Supporting Information

Modulating Oxidative Stress and Bacterial Infection with Carbon Dot-Infused Bioactive Hydrogels for Infected Wound Regeneration

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Experimental section

Swelling ratio study The equilibrium swelling method was used to assess the hydrogel's ability to retain water. To completely remove the water, the PG-PD hydrogels with various compositions (Table 1) were freeze-dried for an entire night. After being weighed, the freeze-dried hydrogels were submerged in 15 mL of distilled water in a beaker. After the surplus surface water was dried with tissue paper, the hydrogels were taken out of the water at regular intervals and weighed. The following formula, where Ww is the weight of the wet hydrogel at a specific moment, and Wd is the weight of the freeze-dried hydrogel, was used to calculate the swelling ratio.

$$Swelling\ Ratio\ \% = \frac{Ww - Wd}{Wd} \times 100 \quad \text{Equation S1}$$

Table S1: List of different compositions of the hydrogel

Sample	Gelatin	PVA	Dopamine	water
PG-PD_0	0.5 g	0.7 g	---	10 mL
PG-$PD_{0.03}$	0.5 g	0.7 g	0.03 g	10 mL
PG-$PD_{0.05}$	0.5 g	0.7 g	0.05 g	10 mL
PG-$PD_{0.1}$	0.5 g	0.7 g	0.1 g	10 mL

Vitro degradation study The hydrogel samples were submerged in separate sets of PBS buffer (pH=7.4) and citrate buffer (pH=5.5) at room temperature to assess the pH-dependent degradation of the PG-PD hydrogels. On different days, the residual weight of the hydrogels was recorded after excess water on their surface was removed with tissue paper. The weight ratio was measured by using the following formula,

$$Weight\ ratio\ \% = \frac{Wt}{W0} \times 100 \quad \text{Equation S2}$$

Where W_t = weight of hydrogel at any time, W_0= initial weight of the hydrogel, respectively.

Antioxidant study of CD The antioxidant property of the carbon dot was measured by using the OH scavenging capability of CD using the Fenton reaction. 1500 µL of salicylic acid

ethanolic solution (1.8 mM) was mixed with 2000 µL of FeSO4 (1.8 mM) solution. The aforesaid mixture was then mixed with the carbon dot solution, followed by 100µL of H_2O_2 (100 mM). Afterwards, the sample solution was incubated for ten minutes at 37 °C. Next, the mixture was centrifuged for three minutes at 5000 rpm to collect the supernatant. A UV-Visible spectrometer was used to detect the supernatant's absorbance at 530 nm, and the following formula was used to compute the free OH radical scavenging,

$$Scavenging\ activity\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad \text{Equation S3}$$

Where A_c = absorbance of sample without carbon dot, A_s = absorbance of sample with carbon dot

Cell culture

Mice skin fibroblast cells NIH-3T3 were originally obtained from ATCC. Cells were maintained in Dulbecco's Modified Eagle Medium with 10% fetal calf serum and 5% penicillin/streptomycin. Cells were cultured in a humidified incubator with 5% CO2 and 37°C.

Biocompatibility of CD, PG-PD, CD@PG-PD

Cytotoxicity Assays

The cytotoxicity of the hydrogel was investigated using the standard MTT assay. Briefly, 1×10^4 cells/well of NIH-3T3 fibroblast were seeded in a 96-well plate. Cells were then treated with varying concentrations (10, 20, 40, 80, and 160 µg/mL) of CD, PG-PD, and CD@PG-PD hydrogels. After 24 and 48h of the treatment period, cells were incubated with MTT dye at 0.5mg/ml for 3 h. Further, the formazan crystals were dissolved by adding 200 µL of isopropanol in each well. The absorbance was measured at 575 nm in a microplate reader. The data were then analyzed to determine the vitality of the cells.

Hemolysis study

Hemolysis assay was conducted to study the blood compatibility of the designed carbon dot and hydrogels. 1ml of blood was collected from the Wistar rats in an anticoagulant tube and centrifuged (1,000 rpm) for 10 min to collect the red blood cells (RBC). After centrifugation,

the red blood cells (RBC) were washed with 10 mmol PBS three times. Then, RBC was diluted 10-fold with PBS before use. Then, 5mg of CD, PG-PD, and CD@PG-PD hydrogel were dispersed in 1ml of PBS solution to prepare samples for the test. Then, for the hemolysis study, 0.8 mL of the sample was mixed with 0.2 mL of RBC and incubated at 37 ℃ for 1 h in triplicate. RBC suspensions incubated with PBS and Triton-X were used as negative and positive controls, respectively. Next, 200 mL of supernatant was collected in a 96-well plate, and the absorbance at 545 nm was measured. The hemolysis rate was calculated using the following formula,

$$Hemolysis\ rate\ (\%) = \frac{Ax - An}{Ap - An} \times 100$$ Equation S4

Where Ax, An, and Ap represent the absorbance of the sample, and the negative and positive controls.

