

Fatty acid-modified microgels: transmission electron microscopy study

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In order to examine modified microgels with transmission electron microscopy (TEM), we developed a sample preparation protocol that employs colloidal gold to label the sample/resin interface. This approach avoids the introduction of additional components to the prepolymer mixture, possibly altering the material properties of the resulting hydrogel. Examination of thinly sectioned hydrogels embedded in resin by TEM revealed that the gold particles clearly outlined the hydrogel/resin interface, facilitating characterization of a fatty acid coating on the hydrogel. Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

Recently there has been widespread interest in microscale actuators based on stimuli-responsive hydrogels that regulate fluid flow by harnessing the volume transitions that these materials experience in response to changes in the local environment (i.e. light,¹ electrical field² and pH^{3–5}). Such components, including autonomous and self-regulating pH-sensitive hydrogel valves, can be positioned easily within microchannels using photopolymerization techniques.^{6–9} Previously, we expanded the repertoire of hydrogel-based components for microfluidic devices by developing lipid and hydrogel hybrids that may amplify a weak chemical signal or function as chemical containers for controlled release.¹⁰ Thin ion-impermeable coatings of lipophilic fatty acids were attached covalently to pH-sensitive hydrogel microstructures (microgels) through an *in situ* process.⁷ When bathed in buffer solutions that caused swelling in unmodified microgels, the modified microgels remained contracted until expansion was induced chemically with buffered detergent solutions.¹⁰

Knowledge of the coating thickness is essential in order to optimize these hybrids for specific applications. However, small non-conductive objects with rough surfaces are not amenable to examination with many surface characterization techniques. Although composition–depth profiles of planar model systems can be acquired with XPS, SIMS or attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), these methods cannot provide a clear picture

of the coating morphology. Furthermore, it is desirable to examine the actual hybrid microstructures instead of planar models in order to identify defects in the coating that may be a consequence of coating formation on the irregular and disordered microgel surface.

Transmission electron microscopy (TEM) can be used to visualize chemically distinct microdomains within non-planar polymers by employing selective electron-dense stains to create contrast between the regions of interest.^{11–13} The core-shell morphology of polymeric microspheres and depth-dependent phase segregation of block copolymers have been observed by imaging cross-sections of the bulk materials.^{13–16} Similarly, inspection of modified microgel cross-sections with TEM is a promising approach to determining the fatty acid coating thickness. Acquiring the appropriate cross-sections necessitates cutting thin microgel slices with an ultramicrotome, which is facilitated by embedding the sample in a resin block.¹⁷ As we report in this paper, the amorphous microgel does not display any features that can be used to distinguish it from the resin, complicating coating thickness determination. We describe a sample preparation protocol that employs colloidal gold to label clearly the sample/resin interface, simplifying characterization of the fatty acid coating.

EXPERIMENTAL

All microgels were made from a monomer mixture of 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA, 1 wt.%) and 2,2-dimethoxy-2-phenylacetophenone (DMPA, 3 wt.%). The mixture was injected into the channel and placed on the stage of an Olympus Epi-Fluorescent microscope (BX-60). A photomask of a 400 µm diameter circle, made of transparency film printed

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on a high-resolution printer (5080 dpi, Linotype Herkules Imagesetter, Heidelberg, Germany), was positioned on top of the channel in the desired location. Once the solution reached a static state, it was polymerized by exposure of the unmasked regions to UV light for 2 min. The light source was a 100 W mercury lamp passed through a near-UV filter cube with a 360–370 nm bandpass (U-MNUA, type BP360-370) and intensified with a $2 \times$ lens. After polymerization, the channel was flushed with methanol and acetone, and dried under nitrogen.

To label the microgel surface, each channel containing a microgel was filled with an aqueous solution of colloidal gold (20 nm diameter, concentration 0.01% as HAuCl_4 , Sigma). After two days, the channel was rinsed with water and acetone to remove the excess gold, then dried under nitrogen for an additional 2 days.

To determine whether the gold particles remained on the surface of unmodified microgels after rinsing, the samples were removed from the channels and coated with carbon using a Denton DV-502A carbon evaporator. Imaging was performed by SEM (Philips XL30 ESEM-FEG) with secondary electron and backscattered electron detectors.

