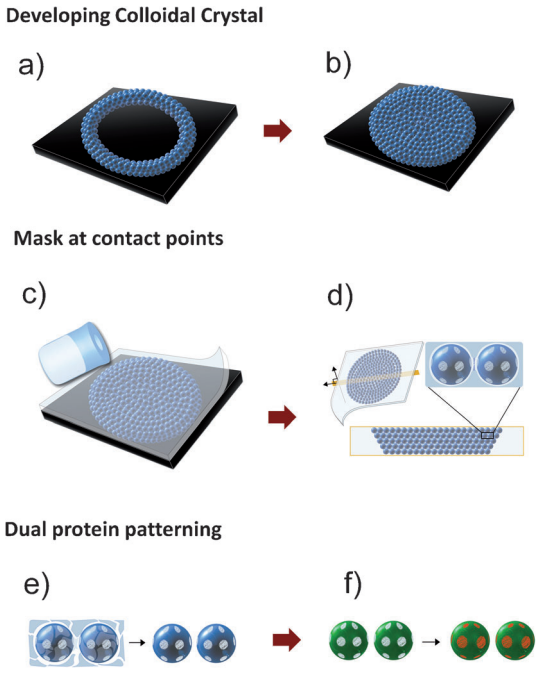

Figure 1. Representation of a method to produce anisotropic microspheres with six regularly spaced patches. Developing the colloidal crystal: a) Colloidal well formation and b) iterative filling with microsphere suspensions. Development of the mask at particle contact points: c) PDMS layering; d) illustration of microspheres with PDMS patches at the contact points. Dual protein patterning: e) Separation of the microspheres from the scaffold; f) labeling of first protein at non-mask region (green) followed by removal of the mask and immobilization of second protein (red).

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[**] We thank the Center for Biological Imaging (University of Pittsburgh) for performing confocal microscopy and scanning electron microscopy experiments. We also thank Mintai Peter Hwang for help with the graphics. This work was funded by the Arnold and Mabel Beckman Foundation.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201101217.

leads to highly ordered and regular colloidal crystals. Next, liquid polydimethylsiloxane (PDMS) is applied to the colloidal crystal. Notably, PDMS is dewetted from the bulk of the particle surface and selectively solidifies at the contact points between microspheres. This wetting/dewetting phenomenon results in an "egg-crate"-like PDMS scaffold with highly interconnected channels around the microspheres. This channel network enables protein labeling inside the scaffold (in which case PDMS patches at the contact points act as a protective mask) and then subsequent removal of the patches to expose unlabeled regions that are uniformly spaced on the surface of the microspheres (Figure 1c, d). Through this approach, any number of bioconjugate strategies could be explored for labeling the two different regions (masked and unmasked regions) of the microsphere. In our initial demonstration of this technique, the exposed surface of the particles was first labeled through an avidin–biotin polyethylene glycol linker (Figure 1f).[15] Subsequently, PDMS patches were removed to expose the protected surface for direct protein labeling using carbodiimide[16] chemistry (Figure 1f). The resulting patterned microspheres have four or six protein-labeled patches, depending upon whether the particle resides on a well edge or is alternatively completely surrounded by neighboring microspheres, respectively.

Microscopy analysis reveals that microsphere orientation and patch formation in the scaffold are conducive to the production of anisotropic labeling on the particle surface (Figure 2). The microstructure of the original "colloidal well" is shown in Figure 2a and the colloidal crystal that results from iterative filling of this well is shown in Figure 2b. Addition of the PDMS mixture (PDMS pre-polymer and curing agent) to the colloidal crystals did not disturb the crystal packing of the microspheres as can be seen in Figure 2c. Upon curing, PDMS appears as dewetted from the particle surface except at the contact points between the microspheres (Figure 2c); this observation is supported by energy dispersive X-ray analysis (EDAX) (Figure S2). As observed in Figure 2c, resulting patches are approximately 10–15 nm thick and 1/5th the diameter of microspheres.

