A Large-Scale Array of Ordered Graphene-Sandwiched Chambers for Quantitative Liquid-Phase Transmission Electron Microscopy

Kitaek Lim, Yuna Bae, Sungho Jeon, Kihwan Kim, Byung Hyo Kim, Joodeok Kim, Sungsu Kang, Taeyeong Heo, Jungwon Park,* and Won Chul Lee*

Liquid-phase transmission electron microscopy (TEM) offers a real-time microscopic observation of the nanometer scale for understanding the underlying mechanisms of the growth, etching, and interactions of colloidal nanoparticles. Despite such unique capability and potential application in diverse fields of analytical chemistry, liquid-phase TEM studies rely on information obtained from the limited number of observed events. In this work, a novel liquid cell with a large-scale array of highly ordered nanochambers is constructed by sandwiching an anodic aluminum oxide membrane between graphene sheets. TEM analysis of colloidal gold nanoparticles dispersed in the liquid is conducted, employing the fabricated nanochamber array, to demonstrate the potential of the nanochamber array in quantitative liquid-phase TEM. The independent TEM observations in the multiple nanochambers confirm that the monomer attachment and coalescence processes universally govern the overall growth of nanoparticles, although individual nanoparticles follow different growth trajectories.

Liquid-phase transmission electron microscopy (TEM) offers a real-time microscopic observation of the nanometer scale for understanding the underlying mechanisms of the growth, etching, and interactions of colloidal nanoparticles. Despite such unique capability and potential application in diverse fields of analytical chemistry, liquid-phase TEM studies rely on information obtained from the limited number of observed events. In this work, a novel liquid cell with a large-scale array of highly ordered nanochambers is constructed by sandwiching an anodic aluminum oxide membrane between graphene sheets. TEM analysis of colloidal gold nanoparticles dispersed in the liquid is conducted, employing the fabricated nanochamber array, to demonstrate the potential of the nanochamber array in quantitative liquid-phase TEM. The independent TEM observations in the multiple nanochambers confirm that the monomer attachment and coalescence processes universally govern the overall growth of nanoparticles, although individual nanoparticles follow different growth trajectories.

Liquid-phase transmission electron microscopy (TEM) offers a real-time microscopic observation of the nanometer scale for understanding the underlying mechanisms of the growth, etching, and interactions of colloidal nanoparticles. Despite such unique capability and potential application in diverse fields of analytical chemistry, liquid-phase TEM studies rely on information obtained from the limited number of observed events. In this work, a novel liquid cell with a large-scale array of highly ordered nanochambers is constructed by sandwiching an anodic aluminum oxide membrane between graphene sheets. TEM analysis of colloidal gold nanoparticles dispersed in the liquid is conducted, employing the fabricated nanochamber array, to demonstrate the potential of the nanochamber array in quantitative liquid-phase TEM. The independent TEM observations in the multiple nanochambers confirm that the monomer attachment and coalescence processes universally govern the overall growth of nanoparticles, although individual nanoparticles follow different growth trajectories.
The advanced liquid cell is fabricated by sandwiching a nanostructured membrane of anodic aluminum oxide (AAO) between layers of graphene (Figure 1a). The AAO membrane is filled with highly ordered uniform nanopores that are formed spontaneously and concurrently, during the anodization process, and the dimension of the nanopore is selectively controllable by changing the anodization parameters (Figure S2, Supporting Information). In this work, we fabricate an exemplary design of the liquid cell, employing the AAO membrane with a pore diameter, interpore distance, and thickness of approximately 100 nm, 125 nm, and 100 nm, respectively.

In Figure 1b, two graphene TEM grids, for the top and bottom layers of the liquid cell, are prepared by transferring graphene onto holey carbon TEM grids by direct transfer. Deionized water is dropped onto the graphene-transferred grid. Thereafter, the AAO membrane, supported on a poly(methyl methacrylate) (PMMA) substrate, is placed onto it, thus encapsulating the liquid sample and bonding with the AAO membrane, simultaneously. The final structure is an array of cylindrical nanochambers, each of which consisted of a nanopore and top/bottom graphene layers with the encapsulated liquid sample (the third inset in Figure 1b). TEM imaging of the liquid sample is facilitated by passing e-beam through the graphene-sandwiched nanochambers. In this work, an aqueous precursor solution of chloroauric acid, octylamine, and cetyltrimethylammonium bromide is imaged to study the growth and diffusion of colloidal gold nanoparticles to demonstrate the application of the developed liquid cell in situ TEM imaging.

