

Supporting Information

Selective Cleavage of Polyphosphoester in Crosslinked Copper based Nanogels: Enhanced Antibacterial Performance Through Controlled release of Copper

**Nagarajan Padmavathy[#], Paresh Kumar Samantaray^S, Lopamudra Das Ghosh[#],
Giridhar Madras[±], Suryasarathi Bose^{#*}**

Experimental

Materials

All reagents were of analytical grade or of the highest purity. Copper acetate, diethylene glycol (DEG) and sodium hydroxide (NaOH) were obtained from Spectrochem, India. 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) was used as received from TCI chemicals, India. Methoxy polyethylene glycol (mPEG) with M_w 2500 g/mol, 3-butyn-1-ol, trimethyl phosphosphine, thiourea (TU), 1,8-diazabicyclo-undec-7-ene (DBU) and methoxy polyethylene glycol (mPEG) with molecular weight \sim 2500 g/mol were procured from Sigma. PVDF with $M_w \sim$ 440,000 g/mol (Kynar 761) and styrene-maleic anhydride with M_w 80000 g/mol (Polyscope) were procured from Arkema. All the solvents and other reagents were obtained from Sd-Fine private limited and used as received.

Characterization Techniques

Characterization of hybrid nanogel and membranes was performed by Fourier transform infrared spectroscopy (FT-IR, PerkinElmer, USA) from 4000–650 cm^{-1} . The morphology of the nanogel particles was studied by transmission electron microscopy (TEM, JEOL JEM) operated at 200 kV. A drop of particle dispersion was mounted onto a copper grid (200 mesh) and the grid was dried at 25° C before imaging. The $^1\text{H-NMR}$ and $^{31}\text{P-NMR}$ spectra for all the samples was recorded on a 400 MHz instrument (Bruker) using CDCl_3 and D_2O as solvents.

To view the surface and cross sectional morphologies of the PVDF/SMA membranes, samples were cryo-fractured in liquid nitrogen and mounted on an aluminum stub and

sputter-coated with gold prior to SEM imaging (SEM ULTRA 55, FESEM with EDS, Carl Zeiss). The water contact angles of the polymeric membranes modified and pristine PVDF/SMA were assessed at 25 °C using FTÅ 200 contact angle goniometer (Dataphysics, Germany) by placing 3 µl water droplets on the samples using the sessile drop method. The contact angles reported in triplicates from three discs and the readings were obtained as soon as the contact angle became static with time.

Size measurements of the nanogels at 25°C were performed using dynamic light scattering (DLS) by Zeta sizer, Malvern, with a laser diode operates at 658 nm. The samples in the glass sizing cell were equilibrated for 30 mins before measurements and analyzed using a log correlator over 120 accumulations for a 3.0 mL sample. The average hydrodynamic radius of the particles was reported from the histograms of 120 accumulations of number distributions.

To determine the molecular weights of the PBYP prepared through macro initiative ring opening polymerization, 50 mg of dried PEG-PBYP was dissolved in THF and analyzed by gel permeation chromatography (GPC, Waters, THF column). Using monochromatic Cu K α radiation (1.541Å), powder X-ray diffraction (XRD) pattern was recorded for CuO NPs on a Bruker diffractometer. To test the contents of Cu ions in the filtered water leaching from the nanogel and in the membranes, the experiments were performed to determine the level of Cu ions from permeate filtered through the CuO-PPE containing PVDF/SMA membrane. At predetermined time intervals, 10 mL of permeate was collected through the membranes for ICP-MS measurements (Inductively Coupled Plasma Mass Spectrometry, QTEGRA, ThermoFischer).

Flux measurements

The separation properties of all the prepared membranes were measured by an inbuilt cross flow setup the pressure of filtration cell was supplied by a booster pump and all the filtration experiments were carried out at the pressures of 0.066MPa to 0.2 MPa (10 to 30 psi). Prior to the measurements, at each pressure, the membranes were stabilized for 30 minutes. The flux volume of filtered water was collected for a certain time and flux was calculated by the following.

$$J = V / A \times t \quad (1)$$

Where, J (L/m²h) is the volume of permeated water, t (h) is the permeation time, and A (m²) is effective area for filtration.

