Introduction

The specific features of two-dimensional (2D) nanomaterials such as their high degree of morphological anisotropy, specific chemical functionalities, and unique surface charges define their biological properties [1]. One of the newest families of 2D material labeled ‘MXenes’ has been extensively studied in this context [2]. Specifically, the name ‘MXene’ reflects the layered stoichiometry of their parent MAX phases i.e. $M_{n+1}AX_n$, where $n = 1, 2,$ and $3, M$ is an early transition metal of the periodic table of

On tuning the cytotoxicity of $\text{Ti}_3\text{C}_2$ (MXene) flakes to cancerous and benign cells by post-delamination surface modifications

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Keywords: MXenes, delaminated $\text{Ti}_3\text{C}_2$, cytotoxicity, reactive oxygen species, mammalian cells, oxidation

Supplementary material for this article is available online

Abstract

Despite intensive research on the application of two-dimensional (2D) materials, including MXenes, in nanomedicine, the knowledge concerning the mechanisms responsible for their observed bio-effects is far from being understood. Here we present insight into the mechanism of toxicity in vitro of the 2D $\text{Ti}_3\text{C}_2$ MXene. The most important results of this work are that using simple, inexpensive, post-delamination treatments, such as ultrasonication or mild thermal oxidation it is possible to ‘tune’ the cytotoxicity of the $\text{Ti}_3\text{C}_2\text{Ti}_2$ flakes. Sonication of $\text{Ti}_3\text{C}_2\text{Ti}_2$ flakes, or sonication followed by mild oxidation in the water at 60 $^\circ$C, renders them selectively toxic to cancer cells as compared to non-malignant ones. It relates to the appearance of superficial titanium (III) oxide ($\text{Ti}_2\text{O}_3$) layer corresponding to the type of post-treatment. The presence of surface-$\text{Ti}_2\text{O}_3$ results in a noticeably higher generation of oxidative stress compared to pristine 2D $\text{Ti}_3\text{C}_2$. Our findings give evidence that the sonication and thermal treatments were successful in changing the nature of the surface terminations on the $\text{Ti}_3\text{C}_2\text{Ti}_2$ surfaces. This study makes a significant contribution to the future rationalized surface-management of 2D $\text{Ti}_3\text{C}_2$ MXene as well as encourages new rationalized applications in biotechnology and nanomedicine.

Bullet points:

1. First study on 2D $\text{Ti}_3\text{C}_2$ MXene superficially oxidized to titanium (III) oxide i.e. $\text{Ti}_2\text{O}_3$.
2. By sonication $\text{Ti}_3\text{C}_2\text{Ti}_2$ MXene flakes followed by mild thermal oxidation in the water at 60 $^\circ$C for 24 h, it is possible to ‘tune’ the toxicity of the flakes to cancerous cell lines.
3. Decreases in cell viabilities were dose-dependent.
4. Highest cytotoxic effect was observed for thermally oxidized samples.
5. The thermally oxidized samples were also selectively toxic towards all cancerous cell lines up to 375 mg l$^{-1}$.
6. Reactive oxygen species generation was identified as a mechanism of toxicity.

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results have revealed that toxicity towards bacteria cells hand, when dimethyl sulfoxide (DMSO) is used in the ments [7, 8], as well as recognition of some theranostic medical [10, 11] and other biological-related [12] applications of Ti3C2 MXene, substantial knowledge of the above mentioned and other intensive studies on the specific bio-effects related to its pristine and/or oxidized surfaces is still far from being satisfactory. The specific bio-action of MXenes [7] has boosted intensive research on their application in nanomedicine, which brought some interesting findings such as the specific selectivity of 2D sheets of Ti3C2 towards cancer cells [5], possibility of photo-thermal treatments [7, 8], as well as recognition of some theranostic [9] and synergistic [10] features. However, despite all the above mentioned and other intensive studies on the medical [10, 11] and other biological-related [12–14] applications of Ti3C2 MXene, substantial knowledge of the mechanisms that are responsible for the observed specific bio-effects related to its pristine and/or oxidized surfaces is still far from being satisfactory.

In our previous studies, we made the case that different levels of reactive oxygen species, ROS, indicated a potential mechanism of higher toxicity against cancerous cells versus normal ones [5, 8]. On the other hand, when dimethyl sulfoxide (DMSO) is used in the process, sulfur, S, appears on MXene surface [5]. Other results have revealed that toxicity towards bacteria cells depends on MXene stoichiometry [15], the lateral size of flakes, as well as exposure time [16, 17].

