## **Supporting Information**

Biopolymer functionalized two-dimensional  $WS_2@TiO_2$  nanocomposite for in vitro biomedical and photocatalytic applications: Facile synthesis and characterization

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## **1.** Materials and methods

# 1.1. Preparation of CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC



Scheme 1 Schematic representation of the synthesis of CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC

#### 1.2. Antibacterial test

The Bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus subtilis* was obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh. The disc diffusion method was used to test the antibacterial activity of the prepared samples in vitro against Gram-negative and Gram-positive bacteria. The cooling medium was poured into sterile Petri plates and left to solidify. Using a sterile swab, the plates with media were seeded with the appropriate microbial suspension. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (NIT-Nitrofurantoin 300 µg for Bacteria) discs. The various concentrations (20 µg, 40 µg, 80 µg, and 120 µg) of the composites were loaded into the wells. The antibacterial test was carried out under visible light condition. The plates were incubated for 24 hours at 37°C. The diameter of the zone formed around the paper disc was measured and expressed in millimeters after the incubation period.

#### **1.3.** Biocompatibility Study

For the biocompatibility study, kidney epithelial (Vero cells) were procured from National Centre for Cell Sciences (NCCS), Pune, India. In this study, the MTT assay was used to assess the cell viability of synthesized CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC against Vero cells. Individual Vero cells were cultured in 75 cm<sup>2</sup> cell culture flasks at 37 C in a 5% CO<sub>2</sub> atmosphere being implanted into 96 well plates (1x10<sup>4</sup>/well) and bred for 24 hours. After that, the cells were cultured for 24 hours with different doses of CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC (6.25 µg/mL –100 µg/mL). Then, in a humid condition, 10 µL of MTT solvent (5 mg/ml) was added to each well, and the

bred time was increased for 4 hours. The development of formazan crystals was dissolved by adding 100  $\mu$ L of DMSO to the solution. The cell viability (%) was calculated using the Eq. (1),

% Cell viability = 
$$\frac{A_{570 nm Tested Cells}}{A_{570 nm} Control Cells} \times 100$$
 (1)

## 1.4. Anticancer analysis

The National Centre for Cell Science (NCCS) in Pune provided the breast cancer cell line (MCF 7) which was cultured in Eagles Minimum Essential Medium with 10% fetal bovine serum (FBS). The cells were kept at  $37^{\circ}$  C, 5% CO<sub>2</sub>, 95% air, and 100% relative humidity. Maintenance cultures were changed twice a week, and the culture medium was changed weekly. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine the anticancer activity of synthesized CS, WS<sub>2</sub>, TiO<sub>2</sub>, and CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC against human breast (MCF-7) malignant cells. At a plating density of 10,000 cells/well, 100 µl of cell suspension were seeded into 96-well plates. After 24 h, the cells were treated with different concentrations of the test samples (6.25 - 100 µg/mL). After 24 h of culture, the cells were incubated for 48 hours with varying amounts of DMSO-dissolved samples. After 48 h, 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well and incubated for 4 h at  $37^{\circ}$ C. The absorbance wavelength of each well was determined by a microplate reader at 570 nm.

% Cell inhibition = 
$$\frac{100 - \frac{A_{570 nm Tested Cells}}{A_{570 nm} Control Cells} \times 100$$
(2)

#### 1.5. Photocatalytic analysis

The photocatalytic activity of the samples was investigated by observing the photocatalytic dye degradation of model organic pollutants MB under visible-light irradiation from a 50 W tungsten halogen lamp. Separately, 0.025 M MB dye was dissolved in 100 ml of deionized water. 6 mg of each of the synthesized composites was added to the dye solutions and sonicated in the dark for 30 minutes to achieve adsorption-desorption equilibrium. The photocatalyst-mixed MB dye solution was then exposed to visible light for 150 minutes at 30-minute intervals. The UV-visible spectra were obtained until the complete degradation of the MB dye occurred. As irradiation time rises, the characteristic absorption peak intensity of MB (664 nm) is reduced.

