3D Printable Collagen-Like Protein Hydrogels via Dynamic Covalent Assembly for Soft Tissue Engineering

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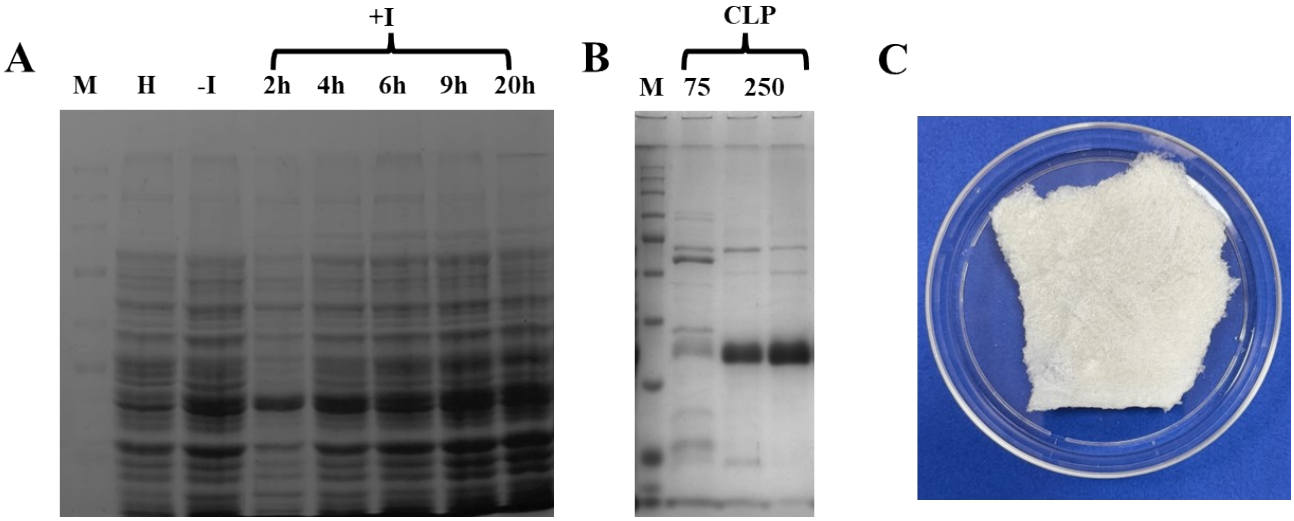

Figure S1. SDS-PAGE analysis showing the expression and purification of the collagen-like protein (CLP). (A) Total cell lysates of *E. coli* BL21 (DE3) host (H) and cells expressing CLP without (-I) and with (+I) IPTG induction at various time points. (B) Purified CLP protein eluted using 75 mM and 250 mM imidazole in the elution buffer. (C) Photograph of lyophilized CLP.

