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Biofunctionalized Two-Dimensional Ti_3C_2 MXenes for Ultrasensitive Detection of Cancer Biomarker

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Abstract

In this work, ultrathin Ti_3C_2 -MXene nanosheets were synthesized by minimally intensive layer delamination methods, and uniformly functionalized with aminosilane ($f\text{-Ti}_3\text{C}_2\text{-MXene}$) to provide a covalent binding for the immobilized bio-receptor (anti-CEA) for label free, ultrasensitive detection of cancer biomarker (carcinoembryonic antigen, CEA). The effect of different redox probes on the electrochemical behavior of $f\text{-Ti}_3\text{C}_2\text{-MXene}$ was investigated and found that hexaammineruthenium ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) is the preferable redox probe for biosensing. The fabricated biofunctionalized $\text{Ti}_3\text{C}_2\text{-MXene}$ exhibits a linear detection range of 0.0001-2000 ngmL^{-1} with sensitivity of $37.9 \mu\text{Ang}^{-1}\text{mLcm}^{-2}$ per decade. The wider linear detection range of our $f\text{-Ti}_3\text{C}_2\text{-MXene}$ is not only higher than previously reported pristine 2D nanomaterials, but is even comparable to other hybrid 2D nanomaterials. We believe that this work opens a new window for development of MXene-based highly sensitive DNA, aptamer, enzyme, antibody, and cell based biosensors, and could be further used in drug delivery application.

Keywords: 2D nanomaterial, Ti_3C_2 -MXene, biosensor, cancer biomarker

1. INTRODUCTION

Ultrathin two-dimensional (2D) nanomaterials possess distinctive set of properties including large surface area and anisotropic electron transport behaviour which make them highly promising transducer materials for applications in biosensing (Ji et al. 2017; Kalantarzadeh and Ou 2015; Tan et al. 2017; Wen et al. 2017). However, these 2D materials usually have drawbacks that limit their performance in biosensors, such as low electrical conductivity (graphitic carbon nitride, MoS_2), hydrophobicity (MoS_2 , graphene), and very stable surfaces that limits incorporation of functional groups to enable transducer surfaces. For example, in the case of graphene, functionalization can only be done in surface defects and edges and MoS_2 is even more difficult to functionalize. Recently, a new group of 2D nanomaterials have emerged that hold the potential to overcome these problems. These materials, known as MXenes, belong to a group of 2D transition metal carbides/carbonitrides that are typically obtained by selective etching of the A-layers from $\text{M}_{n+1}\text{AX}_n$ (MAX) phases (where M is an early transition metal, A is usually group of 13 and 14 element and X is carbon or a combination of C and nitrogen and $n=1-3$). Etching the A-layers results in surface terminations with functional groups such as OH^- , O^{2-} and F^- (Naguib et al. 2011; Naguib et al. 2012; Naguib et al. 2014). These materials have already shown great promise in various applications including electrochemical energy storage (Ahmed et al. 2017; Anasori et al. 2017; Jiang et al. 2018), water desalination (Ren et al.

2015), electromagnetic (Shahzad et al. 2016), catalysis (Ran et al. 2017), gas sensing (Kim et al. 2018) etc. Compared to other 2D materials, MXenes possess a unique combination of excellent electrical conductivity, hydrophilicity, potential for high density incorporation of several functional groups, ultrathin 2D sheet-like morphology as well as excellent ion intercalation behavior which are ideal for electrochemical sensing. So far, biosensors based on MXenes have been focused on the detection of nitrite, pesticides, phenol and H_2O_2 (Liu et al. 2015; Wang et al. 2015a; Wang et al. 2015b; Wu et al. 2018; Zhou et al. 2017). These biosensors used multilayered Ti_3C_2 -MXene systems to immobilize electrochemical active proteins via physical adsorption. This method, although efficient, does not fully utilize the great potential of 2D nanosheet morphology of MXenes to incorporate a high density of surface functional groups. Thus, the sensing performance of physical adsorption methods is limited when detecting low concentration biomarkers, as required by applications such as cancer detection.

