Supporting Information

A Protein-Based Self-healing Hydrogel for Prolonged Antimicrobial Drug Delivery With Synergistic Activity

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Experimental Section

METHODS

Optimization of Parameters for the Synthesis.

To prepare homogeneous solutions with a concentration of 40 mg mL^{-1}, lyophilized BSA powder was dissolved in 10 mM phosphate buffer (pH 7.4) containing 150 mM of sodium chloride. The prepared solutions were then placed in 25 mL Teflon-coated glass vials and subjected to reactions for 15 hours at a temperature of 70°C. The parameters, such as temperature and concentration, have been adjusted for the gelation conditions. We also changed the BSA concentration to create the ideal circumstances for efficient gelation. After the gel was formed, it was put in a desiccator and vacuumed to form the dry gel.

Rheology.

Rheological parameters were measured at room temperature (25°C) using a modular rheometer with a CP-25 plate and a 0.05 mm gap. The measuring system inertia and the motor were calibrated prior to the study. Around 150 µL of hydrogel (50 mg mL^{-1}) hydrogel was taken for the experiment, and the excess was removed To determine the linear viscoelastic range (LVE) of the hydrogels, frequency sweep experiment was performed initially**.** It was conducted over the angular frequency range of 1 to 1000 rad/s keeping a constant shear strain of 1%**.** Subsequently, the amplitude sweep experiment was performed to find out gel to sol transition point. Finally, thixotropy experiment was carried out to check for the self healing property of the hydrogel, the hydrogel was exposed to high 100% and mild 1% strains alternatively, and changes in storage and loss modulus were measured. Also, we measured hydrogel's viscosity in terms of Shear Rate γ (1/s) and viscosity η in (mPa·s) to prove the viscoelastic nature of the hydrogel.

Field Emission Scanning electron microscopy (FESEM) studies.

The FESEM images of hydrogel were taken in the inlens mode using, ZEISS Gemini 360 instrument at an acceleration voltage of 5 KV. Before taking the image, we coated the sample using Au-Pd.

Transmission electron microscopy (TEM) Studies.

The TEM images of hydrogel were taken in the JEOL (JEM -2100) TEM instrument, which is accelerated at 200 kV voltage. Before taking the image, the sample was prepared on a copper grid.

Fluorescent measurement of the BSA hydrogel.

UV-Vis spectra were acquired using the UV-Perkin Elmer Lamda 35 UV-Visible Spectrophotometer. Solutions of Bovine Serum Albumin (BSA) and the hydrogel were prepared in cuvettes with a volume of 3 mL. The spectrophotometer was used to collect UV-Vis spectra spanning a wavelength range from 200 nm to 800 nm.

Fluorescent images of both BSA solution and BSA hydrogel were captured using excitation wavelengths of 395 nm. To achieve excitation, LED light sources emitting at specific wavelengths were employed, and no filters were utilized during the process of image recording. The fluorescence spectra of the BSA protein hydrogel were obtained using a Horiba Jobin Yvon Fluoromax-4-Spectrofluorometer. A 3 mL cuvette containing the BSA hydrogel was scanned from 320 nm to 450 nm with a 10 nm increment for the emission scan. The recorded emission spectra ranged from slightly above the excitation wavelength to 700 nm.

Self-Healing Property.

The BSA hydrogel (50 mg ml^{-1}) was extruded into a mold and molded into circular disk shapes to assess the self-healing characteristic of the hydrogel. The resulting disks were cut into two semicircular disks using a surgical blade. To observe the self-healing properties, one of the

semicircular disks was stained with methylene blue and then placed in contact with the other semicircular disk at a temperature of 25 °C.

Self-destructing nature of the gel.

The BSA hydrogel was prepared following the above-mentioned procedure. Then, the hydrogel was placed in the trypsin medium (1 mg mL-1) to see whether the gel had degraded or not. After that, the medium was incubated at 37° C for the degrading process. It was observed that after 24 hrs, the gel fully degraded in the trypsin solution. We stained the gel with rhodamine 6G chloride dye molecule for the degrading experiment.

