

FACILE FABRICATION OF Ti_3C_2 MXENE NANOSHEETS AND THEIR PHOTOTHERMAL PROPERTIES

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Abstract

Recently discovered two-dimensional (2D) transition metal carbides and nitrides (MXenes) have received tremendous attention because of their unique electrical, optical and chemical properties. These exceptional properties make them a suitable candidate for a variety of applications including multimodal tumor therapy by photothermal effect. In this work, we demonstrate how to reduce the size of 1-5 μm large Ti_3C_2 monolayer MXene sheets into ultrasmall 100-160 nm nanosheets by applying consecutive ultrasonication processes. Different microscopic techniques have been used to visualize the formation of ultrasmall single-layer Ti_3C_2 nanosheets. The as-prepared MXene nanosheets have shown good solubility in water and ethanol. Further, (3-aminopropyl) triethoxysilane (APTES) and poly(3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT:PSS) were utilized for surface modification of the MXene nanosheets to open the possibility of subsequent antibody bio-conjugation. Moreover, PEDOT:PSS improved the photothermal conversion performance of the nanosheets as documented by increasing their temperature from 48.6 $^{\circ}\text{C}$ to 58.1 $^{\circ}\text{C}$ on irradiation by 808 nm wavelength laser. Further in vivo and in vitro studies will be necessary to optimize the photothermal properties of Ti_3C_2 nanosheets.

Keywords: Ti_3C_2 nanosheets, ultrasonication, surface modification, photothermal effect, cell viability

1. INTRODUCTION

Photothermal therapy (PTT) emerged as a non-invasive therapeutic strategy that can kill cancer cells through hyperthermia by converting photon energy into heat energy [1]. Different noble metal nanoparticles, nanorods, nanostars and 2D nanosheets of graphene, reduced graphene and black phosphorous have been utilized as photoabsorbers for PTT application [2-4]. MXenes first described in 2011 are a new class of 2D transitional metal carbides, nitrides, and carbonitrides obtained by etching and delamination of MAX phases [5,6]. MXenes have the formula $M_{n+1}X_nT_x$ where M is the transition metal, X is C and/or N and T is a functional group e.g., -O, -F, -OH [5]. During this short time, different materials of MXenes have been discovered. Owing to ease in surface modification and excellent near-infrared light-absorbing capability, Ti_3C_2 nanosheets distinguish themselves from other MXene nanomaterials [7].

Several synthetic methods have been developed for the production of the biocompatible size range (50-160 nm) of Ti_3C_2 nanosheets. Xuan et al, utilized an organic base tetramethylammonium hydroxide (TMAOH) to etch and delaminate multilayered Ti_3C_2 MXene sheets [8], while Yang et al obtained Ti_3C_2 nanosheets with

the hydrodynamic size range of 91.7 nm by using Tetrapropylammonium hydroxide (TPAOH) organic base [9]. The usage of organic bases can cause complications in biomedical applications. It is still a great challenge to reduce the size of Ti_3C_2 MXene nanosheets by only physical means within a short time while preserving the effect of PTT.

Further, compared to traditionally used gold nanomaterials for PTT, Ti_3C_2 MXene nanosheets have also shown the Localized Surface Plasmon Resonance (LSPR) effect. Ti_3C_2 (MXene) nanosheets, having transition metal element (titanium), exhibit a strong NIR absorption as well as subsequent light-to-heat conversion property enabling effective application in PTT. In this regard, Liu et al. have proved that the LSPR effect of Ti_3C_2 MXene nanosheets can be enhanced by introducing Al-containing moieties in them [10]. Lin et al, in their pioneering work, presented preparation of few-layer Ti_3C_2 MXene nanosheets and their successful application as PTT agent. This work points at the great potential of Ti_3C_2 nanosheets (MXenes) as a novel photothermal agent used for cancer therapy [11]. Complexation in synthetic methods and increase in toxicity of Ti_3C_2 nanosheets required complex surface modifications that cause limitation of such a novel material in biomedical application. Taking these effects into account, cytotoxicity and stability are the two major concerns for the application of Ti_3C_2 nanosheets in PTT. Hence, a suitable Ti_3C_2 surface modification is required to attain improved LSPR effect, lower toxicity, and high stability in physiological systems [12].