Antibacterial study

Gram-positive *S. aureus (S.A.)* and gram-negative *E. coli (E.C.)* bacterial species were used to test the antibacterial activity of CD, PG-PD, and CD@PG-PD hydrogel. First, both bacterial strains were collected from a freezer at -80°C and cultured in nutrient agar plates for 12 hours at 37°C. Each bacterial colony was then separated and cultured for around 7-8 hours at 37°C in an MHB medium. The MHB (negative control) and MHB containing CD, PG-PD, and CD@PG-PD hydrogels were then incubated overnight with 100µL of bacterial inoculum. To monitor the growth of the bacteria, the optical density of the solution was monitored visually during the incubation time. The bacterial colony was developed by spreading 100µL of 5-fold diluted bacterial suspension samples on prepared agar plates and then incubating them for 24 hours at 37 °C. Agar plate photos were taken, and the bacterial colony that developed was manually counted. The hydrogel treatment's antibacterial activity was assessed using the colony-forming unit (CFU) method using the following formula,

$$Survival\ Rate\ (\%) = \frac{(CFU)x}{(CFU)0} \times 100$$ Equation S5

Where $(CFU)_x$ and $(CFU)_0$ represent colony-forming units after 24h and 0h, respectively.

Scratch assay

The scratch assay was used to study the migration of cells *in vitro*. Briefly, NIH-3T3 cell line was seeded in a 6 well plates at a density of 3×10^5 cells/well to promote linear monolayer cell development. Using a sterile pipette tip, the surface was scratched to create a gap (wound mimic). Cell debris was removed by gently washing with PBS solution. PG-PD and CD@PG-PDTG-OC hydrogels were used to treat the scrape. The cell plate was kept in an incubator at 37 °C. Using microscopes, *in vitro* cell migration at the wound site was monitored at various time intervals. The following formula was used to compute the cell migration,

$$Scratch\ closure\ (\%) = \frac{A_0 - At}{A_0} \times 100 \qquad \text{Equation S6}$$

Where A_0, A_t were the scratch areas at 0 h and a particular time.

Haemostatic study

To assess the hydrogel's hemostatic capacity *in vivo*, a Wistar rat model of haemorrhaging liver was generated. Following anaesthesia, the rats's livers were exposed via an abdominal incision, and the bleeding was stopped by placing filter paper underneath the liver. The produced hydrogel matrix was then applied right away to the location of bleeding after a liver puncture was created. The untreated group served as a control. In order to investigate the hydrogel's hemostatic capacity, the time it took to halt bleeding, along with the total blood loss, was then monitored.

In vivo

Male Wister rats, aged 8 weeks, each weighing approximately 200 g were used for *in vivo* studies. Rats were sourced from Rodent Research India Lab, Haryana. All experiments were performed following ethical guidelines of the Animal Ethical Committee (IAEC) of Amity University, Noida with a clearance certificate (CPCSEA/IAEC/AIP/2025/04/01). Rats were housed in individually ventilated cages with a 12 h day/night cycle with provisions for ad libitum food and water. At the end of the experiment, rats were euthanized following carbon dioxide (CO2) asphyxiation as per the guidelines of IAEC.

In vivo wound healing study

For wound healing studies, a full-thickness wound model of wister rats infected with bacteria was created. Briefly, isoflurane was used to anaesthetize 24 wister rats that were randomly

divided into four groups (n=6). The hair was removed from the rear part, and 10 mm biopsy puncher were used to make skin incisions that created a 10 mm diameter wound with 2 mm thickness. Every wound was infected with *S. aureus* (100 µL) for one hour to form infected wounds. Normal saline (control group), PG-PD, CD@PG-PD hydrogels, and commercially available dressing were used to cover the wounds. On days 0, 3, 6, 9, and 12, the wound area was measured, and all dressing materials were replaced once every three days. On day three, skin tissues surrounding the wound site were collected from each group and diluted in PBS solution before being spread on agar plates overnight. The residual bacteria at the wound site were then evaluated by the CFU method. The wound area was monitored, and the pace of wound closure was evaluated using the area of the wound computed with ImageJ.

Histopathological Staining

On day 12, tissues were collected from the wound site. For long-term preservation, the samples were embedded in paraffin and treated in 4 % paraformaldehyde. The tissues were then divided into 5 µm-thick slices that were around 2 cm × 2 cm in size. Hematoxylin and eosin (H&E) staining was subsequently applied to these sections for histological assessment. Additionally, on day 14, important organs such as the heart, liver, spleen, lungs, and kidneys were removed from the mice and subjected to identical analysis using H&E staining.

