ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.,* DOI: 10.1002/adhm.202100206

Tuning Superfast Curing Thiol-Norbornene-Functionalized Gelatin Hydrogels for 3D Bioprinting

*Tobias Göckler, Sonja Haase, Xenia Kempter, Rebecca Pfister, Bruna R. Maciel, Alisa Grimm, Tamara Molitor, Norbert Willenbacher, Ute Schepers**

Supporting Information

Tuning Superfast Curing Thiol-Norbornene-Functionalized Gelatin Hydrogels for 3D Bioprinting

*Tobias Göckler[1], Sonja Haase[1], Xenia Kempter[1], Rebecca Pfister, Bruna R. Maciel, Alisa Grimm, Tamara Molitor, Norbert Willenbacher, Ute Schepers**

[1] Co-first authors

T. Göckler, S. Haase, X. Kempter, R. Pfister, A. Grimm, T. Molitor, Prof. Dr. U. Schepers
Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFG), Hermann-von-Helmholtz-Platz 1,76344 Eggenstein-Leopoldshafen, Germany

Prof. Dr. U. Schepers
Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry (IOC), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany

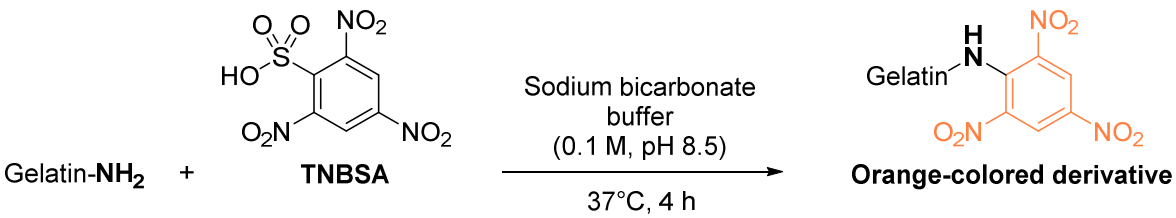
B.R. Maciel, Prof. Dr. N. Willenbacher
Karlsruhe Institute of Technology (KIT), Institute of Mechanical Process Engineering and Mechanics (MVM), Gotthard-Franz-Straße 3, 76131 Karlsruhe, Germany

E-mail: ute.schepers@kit.edu

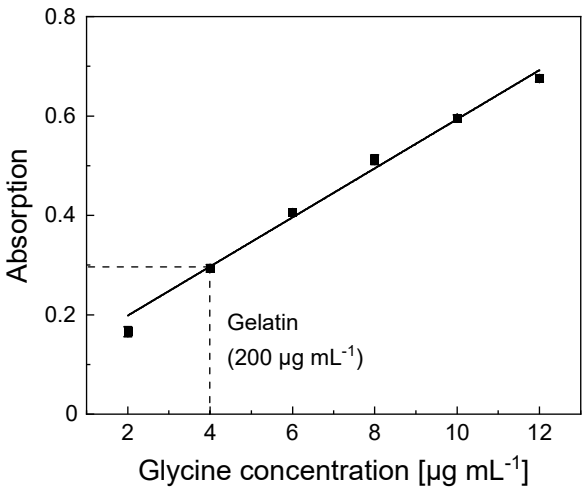
S1. Determination of free amino groups in gelatin

Quantitative assessment of the number of primary amino groups in gelatin was carried out colorimetrically by TNBSA assay using a glycine standard curve (2-12 µg mL^{-1}). TNBSA assay was performed according to an established protocol.[78] Blanks were prepared by adding HCl, followed by the addition of TNBSA reagent. The absorption of each sample was measured at 335 nm (SmartSpec 3000, Bio-Rad Laboratories GmbH, Munich Germany) and a linear trendline was created. From the measured absorption value of a gelatin solution (200 µg mL^{-1}), the average amount of free amino groups per gram of gelatin (type A, gel strength ~300 g bloom) was calculated.