Altogether, there are strong indications that Ti3C2- based MXenes are promising candidates for applications in various biotechnological areas and nanomedicine [5–11]. It is already known that even tiny surface modifications of MXenes surfaces can influence their in vitro and in vivo performance, toxicity, colloidal stability, and circulation within the body [2, 18]. It is however still not clear how their pristine surfaces affect those properties. Also, the presence of TiO2 on the surface of MXenes was recently investigated but only in the context of oxidation stability [19] or resulting photocatalytic properties [20].

Despite all these studies, there is still a lack of comprehensive interdisciplinary studies elucidating the effects of various surface oxidation terminations/layers and its possible origin on the potential cytotoxicity of 2D MXenes. Our hypothesis herein is that particular oxidative post-treatments may play a crucial role in their bio-activity due to the appearance of surface oxides. The main aim of this study was to compare the cytotoxicity of as-received 2D Ti3C2Tz sheets to those that were probe sonicated and those that were first probe sonicated and then mildly oxidized by heating in the water at 60 °C for 24 h. It is important to note that the sonication was followed by thermal treatment to ensure that the lateral sizes of the flakes remained unchanged and thus not a factor in comparing these two sets of samples.

Materials and methods

Synthesis
The formation of MXenes involved etching out aluminum, Al, from the MAX phase, Ti3AlC2, according to the protocol of Ghidiu, et al [21]. The Ti3AlC2 powders were synthesized by mixing titanium carbide, TiC, (Alfa Aesar, 99.5%, 2 µm), aluminum, Al, (Alfa Aesar, 99.5%, 325 mesh), and titanium, Ti (Alfa Aesar, 99.5%, 325 mesh) powders in a molar ratio of 2:1:05:1, respectively. The mixed powders were ball milled at 100 rpm for 24 h and then heated under argon, A, flow at 1350 °C for 2 h. The heating and cooling rates were set at 5 °C min−1. The resulting loosely sintered blocks were ground using a milling bit on a drill press. The milled powders were passed through a 400 mesh (particle size <38 µm) sieve for further experiments.

To convert these powers to MXene, 1 g of LiF is first dissolved in 10 ml of 12 M HCl. Next, 1 g of the Ti3AlC2 powder was slowly added to the solution which was stirred for 24 h at 35 °C at 300 rpm. Subsequently, the solution containing multilayered, ML, MXenes at this stage, was transferred into a centrifuge tube to which 30 ml deionized, DI, water was added. After centrifugation at 3500 rpm for 10 min, the supernatant was discarded, and the washing process was repeated several times until the pH of the solution was near neutral. Then, the solution was sonicated under argon, Ar, flow for 1 h in a bath sonicator. To avoid oxidation, the temperature of the bath was kept below 20 °C using ice. Finally, the solution was centrifuged for 1 h at 5000 rpm, and the supernatant containing MXene flakes (8.5 mg ml−1) was collected and stored under Ar until further use.

The stock dispersion of 2D Ti3C2 MXene sheets was post-treated in two ways. In the first, probe-sonication (Vibra Cell VCX 750, Sonics & Materials Inc., 750 Watt, 20 KHz) in DI water with no Ar shielding, for 5 s, in a periodic mode (1 s work per 1 s break) at a low energy of 300 W (50% of its initial power) to mildly oxidize the 2D Ti3C2 flakes. In the second step, the sonicated flakes were heated in an open container of DI water to 60 °C for 24 h. The as-received, sonicated and thermally oxidized samples will henceforth be referred to as, AR-MX, SO-MX, and TO-MX, respectively (figure 1).

Characterization of the morphology, structure and chemical composition of the surface
The morphologies of the Ti3AlC2 MAX phase and the AR-, SO- and TO-MX flakes were examined using a scanning electron microscope, SEM (LEO 1530, Zeiss), at an accelerating voltage of 2.0 kV. Water dispersions of the samples were first lyophilized using a lyophilizer (ALPHA 2–4 LD plus Christ Lyophiliser).
The resulting powders were directly deposited onto the surface of conductive C-tape and surface-coated with a thin carbon layer prior to SEM analysis. The 2D sheets were additionally examined using a transmission electron microscope, TEM, (Philips CM 20). The layered structure on the cross-section of the individual representative 2D sheets was revealed by high-resolution, HRTEM. Images were taken in dark field mode, as well as, high-angle annular dark-field imaging (HAADF).

