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Research Letter

Hollow Disc and Sphere-Shaped Particles from Red Blood Cell Templates

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Colloidal gold particles with uniform size distributions were fabricated utilizing human red blood cells (RBCs) as templates. The gold shells were charged with a metal chelating agent to prevent flocculation. The procedure described here allows control over the shape of the colloidal particles. Thus, it was possible to fabricate discs and spheres by controlling the osmotic pressure.

1. INTRODUCTION

Colloidal particles with specific shape and function [1, 2] are currently a subject of intense investigation for the development of pigments [3], microcapsules [3, 4], guided drug carriers [5, 6], synthetic opals [7], transparent metals [8], photonic crystals [7, 8], thin-film transistors, organic light emitting diodes [9], solar cells [10, 11], and tunable lasers [12].

Large populations of nonspherical colloidal particles with uniform size distributions have many potential applications. Nonetheless, there are only a few techniques for the creation of nonspherical particles since their formation has been proven difficult. In recent years there has been a considerable effort in the formation of colloidal nanorods of various materials [13, 14]. Another example of nonspherical particles is the formation of oval-shaped polystyrene by applying mechanical force to spherical particles in the solid phase [15].

On the one hand, it is difficult to create uniform populations of nonspherical colloids in the laboratory. On the other hand, nature has been extremely efficient at this task. Moreover, unique shapes of colloidal particles are found throughout nature.

Human blood cells have a biconcave disc shape with an average diameter of 8.1 μm. They are roughly 2 μm thick at the outer edges and 1-μm thick in their center [16]. In a typical person, red blood cells (RBCs) have a coefficient of variation of approximately 11% [16]. Thus, the size distribution in this colloidal population can be considered relatively uniform. The outer surface of a red blood cell is composed of a phospholipid bilayer membrane [16] similar to that of most eukaryotic cells. However, unlike most cells, RBCs do not divide or reproduce themselves. They are fabricated as individual particles elsewhere within the body [16] and are, thus, truly colloidal in nature. The colloidal nature of RBCs and their unique shape motivated us to fabricate metallic colloidal particles using them as templates. Several potential commercial applications are envisioned [1–10].

2. EXPERIMENTAL SECTION

2.1. Materials

All chemicals were obtained from Aldrich Chemical Company and were used as received unless otherwise noted. Gold chloride was prepared as described elsewhere [17]. RBCs were obtained using a diabetic finger lancet. A boric acid and sodium borate isotonic contact lens saline solution was used in all experiments. Samples were centrifuged using a Hamilton Bell Van Guard V6500, which is a fixed speed blood centrifuge. All solutions were prepared with deionized water with a resistivity below 17 M Ω cm.

2.2. Colloidal discs

Colloidal gold discs were prepared at room temperature as follows. Three drops of blood were dispersed in 1.5 mL of saline solution in 2 mL centrifuge tubes. 100 μL of 0.06 M aqueous sodium citrate (used as anticoagulant) followed by 100 μL of 0.006 M aqueous gold chloride were added at once. The mixture was shaken by hand and centrifuged for 60 seconds. The supernatant was decanted and the remaining sediment was quickly redispersed in pure saline solution. The coating process (i.e., addition of aqueous $AuCl_3$) was repeated two additional times. The gold coated RBCs were centrifuged and redispersed several times in 400 μL of 0.035 M aqueous 16-mercaptohexadecanoic acid for the purpose of purification.

2.3. Colloidal gold spheres

Six drops of blood were dispersed in 1.5 ml of saline solution. 120 μL of .06 M aqueous sodium citrate followed by 400 μL of 0.006 M aqueous gold chloride were added at once. After addition of gold chloride, the sample was immediately shaken and centrifuged for 60 seconds. The supernatant was decanted and the remaining sediment was quickly redispersed in pure saline solution The coating process was repeated one additional time. The sediment was redispersed in 1.5 mL of saline solution and 300 μL of aqueous gold chloride. On the last coating cycle, the gold chloride remained in solution one minute before the addition of 400 μL of 0.035 M aqueous 16-mercaptohexadecanoic acid and 50 μL of 7.5 M aqueous sodium hydroxide. The particles were centrifuged and redispersed in deionized water containing 50 μL of aqueous sodium hydroxide. The spheres were centrifuged and redispersed several times in deionized water to purify the samples with 400 μL of the aqueous 16-mercaptohexadecanoic acid. The spheres were then centrifuged and redispersed two additional times and were allowed to sediment for 7 minutes before removing all the supernatant with a pipette, leaving only a small amount of sediment at the bottom. The purified spheres were redispersed in water and were allowed to selfassemble by sedimentation.