In order to evaluate the ultimate potential of MXenes for biosensing this paper proposed for the first time to use single/few-layered MXene (Ti_3C_2) nanosheets for covalent immobilization of bio-receptor (anti-CEA) for the cancer biomarker detection. Highly efficient electrochemical biosensors require highly active transducer surfaces that integrate the biomolecules efficiently while providing faster access to the analytes (Wang et al. 2017). In this context, the morphology of ultrathin 2D nanosheet of single/few layered Ti_3C_2 -MXene with its high density of functional groups offers improved biomolecule loading and faster access to analyte. In addition, covalent immobilization of bio-receptor (enzymes, DNA, proteins, etc.) leads to not only improved uniformity and distribution, but also enables higher density of bound bio-markers that would result in enhanced biosensor performance (Wang et al. 2015d).

In this paper, amino groups are introduced on the surfaces of single/few-layered MXene (Ti_3C_2) nanosheets via chemical means for the covalent immobilization of bio-receptor. The resulting biofunctionalized MXene exhibits wide linear detection range towards carcinoembryonic antigen (CEA), an important cancer biomarker found in colorectal, lung, ovarian, pancreatic, breast and liver cancer patients (Hammarström 1999; Kulpa et al. 2002; Myers et al. 1978; Wanebo et al. 1978). According to WHO report nearly half of all cancer deaths each year are associated with these cancers (WHO 2018). Therefore, monitoring of CEA level may be an interesting alternative for clinical monitoring, prognosis, recurrence possibilities, and screening of hepatic metastases of cancers.

2. Materials and methods

2.1 Materials

All biomolecules including carcinoembryonic antibody monoclonal (anti-CEA), carcinoembryonic antigen (CEA), bovine serum albumin (BSA) and human serum were procured from Sigma Aldrich, USA. Other chemicals including (3-Aminopropyl)triethoxysilane (Alfa Aesar, 98%), 1-ethyl-(dimethylaminopropyl)-carbodiimide hydrochloride (EDC, Sigma Aldrich), N-hydroxysuccinimide (NHS, Sigma Aldrich), lithium chloride (Sigma Aldrich) and buffer salts were of the analytical grade. Millipore purified De-ionized (DI) water was used throughout experiments.

2.2 Synthesis of Ti_3C_2 -MXene

Ti_3AlC_2 MAX phase was purchased from Carbon-Ukraine Ltd. with particle sizes of less than 38 μm . Ti_3C_2 -MXene was synthesized by minimally intensive layer delamination (MILD) method, where manual shaking is enough to obtain larger and high quality single/few layer of MXene. (Sang et al. 2016). First, 2 g of Ti_3AlC_2 (MAX Phase) was gradually added to the

etchant solution, which were prepared similarly to the previous report (Lukatskaya et al. 2017). In brief, the etchant solution was prepared by mixing 1.5 g of LiF into 20 ml 6 M HCl, followed by stirring for 10 min. Ti_3AlC_2 (MAX) was slowly added into the etchant solution at 35 °C and stirred for 24 h. Then etched product was transferred into a falcon tube and washed with DI water under centrifugation (3500 rpm, 5 min) until the supernatant reached a $\text{pH} \geq 6$. Neutralization of pH is an important step to remove acidic and etched waste from Ti_3C_2 -MXene. The obtained product was again mixed with DI water and bath sonicated for 1 h. Next, resultant black color supernatant Ti_3C_2 -MXene nanosheets were collected via centrifugation at 3500 rpm for 5 min.

2.3 Functionalization of Ti_3C_2 -MXene

For functionalization of the as-synthesized ultrathin Ti_3C_2 -MXene nanosheets, (3-Aminopropyl)triethoxysilane (APTES) was used. In a typical experiment, 100 mg suspension of ultrathin Ti_3C_2 -MXene was mixed with 50 ml of ethanol after which 1 ml of APTES was added step wise and kept at room temperature with constant stirring (500 rpm) for 48 h. To remove the unbound APTES molecules, the obtained product was washed with water and ethanol (1:1) mixture. The obtained product was dried at 50°C overnight in a vacuum condition. This product (functionalized Ti_3C_2 -MXene, $f\text{-Ti}_3\text{C}_2$ -MXene) was finely crushed into powder and stored in vacuum for further use. Figure 1a illustrates a schematic representation of the synthesis and functionalization process of Ti_3C_2 -MXene.

