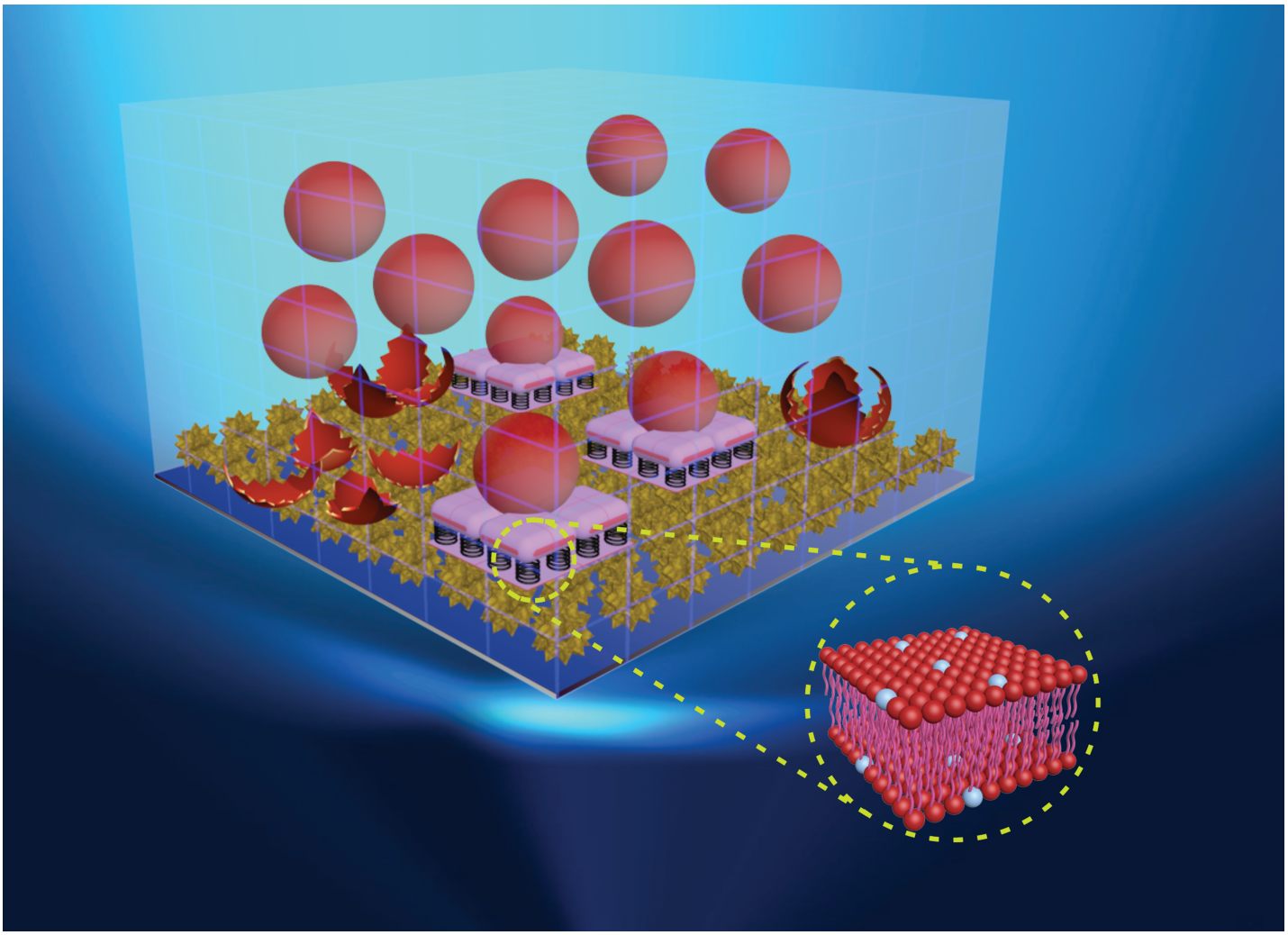

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Title: Soft landing of cell-sized vesicles on solid surfaces for robust vehicle capture/release

Controllable capture/release of lipid giant unilamellar vesicles is achieved, which is expected to open previously inaccessible avenues of research. This work provides a new model for cellular vesicle–substrate interactions, and is capable of resolving the long-term challenge of how to make a GUV suspension encapsulate a target material exclusively on the inside without any traces on the outside.