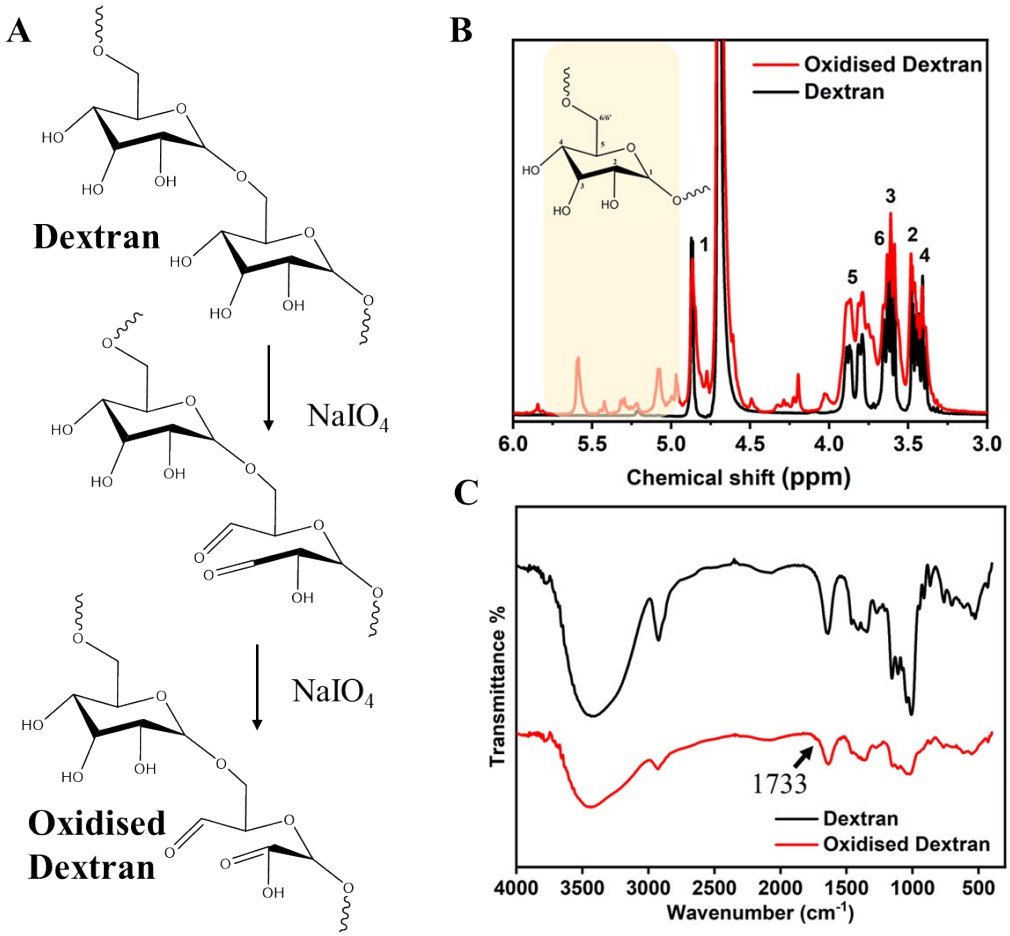

Figure S2. A) Synthesis steps of oxidised dextran. B) ^{1}H NMR spectra of dextran and oxidised dextran (AD). C) FTIR-ATR spectra of dextran and oxidised dextran.

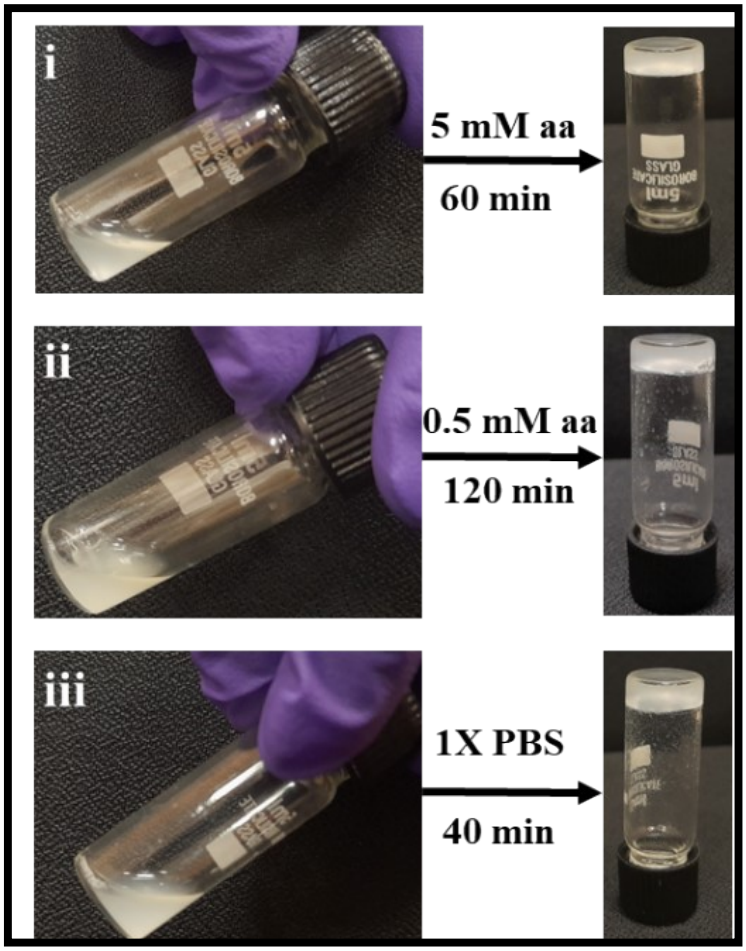

Figure S3. Photographs demonstrated sol to gel transformation of CLP-AD 1:10 in (i) 5 mM acetic acid, pH 6.5 (ii) 0.5 mM acetic acid, pH 6.5; (iii) 1× PBS, pH 7.4.

