Possible autocatalytic reduction of resazurin by MXenes with cultured cells

Anton Roshchupkin; Yevheniia Husak
Biomedical Research Center
Medical Institute
Sumy State University
Sumy, Ukraine
rantad123@gmail.com;
evgenia.husak@gmail.com

Illya Yanko; Ekpe Onyinyechi Peace
Biomedical Research Center
Medical Institute
Sumy State University
Sumy, Ukraine
yanko.ilya.brc@gmail.com
onyipeace99@yahoo.com;

Vitalii Balitskyi
Materials Research Center,
Kyiv, Ukraine
vitalik@mrc.org.ua

Daniil Burduli
Ivan Baginskyi
Materials Research Center,
Kyiv, Ukraine
daniil@mrc.org.ua
ibaginskiy@mrc.org.ua

Oleksiy Gogotsi; Veronika Zahorodna
Materials Research Center, Carbon-Ukraine (Y-Carbon ltd.)
Kyiv, Ukraine
alex@mrc.org.ua
veronika@mrc.org.ua

Maksym Pogorielov; Sergiy Kyrylenko
Biomedical Research Center
Medical Institute
Sumy State University
Sumy, Ukraine
m.pogorielov@gmail.com
kyrylenk@gmail.com

MXenes is a novel class of layered planar compounds with potentially wide application in many fields including in biomedicine [1]. The MXenes have been shown to be generally non toxic for living cells [2]. Metabolic assays, both resazurin redution assay and the tetrazolium dye (MTT) redcution assay are often used to measure viability a of cells. We set out to study cytotoxicity of $Ti_3C_2T_x$ MXenes in cultures of the Chinese hamster ovary (CHO) and human mesenchymal stem (hMSC) cells using resazurin reduction assay (RRA). The RRA generally well collerates with the viability of the cells judged by the number and the visual appearance of the cells observed under light microscope. We confirmed that the MXenes were non toxic for the cells in the range of concentrations up to 5 µg/ml. We also found that MXenes become toxic to the cells at excessive concentrations, which could be well explained e.g. by unspecific interaction of MXenes via their sharp edges [3,4] with the cell membranes.

However, we noticed that at the excessive concentrations of MXenes the results of the RRA went into apparent contradiction with the microscopically observed situation with the cells. As was clearly seen under the microscope, the cells become impaired and the number of surviving cells became substantially diminished at the concentrations of MXenes over 50 µg/ml. However, at high concentrations of MXenes the RRA did not show decreasing number of cells. Instead, the RRA readings were paradoxically even higher thant the readings at the lower MXene concenterations.

We analyzed available literature data and found out that the published results with the cell metabolic assays showed a smililar trend [2], where moderate concentrations of MXenes showed initial cytotoxicity, while their increasing concentrations did not result in corresponding readings of the metablic assay. Unlike in our case with the RRA assay, the MTT assay was used in that particular case. However, these two assays are based on similar principles and both reflect the metabolic state of the cell cultures. Therefore, the results of these two assays could be comparable.

MXenes are known to have reductive capacity (Y. Gogotsi, personal communication). We postulated that MXenes are able to reduce resazurin or the tetrazolium dye autocatalytically. This could explain observed discrepancies in visual appearance and the number of cells and the results of metabolic assays. We set up a simple experiment to clarify that claim. MXenes were mixed with resazuring in culture medium and let stand in the cell culture incubator. We did not observe any reduction of resazurin after 24 hr incubation. However, after 5 days incubation we detected clear autocatalytic reduction of resazurin by MXenes.

The degree of the observed autocatalytic effect was not enough to fully explain the apparent discrepancies in the metabolic assays with the cells. We hypothesize that the increased resazurin reduction can be explained the presence of ceratain inctracellular enzymes, which get released into the culture medium after cell death. We currently investigate possible reasons for this effect. Altogether, we suggest that the results of the cell metabolic assays should be interpreted with care due to possible MXene autocatalytic reduction.

Acknowledgements

Supported by the Ministry of Education and Science of Ukraine (grant 0120U101972); H2020 Marie Skłodowska-Curie Actions (NanoSurf 777926, CanBioSe 778157, NANO2DAY 77810, SALSETH 872370); National Research Fund of Ukraine (2020.02/0223); Erasmus+ JM project 599989-EPP-1-2018-1-UA-EPPJMO-MODULE.

References

[1] M. Xin, J. Li, Z. Ma, L. Pan, and Y. Shi, "MXenes and Their Applications in Wearable Sensors," Front. Chem., vol. 8, no. April, pp. 1–14, 2020, doi: 10.3389/fchem.2020.00297.

[2] A. Rozmysłowska-Wojciechowska et al., "A simple, low-cost and green method for controlling the cytotoxicity of MXenes," Mater. Sci. Eng. C, vol. 111, p. 110790, Jun. 2020, doi: 10.1016/j.msec.2020.110790.

[3] K. Rasool, M. Helal, A. Ali, C. E. Ren, Y. Gogotsi, and K. A. Mahmoud, "Antibacterial Activity of Ti 3 C 2 T x MXene," ACS Nano, vol. 10, p. 12, 2016, doi: 10.1021/acsnano.6b00181.

[4] O. Gogotsi, V. Zahorodna, V. Balitskyi, Y. Zozulya, H. Gogotsi, M. Brodnikovskiy, M. Gubynskyi, S.Fedorov, M. Alhabeb, F.Meng, B. Anasori, Y. Gogotsi "Synthesis and Biomedical Applications of 2d Carbides (MXenes)", Abstract Book of 5th International Conference Nanobiophysics: Fundamental and Applied Aspects, pp. 1-7, October 2-5, 2017, Kharkiv, Ukraine.