As featured in:

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Cite this: *Soft Matter*, 2015, **11**, 3094

Soft landing of cell-sized vesicles on solid surfaces for robust vehicle capture/release†

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Received 8th January 2015
Accepted 4th March 2015

DOI: 10.1039/c5sm00049a

www.rsc.org/softmatter

Based on a concept of a smooth and steady landing of fragile objects without destruction *via* a soft cushion, we have developed a model for the soft landing of deformable lipid giant unilamellar vesicles (GUVs) on solid surfaces. The foundation for a successful soft landing is a solid substrate with a two-layer coating, including a bottom layer of positively charged lysozymes and an upper lipid membrane layer. We came to a clear conclusion that anionic GUVs when sedimented on a surface, the vesicle rupture occurs upon the direct contact with the positively charged lysozyme layer due to the strong coulombic interactions. In contrast, certain separation distances was achieved by the insertion of a soft lipid membrane cushion between the charged GUVs and the lysozyme layer, which attenuated the coulombic force and created a mild buffer zone, ensuring the robust capture of GUVs on the substrate without their rupture. The non-covalent bonding facilitated a fully reversible stimuli-responsive capture/release of GUVs from the biomimetic solid surface, which has never been demonstrated before due to the extreme fragility of GUVs. Moreover, the controllable capture/release of cells has been proven to be of vital importance in biotechnology, and similarity the present approach to capture/release cells is expected to open the previously inaccessible avenues of research.

Introduction

With respect to the frontier research on cell–substrate interaction,[1] the interaction between cell-like lipid vesicles and solid substrates has attracted considerable attention.[2–15] Such interfacial interactions are in fact complex and still not fully deconvoluted,[2] and the development of new models and mechanisms to manipulate the behavior of vesicles on surfaces is challenging. According to their size distribution, lipid vesicles can be classified into small and large unilamellar vesicles (SUVs and LUVs) in the sub-micron scale as well as giant unilamellar vesicles (GUVs) with sizes above one micron. While extensive studies have been conducted on the behaviors of SUVs and LUVs on a substrate through specific linkages, such as electrostatic forces,[3–5] biotin–avidin,[6] DNA hybridization[7] and covalent bonding,[8] similar models for GUVs are scarce and consist of a few instrumentation designs, including micropipettes[9] as well as optical[10] and microfluidic trapping.[11] The reason for this situation is that GUVs have much less stability than SUVs and LUVs;[12] therefore, once the fragile GUVs come into direct contact with a solid surface, two extreme outcomes are possible: either no specific binding to the surface at all, or the quick deformation and rupture on the surface.[13] Accordingly, one prominent question is how to develop a model that can precisely manipulate the behavior of GUVs on a surface while concurrently retaining their stability and acquiring on-demand flexible feedback.

The present study deals with an electrostatic force that has been widely utilized in natural substances. Owing to the poor stability of GUVs, the coulombic force as a major class of non-covalent interactions is usually found to be detrimental for GUVs as they quickly deform and break upon their contact with charged surfaces. Sinner *et al.* presented a suitable method for the immobilization of anionic SUVs and GUVs on cationized bovine serum albumin (BSA)-coated surfaces. However, the strong electrostatic interaction made the fusion, deformation or rupture of GUVs easy, and the work did not address the controllable release of vesicles.[14] Herein, we propose a method in which a biomimetic lipid membrane is introduced between a positively charged surface and anionic GUVs; thus, a steady capture of batch GUVs without coulombic force-induced destruction can be achieved. The release of GUVs could then occur under mild heat stimuli (Scheme 1). As far as we know, this is the first successful example of a controllable capture/release of batch GUVs on a substrate. Our findings not only provide a new insight for GUV-surface interplay, but also help overcome the shortage of conventional physical manipulations