#### 2. Results and discussions

#### 2.1 FTIR Spectroscopy

The FTIR spectra of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and the CS/WS<sub>2</sub>@TiO<sub>2</sub> NC were recorded between 4000 and 400 cm<sup>-1</sup>, as shown in Fig. S1 (a–d). The FTIR spectrum of the CS NPs exhibits a broad peak between 3462 and 3285 cm<sup>-1</sup>, which is attributed to the hydroxyl group (O–H) and N–H stretching vibration of the amino group. The peak at 2782 cm<sup>-1</sup> is caused by –CH<sub>2</sub> stretching. The notable bands at 1645 and 1564 cm<sup>-1</sup> can be attributed to the C=O stretching vibration of amide I and the N–H<sub>2</sub> bending vibration in combination with C–N stretching (amide II), respectively. Similar findings were also noticed by.<sup>1</sup> The spikes at 1412 and 1355 cm<sup>-1</sup> indicate

CH<sub>2</sub> bending and CH<sub>3</sub> symmetrical deformation, respectively. In the CS spectrum, other regular peaks at 1130, 1021, and 892 cm<sup>-1</sup> are identified, which correspond to -C-O- and -C-C stretching, respectively.<sup>2,3</sup> The FTIR spectra of the WS<sub>2</sub> nanosheets (red line) shows a broad band at approximately 3412 cm<sup>-1</sup>, which is linked to the O–H group. The peaks at 1641 and 1458 cm<sup>-1</sup> are linked with the expansion deformation of the hydroxyl group. The characteristic peaks at 726 and 945 cm<sup>-1</sup> are related to W–S and S–S bonds, respectively.<sup>4,5</sup> The FTIR spectra of the TiO<sub>2</sub> NPs (blue line) shows a band at 3454 cm<sup>-1</sup>, which relates to the stretching vibration of the hydroxyl group (O–H) adsorbed on various active sites on the TiO<sub>2</sub> surface. Another band at 1630 cm<sup>-1</sup> is due to the H–O–H bending vibration of physisorbed water.<sup>6</sup> Note that water molecules interact strongly with the TiO<sub>2</sub> surface, resulting in considerable broad OHstretching vibration. The peak at 532 cm<sup>-1</sup> is due to Ti–O stretching in TiO<sub>2</sub>.<sup>7,8</sup> The FTIR spectra of the NC (green line) shows that the bending vibration peaks of  $-NH_2$  are shifted slightly, from 1564 to 1557 cm<sup>-1</sup>, compared to those of pure CS. New vibration peaks at 810, and 640 cm<sup>-1</sup> correspond to the W–S, and Ti–O interactions between CS, tungsten, and titanium, respectively. Furthermore, the peaks of the hydroxyl, carbonyl, and amine groups are shifted to lower wavenumbers. This shift indicates that WS<sub>2</sub> and TiO<sub>2</sub> are widely dispersed in the CS polymer matrix. Also, the FT-IR findings indicate that chitosan was successfully functionalized with WS<sub>2</sub> and TiO<sub>2</sub> via mussel-inspired chemistry and that poly-cationic chitosan interacts with the WS<sub>2</sub> and  $TiO_2$  via the amine groups. Hence, these connections may major impact on the biomedical and photocatalytic applications of the composites.



Fig. S1 (a–d) FTIR spectrum of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and CS/WS<sub>2</sub>@TiO<sub>2</sub> NC was recorded between 4000- 400 cm<sup>-1</sup>

### 2.2 TGA

Fig. S2 depicts the TGA curves of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and the CS/WS<sub>2</sub>@TiO<sub>2</sub> NC between 0 °C and 1000 °C. The TGA curve of CS (black line) indicates three stages of decomposition. The first stage of mass loss occurs between 20 °C and 150 °C and is caused primarily by the loss of a small amount of water embedded in the polymer network. The second stage of mass loss occurs between 220 °C and 340 °C and is related to the decomposition of organic moieties. The third stage of weight loss, at ~380 °C to 600 °C, is due to the decomposition of the polymer.<sup>9, 10</sup> The amount of CS residue at 1000 °C is 26.32%. The TGA curves of WS<sub>2</sub> (red line) and TiO<sub>2</sub> (blue line) show very little mass loss between 0 °C and 1000 ° C because these materials are inorganic.<sup>11,12</sup> The residual amounts of these two inorganic materials at 1000 °C are 80.53% and 82.62%, respectively. The TGA curve of the NC (green line) shows little mass loss compared to the organic material CS. The first stage of mass loss occurs at ~120 °C and is caused primarily by the loss of a small amount of water. The second stage occurs between 210 °C and 320 °C and is related to the decomposition of organic moieties. The third stage, between ~380 °C and 600 °C, is due to the decomposition of the polymer. At 1000 °C, the residual amount of the NC is 60.24%. Note that the integration of WS<sub>2</sub> and TiO<sub>2</sub> NPs into the CS matrix greatly improves the stability of this composite.