Supplementary Figures

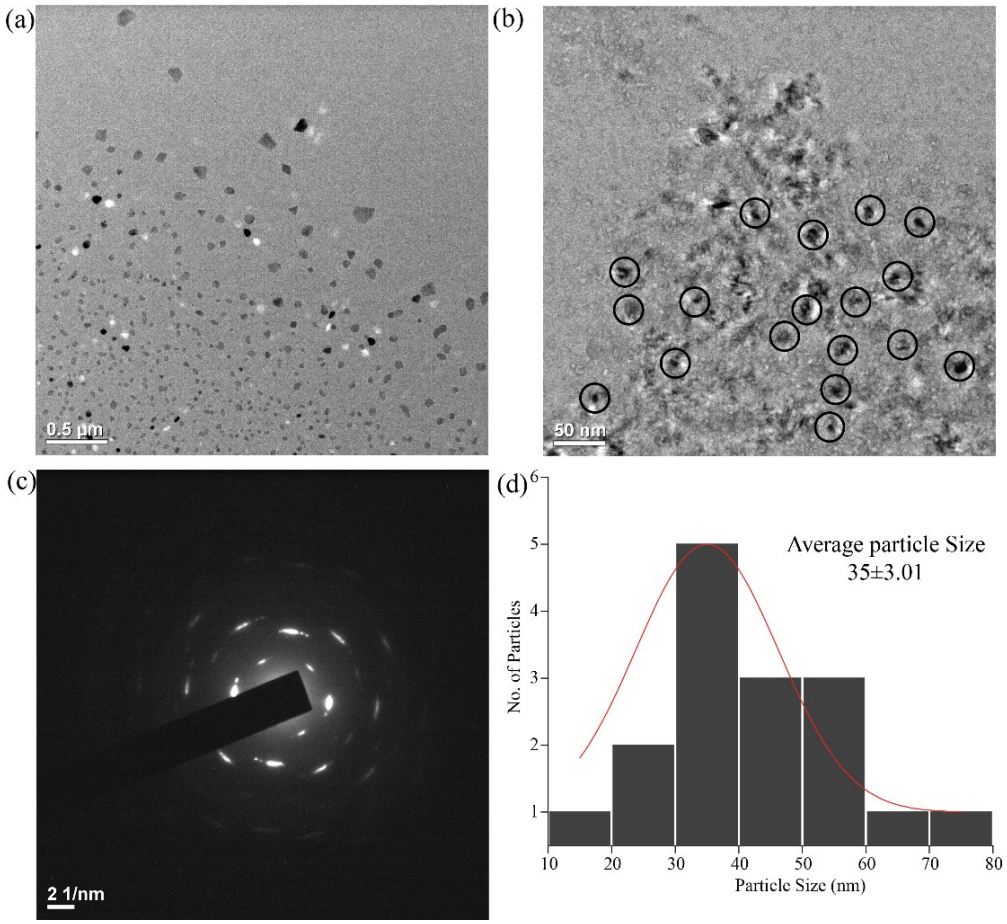

Figure S1. TEM micrographs of synthesized AgNPs within the hydrogel network at different resolutions (a) 0.5 µm, (b) 50 nm, (c) Selected area electron diffraction (SAED), and (d)average AgNPs particle size.

