Supplementary Information

for

Near-instantaneous volumetric printing of complex scaffolds comprised of tough PEG-based hydrogels

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Materials

All substances were used exactly as they were purchased. Linear Polyethylene glycol (PEG, 6, 10 kg/mol, Sigma-Aldrich), glycerol ethoxylate (1 kg/mol, Sigma-Aldrich), 4-arm PEG (2, 20 kg/mol, JenKem technology), 3-Mercaptopropionic acid (99%, Thermo Scientific), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, 95+%, Sigma-Aldrich), sodium hydrogen carbonate (VWR chemicals), magnesium sulfate ($MgSO_4$) (99%, Acros Organics), sulfuric acid (H_2SO_4) (95%, VWR chemicals), toluene (99.8+%, Fisher Scientific), dichloromethane (DCM) (99.8+%, Thermo Scientific), diethyl ether (99.5%, Thermo Scientific), tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCL, 98+%, TCI Europe), 5-norbornene-2-carboxylic acid isomer 97%, (Thermoscientific), N-N′-Dicyclohexylcarbodiimide (DCC) 99% (Arcos organics), 4-Dimethylaminopyridine (DMAP) 99% (Arcos organics); pyridine anhydrous max. 0.003% water (VWR chemicals), 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO, 98%, Sigma-Aldrich), Tartrazine (98+%, TCA America). Human mesenchymal stem cells (hMSCs, Lonza) were maintained in complete media composed of alpha minimal essential media (MEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin (Gibco). Cellular viability was investigated by staining cells with Calcein AM (Invitrogen) and propidium iodide (Miltenyi Biotec). Cytotoxicity of gels was examined using CyQUANT™ LDH Cytotoxicity Assay Kit (Invitrogen). Alexa Fluor® 488 Phalloidin, and DAPI were purchased from Thermo Fisher Scientific.

EXPERIMENTAL SECTION

Synthesis

Synthesis of 3-arm PEG-Thiol (1 kg/mol) or 4-arm PEG-Thiol (2 kg/mol)

Multi-arm PEG-Thiols were synthesized through Fisher esterification using well-established protocols with slight modifications.[1,2,3] Three-arm PEG-Thiol was prepared by dissolving 10 g (10 mmol) of glycerol ethoxylate (1 kg/mol) in 100 mL of toluene. Similarly, for the synthesis of four-arm PEG-Thiol, 10 g (5 mmol) of 4-arm PEG (2 kg/mol) was dissolved in the identical volume of toluene. After the complete dissolution of the PEG, four drops of concentrated H_2SO_4 were introduced, followed by the addition of 3-Mercaptopropionic acid (2 equiv per arm; 6.4 g, 60 mmol, 5.2 mL for 3-arm PEG-Thiol, and 4.2 g, 40 mmol, 3.5 mL for 4-arm PEG-Thiol) to the solution. Subsequently, the solution underwent reflux for 24 hours at 135 °C, utilizing a Dean-Stark apparatus to collect the water produced as the byproduct of the reaction. Afterward, toluene was removed using a rotary evaporator, and the resulting product was dissolved in DCM (100 mL), followed by washing with saturated $NaHCO_3$, 30 mL of deionized water containing TCEP·HCl (0.2 g, 0.7 mmol) and brine. The solution was then dried using $MgSO_4$, and DCM was evaporated on a rotary evaporator. The product was vacuum-dried to yield a transparent, viscous polymer

and stored at -20 ºC. The ^{1}H NMR spectra of 3-arm PEG-Thiol$_{1k}$ and 4-arm PEG-Thiol$_{2k}$ are depicted in **Figure S1** and **Figure S2**, respectively.

Synthesis of linear or 4-arm Norbornene functionalized PEG (6, 10, and 20 kg/mol)

Linear or 4-arm PEG-Norbornenes were synthesized using well-established protocols with slight modifications.[4,5] In a round-bottom flask covered with aluminum foil under nitrogen, 5-norbornene-2-carboxylic acid (10 equiv per arm; 4.6 g, 33.3 mmol for linear PEG$_{6k}$, 2.8 g, 20 mmol for linear PEG$_{10k}$, and 4-arm PEG$_{20k}$) and DCC (5 equiv per arm; 3.4 g, 16.7 mmol for linear PEG$_{6k}$, 2.1 g, 10 mmol for linear PEG$_{10k}$, and 4-arm PEG$_{20k}$) were allowed to react in 25 mL of dry DCM for 1 hour at room temperature. This resulted in the formation of an intermediate product, norbornene carboxylic acid O-acyl-urea, followed by the subsequent generation of norbornene anhydride and the byproduct dicyclohexylurea. The formed norbornene anhydride was then filtered through a fritted funnel. In another round bottom flask, 30 mL of dry DCM was used to dissolve 10 g of dry PEG (linear PEG with a molar mass of 6 or 10 kg/mol or 4-arm PEG with a molar mass of 20 kg/mol) and DMAP (0.5 equiv per arm; 0.2 g, 1.7 mmol for linear PEG$_{6k}$, 0.12 g, 1 mmol for linear PEG$_{10k}$, and 4-arm PEG$_{20k}$). Then, pyridine (5 equiv per arm; 1.3 g, 16.7 mmol for linear PEG$_{6k}$, 0.8 g, 10 mmol for linear PEG$_{10k}$, and 4-arm PEG$_{20k}$) was added to the solution. The filtrate obtained from the first reaction was then added dropwise to this solution. The flask was placed in an ice bath while covered with foil, and the reaction continued for 24 hours under nitrogen. 50 mL of DCM was added to the product and subsequently washed with saturated $NaHCO_3$ and brine. The solution was then dried using $MgSO_4$. The resulting product was concentrated to approximately 20 mL. Subsequently, the solution was added dropwise into cold diethyl ether (500 mL), and the precipitation was repeated (three times). The product was vacuum-dried to obtain a white powder and stored at -20 ºC. The ^{1}H NMR spectrum of linear PEG-Norbornene$_{10k}$ is depicted in **Figure S3**.

Hydrogel preparation

The hydrogels were fabricated by combining multi-arm PEG-Thiol crosslinkers, either 3-arm PEG-Thiol (1 kg/mol) or 4-arm PEG-Thiol (2 kg/mol), with telechelic linear PEG-Norbornenes of approximate molar masses 6 and 10 kg/mol, or 4-arm PEG-Norbornene (20 kg/mol). The hydrogels were prepared with varied polymer contents, ranging from 5 wt % to 45 wt %, using molar ratios of Norbornene to thiol groups at 1:1, 1.25:1, and 2:1. The individual components, PEG-Thiol, and PEG-Norbornene were accurately weighed into separate vials. Initially, the PEG thiol was dissolved in deionized water, and this solution was then added to the vial containing PEG-Norbornene. To this mixture, 0.05 wt % of LAP was added as the photoinitiator for most experiments, although for certain cases, gels were prepared using either 0.15 or 0.3 wt % LAP. After the thorough dissolution of the components and achieving a clear solution, the precursor solutions underwent sonication to eliminate bubbles and were subsequently transferred into the appropriate

molds. UV irradiation (365 nm- 10 mW/cm^2) was applied to cure the solutions. **Figure S4** illustrates the 20 wt % hydrogel solution created using linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ before and after 5 seconds of UV illumination.

Uniaxial compression testing

Uniaxial compression testing was carried out using a Shimadzu AGS-X tensile tester. For the majority of the hydrogels, a 500 N load cell was employed, while gels with a polymer content of 45 wt % were tested using a 5 kN load cell. The hydrogel solutions with varied formulations were prepared following the previously described protocol. These solutions were then poured into PMMA molds to create cylindrical specimens with a diameter of 8 mm and a height of 3 mm (**Figure S5**). Subsequently, the samples were subjected to UV curing for 10 minutes (365 nm, 10 mW/cm²). To ensure thorough crosslinking, the samples were inverted in the UV crosslinker after 5 minutes of UV curing. The hydrogels were compressed to 90% strain at room temperature at a compression rate of 1 mm/min. The elastic modulus (E) was calculated from the slope of the initial linear fitted trendline of the engineering stress/strain data within the 5–20% strain range. At least three samples were measured for each condition, and the results were reported as mean ± standard deviation.

