Supporting Information

Physiological pH-dependent gelation for 3D printing based on the phase separation of gelatin and oxidized dextran

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Experimental Section

Materials. Gelatin (from porcine skin, Type A) was purchased from Sigma-Aldrich. Dextran (M_w = 70 kDa) and sodium periodate ($NaIO_4$) were purchased from Aladdin. Dialysis bags (MWCO 7000) were purchased from Shanghai Luniao company. The syringe filter unit (PES, 0.22 μm pore size) was purchased from Millex. Fetal bovine serum (FBS) was acquired from Sigma and penicillin-streptomycin (Pen-Strep) solution was purchased from HyClone. DME/F-12 (1:1, 1×) cell culture media was purchased from HyClone. Live/Dead staining kit was purchased from Molecular Probes. All other chemicals were of reagent grade and deionized or distilled water was used.

Synthesis of oxidized dextran (Dex-Ald). Dex-Ald was synthesized according to a previously reported procedure.[1] Dextran (70 kDa) was dissolved in deionized water to get 10 wt% solution, then certain mass ratio (dextran:$NaIO_4$ = 2:1) of sodium periodate ($NaIO_4$) was dissolved in deionized water and added into the above solution. After stirring for 8 hrs at room temperature, the reaction was quenched by excess amount of ethylene glycol. Then the solution was dialyzed against deionized water for 3 days, freezed and lyophilized. The structure of oxidized dextran was characterized by ^{1}H NMR. The oxidation degree of the polymers was quantified using hydroxylamine hydrochloride assay as previously described.

Phase separation Assays. Direct microscopy was used to observe the droplet morphology of the gelatin/Dex-Ald solution (10 wt%, weight ratio 1:1, pH 7.4). The solution was pre-warmed at 37 °C and then dropped on the object slide at rt for direct observation. The gelatin was labeled with rhodamine for visualization. To further assess the formation of phase separation, the precursor solution of gelatin/Dex-Ald at different pHs (from 6.0 to 8.0) were measured using a UV/visible spectrophotometer after incubating for 2 hrs. The size distribution of gelatin/Dex-Ald solution under

different conditions (0.01 M PBS, 0.05 M PBS, 0.1 M PBS, 0.01 M PBS with 1% F127 and 0.01 M PBS with 1% SDS) was monitored by DLS (Beckman Coulter Delsa™ Nano C) at 37 °C, where F127 was polyethylene-polypropylene glycol and SDS was sodium dodecyl sulfate.

Rheological Test. Dynamic rheology experiments were performed on HAAKE MARS Rotational Rheometer with parallel-plate (P20 TiL, 20-mm diameter) geometry at different temperature. Time-sweep oscillatory tests were performed at 10% strain (CD mode), 1 Hz frequency and a 0.5 mm gap for several minutes (including pre-mixing time of 1 min). The gel point was determined as the time when the storage modulus (G') surpassed the loss modulus (G''). Frequency-dependent oscillatory tests were performed at 10% strain (CD mode), and a 0.5 mm gap from 0.5 rad/s to 100 rad/s. The strain sweep was performed to confirm that this value was within the linear elastic regime.

Hydrogel Swelling Ratio. Hydrogel precursor solution was prepared in 0.2 mL molds for 12 h at room temperature in order to equilibrium crosslinking of the polymer network. Then, the hydrogels were placed in cell culture inserts (24-well) and submerged in D-PBS under different pH. After swelling 24 h, the change of hydrogels' weight was recorded after drawing off excess water from the surface of the gels. The swelling ratio was calculated as the following formula:

$$Swelling\ Ratio = \frac{W_{Swelling}}{W_0} \times 100\%$$

Where $W_{Swelling}$ is the weight of hydrogels immersed in D-PBS, and W_0 is the initial weight of hydrogels.

Mechanical Test. Mechanical tests were carried out on as-prepared hydrogels using GT-TCS-2000 single column apparatus with capacity of 100 N. For compression tests, hydrogel samples were prepared as cylindrical shape with a diameter of 10 mm and thickness of 3 mm. The speed was set at 1 mm/min.

Hydrogel Degradation. Hydrogel precursor solution was prepared in 0.2 mL molds for 12 h at room temperature to achieve complete gelation. Then, the hydrogels were placed in cell culture inserts (24-well) and submerged in D-PBS at pH 7.4. The rate of degradation for each gel was evaluated as the change of hydrogels weight at predetermined time intervals after drawing off excess water on hydrogels surface.