Membrane fouling assessment

To evaluate the antifouling properties of the modified membranes, static adsorption fouling tests were executed through quantitative analysis with BSA as model protein pollutant. All the tested membranes were cut into regular shape of $2 \times 3 \text{ cm}^2$ and immersed into 50 mL of BSA (0.5 g/L, pH 7.4) phosphate buffer solution, and triplicate samples for each polymer membranes were tested to obtain the mean values.

On the basis of the dynamic filtration fouling test, the water flux recovery ratio (FRR), the irreversible flux decline ratio (IFR) and relative fouled flux ratio (RFR) were employed to evaluate antifouling ability, it should be noticed that the membrane with the higher value of FRR and lower value of IFR indicated the better antifouling properties. The used pollutant solution includes BSA (1g/ L, pH 7.4).

The loop filtration process was executed consists of three steps, first, stable flux (J) was obtained via pure water filtration, second, the feed solution was changed to pollutants solution (BSA) and another stable flux (J_p) was obtained, then a cleaning process was introduced and the membrane was taken out from the filter system to immerse in the phosphate butter for 10 min and rinsed with pure water for 20 min, and reinstalled back into filtration system, the second flux (J_2) of pure water was recorded in the third step. All the FRR, IFR, and RFR values were calculated by the following equations.

$$\text{FRR} = J_2 / J \times 100 \% \quad (2)$$

$$\text{IFR} = (1 - J_2 / J) \times 100 \% \quad (3)$$

$$\text{RFR} = J_p / J \quad (4)$$

Biological Studies

Bacteria culture

The bacterial strains used for present study was *Escherichia coli* (*E. Coli*) (MG 1655) obtained from (NCBS), Bangalore. The strains were cultured at 37°C and revived on Luria-Bertani (LB) broth, supplemented with beef extract plates and yeast. To obtain pure culture, single colony was picked from the agar plate and used for inoculating 5 ml of freshly prepared LB for overnight suspension culture at 37°C in an incubator shaker at 100 rpm. 100 μL of the overnight cultured cell suspension was sub-cultured further in fresh media for 4 h under same incubating conditions.

ROS analysis

The production of ROS by the PVDF/SMA membranes before and after modification was examined using the dichlorofluorescein diacetate dye from Sigma Aldrich. The dye was added to the bacterial suspension and incubated for 30 mins in dark. The fluorescent measurements were obtained by exciting at 485 nm and collected emission signals at 528 nm. For both PVDF-SMA and modified PVDF-PPE-CuO membranes were evaluated and bacterial suspension in PBS was used a control.

MTT Assay

In order to quantify the viability of the HaCAT cells on the permeate collected from the CuO-PPE modified and pristine PVDF/SMA membranes, MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, assay was performed. The cells were cultured on the sterilized polymeric samples kept in 24 well plates at an approximate density of 3×10^3 cells/well with further incubation for 1, 4 and 7 days in 5% CO₂ incubator at 37°C temperature and 95% humidified atmosphere. After the desired period of incubation, the culture media was aspirated and replaced by 0.5mg MTT/ml reagent prepared in DMEM without serum. The enzyme mitochondrial succinate dehydrogenase of metabolically active cell reduces the yellowish water soluble MTT to water insoluble blue-violet coloured end product known as formazan crystals. This reaction rate will be directly proportional to the presence of number of mitochondrial active viable cells on the sample. After Day 1, Day 4 and Day 7 of incubation at 37° C, un-reacted MTT dye was removed and formazan crystals were solubilized by addition of 200µl dimethyl sulfoxide. The optical density (O.D) was quantified spectrophotometrically in an ELISA microplate reader (iMark, Bio-radlaboratories, India) at 595 nm.

For morphological observation of the HaCAT cells on the permeate, cells were fixed with 3.7 % formaldehyde for 30 min and washed twice with PBS (pH 7.2). The cells were permeabilized with 0.1% TritonX-100 for 10 min and washed twice with PBS. Cells were stained for 30 min with 6.6 µM Alexaflor 488 solution (Life Technologies) for F-actin and 14.3 µM DAPI (Life Technologies) for 2 min to stain the nuclei. The cells were imaged using an epi-fluorescence microscope (Olympus IX 53).

