Influence of Binary Microgel Phase Behavior on the Assembly of Multi-Functional Raspberry-Structured Microgel Heteroaggregates

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Abstract

We investigate the influence of microgel composition on phase behavior of binary microgel dispersions using poly(*N*-isopropylacrylamide) microgels cross-linked with 5 mol-% and 1 mol-% *N,N'*-methylenebis(acrylamide), or poly(*N*-isopropylmethacrylamide) microgels cross-linked with 5 mol-% *N,N'*-methylenebis(acrylamide). We then explore the dispersion phase behavior in the context of microgel deposition at a planar interface. These results are then compared to the observed assembly of microgels at curved interfaces, in the form of raspberry-like patchy particles (RLPPs) consisting of a polystyrene core surrounded by a (two-component) microgel shell. Results suggest that microgel composition has a large influence on the ability of binary dispersions to coat planar and curved interfaces. In particular, we demonstrate that binary dispersions of microgels containing higher cross-linker content exhibit decreased packing densities that are very pronounced at a curved interface. To enhance packing density we also explore the use of a two-step coating process to fabricate RLPPs with enhanced control over topography. Development of these complex vehicles is potentially beneficial in the modulation of biological systems where spatial and temporal presentation of molecules can have a large influence on cellular behavior.

Keywords: Heteroaggregation, multi-functional delivery vehicles, microgel phase behavior

Abbreviations

Atomic Force Microscopy
Raspberry-Like Particles
Raspberry-Like Patchy Particles
Scanning Electron Microscopy
Effective Volume Fraction
N-isopropylacrylamide
Poly(*N*-isopropylacrylamide)
N-isopropylmethacrylamide

AFM
RLPs
RLPPs
SEM
 ϕ_{eff}
NIPAm
pNIPAm
NIPMAm

Poly(<i>N</i> -isopropylmethacrylamide)	pNIPAM
<i>N,N'</i> -methylenebis(acrylamide)	BIS
Acrylic Acid	AAc
Sodium Dodecyl Sulfate	SDS
Ammonium Persulfate	APS
1-ethyl-2-[2-dimethylaminopropyl]carbodiimide hydrochloride	EDC
<i>N</i> -hydroxysuccinimide	NHS
4-Aminobenzophenone	AB
Ethanol	EtOH
Dimethylsulfoxide	DMSO
Polystyrene	PS
Phosphate Buffer Saline	PBS

Introduction

Microgels are colloidal hydrogel nano- or microparticles that have been proposed for a variety of applications including tissue engineering scaffolds,¹⁻² drug delivery,³⁻⁴ biomimetic constructs,⁵⁻⁶ and separations.⁷⁻⁸ Microgel dispersions exhibit rich phase behavior due to the softness of the individual colloidal particles.⁹ In particular, the phase behavior of thermoresponsive poly(*N*-isopropylacrylamide) (pNIPAm) microgels has been investigated in detail, revealing that the softness imparted on these colloids increases the thermodynamic parameter of interest, the effective volume fraction, required for crystallization and narrows the coexistence region in comparison to hard spheres.¹⁰ While the phase behavior of single-component pNIPAm microgel dispersions has been investigated in detail,¹¹⁻¹⁴ the phase behavior of binary microgel dispersions has received markedly less attention. Though there has been investigation of other hard sphere colloidal mixtures including latex particles,¹⁵⁻¹⁸ it is reasonable to predict that binary microgel dispersions will exhibit unique behavior due to the softness of the building blocks.

Previously, our lab has investigated the behavior of colloidal crystals composed of two microgel components. In one study, we formed colloidal crystal lattices from pNIPAm microgels containing acrylic acid (AAc) ($R_H \sim 375 \pm 20$ nm) and observed that such crystals were able to seamlessly integrate large, “defect microgels,” ($R_H \sim 873 \pm 66$ nm) doped in at 0.1 wt%.¹⁹ Our lab has also explored how binary microgel dispersions respond at an interface through centrifugal (active) and passive deposition on a

planar glass surface.²⁰ We observed decreases in microgel footprints during deposition for both passive and active methods, which was an indication of how particle-particle repulsion dictated microgel spreading at solid substrates. However, these studies were not designed to capture colloidal phase behavior in solution.