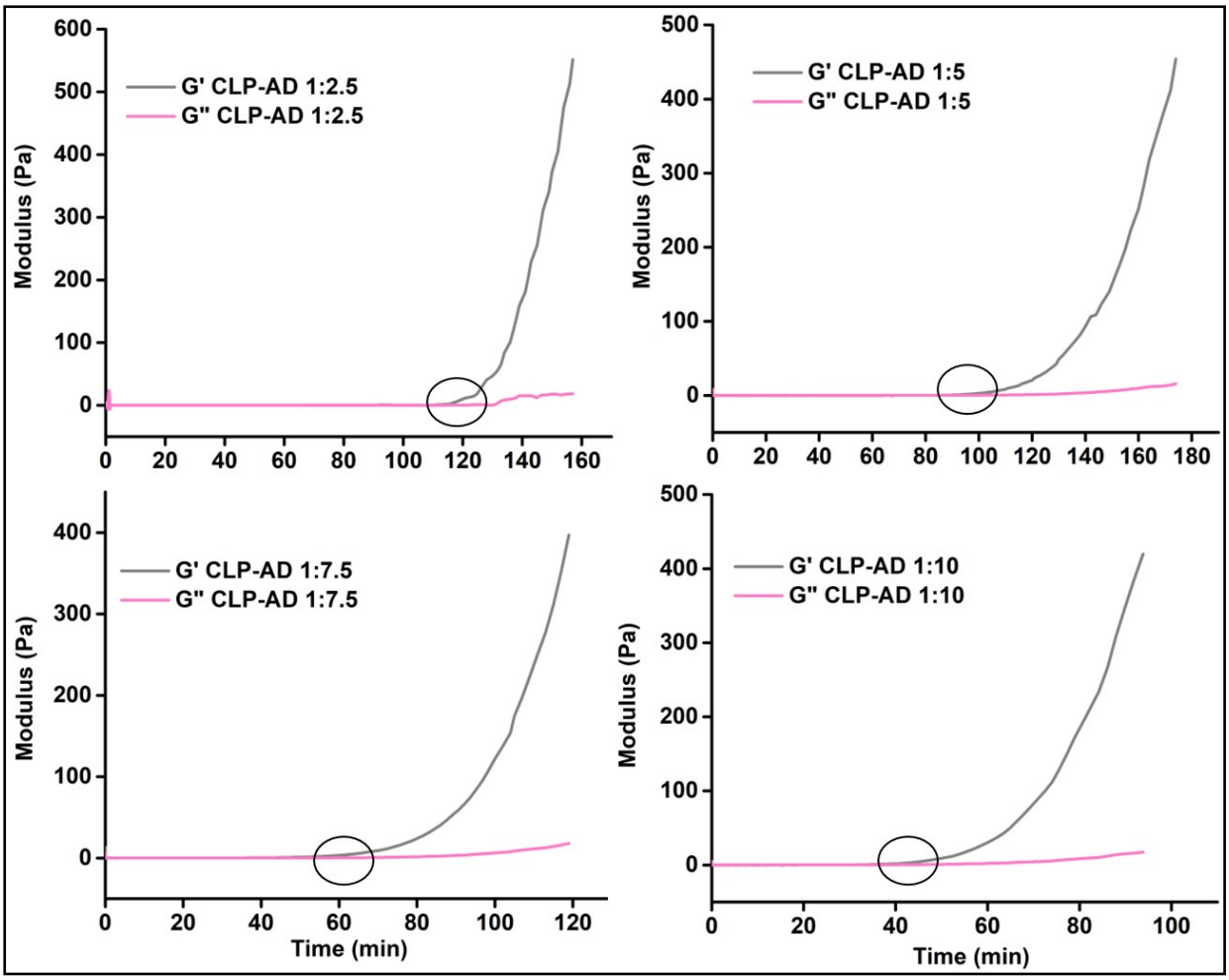

Figure S4. Time sweep experiment shows the formation of the network structure of the hydrogel over time. The crossover points of G′ and G″, marked by a circle, indicated the gelation point.