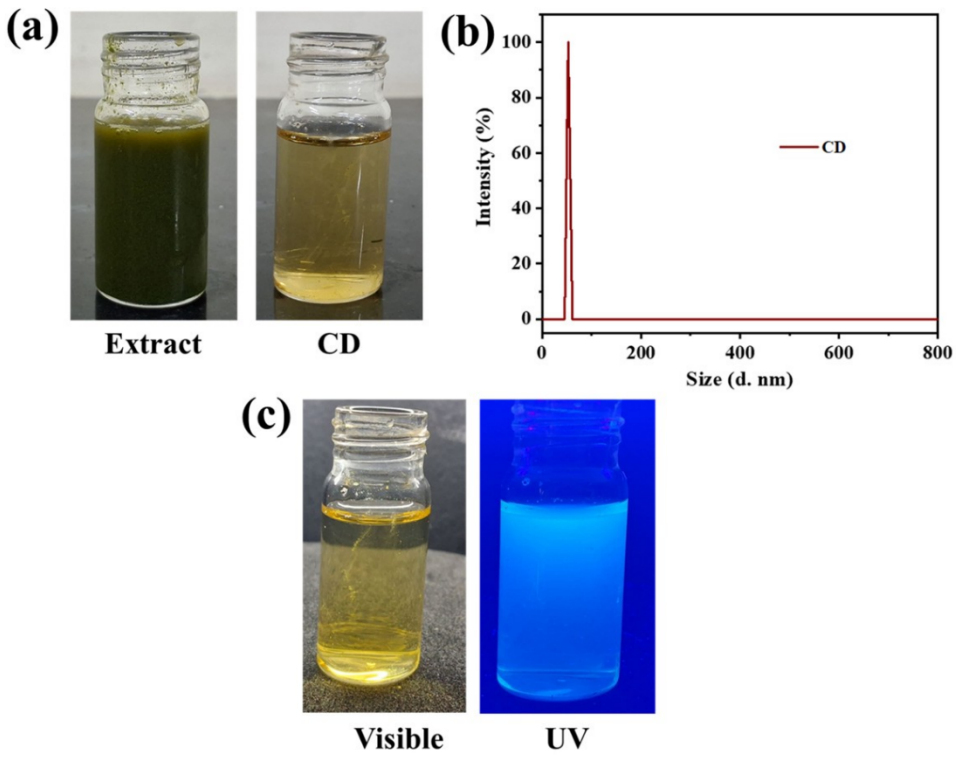

Figure S1. (a) Images of precursor solution and CD solution; (b) DLS size of the CD; (c) CD solution under visible and UV exposure.