In the present contribution, we explore the deposition of binary microgel mixtures with defined effective volume fractions (ϕ_{eff}) onto planar and curved interfaces (**Scheme 1**). We investigate how microgel stiffness influences film formation using a mixture of pNIPAm microgels containing either 1 mol-% or 5 mol-% *N,N'*-methylenebis(acrylamide) (BIS). We also explore how microgel network structure influences film formation from a binary mixture of pNIPAm microgels and poly(*N*-isopropylmethacrylamide) (pNIPMAm) microgels both containing 5 mol-% BIS. Understanding of binary microgel mixtures can shed light on development of complex microgel coatings or microparticles, which are of particular interest for biomedical applications, including drug delivery and tissue engineering scaffolds,²¹ where sensitive biological systems are highly influenced by mechanical properties,²²⁻²⁴ topographical features,²⁵⁻²⁶ microstructure,²⁷⁻²⁸ and spatial/temporal presentation of biomolecules.²⁹⁻³⁰

Scheme 1. (A) Behavior of binary concentrated microgel dispersions was evaluated by placing a drop of highly packed microgel dispersions on coverslips. (B) Behavior of binary concentrated microgel

dispersions at a curved interface was evaluated by fabricating raspberry-like patchy particles using colloidal-phase mediated heteroaggregation.

Materials and Methods

Materials

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received, unless otherwise noted. Reagents *N,N'*-methylenebis(acrylamide) (BIS), acrylic acid (AAc), sodium dodecyl sulfate (SDS), ammonium persulfate (APS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 4-aminobenzophenone (AB), 95% v/v ethanol (EtOH), dimethylsulfoxide (DMSO), sodium chloride (NaCl), (3-aminopropyl)-trimethoxysilane (APTMS) and sodium phosphate monobasic monohydrate were all used as received. The monomers *N*-isopropylacrylamide (NIPAm) and *N*-isopropylmethacrylamide (NIPMAm) were recrystallized from hexanes (VWR international, West Chester, PA) and dried *in vacuo* prior to use. Carboxyl modified polystyrene (PS) spheres were purchased from Polysciences, Inc. (Warrington, PA). Deionized water used in all reactions, purifications, and buffer preparations was purified to a resistance of 18 M Ω (Barnstead E-Pure system), and filtered through a 0.2 μ m filter to remove particulate matter.

Microgel Synthesis

Microgels were synthesized as previously described using precipitation polymerization.³¹ For pNIPAm microgels, the total monomer concentration was 100 mM with molar compositions of 95 mol-% pNIPAm and 5 mol-% BIS [denoted pNIPAm:BIS (95:5)] or 99 mol-% pNIPAm and 1 mol-% BIS [pNIPAm:BIS (99:1)]. For samples containing pNIPMAm, the total monomer concentration was 140 mM with molar compositions of 95 mol-% pNIPMAm and 5 mol-% BIS [denoted pNIPMAm:BIS (95:5)]. Monomer, crosslinker, and surfactant SDS were dissolved in water and filtered to remove any undissolved solids. The solution was placed in a 3-neck round bottom flask equipped with a condenser, heated to 70 °C while mixing with a magnetic stir bar (stir speed 450 RPM) while being purged with N₂ for approximately 1 h.

Finally, 1 mL of the initiator APS was added with a syringe needle. The solution was held at ~70 °C overnight, and then cooled to room temperature. The microgel solution was filtered through glass wool, purified via sedimentation, and lyophilized for storage.

Microgel Characterization

Microgel hydrodynamic radius (R_H) values were determined using a DynaPro Dynamic Light Scattering (DLS) instrument (Wyatt, Technology, Santa Barbara, CA) in high HIS PBS (**Table S1**). Viscometry data were obtained using an Ubbelohde viscometer with a viscometer constant of 0.003121 cSt/s at 21 °C (**Figure S1**). Samples were prepared between 0.05 wt% and 0.25 wt% depending on the microgel composition. At least four weight fractions were used for each microgel composition and three trials were performed at each weight fraction. The swelling ratio, k , was determined by fitting the data to the Einstein-Batchelor equation as described previously³² (**Table S1**). Microgel stiffness was also characterized using AFM nanoindentation. Samples were prepared by creating highly packed microgel dispersions with an effective volume fraction of 0.7. A single drop of this dispersion was deposited onto an APTMS-functionalized glass coverslip and rinsed to remove excess microgels. Samples were hydrated in HIS PBS and interrogated using a silicon nitride tip with a spring constant ~0.16 N/m. For each sample, a minimum of three 32 x 32 point arrays were collected. Representative images are shown.