Figure S1

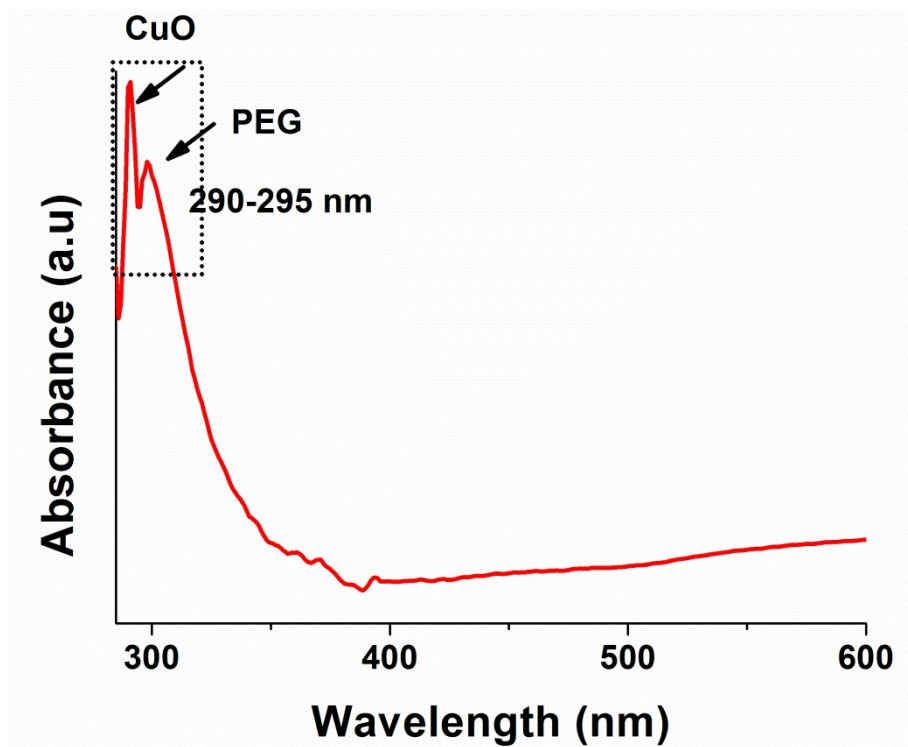


Figure S1. The UV-Visible spectrum of CuO-mPEG in ethanol shows the presence of PEG at 290 nm along with native peak of CuO.