2.4 Fabrication of biosensing platform

A glassy carbon (GC) electrode was used to study the electrochemical performance of APTES functionalized Ti_3C_2 -MXene ($f\text{-Ti}_3\text{C}_2$ -MXene) for cancer biosensing. For this, GC electrode was used and polished with alumina slurry of 0.3 μm and 0.05 μm respectively, then

the electrode was sonicated in the mixture of acetone and ethanol followed by nitrogen drying. Next, $f\text{-Ti}_3\text{C}_2\text{-MXene}$ was sonicated in a mixture solution of ethanol and water containing 0.1 % nafion for 1 h to result in a concentration of 1 mg/mL. 5 μL of the obtained solution was drop casted on GC electrode and kept for drying, the loaded electrode was referred to as $f\text{-Ti}_3\text{C}_2\text{-MXene/GC}$. The solution comprising 10 μL bio-receptor (monoclonal antibody against CEA), 10 μL EDC (0.2M), and 10 μL NHS (0.05M) was prepared. Then 10 μL of thus-obtained solution was drop-casted on $f\text{-Ti}_3\text{C}_2\text{-MXene/GC}$ and kept in a humid chamber for formation of covalent bonding between amino groups of $f\text{-Ti}_3\text{C}_2\text{-MXene}$ and carboxyl groups present on the Fc region of the bio receptor (anti-CEA). The prepared bioelectrode (anti-CEA/ $f\text{-Ti}_3\text{C}_2\text{-MXene/GC}$) was washed with phosphate buffer saline (PBS) to remove any unbound anti-CEA. Next, 1% BSA was used to block unspecific active site on the bioelectrode. Finally the anti-CEA/ $f\text{-Ti}_3\text{C}_2\text{-MXene/GC}$ bioelectrode was washed with PBS and stored in a refrigerator for further studies.

2.5 Characterization

The as-prepared ultrathin $\text{Ti}_3\text{C}_2\text{-MXene}$ nanosheets and $f\text{-Ti}_3\text{C}_2\text{-MXene}$ nanosheets were characterized by X-ray diffractometer (Bruker, D8 ADVANCE, Cu $\text{K}\alpha$, $\lambda = 0.15406 \text{ nm}$), Raman spectroscopy (LabRam Aramis, blue laser with excitation wavelength of 473 nm), transmission electron microscope (Titan G2 80-300 ST, FEI) and X-ray photoelectron spectroscopy (XPS) (Kratos Analytical, AMICUS/ESCA 3400). The cyclic voltammetry at the potential window 0.2 to -0.8V with scan rate 50 mV/s were carried out on a VMP3 multi-channel potentiostat (Bio-Logic science instruments, France) using three electrode electrochemical cell with the modified GC as the working electrode (3 mm^2 working area), platinum wire as the auxiliary electrode, and Ag/AgCl as the reference electrode (aqueous, ALS Co. Ltd, Japan) in phosphate buffer saline (PBS, 50 mM, pH 7.4).

3. Results and Discussion

3.1 Structural, morphological and elemental analysis

Figure 1b shows the XRD pattern of the Ti_3AlC_2 -MAX (pink curve), Ti_3C_2 -MXene (orange curve) and f - Ti_3C_2 -MXene (green curve), respectively. The XRD patterns of the MAX sample matches well with reported Ti_3AlC_2 -MAX (JCPDS NO. 052-0875) (Alhabeab et al. 2017). It is important to note that after exfoliation, the XRD peaks for Ti_3C_2 -MXene exhibit broadening and significant loss of crystallinity due to the removal of aluminum. The broadened (002) peak decreases from 9.7° to 7.5° , indicating an increase in d-spacing (0.9 nm to 1.17 nm) for Ti_3C_2 -MXene layers, when compared with Ti_3AlC_2 -MAX. The other characteristic peaks for Ti_3AlC_2 -MAX become very weak in Ti_3C_2 -MXene as well as f - Ti_3C_2 -MXene. It has been observed that after silane functionalization of Ti_3C_2 -MXene, the 2θ angle shifts to 5.1° , indicating that interlayer spacing increase up to 1.7 nm. This demonstrates the binding of APTES within the layers as well. A recent study on expansion of Ti_3C_2 -MXene interlayer spacing using trimethylalkylammonium cations of varied alkyl chain lengths, support this claim (Ghidiu et al. 2017). The Raman spectrum of the Ti_3AlC_2 -MAX, Ti_3C_2 -MXene and f - Ti_3C_2 -MXene are shown in Figure S1a, supporting information. The characteristic peaks for Ti_3AlC_2 -MAX are located at 123, 201, 271, 630 and 660 cm^{-1} (Presser et al. 2012). The sharp peak at 209 cm^{-1} and the wider peaks centered at ~ 400 , ~ 600 , and 727 cm^{-1} observed after MILD treatment suggest selective etching of aluminum and presence of functional group (-O, -OH, -F). This is consistent with Raman peaks found for Ti_3C_2 -MXene in the literature (Hu et al. 2015; Naguib et al. 2011). After the APTES treatment (f - Ti_3C_2 -MXene), the Raman peaks broaden, and shift from 209 to 204 cm^{-1} .