Preparation of Aminobenzophenone-functionalized PS Cores

Before heteroaggregation, core (polystyrene) particles were conjugated to AB to enable UV coupling of the microgels, which is intended to add stability to the resulting RLPs. Carboxylated-PS core particles were functionalized with AB (25 mM) via carbodiimide coupling using EDC (2 mM)/NHS (5 mM) in DMSO for two hours in the dark on a shaker. PS core particles were purified in EtOH via ten rounds of sedimentation/resuspension.

Single-Step Preparation of Raspberry-Like Patchy Particles

RLPs were prepared using colloidal-phase mediated heteroaggregation. For each sample, varied amounts of each microgel sample were dissolved in 400 μL of HIS PBS and were allowed to equilibrate for a minimum of two days. AB-functionalized PS core particles were re-suspended in HIS PBS and added to the colloidal phase (0.25% w/v, 100 μL) to produce mixed colloidal dispersions with effective volume fractions of approximately 0.7. The solutions were homogenized via vortexing after which the samples were exposed to longwave UV light from a Blak Ray 100 W lamp for 30 min. Samples were purified via sequential sedimentation and resuspension in EtOH.

Preparation of Raspberry-Like Particles

RLPs were prepared using colloidal-phase mediated heteroaggregation. For each sample, 5 mg of microgels were dissolved in varied volumes of HIS PBS. AB-functionalized PS core particles were re-suspended in HIS PBS and added to the colloidal phase (0.25% w/v, 100 μL) to produce mixed colloidal dispersions with effective volume fractions between 0.1-0.6. The solutions were homogenized via vortexing after which the samples were exposed to longwave UV light from a Blak Ray 100 W lamp for 30 min. Samples were purified via sequential sedimentation and resuspension in EtOH. AFM images of these RLPs were used to determine conditions for the two-step preparation of raspberry-like patchy particles and can be found in the Supporting Information (**Figure S2**).

Two-Step Preparation of Raspberry-Like Patchy Particles

RLPs were prepared using colloidal-phase mediated heteroaggregation. Dispersions of pNIPAm:BIS (95:5) and pNIPAm:BIS (95:5) were prepared by suspending microgels in 400 μL of HIS PBS and allowing the particles to solvate for two days. AB-functionalized PS core particles were re-suspended in HIS PBS and added to the pNIPAm:BIS (95:5) colloidal phase (0.25% w/v, 100 μL) to produce mixed colloidal dispersions with an effective volume fraction of 0.30. The solutions were homogenized via vortex after which the samples were exposed to longwave UV light from a Blak Ray 100 W lamp for 30

min. Samples were purified via sequential sedimentation and resuspension in EtOH. Single component RLPs were then suspended in 100 μ L PBS and added to the pNIPAm:BIS (95:5) colloidal phase to produce mixed colloidal dispersions with an effective volume fraction of 0.49. The solutions were homogenized via vortexing, after which the samples were exposed to longwave UV light from a Blak Ray 100 W lamp for 30 min. Samples were purified via sequential sedimentation and resuspension in EtOH.

Colloidal Phase Characterization at a Planar Interface

Concentrated microgel dispersions were prepared by weighing out individual microgel components in the dry state and solvating these mixtures in 10 mM, pH 7.4 phosphate buffer saline (PBS) with 100 mM NaCl high ionic strength (HIS) for several days (minimum of 4 days) on a shaker to establish an equilibrium. These samples were also periodically vortexed to ensure mixing. Glass coverslips were functionalized with 1% APTMS for two hours. Coverslips were washed with deionized water and dried with nitrogen. Using a transfer pipette, a single drop of the concentrated microgel dispersion was applied to the surface of the functionalized coverslip. The coverslip was then immediately washed with deionized water to remove excess solution. These samples were imaged using an MFP-3D Atomic Force Microscope (AFM) system (Asylum Research, Santa Barbara, CA).