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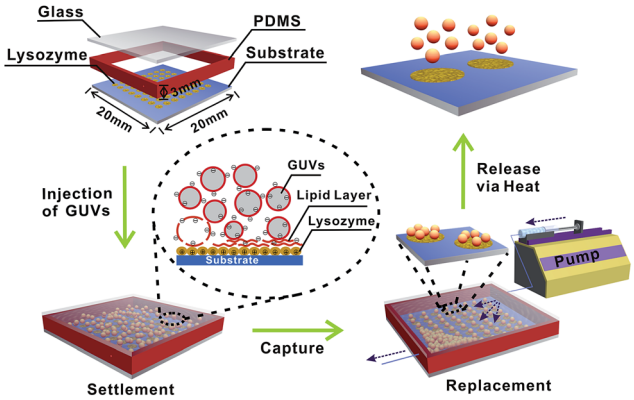
† Electronic supplementary information (ESI) available: Experimental details, optical microscope, zeta potential curve and SEM images, movies. See DOI: 10.1039/c5sm00049a



Scheme 1 The process to achieve controllable capture and release of GUVs on a surface.

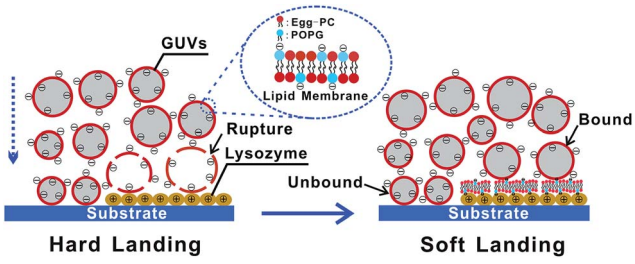
on single GUVs; thus, leading to important possibilities in vesicle-based biotechnology such as microfluidic chips and sensor/reactor arrays.[15]

Results and discussion

As depicted in Scheme 1, this process starts from a designed multilayer surface to provide a mild docking interface for the GUVs. For this purpose, a flat substrate such as glass was first coated with a phase-transited lysozyme layer,[16] which was recently developed in our laboratory to serve as a stable modification priming layer.[17] It has been proven that such a layer provides positive core charges for an effective binding with anionic colloids.[17] Encouraged by this finding, in the present study, we prepared anionic GUVs using a lipid mixture of a negative 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phospho-*rac*-(1-glycerol) sodium salt (POPG) and zwitterionic L-α-phosphatidylcholine (Egg-PC). We found that the resultant anionic GUVs were prone to breaking upon direct contact with the positively charged lysozyme layer (Movie S1,† and Scheme 2). In contrast, the zwitterionic GUVs made by pure Egg-PC without any net charge did not effectively interact with the lysozyme layer (Fig. S1†). Such a rupture of the anionic GUVs differed from what has been observed for SUVs and LUVs, *i.e.*, that the stable adhesion of SUVs or LUVs without breaking could be driven by electrostatic interaction.[3–5] The direct contact with the positively charged surface triggered a strong stimulus in the vesicle membrane, and hence such a hard landing induced the rupture of the fragile GUVs.

As an alternative to hard landing, we envisaged that the insertion of a lipid membrane between the positive surface and the GUVs might give rise to a biocompatible cushion enabling the accommodation of the GUVs without rupture. This was denoted as a soft landing (Scheme 2). A lipid-based soft bed was formed by first deliberately manipulating the hard landing of the GUVs on the lysozyme layer (Movie S1 and Fig. S2†). On a bottom substrate with the positively charged lysozyme array, an aqueous glucose solution containing GUVs with an encapsulated isotonic sucrose solution was introduced through a syringe pump. The glucose/sucrose density contrast accelerated the gravimetrical sedimentation of the GUVs to the bottom substrate. It was found that the vesicles on the surface either quickly broke and fused onto the lysozyme layer upon contact, or were safely deposited without rupture on the area outside the lysozyme layer (Movie S1†). After sedimentation for 30 min, the membrane pieces gradually dominated the whole lysozyme area (Fig. S2†). Subsequently, the GUVs started to stably attach to the lipid membrane-coated lysozyme zone without rupturing. After a certain incubation time, the introduced solution was exchanged with an isotonic glucose solution. We found that the vesicles outside the lysozyme area were actually unbound and easily flushed away by the solution flow, while the vesicles on the lysozyme layer, either from the original residence or immigration from exterior space, could stably be bound (Movie S2 and Fig. S3†). A micropatterned deposition of GUVs then clearly appeared, which was in accordance with the lysozyme array (Fig. 1).