Optical absorption spectra of the 2D sheets of the AR-, SO- and TO-MX flakes were analyzed in the range of 220–1100 nm using a double beam scanning UV–VIS spectrometer (Evolution 220, Thermo Scientific) equipped with an integrating sphere. The measurement parameters were: integration time of 0.3 s; a resolution of 1 nm; and a scanning speed of 200 nm min⁻¹.

Fourier-transform infrared (FTIR) spectroscopy (FT-IR Nicolet iS5 from Thermo Electron), equipped with a diffuse reflectance infrared Fourier-transform (DRIFT) accessory was used to analyze the presence of different chemical species on the surfaces of the AR-, SO- and TO-MX flakes. For the DRIFT measurements, each sample was prepared by careful abrasion in an agate mortar containing 80 mg of dry KBr (Fluka, assay ≥ 99.5%) with the specimen (2 mg) to obtain samples with concentrations ca. 2.5 wt.% in KBr. The samples were then placed in a DRIFT cuvette. Pure KBr was used as a reference for the baseline correction. For all measurements, the spectral resolution was set to 2 cm⁻¹, and each spectrum reported in the average of 30 scans. Vibration modes were collected in the mid-infrared spectral region (with a wave numbers range of 4000–400 cm⁻¹). For data analysis, OMNIC, Thermo Fisher computer program was used.

X-ray photoelectron spectroscopic (XPS) measurements were carried out using a PHI 5000 VersaProbe (ULVAC-PHI) spectrometer with monochromatic Al Kα radiation (hv = 1486.6 eV). The x-ray source operated with a spot size of 100 μm, 25 W and 15 kV. The high-resolution (HR) XPS spectra were collected with a hemispherical analyzer at the pass energy of 117.4 eV and an energy step size of 0.1 eV. The x-ray beam was incident at the sample surface at an angle of 45° with respect to the surface normal, and the analyzer axis was located at 45° with respect to the surface.

Zeta potential analysis as a function of temperature was performed using a NANO ZS ZEN3500 analyzer equipped with a green laser and back-scattered light detector operating at an angle of 173°. The concentration of the tested samples was 60 μg cm⁻³ in distilled water of pH = 7. The temperature-dependence curve was obtained in the 25 °C–60 °C temperature range in steps of 5 °C. Each measurement was repeated 20 times, and the results are expressed as the mean value of the zeta potentials.

**Theoretical calculations**

Calculations were performed within the spin-polarized density functional theory (DFT) [22, 23], as implemented in the Vienna ab initio simulation package (VASP) code [24]. The electron-ion
interaction was modeled using projector augmented wave pseudopotentials (PAW) [25, 26] within the Perdew–Burke–Ernzerhof (PBE) approach to an exchange-correlation function [27]. The effect of Van der Waals forces is included for valence electrons in the exchange-correlation function vdW-DF2 [28]. A plane wave basis set was limited by a kinetic energy cutoff of 400 eV. The Brillouin Zone integration of a $k$-point mesh of $12 \times 12 \times 2$ in the Monkhorst–Pack sampling scheme [29] was used for the $(2 \times 2)$ supercell. The convergence threshold for the energy was set to $10^{-5}$, and the structures were relaxed until the force on each atom was less than 0.01 eV Å$^{-1}$ in all calculations.

Analysis of in vitro cytotoxicity

The cytotoxicity assessed in vitro against human epithelial breast (MCF-10A, American Type Culture Collection, ATCC), breast cancer (MCF-7, ATCC), human immortalized keratinocytes (HaCaT, ThermoFisher), and human malignant melanoma (A375, ATCC) cell lines by measuring the cells’ viability after their exposure to increasing concentrations (0–500 mg l$^{-1}$) of the AR-, SO- and TO-MX samples. The details of the cytotoxicity tests can be found in supplementary information (stacks.iop.org/TDM/7/025018/mmedia).

Results

Typical SEM micrographs of the Ti$_3$C$_2$ layers (figure 2(a)) show them to have non-regular shapes and clearly possess a 2D morphology. The STEM-HAADF image (figure 2(b)) shows in detail the curled morphology of the 2D sheets and lateral dimensions of c.a. several micrometers. The HRTEM, shown in figure 2(c) shows two Ti$_3$C$_2$T$_x$ layers viewed in cross-section. Typical SEM images of the SO- and TO-MX samples presented in figures 2(d) and (e), respectively, show that the two methods resulted in surface oxidation characterized by different morphologies. On the surface of the SO-MX individual nanoparticles of larger sizes can be noticed, whereas the surface of TO-MX is covered by many small nanoparticles.