¹, ~400 to 383 cm⁻¹ and 727 to 739 cm⁻¹, respectively, implying successful aminosilane functionalization.

The detailed surface morphologies of 2D nanosheets of Ti₃C₂-MXene and fTi₃C₂-MXene nanosheets are shown in Figure S1 (b-e) in the supporting information. Very thin, highly transparent, smooth and sheet-like morphology are clearly observed in Figure S1b. This indicates successful synthesis of ultrathin (single to few layer) Ti₃C₂-MXene layers. The darker region corresponds to the folding and overlapping of the ultrathin MXene sheets. After the APTES functionalization, the *f*-Ti₃C₂-MXene sheets overlap onto each other and the smoothness degrades. This is likely due to the shearing forces applied during the stirring (Figure S1c). TEM results of the edges of *f*-Ti₃C₂-MXene reveals stacking of few layers of 2D nanosheets (Figure S1d) and hexagonal structure of the basal planes (Figure S1e). The TEM imaging of an individual *f*-Ti₃C₂-MXene sheet as well as the corresponding elemental map (Figure 1c,d) show uniform distribution of silicon (Si, brown), oxygen (O, green) and nitrogen (N, dark yellow) over the nanosheets revealing homogenous functionalization of Ti₃C₂-MXene via APTES. The elemental mapping of Ti₃C₂-MXene nanosheet (Figure S1f) also indicates the presence of titanium, carbon, oxygen, and fluoride.

3.2 XPS analysis

X-ray photoelectron spectroscopy (XPS) was used to analyze the surface chemical states of Ti₃C₂-MXene and *f*-Ti₃C₂-MXene. The survey spectrum of Ti₃C₂-MXene (Figure S1g) shows the presence of Ti, C, O and F. The presence of O and F indicate the possible surface termination [Ti₃C₂(OH)₂, Ti₃C₂F₂] during etching (Naguib et al. 2011). Moreover, the presence of Si 2p and N 1s peaks in *f*-Ti₃C₂-MXene confirm APTES functionalization (Figure 2a, b). Figure 2c shows deconvoluted XPS spectra of the pristine Ti₃C₂-MXene and *f*-Ti₃C₂-MXene. The C 1s peaks at

281.7 eV and 282.7 correspond to internal C-Ti bond and surface terminated C-Ti bond (C-Ti-O/F), respectively. The other peaks can be assigned to graphitic C-C (284.8 eV) and C-O (286.2 eV) bonds. The weak peaks at high binding energy (288 eV) correspond to the C-F/O-C=O group (Ding et al. 2018; Wang et al. 2016). However after functionalization, there is a decrease in peak intensity of inner C-Ti layer, while an intense peak for C-N at ~286 eV is observed, indicating the presence of APTES over the Ti_3C_2 -MXene. Figure 2d shows the O 1s spectrum of pristine Ti_3C_2 -MXene and *f*- Ti_3C_2 -MXene. The peak at 529.8 and 531 eV correspond to oxygen bonded to surface titanium. The peak at 532.8 eV is assigned to C-Ti-OH moiety (Ding et al. 2018; Halim et al. 2016). Further, the peaks at ~ 534 and ~ 535 eV reflect the presence of water molecules between MXene flakes and O-F_x moiety, respectively (Halim et al. 2016). However, after functionalization, Ti-OH moiety forms a covalent bond with the silicon atom in the APTES (Kim et al. 2016). This is shown in Figure 2d where an intense Si-O peak at 532.4 eV is observed.