A



B



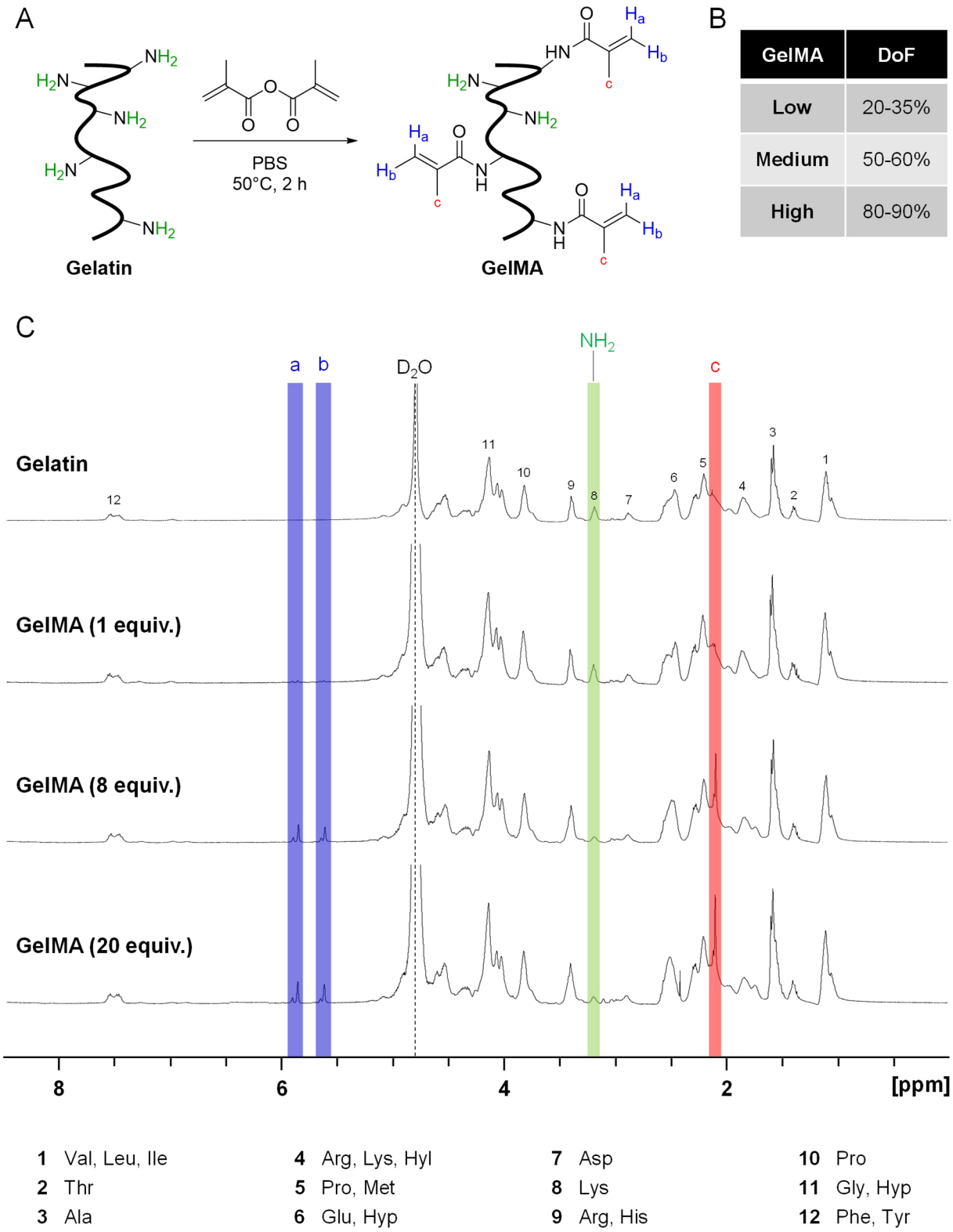
C

$$n\,(NH_2) = \frac{m(Gly)}{M\,(Gly)} = \frac{c(Gly) \times V(Gly)}{M\,(Gly)} = \frac{4\ \frac{\mu g}{mL} \times 0.5\ mL}{75.07\ \frac{g}{mol}} = 0.0266\ \mu mol$$