When the UV–VIS absorbance spectra of the AR-, SO- and TO-MX samples (figure 3(a)) are compared, it is obvious that a much higher absorption peak, near 274 nm, is present for the TO-MXene sample. The AR- and SO-MX samples, on the other hand, are almost identical. Given that anatase absorbs light mainly in the near-UV and UV regions [30], we assume that this absorbance peak is due to a TiO$_2$ and/or Ti$_2$O$_3$ [31]. When the FTIR spectra of the AR-, SO- and TO-MX samples (figure 3(b)) are compared, they appear similar to each other. The absorbance of the TO-MX samples was the highest, while the other two samples were more comparable. The intensities of the OH peaks at 3117 and 1400 cm$^{-1}$ and the Ti–O peak at 703 cm$^{-1}$ were also highest for the TO-MX sample. This increase is presumably due to a higher degree of oxidation in the latter compared to the other two.

The high-resolution XPS spectra in the Ti 2p region, for the AR-, SO- and TO-MX samples are shown in figures 4(a), (c) and (e), respectively. The spectra were deconvoluted assuming three peaks: one at $\approx 455.5$ eV for the C–Ti–O bonds, i.e. that of the O terminated MXene, one at $\approx 456.6$ eV for Ti$^{2+}$ and one
at \( \approx 460 \text{ eV} \) for Ti\(^{4+}\). The results of the de-convolution are listed in table 1. A perusal of these results indicates that, not surprisingly, the TO-MX sample is the most oxidized, followed by the SO one; the least oxidized is the AR-MX sample. Somewhat more surprisingly, however, and for reasons that are not entirely clear the majority Ti oxidation state present at the surfaces of the SO- and TO-MX samples is not Ti\(^{4+}\), but Ti\(^{3+}\). It is important to note here that relatively mild oxidative pre-treatments carried out here apparently were not harsh enough to push the Ti oxidation states to 4\(+\). Why the Ti\(^{4+}\) peak is highest for the AR-MX sample is unclear at this time, and more work is needed to understand why. We note in passing that at \(<20\%\), the Ti\(^{4+}\) fraction is small. The high-resolution XPS spectra in the O 2p region for the AR-, SO- and TO-MX samples are shown in figures 4(b), (d) and (f), respectively. For the AR-MX samples, the major peak is at \( \approx 531.0 \text{ eV} \) that is due to O bonded to a Ti atom on the surface of the MXene block [32]. For the other two samples, the major peak occurs is at \( \approx 532.8 \text{ eV} \). This peak has been attributed to O atoms bonded to a Ti and H atom, viz. Ti-OH or hydrocarbons [32]. In our previous work, we assigned this peak to C=O bonds [33]. At this time, we cannot unambiguously assign this peak to either moiety. Given the FTIR results (figure 3(b)) it is tempting to assign this peak to Ti-OH. The fact that only the TO-MX sample shows large OH vibrations makes such a conclusion valid only for that sample. The detailed XPS data for all three samples are presented in supplementary information.

The evidence presented so far suggests that the mild oxidation incurred during the sonication and subsequent oxidation treatment, oxidize the MXene surfaces. To further confirm this important conclusion, we measured the zeta potentials of all powders as a function of temperature. The results (figure 5) clearly indicate that the zeta potentials of the AR-MX sample are quite different than those of the SO and TO samples that appear to be more similar to each other. These results confirm that the sonication and thermal treatments were successful in changing the nature of the surface terminations on the Ti\(_3\)C\(_2\)T\(_2\) surfaces.

