Supporting Information

Graphene oxide linked sulfonate-based polyanionic nanogels as biocompatible, robust and versatile modifiers to ultrafiltration membranes

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1. Preparation and characterization of graphene oxide (GO)

Graphene oxide (GO) was prepared from natural graphite flakes by a modified Hummers method. Briefly, 5 g graphite and 3.75 g NaNO₃ were placed in a flask. Then, 150 mL H₂SO₄ was added with stirring in an ice-water bath, and then 20 g KMnO₄ was slowly added for over 1 h. The mixture was stirred in an ice water bath for 2 h, followed by a vigorously stirring for 3 days at room temperature. Then, the mixture was diluted with DI water (500 mL) slowly, and the excessive KMnO₄ was decomposed by H₂O₂ (30 wt.%, 15 mL). The insoluble precipitations were removed by centrifugation. The resulted GO solution was filtered and washed with HCl (10 wt.%, 1 L) and DI water for several times to remove the metal ions. The pristine brown GO solution was dialyzed with DI water for 1 week before use to remove any residual salts and acids.

The prepared GO was characterized with several methods. Morphological observation was carried out with a transmission electron microscope (TEM) (JEM-1200EX, JEOL, Japan). Two to three drops of the solution (1 mg/mL) were spread on a 3 mm copper grid and dried at ambient temperature to prepare the TEM sample. A Multimode Nanoscope V scanning probe microscopy (SPM) system (Bruker, USA) was used to obtain atomic force microscopy (AFM) image. The sample was prior prepared by dropping and drying on silicon wafer for the AFM analysis. FTIR spectrum was acquired on a FTIR spectrometer (Nicolet 560, USA) between 500 and 4000 cm⁻¹, using the KBr disk method. A Q500 Thermogravimetric analyzer (TA
instruments, USA) was used to get the thermogravimetric analysis (TGA) curve of the sample under a dry nitrogen atmosphere, and the temperature ranged from 50 °C to 700 °C with a heating rate of 10 °C/min.

**Figure S1.** (A) TEM image and corresponding SAED patterns of GO. (B) Typical AFM image and cross-section analyse of GO. (C) Chemical structure of GO. (D) FTIR spectrum for the prepared GO. (E) The TGA image for the prepared GO.

**2. Morphology of the SPN nanogel**

**Figure S2.** TEM image of the prepared SPN nanogel without adding of GO.
3. Elution experiments and components of the membranes

The obtained membranes were rinsed frequently in DI water for two weeks by refreshing the water every day. And the absorbance curves of the rinsing DI water for different days were detected by an UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan). To further investigate the eluting performances, the newly prepared membranes were extracted for 24 h with a Soxhlet extractor, and the absorbance curves of the extracting solutions for PES/SPN2 and PES/GO-SPN2 were detected by an UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan).

![Graphs showing absorbance curves](image)

**Figure S3.** (A) Absorbance curves after rinsing PES/GO-SPN2 in DI water for different days. (B) Absorbance curves of the extracting solutions for the samples of PES/SPN2 and PES/GO-SPN2. The absorbance curves for the pure nanogel solutions of SPN and GO-SPN were also given for the elution comparison.

The conversion efficiency of the monomers could be calculated by the following equation:

\[
Conversion \, efficiency = \left( \frac{W_{\text{total}} - W_{\text{PES}} - W_{\text{GO}}}{W_{\text{monomers}}} \right) \times 100\% \quad (S1)
\]
where \( W_{\text{total}} \) is the total dry weight of the sample obtained by liquid-liquid phase inversion method; \( W_{\text{PES}} \) is the added weight of PES during the polymerization; \( W_{\text{GO}} \) is the added weight of GO; \( W_{\text{monomers}} \) is the weight of the added monomers.

4. FTIR and TGA curves of SPN nanogel blended PES membrane

![FTIR and TGA curves](image)

**Figure S4.** FTIR spectra (B) and TGA curve (C) of the PES/SPN2 membrane.

5. XPS curves of the GO-SPN nanogel

![XPS curves](image)

**Figure S5.** XPS spectrum for the nanogel of GO-SPN.

6. Porosity test

The porosity of the membranes was measured by a dry-wet weight method using the following equation:\(^2\)
\[
\text{Porosity} = \frac{(W_w - W_{D})}{W_D / \rho_\text{w} + (W_w - W_D) / \rho_\text{w}} \times 100\% \tag{S2}
\]

where \(W_w\) is the wet weight of the sample; \(W_D\) is the dry weight of the sample; \(\rho_\text{w}=1.0\) g/cm\(^3\) is the density of water; \(\rho_\text{P}=1.43\) g/cm\(^3\) is the density of PES.

**Table S1.** The porosities of the membranes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PES</th>
<th>PES/GO</th>
<th>PES/GO-SPN1</th>
<th>PES/GO-SPN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity (%)</td>
<td>71.38±1.06</td>
<td>74.21±0.91</td>
<td>76.78±0.81</td>
<td>77.31±1.08</td>
</tr>
</tbody>
</table>

7. **Viscosity test**

The viscosity of the casting solutions was detected by a rotational rheometer (AR2000ex, TA) with a shearing rate of 100 s\(^{-1}\) and the results are shown in Table S2.