The intrinsic nature for the stable binding of GUVs was further probed using negatively charged particles. It was observed that the deposition of anionic GUVs in the presence of negatively charged polystyrene particles produced the co-



Scheme 2 A schematic of the hard *vs.* soft landings during the sedimentation of GUVs on a lysozyme-primed positive surface.

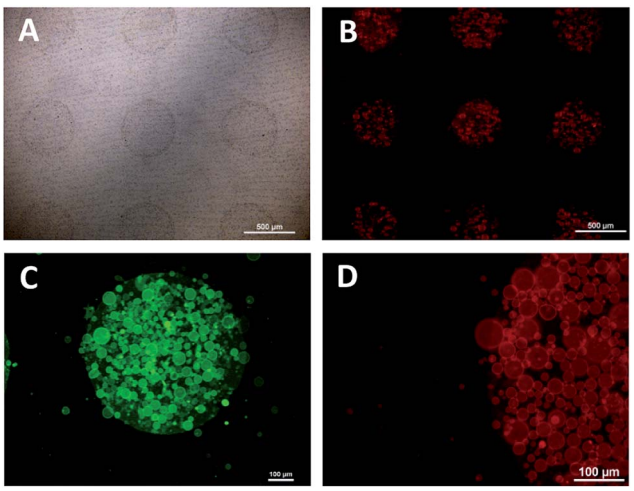


Fig. 1 The site-selective capture of GUVs by the micropatterned lysozyme array. (A) The bright field image of the lysozyme array; (B–D) the fluorescent images of the captured GUVs within lysozyme spots. Lysozyme, 2 mg ml^{-1}; POPG/Egg-PC = 5 : 95 (w/w) with 0.1 mol% $DiIC_{18}$ added for (B and D) or $DiOC_{18}$ added for (C).

adsorption of the particles and GUVs (Fig. S4†). This result was a specific indication that the driving force originated from the electrostatic interaction. Under the shear force, F, along the flow direction, a stable capture of GUVs on the surface could be determined by a force balance exerted on the GUVs (Scheme 3). The maximum static friction force, F_{max}, is defined by $(F_e + G)\mu$, where F_e is the coulombic force between the anionic GUVs and the positively charged lysozyme layer, G is the gravimetric force on the GUVs and μ is the friction coefficient. While G, μ and F are determined by the system factors, F_e could be tuned by the distance r between the GUVs and the lysozyme layer as well as the charge q_i from two interfaces *via*

$$F_e \propto q_1 q_2 / r^2. \quad (1)$$

A stable adsorption of GUVs on the surface could be predicted using the equation:

$$F_{max} = (F_e + G)\mu \geq F. \quad (2)$$

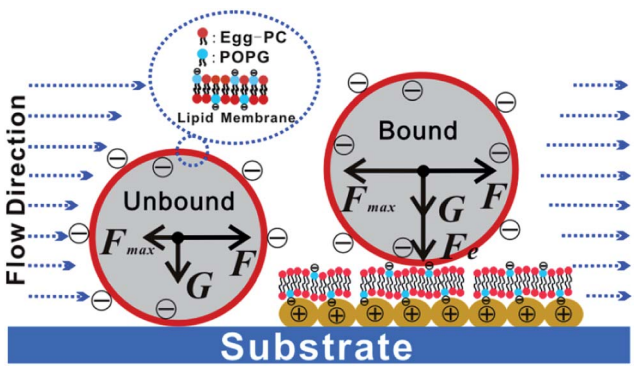
On the contrary, when the GUVs were located on a bare substrate without the charged lysozyme coating, F_e disappeared. As a result, $F > F_{max}$, which led to the GUVs readily slipping off the surface under the directional flow.