The fabricated nanochamber array is observed at different magnifications to obtain an overview of the structures in a whole grid and in a single nanochamber (Figure 1c–g). The area on the TEM grid where AAO is transferred is distinct on the optical images (Figure 1c). Evenly distributed nanochambers with uniform diameters are observed and the identification of the nanochambers fully filled with the liquid samples is possible on TEM imaging at low magnification (Figure 1e,f; Figure S4, Supporting Information). Some of the nanochambers are empty or contain bubbles in encapsulated liquid, but the number of liquid-filled nanochambers is adequate for quantitative and repeated data collection. The thickness of the encapsulated liquid sample is estimated by using electron energy loss spectroscopy in cryo-scanning TEM measurement (Figure S5, Supporting Information). The averaged thickness over 20 nanochambers is 67.9 ± 26.8 nm.

The fabrication process of the liquid cell is illustrated in Figure 1b. Two graphene TEM grids, for the top and bottom layers of the liquid cell, are prepared by transferring graphene onto holey carbon TEM grids by direct transfer. Deionized water is dropped onto the graphene-transferred grid. Thereafter, the AAO membrane, supported on a poly(methyl methacrylate) (PMMA) substrate, is placed onto it. As the water is dried in an oven, the graphene layer and the AAO membrane are bonded by van der Waals force, which is strong enough to produce a hermetic seal that prevents leakage (first inset in Figure 1b).

The PMMA layer is thereafter, removed by immersing the assembled grid in an acetone bath. At that stage, nanosized-wells (nanowells) with an AAO sidewall and a bottom graphene layer (the second inset in Figure 1b) are formed. The fabricated nanowells with the bottom graphene layer are confirmed by TEM images and fast Fourier transform (FFT) patterns (Figure S3, Supporting Information). To load a liquid sample into the nanowells, the liquid sample is dispensed on the top of the AAO membrane, after which another graphene-transferred grid is placed onto it, thus encapsulating the liquid sample and bonding with the AAO membrane, simultaneously. The final structure is an array of cylindrical nanochambers, each of which consisted of a nanopore and top/bottom graphene layers with the encapsulated liquid sample (the third inset in Figure 1b). TEM imaging of the liquid sample is facilitated by passing e-beam through the graphene-sandwiched nanochambers. In this work, an aqueous precursor solution of chloroauric acid, octylamine, and cetyltrimethylammonium bromide is imaged to study the growth and diffusion of colloidal gold nanoparticles to demonstrate the application of the developed liquid cell in situ TEM imaging.
in the nanochamber array affords an effective visual guide for coarse searching and focusing, during the sequential imaging processes of multiple chambers.

As previously noted, one of the major advantages of employing graphene is the enabling of high-resolution observation in liquid-phase TEM. A high-resolution TEM movie obtained with the fabricated nanochamber array, exhibiting a single gold nanoparticle with an initial diameter of 5 nm, is presented in Movie S2, Supporting Information. The atomic lattice fringes of the gold nanoparticle are clearly visible throughout a prolonged period, as shown in the TEM snapshots from Movie S2, Supporting Information and their corresponding FFT patterns (Figure 2a). A single crystalline face-centered-cubic (fcc) structure of the gold nanoparticle is identified by the measured lattice spacings and the angular differences between the bright spots for the (002), (−11−1), and (−111) planes in the FFT patterns. We also observe an in-plane rotational motion of the nanoparticle by monitoring the angular change of the FFT patterns. The orientation of the (002) peak is indicative of this rotational motion, which is plotted in Figure 2b. These results confirm that the developed liquid cell can achieve sub-nanometer spatial resolution in liquid-phase TEM.