Following patch formation, dual labeling of the microsphere surface can be accomplished by conjugating one protein onto the exposed surface followed by removal of the mask and conjugation of a second protein. For the microspheres described here, a solution containing amine-linked biotin (protecting group) was first linked to carboxyl groups on the sphere surface using carbodiimide chemistry. Afterwards, the PDMS mask is removed and a protein (albumin, used as a model protein) was directly immobilized to the newly exposed area. Finally, fluorescently labeled avidin (as another model protein) is introduced to bind to the immobilized biotin. As seen in Figure 2d the resulting dual-labeled particles display patterns of patches that are consistent with the packing that led to a specific pattern of masks. When dual-labeled particles from three independent fabrication procedures were examined under mild flow conditions (to induce rolling), it was observed that $(94\pm7)\%$ of all particles bear four or six patches ($n=100$ particles).

Notably, the general process described above can also be applied to other specific arrangements of particles, producing

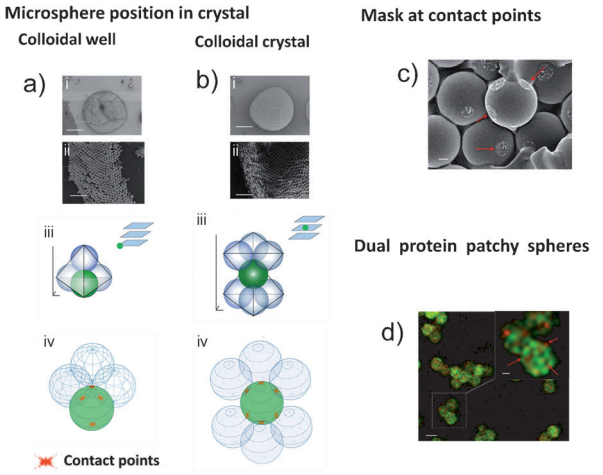

Figure 2. 3D arrangements of microspheres and the resulting anisotropic, dual-labeled particles. a) Visualization of colloidal well microstructure: i) SEM image of a colloidal well (ring) (bar = 1 mm); ii) structure of the edge of the colloidal well (bar = 50 μm); iii) scheme illustrating the packing of microspheres at the edge of the colloidal well (inset: microsphere (green) at the bottom plane (light blue) of the colloidal crystal); iv) the resulting four contact points (red dots) around the single microsphere (green) at the lower plane of the colloidal crystal are likely due to three contacts with neighboring particles and one contact with the underlying substrate. b) A colloidal crystal formed by filling the well: i) SEM image of a colloidal crystal (bar = 1 mm); ii) structure of the central cross-section of the colloidal crystal (bar = 50 μm); iii) scheme illustrating the packing of microspheres at the center of a colloidal crystal (inset: microsphere (green) at middle plane (light blue) of the colloidal crystal); iv) the resulting contact points (red dots) around the single microsphere are all due to contacts with neighboring particles. c) SEM image of particles embedded in the PDMS scaffold with protective patches (red arrows) (bar 1 μm). d) CLSM image of dual-protein-patterned microspheres (bar = 5 μm). The red signal is from albumin rhodamine at patches and green is a result of streptavidin fluorescein. The inset shows an enhanced image of the marked section (inset bar = 1 μm).

a given number of patterns on a particle surface that corresponds with the packing arrangement. For instance, 2D planar particle arrangements can be created on a hydrophilic filter membrane so that excess mask forming liquid solution can be easily drawn off (any remaining liquid will only reside at the contact points between particles). As one example, dilute distributions (particles do not touch their neighbor) of 18 μm particles on a filter (pore size 0.22 μm) will yield microspheres with only one patch on their surface ($(94\pm5)\%$ by counting under mild flow with $n=100$ particles) given that the only contact point of a microsphere is that between itself and the filter surface (Figure 3a(i)). Conversely, aligning particles onto a hydrophilic filter membrane surface (using a 100 μm thin hydrophilic thread dipped in particle suspension) leads to particles with three contact points ($(84\pm7)\%$ by counting with $n=100$ particles) (Figure 3a(iii)) (two polar contacts with the neighboring particles and one contact with the filter surface below). Finally, particles with only two polar patches ($(86\pm8)\%$ by counting with $n=100$ particles) (Figure 3a(ii) and c) can be easily produced by simply utilizing microsphere line orientation (described above) and more