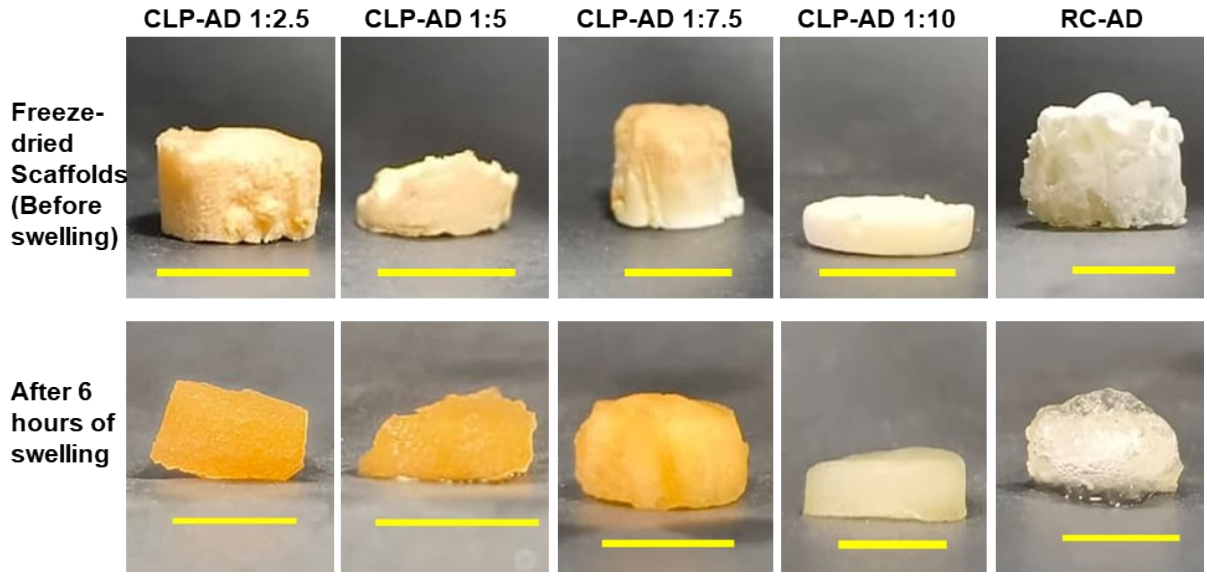

Figure S5. Photographs demonstrated swelling images of the hydrogel. Scale bar represents 5 mm.