Gel fraction and swelling degree

To determine the gel fractions (GF), cylindrical specimens with a diameter of 8 mm and a height of 3 mm using different formulations were fabricated, following the previously mentioned procedure. The gels were dried in a vacuum oven at 50 ºC overnight, and their initial dry weight (W_{d1}) was recorded. Subsequently, the dried gels were immersed in deionized water (3 mL) for two days at room temperature, with regular water changes to remove any unreacted precursors. Following this, the gels were dried again in the vacuum oven at 50 º C and weighed (W_{d2}). Each gel formulation was tested in triplicate, and the results were presented as mean ± standard deviation. Equation (1) was used to calculate the gel fraction.

$$\text{Gel Fraction (\%)} = \frac{W_{d2}}{W_{d1}} \times 100 \quad (1)$$

In order to assess the swelling behavior of various gel compositions, hydrogels with different formulations were first created as previously described. They were then dried overnight at 50 ºC in a vacuum oven, and their initial dry weights were recorded. Then, these gels were placed in distilled water (3 mL) at room temperature with repeated water changes until equilibrium was attained. Equation (2) was used to determine the degree of swelling based on the gels' initial dry weight (W_{d1}) and their weight at equilibrium (W_s). Each gel formulation was tested in triplicate, and the results were reported as the mean ± standard deviation.

$$\text{Degree of swelling (\%)} = \frac{W_s - W_{d1}}{W_s} \times 100 \quad (2)$$

Rheology

Photo-rheology experiments were performed using an Anton Paar 702 rheometer equipped with an 8 mm parallel plate geometry and an OmniCure S2000 light. Following the previously described protocol, several hydrogel precursor formulations were prepared and loaded into the rheometer. Time sweep measurements were performed at a frequency of 10 rad/s and a strain of 0.1% over the course of 240 seconds. UV light was applied from 60 to 240 seconds at a wavelength of 405 nm and an intensity of 10 mW/cm^2 to monitor the crosslinking process. Additionally, photo-rheology experiments were performed using an Anton Paar Physica MCR 302 with a parallel plate (PP25 as the top plate, diameter: 25 mm) and an OmniCure S1500 light source with a 400-500 nm wavelength filter. These measurements were performed at 40 °C with a strain amplitude of 0.1% and an oscillation frequency of 1 Hz. 180 µL of resin was placed between the parallel plates with a gap of 150 µm. UV light was turned on after 60 s, exposing the sample for 180 seconds.

Cellular viability and cytotoxicity

5%, 20% and 45% gels were prepared as mentioned.Prior to cell culture, these gels were sterilized for 15 minutes using UV light (254 nm). Following this, serum free alpha minimal essential media (MEM) was added on top of each gel to allow swelling. After 24 hours, hMSCs were seeded at a density of $2.5 \times 10^4/cm^2$ on top of each gel and on tissue culture plastic as 2D controls. Media was refreshed 48 hours post cell seeding.

After 5 days of culture, conditioned media was collected to measure the release of lactase dehydrogenase (LDH), while the cells were stained to examine viability. Briefly, cells were stained using Calcein AM (1:1000) in serum free media for 30 minutes at 37 ºC. The staining solution was aspirated and propidium iodide (1:100) in PBS was added 5 minutes before imaging using the SlideScanner (Nikon Ti-E) microscope. Images were processed using Fiji (Image J). To measure cytotoxicity of 5, 20, and 45% gels, conditioned media from live control and experimental conditions was collected on Day 2 and Day 5 and centrifuged for 10 minutes at 10,000 g to remove cellular debris. The LDH assay was performed according to the manufacturer's instructions. Conditioned media was mixed with equal volume of the reaction mix and incubated at room temperature for 30 minutes protected from light. The reaction was stopped using the stop solution, and the absorbance was measured at 490 and 680 nm using the CLARIOstar Plus (BMG LABTECH) plate reader. Positive controls for propidium iodide staining and maximum release of lactase dehydrogenase were obtained by treating cells on Day 5 with 1% Triton-X 100 and 1x lysis buffer (kit) respectively for 45-60 minutes. The experiments were performed in triplicates. LDH release on day 2 and 5 was compared using Wilcoxon signed rank-test and a p value

Injectability

In order to assess the injectability of the system, a 20 wt % hydrogel solution comprising linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a 0.05 wt % LAP concentration was prepared. The molar ratio of norbornene to thiol groups was 1:1. A deep cavity was made in a porcine bone. The hydrogel resin was injected into the tissue's defective area with an 18G needle (1.2 mm) under simultaneous UV light (365 nm) illumination. A full minute of UV irradiation was used to ensure the entire deep area (~ 1 cm) was fully cured. One drop of blue food coloring was added to the hydrogel solution during resin preparation to enhance visualization.

Injectability studies were also carried out utilizing cell-laden hydrogel solutions to investigate the viability of the cells after injection. ATDC5 chondrocyte cells (RIKEN cell bank, Japan) were cultured in Dulbecco's Modified Eagle's Medium-F12 (DMEM-F12, high glucose) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) P/S at 37 °C in a humidified incubator with 5% CO_2. The medium was changed every 2–3 days, and the cells were passaged at 80% confluence.

20 wt % hydrogels were prepared using linear PEG-Norbornene$_{10k}$ combined with a 4-arm PEG-Thiol$_{2k}$ crosslinker at a LAP concentration of 0.05 wt %. In all conditions, cysteine-RGD peptide was added at the final concentration of 1 mM. Using the previously described protocol, hydrogel solutions were prepared in cell culture media (rather than water) containing P/S. ATDC5 chondrocyte cells were re-suspended in the hydrogel solution to obtain a concentration of 5×10^6 cells/mL. Then, approximately 100 µL of the cell-laden solution was injected into each well of a flat-bottom black 48-well plate through 1 mL syringes with pink nozzles (18G needles). Subsequently, these hydrogels were photo-cured at 365 nm (10 mW/cm^2) for 1 minute. Fresh media was added to the gels.

Cell viability was assessed after 1, 7, and 28 days using Live/Dead Fixable kit (Thermofisher LIVE/DEAD™ Fixable Far-Red Dead Cell Stain Kit- Catalog Number: L34973, 80 Assays) according to the manufacturer's instructions. The gels were washed twice with PBS; then, cells were stained with the dye solution in PBS (0.5 µL dye in 500 µL PBS). The gels were incubated at room temperature for 30 minutes in the dark, washed once with PBS, and fixed with paraformaldehyde (PFA). Cell morphology was visualized on days 1, 7, and 28 by staining the actin filaments in the cell body with Alexa Fluor® 488 Phalloidin and the cell nucleus with DAPI. The gels utilized for the Live/Dead Fixable were rinsed with PBS (400 µm). Following the addition of 0.1% Triton solution (400 µm), the gels were incubated at room temperature for 30 minutes in the dark and then washed with PBS. Subsequently, BSA (0.1% (w/v)) was added to the samples, and the gels were incubated for 1 hour, followed by rinsing with PBS. The cells were then stained using DAPI (1:500) and Phalloidin 488 (1:250) solutions in PBS and incubated for one hour

at room temperature. The stained cells were imaged using a fluorescent microscope to determine their viability and morphology.

Digital light processing (DLP)

Using a BIOLUX custom-built DLP printer, one-layer experiments known as the gelation test measurements were performed to evaluate the system's capability for processing via DLP. Various hydrogel resins containing linear or 4-arm PEG-Norbornene (6, 10, and 20 kg/mol) and 4-arm PEG-Thiol crosslinker (2 kg/mol) were prepared at a 0.05 wt % LAP concentration, using the previously described protocol. The total polymer concentrations of the resins were either 20 or 25 wt %. The molar ratio of norbornene to thiol groups was 1:1. In these experiments, 600 µL of the prepared photo-resins were loaded onto a macroscopic glass slide positioned on the glass stage of the DLP printer. With the aid of a 405 nm laser, a pattern consisting of a square frame with a smaller solid square inside was projected eight times onto the various locations of the glass slide. The duration of light exposure varied for each pattern, ranging from 2 to 9 seconds.

In order to investigate the system's capacity for 3D structure fabrication using DLP, 20 wt % hydrogel photo-resins were prepared using linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ crosslinkers with a 0.3 wt % LAP concentration. The molar ratio of norbornene to thiol groups was 1:1. Tartrazine as a photo-absorber was added to the photo-resins at a final concentration of 1 mM. Using DLP, the object was fabricated layer by layer. Each layer height was set at 100 µm, and the printing speed was fixed at 2 mm/s. Samples were exposed to 405 nm light (10 mW/cm^2), with the first layer being cured for 15 seconds and the subsequent layers being exposed for 3 seconds each. To remove any unreacted resin, printed objects were rinsed with 70% ethanol once printing was finished.