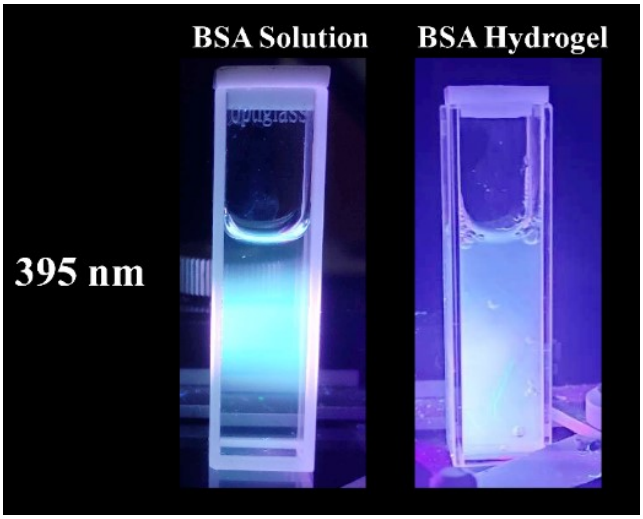

Figure S2. Fluorescence photograph of BSA solution and BSA hydrogel excited at 395 nm.

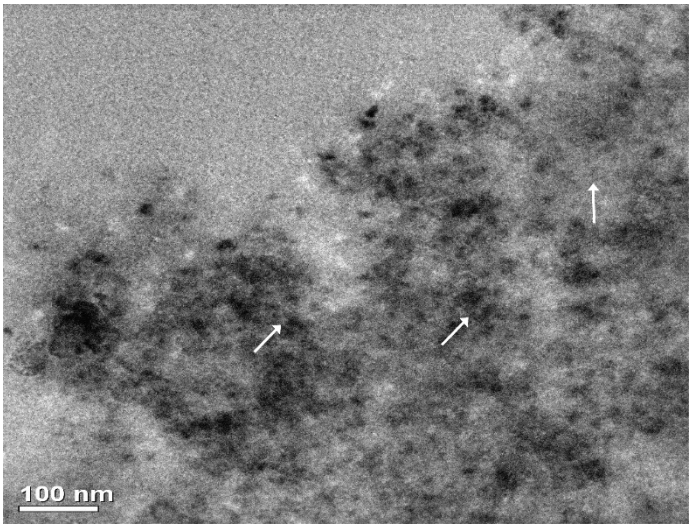

Figure S3. TEM micrographs of synthesized BSA hydrogel at resolutions 100 nm.