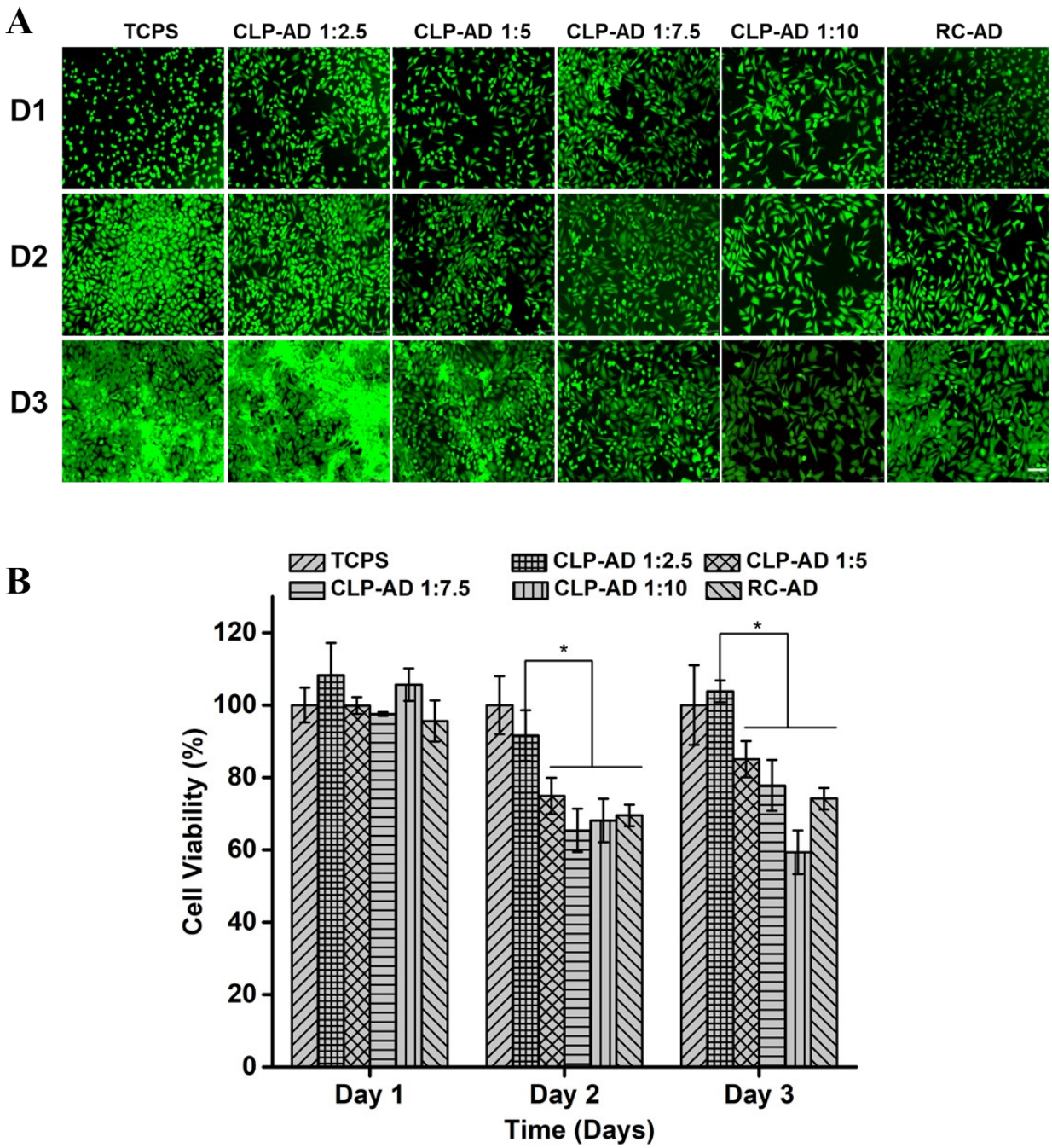

Figure S6. In vitro cytotoxicity test by extract method. A) Fluorescence images showing live/dead staining of cells treated with CLP-AD extracts after 24, 48, and 72 hours of incubation. B) MTT assay graph showing the percentage of cell viability following treatment with CLP-AD extracts. Cells seeded on tissue culture polystyrene (TCPS) without extract treatment was used as the cell control. Data were normalized to the cell control. Scale bar = 100 µm. [ns = non-significant, *$p < 0.05$].