Fig. S2 TGA spectra of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC

# 2.3. Surface morphology and elemental composition analysis



Fig. S3 (a, b) FE-SEM images of CS NPs at different magnifications

Table S1: The elemental c	omposition of CS/WS <sub>2</sub> @TiO <sub>2</sub> com	posite in EDS spectrum
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Element	Weight %	Atomic %
СК	63.82	73.84
NK	05.48	04.52
ОК	04.12	03.68
WК	10.76	07.85
S K	07.18	05.33
Ti K	08.64	04.78



Fig. S4 (a-f) Elemental mapping images of CS/WS<sub>2</sub>@TiO<sub>2</sub> NC



Fig. S5 Selected area electron diffraction (SAED) patterns of CS/WS<sub>2</sub>@TiO<sub>2</sub> composites.

# 2.4. Antibacterial activity



Fig. S6 (a-d) Antibacterial activity of CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC against different bacteria

Table S2: Antibacterial assay of CS/WS<sub>2</sub>@TiO<sub>2</sub> composite against *S. aureus*, *B. subtilis*, *K. Pneumoniae*, and *E. coli* microbes obtained by Agar diffusion method

Antibacterial activity			
Microorganism	Concentration	Zone of Inhibition	
	(µg/mL)	(mm)	
	20 µg	20	
S. aureus	40 µg	21	
	80 μg	23	
	120 µg	24	
	20 µg	13	
B. subtilis	40 µg	14	
	80 µg	16	
	120 μg	19	
	20 µg	12	
E. coli	40 µg	14	
	80 µg	16	
	120 µg	17	
	20 µg	23	
K. Pneumoniae	40 µg	24	
	80 µg	25	
	120 µg	27	

The morphology and oxidative stress are key attributes in the antibacterial activity of nanomaterials. Therefore, it is essential to thoroughly assess the possibility of cellular oxidative stress caused by the NC. Oxidative stress can occur through ROS-mediated and ROS- independent pathways. This research considers ROS-mediated oxidative stress, which is induced by ROS-generated composite-based nanomaterials.<sup>13, 14, 15</sup> The antibacterial mechanism is illustrated in Fig. S7. The antibacterial properties of composites have been related to NPs interactions with sulfur-containing proteins on the cell membrane causing cell wall destruction. The prepared composites have a positive charge (the WS<sub>2</sub> nanosheets release W<sup>4+</sup> ions, the TiO<sub>2</sub> NPs release Ti<sup>4+</sup> ions, and CS also has a positive charge). The high electrostatic interaction between positive metal ions and bacterial cells aided composite adsorption on the cell surfaces. The CS/WS<sub>2</sub>@TiO<sub>2</sub> NC deposited on the cell surface prevented the cell membrane from moving freely, leading to membrane damage and cytoplasmic leakage. Consequently, smaller NPs were more harmful to bacteria but did not harm eukaryotic cells. These findings suggest that these nanomaterials might be useful as antimicrobial agents in biomedical applications.



Fig. S7 Antibacterial mechanism of the synthesized CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC

#### 2.5. Biocompatibility

Biocompatibility is a significant factor in whether nanomaterials can be used successfully for biological applications such as bioimaging and theranostics. The biocompatibility of the CS/WS<sub>2</sub>@TiO<sub>2</sub> NC with normal cell lines was evaluated to investigate its suitability for use as an anticancer agent. Kidney epithelial cells (Vero cells) were employed as normal cell line models. The viability of the Vero cells was tested after they were mixed with CS, WS<sub>2</sub>, TiO<sub>2</sub>, and the CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC at concentrations of 6.25 to 100 µg/mL. A conventional MTT assay was used to assess the cytotoxicity of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and the NC (see Fig. S8). The cell viability values for the NC in the 6.25–100 µg/mL range are 97.06%, 95.45%, 91.75%, 87.32%, and 83.56%, respectively. The viabilities of cells incubated with the NC all exceeded 80% as the NC concentration was increased, demonstrating the good biocompatibility of the composites. As a result, the CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC materials are non-toxic, making them ideal candidates for biomedical applications.



