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Cytotoxicity of Group 5 Transition Metal Ditellurides (MTe_2; M=V, Nb, Ta)

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Abstract: Much research effort has been put in to study layered compounds with transition metal dichalcogenides (TMDs) being one of the most studied compounds. Due to their extraordinary properties such as excellent electrochemical properties, tuneable band gaps and low shear resistance due to weak van der Waals interactions between layers, TMDs have been found to have a wide application such as electrocatalyst for hydrogen evolution reactions, supercapacitors, biosensors, field-effect transistors (FETs), photovoltaic and lubricant additives. In very recent years, Group 5 transition metal ditellurides have received immense amount of research attention. However to date, little has been known of the potential toxicities posed by these materials. As such, we conducted the cytotoxicity study by incubating various concentrations of the Group 5 transition metal ditellurides (MTe_2; VTe_2, $NbTe_2$, $TaTe_2$) with human lung carcinoma epithelial A549 cells for 24 hours and the remaining cell viabilities after treatment was measured. Our findings indicate that VTe_2 is highly toxic while $NbTe_2$ and $TaTe_2$ are deemed to exhibit mild toxicities. This study constitutes an exemplary first step towards the understanding of the Group 5 transition metal ditellurides' toxicity effects in preparation for their possible commercialization in the future.

Introduction

Ever since the first isolation of graphene from graphite by Novoselov et al. in 2004,[1] two-dimensional (2D) nanomaterials have gained intensive academic and industrial research interest due to their extraordinary and unique properties. To date, a vast range of 2D nanomaterials have been reported, such examples include transition metal dichalcogenides (TMDs), graphitic carbon nitride (g-C_3N_4), black phosphorus and hexagonal boron nitride (h-BN).[2-8] Among 2D nanomaterials, TMDs are deemed to be one of the most studied layered compounds due to their extraordinary electrochemical properties.[9-12]

TMDs have a general chemical formula of MX_2, whereby M represents a transition metal (for instance Ti, V, Nb, Ta, Mo, W and so on) of a +4 oxidation state and X is a chalcogen (S, Se or Te) with a -2 oxidation state.[9,13,14] Different permutations of these elements give rise to approximately 60 different TMDs, where two-thirds of these compounds are reported to assume layered structures. Generally, transition metals from Group 4–7 generate compounds that are predominantly layered while some Group 8–10 transition metals give rise to three-dimensional crystal compounds.[14-16] Many TMDs possess a layered structure akin to graphite and within one layer of TMDs, the transition metal is sandwiched between two chalcogen, hence resulting in the MX_2 stoichiometry. It has been reported that the bonds within each layer are covalent, whereas the bonds between two different MX_2 layers are typically held by van der Waals forces of interactions, thus allowing exfoliation of TMDs down to single layers.[9,13-15,17-19]

The unique and advantageous properties of TMDs such as large surface area, tunable band gaps, stability against photocorrosion and low shear resistance due to weak van der Waals interactions between layers have attracted much research attention and led to numerous and diversified applications of TMDs.[13,14,20,21] Such applications include electrocatalytic hydrogen evolution, high performance electrochemical supercapacitor, biosensors, field-effect transistors (FETs), photodetectors, heterostructure junctions, photovoltaics and lubricant additives.[5,9,13,14,17-32] In recent years, enormous efforts have been devoted to explore the different applications of Group 5 transition metal ditellurides with VTe_2 being deemed to be a promising HER electrocatalyst,[33] $NbTe_2$ being reported to be an excellent lubricant additive[20] and $TaTe_2$ having potential applications as a supercapacitor.[34] However to date, little has been known of the potential toxicities posed by Group 5 transition metal ditellurides. There is still much to address for the understanding of the toxicological behaviour of TMD materials especially for bulk Group 5 ditelluride TMDs, which, to our knowledge has been undetermined. This gap in literature has to be filled in order to inform users of the potential health implications posed in view of their possible implementation in industries.