The microgels were modified with a fatty acid coating using the previously reported procedure.^{10,18} Briefly, a 50 ml syringe was charged with 0.1 M oleoyl chloride, 0.1 M triethylamine (NEt_3) and 0.01 M 4-(dimethylamino)pyridine (DMAP) dissolved in benzene. The syringe was fitted with a 16-gauge syringe needle with silicone tubing (1.58 mm ID, 2.41 mm OD, HelixMark) connecting the needle to the inlet of a channel that contained a microgel. Another piece of tubing was connected to the channel's outlet and run to a waste container. The syringe was placed in a Harvard PHD programmable syringe pump and the solution was pumped through the channel at a rate of 5 ml h^{-1} until the syringe was half empty ($\sim 4.5 \text{ h}$). The channels were cleaned by rinsing with methylene chloride, the tubing and needles were replaced and the pump was restarted so the solution flowed through the channel in the opposite direction than that previously employed for the remainder of the modification process. Channels were flushed with methylene chloride to remove unreacted reagents and placed on a nitrogen line to dry.

Modified and unmodified (control) microgels were carefully removed from the glass channels and soaked in an aqueous osmium tetroxide solution (OsO_4 , 1 vol.%) for 1 h at room temperature. The samples were rinsed with water to remove the excess OsO_4 , dehydrated through a graded series of ethanol and propylene oxide¹⁷ and embedded in LX-112 resin (Ladd Research Industries). The region of the resulting epoxy block that contained the microgel was cut with a rotary tool and glued to the remaining resin block such that sectioning on the microtome would produce radial cross-sections of the microgel. The resin block was trimmed, cut into ultrathin sections (90 nm) with a diamond knife (Micro Star Technologies) on a Leica Ultracut UCT ultramicrotome and collected on copper grids (400 mesh). Samples were examined using a Philips CM200 TEM at 120 kV.

RESULTS AND DISCUSSION

Previously we reported the *in situ* modification of pH-sensitive microgels with a fatty acid coating, which enabled them to remain contracted when bathed by elevated buffer solutions that triggered the expansion of unmodified microgels. Although the presence of the hydrophobic coating has been confirmed with fluorescent lipophilic dyes, this technique lacked the resolution to determine accurately the thickness of the fatty acid layer.¹⁸ Therefore, we have developed a method to prepare the samples for examination with TEM.

There are several steps employed for the preparation of organic samples for TEM analysis. The sample must maintain its structural integrity under a range of environmental conditions, so treatment with a fixative is commonly the first step. Next, because TEM images are produced by the selective transmission of electrons through a sample, electron-transparent organic materials are selectively labelled with electron-dense stains to provide contrast in the resulting images. Thick objects also must be sectioned with an ultramicrotome to allow for adequate electron transmission with minimal inelastic scatter, which compromises image quality. Embedding the object within a resin block facilitates the sectioning process.

Initially, sample preparation was performed as follows. The microgels were modified with an unsaturated fatty acid derivative, oleoyl chloride (Fig. 1), to create a coating that was compatible with the common fixative, OsO_4 . Both modified and unmodified (control) microgels were carefully removed from the glass channels and soaked in an aqueous OsO_4 solution for 1 h at room temperature. The OsO_4 served as a fixative and selectively stained the fatty acid coating (Scheme 1). After embedding the microgels in epoxy resin following standard protocols,¹⁷ the blocks were trimmed so that sectioning would produce ultrathin cross-sections of the cylindrical microgels. In our initial trials, however, examination of these sections with TEM did not produce suitable images for coating thickness determination because the interface between the microgel and resin could not be identified clearly (Fig. 2).

To rectify this problem, we attempted to label the microgels with colloidal gold. Because the addition of an aqueous colloidal gold suspension to the prepolymer cocktail

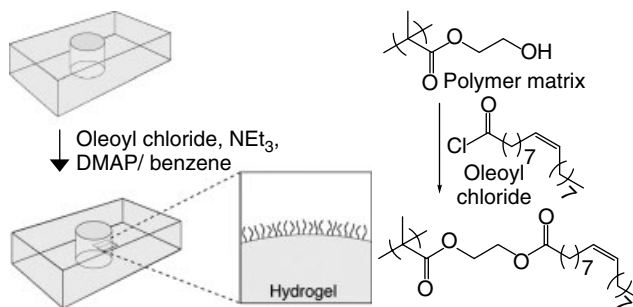
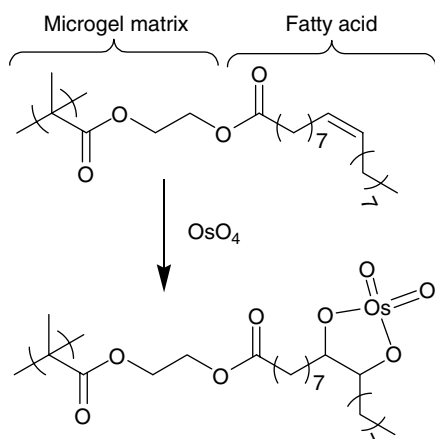


Figure 1. Simplified schematic representation of the modification process and chemical reaction. Fatty acid chlorides are covalently attached to the microgel matrix through an ester linkage.