$$c\left[\frac{\mathbf{mmol\ (NH_2)}}{\mathbf{g\ (Gel)}}\right] = \frac{n\,(Gly)}{m\,(Gel)} = \frac{n\,(Gly)}{c(Gel) \times V(Gel)} = \frac{0.0266\ \mu mol}{200\ \frac{\mu g}{mL} \times 0.5\ mL} = \mathbf{0.266}\ \frac{\mathbf{mmol}}{\mathbf{g}}$$

Figure S1. A) TNBSA reaction. B) Glycine standard curve (2-12 µg mL^{-1}). C) Calculation of free amino groups per gram of gelatin (type A, gel strength ~300 g bloom) from the absorption of a gelatin solution (200 µg mL^{-1}).

S2. Degree of functionalization of GelMA



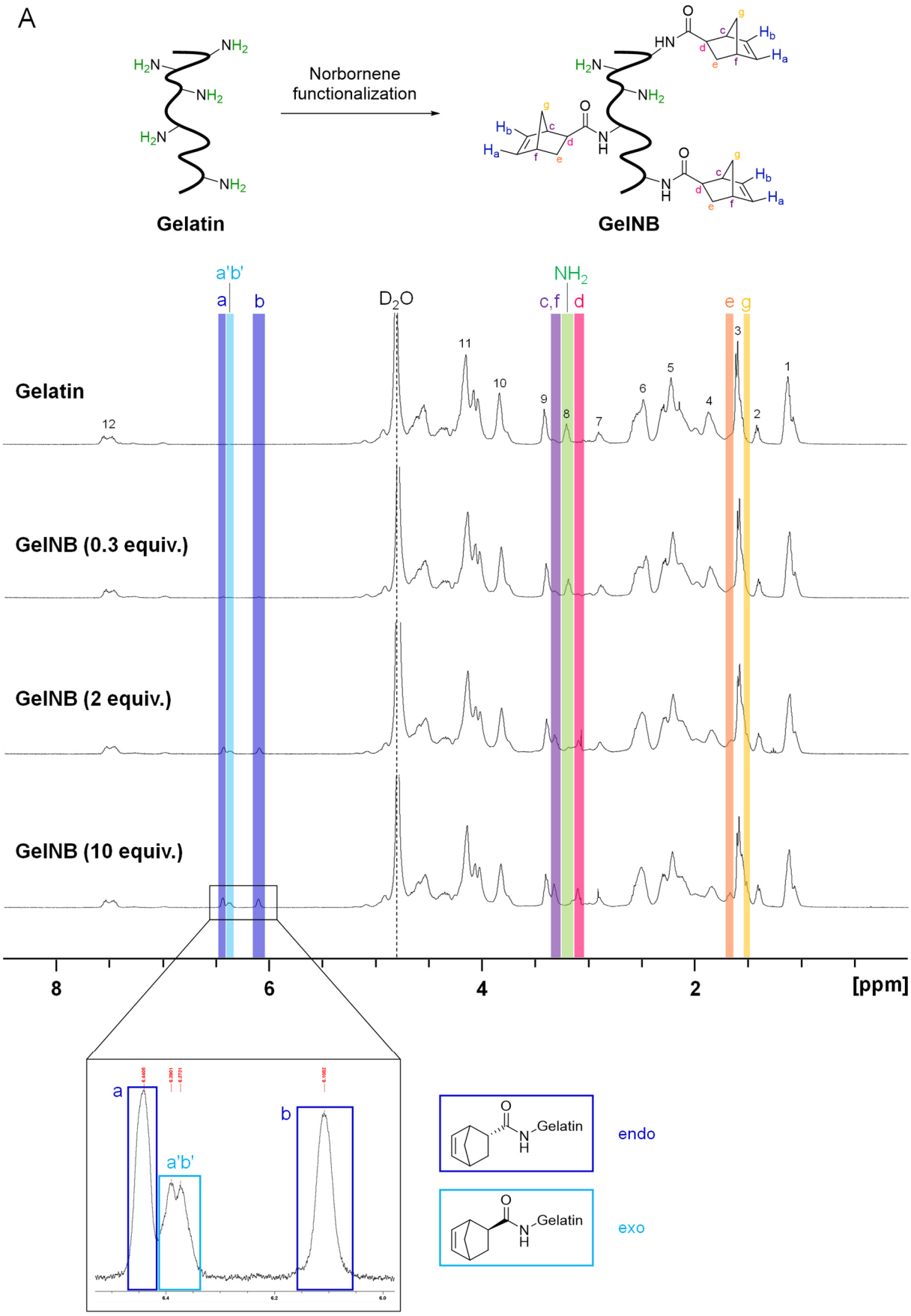
GelMA	DoF
Low	20-35%
Medium	50-60%
High	80-90%

