Supporting Information

High Refractive Index Inorganic-Organic Interpenetrating Polymer Network (IPN) Hydrogel Nanocomposite Toward Artificial Cornea Implants

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Materials: Zinc acetate dihydrate ($Zn(Ac)_2{\cdot}2H_2O$, 98%, Alfa-Aesar), mercaptoethanol (ME, 98%, Alfa-Aesar), thiourea (99%, Alfa-Aesar), 2-hydroxyethyl methacrylate (HEMA, 97%, Sigma-Aldrich), acrylic acid (AA, 99.5%, Alfa-Aesar), 1,4-diisocyanatobutane (97%, Sigma-Aldrich), 2-hydroxy-2-methylpropiophenone (Darocur 1173, 97%, Sigma-Aldrich), triethylene glycol dimethacrylate (TEGDMA, 97%, Sigma-Aldrich), dibutyltin dilaurate (95%, Alfa-Aesar), bovine serum albumin (Sigma-Aldrich), bicinchoninic acid solution (Sigma-Aldrich), copper (II) sulfate solution (Sigma-Aldrich, 4% (w/v) prepared from copper (II) sulfate pentahydrate) and bovine serum albumin (Quick Start™, standard set, BIO-RAD) were purchased and used without further purification. N,N-dimethylformamide (DMF, Fisher Scientific) was of HPLC grade and purified by vacuum distillation prior to use. All other solvents were of analytical grade and were used as-received.

ATCC (American Type Culture Collection, Rockville, MD, USA) cell lines, PCS-200-010 (primary epidermal keratinocytes; normal, human, neonatal), PCS-200-030 (dermal cell basal medium) and PCS-200-040 (keratinocyte growth kit, containing PCS-999-005 (apo-transferrin),

PCS-999-023 (rh TGF-α), PCS-999-022 (rh insulin), PCS-999-008 (epinephrine), PCS-999-015 (L-glutamine), PCS-999-013 (hydrocortisone) and PCS-999-009 (extract-p)) were obtained from Bio-REV PTE LTD. Primary epidermal keratinocytes (normal, human, neonatal, PCS-200-010) were maintained in 75 cm^2 tissue culture polystyrene flasks in complete medium consisting of dermal cell basal medium (PCS-200-030) and keratinocyte growth kit (PCS-200-040) supplemented with 10% fetal bovine serum (FBS).

Characterization of ZnS NPs: Transmission electron microscopy (TEM) was carried out using a JEOL-3010 microscope with an accelerating voltage of 200 kV. The TEM specimen were prepared by dissolving a few milligram of the ZnS NPs powder in DMF to form a homogeneous solution and by putting a drop of the solution on the copper grids coated with carbon film. The X-ray diffraction (XRD) data were collected on a Bruker AXS D8 X-ray diffractometer with a Cu Kα (λ = 1.5406 A°) source, operated at 40 kV and 20 mA at 293 K. The 2θ scanning range was from 20^o to 70^o with a step size of 0.02^o and at a scanning speed of 1 degree/min. Fourier transform infrared (FTIR) spectra were recorded on a Bio-Rad digilab FTS 3100 spectrometer. The FTIR pellets were made from approximately 2 mg of the sample and 100 mg of KBr. Thermogravimetric analysis (TGA) was carried out in a PerkinElmer Diamond TG/DTA instrument at a heating rate of 10 °C/min from ambient temperature to 700 ^oC under a flow of nitrogen at 200 mL/min. The weight percentages of carbon, hydrogen, sulfur and nitrogen were measured in an Elementarvario CHNS elemental analyzer. The weight percentage of zinc was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) on a Perkin Elmer ICP Optima 2000DV machine.

NMR measurement: The ^1H NMR measurement for ZnS/ME and ZnS/HEMA was carried out in a Bruker 300MHz NMR spectrometer using DMSO-D6 as the solvent.

UV-vis measurement. The UV-vis measurement of a thin pieces of flat film of ZnS/PHEMA/PAA IPN hydrogel was carried out in a Shimadzu UV-2450 spectrometer with a wavelength range of 275-700 nm.