Volumetric 3D printing (VAM)

Volumetric printing was carried out using the Tomolite (405 nm light source) from Readily3D. Linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ crosslinkers were selected as building blocks for object fabrication via VAM. A 1:1 molar ratio of norbornene to thiol terminal reactive groups was used to prepare 45 wt % resin formulations with a 0.3 wt % LAP concentration. TEMPO, acting as a radical scavenger, was added to the photo-resins at a 1:10 or 1:5 mass ratio to LAP. PEG-Thiol$_{2k}$ and PEG-Norbornene$_{10k}$ were individually weighed in separate vials. LAP was precisely weighed and added to the vial containing PEG-Norbornene$_{10k}$. Subsequently, PEG-Thiol$_{2k}$ was dissolved in deionized water, and this solution was then introduced into the vial containing PEG-Norbornene$_{10k}$ and LAP. TEMPO was added to this mixture (from a stock solution of 2 mg mL^{-1} in deionized water). Several cycles of vortexing and sonication were applied until complete dissolution and bubble elimination occurred. Next, the resin was transferred into a vial for

volumetric printing. Various complex structures were created with illumination at 405 nm with an average light intensity of 9.4 mW/cm^2 using different light doses. After printing, the unreacted resin in the vial was carefully and gradually removed using a glass pipet. A small amount of water was added to the vial to facilitate the resin removal process, making the unreacted resin less viscous. Next, the printed structures were carefully removed from the vial and washed with water to eliminate unreacted resin. Notably, no post-printing curing was needed for any fabricated structures, regardless of the geometry, due to thiol-norbornene chemistry's rapid and selective nature. Different steps of post-printing are illustrated in **Figure S6**.

Crosslink density calculations:

The effective crosslink density of the polymer networks was estimated from oscillatory shear rheology measurements using the theory of ideal elastic (rubber-like) networks. In this framework, the shear storage modulus in the plateau region (G′) is directly related to the number density of elastically active network chains (eq 3).

$$\nu_e = \frac{G}{RT} \quad (3)$$

Here, G' is the storage modulus in the rubbery plateau, v_e is the effective crosslink density (mol·m^{-3}), R is the universal gas constant, and T is the absolute temperature (K). The storage modulus values used for these calculations were taken from the linear viscoelastic regime at low frequencies, where the material exhibits a frequency-independent plateau. All measurements were performed at 298 K, and the corresponding temperature was used in the calculations.

Crosslinking density calculations based on swelling

The crosslink density of the different hydrogel formulations was calculated from the equilibrium swelling data using the Flory–Rehner equation. The swelling factor (Q_m) was calculated using equation (4), where W_d is the initial dry weight of the gel and W_s is the weight at equilibrium (after swelling in water):

$$Q_m = \frac{W_s}{W_d} \quad (4)$$

Q_m was then used to calculate the volume fraction of polymer ($\boldsymbol{v}_2$), according to equation (5), where ρ_p is the density of the dry gel (1.12 g/cm^3 for PEG) and ρ_s is the density of the solvent (1 g/cm^3 for water):

$$\upsilon_2 = \frac{1}{1+Q_m \cdot \frac{\rho_p}{\rho_s}} \quad (5)$$

The volume fraction of polymer was then used to calculate the crosslink density, together with the molar volume of the solvent (V_1, 18 cm^3/mol for water), and the polymer-solvent interaction parameter (χ_1) (0.426 for PEG-water). The crosslink density (ν_e) is calculated according to the Flory-Rehner equation eq 6.

$$\nu_e = -\frac{1}{V_1} \cdot \frac{\ln(1-\upsilon_2)+\upsilon_2+\chi\upsilon_2^2}{\upsilon_2^{1/3}-\frac{\upsilon_2}{2}} \quad (6)$$

The cross-link density here assumes ideal swelling and a uniform, homogeneous network. Therefore, the contribution of finite chain length (M_n^{-1}) is neglected in the calculation. While this assumption of an ideal network may be associated with some uncertainty, the magnitude of the crosslink density extracted from swelling data is roughly (factor 2–3) in agreement with the rheological measurements and the theoretical values from the building blocks.

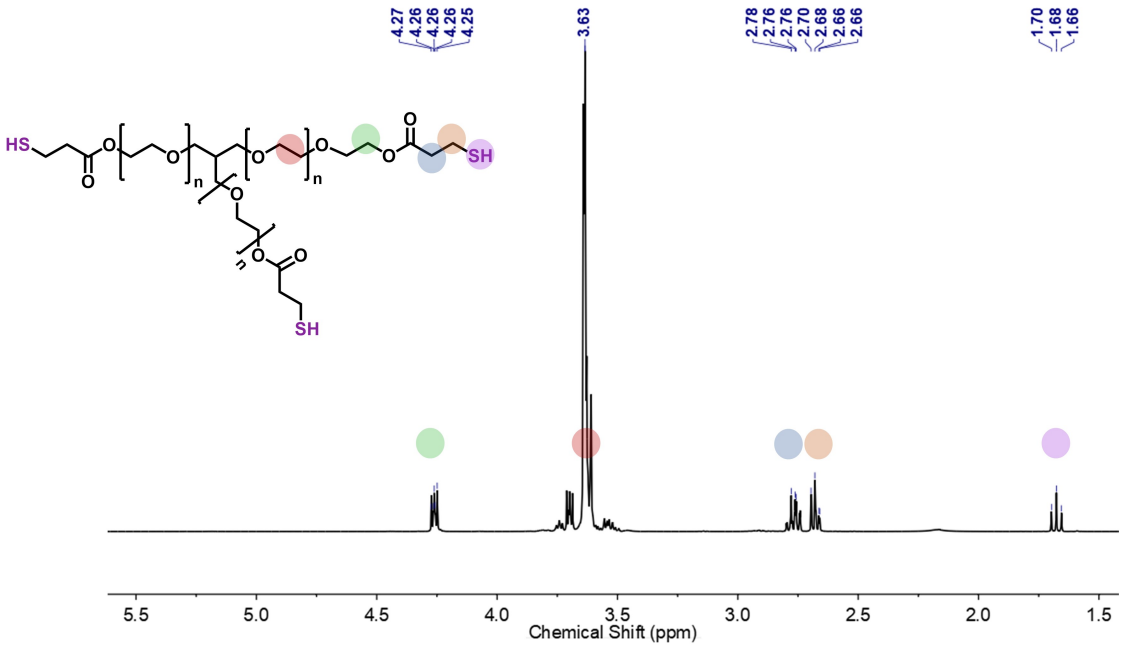

Figure S1. ^{1}H NMR spectrum of 3-arm PEG-Thiol (1 kg/mol) in $CDCl_3$ (400 MHz).

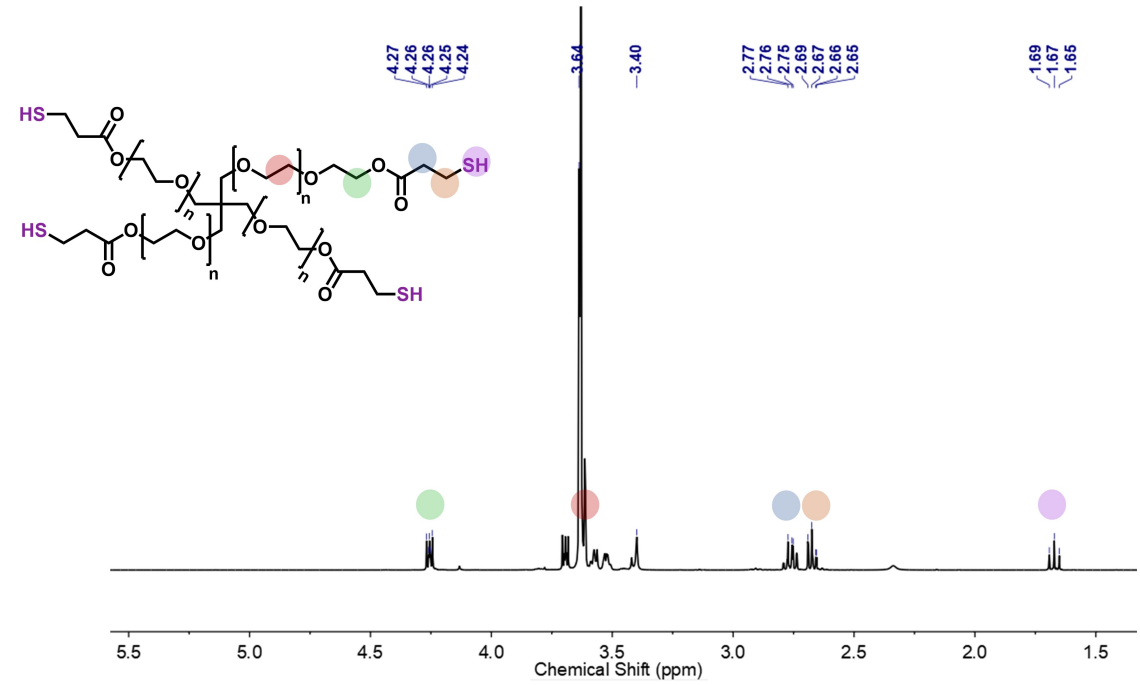

Figure S2. ^{1}H NMR spectrum of 4-arm PEG-Thiol (2 kg/mol) in $CDCl_3$ (400 MHz).

