Application Note

Poseidon Select™

Real-Time Imaging of Nanoparticle Movement within Glioblastoma Cells

Introduction

The development of in situ liquid transmission electron microscopy (TEM) has enabled the study of dynamic real-time processes and behaviors in the electron microscope. Traditionally, due to the high vacuum conditions required to maintain the electron beam, conventional preparation of biological samples for TEM imaging has required that samples be dried, chemically fixed or embedded in ice or resin, which arrests dynamic cellular processes, thus preventing real-time imaging. Protochips' Poseidon system is a dedicated in situ liquid TEM imaging platform that isolates hydrated samples between two microchips (E-chips), which each contain a membrane of 50 nm thick silicon nitride. With the Poseidon system, significant sample preparation related artifacts are avoided, and researchers can study dynamic behavior and mechanisms in the sample's native liquid environment at a resolution of a few nanometers.

Glioblastoma is the most common and lethal form of malignant brain cancer and nanoparticle-based therapies represent a novel and promising approach in the treatment of this deadly disease. In order to better understand how nanoparticles interact with cancer cells, Dr. Deborah Kelly's lab at Virginia Polytechnic University in Roanoke, VA used Protochips' Poseidon system to study the real-time interactions between gold nanorods and glioblastoma stem cells using in situ liquid TEM [1].

The Poseidon system has been used previously to obtain in situ liquid snapshots of nanoparticles, such as gold and quantum dots, on the surface of, and internalized in liquid immersed eukaryotic cells [2,3]. However, due to the size and thickness of the cell lines used, these studies were all performed using high angle annular dark field scanning transmission electron microscopy (HAADF STEM) rather than conventional TEM imaging. The cells in this study were imaged in TEM mode, which is possible because of their relatively small size compared to other eukaryotic cell lines, which enabled thin liquid layers to be used.

Experiment

Non-adherent cell lines, such as the glioblastoma stem cells used in this study (GSC9-6), prefer to grow in suspension in serum-free stem cell culture media,

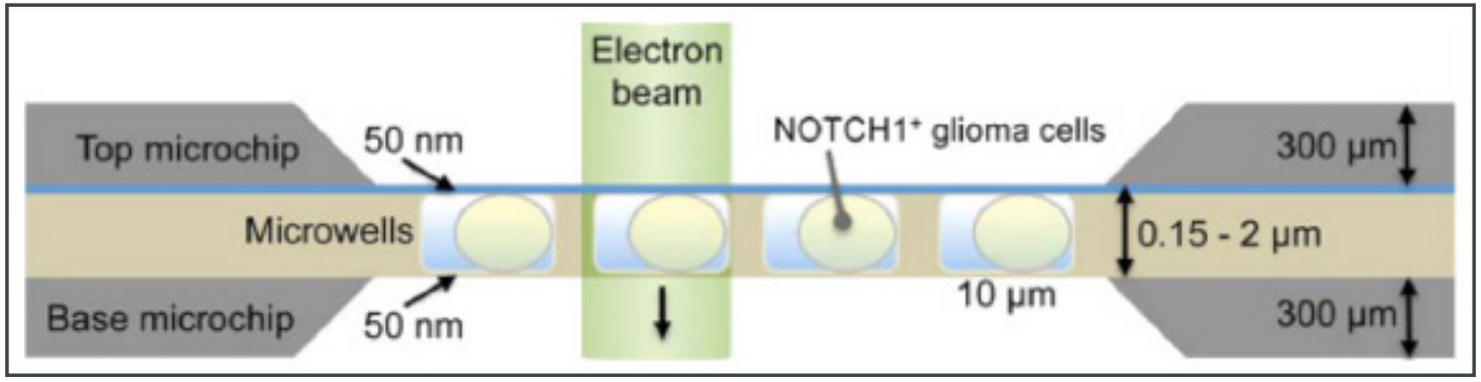

Figure 1: Cross section of the imaging Poseidon imaging chamber used in this experiment. Glioblastoma cells are confined within 10 x 10 µm² wells on the surface of the bottom Echip.

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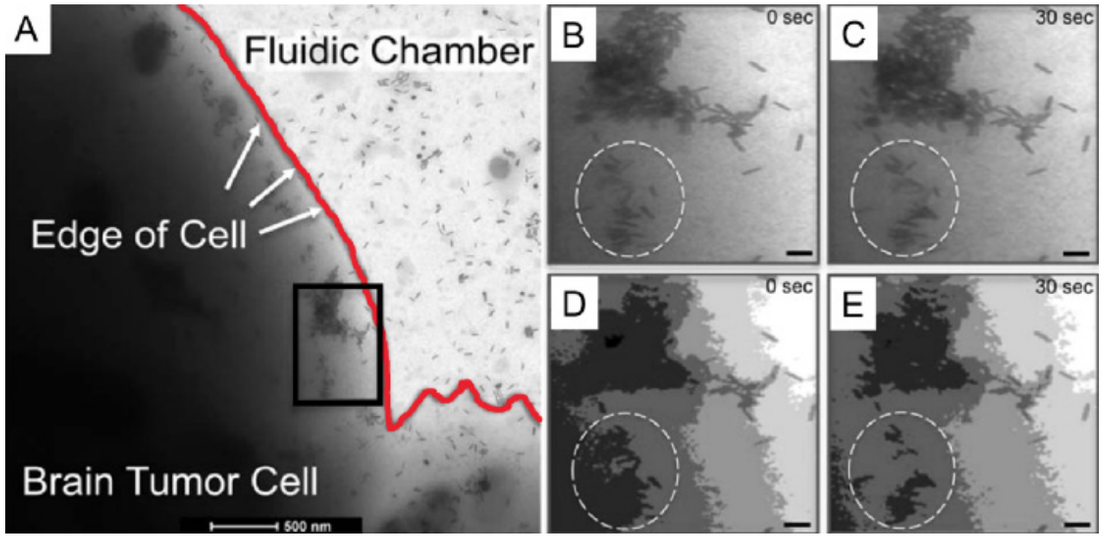

