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Synthesis of dual-phase Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers for efficient adsorption of SARS-CoV-2

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes worldwide COVID-19 pandemic, is a great threat that has taken millions of the people lives [1–3]. This virus, similar to other kinds of coronaviruses (e.g., SARS-CoV-1, MERS), is a respiratory RNA virus spreading in population by abundant mediators, such as air, water, food, touched things, and so on [4–6]. It is very important to develop efficient strategies to remove this virus and to capture it for detection from these mediators, especially from air and water.

Nanomaterials exhibited high surface areas and adjustable surface affinity to viruses. Adsorption of viruses by nanomaterials is an exciting topic to remove viruses for protecting human bodies from life-threatening infections, and to enrich them for sensitive detection [7,8]. A series of nanomaterials, such as polymer-based nanomaterials, natural nanofibers, graphene nanofilms, and so on [9–11]. However, it remains a great challenge to develop inorganic nanomaterials for efficient adsorption of viruses, especially SARS-CoV-2.

In this study, we synthesized a series of titanium oxide nanofibers, including dual-phase Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers and single-phase Ti$_3$O$_11$ nanofibers, by using hydrothermal methods and electrospinning-hydrogen reduction method. Protein and phospholipid adsorption assays were performed to evaluate the affinity of the nanofibers to model biomolecules. Adsorption of the nanofibers to SARS-CoV-2 pseudovirus was further detected to investigate the virus adsorption ability of the nanofibers. This study revealed that the dual-phase Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers are promising platforms capturing the dangerous SARS-CoV-2 to avoid its infection and to capture it for virus detection.

2. Materials and methods

2.1. Synthesis and characterization of the nanofibers

The Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers were synthesized using the Shi’s hydrothermal method with modification [12]. Briefly, 20 mL NaOH solution (containing 12 g NaOH in dH$_2$O) and 8 mL Ti(SO$_4$)$_2$ solution (containing 1.07 g Ti(SO$_4$)$_2$ in dH$_2$O) were mixed by magnetic stirring. The mixture was added in a 50-mL Teflon-lined stainless-steel reactor, followed by heated at 180 °C for 48 h, obtaining the Na$_2$Ti$_3$O$_7$ nanofibers. The nanofibers were then treated by 1 mol/L HCl for 1 h, obtaining the H$_2$Ti$_3$O$_7$ precursor. 100 mg of the precursor were mixed with 100 mg of polydopamine at Tris buffer (pH = 8.5) by magnetic stirring for 24 h. The coated precursor was then harvested by centrifugation and calcinated at 1000 °C for 1 h at a tube furnace, obtaining the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers. The Ti$_3$O$_11$ single-phase nanofibers were synthesized by electrospinning-hydrogen reduction method [13]. The synthesized nanofibers were characterized by transmission electron microscopy (TEM, Tecnai G² F-20, FEI, USA), X-ray diffraction (XRD, D/
max-2500, JAPANSCIENCE, Japan), Brunauer-Emmett-Teller (BET) analyzer (Micromeritics Automatic Surface Area Analyzer Gemini 2360, Shimadzu, Japan), Fourier transformed infrared spectra (FT-IR, FTS6000, Bio-rad, USA), and X-ray photo-electron spectroscopy (XPS, Kratos Analytical Ltd., Axis Ultra DLD, UK).

2.2. Protein and phospholipid adsorption assays of the nanofibers

The adsorption ability of the nanofibers to BSA and PE was evaluated according to our previous methods [14]. BSA adsorption was performed in 10 mL of distilled water, with the initial nanofiber concentration and BSA concentration of 500 mg/L in 40-mL vials. The vials were gently shaken at 37°C for 24 h, followed by centrifugation to remove the nanofibers. BSA concentration of the supernatants were measured by a Coomassie brilliant blue assay. PE adsorption was performed in 10 mL of 1:1 chloroform/methanol, with the initial nanofiber and PE concentration of 500 mg/L in 40-mL vials. After 24 h of shaking at 37°C, the suspensions were centrifuged, and PE concentration of the supernatants were measured using a LC–MS system (LCMS-2020, Shimadzu, Japan).

2.3. Virus adsorption assays of the nanofibers

To evaluate the adsorption ability of the nanofibers to SARS-CoV-2 pseudovirus, 100 μL of SARS-CoV-2 pseudovirus solution (FUBIO, China) were diluted in 10 mL dH₂O, and then 100 mg of the nanofibers were added. The mixture was shaken for 30 min and filtered. The filtered solids were stained by the antibody of the SARS-CoV-2 spike protein, followed by confocal microscope observation. The filtered liquid was used for RNA extraction in a nucleotide extractor (QIAcube HT, USA). The concentration of SARS-CoV-2 pseudovirus was also detected by the QX200 Droplet Digital PCR (ddPCR) System (Bio-Rad, USA), using the SARS-CoV-2 detecting primers COVID19-F (CAATGCTGCAATCGTGCTAC) and COVID19-R (GTTCGGACTACGTGATGAGG) [15].