1	Val, Leu, Ile	**4**	Arg, Lys, Hyl	**7**	Asp	**10**	Pro
2	Thr	**5**	Pro, Met	**8**	Lys	**11**	Gly, Hyp
3	Ala	**6**	Glu, Hyp	**9**	Arg, His	**12**	Phe, Tyr

Figure S2. A) Chemical modification of gelatin to GelMA using varying amounts of methacrylic anhydride (1 equiv., 8 equiv., 20 equiv.). B) Degree of functionalization (DoF) of GelMA (Low, Medium, High) determined by TNBSA assay. C) ^{1}H NMR spectra (400 MHz, D_2O, 315 K) of unmodified gelatin and GelMA hydrogel precursors of varying DoF (Low, Medium, High).

S3. ^{1}H NMR spectra of hydrogel precursors GelNB and GelS

NMR measurements were performed using a 400 MHz NMR spectrometer (AVANCE 400, Bruker, Germany). Chemical shifts are given in parts per million (δ/ppm) downfield from tetramethylsilane (TMS) and referenced to D_2O (4.80 ppm) as internal standard. The description of signals includes: *s* = singlet, *bs* = broad singlet. ^{1}H NMR spectra of gelatin, GelMA, GelNB and GelS were recorded at a temperature of 315 K to avoid gelation of the sample during the measurement.


A
Norbornene functionalization
Gelatin
GelNB
a'b'
a
b
D2O
NH2
c,f
d
e
g
Gelatin
GelNB (0.3 equiv.)
GelNB (2 equiv.)
GelNB (10 equiv.)
8
6
4
2
[ppm]
endo
exo