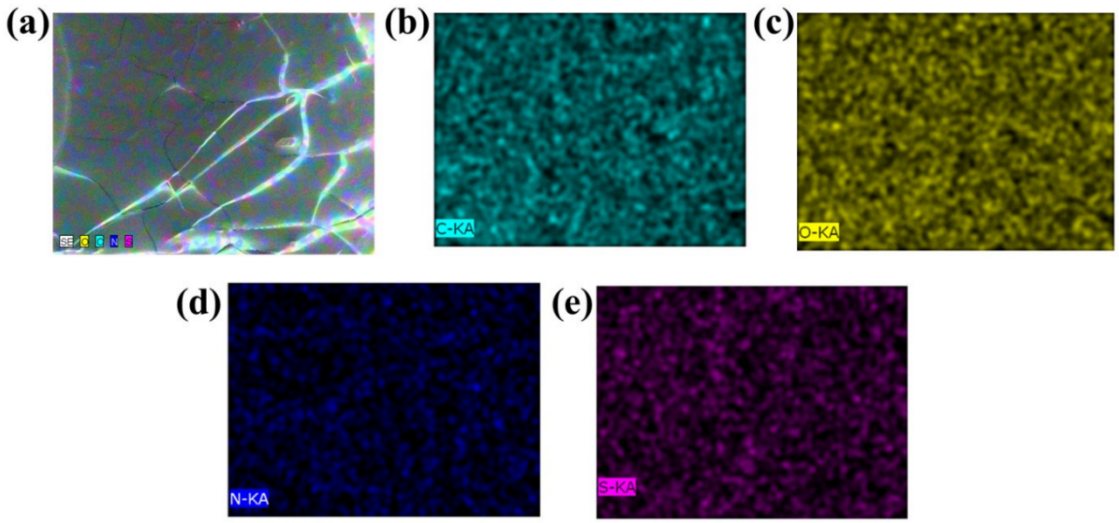

Figure S2. (a-e) EDS elemental analysis CDs

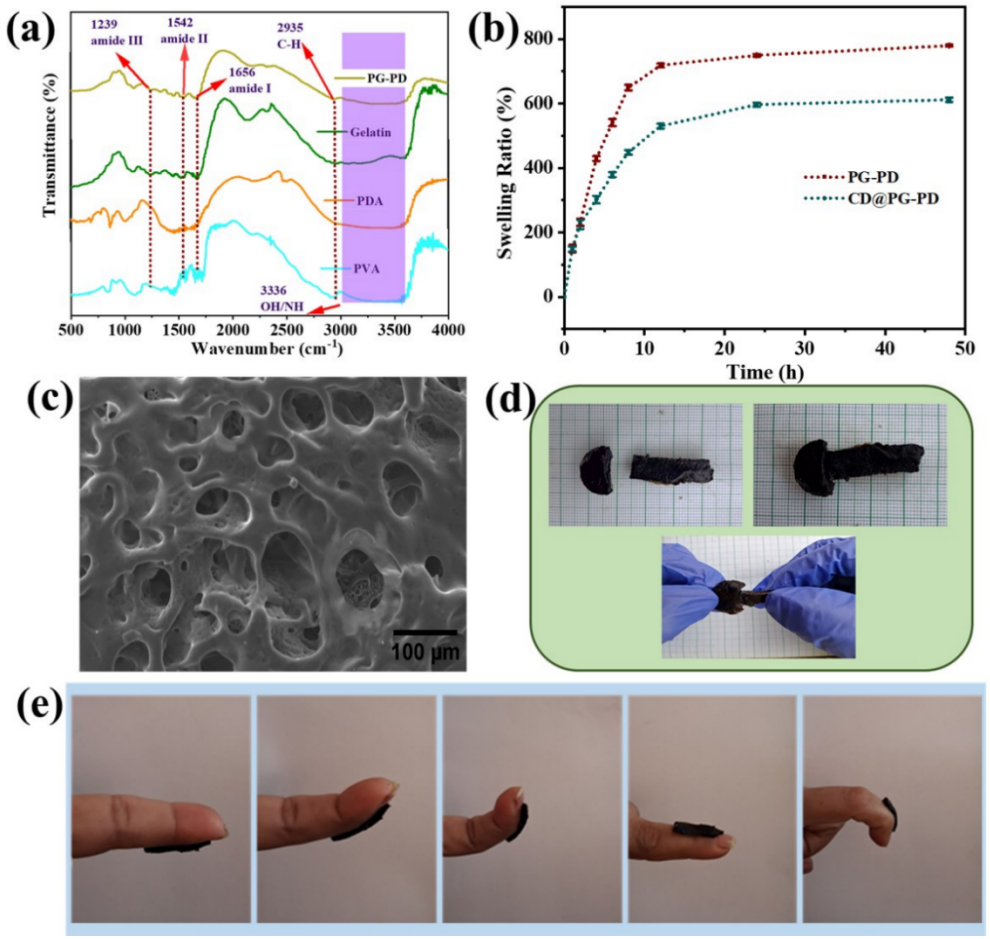

Figure S3. (a) FTIR analysis of the hydrogel; (b) Swelling study of PG-PD, CD@PG-PD; (c) SEM image of CD@PG-PD; (d) self-healing of CD@PG-PD; (e) adhesion of the CD@PG-PD on human skin at different angles.

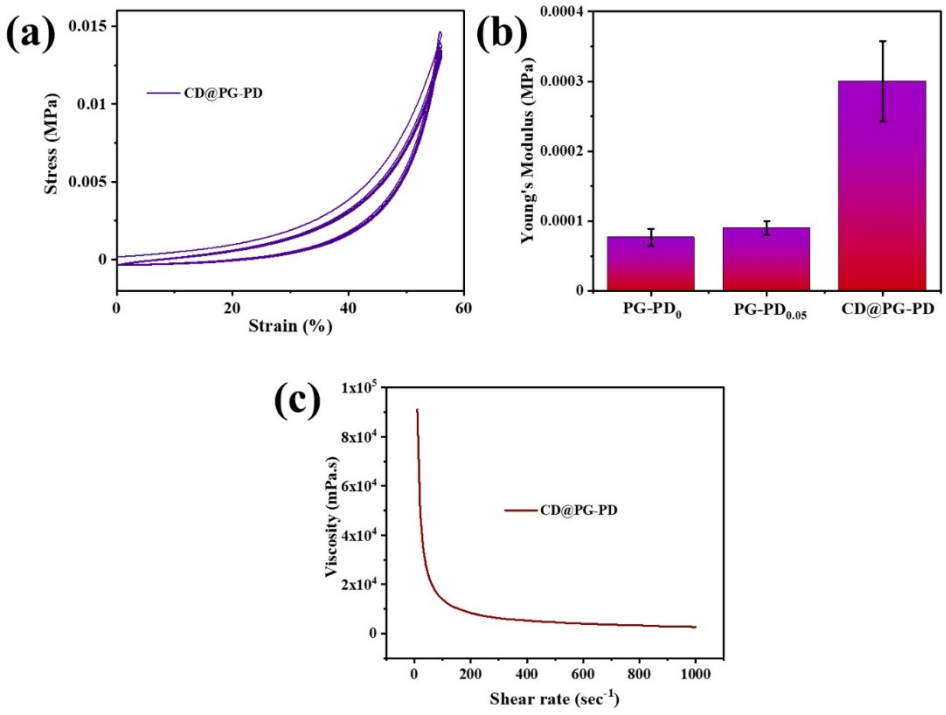

Figure S4. Cyclic stress-strain study of CD@PG-PD; (b) Young's modulus comparison of hydrogels; (c) shear thinning study of CD@PG-PD.