Scheme 1

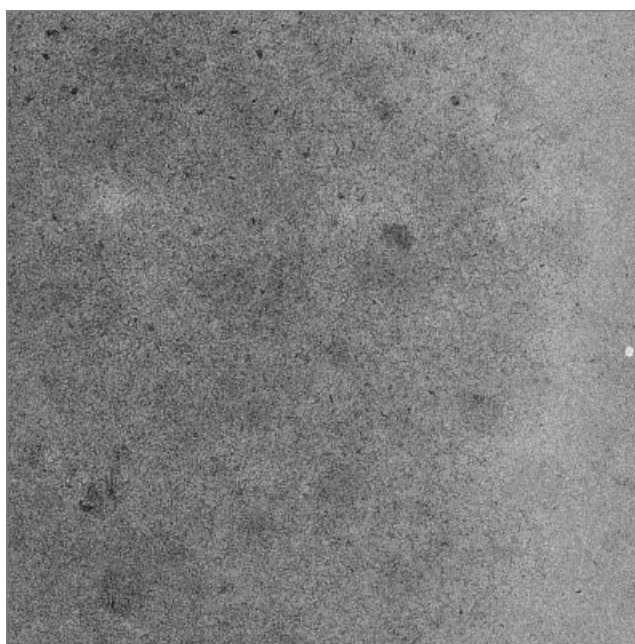


Figure 2. Transmission electron microscopy image of thin microgel section. The microgel/resin interface could not be identified.

was expected to alter the porosity of the resulting microgels,¹⁹ we tried to incorporate gold particles within the microgel

matrix simply by soaking the unmodified microgels in an aqueous colloidal gold suspension prior to creation of the fatty acid coating. Examination of the microgels using the backscattered electron detector of the scanning electron microscope revealed that the gold particles were present on the microgels even after extensive rinsing (Fig. 3). However, SEM could not be used to determine the depth that the gold particles infiltrated the microgel matrix.

The sample preparation protocol was repeated using microgels that were incubated in an aqueous colloidal gold solution prior to modification with oleoyl chloride. Transmission electron microscopy imaging revealed that the 20 nm diameter gold particles did not penetrate into the microgel matrix, but instead outlined the microgel/resin interface (Fig. 4A). The irregular microgel surface was clearly visible, and even crevices of submicron dimensions on the microgel surface were demarcated by the gold particles (Fig. 4B).

Detection of the microgel/resin interface facilitated identification of the fatty acid coating, which was selectively stained with OsO_4 and located at the periphery of the microgel with a thickness of $\sim 7000 \mu\text{m}$ for the sample displayed in Fig. 5A. Further studies are under way to determine the variability in thickness of this layer. Likewise, the location of the microgel/resin interface of embedded unmodified (control) microgels was clearly labelled by the gold particles, although these samples lacked the fatty acid coating and therefore the contrast produced by the OsO_4 stain (Fig. 5B).

The sample preparation methodology described above clearly marks the microgel/resin interface with gold nanoparticles simply by soaking the samples in a colloidal gold solution. The gold particles remain on the surface of the microgel even after extensive rinsing because they are most likely physically entrapped within the outermost chains of the microgel, which is facilitated by the slight expansion of the hydrogel network that occurs when the structure is exposed to water. (Although we did not attempt to control the quantity of gold deposited on the sample surfaces, we suspect that it is possible by varying the concentration of gold particles in solution.) Therefore this labelling technique should be applicable to other hydrogels that have similar hydration properties. However, microgels that expand in elevated pH solutions are incompatible with this protocol because the colloidal gold solution is buffered to stabilize