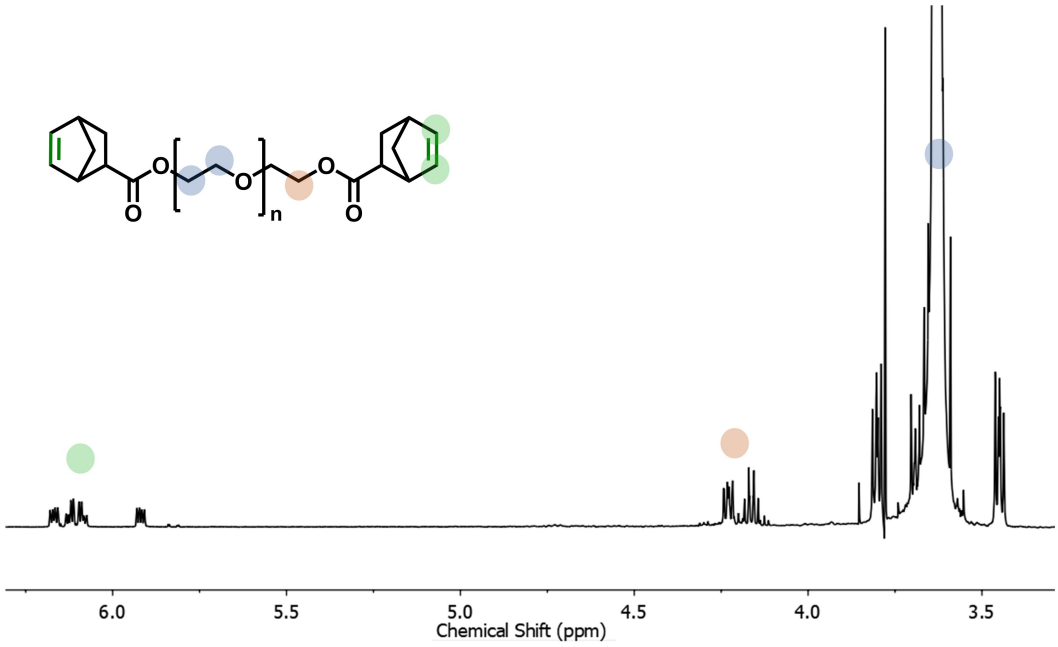

Figure S3. ^{1}H NMR spectrum of linear PEG-Norbornene (10 kg/mol) in $CDCl_3$ (400 MHz).

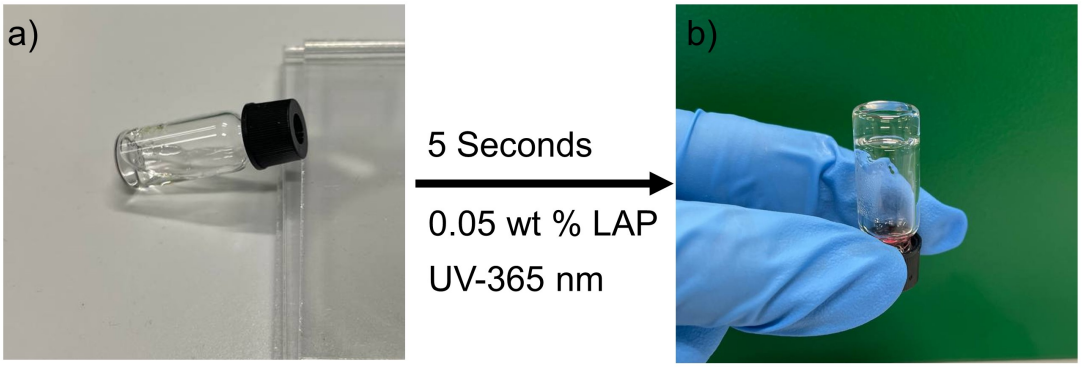

Figure S4. 20 wt % hydrogel solution created with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ crosslinker at a LAP concentration of 0.05 wt %: a) Before the gelation process, and b) after 5 seconds of UV illumination (365 nm).

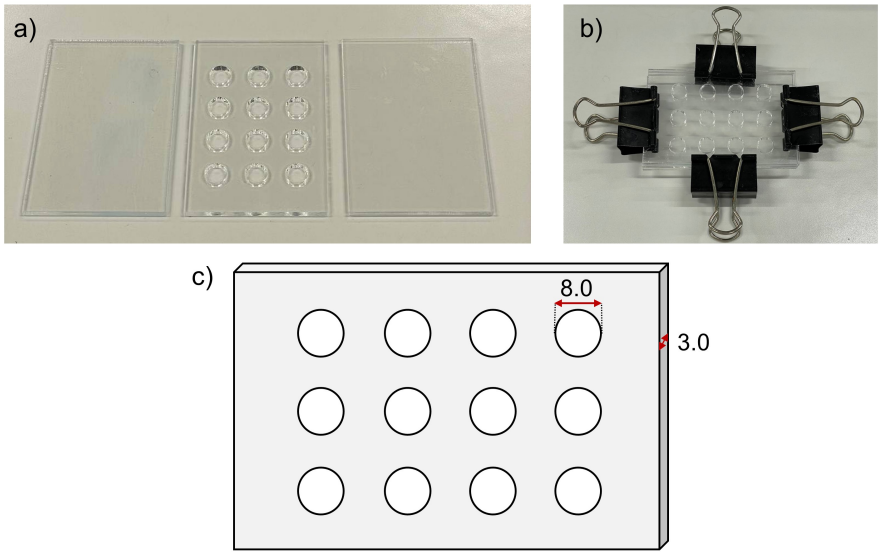

Figure S5. Representation of the homemade molds utilized for sample preparation for compression testing, with dimensions specified in millimeters.

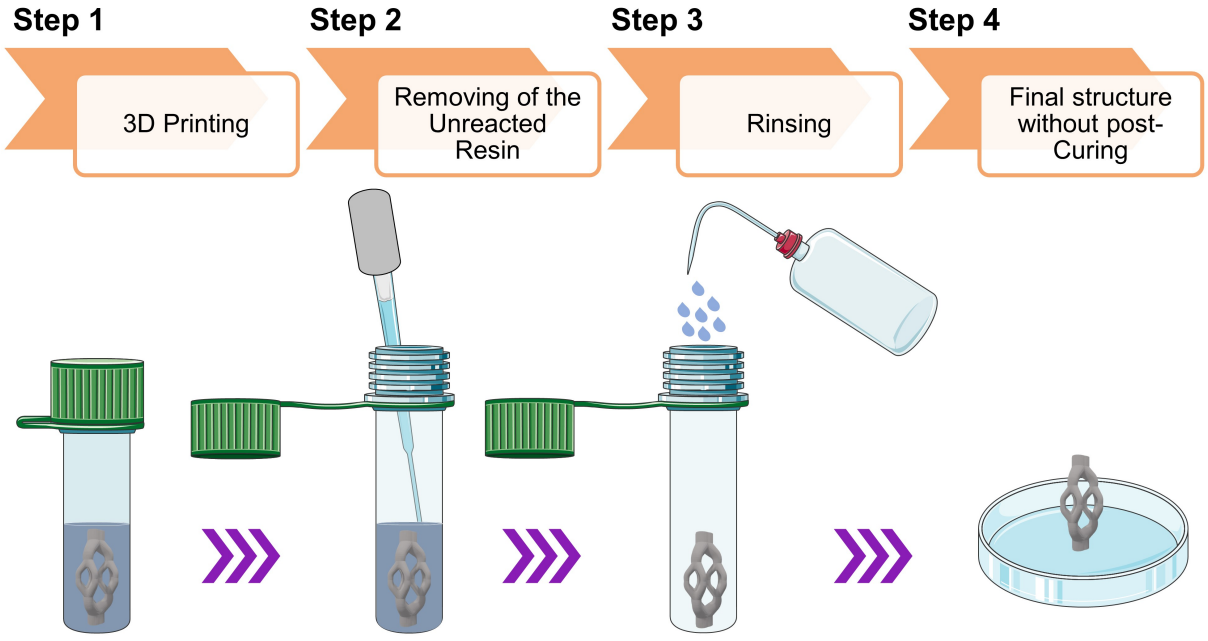

Figure S6. Post-printing procedure after volumetric printing of various geometries.