Some previous experimental studies also suggested that the oxidation of Ti\(_3\)C\(_2\) sheets surface with TiO\(_2\) influence their stability [34]. The formation of anatase TiO\(_2\) phase occurs for flakes regardless of their thickness, and thus, independently of the number of layers constituting the flakes. It has been experimentally shown that the formation of the anatase phase starts at the surface and proceeds to encapsulate the entire structure [34]. To investigate this problem further, in particular, to shed light on the microscopic quantum mechanical mechanisms of Ti\(_4\)O\(_3\) phase formation,
and to judge the stability of the Ti$_3$C$_2$ sheets covered with Ti$_3$O$_4$, we carried out first principle calculations in the framework of the DFT on the basis of the theoretical and experimental studies demonstrating that the surface of 2D Ti$_3$C$_2$ sheets is mostly terminated by oxygen groups [35–39]. To model the earliest stages of formation of Ti$_3$O$_4$ on the surface of Ti$_3$C$_2$ MXene we focus on the formation of Ti$_3$C$_2$(O$_2$Ti)$_n$-O structure whereas Ti$_3$C$_2$ is the pristine surface of the MXene; (O$_2$Ti)$_n$ is the early-stage surface oxidation layer; and –O is the upward oxygen willing to form a bond with the model surface i.e. formation of Ti–O bond. This process is modeled employing the supercell geometry with the surface represented by a slab consisting of two layers (2L) of Ti$_3$C$_2$O$_2$ (see figure 6(a)).

We examine three different oxygen adsorption sites to the Ti$_3$C$_2$(O$_2$Ti)$_n$ surface, namely, the above Ti atom from the oxidized layer, Ti atom from Ti$_3$C$_2$, and O atom from the oxidized surface. These sites are shown schematically in figure 6(b) and denoted by numbers 1, 2, 3, respectively. For the details of the case 4 and the theoretical calculations see the supplementary information. Results indicate that titanium sites are more energetically preferable for O adsorption than the oxygen or carbon sites, even for the case, where Ti atom is deeper to the surface (site 2). Due to the fact, that the Ti–O bonds are the building blocks of the Ti$_x$O$_y$ structure, we conclude that the formation of the Ti$_3$O$_4$ layer at the surface of 2D Ti$_3$C$_2$ structure is plausible and it is beneficial for increasing the stability of the MXene.

In the remainder of this section, we report on the most important results of this work, viz. the cytotoxicity results. As noted above, the cytotoxicity was assessed in vitro using human epithelial breast (MCF-10A),

Figure 4. XPS spectra resolved in the range of binding energies corresponding to O1s and Ti 2p groups of signals obtained for (a) and (b) AR- (c) and (d) SO-, and (e) and (f) TO-MX samples.
A perusal of these results indicates that:

(i) All three MXene compositions, AR- (figure 7(a)), SO- (figure 7(b)) and TO-MX (figure 7(c)) are toxic to all cell lines. However, the malignant cells are more severely attacked. The normal cells fare better even at the highest MXene concentrations. It should be stressed that the dose of the tested material can be described as non-toxic if the cells’ viability after 24 h of exposure is above 70% [40].

(ii) The toxicities are not equal, however. Comparing figures 6(a)–(c) it is clear that, with one notable exception (see below), the TO-MX samples are the most lethal, followed by the SO- samples and lastly, the AR-MX samples. The exception is that the AR-MX flakes, at a concentration of 375 mg l$^{-1}$, and higher, are particularly toxic to MCF-7 cells (see figures 7(a) and (d)), while being relatively benign to the MCF-10 cells.

(iii) The higher the MXene concentrations the more toxic they are.

(iv) At concentrations of 125 mg l$^{-1}$, the SO-MX and TO-MX are lethal to A375, while being relatively benign to HaCaT cells.

**Discussion**

The obtained materials were characterized using SEM, TEM, UV–VIS, FTIR, XPS, as well as in vitro biological assays. Detailed characterization of the oxidized samples indicated the presence of a surface-oxidation layer composed mostly of titanium(III) oxide, i.e. Ti$_2$O$_3$, i.e. the majority Ti oxidation state...
present at the surfaces of the SO- and TO-MX samples is not Ti$^{4+}$, but Ti$^{3+}$. It is important to note here that relatively mild oxidation pre-treatments carried out here apparently were not harsh enough to push the Ti oxidation states to 4+. Theoretical first principle studies revealed that the oxidization process to Ti$_2$O$_3$ leads to the formation of the stable covalent bonds of Ti–O at the Ti$_3$C$_2$ surface. The Ti–O bonds are the building blocks of the Ti$_2$O$_3$ structure, which can be regarded as naturally forming at the Ti$_3$C$_2$ surface.