3.3 Electrochemical characterization

Next, the analyses of the resulting functionalized layers using cyclic voltammetry (CV) are presented. CV was conducted to investigate the electrochemical redox properties of *f*- Ti_3C_2 -MXene layers used in modified glassy carbon (GC) electrodes. Details are provided in the experimental section. For the analyses, 5mM potassium ferro-ferricyanide ($[\text{Fe}(\text{CN})_6]^{-3/-4}$) was used as a redox probe in a PBS solution (Figure S2a, supporting information). It has been observed that for positive potentials the $[\text{Fe}(\text{CN})_6]^{-3/-4}$ is oxidized and reduced, but after the first cycle the electrochemical oxidation current density becomes unstable and drops successively. This sudden drop in oxidation current density after the first cycle may be due to oxidation of Ti_3C_2 -MXene layers at higher potentials (-0.1 V to 0.8 V), resulting in the formation of TiO_2

over the MXene sheets (Lorencová et al. 2017). To overcome this problem, hexammineruthenium (III) chloride ($[\text{Ru}(\text{NH}_3)_6]^{+3}$) was used as a redox probe because it can be oxidized and reduced at lower potential windows. Figure S2b (supporting information) shows the cyclic voltammogram for *f*- Ti_3C_2 -MXene in PBS buffer with and without redox probe ($[\text{Ru}(\text{NH}_3)_6]^{+3}$) at -0.8 to 0.2 V. The improved and stable electrochemical current density suggests that $[\text{Ru}(\text{NH}_3)_6]^{+3}$ is a suitable redox probe for *f*- Ti_3C_2 -MXene. Next, *f*- Ti_3C_2 -MXene/GC electrodes were further biofunctionalized (anti-CEA) to detect cancer biomarker, CEA (details are provided in experimental section). A schematic representation of the electrode surface and redox probe interaction is shown in Figure 3. Figure 4a shows the CV results conducted on *f*- Ti_3C_2 -MXene/GC, anti-CEA/*f*- Ti_3C_2 -MXene/GC and BSA/anti-CEA/*f*- Ti_3C_2 -MXene/GC electrodes. It can be seen that the magnitude of the electrochemical current density for anti-CEA/*f*- Ti_3C_2 -MXene/GC electrode ($\sim 2.63 \text{ mA/cm}^2$) is higher than that of APTES functionalized *f*- Ti_3C_2 -MXene/GC electrode ($\sim 1.36 \text{ mA/cm}^2$). This can be attributed to the efficient immobilization and good orientation of anti-CEA over the *f*- Ti_3C_2 -MXene resulting in better electron transfer between the solution and electrodes. Also, the electrostatic charge present on the antibodies ($-\text{NH}_2$) may also contribute to better electron diffusion (Kumar et al. 2015). A decrease in magnitude of electrochemical current density ($\sim 2.16 \text{ mA/cm}^2$) is observed after BSA immobilization onto the anti-CEA/*f*- Ti_3C_2 -MXene/GC electrode. This is due to blockage of unspecific active sites on anti-CEA/*f*- Ti_3C_2 -MXene/GC electrode which hinders the permeability of redox probe ($[\text{Ru}(\text{NH}_3)_6]^{3+}$).

The kinetics of the *f*- Ti_3C_2 -MXene/GC electrode was investigated under different scan rates from 10 to 120 mV/s (Figure 4b). The cathodic and anodic current density increased linearly with the square root of the scan rate (Figure 4c) indicating that the electrochemical

reaction was diffusion controlled, following equations 1 and 2 below. The diffusion coefficient (D) of the redox probe ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) for the *f*-Ti₃C₂-MXene/GC electrode was obtained through Randles-Sevcik equation and calculated to be $5.5 \times 10^{-5} \text{ cm}^2/\text{s}$.

$$\begin{aligned} I_{pc(f\text{-Ti}_3\text{C}_2\text{-MXene/GC})} &= [0.306 \text{ mA}(\text{smV}^{-1}) \times (\text{scan rate}[\text{mVs}^{-1}])^{1/2}] - 0.536 \text{ mA}, R^2 \\ &= 0.99 \end{aligned} \quad \dots\dots\dots (1)$$

$$\begin{aligned} I_{pa(f\text{-Ti}_3\text{C}_2\text{-MXene/GC})} &= [-0.232 \text{ mA}(\text{smV}^{-1}) \times (\text{scan rate}[\text{mVs}^{-1}])^{1/2}] - 0.230 \text{ mA}, R^2 \\ &= 0.99 \end{aligned} \quad \dots\dots\dots (2)$$

