Visualisation of $Ti_3C_2T_X$ MXenes in eukaryotic cells by Transmission Electron Microscopy

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MXenes is a novel class of 2D nanomaterials, which draw increasing attention in various fields [1]. MXenes were reported to have exceptional photothermal conversion efficiency [2], and that is why they are considered to be promising in biomedicine for treatment of cancer using photothermal therapy approach [3]. Consequently, we investigated interaction of $T_3C_2T_x$ MXenes with eukaryotic cells in cultures. In the preliminary experiments we learned that MXenes become closely associated with the cells. However, we still do not know in which cellular compartment they are located. Here we describe our attempts to study localization of MXenes in Chinese hamster ovary (CHO) cells using transmission electron microscopy (TEM).

We used TEM metal grids with vacuum deposited carbon support films. However, traditionally used Cu grids were too toxic for the cells. We then attempted to grow cells on the carbon film sprayed on the plastic culture dish. We postulated that the pieces of such plastic along with the film with the fixed cells could be dissolved in chloroform, releasing the film with the firmly attached cells. We tried to dissolve the plastic on the interface of chloroform and water, with the idea that the carbon film would float to the water surface, where it could be caught by the grid. However, the dissolved plastic was forming thin layers on top of water and prevented catching the carbon films. We then tried to dissolve the plastic in chloroform only. It was indeed possible to locate the released film in the transmission light and catch it with the grids. However, such approach gave unsatisfactory results due to intensive jamming of the carbon films with the cells.

We then looked for a way to overcome toxicity of the Cu grids. We assessed cell toxicity of the grids made out of nickel and palladium. We found that the Ni grids were also very toxic to the cells. However, the cells tolerated the presence of the Pd grids well enough to grow the cells on them. We then deposited the carbon films on the Pd grids, grew the cells on the grids with already deposited carbon support films, and incubated the cells with MXenes. The cells were fixed with freshly prepared 2.5% glutaraldehyde in PBS, dehydrated in increasing concentrations of ethanol and air dried. The cells on the grids were then double contrasted with uranyl acetate and lead citrate as described [4] with modifications. The cells were then investigated by a transmission electron microscope ПЕМ-125К (JSC Sumy Electron Microscopes, Sumy, Ukraine) at 90 kV voltage and 0.5 μm spot size.

We found that the MXene flakes can indeed be visualized within the cultured CHO cells (Fig. 1). Currently we investigate in which particular cellular compartment the MXenes are located within the cells.

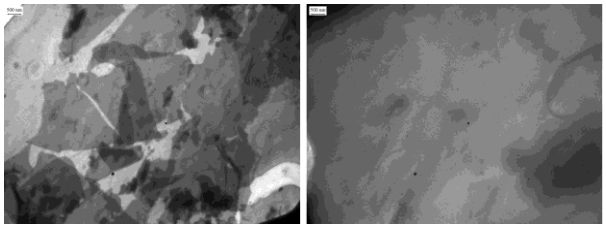

Fig. 1. TEM images of CHO cells with/without MXenes. Scale bar = 500 nm.

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References

[1] B. Anasori and Y. Gogotsi, "2D Metal Carbides and Nitrides (MXenes): Structure, Properties and Applications", Springer International Publishing, 2019, pp. 3–12.
[2] D. Xu, Z. Li, L. Li, and J. Wang, "Insights into the Photothermal Conversion of 2D MXene Nanomaterials: Synthesis, Mechanism, and Applications," Advanced Functional Materials, vol. 30, no. 47. Wiley-VCH Verlag, p. 2000712, Nov. 01, 2020, doi: 10.1002/adfm.202000712.
[3] S. Pan et al., "2D MXene-Integrated 3D-Printing Scaffolds for Augmented Osteosarcoma Phototherapy and Accelerated Tissue Reconstruction," Adv. Sci., vol. 7, no. 2, Jan. 2020, doi: 10.1002/advs.201901511.
[4] L. Graham and J. M. Orenstein, "Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research," Nat. Protoc., vol. 2, no. 10, pp. 2439–2450, Oct. 2007, doi:10.1038/nprot.2007.304.