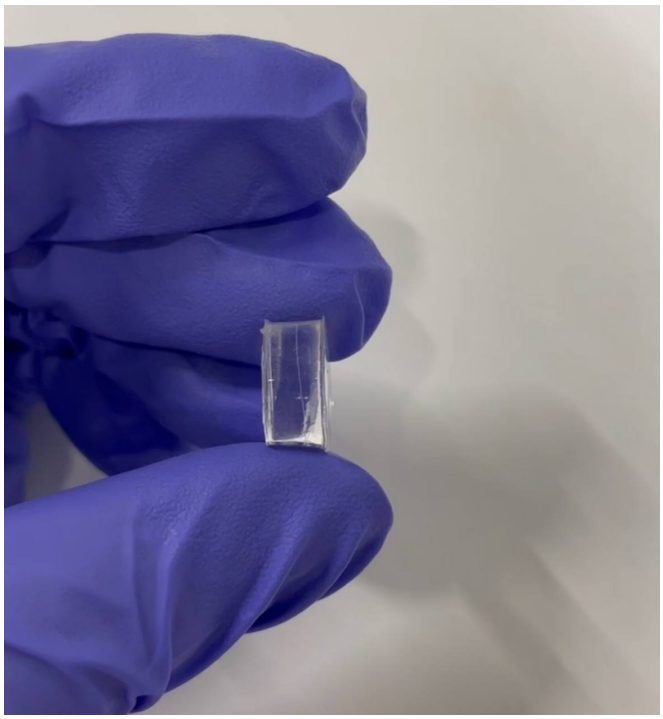

Figure S7. Crack formation along the side of the 20 wt % hydrogel composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ after 90% deformation.

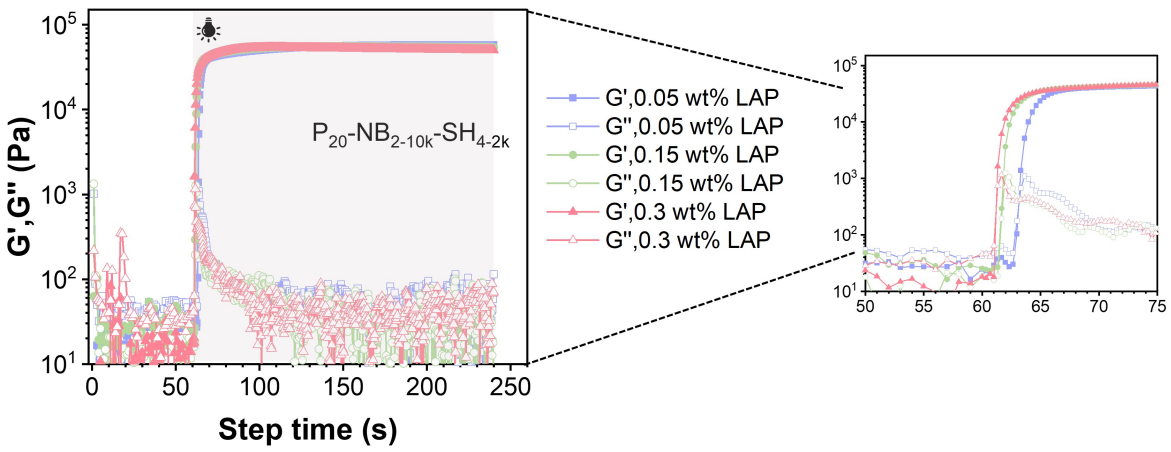

Figure S8. Effect of LAP concentration on the gelation kinetics of the 20 wt % hydrogels made with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$.

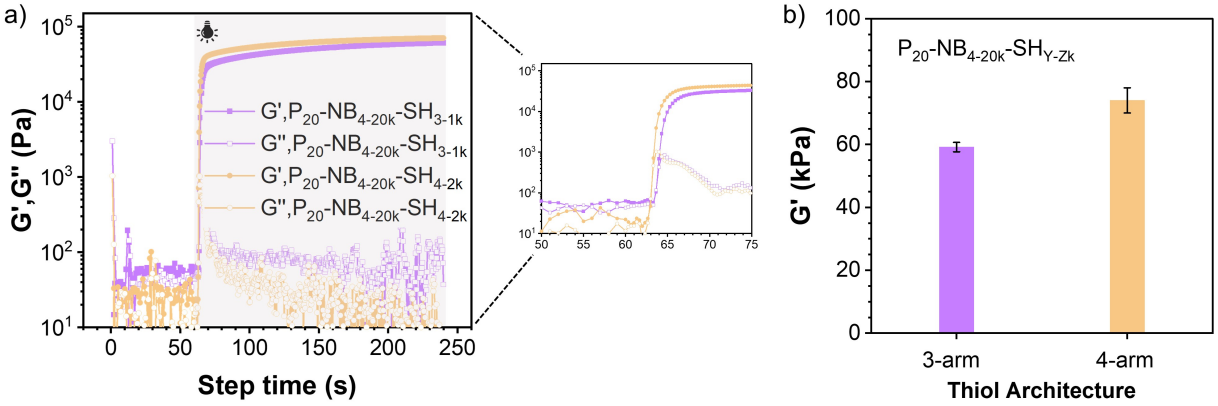

Figure S9. a) G′ and G″ versus time curves and b) the plateau G′ values of the 20 wt % hydrogels made with 4-arm PEG-Norbornene$_{20k}$ and combined with either 3-arm PEG-Thiol$_{1k}$ or 4-arm PEG-Thiol$_{2k}$, at a LAP concentration of 0.05 wt %.

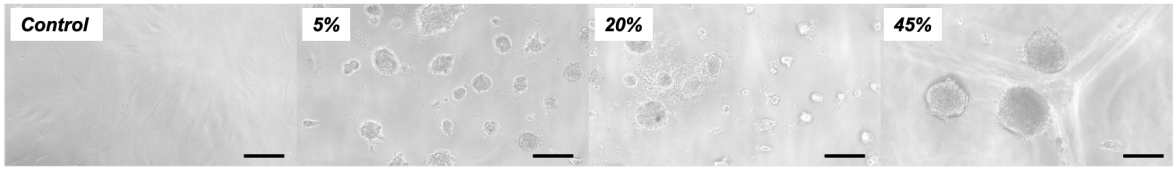

Figure S10. Hydrogels support hMSC adhesion. Representative brightfield images of hMSCs 24 h after seeding on the gels. Scale bar = 150 µm (n = 3; technical replicates).

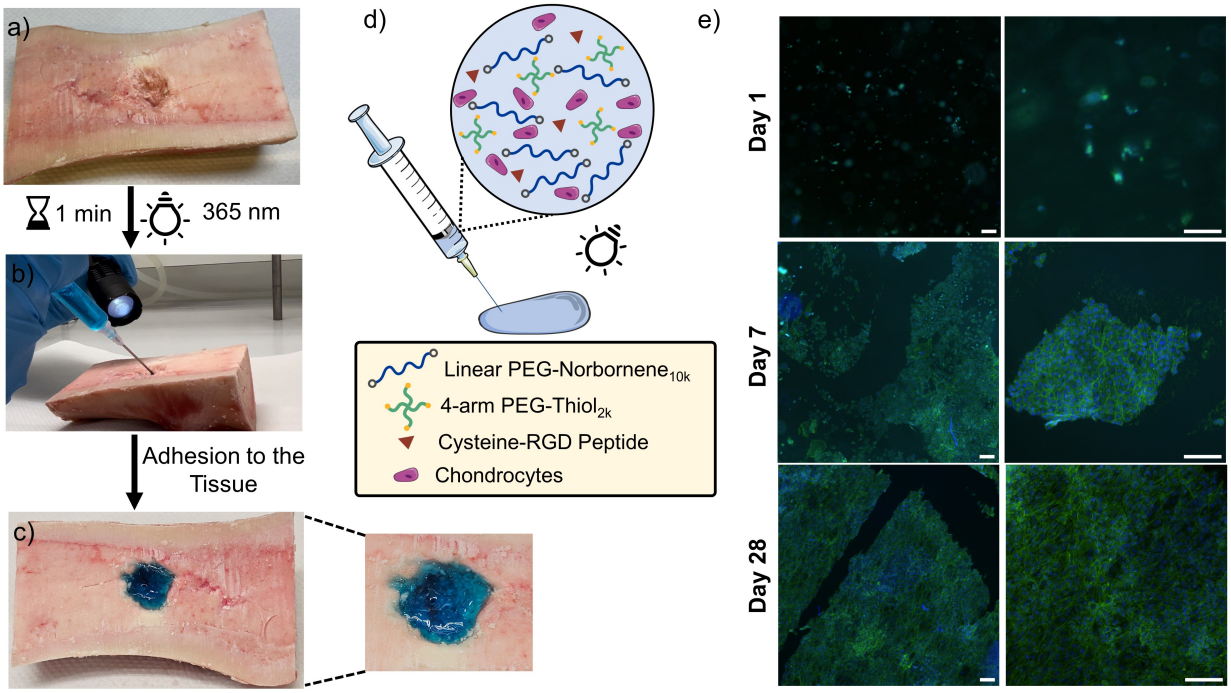

Figure S11. a) Defected area of the animal tissue (porcine bone). b) Injection of the 20 wt% hydrogel solution composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at an LAP concentration of 0.05 wt % into the defective area under 365 nm light illumination for 1 minute. c) Hydrogel formation in the injured region with a strong adhesion to the surrounding tissue. d) Schematic representation of cell-laden hydrogel solution composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at an LAP concentration of 0.05 wt % used for injection experiments. The solution contains cysteine-RGD at a final concentration of 1 mM. e) The morphology of ATDC5 chondrocyte cells following injection of 20 wt % cell-laden hydrogel solutions prepared with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at LAP concentrations of 0.05 wt % and containing 1 mM of cysteine-RGD using 18G needles. The images were taken at varying magnifications after 1, 7, and 28 days. DAPI and phalloidin are represented by blue and green colors, respectively. The cells were mainly inside the gels on day one, but on day 7, and particularly on day 28, the cells were at the bottom of the well-plates. Scale bars: 200 µm.