3D printing of gelatin/Dex-Ald hydrogel. Gelatin (5 wt%) and Dex-Ald (5 wt%) were

dissolved into D-PBS (pH 7.4) to get precursor solution with or without HDFs cells (1×10^6 cells mL^{-1}). Then, the pre-gel solution was loaded into extrusion cartridges, which were placed on the printing carriage of 3D printer for extrusion through a 200 μm needle. The cartridges were cooled to 18 °C within 5 minutes to make gel-phase bioinks. Then, the gels were printed on an EnvisionTEC 3D-Bioplotter, whose printing pressures and speeds were pre-set. Gels were typically printed by applying a pressure of 0.5-1 bar at a printing speed of 2 mm s^{-1} onto glass. After printing and incubating for certain time, the 3D construct was conducted for further measurement. For observing cell viability, live/dead staining was performed to differentiate living cells and dead cells. Living cells were stained with calcein-AM (green) while dead cells were stained with PI (red). Cells encapsulated in the hydrogels were imaged on an A1R Nikon confocal microscope with 4× or 10× air objective.

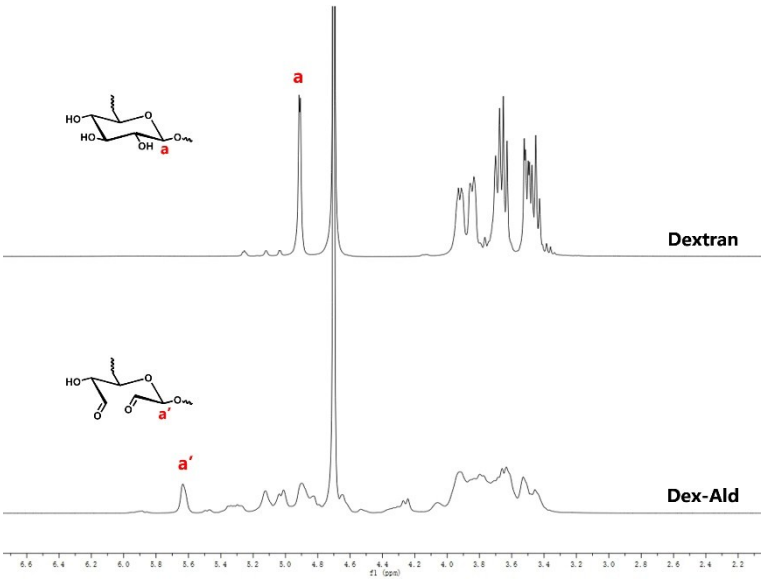

Figure S1. ^{1}H NMR spectra of Dex-Ald compared with Dextran. Obvious shift of proton from peak a to a' was observed in the spectra. The ^{1}H NMR spectrum of Dextran contains several multiplets in the range from 3.4 to 4.0 ppm corresponding to the protons of the Dextran subunits (up). Despite of the fact that the aldehyde proton signal was not observed in expected interval at 9.0-9.5 ppm, the presence of peaks in the range from 4.2 to 5.6 ppm can be taken as indirect evidence of the conversion of aldehyde groups into the more stable hemiacetals (down).

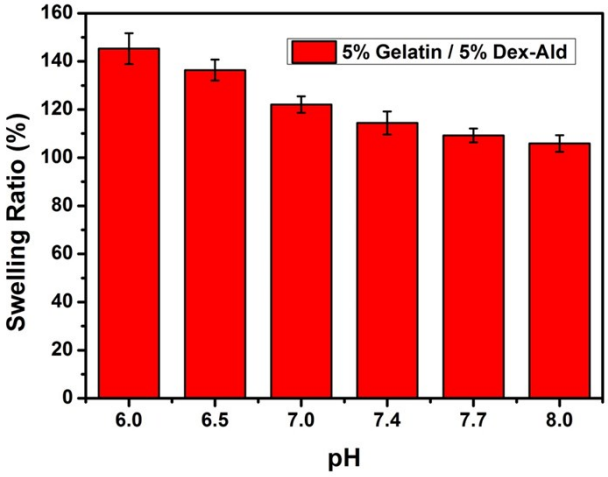

Figure S2. The swelling ratio profile of gelatin/Dex-Ald hydrogel (10 wt%, weight ratio 1:1) under different pH (n=3 for each pH).

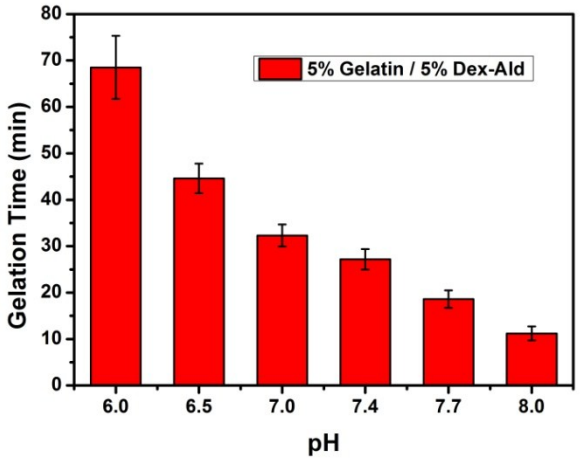

Figure S3. Gelation time of gelatin/Dex-Ald hydrogel (10 wt%, weight ratio 1:1) at 18 ^{o}C under different pH (n=3 for each pH).

