Probing the growth and biodegradation of nanomaterials with liquid transmission electron microscopy

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1. INTRODUCTION

Liquid-cell transmission electron microscopy (TEM) has been recently implemented on an aberration-corrected microscope at Paris Diderot University. This *in situ* technique consists in imaging the dynamics of nano-objects in an encapsulated liquid solution within an electron-transparent microfabricated cell.[1] These recent advances in the micro-fabrication of liquid cells allow investigating complex phenomena that arise at the liquid-solid interface such as the nucleation and growth of nanocrystals,[2] NPs interaction,[3] electrochemical reaction,[1] and biological processes.[4] Here, we have exploited liquid cell TEM to reveal the growth mechanisms of gold nanoplates [5] and for the first time, to study the biodegradation processes of carbon nanotubes.

2. RESULTS

2.1 Methods

The liquid cells commercialized by Protochips Inc. consist of two silicon wafers with dimensions of 2 * 2 mm and 4.5 * 6 mm, called the small and large E-chips respectively (Fig. 1). Each E-chip has one 550 µm * 50 µm window covered by a 30 nm thick Si_3N_4 amorphous film. The growth of gold nanoplates were performed in $HAuCl_4$ aqueous solution (1 mM), whereas the nanotube biodegradation were followed in water and ethanol. A drop of solution was squeezed in between the two E-Chips in a volume defined by the thickness of the gold spacers on the small E-Chip (150 nm in our case). The entire chamber was then closed by the lid of the holder tip resulting in a vacuum sealed liquid-cell. As illustrated in Fig. 1, the impermeability of the liquid cell is ensured by two concentric O-rings. Both experiments were realized in static mode (no flow) but the composition of the environment can be controlled with a micro-fluidic system which enables to mix different reaction solutions at the observation window.

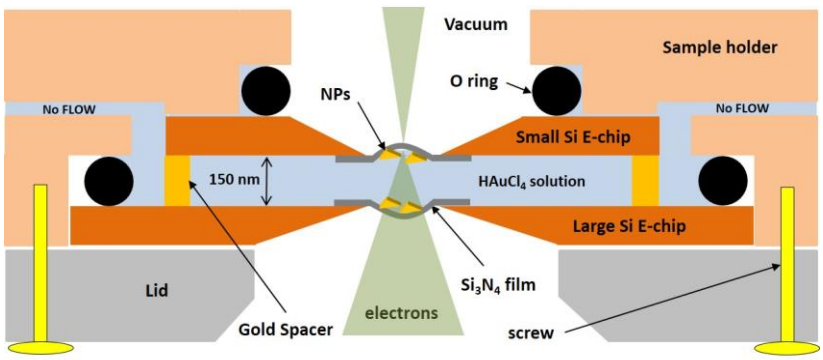

Figure 1. Schematic cross section of the sealed liquid cell in the JEOL ARM microscope.

2.2 Unrevealing the growth of gold nanoplates

The growth of colloidal nanoparticles is simultaneously driven by kinetic and thermodynamic effects that are difficult to distinguish. We have exploited in situ scanning transmission electron microscopy in liquid to

study the growth of Au nanoplates by radiolysis and unravel the mechanisms influencing their formation and shapes (Fig. 2). The electron dose provides a straightforward control of the growth rate which allows quantifying the kinetic effects on the planar nanoparticles formation. Indeed, we demonstrate that the surface-reaction rate per unit area has the same dose-rate dependent behavior than the concentration of reducing agents in the liquid cell. Interestingly, we also determine a critical supply rate of gold monomers for nanoparticle faceting, corresponding to three layers per second, above which the formation of nanoplates is not possible because the growth is then dominated by kinetic effects. At lower electron dose, the growth is driven by thermodynamic and the formation and shape of nanoplates are directly related to the twin-planes formed during the growth. [5]