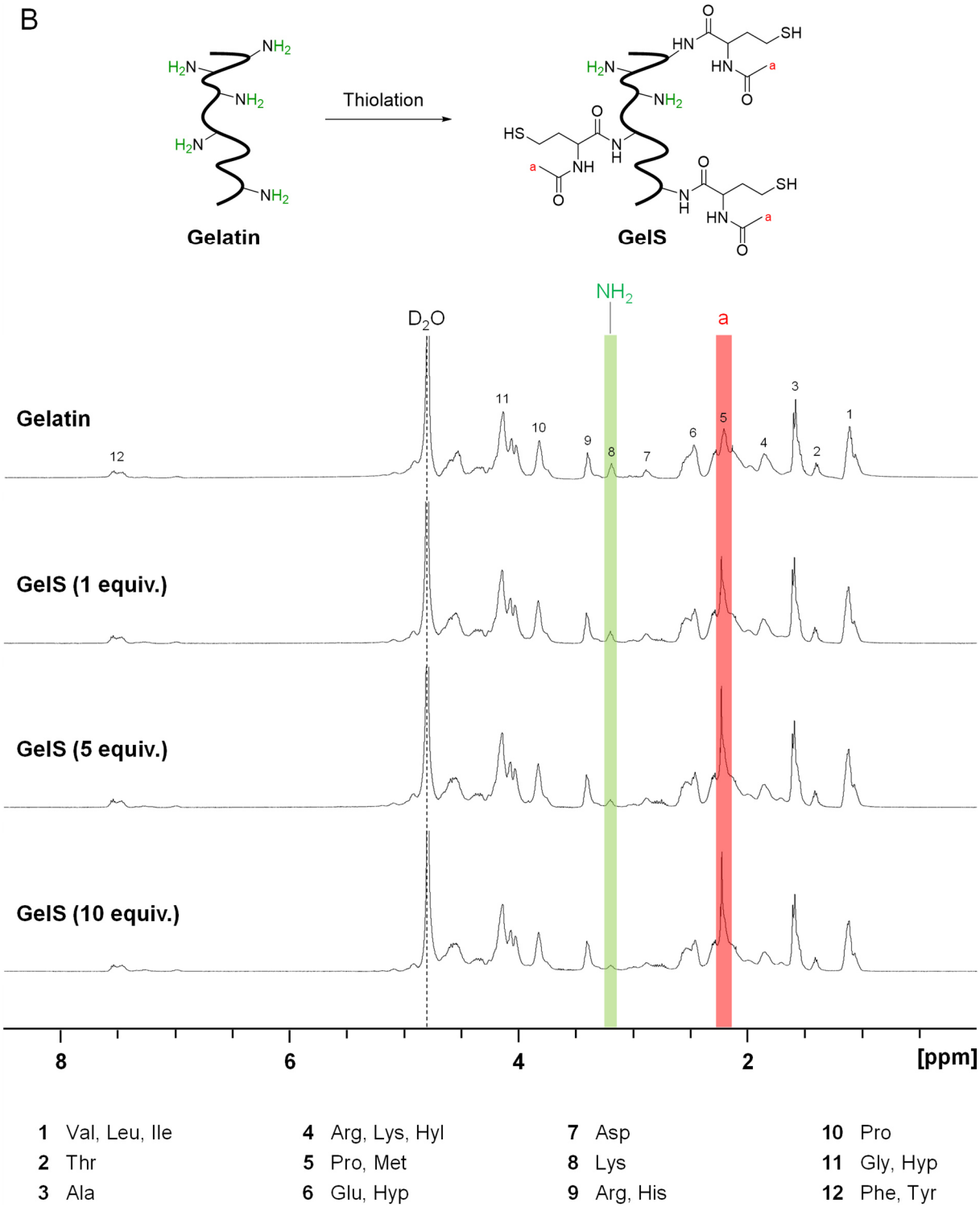


Figure S3. ^{1}H NMR spectra (400 MHz, D_2O, 315 K) of unmodified gelatin and hydrogel precursors of varying DoF. A) GelNB (Low, Medium, High) and B) GelS (Low, Medium, 10 equiv. AHCT).

S4. Frequency sweeps of GelNB/GelS hydrogels (Low, Medium, High) and their precursor solutions

Dynamic shear oscillatory measurements were performed with a parallel-plate rheometer (PP25, MCR 501, Anton Paar, Ostfildern-Scharnhausen, Germany) for crosslinked GelNB/GelS hydrogels (Low, Medium, High) at 37 °C and their uncrosslinked precursor solutions at 14 °C. Frequency sweeps from 0.1 rad s^{-1} to 100 rad s^{-1} were measured at a constant amplitude of 1 Pa and a plate-to-plate distance of 1 mm.

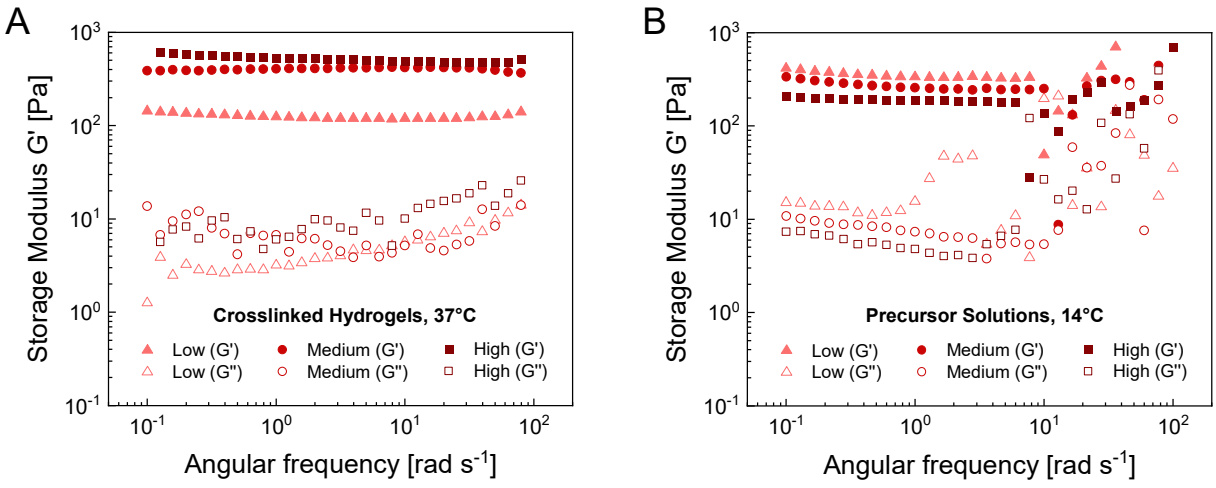


Figure S4. Frequency sweeps (0.1 rad s^{-1} to 100 rad s^{-1}) of A) photocrosslinked GelNB/GelS hydrogels (Low, Medium, High) at 37 °C and B) their uncrosslinked, but physically gelated precursor solutions at 14 °C.

S5. Cell viability analysis of 3D bioprinted NHDF using GelNB/GelS bioinks

Live/dead staining of NHDF with calcein-AM (4 µg mL^{-1}) and propidium iodide (20 µg mL^{-1}) in GelNB/GelS bioinks (Low, Medium, High) was performed 1, 7, and 14 days post-printing. Confocal microscopy (Leica TCS SPE, Leica Microsystems, Wetzlar, Germany) was used to record z-stacks of 300 µm thickness (step size: 5 µm), which were converted into 3D images with LasX software. 20 images were taken per hydrogel and the number of live and dead cells was counted using Python software. Viability was determined by the percentage of live cells over the total cell count.

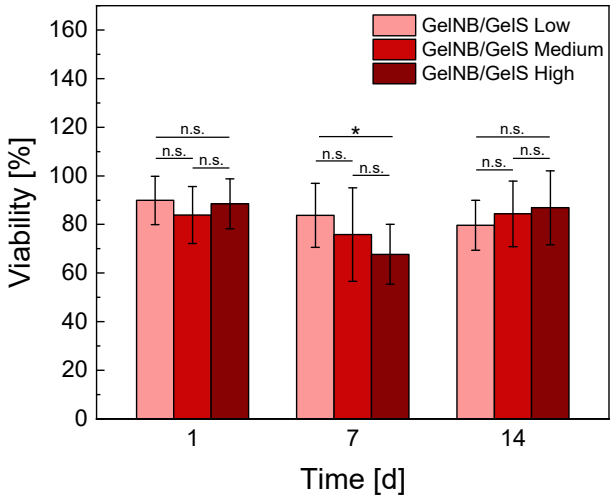


Figure S5. Post-printing cell viability analysis of 3D bioprinted NHDF using GelNB/GelS bioinks (Low, Medium, High) over a period of 14 days. Viability was determined by the percentage of live cells over the total cell count. Data were presented as mean ± SD and statistically evaluated by one-way ANOVA. *, **, *** represent $p < 0.05$, 0.01, and 0.001, respectively.