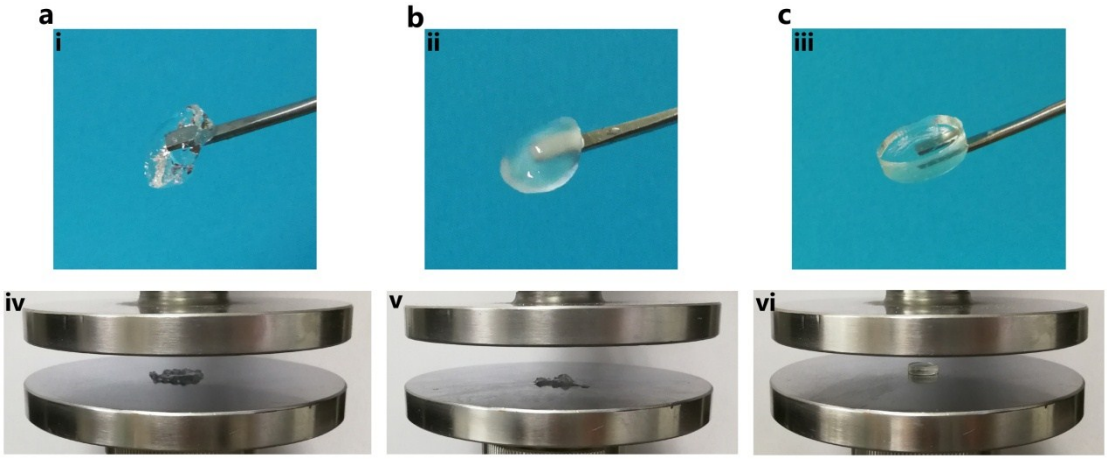

Figure S4. Graphs of stress-strain test (60 % strain) of gelatin (i, iv), gelatin/Dex-Ald (5 min) (ii, v) and gelatin/Dex-Ald (2 hrs) (iii, vi) hydrogels.

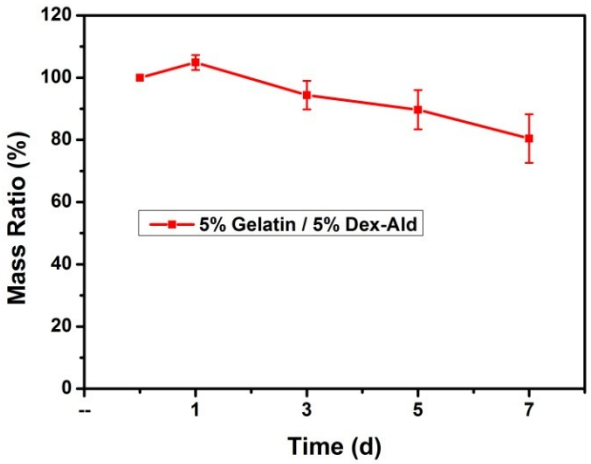

Figure S5. The degradation profile of gelatin/Dex-Ald hydrogel (10 wt%, weight ratio 1:1, pH 7.4) (expressed in terms of the gel mass at different time points to the initial mass).

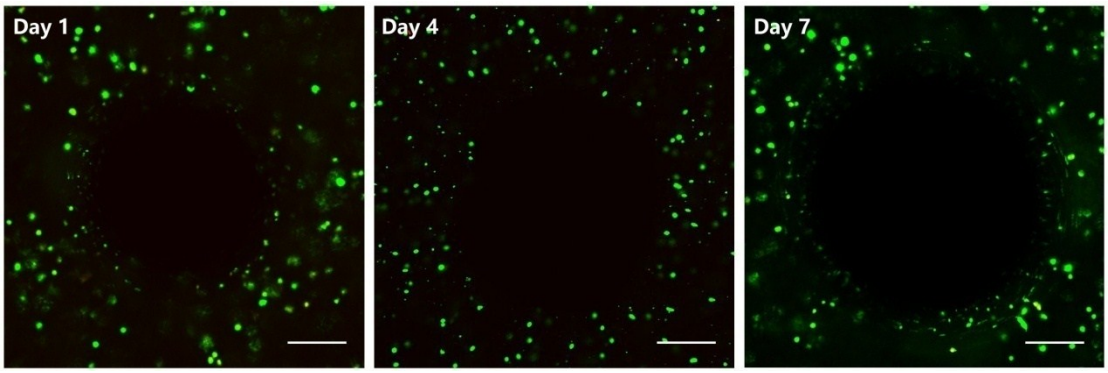

Figure S6. Confocal images of HDFs in 3D printed hydrogel stained with a live/dead reagent at day 1, day 4 and day 7. Live cells fluoresce green, whereas dead cells emit red. Scale bar: 200 μm.

References

(1) Zhang H.; Qadeer A.; Chen W., *Biomacromolecules* **2011**, *12*, 1428-1437.