Figure S2

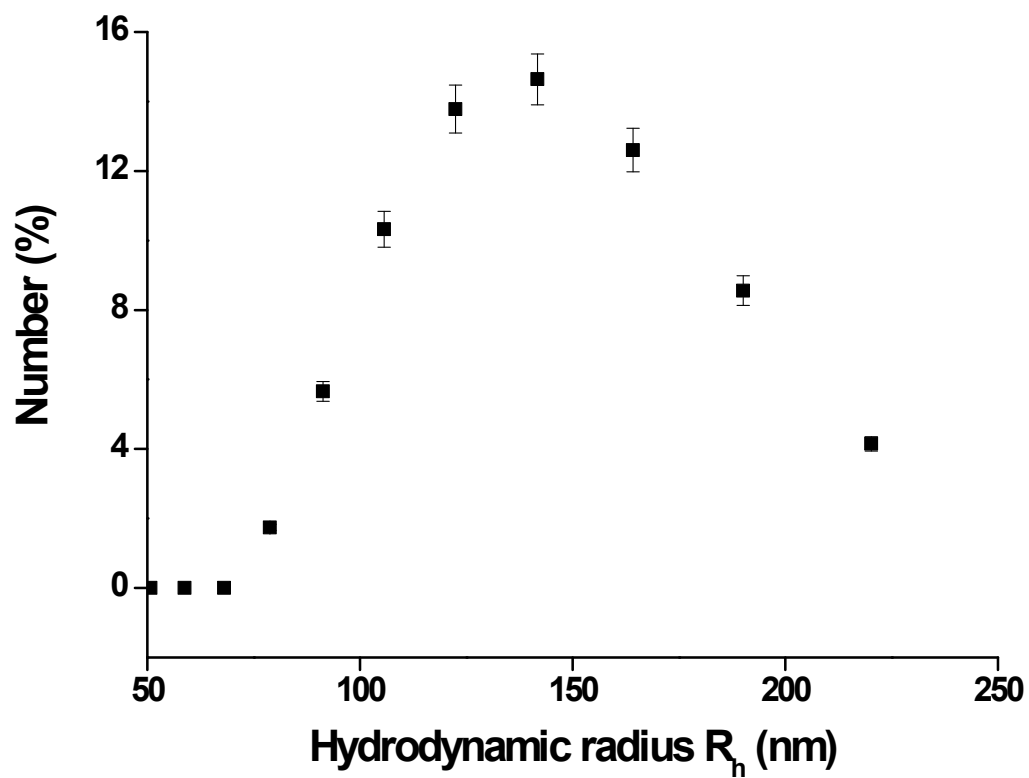
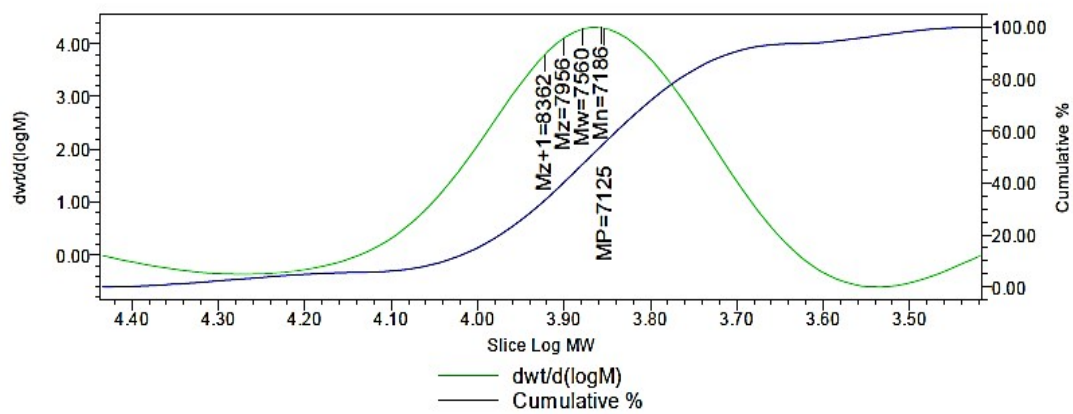


Figure S2. The hydrodynamic radius of the as prepared nanogel in water.

Figure S3



GPC Results

Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1	7186	7560	7125	7956	8362		1.052151		

Figure S3. The gel permeation chromatogram of the nanogel synthesized in the absence of copper oxide but with mPEG as initiator