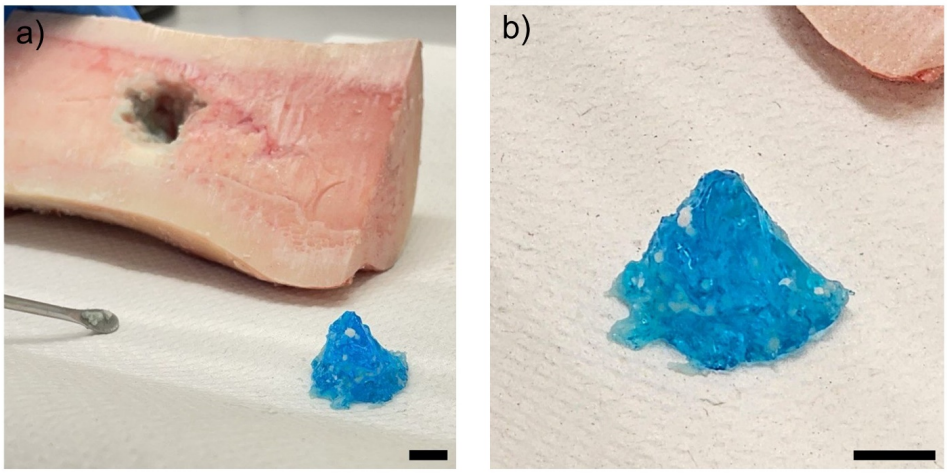

Figure S12. a–b) Injected 20 wt % hydrogel made with PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ after one minute of UV curing and removal from the defective area of a porcine bone. One drop of blue food coloring was added to the hydrogel solution to enhance visualization. Scale bars: 5 mm.

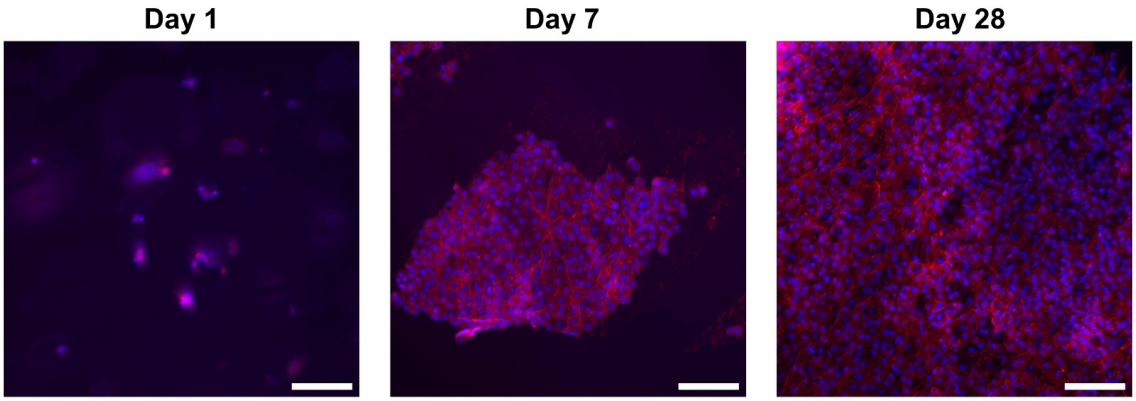

Figure S13. The viability of ATDC5 chondrocyte cells derived from the live-dead fixable staining for days 1, 7, and 28 following injection of 20 wt % cell-laden hydrogel solutions prepared with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at the LAP concentration of 0.05 wt % and containing 1 mM of cysteine-RGD using 18G needles. The blue dots denote DAPI, while the red color shows the fixable dead stain. The cells were mainly inside the gels on day one, but on day 7, and particularly on day 28, the cells were at the bottom of the well-plates. Scale bars: 200 µm.

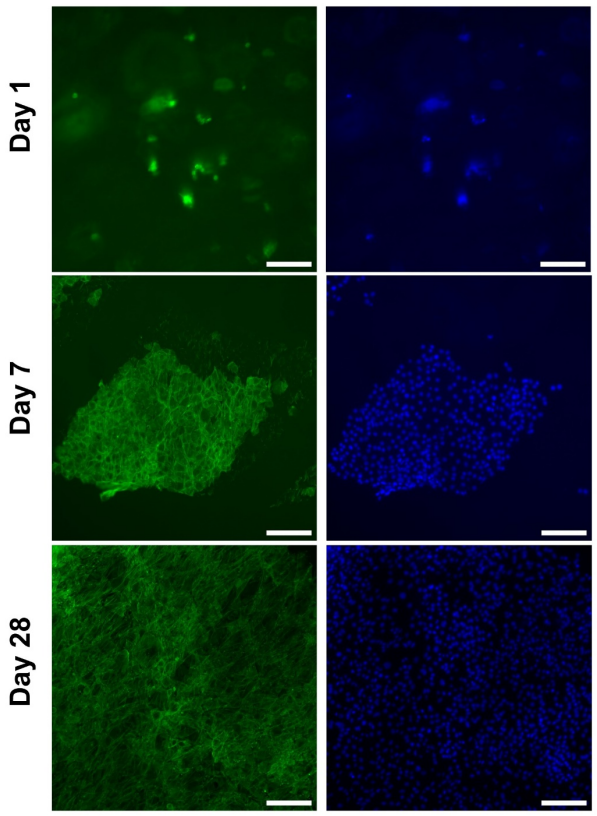

Figure S14. The morphology of ATDC5 chondrocyte cells following injection of 20 wt % cell-laden hydrogel solutions prepared with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at the LAP concentration of 0.05 wt % and containing 1 mM of cysteine-RGD using 18G needles. The images were taken after 1, 7, and 28 days. DAPI and phalloidin are represented separately by blue and green colors, respectively. The cells were mainly inside the gels on day one, but on day 7, and particularly on day 28, the cells were at the bottom of the well-plates. Scale bars: 200 μm.

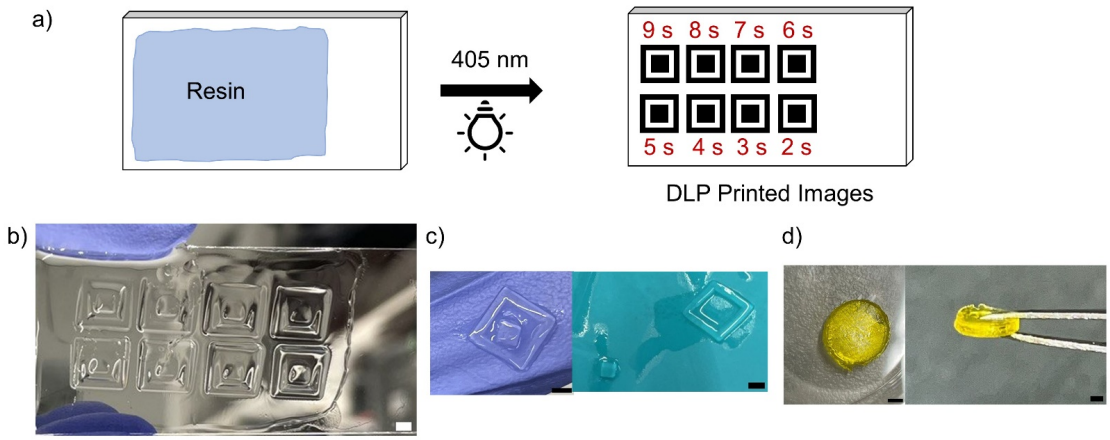

Figure S15. a) Representation of the gelation time measurement was performed using a DLP printer. A pattern consisting of a square frame with a smaller solid square inside was projected eight times in succession onto the various locations of a microscope glass slide. The duration of light exposure (405 nm) varied for each pattern, ranging from 2 to 9 seconds. b) Printed patterns on a microscopic glass slide using 20 wt % hydrogel resin made with linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.05 wt %. c) One-layer DLP printed patterns exhibiting high resolution and good mechanical integrity achieved after 2 seconds of light illumination using 20 wt % hydrogel resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.05 wt %. e) A DLP printed disc (8 mm in diameter and 2 mm in height) composed of 20 layers, each 100 µm thick, using 20 wt % hydrogel resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.3 wt % and 1 mM tartrazine. The first layer was exposed to light for 15 seconds, followed by subsequent layers being irradiated for 3 seconds each. Scale bars: 2 mm.

