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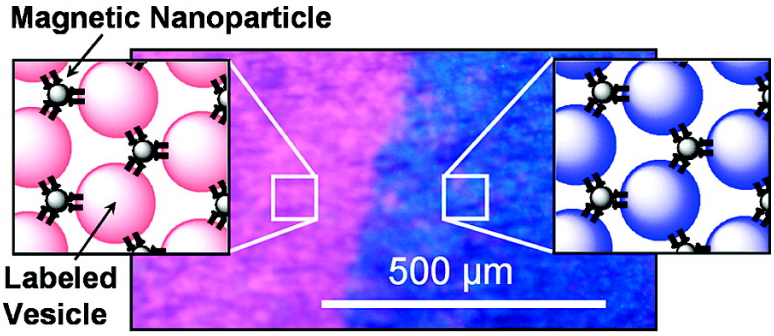

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Magnetic Assembly and Patterning of Vesicle/Nanoparticle Aggregates

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Biomaterials that replicate the structural and functional complexity of tissue have thus far proved to be elusive. Cell−cell adhesion within tissue has been recreated using vesicle−vesicle recognition, but the resulting assemblies have been unstructured conglomerates.[1] Nonetheless, structured assemblies of vesicles will have great potential as biomaterials[1e,f] since they can host integral membrane proteins (IMPs) and compartmentalize incompatible reagents. For example, patterned assemblies of IMP-containing vesicles would be useful for high-throughput screening of drugs[2] and, given that vesicles can be used as "nanoreactors",[3] patterned vesicle assemblies could become tissue-mimetic "nanofactories" that sequentially process biomolecules. Creating tissue-mimetic structures from vesicles currently requires direct manipulation of vesicles with micropipettes[4] but building large structures using this approach would be laborious. Alternatively, magnetic labeling of vesicle aggregates should be a versatile method for noninvasive spatial control of these biomaterials. Furthermore, combining biocompatible magnetic nanoparticles[5] with vesicle technology has already been exploited for tissue engineering,[6] magnetic bioimaging,[7] and drug delivery.[8]

Herein we report the creation of vesicle aggregates cross-linked by magnetite nanoparticles (Figure 1), which are susceptible to external magnetic fields. Magnetospatial manipulation of different populations of vesicle aggregates afforded patterned assemblies of vesicles with potential bionanotechnological applications.

Cross-linking of the vesicles by magnetic nanoparticles was designed to be reversible; the particles should not insert into or otherwise affect the integrity of the vesicle membrane.[9] To ensure reversibility, we used multiple weak interactions to link the vesicle and nanoparticle surfaces together. We retained the Cu(IDA)-histidine binding motif employed in previous work,[10] but added a catechol surface anchor to bind strongly to the surface of Fe_3O_4 nanoparticles.[11] Catechol-terminated histidine ligand **1** was synthesized from *N*-acetylhistidine and dopamine,[11] while Fe_3O_4 nanoparticles were synthesized hydrothermally[12] and histidine-coated by sonication with 12 mm **1** in methanol. Transmission electron microscopy (TEM) showed the coated particles ([**1**-MNP]) (Figure 2a) had an average diameter of 10 ± 2 nm; elemental analysis showed the coated particles were 12% w/w **1**, corresponding to (80 ± 20) % coverage of the nanoparticle surface with histidyl groups.[13] Vesicles (∼800 nm diameter) with 5% mol/mol of lipid H_2**2** (1 mm H_2**2**) in dimyristoyl phosphatidylcholine (DMPC) mixed 50% mol/mol with cholesterol ([H_2**2**-DMPC/chol] vesicles) were formed by extrusion; subsequent addition of copper(II) afforded [Cu(**2**)-DMPC/chol] vesicles quantitatively.[10a,13]

The aggregation of vesicles can be inferred from increases in turbidity and/or directly observed using fluorescence microscopy. Addition of a suspension of [H_2**2**-DMPC/chol] vesicles to a suspension of [**1**-MNP], at a 1:1 ratio of H_2**2** to ligand **1**, resulted in no turbidity increase. However, the addition of copper(II) (1 equiv) gave a rapid increase in turbidity, and the formation of large