Similarly, the electrochemical kinetics of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC electrode was also investigated (Figure S2 c, d). The cathodic and anodic current density also increased linearly with the square root of scan rate indicating also diffusion controlled reactions. This reaction allows facile electron transfer from the medium to the electrode per equation 3 and 4:

$$\begin{aligned} I_{pc(\text{BSA/anti-CEA/}f\text{-Ti}_3\text{C}_2\text{-MXene/GC})} &= [0.346 \text{ mA}(\text{smV}^{-1}) \times (\text{scan rate}[\text{mVs}^{-1}])^{1/2}] - 0.360 \text{ mA}, R^2 = 0.99 \\ &\dots\dots\dots (3) \end{aligned}$$

$$\begin{aligned} I_{pa(\text{BSA/anti-CEA/}f\text{-Ti}_3\text{C}_2\text{-MXene/GC})} &= [-0.266 \text{ mA}(\text{smV}^{-1}) \times (\text{scan rate}[\text{mVs}^{-1}])^{1/2}] - 0.063 \text{ mA}, R^2 = 0.99 \\ &\dots\dots\dots (4) \end{aligned}$$

Next, the surface concentration of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC electrode was calculated from the plot of I_{pc} vs. v using the Brown-Anson model as given in equation 5 (Brown and Anson 1977) :

$$I_p = n^2 F^2 \gamma A \nu (4RT)^{-1} \dots\dots\dots (5)$$

Where I_p indicates the peak current, n the number of electrons transferred in the redox process (1), F is the Faraday constant (96485 C mol^{-1}), γ is the surface concentration of the absorbed electro-active species, A is the surface area of the electrode, ν is the scan rate (V/s), R is the gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) and T is the temperature (298 K). From this equation, the surface concentration of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC bioelectrode was calculated to be $2.5 \times 10^{-8} \text{ mol cm}^{-2}$.

Figure 4d shows the electrochemical response of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC electrode evaluated using CV (0.2 to -0.8V, scan rate 50 mV/s) as a function of CEA concentration ranging from 0.0001 ngmL⁻¹ to 2000 ngmL⁻¹ in a PBS solution (50 mM, pH 7.4, 0.9% NaCl) containing 5mM [Ru(NH₃)₆]³⁺ as a the redox probe with incubation time of 15 minutes. The enlarged view of the response study (Figure 4e) shows that the magnitude of the electrochemical current density decreases as the CEA concentration increases from 0.0001 ngmL⁻¹ to 2000 ngmL⁻¹. This is due to specific binding between CEA and the bio-receptor (anti-CEA, monoclonal) that indicates the successful formation of the immune-complex at the electrode/electrolyte interface. This results in reduced charge transfer via the redox probe, [Ru(NH₃)₆]³⁺ leading to a reduction in the electrochemical current density. Figure 4f shows a calibration curve between the magnitude of electrochemical current density and the CEA concentration. A linear relationship is observed between the magnitude of electrochemical current density and the logarithmic value of the CEA concentration in the range of 0.0001-2000 ngmL⁻¹ with regression coefficient of 0.992. The linear relationship could be depicted by the equation, $I_p \text{ (mA)} = 1.695 - 0.0379 \times \log C_{\text{CEA}} \text{ (ng/mL)}$, with detection limit of 0.000018 ngmL⁻¹

(Detection limit = $3S_a/b$, where S_a is standard deviation of the response and b is the slope of calibration curve). The reproducibility was evaluated by running this test 3 times. The error bars included were based on the standard error of these 3 tests. The sensitivity of the fabricated BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC bioelectrode estimated from the slope of the curve was found to be $\sim 37.9 \mu\text{A} \cdot \text{ng}^{-1} \cdot \text{mL} \cdot \text{cm}^{-2}$ per decade. The characteristics of the *f*-Ti₃C₂-MXene based CEA biosensor was compared with other 2D nanomaterials and their composites (Table 1). This comparative study clearly reveals that *f*-Ti₃C₂-MXene based immobilization matrix shows wide linear detection range ($0.0001\text{--}2000 \text{ ng mL}^{-1}$) compared to recent reported hybrid 2D nanomaterials such as AgNPs/MoS₂/rGO (Wang et al. 2018), Pt-PdNPs/N-GQDs@AuNPs (Yang et al. 2017), β -cyclodextrin/rGO (Tian et al. 2016), MoS₂-AuNPs (Wang et al. 2015c), GO/MWCNTs-COOH/AuNPs@CeO₂NPs (Pang et al. 2015), rGO-AuNPs (Liu et al. 2014) and g-C₃N₄-AuNPs (Chen et al. 2014). A control experiment was performed as a function of CEA concentration (Figure S2e) without bio-receptor (anti-CEA) to investigate the selectivity of the electrode. No significant change in electrochemical current density response was observed indicating that the CEA antigens specifically interact with anti-CEA present on the surface of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC bioelectrode.