Characterization of Raspberry-Like Patchy Particles and Raspberry-Like Particles

Microparticles were imaged using a NOVA 200 Focused Ion Beam/ Scanning Electron Microscope system (FEI, Hillsboro, OR) to assess the resulting RLP microgel coverage. Samples were prepared by placing a drop of purified microparticles dispersed in EtOH on copper tape adhered to a metal stub. The samples were coated with gold/palladium for 2-3 min at 25 V using a Hummer V Sputterer (Anatech USA, Union City, CA).

Image Analysis

Fourier analyses of images were performed using the FFT algorithm in ImageJ (v1.48, NIH). The resultant frequency-domain images were then processed using the ImageJ exponential transform (Exp) to rescale the intensities.

Results and Discussion

Previously, our lab has developed a method to produce core-shell microgel heteroaggregates called raspberry-like particles (RLPs) where ABP-functionalized PS cores are immersed in a concentrated dispersion of microgels; this dispersion is irradiated with ultra-violet light to covalently bond a microgel shell atop the PS core.³³ This method is more versatile than previous methods because it eliminates the need for Coulombically-driven assembly. We have demonstrated the colloidal-phase mediated heteroaggregation is a robust approach using a variety of core and shell components with limited constraints due to synthesis conditions. In addition to being a useful fabrication method, this approach presents a unique avenue towards the exploration of microgel interactions at a curved interface. We have seen that microgel stiffness, microgel network structure, and effective volume fraction of the colloidal phase influence particle interactions at a curved interface.³² Recently, we have become interested in understanding how binary mixtures of microgels behave at such an interface. In particular, we are interested in understanding the influence of the mechanical stiffness of microgels on a binary mixture and the influence of microgel network structure on binary microgel dispersion interfacial behavior. Previous microgel degradation studies in our lab have revealed that microgel network structure is dictated in some cases by the parent monomer.³⁴ These studies indicate that microgels composed of pNIPAm exhibit a heterogeneous cross-linker distribution, containing a densely cross-linked core surrounded by a loosely cross-linked periphery. In contrast, microgels composed of pNIPMAm exhibit a more homogeneous cross-linker distribution. In addition, varying cross-linker density has been demonstrated to be another facile route to tune microgel network structure as well as subsequent mechanical properties such as the Young's modulus and bulk modulus.³⁵⁻³⁷ Herein, we investigate the coating behavior of binary microgel dispersions containing microgels with varied monomer and varied cross-linker content. We explore a

binary mixture of pNIPAm microgels composed of either 1 mol-% or 5 mol-% BIS and another binary mixture composed of 95 mol-% pNIPAm or pNIPMAm with 5 mol-% BIS.

Binary dispersion compositions were selected based on previous studies of colloidal-phase mediated heteroaggregation using the same microgel compositions; these previous studies demonstrated that pNIPAm microgels are capable of producing well-coated RLPs at a wide range of effective volume fractions (regardless of cross-linker concentration) while pNIPMAm microgels containing 5 mol-% BIS produce well-coated RLPs over a limited range of effective volume fractions.³² In the present study, we investigated the microgel arrangement within the colloidal phase by depositing a sessile drop of each highly packed binary microgel dispersion (**Table 1**) onto a glass substrate and immediately washing away the excess solution (**Scheme 1**). Due to the high effective colloidal volume fraction employed in these studies, we assume that the microgels have very short diffusion paths within the dispersed phase, resulting in a diffusion path-length of almost zero at the glass interface. As a result, the interfacial arrangement of the phase should be frozen at the planar interface. AFM imaging of these experiments reveals that the ability to coat a planar substrate is influenced by the microgel components (**Figure 1**).