Herein, we have successfully achieved a 100-160 nm size range of Ti_3C_2 nanosheets without the addition of any organic bases. Ultrasonication methods were utilized for the cutting of 1-5 μm single layer Ti_3C_2 MXene sheets into 100-160 nm Ti_3C_2 nanosheets. Further, (3-aminopropyl) triethoxysilane (APTES) were functionalized on the surface of Ti_3C_2 MXene nanosheets that further attached with NHS-PEG-Biotin through a chemical bond. This surface modification not only reduces the toxicity of Ti_3C_2 nanosheets but also causes great stability in different solvents including water, ethanol, and phosphate buffer solution (PBS). Interestingly, the LSPR effect of Ti_3C_2 MXene nanosheets is enhanced by utilizing poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), which can further be attached with poly(ethylene glycol) moieties and can be utilized for the NIR-II PTT effect.

2. EXPERIMENTAL

2.1. Preparation of Ti_3C_2 nanosheets

Single layer micro size Ti_3C_2 MXene sheets (1-5 μm) were synthesized by using mixed acids (HCl+LiF) to etch Aluminum (Al) from Ti_3AlC_2 [13]. The cutting of as prepared larger Ti_3C_2 sheets into smaller ones was performed by utilizing subsequent bath and probe sonication. First, bath sonication (Sonorex RK 510 H, 35 kHz, Bandelin) was applied for 24 h followed by 4 h probe sonication (Sonopuls HD 2070, 20 kHz, Bandelin) placed in the ice bath. The nanosheets were centrifuged (Model 3-30K, Sigma centrifuge) with 8000 RPM for 40 min at 20 °C. The residue was collected and vacuum dried overnight. Next, the fully dried nanosheets were dissolved in 5 ml ethanol. The nanosheets were characterized by Atomic Force Microscopy (Bruker, Multimode 8), Scanning electron microscopy (manufacturer), Zetasizer Nano ZS 90 (Malvern Panalytical).

2.2. Surface modification of Ti_3C_2 nanosheets

First, we have prepared amino-functionalized MXene sheets (MXene-NH₂). 5 ml MXene solution (0.4 mg/mL) and 10 μl APTES (Sigma Aldrich) dissolved in 1 ml ethanol were mixed on a laboratory shaker for 24h. Next, 1 mg of NHS-PEG-Biotin (Sigma Aldrich, 1469.72 MW) was dissolved in 1 ml ethanol and poured into the MXene-NH₂ solution. The mixture was sonicated for 24 h, followed by vacuum drying overnight. The obtained solvent-free MXene-PEG-biotin powder was dissolved in PBS solution and stored for further use at 4 °C.

Secondly, we have prepared PEDOT:PSS modified nanosheets (MXene-PEDOT:PSS). Similarly as in the previous functionalization procedure, the PEDOT:PSS (50 μL , OSSILA) was mixed with Ti_3C_2 nanosheets in

deionized water and left on a laboratory shaker for 24 h. Next, the solution was centrifugated at 10.000 RPM for 20 min, then the residue was collected and dissolved in DI water for further use.

2.3. Photothermal effect

The absorbance measurements were realized by a SolidSpec-3700 UV-VIS-NIR spectrophotometer (Shimadzu, Japan), using an integrating sphere. Deionized water was used for the baseline correction before the measurements and also as a reference sample during the measurements. 100 μ l of pure and surface modified Ti_3C_2 nanosheets were used in a transparent quartz cell and irradiated with an 808 nm laser (0.430 W). The temperature changes were recorded with a K-type thermocouple coupled to an Extech SDL200 data-logging thermometer.

