

Supplementary Information

Titanium dioxide/Graphene oxide blending into Polyethersulfone Hollow Fiber Membranes improves Biocompatibility and Middle Molecular weight Separation for Bioartificial Kidney and Hemodialysis applications

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1. Synthesis of Graphene Oxide (GO)

Graphene oxide (GO) was synthesized using a modified Hummers method as described in the research paper ¹. Initially, 5 g of graphite powder and 2.5 g of sodium nitrate (NaNO₃) were mixed in 108 mL of sulfuric acid (H₂SO₄) and 12 mL of phosphoric acid (H₃PO₄) in an ice bath for 15 minutes. Subsequently, 15 g of potassium permanganate (KMnO₄) was gradually added while maintaining the temperature below 5°C, and the mixture was stirred for 2 hours to facilitate oxidation. The reaction mixture was then transferred to a 40°C water bath and stirred for 1 hour, followed by heating in a 98°C oil bath. To ensure complete oxidation, 280 mL of deionized (DI) water was added, and stirring continued for another hour. The reaction was terminated by adding 15 mL of 30% hydrogen peroxide (H₂O₂), after which the mixture was cooled to room temperature. The resulting suspension was centrifuged and subjected to multiple washing cycles with DI water and 5% hydrochloric acid (HCl) until a neutral pH was achieved. The final GO product was dried in a vacuum oven at 90°C, yielding GO powder. [Figure S1](#) shows the pictorial representation of synthesis of graphene oxide by modified hummers method.

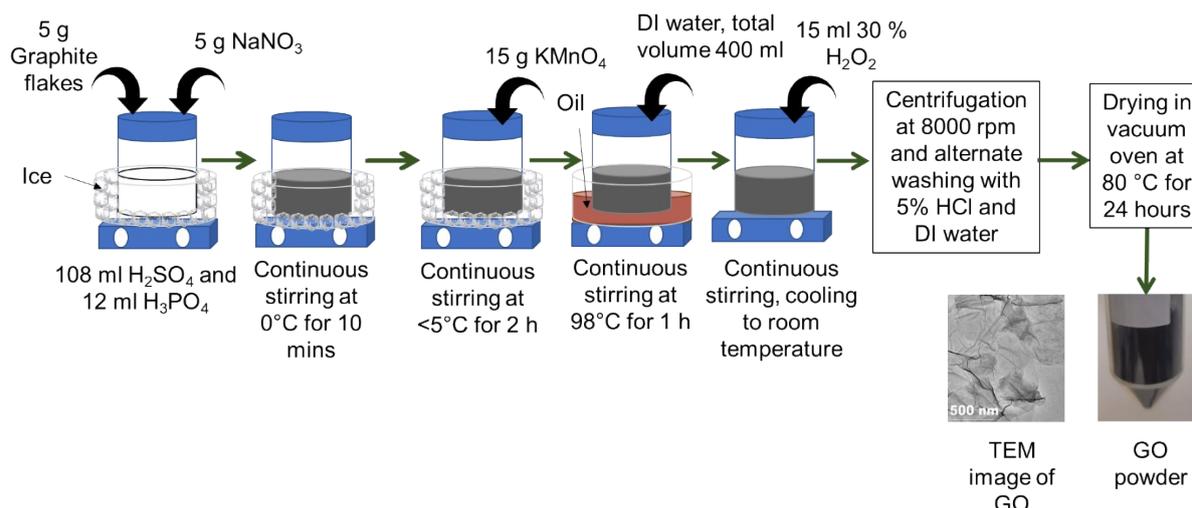


Figure S1: Synthesis process of Graphene Oxide by modified hummers method.

2. Synthesis of TiO_2/GO nanocomposite by hydrothermal treatment

A 2 mg/mL aqueous dispersion of graphene oxide (GO) was prepared by dispersing 200 mg of GO in 100 mL of Milli-Q water, followed by stirring at 250 rpm for 30 minutes and ultrasonication for 1 hour. TiO_2 (100 mg) was then added to achieve a GO/TiO_2 ratio of 2:1, with subsequent stirring for 30 minutes and ultrasonication for 1 hour. The colour of the dispersion changed from black to grey, indicating interaction between GO and TiO_2 . The dispersion was transferred to a teflon-lined stainless-steel autoclave and heated at 180°C for 8 hours in a vacuum oven. After cooling to room temperature, the dispersion underwent ultrasonication and centrifugation at 8000 rpm for 30 minutes before being stored at -80°C for freeze-drying.² [Figure S2](#) shows the synthesis protocol of TiO_2/GO nanocomposite.