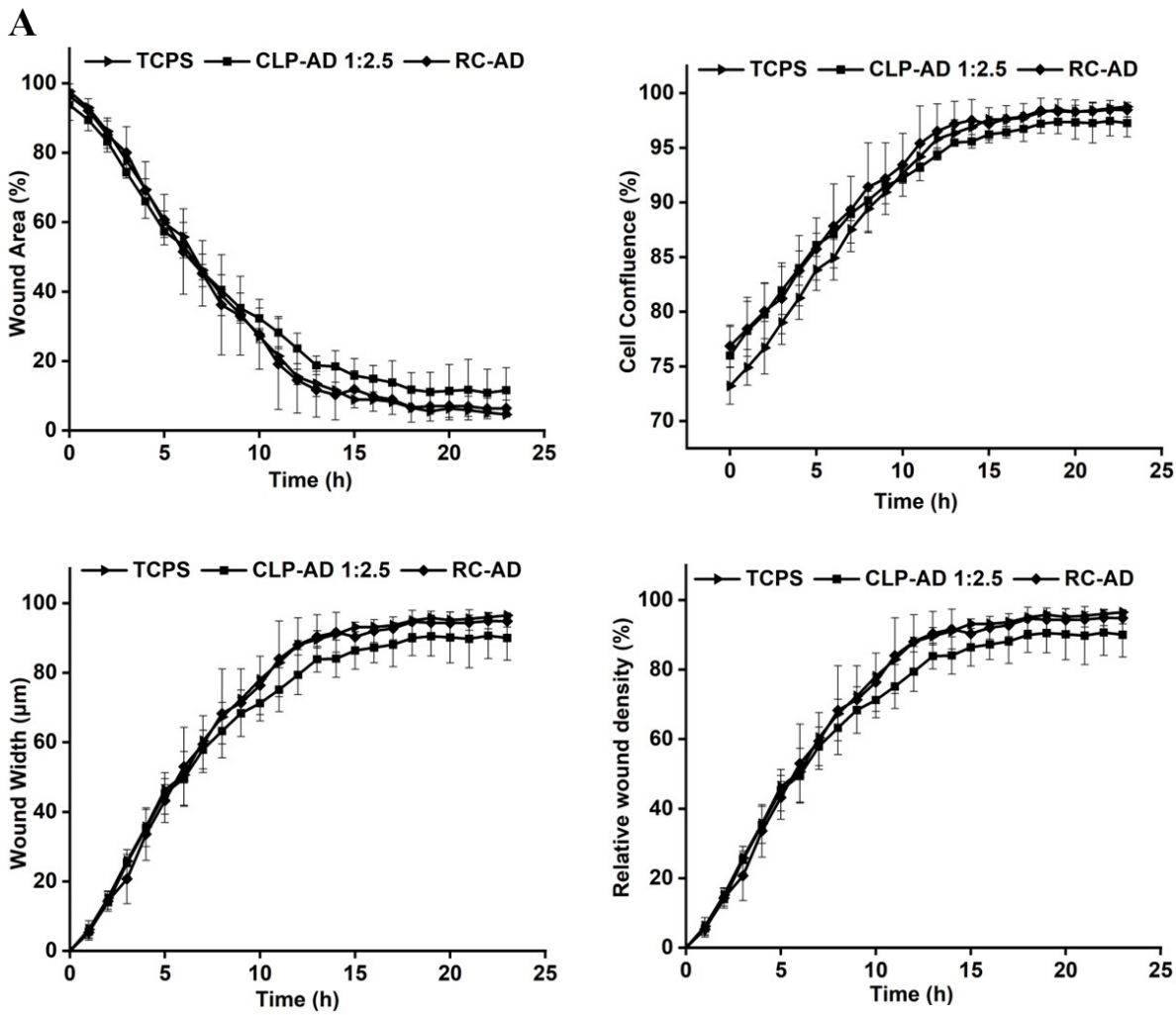

Figure S7. A) Wound area, cell confluence, wound width and relative wound density was quantified using the Julistage wound scratch analysis software and compared with untreated controls (TCPS).