2.4. Cell viability test

The HCT116 (CCL-247, human colorectal carcinoma) cells were seeded into 96 well plates in a concentration of 10 thousand cells per well. For each sample, we have used 6 parallel wells. Cells were cultivated in DMEM medium with stable 2 mM L-glutamine (Biochrom) supplemented with 10% fetal calf serum (FCS, Biochrom) and gentamicin 80 μ g/ml (Lek). Cells were incubated in a humidified atmosphere at 37 $^{\circ}$ C in the presence of 5 % CO_2 for 24 h. The cells were moved to hypoxic workstation Ruskin Invivo2 300 (Ruskin) with 1 % O_2 and 5 % CO_2 for 24 h to induce the expression of CAIX protein and other hypoxia-inducible proteins. After 24 h in hypoxia, 40 μ l of the samples were added to the media and incubated further for 24 h or 48 h. Cell viability assay was done by Cell Titer blue viability assay (Promega) according to manufacturer's instructions. 20 μ l of Cell titer blue reagent were added and incubated for 1 hour at 37 $^{\circ}$ C in the presence of 5 % CO_2 . The fluorescence was measured at 590 nm (Synergy H4 microplate reader, BiTek) and the fluorescence and percentage of viable cells were determined. Each sample analysis was done in hexaplets and the standard deviation and T-test were determined.

3. RESULTS AND DISCUSSION

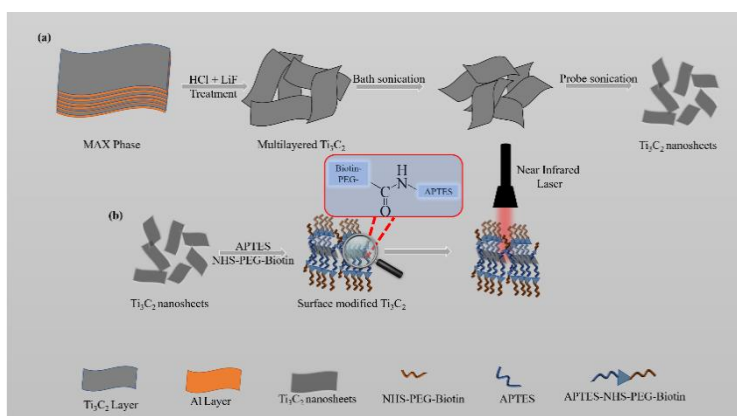


Figure 1 (a) Schematic illustration of a synthetic process for Ti_3C_2 MXene nanosheets including mixed acids-based etching, ultrasonication exfoliation, and cutting. (b) Subsequent APTES-PEG-Biotin surface modification of Ti_3C_2 nanosheets and their photothermal response to NIR light.

The scheme of the preparation of the MXene nanosheets is illustrated in **Figure 1(a)**. To achieve smaller size Ti_3C_2 MXene nanosheets compatible with the biomedical application, two subsequent ultrasonication processes were applied on the MXene sheets. Traditionally the liquid phase exfoliation method uses sonication to create the force necessary to exfoliate 2D materials. Since, in our experiments, we apply sonication on already single- and few-layer MXene sheets. Hence, further mechanical agitation by sonication is leading to

size reduction. Firstly, the bath sonication of the larger Ti_3C_2 MXene sheets (1-5 μm) causes the cutting of the sheets into 500-700 nm diameter sheets **Figure 2(a)**. Then probe sonication further reduces the nanosheets into considerably smaller sizes (100-160 nm). The size of the nanosheets both after bath and probe sonication was confirmed by AFM (see **Figure 2 (b)**). The size distribution was estimated from SEM micrographs taken from drop-casted samples on the silica substrate of the MXene solution (see **Figure 2(c-d)**). The SEM images were evaluated by ImageJ software to acquire the lateral size of the sheets. The mean value of the lateral size is 100-160 nm **Figure 2(d)**. From the size distribution study of the sonicated MXene solution, we can conclude that it was required to combine both bath and probe sonication for proper size reduction.