We investigate the detailed pathways of the growth of the colloidal gold nanoparticles in a nanochamber by in situ TEM (Movie S3, Supporting Information). Three exemplary growth trajectories are captured in the liquid cell (Figure 2c,d). Initially, the three gold nanoparticles possess circular diameters of 2–3 nm. Noticeable increases in the diameters of the nanoparticle occur in the first 20 s, after which the growth slows down. The diameters of the two nanoparticles (NP1 and 2) increase continuously with time, indicating that the growth by monomer attachment[5,14] is dominant in the two cases throughout the period. Conversely, the growth of the third nanoparticle (NP3) involves a sudden increase in the diameter by ≈2 nm from 3 to 4 s. This sudden increase is due to the coalescence.[5,15] The detailed process of the coalescence, including neck formation, is presented in Figure S6, Supporting Information. The growth of the nanoparticle halts briefly just after coalescence in a process called the relaxation period.[5] Thereafter, the growth proceeds by monomer attachment. The above observations confirm that the growth of colloidal metal nanoparticles proceeds by two mechanisms: monomer attachment and particle coalescence, consistent with previous liquid-phase TEM investigations.[5,14,15]

The developed liquid cell affords repeated observations of nanoparticle growth and dynamics in multiple nanochambers in the same chemical conditions by carrying out consecutive imaging with the constant electron beam radiation.
Concentrations of radiolysis species in a given electron beam dose rate rapidly reach to the steady-state within 1 ms and maintain it thereafter (Figure S7, Supporting Information). The repeated observations facilitate numerous data collection. The independent observations of the generation and growth of gold nanoparticles in four different chambers of a liquid cell for 30 s are shown in the in situ movies (Movies S4–S7, Supporting Information). Enlarged TEM image frames extracted from the movies are shown in Figure 3a. The diffusion trajectories of the representative particles are indicated by a color gradient for time evolution from 0 (white) to 30 s (colored). The movement of the nanoparticles is analyzed by their mean square...
displacement (MSD). The linearity expressed in the log–log plot of MSD indicates that the nanoparticles are dwelling in a fluid, following the Brownian motion.[16] The measured diffusion coefficient (D, in Figure 3b) is in the range of 1.9–9.6 × 10⁻² nm² s⁻¹ (Supporting Information). Over the same period (0–30 s), the growth of the particles also occurs in each nanochamber. The distribution of diameters of the nanoparticles observed in each chamber can be measured at different periods of the growth, as shown in Figure 3c. The growth patterns observed in the different chambers are not identical. However, they share a similar general trend. The changes in the average size and the number of nanoparticles are tracked, as shown in Figure 3d. In the early stage (0 to ~10 s), the average size of the nanoparticles slightly decreases because of the continuous generation of small clusters. Subsequently, the average size continuously increases to a level for a prolonged period. The number of particles decreases at this later stage, implying that coalescences are frequent as an alternative growth mechanism. The multi-chamber observations in a fluid, following the Brownian motion.[16] The measurements from multiple chambers (Figure 3e–g), while it is unclear in a limited measurement from an individual nanochamber (Figures S8 and S9, Supporting Information), emphasizes the importance of the large-scale data collection from multi-chambers to correctly understand the behaviors of nanoparticles. The positive relationship implies that the surface reactions are highly correlated with the effective viscosity and relevant transport dynamics around the individual nanoparticles.

In this work, we developed a novel type of TEM liquid cell by sandwiching an AAO membrane between graphene layers. For the demonstration of its potential application in quantitative data acquisition and analysis, we performed TEM observations on colloidal gold nanoparticles dispersed in a liquid, with the fabricated nanochamber array. Our study confirmed that the diffusion dynamics and growth pathways of the colloidal nanoparticles can be studied in multiple chambers of a liquid cell while maintaining the high-resolution capability of graphene liquid-cell TEM.

**Experimental Section**

The detailed experimental process is available in the Supporting Information.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

K.L., Y.B., and S.J. contributed equally to this work. This work was mainly supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2018R1D1A1B07050575 and 2019R1F1A1059099). S.J., K.K., and W.C.L. acknowledge the support from the convergence technology development program for bionic arm through the NRF, funded by the Ministry of Science & ICT (MSIT) (No. 2015M3C1B2052811). Y.B., B.H.K., J.K., S.K., and J.P. acknowledge the financial support from the Institute for Basic Science (IBS-R006-D1), the NRF grant funded by the Korean Government (MSIT) (Nos. NRF-2017R1A5A1013635 and NRF-2019M3E6A1064877), and Korea Toray Science Foundation. B.H.K. and J.P. acknowledge the support from the Samsung Science and Technology Foundation under Project number S5TF-BA1802-08 for method development, fabrication, and characterization.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

anodic aluminum oxide, graphene liquid cells, in situ TEM, liquid-phase TEM

Received: April 28, 2020
Revised: June 28, 2020
Published online:


