Supplementary Information (SI) for Soft Matter.
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Electronic Supplementary Information

Functionalized amino acid-based injectable hydrogel for sustained drug delivery

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Instrumentation

Field Emission Scanning Electron Microscopy (FESEM)

The microstructure of freeze-dried hydrogels was captured by using FESEM (FESEM Supra 55, Make – Zeiss, Germany) operating at an accelerating voltage of 5 kV and an emission current of 10 μA. Hydrogel disks were shaped in a 24-well plate and freeze-dried. Lastly, samples were sputter-coated with platinum before FESEM observation.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H and ^{13}C NMR spectra of Gly-MA and *cl*-β-CD-g-p(Gly-MA) hydrogel were recorded by a 400 MHz NMR Bruker Avance III HD (400 MHz) spectrometer using DMSO-d_6 as a solvent.

UV-visible Spectroscopy

UV-visible spectrophotometer (Shimadzu, Japan: Model: UV 1800) was used to record all UV-visible spectra.

Evaluation of *in vitro* cytocompatibility

MTT assay

The newly synthesized *cl*-β-CD-*g*-p(Gly-MA) hydrogel was washed thoroughly using PBS thrice to remove the unwanted impurities and sterilized using UV light for 2 hours. On the other hand, HeLa and L929 (both the cell lines were obtained from National Centre for Cell Science, Pune) cells were cultured in DMEM media (Gibco) supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin, in a 5% CO_2 atmosphere at 37 °C. After 3 days, cells were detached from tissue culture flasks using trypsin (Gibco). Equal numbers of cells (5000 cells/well in 200 μL) were seeded onto the cl-β-CD-g-p(Gly-MA) hydrogel surface in 96-well plates. Cell density was measured using an automatic cell counter (Countess® II, Life Technologies). The MTT assay was performed on cl-β-CD-g-p(Gly-MA) hydrogel and control (TCP) after 24 and 72 h. MTT reagent (Sigma Aldrich, US) was added to each well at a final concentration of 0.5 mg/mL and incubated at 37 °C for 4 h. The media was then removed, and 150 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. After a 15-minute incubation at 37 °C, absorbance was measured using a Thermo Scientific Multiskan FC microplate reader with a 620 nm filter. Cell viability was calculated using (Eq S1)[1, 2]

$$\% \text{ Cell viability} = \frac{OD_{620}(\text{Sample}) - OD_{620}(\text{Blanck})}{OD_{620}(\text{Control}) - OD_{620}(\text{Blanck})} \times 100 \text{----------Eq (S1)}$$

Live-dead assay

Cell viability on the hydrogel seeded with L929 cells was assessed using the live/dead assay (Invitrogen, USA) according to the manufacturer's protocol. The hydrogel samples were UV-sterilized for 2 h. After overnight incubation in culture media, approximately 5000 cells per well were seeded onto the scaffolds and incubated at 37 °C for 24 and 72 h. After the incubation periods, the media was discarded. Live/dead staining dye was applied, and the scaffolds were incubated at 37 °C for 20 minutes. Fluorescence images were captured using an inverted fluorescence microscope (Carl Zeiss, Germany) with excitation filters of 450–490 nm (green, Calcein AM) and 510–560 nm (red, ETD-1) using ZEN software.[1]

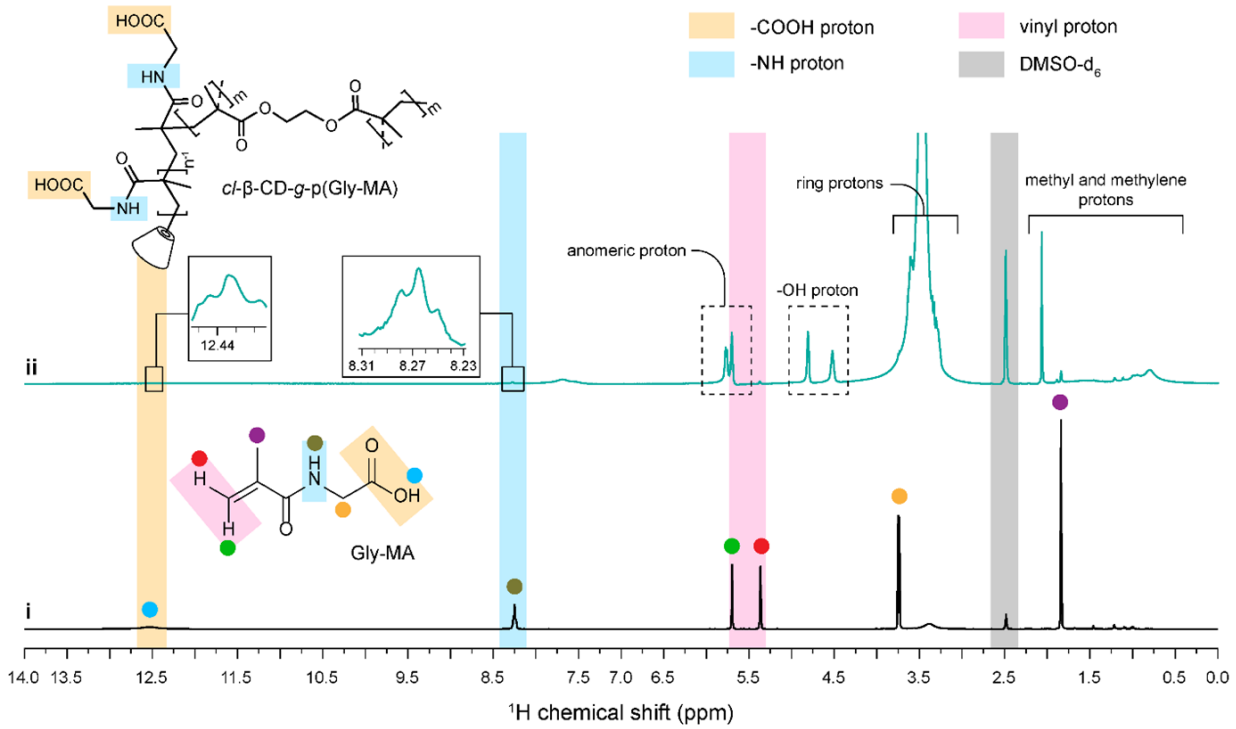

Fig. S1 ^{1}H NMR spectra of (i) Gly-MA and (ii) *cl*-β-CD-*g*-p(Gly-MA) hydrogel. [Reused from Fig. 2 of Ref. 3]

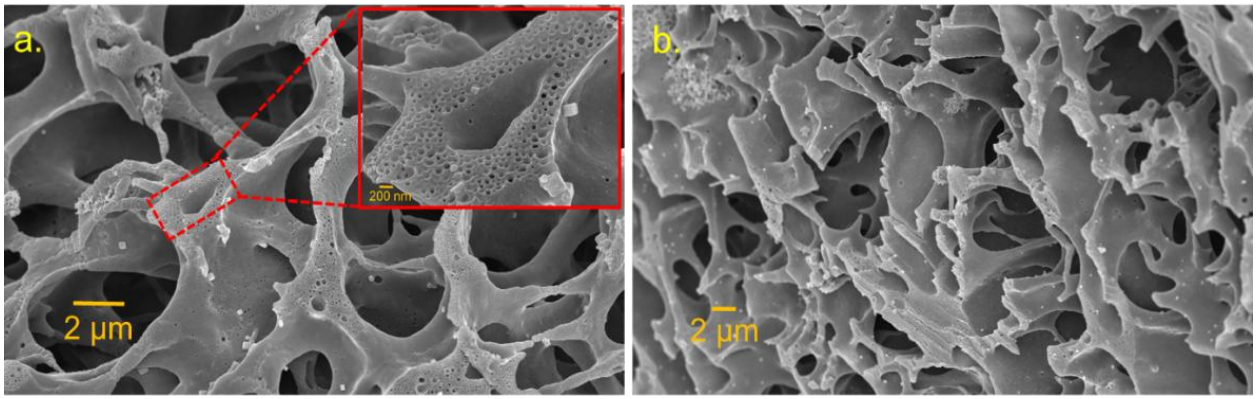

Fig. S2 (a) FESEM image of the developed injectable hydrogel *cl*-β-CD-*g*-p(Gly-MA) (b) Cross-sectional image of the pores of the hydrogel. [Reused from Fig. 2 of Ref. 3].

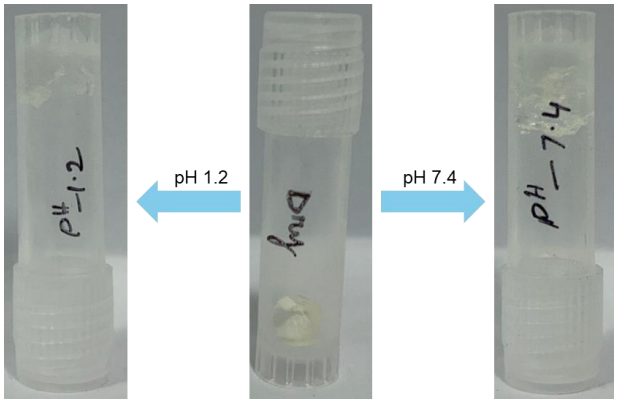

Fig. S3 Digital images of dried and swollen hydrogel at pH 1.2 and 7.4.

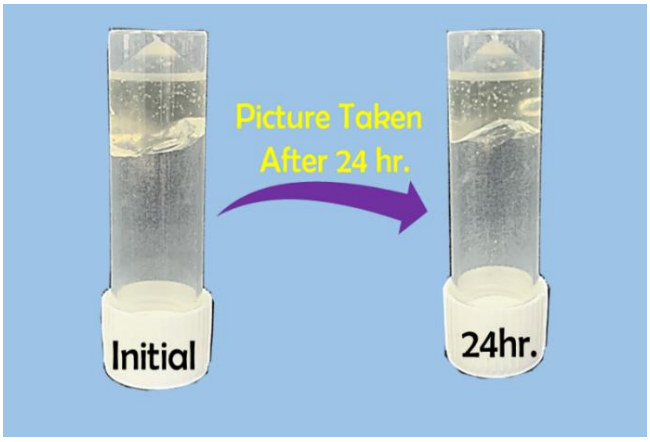

Fig. S4 Inverted picture of the hydrogel in a vial for 24 h. [Reused from Fig. 4b of Ref. 3]

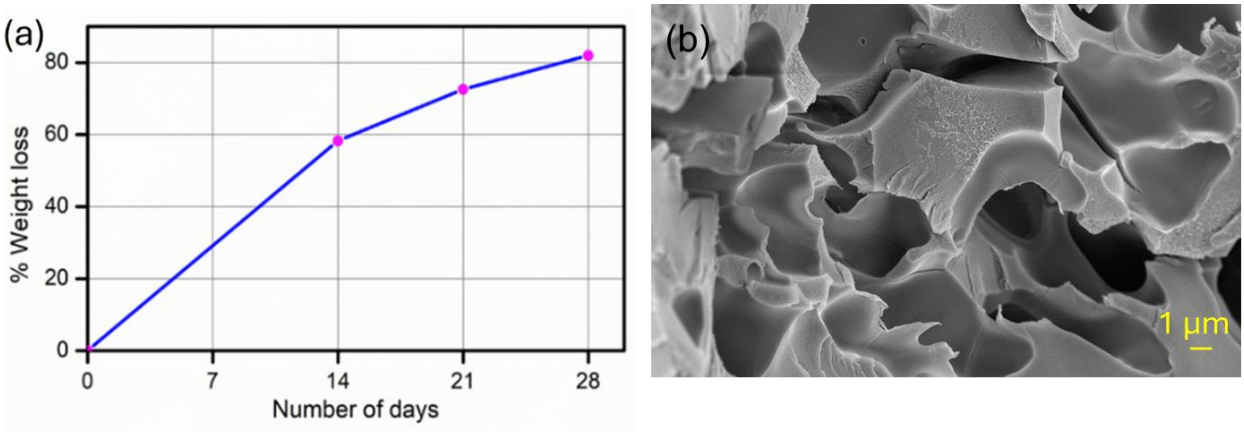

Fig. S5 (a) Weight loss vs time plot confirming *in vitro* enzymatic degradation of the gel. [Reused from Fig. 3 of Ref. 3]. (b) FESEM morphology of the 28 days degraded freeze-dried hydrogel.

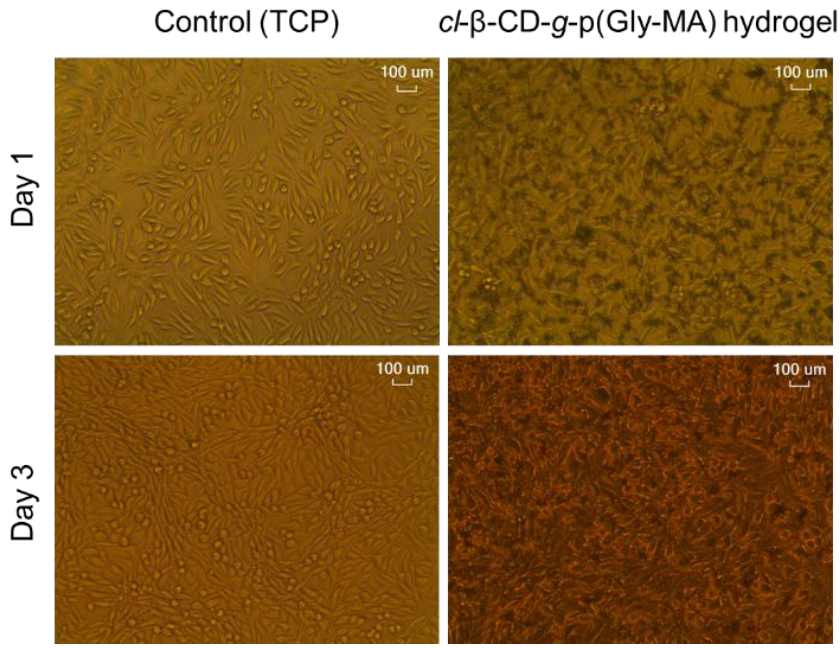

Fig. S6 Bright-field images of 3T3 cells seeded on the hydrogel and TCP after days 1 and 3.

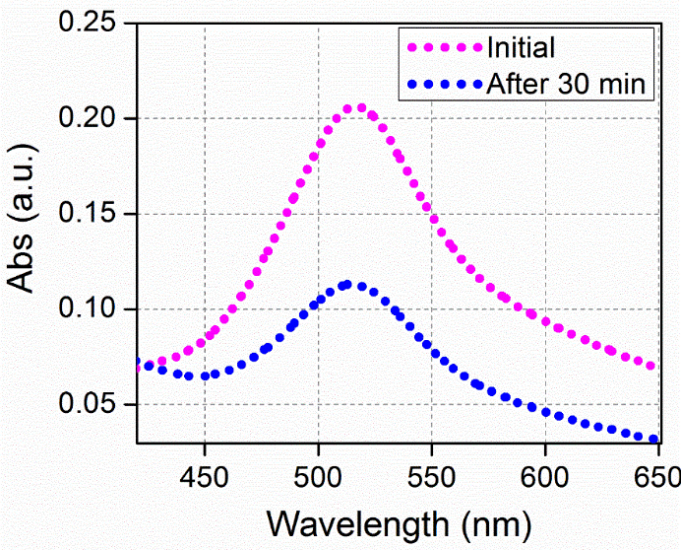

Fig. S7 UV-visible spectra for DPPH peak reduction in the presence of the synthesized hydrogel.