The distance r in eqn (1) is a decisive factor to control the F_e and the landing of GUVs. When the anionic GUVs approached the positively charged lysozyme layer, the very short r quickly enhanced F_e and the resultant strong F_e induced the deformation and rupture of the GUVs. In addition, when the lipid membrane was inserted between the GUVs and the lysozyme layer, r actually increased by 3–5 nm since the lipid membrane prepared by the fusion of vesicles onto the substrate had a well-defined lipid bilayer thickness.[18] By this increase of r, the coulombic interaction F_e between the GUVs and the lysozymes was attenuated, which thus decreased the damage to the GUVs, all the while maintaining a stable anchoring strength to tether the GUVs at $F_{max} > F$. In addition to r, the same material as the GUVs is adopted by the membrane and the biocompatibility is also accounted for in the mildness of such lipid membrane cushion towards the GUVs. The importance of this inserted lipid membrane interlayer was further proved by the control experiment. When the abovementioned regular lipid membrane was replaced with a dry lipid film by a simple deposition and evaporation of the lipid solution in chloroform on the substrate, the resultant lipid film with uncontrolled thickness and disordered organization did not provide a suitable and robust platform to modulate the electrostatic interaction between the lysozyme layer and the GUVs (Fig. S5†).

The charge, q_i, in eqn (1) is another factor that controls the F_e and consequent GUVs landing, which was mainly tailored by the lysozyme and POPG concentration. A high lysozyme concentration resulted in a denser particle distribution (Fig. S6†) and an elevated zeta potential on the surface, which could be positively correlated to the amount of captured GUVs (Fig. S7†). However, an overly high lysozyme concentration, *e.g.*, 10 mg ml^{-1}, induced inferior capture results than those obtained at low concentrations (Fig. S8†) since the overly strong electrostatic interaction would exceed the maximum tolerance for the GUVs. Unlike the lysozyme layer, the POPG is equally distributed in both the GUVs and the lipid membrane interlayer; therefore, the electroscreening effect from the POPG in the lipid interlayer would disturb the F_e induced by the POPG in GUVs (Fig. S7†). Notable electroscreening effect was observed at the POPG percentage of 10%. In this case, for all of the lysozyme concentrations, a low capture amount of GUVs was found. A significantly elevated capture amount was obtained after incomplete electroscreening was achieved, using a POPG content below 10%. Specifically, at the range of lysozyme concentration of 1–10 mg ml^{-1}, 5% POPG gave a higher capture amount than 1%, because the elevation of F_e at 5% was still stronger than that at 1% after compromising the amplification on the electroscreening raised by the increase of POPG content in the lipid interlayer. At a lysozyme concentration below 1 mg ml^{-1}, a higher capture amount was obtained at 1% POPG than 5%, since under such low lysozyme concentration, the increase in F_e at 5% became less than that at 1%, after including the electroscreening effect. In the present study, the salt-induced electroscreening could not be explored as the sudden change on the ionic strength and osmotic pressure easily induces the rupture of the GUVs.

The q from the lysozyme layer was also affected by the solution pH because it could trigger a remarkable change in the protonation state of amines of the lysozyme molecules. When the pH changed from acidic to basic, the surface zeta potential of the lysozyme layer gradually transited from positive to negative, which was in accordance with the change from a protonated to a deprotonated status (Fig. S9†). This tendency was directly correlated to the amount of captured GUVs, indicating that a pH of 5.9 favored capture, while a pH of 8.0 weakened it.

Unlike most vesicle immobilization methods that are usually unable to reversibly release the vesicles or those that rely on complex designs to achieve this, the present method for GUVs capture can be simply reversed to release batch GUVs from the surface through mild heat input, thanks to the thermo-sensitivity of the coulombic interaction (Fig. S10†). A typical release process is illustrated in Fig. 2. Upon heating at 37 °C, the



Scheme 3 The analytical graph of the force for GUVs on the lipid membrane-deposited lysozyme layer.

immobilized GUVs' micropattern was blurred gradually due to the GUVs release, and subsequently the GUVs floated above the surface. When the chamber was inversed, the floating vesicles moved further away from the lysozyme area. A clear lipid membrane pattern was finally recovered without any remaining vesicles. The released vesicles were found to be suspended in a new solution and their quantity and size distribution were similar to that of before the capture (Fig. 2 and S11†).