RI measurement and swelling studies: The refractive index (RI) of the IPN hydrogel nanocomposite at the wavelength of 589 nm was measured on a NAR-4T abbe refractometer at 25 °C, using methylene iodide containing sulfur solution (for dry state) and monobromonaphthalene (for hydrated state) as contact liquids. The sample was cut to 20–30 mm in length, around 8 mm in width and 3–10 mm in height, followed by washing and surface polish. The RIs of three duplicate cuts from the same sample were measured for consistency check. The equilibrium water content of the ZnS/PHEMA/PAA IPN hydrogel was estimated by comparing the dry and the swollen weights. The swollen gel soaked in deionized water was taken out and petted dry for measuring of the weight at regular time intervals until equilibrium was attained. The equilibrium water percentage was calculated using Equation (1):

$$W\% = (W_s - W_d) / W_s \times 100\% \qquad (1)$$

where W_s and W_d are the weights of swollen and dry nanocomposites, respectively.

Cytotoxicity studies: The *in vitro* cytotoxicity studies were conducted using a transwell cell culture system. Cells were incubated in an environment at 37 ^oC and 5% CO_2 for 4 d to allow cellular attachment and growth before further experiments. The IPN hydrogel was cut into small circular pieces followed by sterilization in ethanol and water mixture (70:30 of volume ratio) at

room temperature for 1 d. Before cell seeding, the IPN hydrogel was soaked with sterilized phosphate buffer (PBS) solution for 1 h and then immersed on polycarbonate filter inserts in 24-well tissue culture polystyrene (TCPS) for indirect contact with primary epidermal keratinocytes. Primary epidermal keratinocytes and the culture medium were then added to the plate wells. TCPS well was used as a positive control and PHEMA/PAA hydrogel without ZnS NPs was used for comparison. The medium was changed every 2 d. The seeding density was 1.0×10^5 cells/mL. Cells were stained by LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Molecular Probe), containing calcein AM and ethidium homodimer (EthD-1) for identification of live and dead cells, respectively. After a certain period of cell seeding time ranged 1-7 d, the medium was discharged and the culture was washed with PBS. Then ''Live/Dead'' solution containing 2 μM of calcein AM and 4 mM of EthD-1 was added and the mixture was incubated at 37 ^{o}C for around 20 min. The populations of live and dead cells in the stained cultures were analyzed using fluorescent microscopy (Axiovert 200 motorized inverted microscope system).

Methyl tetrazolium (MTT) assay: MTT assay was used to examine mitochondrial function and cell proliferation. MTT solution with 5 mg/mL concentration was prepared by dissolving thiazolyl blue tetrazolium bromide in PBS. After the cells were cultured for certain time periods (1 d, 3 d, 5 d and 7 d), 100 μL of MTT solution was added to the culture medium in each well of the 24-well plate containing IPN hydrogel nanocomposite. The culture media were incubated on a shaker at 37 °C for 4 h for color development. The medium was removed after 4 h and DMSO was added to dissolve the formazan formed. The plate was incubated for another 2 h. After that, the DMSO with solubilized formazan was transferred to a 24-well assay plate and absorbance was measured using a microplate reader (Biorad) at a wavelength of 490 nm. Three parallel readings were averaged for each sample.

Protein adsorption study: Fluorescein isothiocyana (FITC)-labeled bovine serum albumin (BSA) was adopted in the protein absorption studies on the ZnS/PHEMA/PAA hydrogel. An appropriate amount of FITC-labeled BSA was dissolved in PBS solution to prepare a FITC-BSA solution with a concentration of 3 mg/mL. The ZnS/PHEMA/PAA IPN hydrogel was cut into small circular pieces and soaked in the PBS solution for 1 h. The samples were then immersed in the FITC-labeled BSA solution and incubated on a shaker at 37 °C for 4 h. After incubation, the surfaces of the IPN hydrogel was thoroughly washed with PBS solution and the imaging experiment was conducted on an Olympus IX71 inverted microscope, photometrics CoolSNApcf cooled CCD.

Cell adhesion study: The procedures for cell culture and the treatment of IPN hydrogel were the same as above. IPN hydrogel were placed into 24-well TCPS and washed three times with PBS and one time with the cell media. The wells were seeded with cells followed by incubation at 37 ^{o}C for 24 h. The seeding density was 1.0×10^5 cells/mL. After incubation, the surfaces of the wells were washed three times with the cell media and cell adhesion was assessed in a phase contrast microscopy (Pascal 5 confocal microscope system).