Clearly, sonication of the Ti$_3$C$_2$T$_x$ flakes, or sonication followed by mild oxidation in water at 60 °C, renders them selectively toxic to cancer cells as compared to non-malignant ones. Based on the results shown in figure 7 the TO-MX flakes at a concentration of 62.5 mg l$^{-1}$ are lethal to A375, but not to HaCaT. We also noticed that there are two toxicological cases i.e. (1) malignant cells’ viability is reduced and associated with low ROS level as well as, (2) high ROS generation concomitant with low viability loss for normal cells. Toxicity effects related to malignant cells are closely related to probable physicochemical interactions with large flakes. This effect is visible especially for MCF-7 cells in figure 7(d). On the other hand, high ROS generation with low viability loss and short incubation times indicate that if the interaction time with the AR-MX flakes would be prolonged, then the viability of cells would be further reduced due to the oxidative stress.

The probable cause of the observed effect might be the presence of Ti$_2$O$_3$ on the surface of the 2D flakes. It should be noted that the vast majority of the available data corresponds to TiO$_2$ in the crystalline phase of anatase which is one of the most commonly used materials and has thus been the subject of significant research regarding toxicity and safety. Ren and coworkers [41] have reported the cytotoxicity of pure nano-TiO$_2$ anatase towards human immortalized keratinocytes. The viability of cells showed a significant decrease in a concentration-dependent manner. Zeng et al [42] confirmed that nano-TiO$_2$ could pose significant cytotoxicity to human umbilical vein endothelial cell cultures, even at the lowest tested concentration (1 mg l$^{-1}$). Some data available on nanosized Ti$_2$O$_3$ however, suggest that it is biocompatible [43].

As can also be observed in the SEM images (see insets in figures 2(d) and (e)), the sizes of the surface Ti$_3$O$_3$ particles present on the 2D Ti$_3$C$_2$ flakes are also in nanoscale. Additionally, significant differences
A M Jastrzębska et al. can be noticed between the surfaces of the SO- and TO-MX samples. The XPS results reveal that TO-MX has also a larger amount of Ti$_2$O$_3$ on its surface which can be directly related to the higher toxicity of this material.

It should be additionally noted that the observed differences in toxicity were not associated with flakes aggregation in the in vitro nutritive media. By using dynamic light scattering (DLS) and zeta potential studies (see supplementary information, figure S5, table S4, respectively) we confirmed the high stabilities of the tested materials and that there was no changes in their stabilities. These results additionally support the influence of titanium oxides on the toxicity of the MXene flakes.

It should be also noted that there is a meaningful set of in vitro data available for surface-modified MXenes (table 2) [2, 18] (also see supplementary information, table S5). It is however still not clear how MXenes’ pristine surfaces affect their basic biological properties. Also, the presence of TiO$_2$ on the surface of MXenes was recently investigated but only in the context of oxidation stability [19] or resulting photocatalytic properties [20]. The available toxicological data for the pristine surface of Ti$_3$C$_2$T$_x$ was compiled and are shown in table 2 in the context of the results obtained here. From these results it is clear that TO-MX sample is toxic to a strong majority of the analyzed cells. The multilayered Ti$_3$C$_2$T$_x$ structure was toxic towards two cell lines in comparison to AR-MX (only one cell line). However, many differences can be noticed between those two MXenes including not only their surface chemistries defined by XPS but also their structures. The surfaces of the multilayered Ti$_3$C$_2$T$_x$ were also covered with a small amount of oxide layer due to harsh delamination method used here [5]. These results additionally...
Table 2. Comparison of the in vitro toxicological data for 2D Ti₃C₂Tx.

| MXene Structure  | Delamination method | Etching method | Delamination agent | Elements detected by XPS | Type of TiₓOᵧ on the surface (on basis of XPS) | Post-treatment | Investigated cell cultures | Toxic (viability loss below 70% with MTT test at a concentration of 205 mg L⁻¹) | ROS level above 120% at a concentration of 250 mg L⁻¹ | Ref. |
|------------------|---------------------|----------------|--------------------|--------------------------|----------------------------------|---------------|-----------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------|
| Ti₃C₂Tx          | Multilayered        | HF etching + probe sonication | DMSO               | Ti, C, O, F, Al, S       | TiO₂ (a significant amount)      | No            | MRC-5                       | No                                                                            | —                                                                            | [5]  |
| Ti₃C₂Tₓ          | N.A.                |                |                    |                          | TiO₂ (traces)                    | No            | MCF-10A                     | —                                                                            | —                                                                            |      |
| Ti₃C₂Tₓ          | SO-MX               | Few-layered    | LiF/HCl etching + sonication | Ti, C, O, F, Cl, Si, Li  | Ti₂O₃ (a significant amount)      | Sonication in water | MCF-10A - Ti₂O₃ (a significant amount) | —                                                                            | —                                                                            |      |
| Ti₃C₂Tₓ          | TO-MX               |                |                    |                          | Ti₂O₃ (a significant amount)      | Thermal treatment in water       | MCF-10A                     | —                                                                            | —                                                                            |      |
confirm our assumption that the presence of Ti$_2$O$_3$ on the MXene surfaces results in higher toxicity towards malignant cells in comparison to only TiO$_2$.