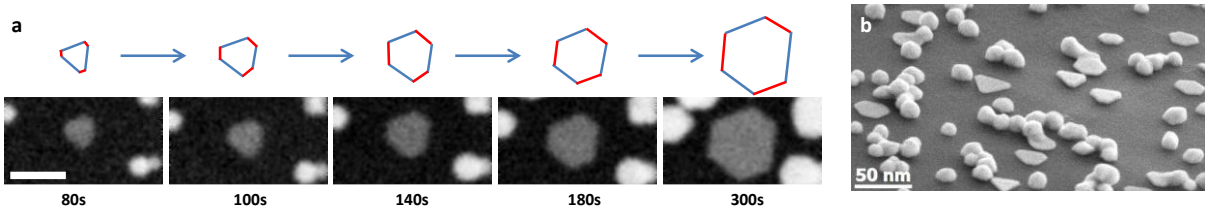

Figure 2. (a) In situ follow-up of the growth of a planar nanohexagon revealing a transition from a triangular to a hexagonal shape. The scale bar corresponds to 100 nm (b) Ex-situ SEM image confirming the formation of 3D facetted NPs and very high aspect ratio platelets on the liquid-cell.

2.3 Biodegradation mechanisms of carbon nanotubes

Carbon nanotubes (CNT) are one of the most promising nanomaterials for pharmaceutical applications, but, as many nano-objetcs, their life cycle in the organism remains unclear. In the last years, the study of CNT biokinetics (translocation, biodistribution and clearance) have shown that CNT are degraded in macrophage cells before to be eliminated by the organism. However, the chemical and structural processes involved in the degradation of the graphitic structures in cellular media are not elucidated. We used liquid-TEM to demonstrate the ability of reactive oxygen species (ROS) such as hydroxyl radicals, which can be produced by macrophages under stress, to destroy CNT (Fig. 3). The degradation mechanisms observed *in situ* allows explaining the resulting structure of CNT after degradation by macrophages *in vitro*. These nanoscale *in situ* analyses, together with the study of the biological response of macrophages to CNT digestion by quantitative PCR, provide nex insight into the clearance processes of CNT in cells, which involve ROS produced by NOX2 enzyme complexes.

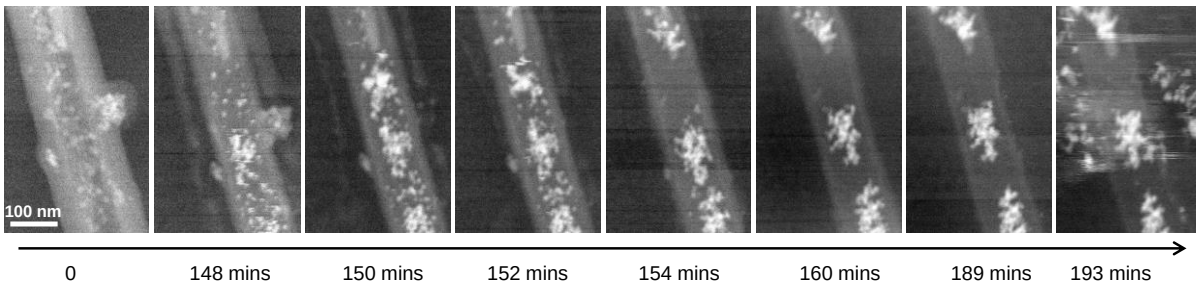

Figure 3. In situ follow-up of the degradation of carbon nanotube filled with iron-oxide NPs due to ROS.

3. CONCLUSION

These two studies illustrate that liquid-cell TEM is a relevant technique to study dynamical processes at the interfaces between liquids and solids, opening many avenues in both materials and life sciences.

REFERENCES

[1] MJ. Williamson et al. *Nat. Mater.* **2**, 532-536 (2003)
[2] H. Liao et al. *Chem. Commun.* **49**, 11720-11727 (2013)
[3] Y. Liu et al. *J. Am. Chem. Soc*. **135**, 3764-3767 (2013)
[4] N. de Jonge et al. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 2159-2164 (2009)
[5] D. Alloyeau et al. *Nanoletters* 10.1021/acs.nanolett.5b00140 (2015)
[6] D. Elgrabli et al. *Part. fibre. Toxicol.* **5**, 20 (2008)