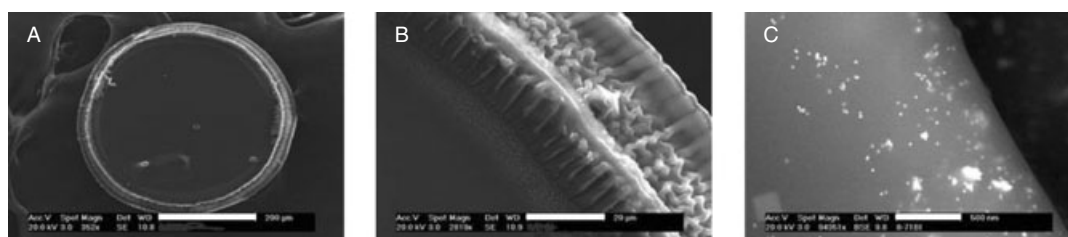


Figure 3. The microgels were soaked in colloidal gold and removed from the glass microchannels for SEM imaging. (A) Top view of a microgel. This surface had been in contact with the top of the glass channel prior to removal from the channel. Scale bar is 200 μm . (B) A SEM image of the edge of the microgel. This is the region that would be coated with fatty acids during subsequent modification. Scale bar is 20 μm . (C) When the backscattered electron detector was used to examine the sample, the gold particles were visible as bright spots in the image. Scale bar is 500 nm.

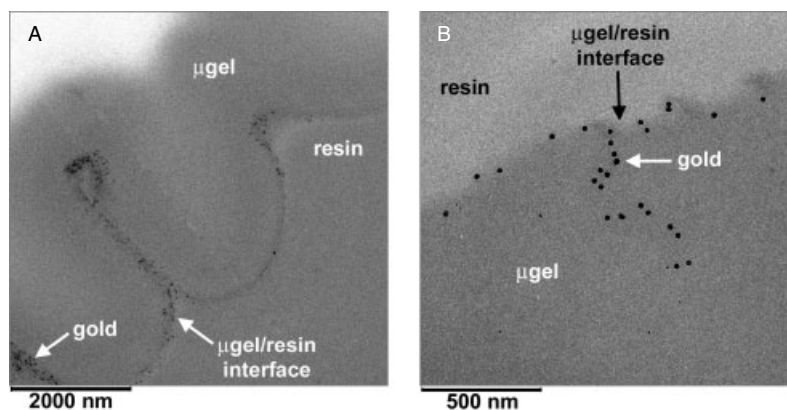


Figure 4. Transmission electron microscopy images of modified microgel cross-sections. The gold particles did not infiltrate the microgel matrix, but lined its periphery instead, facilitating identification of the microgel/resin interface. (A) The irregular surface of the microgel is clearly indicated by the gold particles. (B) Even tiny crevices on the microgel surface were visible.

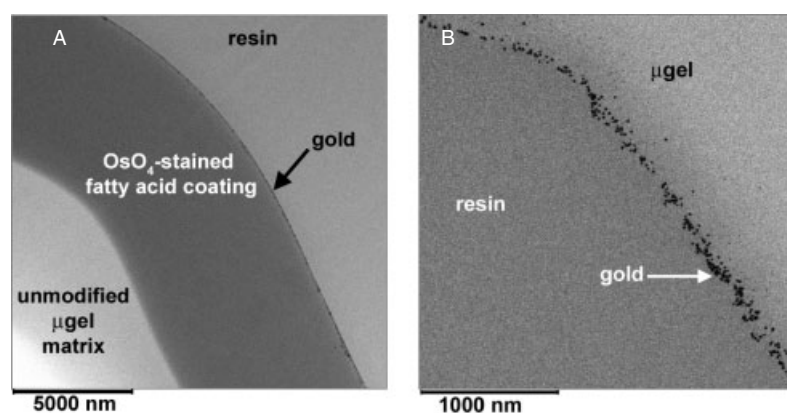


Figure 5. The microgel/resin interface is labelled with gold particles. (A) The fatty acid coating appears as a dark region at the microgel/resin interface due to selective staining with OsO_4 . (B) Unmodified microgels are devoid of contrast at the microgel/resin interface because OsO_4 does not significantly stain the microgel matrix.

the suspension. These hydrogels expand during incubation in the gold solution, which complicates the subsequent sectioning step.

In conclusion, a sample preparation methodology that employs colloidal gold to mark clearly the sample/resin interface was developed for the examination of modified microgels by TEM. The thickness of the fatty acid coating was $\sim 7000 \mu\text{m}$, however further studies are required to determine how this thickness varies within each sample and among different samples. The approach described is advantageous because it was not necessary to introduce additional substances to the prepolymer mixture to label the microgel matrix, which may alter the structure and materials properties of the resulting hydrogel object.¹⁹ This sample preparation protocol will be useful to image small, non-conductive, three-dimensional structures, including the hydrogel-based components that are becoming popular in microfluidic devices.

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