Figure 2: Glioblastoma cell incubated for 60 minutes with gold nanorods imaged *in situ* in liquid with TEM. (A) The edge of the cell outlined in red. Gold nanorods are present in the surrounding fluid and internalized within the cell itself. (B) Magnified TEM image of the regions indicated by the black rectangle. (C) Same region shown in B after 30 seconds has elapsed. (D) Region shown in B, with threshold adjusted to show electron density. (E) Region shown in C with threshold adjusted to show electrondensity.

and cannot be cultured directly on the surface of the E-chip membrane. Thus it was necessary to develop a way to immobilize the cells on the E-chip window to prevent long-range diffusion of the cells from the imaging area during capture of the image series. To prevent the glioblastoma cells from diffusing freely during the image process, the Kelly lab used a two-strategy approach. First, they utilized E-chips that contained integrated 10 x 10 µm2 microwells etched into the surface of the SiN membrane. (E-chip Part #EPT-42A1). Each microwell on the E-chip was large enough to accommodate an individual cell, and confine it within the imaging area, as shown in Figure 1. Second, the GSC9-6 cell line expresses the surface receptor NOTCH1, and this receptor was used to tether the cell to the surface of the E-chip. Using an affinity chemistry approach the microwell E-chip was modified so NOTCH1 receptor would bind to the membrane surface. Monoclonal antibodies against the NOTCH1 receptor were attached to the surface of the microwell E-chip by incubating them with a solution of protein A (0.01mg/mL) followed by monoclonal antibodies (0.01mg/mL). Next, an aliquot of five thousand cells in a 5 µL droplet of buffer was placed on the microwell E-chip and incubated for 2 minutes. Control E-chips not treated with monoclonal antibodies did not result in any cellular binding to the surface. Following the cell capture process, E-chips containing glioblastoma cells were incubated with a solution of polyvinyl-pyridine (PVP) coated gold nanorods in water for up to 60 minutes. After incubation with the nanorods, the liquid chamber was assembled in the tip of the Poseidon system holder for TEM imaging. Thirty second long image series were obtained at each region of interest using an electron dose <0.5 electrons / Å at 0.25 second intervals between frames. Images were recorded using an FEI Spirit Bio-Twin TEM equipped with a LaB6 filament operating in bright field TEM mode at 120 kV.

Gold nanorods were mobile within glioblastoma stem cells as shown in Figure 2, after a twenty minute incubation period, when the nanorods penetrate the cell. The edge of the cellular regions is indicated by the

red line in Figure 2A. A magnified view of the region indicated by the black rectangle is shown in panel 2B-E. Gold nanorods, highlighted by the dashed, white circle change their position within the internal structure of the cell over a period of thirty seconds. The movement of these particles within the cell can be more clearly seen in the contour plots in panel 2D-E. Multiple regions of within a set of 10-12 cells were imaged during the study, during which nanoparticles were observed to enter into, and be expelled from the cellular membrane. A series of images compiled into a movie shows the dynamic nature of the nanorods within the tumor cells (http://pubs.acs.org/doi/suppl/10.1021/nl504481k/suppl_file/nl504481k_si_004.mov). When the incubation period was increased to 60 minutes, there was a greater internalization of the gold rods.

Conclusions

Due to their small size, glioblastoma cancer cells can be imaged in thin liquid layers using TEM mode rather than STEM. Metal nanoparticles, such as gold nanorods, can be resolved in thin cellular regions, such as the edge of the cell using a 120 kV acceleration voltage. The researchers observed nanoparticles moving within, entering and exiting the cell over a time period of several minutes — an advancement over previous *in situ* studies which were limited to single snapshots of chemically fixed cells [2-3]. This work demonstrates that *in situ* liquid TEM is a powerful method for real-time visualization of the interactions between nanoparticles and their cellular targets and therapeutic efficacy assessments. Tethering non-adherent samples to the surface of the E-chip's SiN membrane reduces the time required to locate and focus on the sample, therefore reducing the cumulative electron dose to which the sample is exposed. Users have employed this strategy to study a diverse range of samples, including cells, viral complexes, and proteins [4,5]. For information about how Poseidon Select can be utilized for your application, please contact Protochips at 919-377-0800 or visit us at protochips.com.

References

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