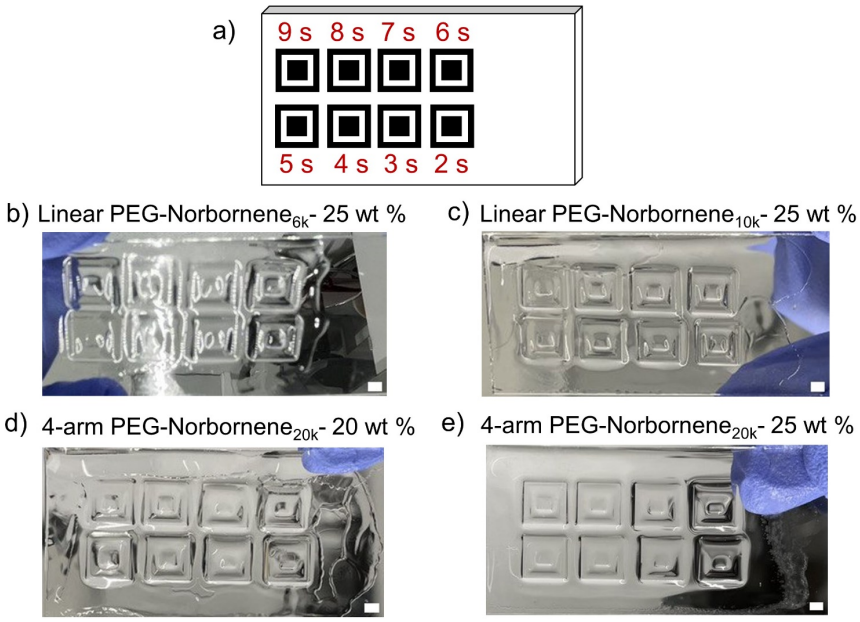

Figure S16. a) Evaluation of the gelation test was done using a DLP printer. A pattern consisting of a hollow square with a smaller solid square inside was projected eight times onto the various locations of the glass slide. The duration of light exposure varied for each pattern, ranging from 2 seconds to 9 seconds. Images of the printed patterns on the microscopic glass slide for: b) 25 wt % hydrogel resin made using linear PEG-Norbornene$_{6k}$ and 4-arm PEG-Thiol$_{2k}$; c) 25 wt % hydrogel resin made using linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$; d) 20 wt % hydrogel resin made using 4-arm PEG-Norbornene$_{20k}$ and 4-arm PEG-Thiol$_{2k}$; and e) 25 wt % hydrogel resin made using 4-arm PEG-Norbornene$_{20k}$ and 4-arm PEG-Thiol$_{2k}$. Scale bars: 2 mm.

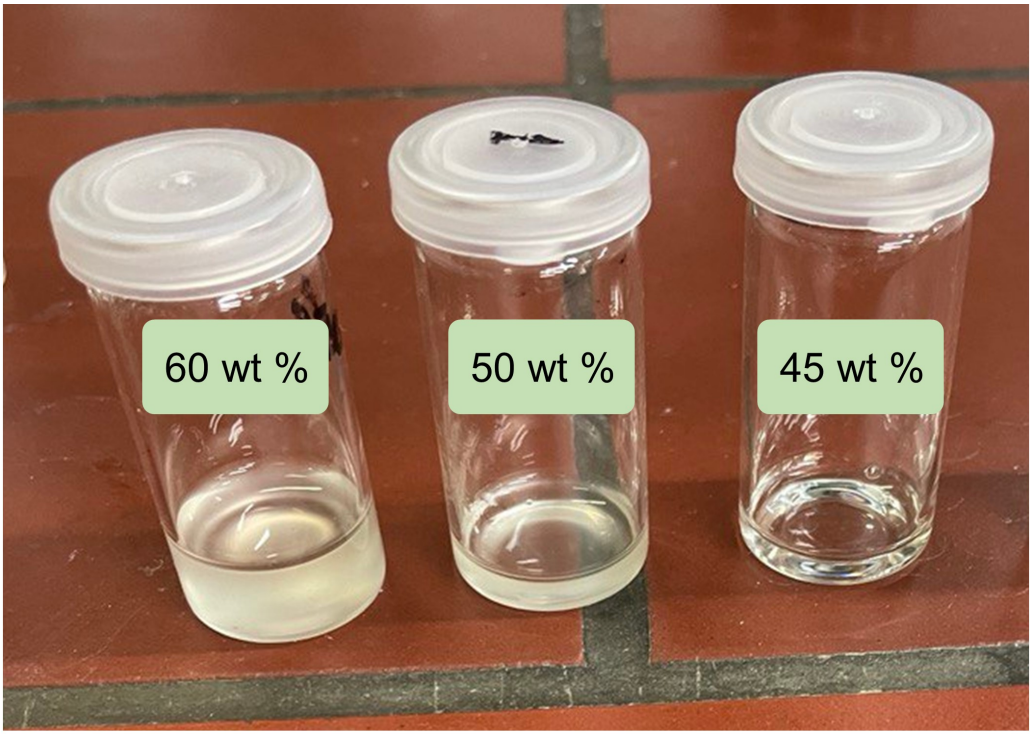

Figure S17. Hydrogel photo-resins, with final polymer contents of 45 wt %, 50 wt %, and 60 wt %, prepared using linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.3 wt %, and a 1:10 mass ratio of TEMPO relative to LAP.

Figure S18. Volumetric printing of a meniscus using 45 wt % resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.3 wt %, and a 1:10 mass ratio of TEMPO relative to LAP. a) Model; and b–c) printed meniscus at 250 mJ/cm^2 light dose.

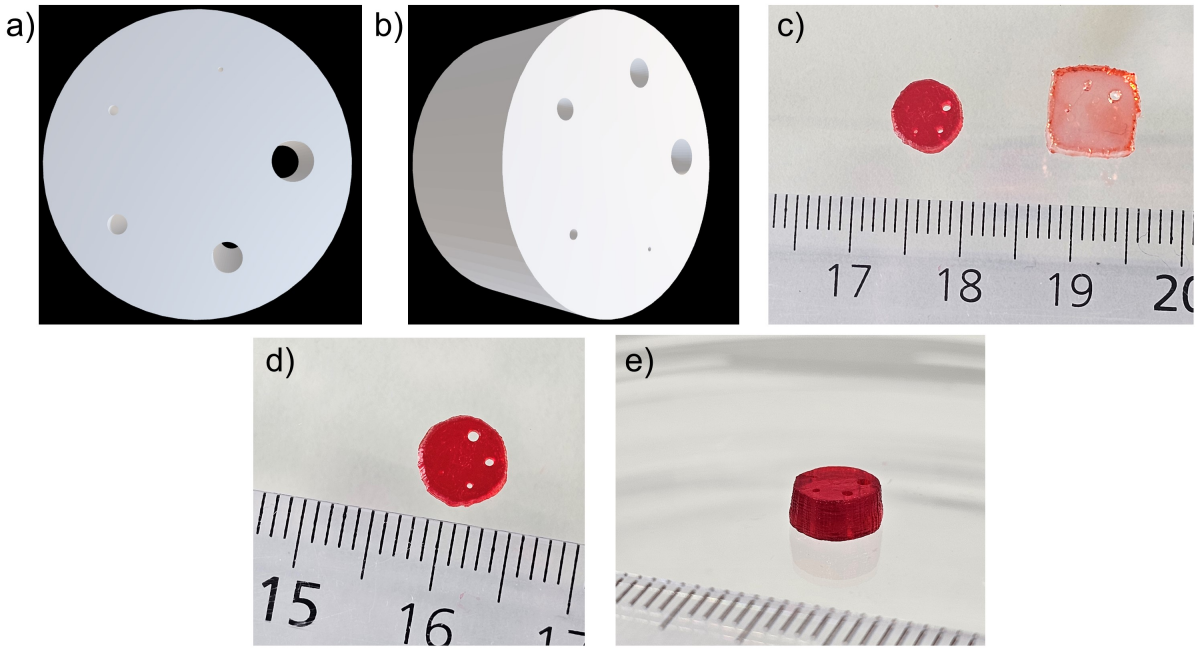

Figure S19. Volumetric printing of discs with five channels of varying sizes using 45 wt % resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.3 wt %, and a 1:10 mass ratio of TEMPO relative to LAP. a–b) Model; c) printed disc with a 275 mJ/cm^2 light dose (left) and printed disc at a light dose of 300 mJ/cm^2 (right, over-cured); and d–e) printed disc with a light dose of 275 mJ/cm^2 from various viewpoints.