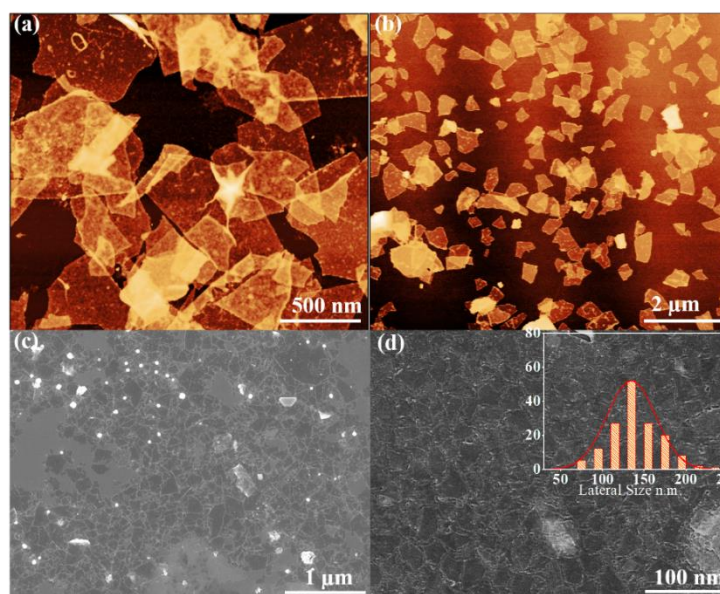


Figure 2. AFM images of (a) after 24 h bath sonicated Ti_3C_2 MXene nanosheets, and (b) bath (24 h) and probe (4 h) sonicated Ti_3C_2 nanosheets. SEM images of (c) 24 h bath sonicated Ti_3C_2 MXene nanosheets, and (d) 24 h bath and 4 h probe sonicated Ti_3C_2 MXene nanosheets: Inset is showing the size distribution of 150 nanosheets done by using ImageJ software.

The MXene nanosheets were functionalized to further explore their potential in biomedical application (see **Figure 1(b)**). We performed amino-functionalization via APTES, which resulted in $-\text{NH}_2$ terminals on the surface. Further, the MXene- NH_2 was PEGylated since polyethylene glycol is well known for its protein resistance and biocompatibility. Moreover, the biotin on the PEG terminal offers the opportunity for subsequent antibody bio-conjugation. Furthermore, we also prepared PEDOT:PSS functionalized nanosheets. The prepared MXene nanosheets before and after functionalization have shown high stability. The zeta-potential of as-prepared and surface modified MXene nanosheets was -50.8 mV and -45.3 mV respectively.

To verify that our treatment did not diminish the optical and photothermal properties of the MXene sheets, we studied them with UV-VIS spectrophotometry and checked the photothermal performance at 808 nm. From the UV-vis absorption spectra, it can be seen clearly that Ti_3C_2 nanosheets show absorption in the NIR region **Figure 3(a)**. Further, the optical absorption scaled linearly with a concentration upon the dilution of pure Ti_3C_2 MXene nanosheets. The absorption properties of optically active materials are dependent on size and surface chemistry. The modification of the surface of Ti_3C_2 MXene nanosheets with APTES/NHS-PEG-Biotin and PEDOT:PSS resulted in the broadening of the absorbance peak in the NIR region **Figure 3(b)**. These absorbance properties of pure nanosheets and surface-modified Ti_3C_2 nanosheets suggest that the presented materials could be potential photothermal agents.

The strong absorption of Ti_3C_2 nanosheets in the NIR range made possible their application as a photothermal agent in cancer therapy [8]. It can be seen clearly in **Figure 3(c,d)** that both pure MXene and MXene-PEDOT:PSS nanosheets have very good photothermal cycling stability under 808 nm laser irradiation. The temperature of pure Ti_3C_2 nanosheets dispersion was increased to about 20°C within the first 4 min of irradiation **Figure 3(e)**, and the highest temperature achieved was 48.6°C. However, it was surprising to observe that by mixing PEDOT:PSS with Ti_3C_2 nanosheets the temperature increase was faster and higher to about 10°C as compared to pure Ti_3C_2 nanosheets **Figure 3(f)**. Hence, the manipulation of photothermal properties of MXene nanosheets can be done by modifying their surfaces with different surfactants.