Fig. S8 Cell viability of CS, WS<sub>2</sub>, TiO<sub>2</sub>, and CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC against Vero cells

# 2.7. Anticancer activity

Table S3: Anticancer activity of CS,  $WS_2$ ,  $TiO_2$ , and  $CS/WS_2@TiO_2$  composite tested with MCF-7 cancer cells at various concentrations obtained by MTT assay method

S. No.	Tested Samples	Concentration (µg/mL) % Cell inhibition	
1	CS	6.25	7.53
		12.5	18.32
		25	34.64
		50	45.81
		100	52.45
2	WS <sub>2</sub>	6.25	10.46
		12.5	31.97
		25	44.37
		50	54.06
		100	67.08
3	TiO <sub>2</sub>	6.25	9.33
		12.5	21.31
		25	42.08
		50	51.16
		100	63.59
4	CS/WS <sub>2</sub> @TiO <sub>2</sub>	6.25	12.24
		12.5	33.91
		25	48.42
		50	61.03
		100	74.72

The metal NPs in the composite polymer bind to and penetrate the negatively charged cancer cell membrane, disrupting cell metabolic activity and causing cell death. Thus, many studies have found that ROS, oxidative stress, and mitochondrial dysfunction are important anticancer cytotoxic mechanisms. In biological systems, ROS [primarily superoxide radicals (O\*<sub>2</sub>), hydroxyl radicals (\*OH), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)] are routinely generated and discharged.<sup>16,17</sup> Fig. S9 shows the anticancer mechanism of the CS/WS<sub>2</sub>@TiO<sub>2</sub> NC. The negatively charged cancer cell membrane links with the NC. Positively charged W<sup>+</sup> and Ti<sup>+</sup> ions released from the NC interact with tumor cells (which are negatively charged), resulting in damage to proteins, lipids, DNA, and mitochondria, and eventually tumor cell mortality.



Fig. S9 Anticancer mechanism of the synthesized CS/WS<sub>2</sub>@TiO<sub>2</sub> hybrid NC.

## 2.8. Photocatalytic activity



Fig. S10 (a–c) UV absorbance spectra of CS,  $WS_2$ , and  $TiO_2$  against MB with different time intervals

**Table S4:** A comparative analysis of photocatalytic performance of the materials developed in

 this research and other recently reported heterojunction composite nanostructures

Material	Dye	Dye degradation (%)	Time taken for degradation (min)	Ref.
MoS <sub>2</sub> /TiO <sub>2</sub>	RhB	81.8	180	18
MoS <sub>2</sub> /TiO <sub>2</sub>	MO	89.1	130	19
MoS <sub>2</sub> /g-C <sub>3</sub> N <sub>4</sub>	MO	60	300	20
GO/TiO₂	MO	85	240	21
TiO <sub>2</sub> /Zn <sub>0.5</sub> Cd <sub>0.5</sub> S	MB	94	240	22
MoS <sub>2</sub> /ZnO	MB	81	80	23
CS/WS <sub>2</sub> @TiO <sub>2</sub>	MB	89.4	150	This work

The photocatalytic activity is known to be affected by many factors, including the light absorption capacity, separation efficiency of photogenerated electrons and holes, and photogenerated hole oxidation ability. Fig. S11 illustrates the photocatalytic mechanism, which involves charge transfer during the degradation of MB dye by the CS/WS<sub>2</sub>@TiO<sub>2</sub> catalyst. Under visible light illumination, electrons in the SC jump from the valence band (VB) to the conduction band (CB). WS<sub>2</sub> is an SC with a narrow bandgap (1.43 eV), whereas with the broad band gap of anatase TiO<sub>2</sub> (3.2 eV). Electrons in the VB of WS<sub>2</sub> can be stimulated to the CB by visible light, causing holes in the VB. By contrast, a small number of electrons in TiO<sub>2</sub> could be stimulated from the VB to the CB. The photoinduced electrons in the WS<sub>2</sub> CB are shifted to the TiO<sub>2</sub> CB, which serves as a photoelectron receiver. The electrons can be captured by oxygen  $(O_2)$  and converted to superoxide radical anions ( $\bullet O_2^-$ ), which can then react with a contaminant. Furthermore, the holes in the WS<sub>2</sub> VB can react with H<sub>2</sub>O to form hydroxyl radicals, which can then react with the dye.<sup>24, 25</sup> In photocatalytic reactions, these reactive radicals can efficiently convert MB to  $H_2O$  and  $CO_2$ , as well as other harmless molecules.



Fig. S11 Photocatalytic mechanisms describing charge transfer during the degradation of dye MB by  $CS/WS_2@TiO_2$  catalyst.

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