Figure S4

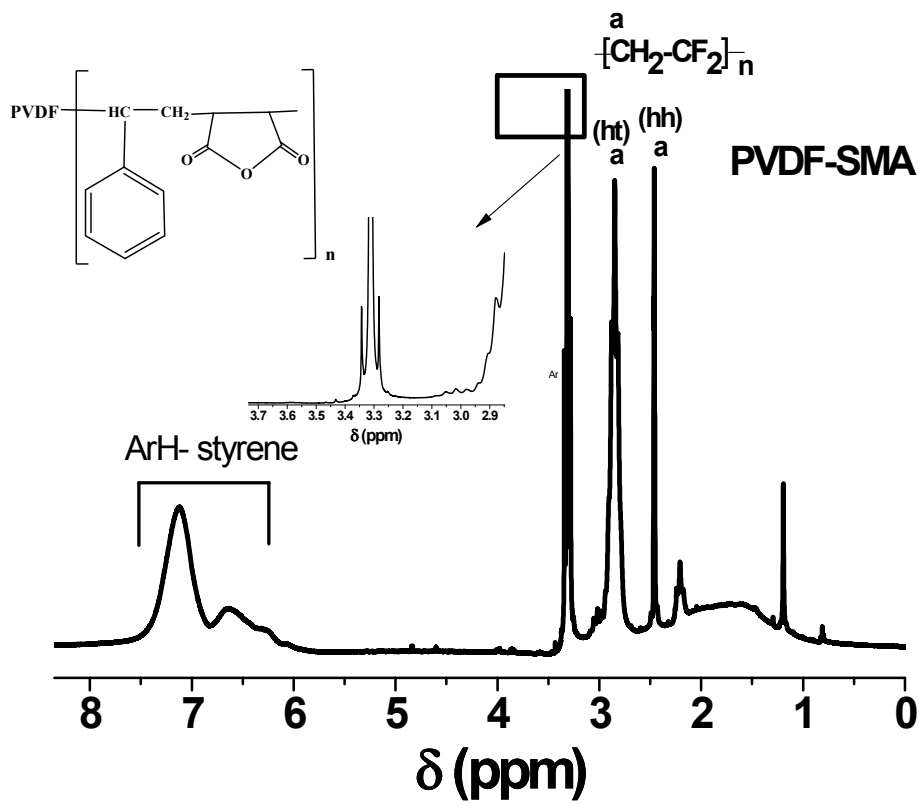
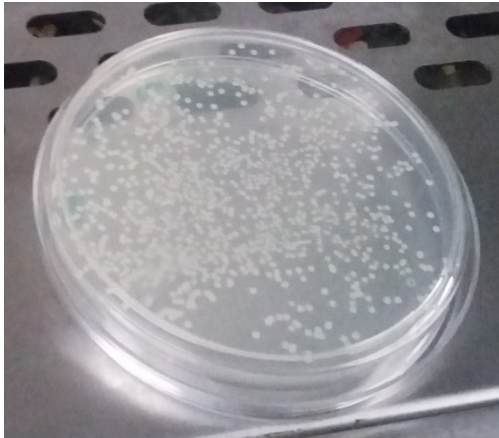


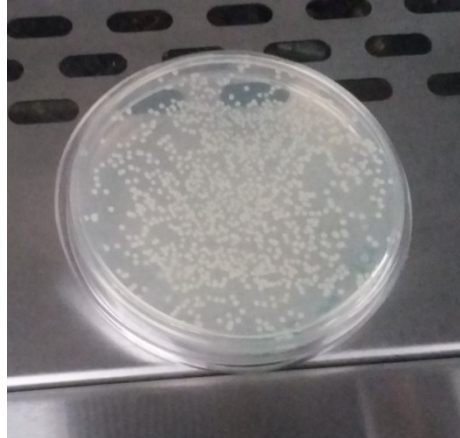
Figure S4. ^1H NMR spectrum of neat PVDF-SMA membrane dissolved in d_6 -DMSO