**Conclusions**

The biological activity of the new family of two-dimensional (2D) materials, called ‘MXenes’ has been studied extensively over the past several years. Despite this effort on the application of 2D Ti$_3$C$_2$ MXene in nanomedicine, there is still a lack of substantial knowledge on the mechanisms responsible for the observed bio-effects. The aim of this study was to shed light on the effect of the surface oxidation layers and/or nanoparticles, on the surface of 2D Ti$_3$C$_2$ MXene sheets on their cytotoxicity. The Ti$_3$C$_2$ MXene sheets were obtained using a LiF/HCl delamination method and were probe-sonicated (SO-Ti$_3$C$_2$) and subsequently heated (TO-Ti$_3$C$_2$) in water to oxidize their surfaces. Our findings give evidence that the sonication and thermal treatments were successful in changing the nature of the surface terminations on the Ti$_3$C$_2$T$_z$ surfaces. Specifically, in this work, we show for the first time that:

1. Sonicating Ti$_3$C$_2$ in an aqueous suspension open to the air for tens of seconds increases the fraction of Ti in the 3+ state. We further show that if these sonicated flakes are then heated in water at 60 °C for 24 h the fraction of Ti in the 3+ state increases further. These results are important in their right since they are the first example of using simple, inexpensive, scalable, post-delamination processes to increase the fraction of Ti in the 3+ state. Note this oxidation state in important in many biomedical and catalytic functions.
2. These simple, post-delamination processes have a dramatic effect on the toxicity of the Ti$_3$C$_2$ flakes to cancerous and non-cancerous cell lines. In essence, we can now ‘tune’ the Ti$_3$C$_2$ flakes to kill cancerous cells and spare non-cancerous ones.
3. Reactive oxygen species generation is one mechanism of toxicity.

The results of the present study provide key knowledge regarding the probable Ti$_2$O$_3$-related mechanism of biological activity of the 2D Ti$_3$C$_2$ MXene sheets, as well as the role of Ti$_2$O$_3$ in the stabilization of the material. It can be concluded that by sonicating Ti$_3$C$_2$T$_z$ MXene flakes followed by mild thermal oxidation in water at 60 °C for 24 h, it is possible to ‘tune’ the toxicity of the flakes to cancerous cell lines. Decreases in cell viabilities were dose-dependent and the highest cytotoxic effect was observed for thermally oxidized samples. The thermally oxidized samples were also selectively toxic towards all cancerous cell lines at concentrations of ~375 mg l$^{-1}$ or higher. The presence of surface-Ti$_2$O$_3$ also resulted in a noticeably higher generation of reactive oxygen species compared to the pristine 2D Ti$_3$C$_2$. It was also recognized as a key factor that induces oxidative stress in analyzed cells which inescapably leads to the reduction of their viability.

**Acknowledgments**

The study was accomplished thanks to the funds allotted the National Science Centre, within the framework of the research project ‘SONATA BIS 7’ no. UMO-2017/26/E/ST8/01073 (in the frame of materials characterization, mathematical modeling, and biological studies) as well as project ‘SONATA 7’ no. UMO-2014/13/D/ST5/02824 (in the frame of synthesis of starting material i.e. pristine 2D sheets of Ti$_3$C$_2$ MXene using LiF/HCl method). MB is funded by the National Science Centre grant no. UMO-2016/23/D/ST3/03446. Access to computing facilities of PL-Grid Polish Infrastructure for Supporting Computational Science in the European Research Space and of the Interdisciplinary Center of Modeling (ICM), University of Warsaw is gratefully acknowledged. MWB was funded by National Science Foundation, (DMR 1740795).

**Competing interests**

The authors declare no competing interests.

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