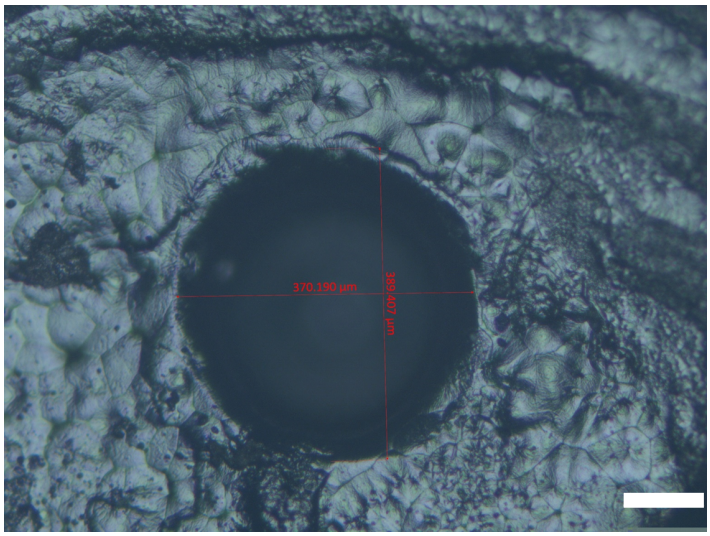

Figure S20. The smallest channel size in the perfusate structure was created by volumetric printing of a 45 wt % resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$. Scale bar: 100 µm.

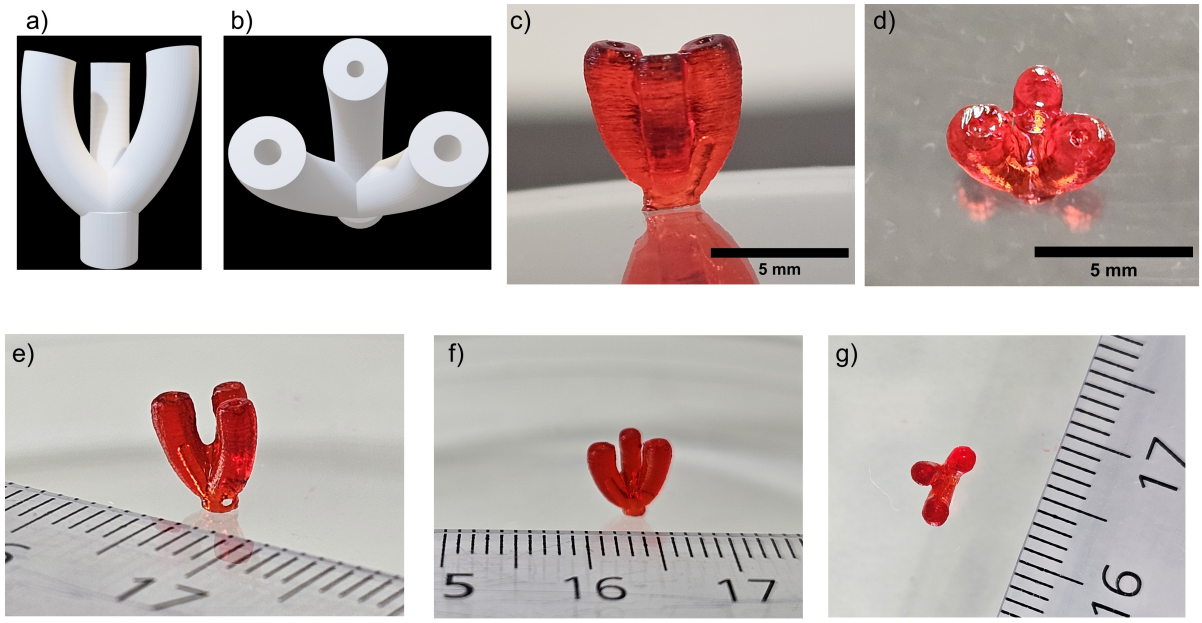

Figure S21. Volumetric printing of tubular structures featuring three perfusable channels using 45 wt % resin composed of linear PEG-Norbornene$_{10k}$ and 4-arm PEG-Thiol$_{2k}$ at a LAP concentration of 0.3 wt %, and a 1:10 mass ratio of TEMPO relative to LAP. a–b) Model; c–d) printed structure at a light dose of 310 mJ/cm^2 (over-cured); and e-g) printed structure at a light dose of 290 mJ/cm^2.

Table S1. Overview of the hydrogel formulations presented in this study.

*Hydrogel Code**	*Polymer Content*	*PEG-Thiol*	*PEG-Norbornene*	*[NB]:[SH]*
P_{5}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	5 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{10}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	10 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{15}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	15 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	20 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{25}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	25 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{45}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	45 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-1.25:1	20 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[1.25]:[1]
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-2:1	20 wt %	Linear,10 kg/mol	4-arm, 2 kg/mol	[2]:[1]
P_{20}-$NB_{2\text{-}6k}$-$SH_{4\text{-}2k}$	20 wt %	Linear,6 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{20}-$NB_{4\text{-}20k}$-$SH_{4\text{-}2k}$	20 wt %	4-arm,20 kg/mol	4-arm, 2 kg/mol	[1]:[1]
P_{20}-$NB_{2\text{-}10k}$-$SH_{3\text{-}1k}$	20 wt %	Linear,10 kg/mol	3-arm, 1 kg/mol	[1]:[1]
P_{20}-$NB_{2\text{-}6k}$-$SH_{3\text{-}1k}$	20 wt %	Linear,6 kg/mol	3-arm, 1 kg/mol	[1]:[1]
P_{20}-$NB_{4\text{-}20k}$-$SH_{3\text{-}1k}$	20 wt %	4-arm,20 kg/mol	3-arm, 1 kg/mol	[1]:[1]

*The formulations utilize the following abbreviations: $P_X NB_{M\text{-}Nk}$-$SH_{Y\text{-}Zk}$, where X signifies polymer content, M and N respectively indicate the number of arms and the approximate molar mass in kg/mol for PEG-Norbornene, and Y and Z respectively represent the number of arms and the approximate molar mass in kg/mol for PEG-Thiol.

Table S2. Summary of the mechanical properties of the hydrogels assessed through compression testing.

Hydrogel Code	*Elastic Modulus (kPa)*	*Maximum Stress (kPa)*
P_5-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	30.0 ± 2.0	1501.2 ± 15.0
P_{10}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	142.0 ± 2.2	3482.0 ± 156.3
P_{15}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	253.2 ± 7.0	4576.1 ± 215.3
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	410.0 ± 1.5	5994.0 ± 158.0
P_{25}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	634.5 ± 22.3	7416.4 ± 79.0
P_{45}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	1951.6 ± 50.2	22591.1 ± 171.3
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-1.25:1	160.5 ± 4.7	4258.5 ± 163.1
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-2:1	6.0 ± 1.0	376.1 ± 27.0
P_{20}-$NB_{2\text{-}6k}$-$SH_{4\text{-}2k}$	520.6 ± 5.6	8330.3 ± 405.4
P_{20}-$NB_{4\text{-}20k}$-$SH_{4\text{-}2k}$	474.2 ± 7.5	6588.4 ± 58.7
P_{20}-$NB_{2\text{-}10k}$-$SH_{3\text{-}1k}$	229,7± 3.5	4116.6 ± 430.0
P_{20}-$NB_{4\text{-}20k}$-$SH_{3\text{-}1k}$	272.6± 16.7	5792 ± 106.7

Table S3. Summary of the gelation time and post-curing storage modulus for the hydrogels investigated in this study.

Hydrogel Code	*LAP (wt%)*	*Gelation time (s)*	*Storage modulus (kPa)*
P_{15}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.05	4.08 ± 0.19	38.7 ± 0.5
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.05	3.31 ± 0	60.2 ± 2.2
P_{25}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.05	2.98 ± 0	90.5 ± 2.3
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-1:1	0.05	3.31 ± 0	60.2 ± 2.2
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-1.25:1	0.05	3.86 ± 0.19	27.5 ± 0.5
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$-2:1	0.05	4.52 ± .38	1.6 ± 0.1
P_{20}-$NB_{2\text{-}6k}$-$SH_{4\text{-}2k}$	0.05	2.43 ± 0.5	80.0 ± 2.1
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.05	3.31 ± 0	60.2 ± 2.2
P_{20}-$NB_{4\text{-}20k}$-$SH_{4\text{-}2k}$	0.05	3.22 ± 0.41	74.0 ± 4.0
P_{20}-$NB_{2\text{-}10k}$-$SH_{3\text{-}1k}$	0.05	3.75± 0.19	46.9 ± 2.5
P_{20}-$NB_{4\text{-}20k}$-$SH_{3\text{-}1k}$	0.05	4.19± 0.38	59.2 ± 1.5
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.15	1.66 ± 0	54.6 ± 0.7
P_{20}-$NB_{2\text{-}10k}$-$SH_{4\text{-}2k}$	0.3	1.33 ± 0	52.4 ± 2.0

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