This is a first report of a controllable capture and release of GUVs by a stimuli-responsive surface, and this method is expected to lead to research areas that were previously inaccessible. For instance, when encapsulating target compounds in GUVs during the classical preparation process, the unencapsulated materials outside the GUVs are barely removed. The typical purification methods adopted for SUVs and LUVs, such as dialysis, column chromatography, centrifuge spinning and membrane extrusion, would cause the fragile GUVs to break and often result in an incomplete purification,[19] due to the change of osmotic pressure and ionic strength in the GUVs population. In the light of this background, the present capture/release of GUVs has a great potential to fully resolve this limitation because the capture-release cycle can actually transfer the GUVs population safely from an old solution to a new one. We demonstrated this by encapsulating the anti-tumor drug doxorubicin (DOX) and the water-soluble dye 6-carboxyfluorescein (Fig. 3). Before the capture and release, the GUVs grown in a DOX solution could not be differentiated from the background under fluorescence microscopy, due to almost equivalent fluorescence signals emitted from DOX in both the interior and exterior space of the GUVs. In contrast, a clear GUV image could be observed by switching the fluorescence to DIC mode (Fig. S12†). After the capture and release of GUVs in a new, clean solution without any DOX added, the DOX as a representative molecular indicator could be exclusively encapsulated only inside the GUVs with a traceless background outside the GUVs, showing the characteristic DOX fluorescence selectively from the inside of the GUVs (Fig. 3A). The preparation of DOX-loaded GUVs with a pristine background is beneficial for the smart release of DOX from the GUVs on demand, which is desirable in the field of vesicle-based micro/nano-carriers.[20] The potential strategies to achieve this aim involve the input of some stimuli, such as electropulse,[21] ultrasound,[22] pH,[23] temperature,[24] light,[25] polymers[26] and biomolecules,[27] as well as nanoparticles.[28]

From Fig. 3A, one could find that more DOX were enriched in the membrane wall than in the lumen. This phenomenon was attributed to the intrinsic hydrophobicity of DOX, which induces an enhanced distribution of DOX in the hydrophobic inter-space of the lipid bilayer. In order to further study the location of the entrapped materials, especially hydrophilic molecules, the encapsulation of the water-soluble 6-carboxyfluorescein was studied by this method. After the capture/release cycle, the GUVs grown in the aqueous solution of 6-carboxyfluorescein clearly indicated that the target material in the solution could spread homogeneously in the lumen of the vesicles (Fig. 3B). Moreover, upon either the capture of GUVs on the surface or the release of the GUVs from the surface, there was no detectable fluorescent signal observed around the outer sides of the GUVs, indicating little leakage of the fluorescent dye from the interior of the GUVs (Fig. S13†). These results reflected that the membrane integrity of GUVs could be conserved after the capture and release from the surface. Furthermore, during the capture and release of GUVs (Movie S1 and S2† as well as

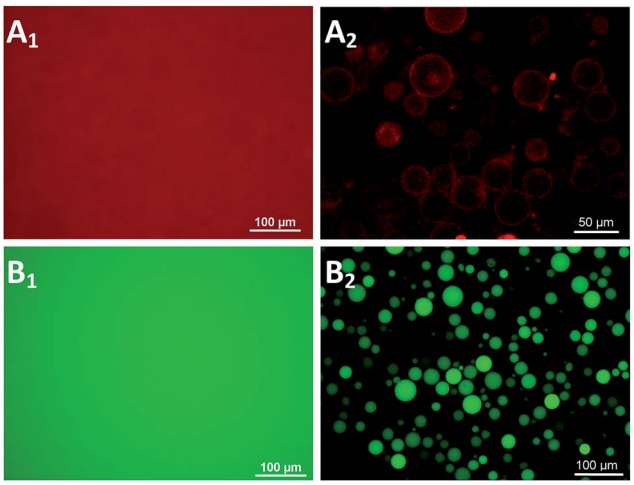


Fig. 3 The encapsulation of DOX (A_1 and A_2) and 6-carboxyfluorescein (B_1 and B_2) in GUVs by the capture/release of GUVs from the lysozyme-primed substrate. (A_1) and (B_1) show the GUVs grown in a sucrose solution of DOX (A_1) (25 μg ml^{-1}) or 6-carboxyfluorescein (B_1) (10 μg ml^{-1}); (A_2) and (B_2) present the GUVs with DOX (A_2) or 6-carboxyfluorescein (B_2) encapsulated inside after the capture-release cycle. The fluorescence emitted from DOX (red) or 6-carboxyfluorescein (green) was only observed in the interior space of GUVs. In these cases, no fluorescent dyes ($DiIC_{18}$ or $DiOC_{18}$) were added. Lysozyme, 5 mg ml^{-1}; POPG/Egg-PC = 10 : 90 (w/w); the images were taken under a fluorescence microscope.