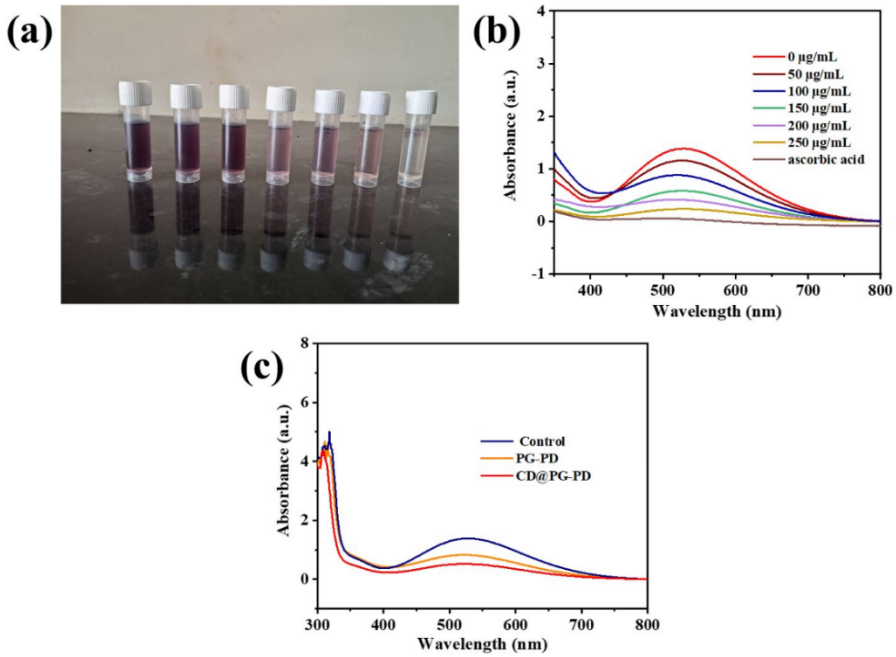

Figure S5. (a) Gradual lighting of purple colour with an increase in concentration of CD; (b,c) Absorption spectra of ·OH mixture with different carbon dot solutions and hydrogels, respectively.

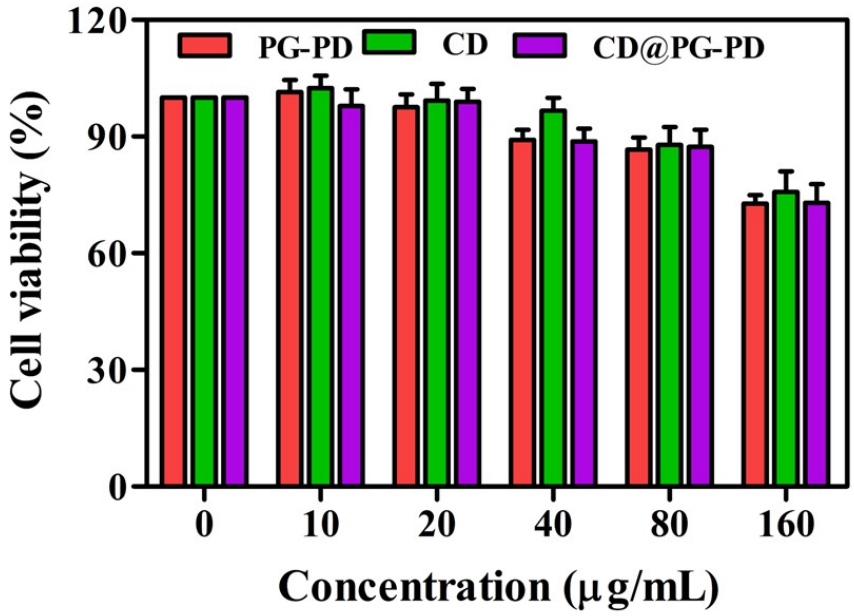

Figure S6. MTT assay after 48 h.

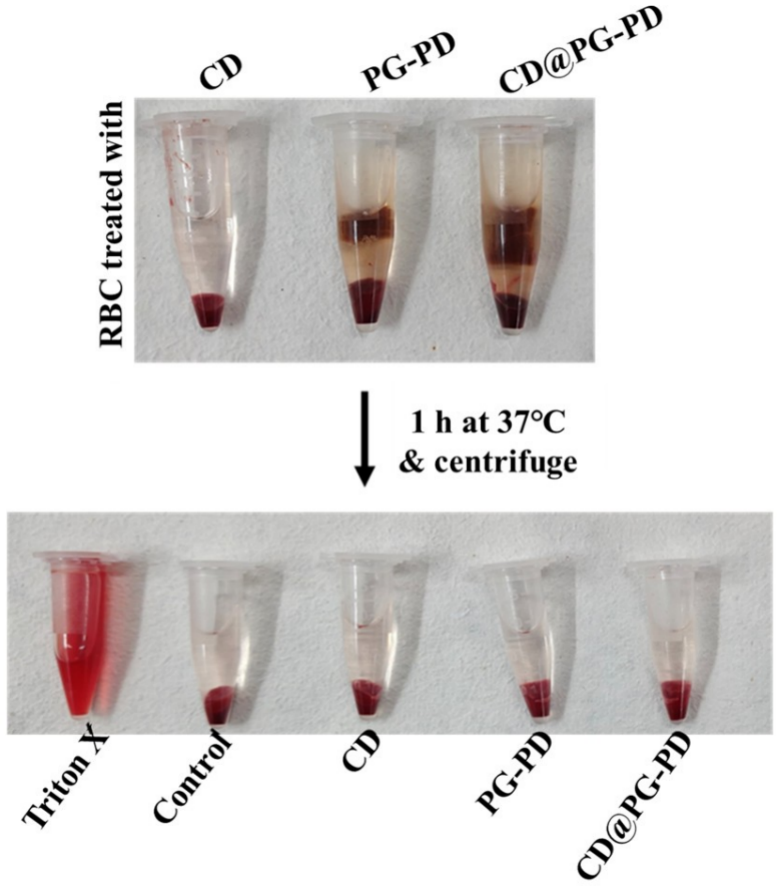

Figure S7. Hemolysis study of CD, PG-PD and CD@PG-PD.