As such, we conducted the cytotoxicity study of the bulk Group 5 transition metal ditellurides (MTe_2; VTe_2, $NbTe_2$, $TaTe_2$) with A549 human lung carcinoma epithelial cells. The cell line was specifically chosen as the lungs is one of the main potential target organ during the processing of nanomaterials[10,35-39] and it is one of the most used cell types in inhalation toxicity studies allowing easy comparison between our results obtained with other reports.[10,35] The remaining cell viability of the A549 cells after 24 h incubation with various MTe_2 concentrations was measured and analyzed using the water-soluble tetrazolium salt (WST-8) assay. Furthermore, control experiments were also conducted in the absence of cells to check for possible sources of particle-induced interferences between MTe_2 with cell viability

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assay markers which could cause false positive or false negative results to be produced. [39-41]

Results and Discussion

Material characterisation

It has been reported that physicochemical properties such as size and chemical compositions could contribute to the nanomaterials' toxicities.[42 - 45] Hence prior to assessing the cytotoxicity of the Group 5 transition metal ditellurides, characterization of the materials were necessary and carried out through scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and dynamic light scattering (DLS).

As seen in Figure 1, through the SEM and STEM images it is deduced that all samples (VTe_2, $NbTe_2$ and $TaTe_2$) display structural features typical of bulk materials. VTe_2 and $TaTe_2$ are predominantly seen to be layered structures while $NbTe_2$ manifest in irregular shapes. Furthermore, the STEM images reveal a wide range of particle sizes ranging from 80 nm to over 3 µm in lateral dimension.

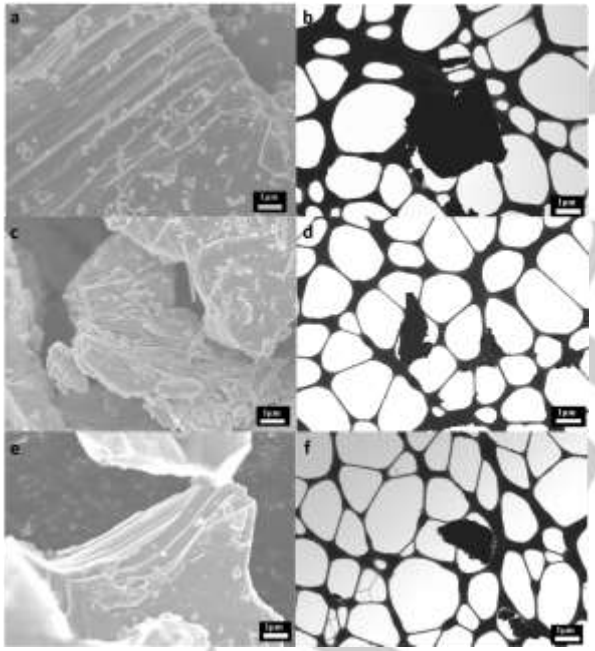

Figure 1. SEM (a,c,e) and STEM (b,d,f) images of VTe_2, $NbTe_2$, $TaTe_2$ respectively. The scale bars represent 1 µm.

Another characterization technique used was the EDX, which provides an elemental mapping of the elements present and their distribution throughout the material. The SEM-EDX images in Figure 2 suggest that the Group 5 transition metals and telluride are well distributed in the materials and that the primary difference present is the transition metal element.

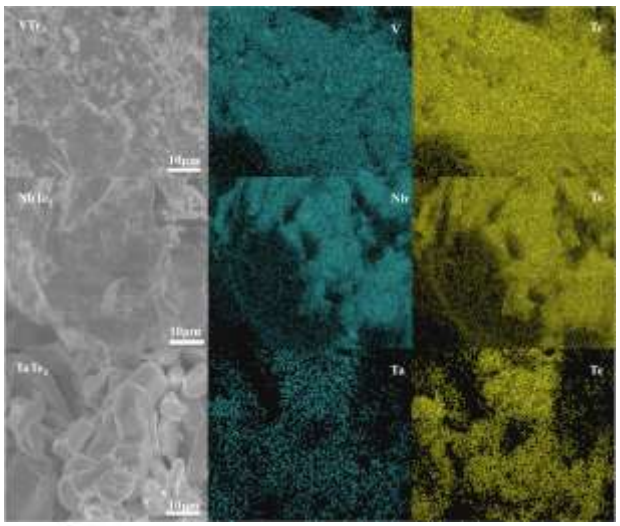

Figure 2. SEM-EDX images of bulk Group 5 ditellurides (VTe_2, $NbTe_2$ and $TaTe_2$). The scale bars represent 10 µm.