Zn^{2+} leaching detection: The ZnS/PHEMA/PAA IPN hydrogel was incubated in the cell culture medium (a phenol-red free medium) for 4 h. After incubation, 1 mL of cell medium was taken out and added with 10 μL of a Zn-sensitive fluorescent dye (Fluozin3, Invitrogen, 0.06 mM) for the fluorescence measurement. The fluorescence spectra were collected on Fluoromax-3 (Horiba Sientific) (λ_{ex}=494 nm).

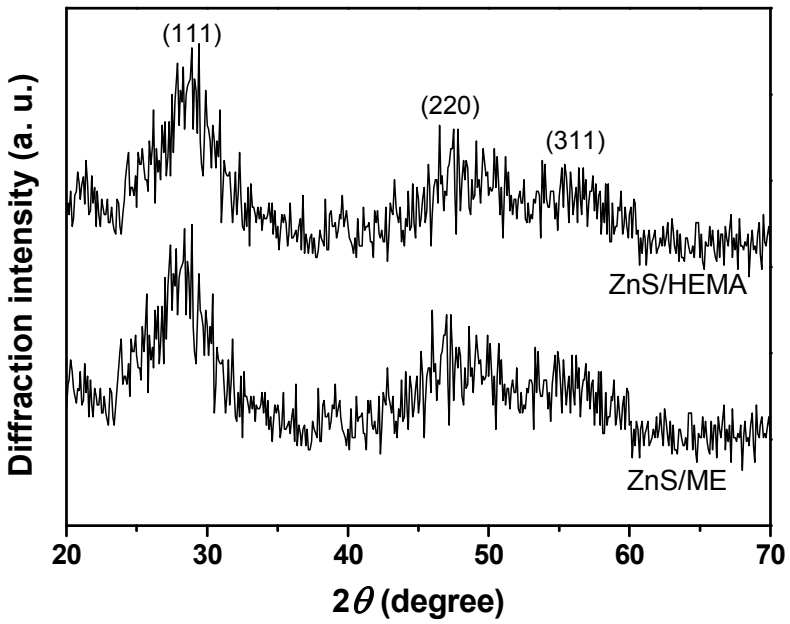

Figure S1. XRD patterns of ME and HEMA capped ZnS NPs.