**Table S2.** The viscosity for the casting solutions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PES</th>
<th>PES/GO</th>
<th>PES/GO-SPN1</th>
<th>PES/GO-SPN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (mPa·s)</td>
<td>603.8±23.5</td>
<td>857.3±31.7</td>
<td>1125.4±45.8</td>
<td>1442.3±56.9</td>
</tr>
</tbody>
</table>

8. **The pH-dependent size of nanogel**

**Table S3.** The size of the nanogel GO-SPN at different pH value measured by dynamic light scattering.

<table>
<thead>
<tr>
<th>pH</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (nm)</td>
<td>40.3± 3.8</td>
<td>66.4± 5.4</td>
<td>117.5± 9.5</td>
<td>154.9± 13.4</td>
<td>235.3± 18.6</td>
<td>348.4± 24.7</td>
</tr>
</tbody>
</table>
9. ELISA

Contact activation and complement activation are important parameters to evaluate the reaction or interaction between blood and materials; they are also direct methods to evaluate the blood compatibility of material. Commercial enzyme-linked immunosorbent assays (ELISA) were used to evaluate the contact activation, including platelet activation (Platelet Factor 4 (PF4), Boatman Biotech Co., Ltd, China) and coagulation cascade activation (thrombin-antithrombin III complex (TAT), Enzygnost TAT micro, Assay Pro, USA), as well as the complement activation (C3a and C5a, BD Opt EIA™, BD Co., Ltd, US) for the prepared membranes. The whole blood (collected with special blood collection tube containing EDTA-K2) incubated with the membrane for 2 h was centrifuged for 15 min at 1000 g (4 °C) centrifugal force to obtain the testing plasma. Then, the detections were carried out according to the respective instruction manuals.
Figure S6. (A) Thrombin–antithrombin (TAT) concentrations for the samples. (B) Platelet factor 4 (PF4) concentrations for the samples. (C) C3a concentrations for the samples. (D) C5a concentrations for the samples. Values are expressed as means ± SD, n = 3.

10. Cytocompatibility

Human umbilical vein endothelial cells (HUVECs) were grown in R1640 medium supplemented with 10% fetal bovine serum (Hyclone, USA), 2 mmol L-glutamine and 1 vol.% antibiotics mixture (10,000 U penicillin and 10 mg streptomycin). Cultures were maintained in a humidified atmosphere of 5% CO$_2$ at 37 °C (Queue Incubator, France). Confluent cells were detached from the culture flask with sterilized PBS and 0.05% trypsin/EDTA solution. The culture medium was changed every day.$^4$

For fluorescence staining (fluorescein diacetate/propidium iodide (FDA/PI)), after culturing for 48 h, the culture polystyrene plate was washed three times by PBS solution, then 400 µL FDA (10 µg/mL, PBS) was added to each well, and the cell culture plate was placed into an incubator (37 °C, 5% CO$_2$). After incubating for 5 min, 200 µL PI (20 µg/mL, PBS) was added, and then the cell culture plate was washed three times by PBS solution. The whole experimentation was manipulated free from light. Then the cell culture plate was enwrapped in silver paper, and a fluorescence microscope (Olympus IX53, Japan) was used to observe the fluorescence images of the samples.
For confocal laser scanning microscopy observation, after culturing for 48 h, the cells were immediately rinsed with 37 °C PBS and fixed in 4 wt.% paraformaldehyde for 10 min. Then the cells were washed with PBS and further treated with 0.5% Triton X-100 in PBS for 5 min. The cellular actins were stained with Rhodamine-phalloidin (100 nM) in the dark for 30 min. The nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI, 5 μg/mL) for 5 min. Subsequently, the stained cells were imaged with a confocal laser scanning microscope (CLSM, Leica).

The viability of the cells was determined by MTT assay after cell culture for different times (2, 4 and 6 day). The cells cultured in wells without PES membranes served as control in this study. For MTT assay, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well and incubated for 4 h at 37 °C. Mitochondrial dehydrogenases of viable cells selectively cleave the tetrazolium ring, yielding blue/purple formazan crystals. Then, 400 μL of ethanol was added to dissolve the formazan crystals. Thus, the quantity of the formazan dissolved in the ethanol reflects the level of cell metabolism. The solution was shaken homogeneously for 15 min. The sample solution was aspirated into microtiter plates, and the optical density was read in a Microplate reader (model 550, Bio-Rad) at 492 nm. The difference among individual groups was evaluated using the Student’s t-test, and the level of significance was chosen as P < 0.05.

11. Mechanical property tests of the prepared membrane samples
Figure S7. The tensile strain of the membrane samples (FSM and HFM).

12. Characterization of the Ag-nanoparticle loaded membranes

Figure S8. (A) EDX mapping analysis of the Ag-nanoparticle loaded membrane PES/GO-SPN2-Ag. (B) XPS spectrum of the Ag-nanoparticle loaded membrane PES/GO-SPN2-Ag.
References