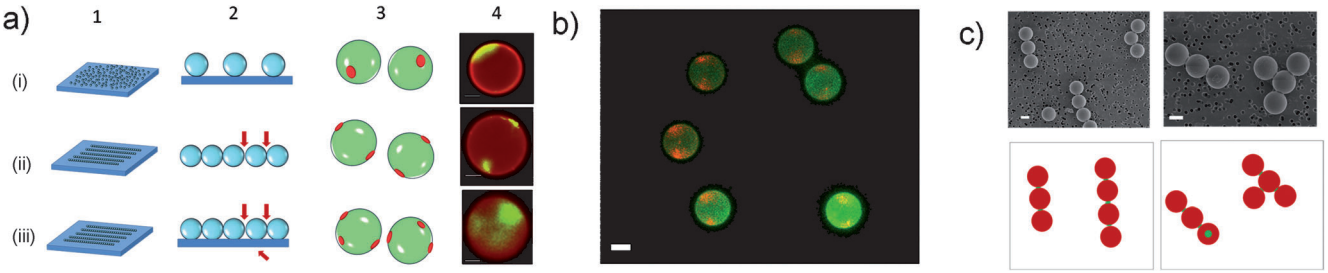

Figure 3. Versatility and application: a) Various 2D planar arrangements and corresponding patchy microspheres. 2D particle arrangements leading to: i) one, ii) two, and iii) three patches on microspheres. Column 1: Arrangement of particles on a substrate (filter membrane) surface; column 2: scheme of a 2D particle arrangement; column 3: scheme of contact points (red dots); column 4: zoomed fluorescent images (bar = 1 μm in each) of representative dual-protein-patterned microspheres (albumin rhodamine (red) at the bulk region and the streptavidin fluorescein (green) at the patches). b) Fluorescent micrograph field showing reverse labeling of patchy particles (i.e. fluorescein (green) at the bulk region and two polar, rhodamine (red)-labeled patches) (bar = 10 μm). c) Predictable orientations of microspheres can be produced based on the memory created with patches composed of biotin by using streptavidin "glue". Top row: SEM image of microspheres connected by streptavidin–biotin bridges between adjacent microspheres. Particles tend to orient into a single file line when particles with one patch and two patches are mixed in a 1:3 ratio (left) while T connectors can be identified when particles with one, two, and three patches are mixed together in a 2:3:1 ratio (right) (bar = 10 μm). Bottom row: scheme illustrating the orientation of the particles (red) and the patches (green) corresponding to the images directly above them.

hydrophobic filter membrane (Figure S4). In the case of 18 μm microspheres the patch dimension was 1/6th the diameter of the particles (approximately 3 μm).

As a simple application of the anisotropic particles, we wished to see if it was possible for certain ratios of particles with various numbers of "glue-like" patches could spontaneously form the lines or connectors (such as those hypothesized by Pawar et al.).[13] To this end, particles with 2D planar patch orientations (described above) were differentially labeled with tethered biotin at the patches and albumin on the remaining surface. In this way, particles in solution would flow freely around one another until streptavidin is added to the solution (Figure S5). If enough random contact could be produced, particles could theoretically self-assemble to predictably form various 2D planar shapes (according to the "memory"-based information programmed into the particles during fabrication).[17] For instance, when small quantities of particles with one patch and large quantities of particles with two polar patches are added together with streptavidin, we observed a spontaneous formation of lines (both curved and straight) (Figure 3c). In the same way, addition of small quantities of particles with three patches could produce structures that resemble T-connectors with the three-patch-particle serving as the junction of the T. The formation of 1D and 2D structures in presence of avidin (external stimuli) is an example of how oriented patterns can spontaneously assemble without any particular thermodynamic or kinetic control.

In summary, we have demonstrated a new method to achieve ordered and regular patterns on a microsphere surface, generating anisotropic "patchy" particles. This method is particularly attractive for patterning soft molecules onto relatively "hard" microspheres. Beyond the applications described above, the techniques demonstrated here could also be explored for various other applications such as printing or painting.

Received: February 17, 2011
Revised: May 4, 2011
Published online: August 1, 2011

Keywords: Janus particles · patchy particles · self-assembly · soft matter · surface anisotropy

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