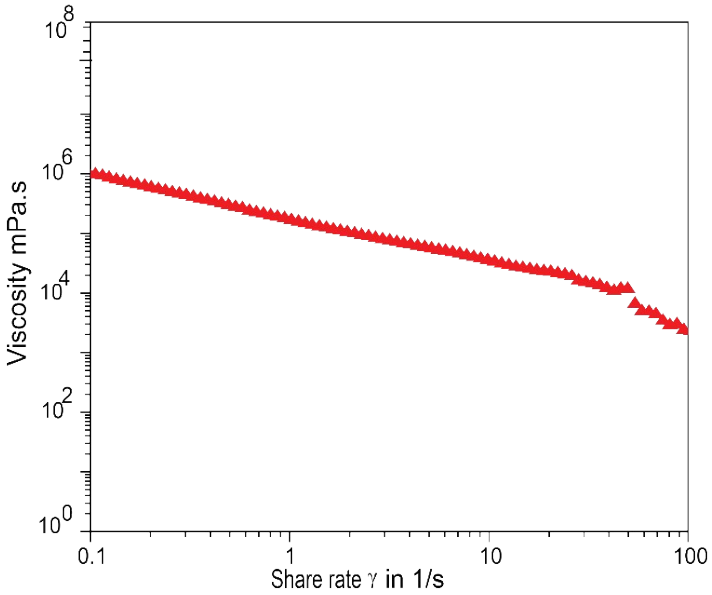

Figure S4. Viscosity graph of BSA hydrogel

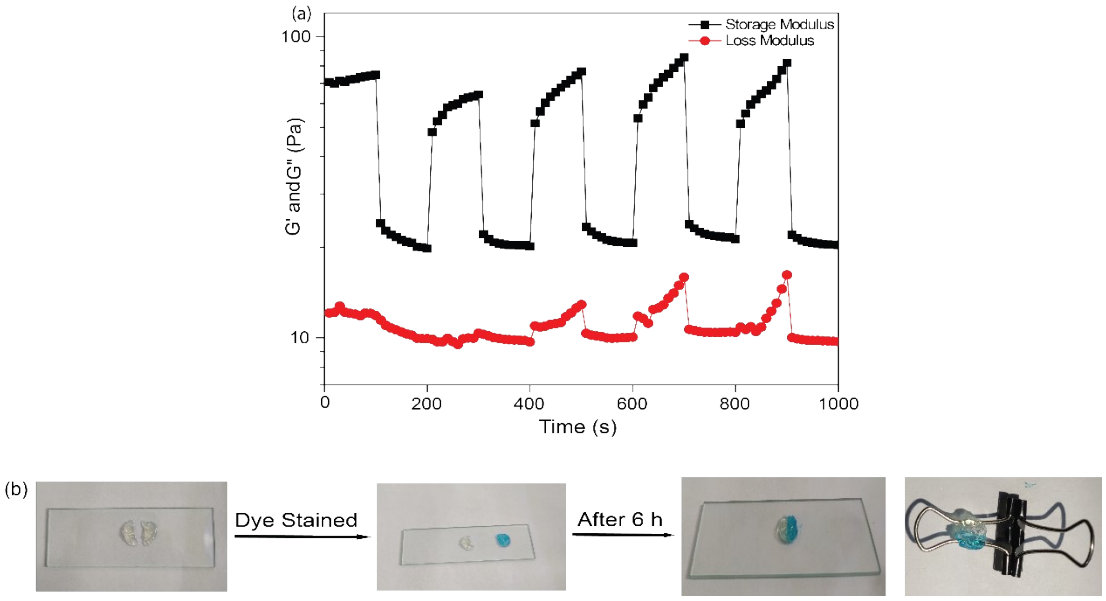

Figure S5. (a) Cyclic dynamic strain sweep experiment to show the thixotropic behaviour of the hydrogel. (b) Self-healing process of hydrogel over a period of 6 h at 25°C after keeping together the stained (with methylene blue) and unstained (colorless) counterparts of the hydrogels.

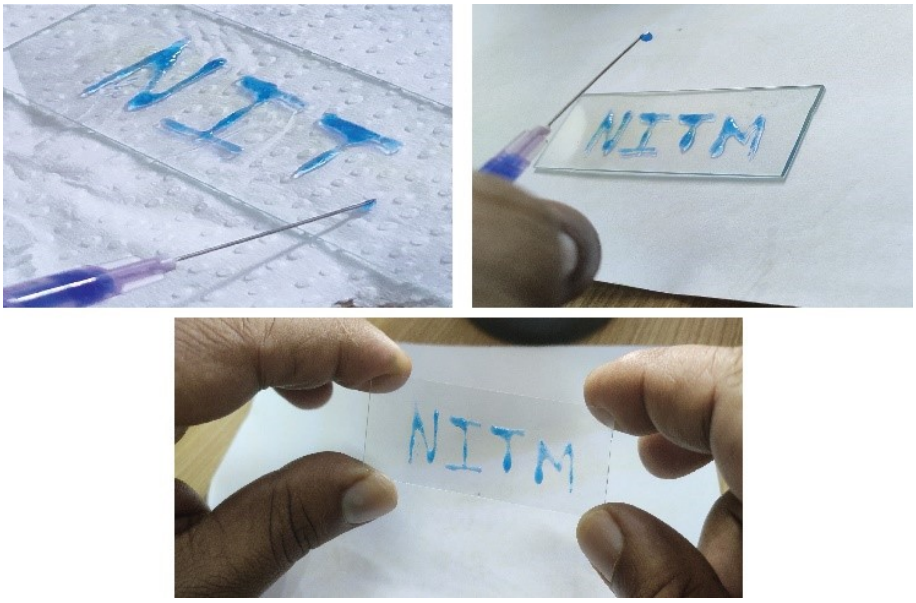

Figure S6. Photographs showing injectable properties of synthesized BSA hydrogel (the hydrogel stained with methylene blue).