Figure S5

E.coli



PVDF-SMA



PVDF-PPE-CuO

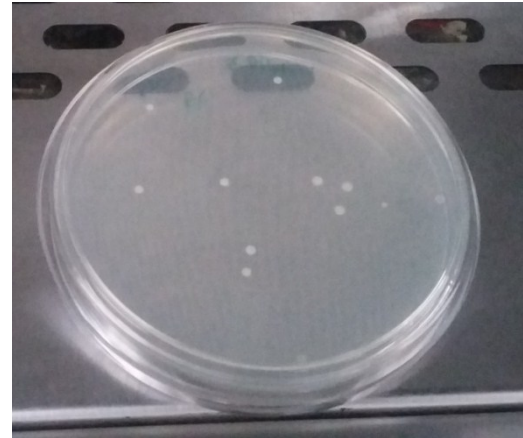


Figure S5. Visual photographs of the colonies grown /inhibited by the membranes

Figure S6

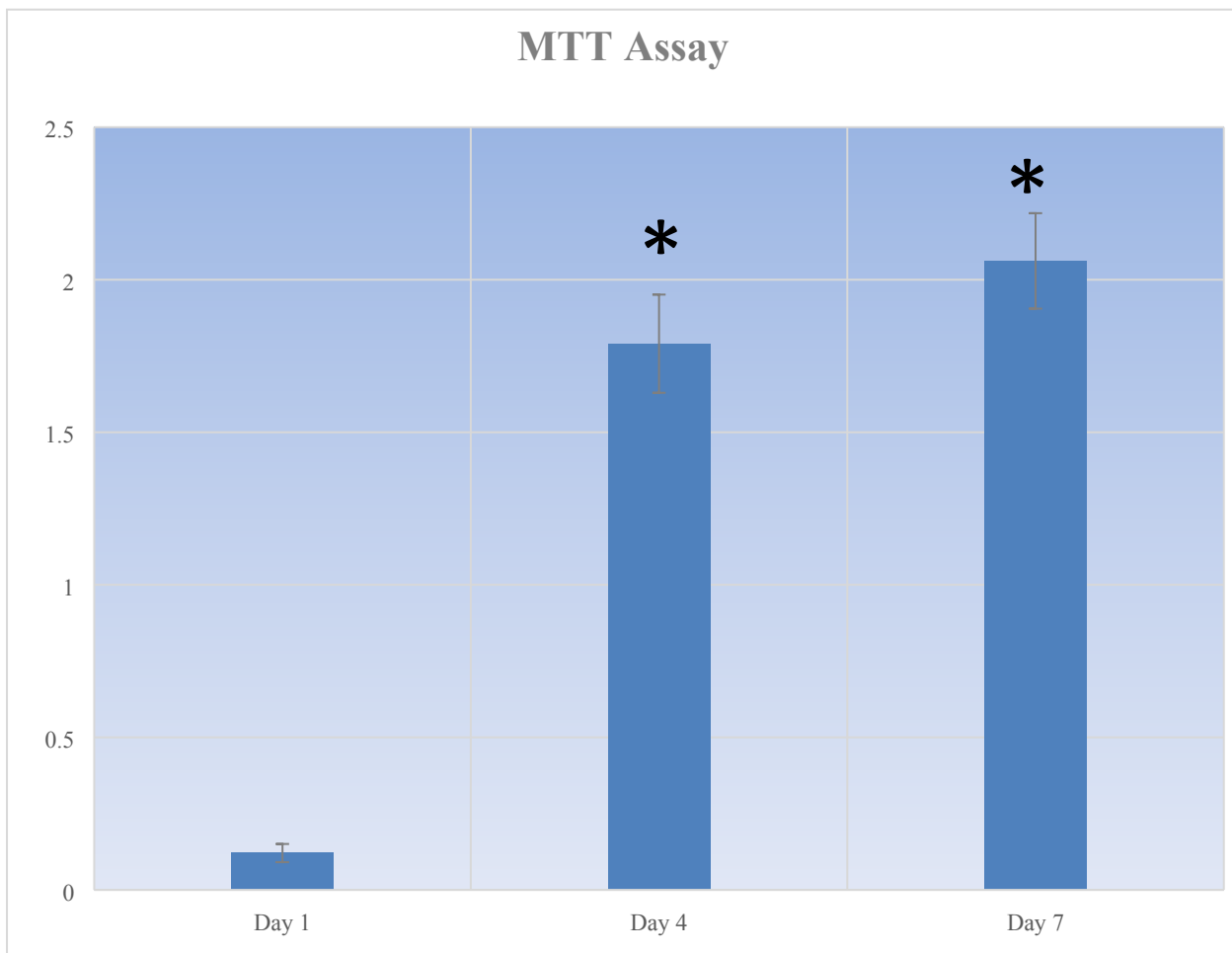


Figure S6. MTT assay of the permeate filtered through PVDF-PPE-CuO membrane and tested against epithelial cells. The absorbance was measured at 595 nm.

Table S1

Time (days)	Release of Copper from the membrane	
	%	ppm
0	0	0
3	0.20 ± 0.05	0.035 ± 0.007
7	0.25 ± 0.05	0.045 ± 0.007
10	0.36 ± 0.05	0.065 ± 0.007
12	0.47 ± 0.01	0.08 ± 0.006
15	0.55 ± 0.03	0.095 ± 0.007
18	0.56 ± 0.04	0.11 ± 0.005
21	0.55 ± 0.05	0.12 ± 0.01
30	0.57 ± 0.06	0.125 ± 0.006
4h- bacterial cells	9.6 ± 0.1	1.6 ± 0.3

Table S1: Copper release studies from the PVDF-PPE-CuO membrane by ICP-MS analysis up to 30 days in water and in bacterial cells (10^4 CFU/mL) incubated for 4h. One-way Anova was carried out for statistical analysis.