XRD data (see Supporting Information, Figure SI-1) shows single phase purity for all ditellurides with a strong preferential orientation along (00l) direction, which originates from the layered character of the materials. It was also determined that all phases are monoclinic structures with C2/m space group. The particle size distribution measurement performed by DLS (see Supporting Information, Fig SI-2) shows that particles from all three materials exhibit multimodal character which corresponds to the wide range of sizes determined from STEM. However, the DLS size ranges differ from the STEM measurements, where DLS data shows a dominant distribution peak with sizes in the range of 500–1000 nm and two smaller fractions with size around 100 and 200 nm. This narrower size range from DLS measurements could be attributed to the faster settling of the larger particles.

Cytotoxicity Assessment

Upon the successful characterization of the materials, we proceeded to determine the toxicity effect of these Group 5 transition metal ditellurides towards A549 cells. To study the materials' toxicity, A549 cells were exposed to the test materials and the remaining cell viabilities were measured using WST-8 assay. WST-8, a mitochondrial activity-based assay, works on the principle that soluble formazan is produce due to cellular reduction by dehydrogenase activities in viable cells. [10,46-48] By normalising the absorbance intensities obtained with a control setup, where cells are not treated with any nanomaterials, the extent of toxicity exhibited by the Group 5 ditellurides TMDs towards A549 cells could be determined. Data collected from the WST-8 assay is illustrated in Figure 3.

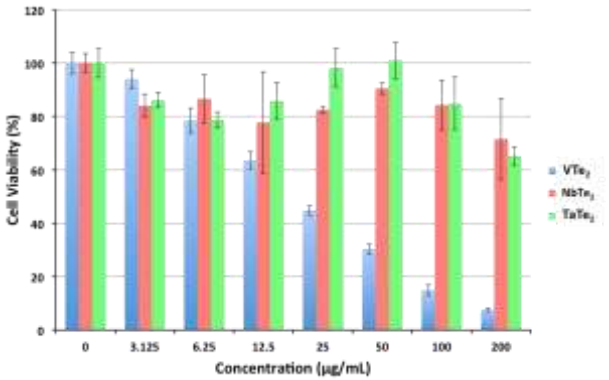

Figure 3. Cell viability percentages measured using WST-8 assay upon 24h incubation of different concentrations of Group 5 transition metal ditellurides with A549 cells.

As depicted in the data above, a dose-dependent trend for VTe_2 was observed. Furthermore, VTe_2 exhibited significant cytotoxicity towards A549 cells, whereby even at low concentrations of 25μg/mL more than 50% of the cells were not viable and at the highest concentration of 200 μg/mL less than 10% of the cells remained viable. This result is in line with previous toxicity studies conducted on exfoliated VTe_2, which demonstrated that VTe_2 was highly toxic.[10] On the other hand, the percentage cell viability for $NbTe_2$ and $TaTe_2$ remained relatively high (≥ 60%) even at high nanomaterial concentrations above 50μg/mL, indicating that these two Group 5 transition metal ditelluride induce low toxicological effects towards the A549 cells. Therefore, in this study the degree of cytotoxicity displayed by the bulk Group 5 transition metal ditellurides can be deemed as VTe_2 being the most toxic while $NbTe_2$ and $TaTe_2$ are of comparable toxicity.