Table 1. Compositions for Binary Microgel Dispersions

Label	ϕ_{eff}	ϕ_{eff} of Components		
		pNIPAm:BIS (99:1)	pNIPAm:BIS (95:5)	pNIPMAm:BIS (95:5)
A1	0.70	0.14	0.56	0
A2	0.69	0.27	0.42	0
A3	0.69	0.41	0.28	0
A4	0.68	0.54	0.14	0
B1	0.68	0	0.28	0.40
B2	0.70	0	0.35	0.35
B3	0.72	0	0.42	0.30
B4	0.72	0	0.49	0.23

Figure 1. (A) AFM images of binary microgel dispersions with varied ratios of 1 mol-% and 5 mol-% BIS cross-linked pNIPAm microgels deposited onto a functionalized coverslip. (B) AFM images of binary microgel dispersions with varied ratios of pNIPAm or pNIPMAm microgels containing 5 mol-% BIS deposited onto a functionalized coverslip. Binary microgel dispersion compositions used for this investigation are listed in **Table 1**. Each scan is 20 μm by 20 μm .

For the binary dispersions containing pNIPAm microgels alone, we see that the microgels are able to effectively interact with the substrate and form robust, ordered coatings (**Figure 1A**). In these images, the brighter particles correspond to the 5 mol-% BIS microgels and the darker particles correspond to the 1 mol-% BIS microgels. As we decrease the volume fraction of 5 mol-% BIS pNIPAm microgels (moving from left to right in **Figure 1A**), the surface coverage of these microgels decreases, as well. For this binary microgel dispersion, the overall composition of the dispersion clearly dictates the composition of the coating. In all cases, the microgels appear to have a high packing efficiency while also exhibiting some degree of order and phase segregation.

Next, we create distinct microgel dispersions using the same pNIPAm microgels containing 5 mol-% BIS to which we add a new secondary component, pNIPMAm microgels containing 5 mol-% BIS (**Figure 1B**). These pNIPAm/pNIPMAm microgel dispersions contain microgels with different network

structures, but similar cross-linker contents. In these images, the pNIPAm microgels appear larger than the pNIPMAm microgels. For these coatings, in contrast with the data shown in **Figure 1A**, decreasing the volume fraction of pNIPAm microgels with 5 mol-% BIS (from right to left in **Figure 1B**) does not correspond to an obvious decrease in the apparent surface coverage of the microgel. In addition, the regions of the coating rich in pNIPMAm microgels are broken up into small, ordered domains. What is even more striking is the decreased packing exhibited by the pNIPAm microgels in the pNIPAm/pNIPMAm coatings. Here, pNIPAm microgels disrupt the order of the pNIPMAm regions while also exhibiting a low tendency to form single-component domains. This behavior is dissimilar from the previous experiment where the coating regions rich in pNIPAm microgels containing 5 mol-% BIS exhibited homogenous packing around the softer pNIPAm microgels (**Figure 1A**). These coatings exhibit domains of ordered pNIPMAm microgels with little or no order in regards to the pNIPAm microgels. In this set of coatings, microgels appear less packed and less ordered overall. Fast Fourier transforms of the data also suggest differing degrees of order in the two sets of experiments (**Figure S3**). In addition, these data suggest that the overall phase composition does not dictate the composition of the coating that is deposited. When compared to one another, these sets of experiments suggest that simply changing one component of the microgel dispersion causes the 5 mol-% BIS pNIPAm microgels to become less effective at coating a planar substrate.

Microgel dispersion composition clearly influences the ability to form ordered coatings. We find it surprising that the binary pNIPAm microgel dispersions create ordered coatings with apparent phase segregation at the interface, as the two microgels are chemically very similar. Though the cross-linker, BIS, is more hydrophobic than the pNIPAm, it is likely core-localized due to the reactivity ratio of pNIPAm and BIS, which should result in the two different microgels (1% and 5% BIS) presenting similar surface chemistries. Thus, this difference in chemical composition does not provide an obvious explanation for the apparent interfacial phase segregation. Moreover, the apparent lack of interfacial phase segregation in the pNIPAm/pNIPMAm microgel dispersions, where the chemical differences are more significant, would suggest another factor may be influencing the coating process. The decreased

coating ability for the pNIPAm/pNIPMAM microgel dispersions, in particular, could be a result of using stiffer microgel particles; as microgel stiffness increases their ability to deform decreases, which could lead to diminished coating ability. Furthermore, recent studies in our lab have elucidated the importance of microgel mechanical properties in a variety of contexts and we suggest that microgel stiffness may influence the phase behavior observed here for the binary microgel dispersions at an interface.