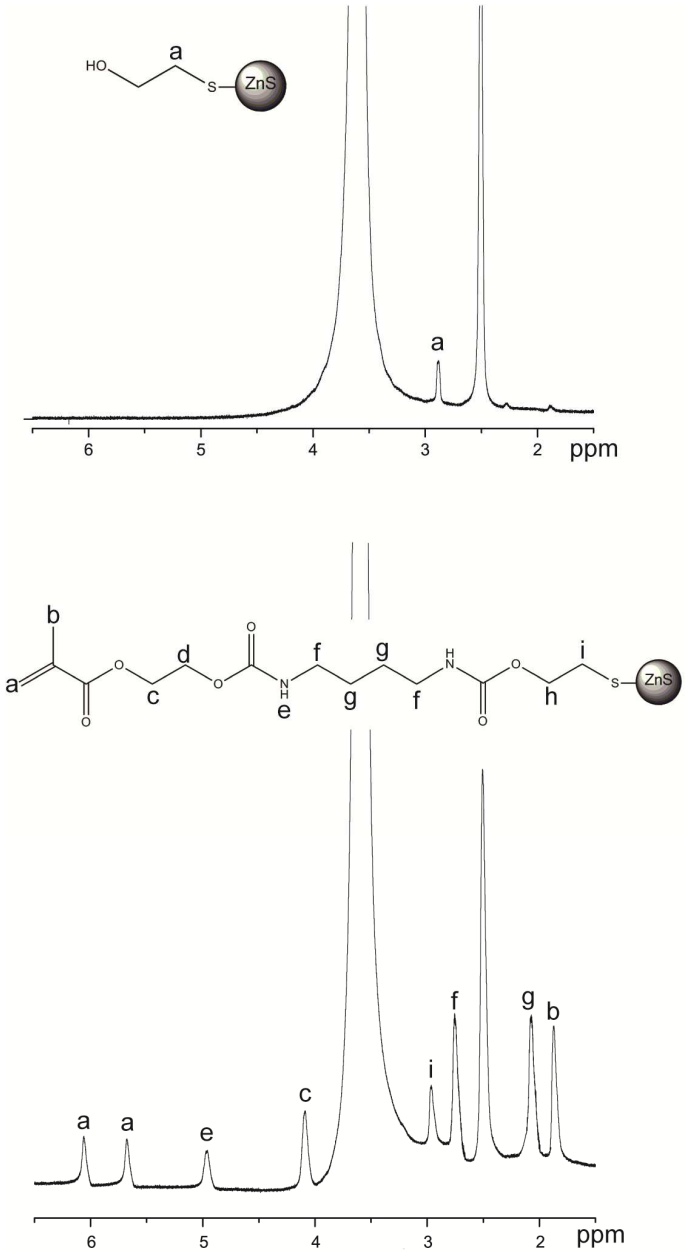

Figure S2. ^{1}H NMR spectra of ZnS/ME and ZnS/HEMA.

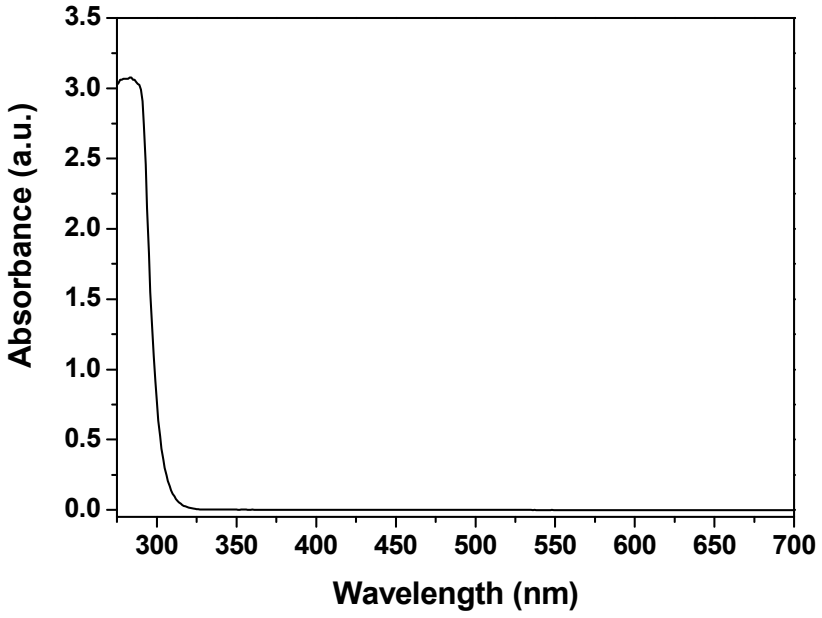

Figure S3. UV-vis absorption spectrum of ZnS/PHEMA/PAA IPN hydrogel nanocomposite.

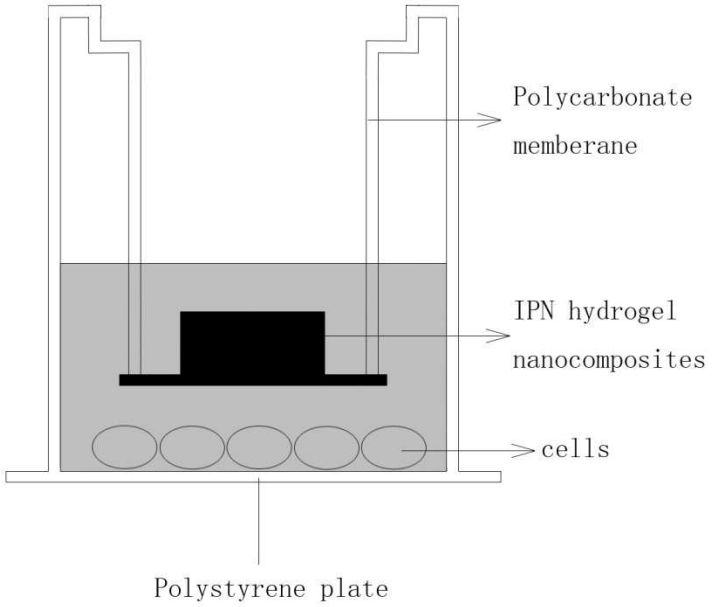

Figure S4. Schematic representation of transwell cell culture with indirect contact with cells for *in vitro* cytotoxicity study of IPN hydrogel nanocomposites.