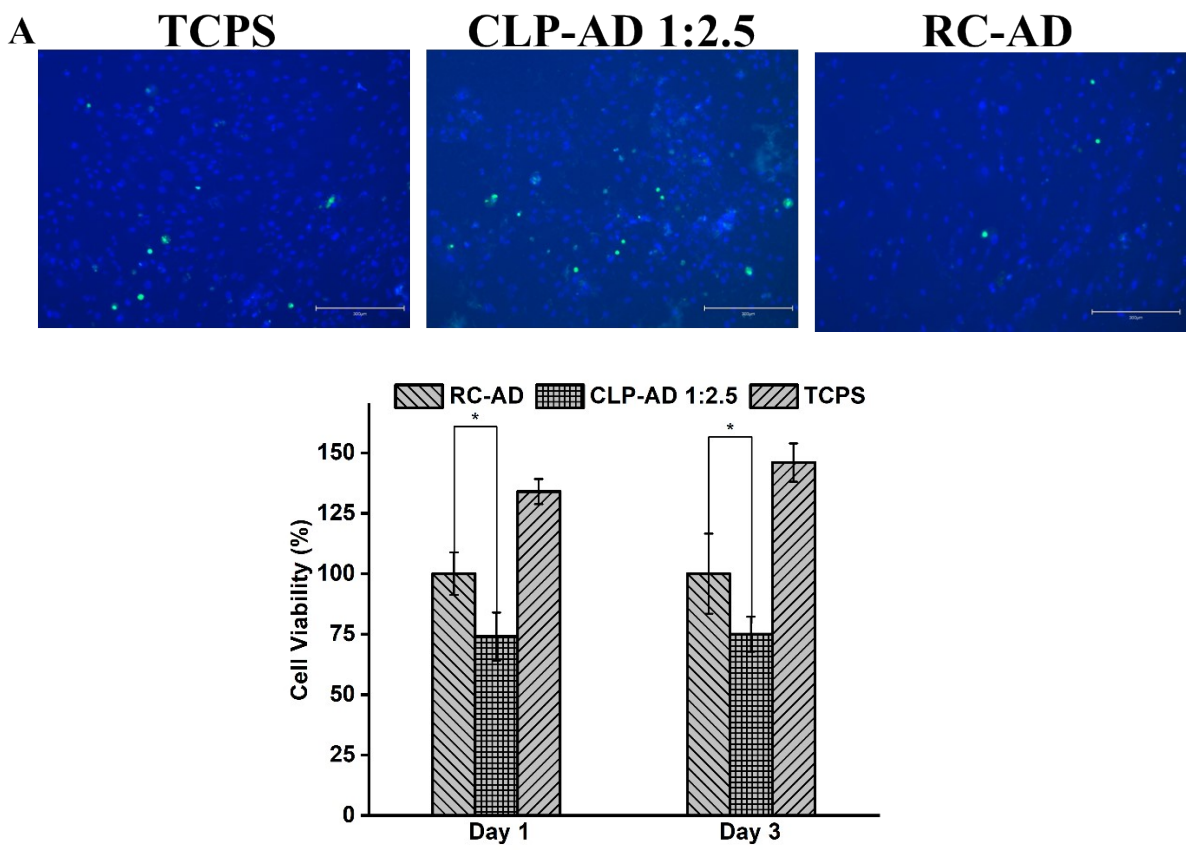

Figure S8. Fluorescence images of live/dead-stained umbilical cord-derived mesenchymal stem cells (UC-MSCs) cultured for 3 days on CLP-AD, RC-AD hydrogels, and cell control (TCPS). Cells were stained using NucBlue and NucGreen ReadyProbes. NucBlue stains the nuclei of all cells (live and dead) by binding to DNA and fluoresces blue upon UV excitation at 360 nm (emission at 460 nm). NucGreen is impermeable to intact cell membranes and selectively stains the nuclei of dead cells, emitting bright green fluorescence. Scale bar = 300 µm. *In vitro* cytotoxicity test by direct culture of Umbilical cord derived stem cells (UC-MSCs) on hydrogel surface for 3 days. Cells seeded on RC-AD hydrogel was used as control. Data normalized to the control. [ns = non-significant, *$p < 0.05$].

Table S1. Average pore size of CLP-AD hydrogels.

Groups	**Average pore size (μm)**
CLP1%-AD1%	Irregular
CLP1%-AD2.5%	143.84 ± 17.88
CLP1%-AD5%	84.32 ± 15.16
CLP1%-AD7.5%	110.20 ± 21.12
CLP1%-AD10%	87.74 ± 12.3
RC-AD	147.75 ± 30.66

Table S2. Summary of Hydrogel Optimization with Different Concentrations of CLP and AD

AD (%)	CLP (mg/mL)	Buffer	pH	Inference	Gelation time
10	10	0.5 mM Acetic acid	6.5	Hydrogel	2 h
	10	5 mM Acetic acid		Hydrogel	1 h
	10	1X PBS	7.4	Hydrogel	40 min
10	1.25	1X PBS	7.4	Soluble	-
	1.75			Soluble	-
	2.5			Soluble	-
	3.125			Soluble	-
	3.75			Soluble	-
	4.375			Soluble	-
	5			**Unstable gel**	7 h
10	10	1X PBS	7.4	Hydrogel	40 min
7.5	10			Hydrogel	1 h
5	10			Hydrogel	2 h
2.5	10			Hydrogel	5 h
1	10			**Unstable gel**	O/N
2.5	10	1X PBS	7.4	Hydrogel	5 h
	20			Hydrogel	4 h
	25			Hydrogel	3.5 h
	50			Hydrogel	3.5 h
1	10	1X PBS	7.4	Unstable gel	O/N
	20			Hydrogel	9 h
	30			Hydrogel	8 h
	40			Hydrogel	8h