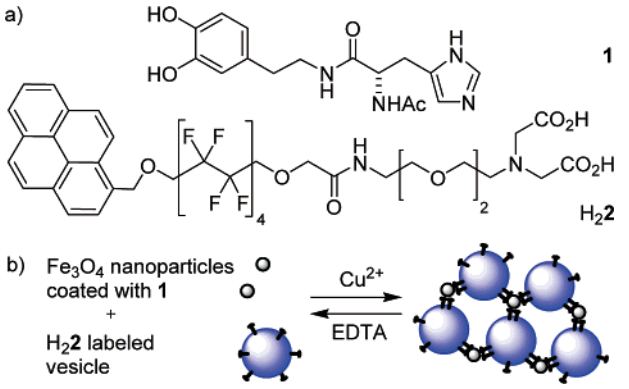

Figure 1. (a) Structures of histidine ligand **1** and adhesive lipid H_2**2**; (b) reversible cross-linking of doped vesicles by coated Fe_3O_4 nanoparticles.

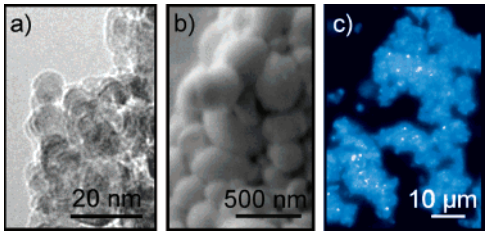

Figure 2. (a) Representative FEG-TEM micrograph of [**1**-MNP] nanoparticles; (b) representative cryo-ESEM of a [**1**-MNP]/[Cu(**2**)-DMPC/chol] nanoparticle/vesicle aggregate; (c) representative *epi*-fluorescence micrograph of [**1**-MNP]/[Cu(**2**)-DMPC/chol] aggregates.

vesicle aggregates was confirmed by cryo-environmental scanning electron microscopy (cryo-ESEM) and *epi*-fluorescence microscopy. The former showed conglomerates of rounded, intact vesicles with sizes from 200 to 800 nm (Figure 2b), while the latter showed <1 μm diameter vesicles cross-linked into large (>20 μm diameter) aggregates (Figure 2c). Energy dispersive X-ray analysis on the nanoparticle/vesicle aggregates showed strong co-localization of signals from copper and iron, consistent with Cu(**2**) binding to the surface of [**1**-MNP].[13]

As hoped, the formation of these nanoparticle/vesicle aggregates was reversible; addition of EDTA (1 equiv) removed the copper(II) and redispersed the vesicles. Both **1** and Cu(**2**) were required for aggregation; uncoated Fe_3O_4 nanoparticles were unable to cross-link [Cu(**2**)-DMPC/chol] vesicles, while undoped [DMPC/chol] vesicles were not cross-linked by [**1**-MNP]. Crucially, the vesicles remained intact after aggregation; immediately after mixing with copper(II) and [**1**-MNP] nanoparticles, only 6% of sulforhodamine B (SRB) encapsulated within [H_2**2**-DMPC/chol] vesicles was released, and there was no further release of SRB over the next 90 min.[13]

Ordinarily, sedimentation of the nanoparticle/vesicle aggregates was very slow, taking over 12 h to complete. However, application of a 5 kG magnet rapidly sedimented the nanoparticle/vesicle

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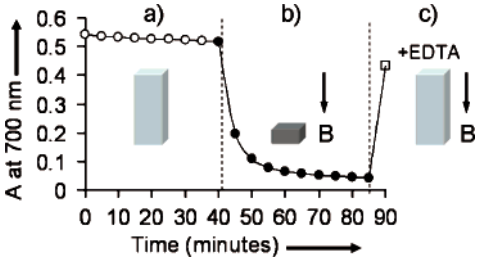

Figure 3. Change in turbidity with time of a suspension of [**1**-MNP]/[Cu(**2**)-DMPC/chol] aggregates (a) under gravity (○), (b) in the presence of a 5 kG magnet (●), and (c) after the addition of EDTA (□).