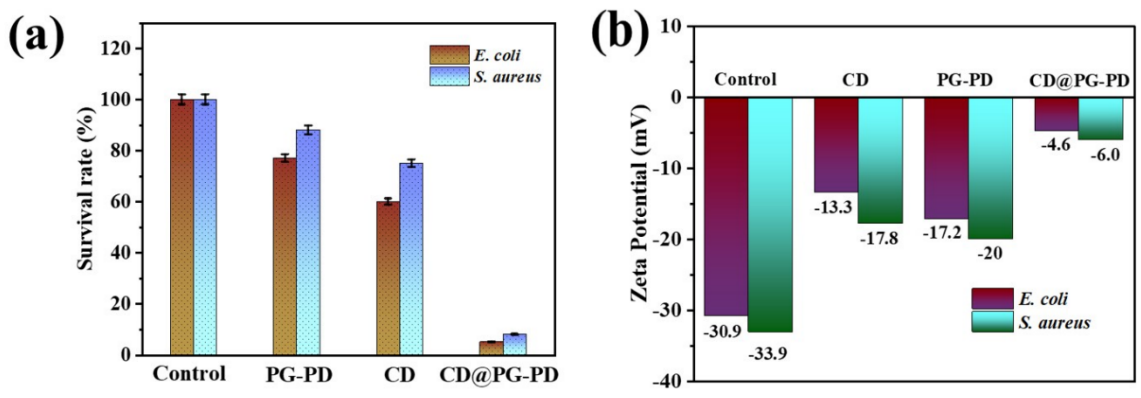

Figure S8. (a) Bacterial survival rate; (b) Zeta potential of bacterial suspension for different incubation samples.

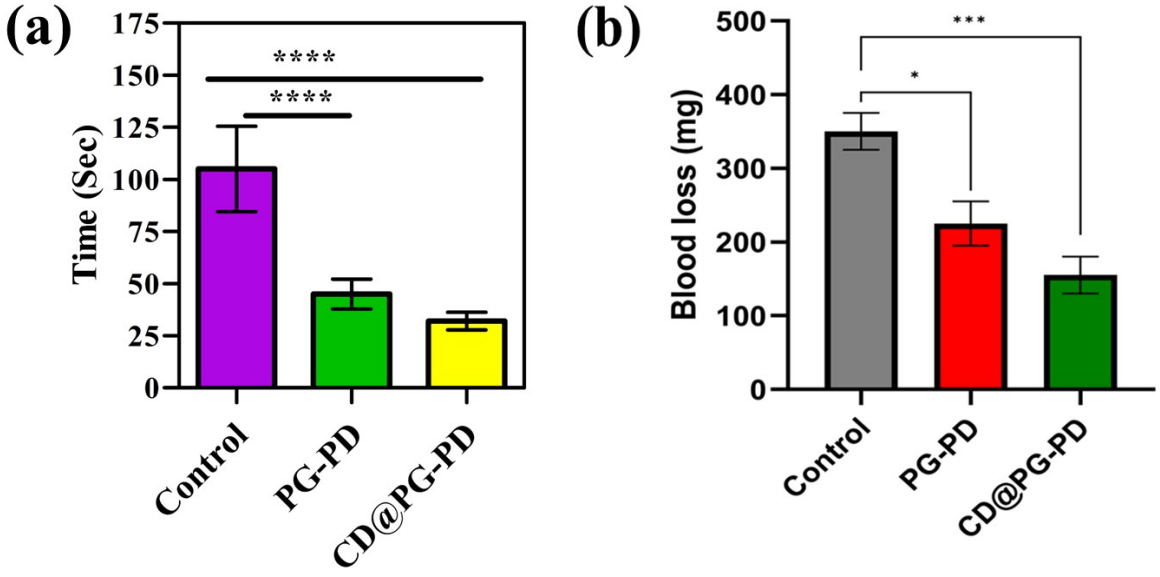

Figure S9. (a) Hemostasis time after the application of different hydrogels; (b) Comparison study of blood loss after the application of hydrogels.