We next performed AFM nanoindentation studies to determine the Young's moduli of the microgels (**Figure 2**). These studies reveal that pNIPAm:BIS (99:1) microgels are the softest, with a Young's modulus of <50 kPa. In comparison, the pNIPAm:BIS (95:5) microgels exhibited varying Young's moduli due to the core-localization of the 5 mol-% BIS, leading to a Young's modulus of ~125 kPa in the core and ~50 kPa in the surrounding shell. Microgels composed of pNIPMAM:BIS (95:5) have a similar Young's modulus around 150 kPa. However, nanoindentation studies of these microgels exhibit more heterogeneity, which could be caused by less efficient microgel packing on the surface of the coverslip, as well as a decreased ability to spread on the surface, resulting in sampling of the underlying substrate. These nanoindentation studies suggest that pNIPAm:BIS (99:1) microgels are at least three times softer than the pNIPAm:BIS (95:5) and pNIPMAM:BIS (95:5) microgels. These studies also suggest that the pNIPAm:BIS (95:5) and pNIPMAM:BIS (95:5) microgels have mechanical properties that are more similar to each other than to the pNIPAm:BIS (99:1) microgels.

Figure 2. The Young's moduli for (A) pNIPAm:BIS (99:1), (B) pNIPAm:BIS (95:5), and (C) pNIPMAm:BIS (95:5) generated in 32 x 32 arrays through AFM nanoindentation studies. Each scan is 5 μm x 5 μm .

We next explored the behavior of microgel mixtures at a curved interface through colloidal-phase mediated heteroaggregation with PS beads, resulting in the development of raspberry-like patchy particles (RLPPs), in order to determine whether interfacial rearrangement of the colloidal phase occurs on the surface of the PS core particles. In these studies, PS core particles were immersed in the same binary microgel dispersions used in the planar studies in order to template the core particle with microgels. The difference in degree of ordering and phase segregation at a curved interface can be seen by comparing the likelihood of finding two distinct microgel types sitting next to one another. RLPPs fabricated using the dispersions of pNIPAm microgels with varying BIS contents, resulted in particles with packed, ordered coatings; phase segregation was also apparent at the PS interface (**Figure 3A**). In this case, each microgel type appears to be ordered in large areas, suggesting these microgels prefer to interact with microgels of the same composition. In contrast, when using the pNIPAm/pNIPMAm microgel dispersions to fabricate RLPPs, particles exhibited poor coating and phase segregation was not as obviously present at the PS interface (**Figure 3B**). In this case, the likelihood of finding two distinct microgel types sitting next to each other on the PS surface appears to be higher than the coatings produced using the pNIPAm microgel dispersions. These studies revealed that RLPP microgel coverage distribution was consistent with planar experiments, suggesting that interfacial reorganization of the colloidal phase does not occur to a greater or lesser degree when a curved interface is introduced into the system.

Figure 3. (A) SEM images of RLPs fabricated using binary microgel dispersions with varied ratios of 1 mol-% and 5 mol-% BIS cross-linked pNIPAm microgels deposited onto a functionalized coverslip. (B) SEM images of RLPs fabricated using binary microgel dispersions with varied ratios of pNIPAm or pNIPMAm microgels with 5 mol-% BIS deposited onto a functionalized coverslip. PNIPAm microgels with 5 mol-% BIS are false-colored blue for clarity. False-coloring was assigned qualitatively by magnifying images to trace the outlines of microgels and fill them in. The original images can be found in the Supplementary Information (**Figure S4**). Binary microgel dispersion compositions used for this investigation are listed in **Table 1**. Scale bars represent 2 μm .