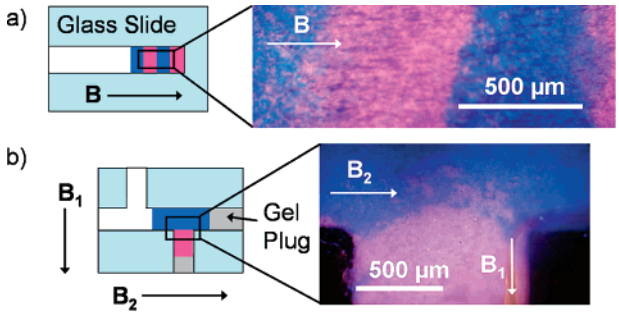

Figure 4. Fluorescence micrographs showing magnetically compressed assemblies of [**1**-MNP]/[Cu(**2**)-DMPC/chol] aggregates on glass slides: (a) bands of aggregates with rhodamine-labeled membranes (red) and unlabeled membranes (blue); (b) a microflow cell with aggregates in separate chambers, either encapsulating SRB (red) or SRB-free (blue).

aggregates, leaving a clear supernatant within 10 min (Figure 3). Removal of the copper(II) by addition of EDTA (1 equiv) resuspended the vesicle plug, and the vesicles could not be resedimented magnetically. This magnetic manipulation did not rupture the vesicles; magnetic sedimentation of vesicle/nanoparticle aggregates encapsulating SRB did not release any SRB, even after 30 min. The ability to separate vesicle/nanoparticle aggregates from nonmagnetic vesicles also showed that Cu(**2**) and [**1**-MNP] did not exchange between the vesicles.[13]

Biomaterials with discrete regions of labeled biocompatible surfaces and encapsulated volumes can be created by magnetically molding these vesicle/nanoparticle aggregates into vesicle assemblies. Such patterns of vesicles or bilayers on surfaces have recently been intensively investigated as interfaces between microelectronic devices and complex cellular assemblies.[2,14]

To demonstrate the potential of our methodology, bands of membrane-labeled vesicle/nanoparticle aggregates were magnetically assembled on glass slides. A 50 μL chamber was filled with a suspension of [**1**-MNP]/[Cu(**2**)-DMPC/chol] aggregates, which were surface-labeled with rhodamine-DHPE. The aggregates were compressed by applying a 5 kG magnet at one end of the chamber, leaving behind a clear supernatant. While maintaining the applied field, this supernatant was removed, and the compacted band washed with buffer solution several times. Then a second population of unlabeled magnetic nanoparticle/vesicle aggregates (without rhodamine-DHPE) was added, magnetically compacted and washed. Repetition of this sequence several times provided a vesicle assembly composed of bands of vesicles with labeled and unlabeled surfaces (Figure 4a).

Similarly, vesicles with encapsulated reagents could be patterned without releasing their contents. A microflow cell with two chambers terminated with plugs of calcium alginate gel was constructed on a glass slide. The same cycle of magnetic compaction and washing was used, but the sequential use of perpendicular magnetic fields allowed two chambers of the flow cell to be filled with different nanoparticle/vesicle assemblies (Figure 4b). In this case the vesicles either encapsulated SRB (red) or were SRB-free (blue fluorescence), but potentially the vesicles within the assemblies could encapsulate cytokines or have membrane-bound receptors/enzymes. The formation of this system was reversible; both the gel plugs and the vesicle assemblies could be dispersed by the addition of EDTA.

The next step will be to cross-link these vesicle assemblies into robust patterned materials, which will be tested as tissue culture scaffolds[15] and as bionanotechnological cell/surface interfaces.[14]

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Supporting Information Available: Syntheses and characterization of **1** and [**1**-MNP]. Spectroscopic analyses and magnetic manipulation of vesicle/nanoparticle aggregates. Studies of SRB encapsulation within vesicle/nanoparticle aggregates. This material is available free of charge via the Internet at http://pubs.acs.org.

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