3. Results

3.1. Characterisation of as-synthesized nanofibers

The Ti₃O₅/Ti₄O₇ titanium oxides were synthesized by calcination of the polydopamine-coated H₃Ti₃O₇ precursor. Both SEM and TEM observation showed that the products had regular nanofiber morphology, with the diameter of 50 – 100 nm, and the length of 3–10 μm (Fig. 1a, b). High-resolution TEM further showed an interplanar spacing of 175.6 pm in the nanofibers (Fig. 1b). XRD analysis showed that the nanofibers had mixed peaks of Ti₃O₅ (JCSPD# 82-1138) and Ti₄O₇ (JCSPD# 77-1390) (Fig. 1c), confirming the dual phase of the obtained nanofibers. XPS analysis revealed the main element compositions of the nanofibers, i.e., Ti and O, together with trace amounts of C, N, and Na (Fig. 1d, Fig. S1). Moreover, FT-IR analysis confirmed the presence of Ti-O (stretching vibration peak at 1636 cm⁻¹) and –OH (asymmetry and symmetrical stretching binding, 3179.56 cm⁻¹) (Fig. 1e). These results revealed the successful preparation of the dual-phase Ti₃O₅/Ti₄O₇ nanofibers. Brunauer-Emmett-Teller (BET) analysis further showed that the Ti₃O₅/Ti₄O₇ nanofibers had a BET surface of 14.5 m²/g (Table S1).

As the control nanofibers, two kinds of Ti₆O₁₁ single-phase nano-fibers (i.e., Ti₆O₁₁-1 and Ti₆O₁₁-2) were prepared by the electrospinning method. TEM observation, XRD and XPS characterization indicated that both kinds of the control titanium oxides had the similar nanofiber morphology (Fig. S2a), the XRD pattern of Ti₆O₁₁ (JCSPD# 85-1058, Fig. S2b), and the main composition of Ti and O (Fig. S2c). BET analysis further revealed that Ti₆O₁₁-1 and Ti₆O₁₁-2 had a BET surface of 235.9 m²/g and 36.0 m²/g (Table S1), respectively.

3.2. High affinity of the Ti₃O₅/Ti₄O₇ nanofibers to model biomolecules

Proteins and phospholipids are two main components on the surface of SARS-CoV-2. To compare the affinity between the nanofibers and biomolecules, two model molecules, i.e., the model protein BSA and the model phospholipids were chosen for adsorption assays. After co-
incubation of the nanofibers and BSA, the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers adsorbed much higher levels of BSA than the two kinds of control Ti$_6$O$_{11}$ nanofibers (155 mg/g versus 44 ~ 81 mg/g, Fig. 2a). Similarly, the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers also adsorbed much PE than the two kinds of Ti$_6$O$_{11}$ nanofibers (420 mg/g versus 128 ~ 255 mg/L, Fig. 2b). These results indicated that the dual-phase nanofibers had high affinity to both proteins and phospholipids, which may facilitate their adsorption to virus particles. The high affinity of the dual-phase nanofibers may be attributed to their surface vacancies that facilitate the nanofiber surfaces strongly binding the biomolecules.

3.3. Efficient adsorption of the SARS-CoV-2 pseudovirus by the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers

The high affinity of the dual-phase nanofibers suggested its high adsorption ability to the SARS-CoV-2 virus. To confirm this, a SARS-CoV-2 pseudovirus, which exposes the critical spike protein (S protein) on the virus surface and has similar surface properties of the real SARS-CoV-2, was used to assess the capacity of the nanofibers to adsorb SARS-CoV-2. After co-incubation of the nanofibers with the SARS-CoV-2 pseudovirus, the nanofibers were filtered and stained by FITC-tagged anti-S protein antibodies. Confocal microscopy revealed that the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers displayed strong FITC fluorescence on the surface (Fig. 3a). In contrast, the two kinds of control Ti$_6$O$_{11}$ nanofibers had only weak FITC fluorescence (Fig. 3a).

To confirm the strongest virus adsorption capacity of the dual-phase nanofibers among the three kinds of nanofibers, droplet digital PCR analysis of the virus in the filtered liquid were further performed. The results demonstrated that the filtered liquid after adsorption by Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers had much lower levels of virus concentrations than that after adsorption by Ti$_6$O$_{11}$ (25% versus 55~91%, Fig. 3b, c). These results revealed that the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers had much higher virus-adsorption capacity than Ti$_6$O$_{11}$. Besides, since abundant viruses had similar protein and lipid compositions to SARS-CoV-2, the Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers may also have high adsorption capacity to other kinds of viruses.

In conclusion, this study synthesized the dual-phase Ti$_3$O$_5$/Ti$_4$O$_7$ nanofibers by precursor preparation, polydopamine coating and furnace
calcination for efficient adsorption of the SARS-CoV-2 virus. The obtained dual-phase nanofibers exhibited much higher affinity to both the model molecules BSA and PE, and to the SARS-CoV-2 pseudovirus than the single-phase TiO$_2$ nanofibers. This study provided a novel strategy of SARS-CoV-2 adsorption by inorganic titanium oxide nanomaterials for avoiding its infection and for capturing it during rapid detection.

**CRediT authorship contribution statement**

Zhanlin Ding: Methodology, Software, Writing - original draft, Writing - review & editing. Hong Wang: Data curation, Investigation. Zhe Feng: Software, Validation. Meiqing Sun: Conceptualization, Investigation, Supervision.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgement**

This work is supported by Project of Science and Technology Development in Wuqing District, Tianjin (WQKJ202067).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2021.130167.

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