One plausible reason that could account for VTe_2 being more toxic is that VTe_2 might produce more reactive oxygen species (ROS) than $NbTe_2$ and $TaTe_2$. Studies have suggested that solid surfaces could directly interact with biological target molecules or indirectly interact with H_2O_2 or O_2, by transfering electron or H^+ to/from solid surfaces to produce ROS. These ROS may in turn cause oxidative stress to the cells and damage cellular proteins, lipids and nucleic acids.[49, 50] A study by Chia et al., demonstrated that the heterogeneous electron transfer (HET) rate of VTe_2 is significantly faster than $NbTe_2$ and $TaTe_2$. A faster HET rate indicates that a lower overpotential is required for an electrochemical reaction to occur,[33] hence more oxidation/reduction reactions could have occurred on the VTe_2 solid surfaces to produce more ROS, causing VTe_2 to be significantly more toxic.

From the EDX images in Figure 2, the main fundamental difference between the materials is the transition metals present, hence one could deduce that the different transition metal present in the Group 5 transition metal ditellurides is the main determining factor for the cytotoxicity properties. The trend observed in this study coincides with the trend from toxicities study of the Group 5 transition metal (IV) compounds, whereby it was observed that in general vanadium compounds are the most toxic among the three elements while niobium and tantalum compounds are considered to be relatively non-toxic.[51] Toxicity studies on rats have shown that the LD_{50} values for vanadium compounds ranges from a few tens to hundreds mg/kg body weight[52, 53] while the reported LD_{50} values for niobium and tantalum compounds ranges from several hundreds to thousands mg/kg body weight.[54-56]

Nanomaterial Induced Interference

Several studies have reported that nanomaterials could interact with the viability markers in cell viability assays, thus causing false readings to be produced.[39-41] As such, control experiments were performed in the absence of cells to check for possible sources of particle-induced interferences, to determine the suitability of the assays. Therefore, we set out to determine possible forms of particle-induced interferences for two commonly used assays which has similar working mechanisms, WST-8 and methyl thiazolyldiphenyl-tetrazoliumbromide (MTT) assays.

One possible interference induced by the nanomaterials on WST-8 is that the nanomaterials have the ability to reduce the active tetrazolium salt to form formazan, thereby generating false positive results and cause an overestimation in the number of viable cells.[57-59] In order to check for any interference, a control experiment was performed by incubating varying concentrations of nanomaterials with WST-8 assay for an hour in a cell free condition to check for possible formazan production through nanomaterials induced reductions. Figure 4 details the data of the WST-8 control experiments.

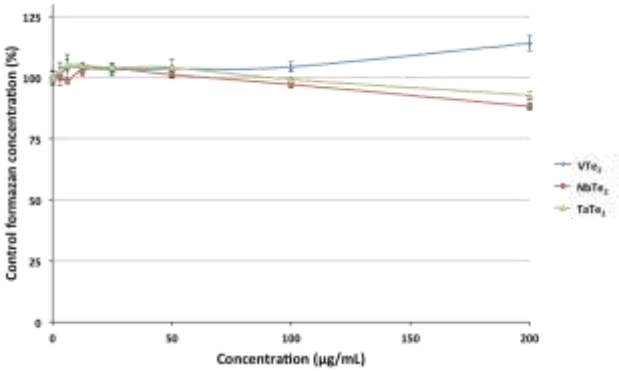

Figure 4. WST-8 control experiment: Formazan generated upon an hour of WST-8 incubation with varying concentrations of Group 5 ditelluride materials.

It can be observed that there was an insignificant nanomaterial induced reduction of the WST-8 by the bulk Group 5 ditellurides, where the amount of formazan produced after an hour of incubation with the nanomaterials were within the ±15% of the quantity measured from the control containing only 10% WST-8. Furthermore during the cell viability assay, a washing step was introduced to minimize the nanomaterial induced interference prior to the introduction of the assay reagent, hence we believe that the interference caused by the Group 5 ditellurides is insignificant.

Apart from using WST-8 assay, another commonly used MTT assay was also considered for use as a cell viability assay in this study. MTT working principle is similar to WST-8 such that in the presence of viable cells, MTT will be converted to purple colored formazan crystals with an absorbance maximum near 570 nm. There exist two possible interferences induced by the nanomaterials on MTT. Firstly, similar to WST-8, the nanomaterials could reduce the active tetrazolium salt to form formazan on its own, thereby generating a false positive result.