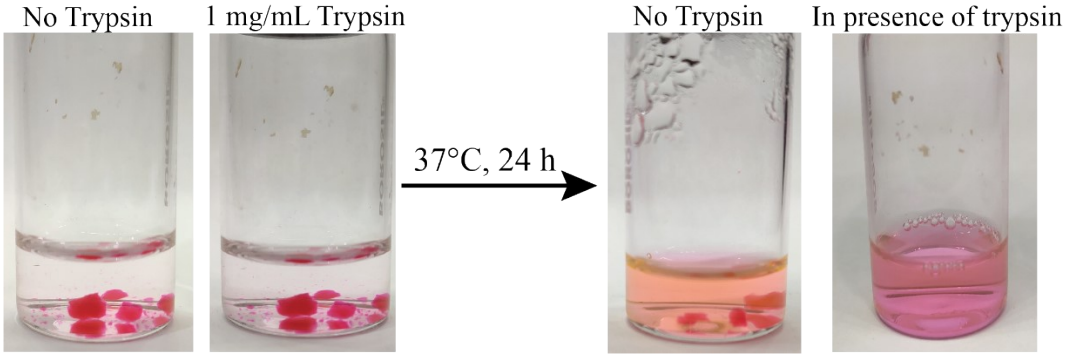

Figure S7. Picture showing the self-destructing nature of the gel in 20 mM phosphate buffer at pH 7.4, 24 h, 37°C. Gel with trypsin (1 mg/mL) completely degraded itself (right sample in the photographs) when compared to hydrogel without trypsin (right sample in the photograph). The gel was stained with Rhodamine 6G.