For the pNIPAm/pNIPMAm dispersion, microgels do not appear to pack closely at either the planar or curved surfaces. Additionally, we found that it is difficult to control the ratio of microgels at the interface based on the ratio of components in the colloidal mixture. In other words, the composition of the dispersion is not accurately reflected in the composition of the coating. We investigated the use of a new two-step process to coat a PS core particle with each microgel population separately for comparison. In this process, we first immersed the PS core particles in a pNIPMAm microgel colloidal phase ($\phi_{\text{eff}}=0.3$) to create a population of raspberry-like particles (RLPs) with a sparse microgel coating (**Figure 4A**). After purification and recovery, we then immersed these RLPs in a pNIPAm colloidal phase to create

RLPPs (**Figure 4B**). This method results in a population of RLPPs with a high overall packing density as visualized through SEM imaging. Although the RLPP population is well-coated as a whole, surface topography is not homogeneous throughout the entire RLPP population (**Figure 4C**). These results suggest that this method may offer enhanced control over microgel packing at a curved interface, but that challenges may still exist in terms of population uniformity. Optimization of volume fractions used in the two-step method could further enhance control over topographical features by creating an RLP population of evenly coated particles to template RLPP assembly.

Figure 4. RLPPs fabricated using a two-step coating process where (A) a pNIPAm microgel coating is first applied, (B & C) followed by addition of the pNIPAm microgel coating resulting in particles with high microgel packing. All scale bars are 2 μm .

Architectural control over composite microparticles has become increasingly important for the development of functional materials. Hybrid colloids offer facile routes for unique functionality tailored through design of vehicles with complementary pairing of materials possessing multiple properties or characteristics.³⁸⁻⁴⁰ For example, Park *et al.* developed magnetic hybrid colloids with silver nanoparticles

localized on the surface for use as an antiviral.⁴¹ Though silver nanoparticles have previously been demonstrated as an antiviral there are still concerns regarding the harmful effects of the uncontrolled release of these nanoparticles into the environment. Development of complex vehicles such as RLPPs are well-suited for encapsulation or delivery within sensitive biological systems where both spatial and temporal control over presentation of chemical and mechanical cues can have a large impact on cellular fate and function.⁴²⁻⁴⁷ The influence of microgel mechanics has become increasingly important in development of functional, tunable biomaterials as researchers have demonstrated sensitivity of biological systems to mechanical properties, which we have previously explored here.⁴⁸ The use of microgels for encapsulation and delivery has been extensively characterized, demonstrating the high degree of synthetic control that influences molecular payload and release rates.⁴⁹⁻⁵¹ In such systems, one can envision a vehicle that is able to deliver multiple molecules at multiple rates, perhaps controlled by cross-linker content and/or charge. In contrast, one could also envision a thermoresponsive pNIPAm/pNIPMAm vehicle that is utilized to trigger release at multiple temperatures or even to sequester biomolecules from the surrounding environment. In addition, we are currently exploring the use of RLPs for development of tissue engineering scaffolds. Use of RLPPs for such applications could provide avenues for controlled functionalization of scaffolds with biomolecules using vehicles containing one population of microgels that allows for facile bioconjugation. Incorporation of such systems into three-dimensional microgel scaffolds also offers the potential for exploration of cellular responses to three-dimensional presentation of mechanical cues and/or topographical features.

Conclusions

A series of binary microgel coatings were constructed on planar and curved substrates in order to examine the relationship between binary microgel dispersions components and coating topography. Binary systems of pNIPAm microgels with varying BIS content exhibit robust, ordered coatings with apparent phase segregation at both planar and curved interfaces. In contrast, binary dispersions of microgels with constant BIS content composed of either pNIPAm or pNIPMAm exhibit poor, disordered

coatings with little phase segregation at either planar or curved interfaces. Similarities in coating behavior at both planar and curved interfaces suggest that binary microgel dispersions do not exhibit interfacial reorganization at curved interfaces during colloidal-phase mediated heteroaggregation. The ability of the pNIPAm microgels containing 5 mol-% BIS to coat a substrate is influenced by the other microgel component in a binary microgel dispersion. While some binary microgel dispersions lead to coating compositions that are predictable based on the dispersion composition, others do not. To improve coating coverage and predictability, we establish the use of a two-step coating process for enhanced control over microgel packing at a curved interface and reduced presence of bare PS. The RLPP constructs we have developed serve as unique tri-heteroaggregate building blocks that can function alone or be assembled for development of complex biomaterials.

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