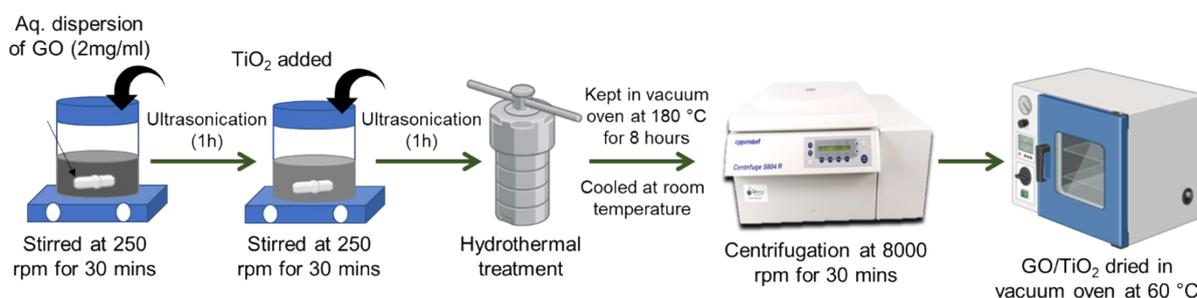


Figure S2: Synthesis procedure of TiO_2/GO nanocomposite by hydrothermal treatment

3. Physicochemical characterization of TiO₂/GO nanocomposite

SEM, TEM and XRD pattern of TiO₂/GO nanocomposite is shown in [figure S3](#). SEM and TEM micrographs of the TiO₂/GO nanocomposite demonstrate that TiO₂ nanoparticles were successfully decorated onto the GO nanosheets, confirming the effective incorporation of TiO₂ onto the GO surface.

The XRD pattern of GO/TiO₂ also showed similar peaks as that of TiO₂ indicating no change in the crystal structure of TiO₂. No peak of GO was observed in the XRD pattern due to low GO content or due to low intensity of diffraction.³

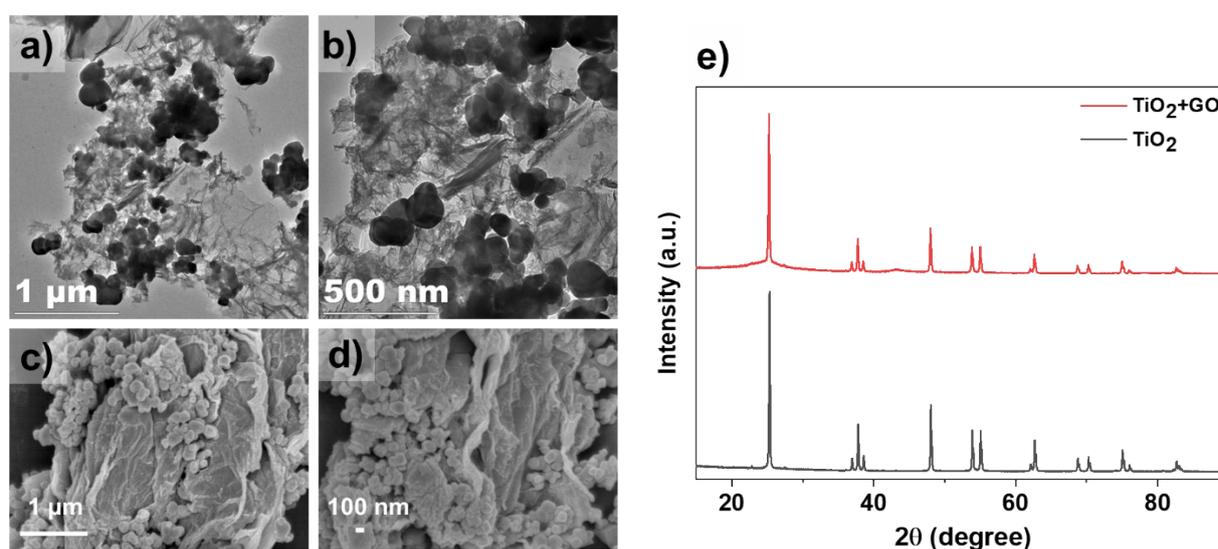


Figure S3: a-b) TEM micrographs, c-d) SEM micrographs, e) XRD pattern of TiO₂/GO nanocomposite. SEM and TEM micrograph showed successful decoration of TiO₂ nanomaterial on graphene oxide (GO) nanosheets. XRD pattern of TiO₂/GO nanocomposite showing no change in TiO₂ peak, and no GO peak due to low intensity of diffraction.

The synthesis of TiO₂/GO nanocomposites via hydrothermal treatment typically results in the reduction of graphene oxide (GO) to reduced graphene oxide (rGO), which significantly decreases the material's hydrophilicity. Synthesis by this method is also tedious and time-consuming. However, for our application, an additive that enhances hydrophilicity is essential. Therefore, we explored a more straightforward and efficient approach. In a study conducted by Zhang et al., TiO₂/GO nanocomposite was successfully synthesized using a facile ultrasonication method, involving the direct mixing of TiO₂ nanoparticles with a GO dispersion, providing a simpler and more feasible alternative.⁴

In our study, we have also followed the similar protocol by direct incorporation of TiO₂ and GO nanomaterial into polymeric solution to fabricate TiO₂/GO incorporated HFMs.