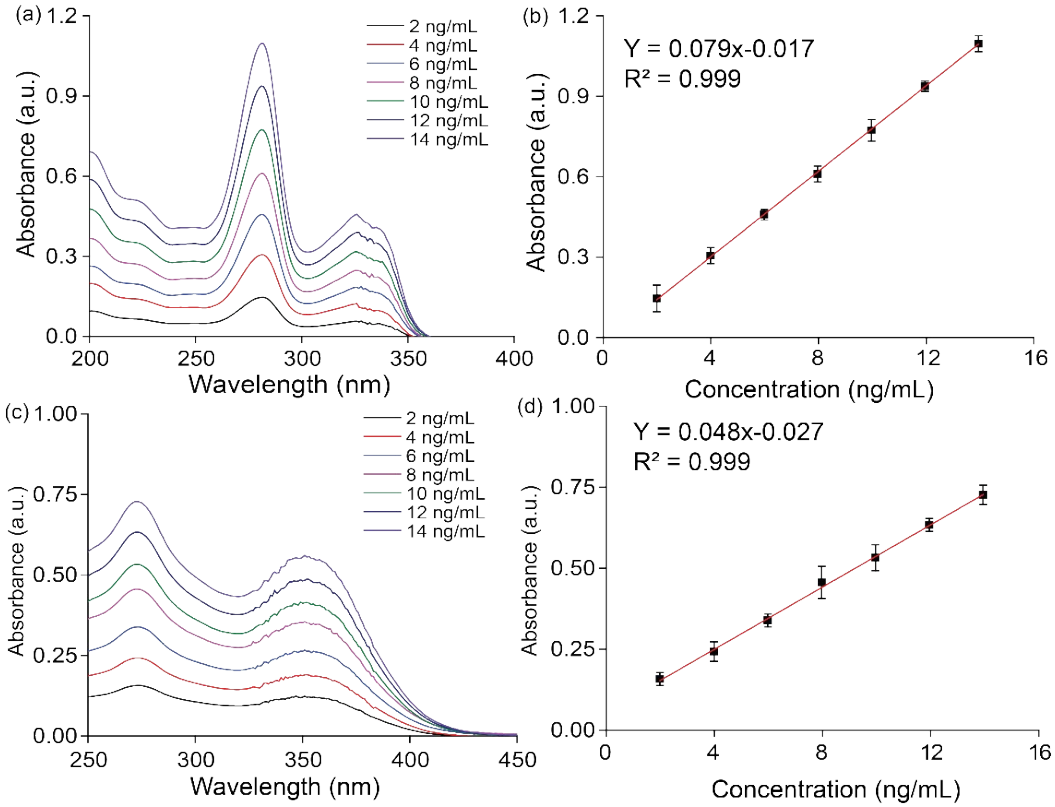

Figure S8. UV-Vis spectra of (a) lomefloxacin at 280 nm and (b) calibration curve of lomefloxacin, (c) UV-Vis spectra of doxycycline at 272 nm, (d) calibration curve of lomefloxacin

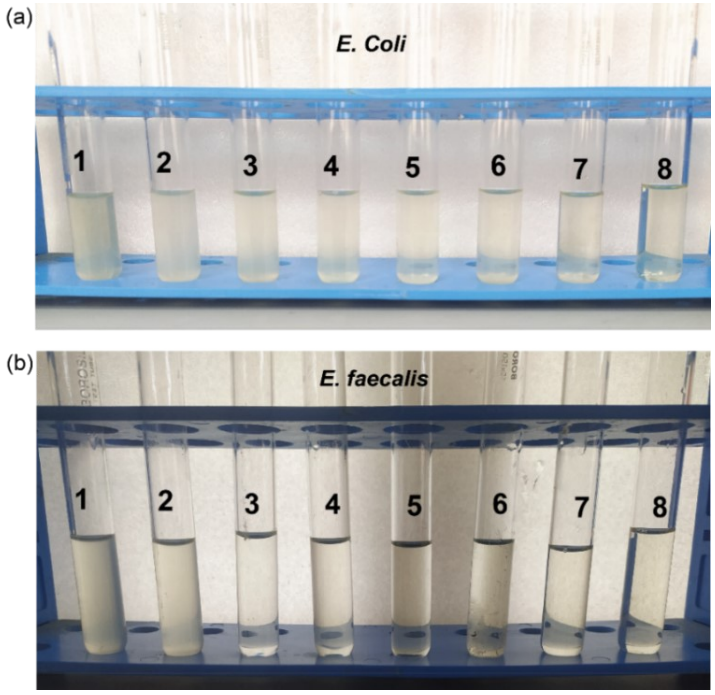

Figure S9. MIC values of the antibiotic loaded hydrogel (a) *E. coli* and (b) *E. faecalis* at various concentrations {1-bacteria, 2- Hydrogel,{(3-8)-antibiotic loaded hydrogel), 3- 1 μg mL^{-1}, 4- 2 μg mL^{-1}, 5- 4 μg mL^{-1}, 6- 6 μg mL^{-1}, 7- 8 μg mL^{-1} , 8-12 μg mL^{-1} }.