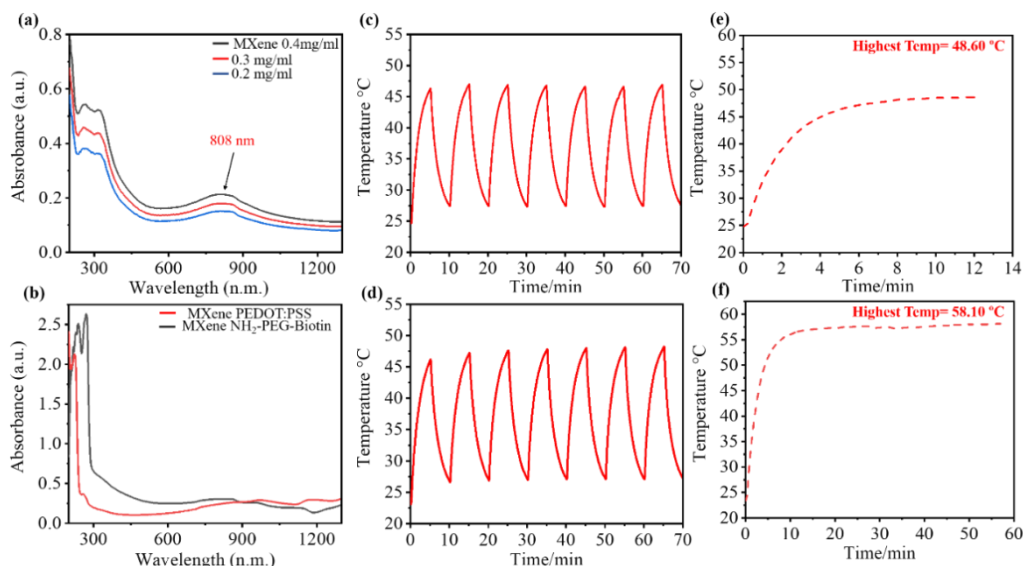


Figure 3. UV-visible absorption spectra of (a) MXene nanosheets and with two dilutions, (b) surface modified MXene with PEDOT:PSS and APTES/NHS-PEG-Biotin. Photostability test of (c) pure, and (d) MXene-PEDOT:PSS nanosheets in DI water under 808 nm laser irradiation. Photothermal performance of (e) pure, and (f) MXene-PEDOT:PSS nanosheets.

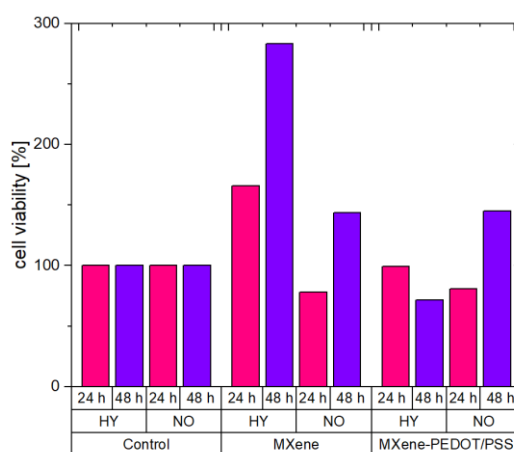


Figure 4. Cytotoxicity of MXene and MXene-PEDOS:PSS nanosheets regarding HCT-116 cell line.

Besides having outstanding photothermal properties, the other key feature for biomedical nanomaterial design is the cytotoxicity of the material. Our results in **Figure 4** revealed that pure Ti_3C_2 and MXene-PEDOS:PSS nanosheets do not cause any damage to the cells and therefore are nontoxic. In normoxic (21 % O_2) conditions after 24 h, the viability for pure Ti_3C_2 nanosheets is 78 % while for PEDOT:PSS modified Ti_3C_2 nanosheets

results out 80 %, after 48 h it is even more than the control. In hypoxic (1 % O₂) conditions after 24 h and 48 h, pure Ti₃C₂ nanosheets have even better viability than the control, and the values of MXene-PEDOT:PSS are similar to the control.

4. CONCLUSION

In summary, an organic solvent-free ultrasonication-based approach was designed to get a highly uniform and biocompatible size range of Ti₃C₂ nanosheets (100-160 nm). The cutting of larger size (1-5 μm) Ti₃C₂ MXene sheets into the anticipated smaller size was achieved by consecutive bath and probe sonication. The attained Ti₃C₂ MXene nanosheets were stable in water for more than three weeks. The surface modification of Ti₃C₂ nanosheets by NHS-PEG-Biotin and PEDOT:PSS were studied. While the usage of NHS-PEG-Biotin can later easily facilitate a specific antibody's binding, in the case of PEDOT:PSS surface functionalization, we observed enhanced photothermal conversion performance under 808 nm irradiation. Cell viability tests have shown that Ti₃C₂ nanosheets and PEDOT:PSS modified Ti₃C₂ nanosheets are nontoxic to the tested cell lines. We believe that this work will provide a feasible approach for the fabrication of biomedical application-worthy Ti₃C₂ nanosheets.

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