4. Confocal micrographs of modified HFMs

Confocal micrographs of modified HFMs (TG(0.5/1.5), TG(1/1), TG(1.5/0.5), 2T, and 2G) is shown in [figure S4](#). TiO₂/GO incorporated HFMs showed dense cells attached on its surface promoting both spheroid formation and spreading and attachment of cells. This result is in agreement with our earlier study in which TiO₂ PES HFMs promotes spheroid formation.⁵ In the earlier studies we observe that GO PES HFMs support enhanced cells attachment of various cell lines.

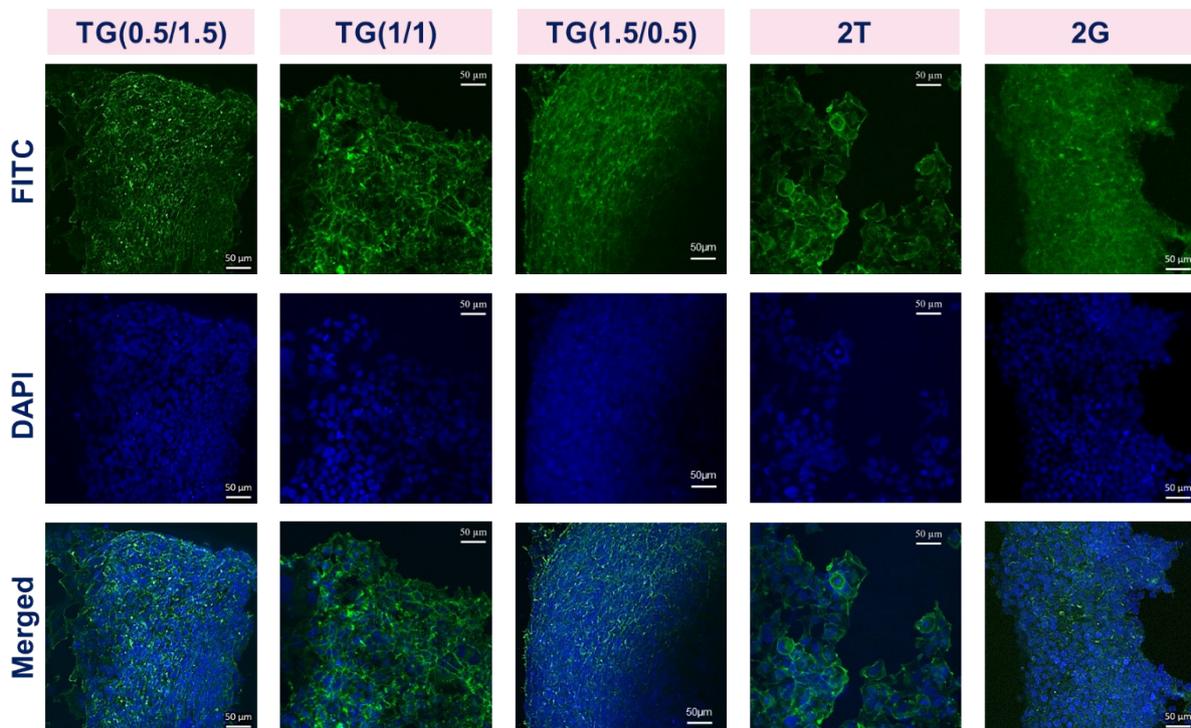


Figure S4: Confocal micrographs of modified HFMs on day 8 depict green fluorescence from FITC and blue fluorescence from DAPI, and the merged image demonstrating the colocalization of both signals. The TiO₂/GO-incorporated HFMs exhibit enhanced cell attachment and spheroid formation, highlighting the synergistic effect of TiO₂ and GO in promoting improved cellular interactions and growth.

5. Hemolytic range for biomaterials

[Table S1](#) shows the hemolytic range for biomaterials to be utilized for blood contacting application as per ASTM F-756-08 standards. HFMs above 5% hemolysis percent are not suitable to use for implants and blood contacting applications.

Table S1: Table showing the hemolysis range for biomaterials (from ASTM F-756-08)

% Hemolysis	Evaluation of hemolytic properties
0-2	Absence of hemolytic effect
2-5	Mild hemolytic
>5	Hemolytic

References

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- 3 M. Safarpour, V. Vatanpour and A. Khataee, *Desalination*, 2016, **393**, 65–78.
- 4 L. Zhang, W. Yu, D. Ma, Z. Sun and Q. Zhang, 2016, **63**, 973–977.
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