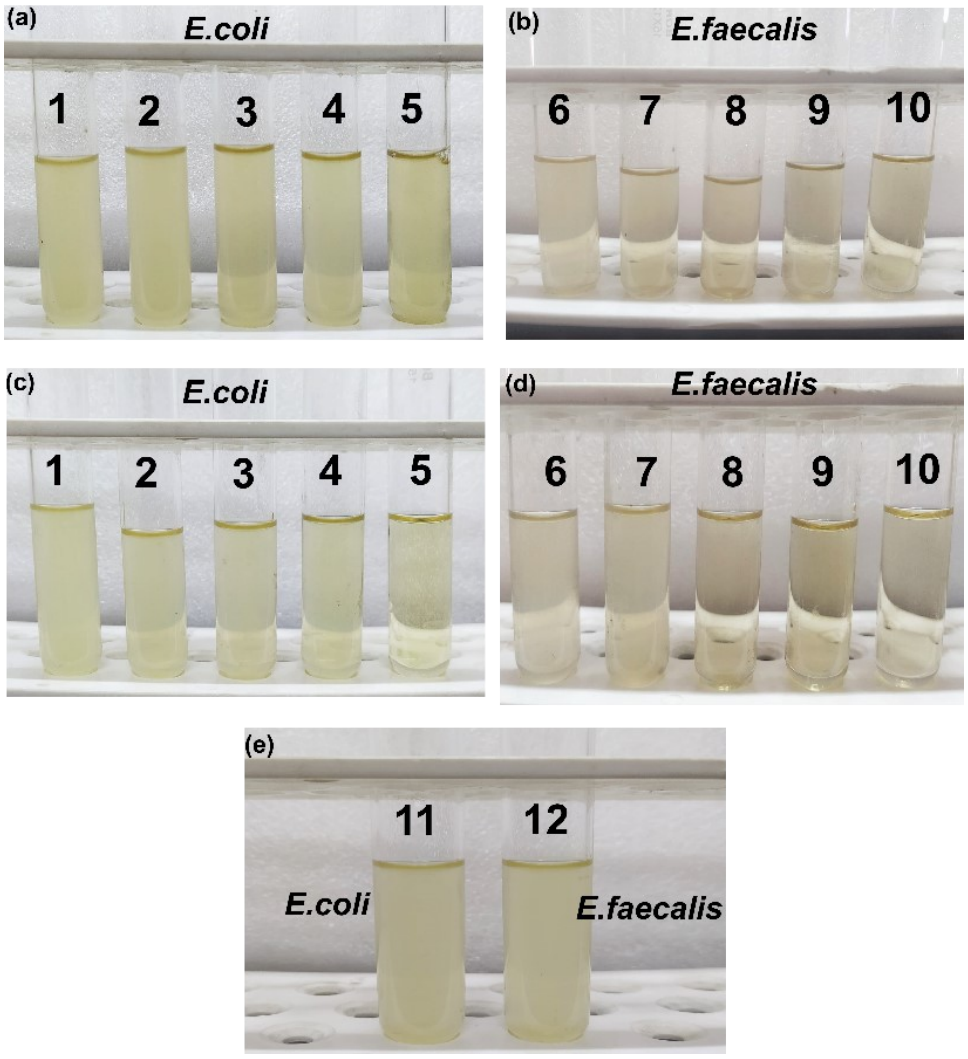

Figure S10. MIC values of the nanoparticles loaded hydrogel (a) *E. coli*, (b) *E. faecalis* at various concentrations{(1-5)-nanoparticles loaded hydrogel)}, 1- 2 μg mL^{-1}, 2- 4 μg mL^{-1}, 3- 6 μg mL^{-1}, 4- 8 μg mL^{-1}, 5- 12 μg mL^{-1}}and {(6-10)-nanoparticles loaded hydrogel) 6- 2 μg mL^{-1}, 7- 4 μg mL^{-1}, 8- 6 μg mL^{-1}, 9- 8 μg mL^{-1}, 10- 12 μg mL^{-1}}. MIC values of the antibiotics nanoparticles-loaded hydrogel (c) *E. coli*, (d) *E. faecalis* at various concentrations {{(1-5)-antibiotics nanoparticles-loaded hydrogel)}, 1- 2 μg mL^{-1}, 2- 4 μg mL^{-1}, 3- 6 μg mL^{-1}, 4- 8 μg mL^{-1}, 5- 12 μg mL^{-1}}and {(6-10)-anibiotics nanoparticles loaded hydrogel) 6- 2 μg mL^{-1}, 7- 4 μg mL^{-1}, 8- 6 μg mL^{-1}, 9- 8 μg mL^{-1}, 10- 12 μg mL^{-1}. (e) the control experiments (with hydrogel only) for both the bacteria.