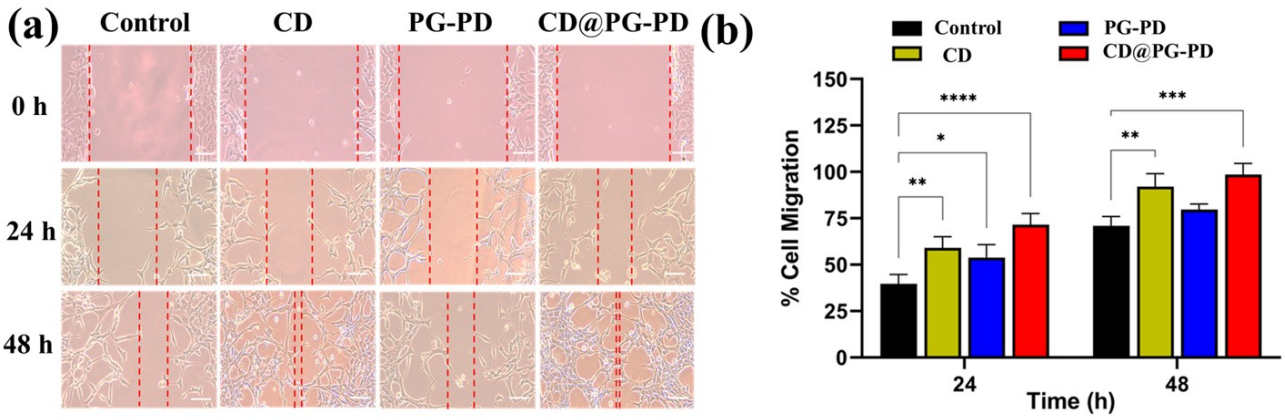

Figure S10. (a,b) Scratch assay and area closure analysis of PG-PD, CD@PG-PD hydrogel, respectively.

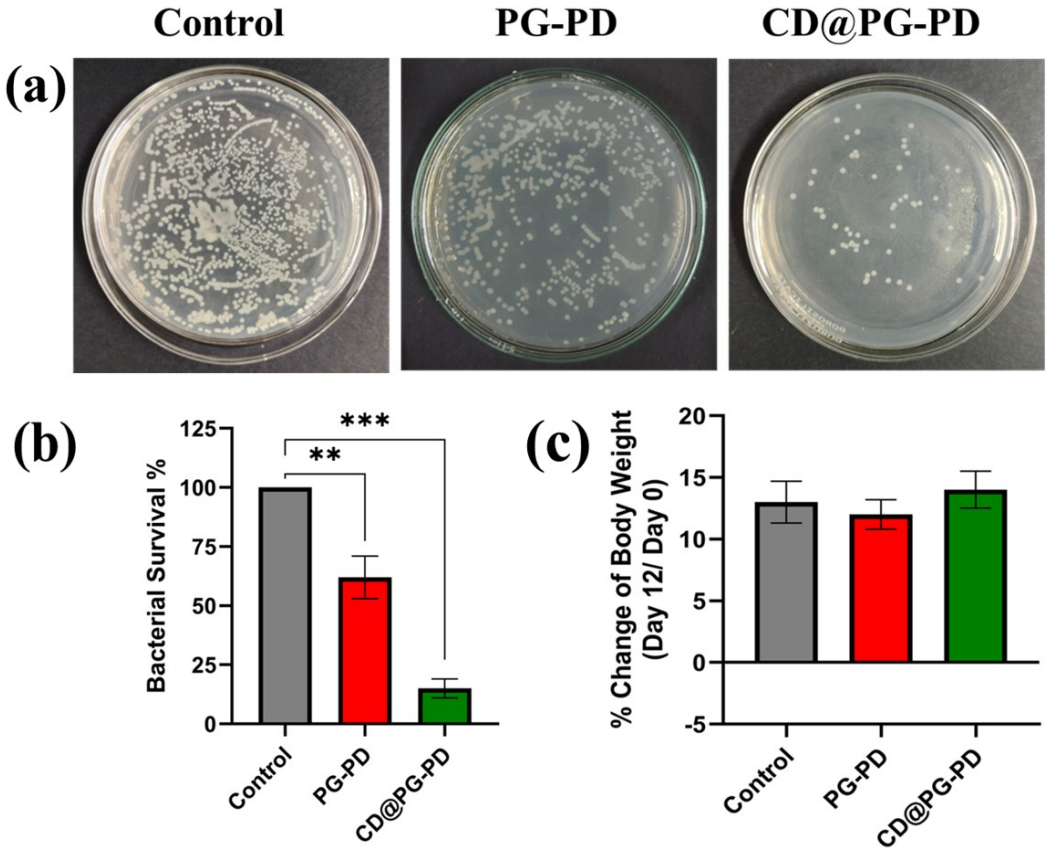

Figure S11. (a,b) Analysis of residual bacterial content in wound tissue exudate on day 3; (c) Percentage body weight change of rats after different treatments.