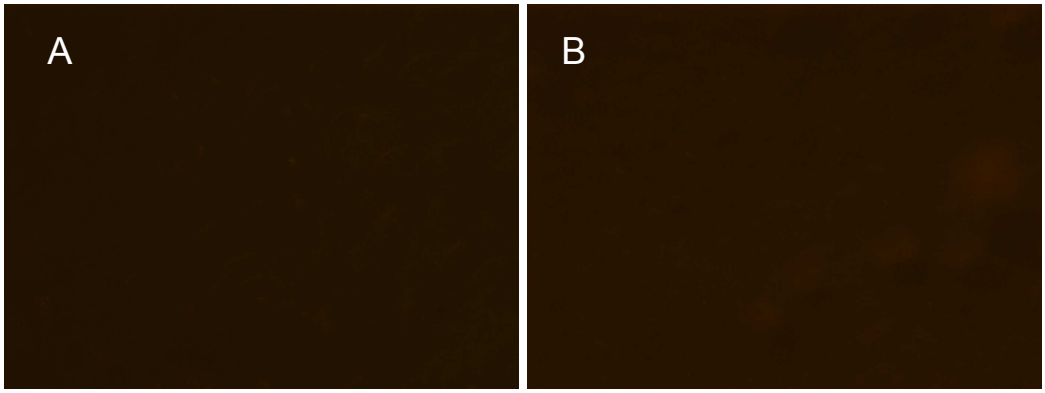

Figure S5. Fluorescent images of ZnS/PHEMA/PAA IPN hydrogel nanocomposite (A) after protein adsorption study, and (B) control sample without protein study.

Figure S6. Cell adhesion on ZnS/PHEMA/PAA IPN hydrogel nanocomposite at 10 × magnification after 24 h incubation with primary epidermal keratinocytes.

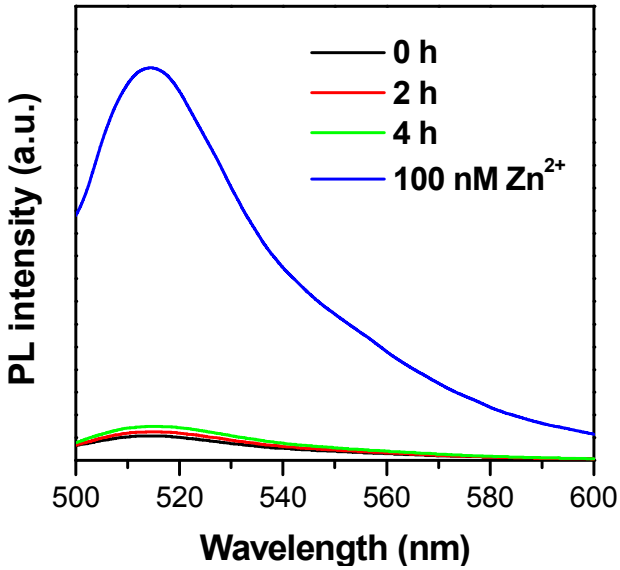

Figure S7. PL spectra of cell culture medium after incubation with ZnS/PHEMA/PAA IPN hydrogel for different time and being added with a Zn-sensitive fluorescent dye (Fluozin3).

Table S1 Chemical analysis of the ME and HEMA capped ZnS NPs and the dry IPN nanocomposite.

Sample	Weight%	C	H	S	N	Zn
ZnS/ME	Measured[a]	9.82	2.24	35.74	-	43.26
	Calculated[b]	10.95	2.30	35.95	-	43.51
ZnS/HEMA	Measured[a]	31.36	4.34	16.39	5.72	19.86
	Calculated[b]	32.82	4.46	16.58	5.89	20.07
ZnS/PHEMA/PAA	Measured[a]	37.63	4.88	12.58	4.54	15.42
	Calculated[b]	37.12	4.75	12.44	4.42	15.05

[a]Data obtained from CHNS and ICP analyses. [b]Estimated from TGA weight loss data.