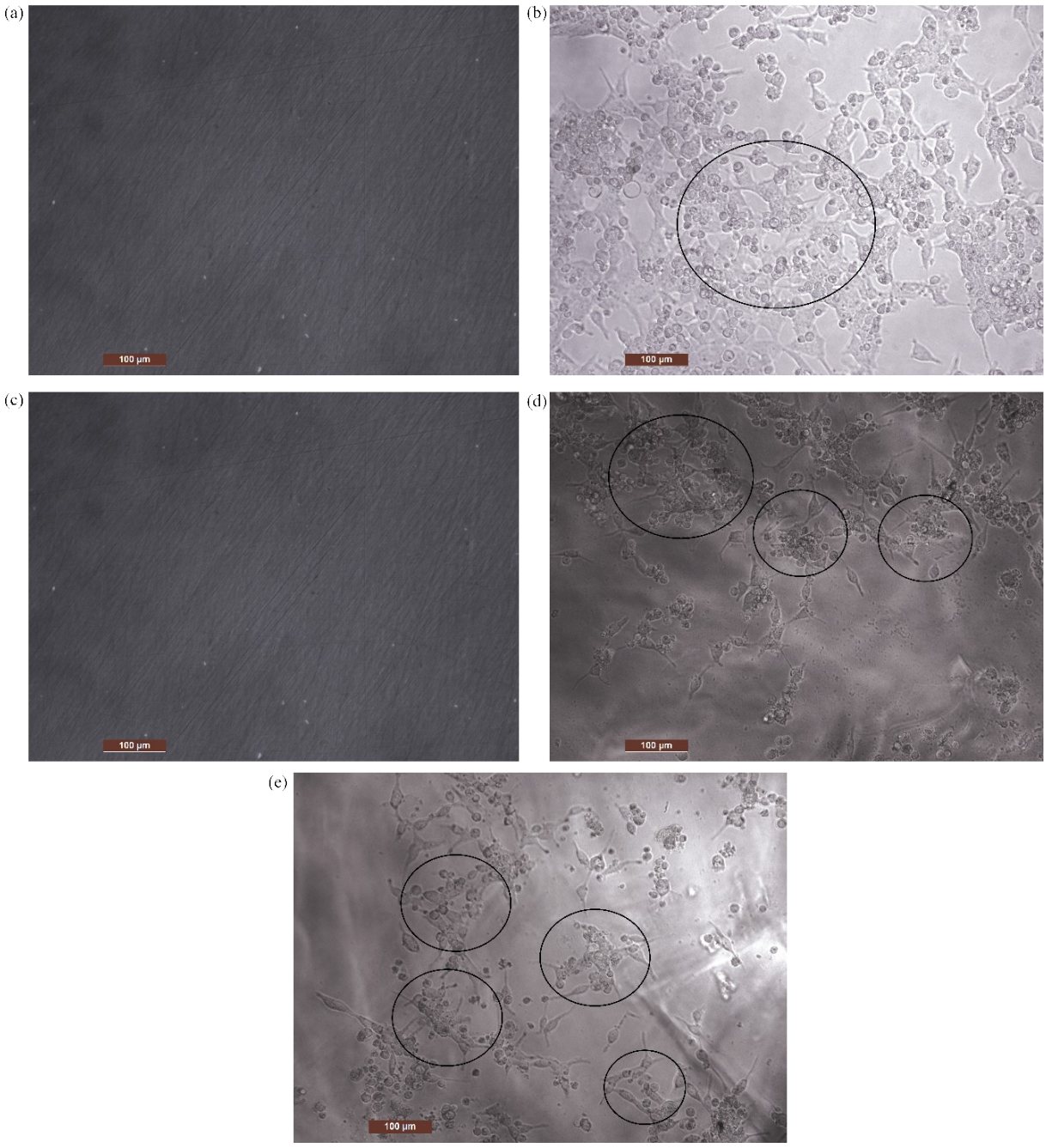

Figure S11. Fluorescence micrographs (20X) of HEK 293 cells were cultured on the top of an uncoated and BSA hydrogel-coated polystyrene plate. Micrographs of (a) polystyrene plate, (b) cells cultured on top of polystyrene plate after 36 hours, (c) BSA hydrogel coating on the top of the polystyrene plate, and (d),(e) micrographs of cells cultured on hydrogel coated polystyrene plate after 36 hours.