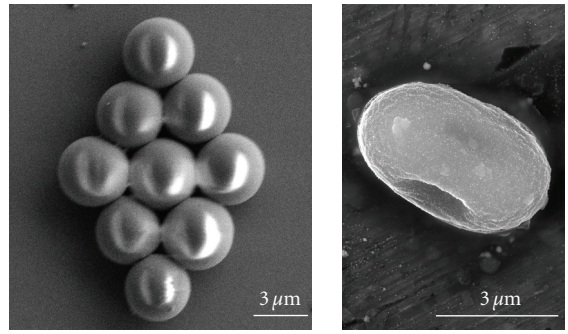
2.4. Sintering of the metal layers

The metal-coated discs were thermally sintered in a vacuum tube furnace as described: the gold-coated cells were heated at 80°C in air for 4 hours and then placed under vacuum. Once under vacuum, the temperature was increased by 10°C per hour for 4 hours. Temperature was held at 120°C for 5 hours and then raised to 500°C, at a rate of 50°C per hour. The samples were kept at 500°C for 10 hours and then cooled to room temperature at a rate of 50°C per hour.

3. RESULTS AND DISCUSSION

3.1. Self assembly and sintering

Colloidal metal disc-shaped particles were self-assembled by sedimentation and then slowly dried in air (Figure 1(a)).



(a) (b)

Figure 1: (a) Colloidal gold discs self-assembled on a silicon substrate prior to sintering. (b) A hollow colloidal gold replica of a red blood cell on a silicon substrate after sintering at 500°C.

Table 1: Elemental composition of gold chloride-coated red blood cells as determined by energy dispersive X-ray spectroscopy (EDX) after sintering.

Element	Weight %	Atomic %
Au	40.07	8.86
Cl	3.34	4.11

Once the particles were dried, they were slowly sintered as described in the experimental section (Figure 1(b)).

Gold chloride is converted to metallic gold and chlorine gas at temperatures above 350°C [17]. The sintering process should therefore result in the thermal conversion of virtually all of the remaining gold chloride to metallic gold. This was verified by energy dispersive X-ray (EDX) (Table 1). Accordingly, chlorine was present in a ratio far below its composition in gold chloride This was evident in both the atomic and weight composition.

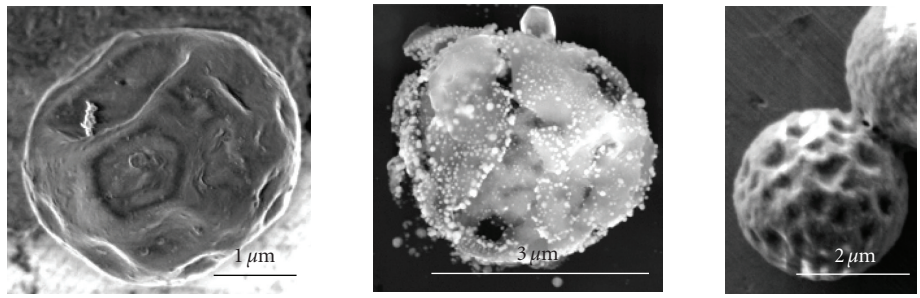
When particles with thick metal shells were sintered, the shells fused at their interfaces causing widening between the necking of the shells (Figure 2(a)).This suggests that there was excess gold chloride remaining in the metal shells before sintering allowing them to neck together during the thermal process, which was significantly below 1064°C, the melting temperature of gold.Roughening of the particles' surface was observed after sintering (Figure 2(a)). Interestingly, the gold-coated particles that were sintered have dodecahedral-shaped nodules visible on the surface of the particles, which were not present prior to sintering (Figure 2(b)). It would be interesting to investigate the formation and evolution of these dodecahedral aggregates on the particles surface during the sintering process, while the gold chloride converts to metallic gold.

3.2. Properties of the gold shells

The coating process described in the experimental section outlines the optimum procedure to create the thinnest shell necessary to stabilize the particles. This allowed us to dry the samples and obtain SEM images without bursting (Figure 2(c)) or collapsing (Figure 3(a)) the shells. The

(a) (b) (c)

Figure 2: (a) Gold discs with a thick layer of gold prior to sintering at 500°C, (b) a 300–400 nm thick layer of gold deposited on the blood cells from repeated coatings of gold prior to sintering. The arrows indicate excess colloidal gold, (c) an SEM image of a red blood cell that bursts because the gold shell was too thin to contain it.



(a) (b) (c)

Figure 3: (a) Collapsed gold-coated RBC disc. (b) A thermally shattered hollow gold disc with a 50–120 nm thick layer of gold. (c) A doublet formed from two thinly coated spheres.

thickness of this gold layer was found to be approximately 30–120 nm thick. This was determined by examining the remains of particles in the SEM that were shattered by thermal shock (Figure 3(a)).