3.4 Reproducibility, stability and serum sample studies

The reproducibility of the biofunctionalized *f*-Ti₃C₂-MXene (BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC) was also investigated (Figure S2, f) using 0.1 ng mL^{-1} CEA and measuring the electrochemical current density response of four different bioelectrodes with a constant surface area. The relative standard deviation was less than 10% with mean value of 1.74 mA/cm^2 . Further, each measurement was repeated 5 times for each bioelectrode and the error bars were included accordingly. Previous studies found that Ti₃C₂-MXene flakes degrade gradually either

in humid air or water and form cloudy-white colloidal solutions containing primarily anatase (TiO₂) (Zhang et al. 2017). Therefore, handling and storage of Ti₃C₂-MXene is crucial to maintain its characteristics; thus, a shelf lifetime study of BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC was performed in the presence of CEA (0.1 ng mL⁻¹) at regular interval of time (Figure S2, g). The study reveals a loss of 20% activity for biofunctionalized *f*-Ti₃C₂-MXene surface in 7 days. To validate the performance of the bio-functionalized Ti₃C₂-MXene the electrochemical response studies of the bio-electrodes (BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC) with spiked human sera sample at 10, 50, 100 and 250 ngmL⁻¹ were performed (Table 2). The observed results exhibited acceptable recovery of CEA in serum samples.

Conclusions

We have demonstrated a label-free and highly sensitive electrochemical biosensor for CEA detection, based on two-dimensional Ti₃C₂-MXene nanosheets. The Ti₃C₂-MXene nanosheets were synthesized by MILD method and uniformly functionalized with APTES for the covalent immobilization of anti-CEA. The fabricated bioelectrode (BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC) exhibits a wider linear detection range of 0.0001-2000 ngmL⁻¹ with sensitivity of ~37.9 μAng⁻¹ mLcm⁻² per decade. The CEA concentration has been estimated in serum samples by using the BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC bioelectrode to evaluate the feasibility of the sensor. The effort should be made to improve the stability of the sensor by prevention of Ti₃C₂-MXene oxidation. We believe that the proposed ultrathin 2D nanomaterial (Ti₃C₂-MXene) could be utilized for development of highly sensitive DNA, aptamer, enzyme, antibody, and cell based biosensor.

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Fig 1. (a) Schematic illustration of Ti_3C_2 -MXene functionalization. Aluminum layered is etched from the Ti_3AlC_2 -MAX phase, which delaminates into 2D nanosheets; the sheets consist of two layers of carbon sandwiched between three layers of titanium with the surface of titanium randomly terminated with $-\text{OH}$, $-\text{O}$, and $-\text{F}$ functional groups. Aminosilane (APTES) is then used to functionalize the Ti_3C_2 -MXene surface. **(b)** XRD pattern of the Ti_3AlC_2 -MAX, Ti_3C_2 -MXene and functionalized Ti_3C_2 -MXene ($f\text{-Ti}_3\text{C}_2$ -MXene). The (002) peak of $f\text{-Ti}_3\text{C}_2$ -MXene shifts towards lower angles indicating an increase in d-spacing, when compared to the Ti_3C_2 -MXene. This implies that the Ti_3C_2 -MXene free surface groups (especially oxide) undergo silanization process. **(c-d)** TEM image of a single $f\text{-Ti}_3\text{C}_2$ -MXene sheet and corresponding elemental map showing the uniform distribution of silicon (Si, brown), oxygen (O, green) and nitrogen (N, dark yellow), and revealing the homogenous functionalization of Ti_3C_2 -MXene with APTES.

Fig 2. XPS spectra showing the chemical states of silicon, carbon, nitrogen and oxygen on the surface of $\text{Ti}_3\text{C}_2\text{-MXene}$ and $f\text{-Ti}_3\text{C}_2\text{-MXene}$. (a-b) The presence of Si 2p and N 1s in $f\text{-Ti}_3\text{C}_2\text{-MXene}$ indicates successful APTES functionalization. The oxide moieties in the $\text{Ti}_3\text{C}_2\text{-MXene}$ [C-Ti-(OH)] undergo silanization process. (c) In the C 1s region, the spectrum is deconvoluted into characteristic peaks, after functionalization a decrease in peak intensity of inner C-Ti layer and a high intensity peak of C-N appear at around 286 eV. (d) In the O 1s region, the fitted peaks of C-Ti-OH (532.8 eV) is replaced by the large intensity peak of C-Ti-OSi (532.4 eV) after silanization.