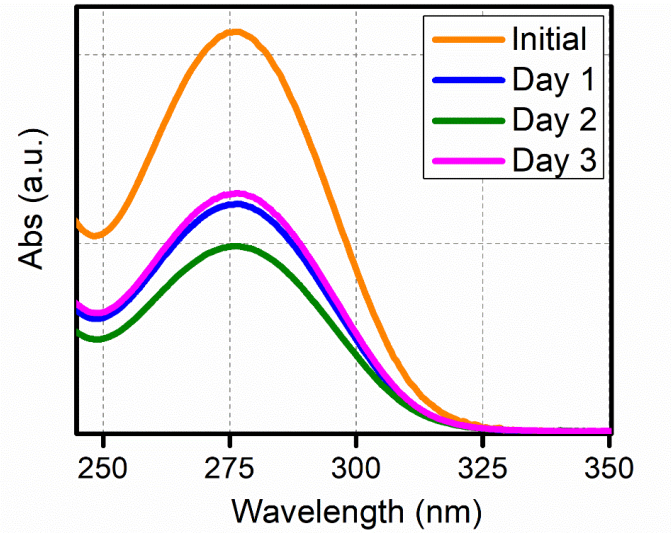

Fig. S8 UV-visible spectra for *in vitro* drug loading pattern.

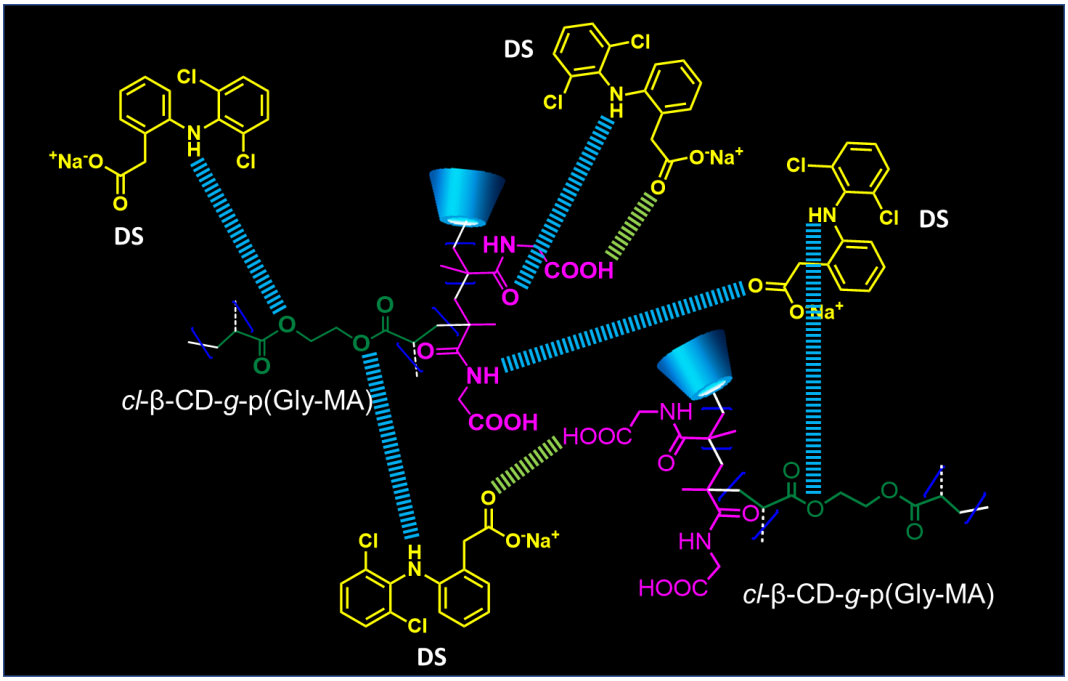

Fig. S9 Feasible interactions between *cl*-β-CD-*g*-p(Gly-MA) hydrogel and DS drug.

Table S1. % loading and % entrapment of DS in *cl*-β-CD-*g*-p(Gly-MA) hydrogel.

Matrix	Time (h)	% Loading	% Entrapment
cl-β-CD-*g*-p(Gly-MA)	24	18	39.2
	48	**23.4**	**55.3**
	72	19.2	38.9

Reference

1. A. Roy, P. Guha Ray, K. Manna, C. Banerjee, S. Dhara and S. Pal, Biomacromolecules, 2021, 22, 5256-5269.

2. A. Roy, K. Manna, P. G. Ray, S. Dhara and S. Pal, ACS Applied Materials & Interfaces, 2022, 14, 17065-17080.

3. A. Roy and S. Pal, IN Patent Application No. 202331064343, 2023.