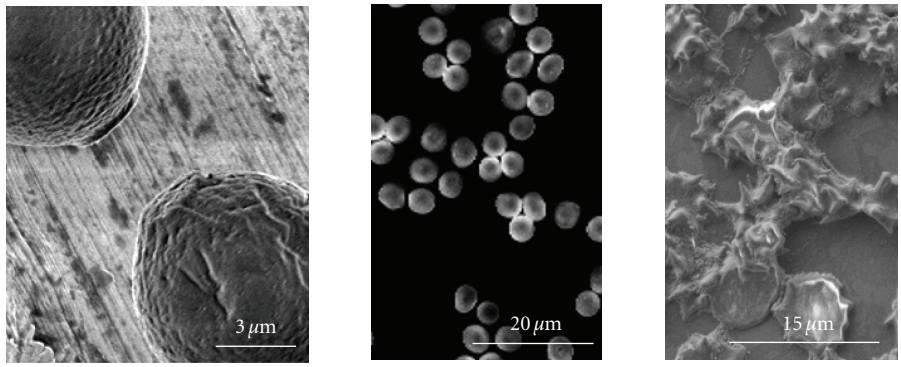
Once the cells were encapsulated with a layer of gold, they could be further coated by placing them in solutions that had much higher concentrations of gold chloride. The particles could also be left in these plating solutions for any desired amount of time since they were completely coated and indifferent to osmotic changes in solution (Figure 2(b)). This allowed the formation of much thicker gold shells and it resulted in reproducible control of the thickness of the gold shells.

3.3. Red blood cells

The cell membrane of an RBC is permeable to water, but only relatively permeable to ions [16]. Thus, the volume of the individual cells can quickly change due to flux of water across the membrane arising from a change in the osmotic pressure across the cells membrane [16, 18]. When the osmotic pressure drives water out of the interior of the cell, the volume of the cell contracts and the cell is said to be crenated. When the osmotic pressure drives water into the cell, its volume expands [19]. This allowed us to modify the shape.

The membrane of a red blood cell is relatively elastic and the volume of the cell can increase from 30% to 40% before bursting in a process called hemolysis [16, 19, 20]. When hemolysis occurred in our samples, it was evident because the samples turned blood red. This red color did not sediment when centrifuged during the coating process and was a very convenient indicator of how the coating process was proceeding. After hemolysis, the membrane of a red blood cell will often reclose or remain stable. These blood cells are called ghosts. Many interesting ghosts were found in our samples and were prevalent when the layer of gold was very thin or coated using a process where the gold layer was deposited too slowly.

Under normal physiological conditions the osmotic pressure across an RBC membrane is about 3.4 times stronger than the atmospheric pressure [19]. The osmotic sensitivity of the cells makes the coating process a challenging time-dependent task that is both pH and electrolyte sensitive. The coating process appears to be a time-sensitive one and only attempts using a quick coating process resulted in reproducible work. A failed coating attempt usually resulted in hemolysis of virtually all of the cells. The speed of the coating process was controlled by the concentration of gold chloride, which also affected the osmotic pressure. The increased osmotic pressure (resulting from excess solution) allowed the formation of spherical gold-coated RBCs (Figure 3(c)).



(a) (b) (c)

Figure 4: (a) A previous doublet formed from two gold spheres that had been forced apart. The arrows indicate where the two spheres were joined. (b) A wide-field SEM image of colloidal disc-shaped particles. (c) An SEM image of dried untreated RBCs on a silicon substrate.

3.4. Colloidal stabilization and mechanical properties

In order to stabilize the colloidal particles, their surfaces needed to be electrostatically charged. This was achieved using the following multistep process. First, the particles were charged using 16-mercaptohexadecanoic acid (a metal chelating agent) [21]. Addition of the chelating agent resulted in the removal of small unwanted gold particles (Figure 2(b)) during the rinsing process described in the experimental section. Thus, they remained dispersed after centrifuging and were decanted during the purification procedure. This also greatly reduced the number of doublet spheres found in the samples (Figure 3(b)). This means that the surfaces of the gold-coated cells must remain relatively malleable after the coating process. This malleability was observed in some of the SEM images of the gold-coated cells prior to sintering (Figure 4(a)). For comparison, an SEM image of dried and uncoated red blood cells is included as a control (Figure 4(c)).

4. CONCLUSION

Hollow gold colloidal disc and sphere-shaped shells were fabricated by encapsulating human red blood cell templates in gold chloride shells. The thickness of the gold shell and the shape of the resulting particles were found to be controllable during the encapsulating process. Coated red blood cells were electrostatically charged, self-assembled and the residual gold chloride was converted to metallic gold by sintering under vacuum at 500°C.

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