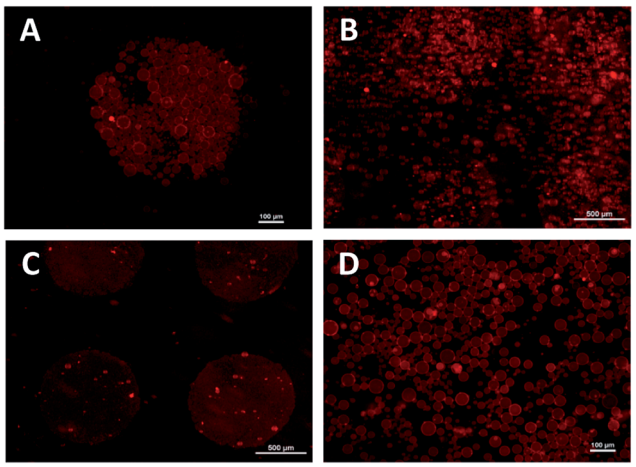


Fig. 2 The release of GUVs from the lysozyme array. (A) The GUVs captured by the lysozyme area before release; (B) the blurred GUV pattern on the lysozyme area upon heating and subtle shaking for 4 h; (C) the lysozyme area after the complete release of the GUVs; (D) the released GUVs in a new solution. Lysozyme, 2 mg ml^{-1}; POPG/Egg-PC = 5 : 95 (w/w) with 0.1 mol% $DiIC_{18}$ added; the images were taken under a fluorescence microscope.

other figures), we did not detect deformed or tubulated GUVs. From the *z*-stack confocal image (Fig. S14†), it was further found that the GUVs captured and released by the surface were not predominantly collapsed but remained nearly entirely spherical.

Conclusions

In conclusion, we have demonstrated that a controllable manipulation of remote coulombic interaction between GUVs and a positively charged lysozyme-primed surface could be achieved for the reversible capture and release of GUVs. The biocompatible lipid membrane as the middle buffer zone between the GUVs and the surface was the key factor to achieve such a delicate balancing between coulombic-driven attraction and flow-driven desorption forces without vesicle rupture.

This study provides a new model for cellular vesicle–substrate interaction, and is capable of resolving the long-term challenge of how to make a GUV suspension encapsulate a target material exclusively on the inside without any traces on the outside. This research could open a door to a new field that has not been accessed before, and pave the way toward using GUVs as protocells or microreactors.

Acknowledgements

P. Y. thanks the funding of the grants 21374057, 51303100 from the National Natural Science Foundation of China (NSFC), the Fundamental Research Funds for the Central Universities (GK201502001, GK201301006), the 111 Project (B14041) and Program for Changjiang Scholars and the Innovative Research Team in University (IRT_14R33). W. Z. thanks the support of the Project funded by China Postdoctoral Science Foundation (2014M560747) and the Scientific Research Program by Shaanxi Provincial Education Department (14JK1801).

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16 In Fig. 1 and 2, there is a non-fouling comb copolymer {poly(methyl methacrylate) (PMMA)-*b*-poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA)} inserted between the pristine glass substrate and the lysozyme layer. The role of the non-fouling polymer layer was not specially correlated to the observed capture/release behavior, but potentially corresponding to a biocompatible platform with a low non-specific adsorption of biomolecules. Such a substrate is beneficial for the future study on cell-inspired biomimetic research. A shortcoming of the non-fouling polymer substrate is that such type of a material easily adsorbs organic molecules, *e.g.* DOX. Consequently, this method was used to encapsulate DOX or 6-carboxyfluorescein in GUVs on a glass substrate without the use of such a non-fouling polymer layer (Fig. 3). See the details in the ESI.†
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