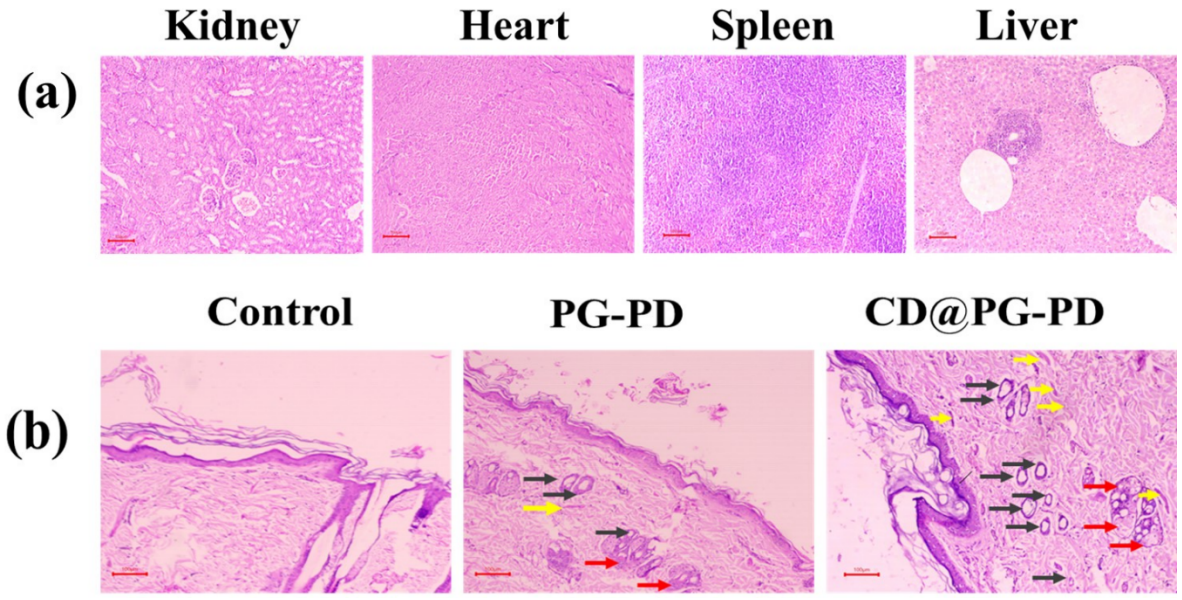

Figure S12. (a) H&E staining of major rat organs to study the biocompatibility of CD@PG-PD hydrogels in vivo; (b) H&E-stained skin tissue sections on day 12 after the treatment of hydrogels. Arrows indicate: black- hair follicles, yellow- collagen fibre, red-Sebaceous Glands

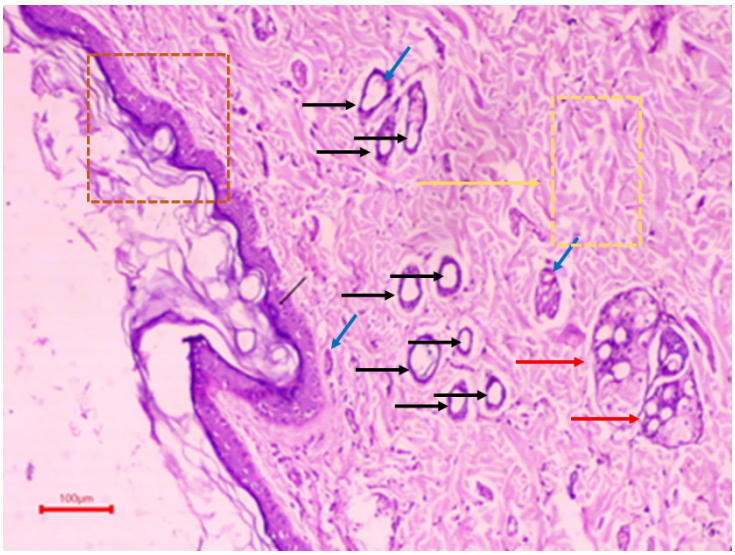

Figure S13. H&E stain tissue analysis of the CD@PG-PD treated group. Arrows indicate: black- hair follicles, yellow- collagen fibre, red-Sebaceous Glands, blue- blood vessel.

Table S2: Comparison study of carbon dot precursor, biocompatibility, scratch assay study, hemostasis time, antibacterial study, antioxidant study and wound healing applications of CD@PG-PD.

CD precursor	gel matrix	Cross linker	biocompatibility	Scratch Assay study	Hemostatic time	Antibacterial activity	OH Scavenging	Wound healing	Ref
ammonium citrate, polyethylenimine	hyaluronic acid, gelatin	BDDE	80 % (50 µg/mL)	-	-	E.C- 99 % S.A- 97 %	-	90 % (11 days)	1
green tea	PVA, PEDOT: PSS,	-	90%	95 %-48 h	-	E.C- 95 % S.A- 96 %	80 % (50 µg/mL of CD)	93 % (18 day)	2
lignin	Methacrylamide-gelatin	UV light	24h- 90 %	-	-	Disk diffusion method	-	-	3
Glutathione, formamide/Zn	Pluronic F-127, Glucose oxidase	thermosponsive	85 %	93.34%	-	-	41 %- (50 µg/mL CD)	96.28% (10 day)	4
Kappa-carrageenan, Casein-Ag	acrylic acid, chitosan	EDC/NHS coupling	good	Good Migration rate-2.4 µm/h	-	Disk diffusion method 30 mm	71%	-	5
Garlic & curry leave extract	**PVA, Gelatin, Polydopamine**	**-**	**MTT 24h-82 % 48h-72 % (160 µg/mL)**		**45 sec**	**E.C- 95 % S.A- 92 %**	**64 % (100 µg/mL of gel) 83 % (250 µg/mL of CD)**	**92 %(12 day)**	**This work**

References:

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