Secondly, the nanomaterials could interfere through binding with the formazan crystals formed thereby generating a false negative result and cause an underestimation in the number of viable cells.[41,57-62] To investigate whether the nanomaterials could induce a reduction of the active tetrazolium salt, different concentrations of the Group 5 ditelluride was exposed with the MTT assay in a cell free environment. To study the probable binding effect, ascorbic acid was added to different concentrations of nanomaterials to induce the formazan crystals formation from the MTT assay. If the nanomaterial binds to the formazan crystals, one would expect to see a decrease in the percentage of formazan with increasing amount of the test materials. The result from the MTT control experiments is illustrated in Supporting Figure SI-3.

It was found that high amount of interference was observed between VTe_2 with the MTT assay whereby the amount of formazan produced increase significantly to approximately 280% at the highest concentration tested while nanomaterial induced reduction of the MTT by $NbTe_2$ and $TaTe_2$ is insignificant. It was also revealed that there is minimal binding of the test materials with the formazan crystals. Due to significant amount of tetrazolium salt reduction by the VTe_2, it is deemed that the MTT assay would not be a fair assay to compare the toxicity between the Group 5 ditelluride materials and hence MTT cell viability assay was omitted in this study.

Comparison with other TMDs

Apart from studying the cytotoxicity of Group 5 transition metal ditellurides, the toxicological data obtained could also be used to do a comparison study with other TMDs for a better understanding of their relative toxicities. By comparing our data with those reported for the widely used Group 6 TMDs (namely MoS_2, WS_2 and WSe_2)[63] under similar conditions (such as undergoing a 24 h treatment of materials with A549 cells), we found that the Group 5 transition metal ditellurides are generally more toxic than the Group 6 TMDs. However an exception was observed in WSe_2 where it is more toxic than the Group 5 transition metal ditellurides with only VTe_2 being more toxic than WSe_2 (Table 1).

Table 1. Normalized cell viability percentages measured using WST-8 assays, after 24h exposure with 200μg/mL of TMDs. Data for Group 6 TMDs are obtained from reference 63.

Materials	Cell Viability (%)					
	MoS_2	WS_2	WSe_2	VTe_2	$NbTe_2$	$TaTe_2$
WST-8 Assay	80.7	83.6	45.0	7.5	71.6	65.1

Additionally, we also compared the effects of transition-metal element within the same group in determining the toxicological behaviour of TMD materials. For example, between MoS_2 and WS_2, which share the same chalcogen element, an increase in cell viabilities was observed down the transition metal group. A similar trend was also observed in our study where the cell viability generally increases down the transition metal group, suggesting that the toxicities of the TMDs could generally decreases down the transition metal group. However, more studies have to be conducted for verification.

Conclusions

With Group 5 transition metal ditellurides gaining an immense amount of academic and industrial research interest, there is a need to investigate the potential toxicity effects posed in view of their possible implementation in industries. This paper investigated the cytotoxicity effects of the Group 5 ditellurides TMDs towards A549 cells using WST-8 assay. The findings of this study indicate that VTe_2 is highly toxic and is affected by the concentration of VTe_2 while $NbTe_2$ and $TaTe_2$ are both mildly toxic with comparable results. Control experiments of WST-8 and MTT were carried out to determine the interference posed by the test materials and the suitability of the assays. Results from the WST-8 suggest minimal nanomaterial induced reduction of the WST-8 indicating that there was insignificant interference posed. On the other hand, MTT data shows a high degree of interference with VTe_2, making MTT assay an unreliable cell viability assay for fair comparison between the Group 5 transition metal ditellurides and was omitted from this study. In our view, these results constitute an excellent initial step towards the understanding of the toxicity effects of the bulk Group 5 transition metal ditelluride. However, more studies have to be conducted to explore the mechanisms behind the biological responses to nanomaterial exposures to have a full understanding of the toxicological effect of Group 5 ditellurides TMDs.

Experimental Section

Full details of the experimental procedures can be found in the Supporting Information.

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Keywords: cytotoxicity • ditellurides • transition metal dichalcogenides • layered compounds

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