Fig 3. Schematic of the electrochemical CEA detection mechanism.

Fig 4. (a) Cyclic voltammogram obtained of different modified GC electrode ($f\text{-Ti}_3\text{C}_2\text{-MXene}$, anti-CEA/ $f\text{-Ti}_3\text{C}_2\text{-MXene}$ and BSA/anti-CEA/ $f\text{-Ti}_3\text{C}_2\text{-MXene}$) vs Ag/AgCl in PBS (pH 7.4, 5mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$). (b) Cyclic voltammogram of $f\text{-Ti}_3\text{C}_2\text{-MXene}$ /GC electrode at varying scan rates from 10-120 mV/s . (c) Randles-Sevcik plot of $f\text{-Ti}_3\text{C}_2\text{-MXene}$ /GC electrode at varying scan rates from 10-120 mV/s . (d) Electrochemical response studies of BSA/anti-CEA/ $f\text{-Ti}_3\text{C}_2\text{-MXene}$ /GC electrode at different concentration of CEA (0.0001-2000 ng/mL). (e) Enlarge view of response studies. (f) Calibration plot between the magnitudes of current recorded and log of CEA concentration.

Table 1: Comparison of present work with other reported 2D nanomaterials for CEA detection

Immobilization Matrix	Detection Technique	Linear Detection Range (ngmL ⁻¹)	Detection limit (ng mL ⁻¹)	Labeling	Shelf life (days)	References
AgNPs/MoS ₂ /rGO	Chronoamperometry	1.0×10^{-5} to 100	0.0000016	No	-	(Wang et al. 2018)
Pt-Pd NPs/N-GQDs@Au NPs	Chronoamperometry	5×10^{-6} to 50	0.000002	No	16	(Yang et al. 2017)
β -cyclodextrin/ rGO	Chronoamperometry	1.0×10^{-3} to 100	0.0003	Yes	28	(Tian et al. 2016)
MoS ₂ -Au NPs	DPV	1.0×10^{-3} to 50	0.0003	Yes	30	(Wang et al. 2015)
GO/MWCNTs-COOH/Au NPs@CeO ₂ NPs	Electro-chemiluminescence	5.0×10^{-2} to 100	0.02	No	15	(Pang et al. 2015)
rGO-Au NPs	SWV	1.0×10^{-2} to 100	0.003	Yes	-	(Liu et al. 2014)
g-C ₃ N ₄ -Au NPs	Electro-chemiluminescence	2.0×10^{-2} to 80	0.007	No	14	(Chen et al. 2014)
<i>f</i> -Ti ₃ C ₂ -MXene	CV	1.0×10^{-4} to 2000	0.000018	No	7	Present work

Abbreviation

Ag NPs: silver nanoparticles, MoS₂: molybdenum disulfide, rGO: reduce graphene oxide, Pt-Pd NPs: platinum-palladium nanoparticles, N-GQD: nitrogen doped graphene quantum dot, Au NPs: gold nanoparticle, COOH-MWCNTs: carboxylic functionalized multiwalled carbon nanotubes, CeO₂ NPs: Cerium oxide nanoparticle, SWV: square wave voltammetry, g-C₃N₄: graphitic carbon nitride, CV: cyclic voltammetry

Table 2: Determination of carcinoembryonic antigen concentration in serum samples using a BSA/anti-CEA/*f*-Ti₃C₂-MXene/GC electrode

Concentration (ngmL ⁻¹)	Amperometric current density (mA/cm ²) obtained with standard CEA sample	Amperometric current density (mA/cm ²) obtained with spiked CEA sample	Recovery %
10	1.668	1.690	101.3
50	1.643	1.646	100.2
100	1.623	1.606	98.9
250	1.600	1.580	98.8

Highlights

- Two-dimensional Ti₃C₂-MXene nanosheets were synthesized by the MILD method.
- Single/few layered MXene nanosheets were functionalized via APTES (*f*-Ti₃C₂-MXene).
- The electrochemical behavior of *f*-Ti₃C₂-MXene was explored.
- *f*-Ti₃C₂-MXene was biofunctionalized for electrochemical detection of CEA.
- It exhibits a wide linear detection range of 0.0001-2000 ngmL⁻¹.