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Inverse thermogelation of aqueous triblock copolymer solutions into macroporous shear-thinning 3D printable inks

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Abstract:

Amphiphilic block copolymers that undergo (reversible) physical gelation in aqueous media are of great interest in different areas including drug delivery, tissue engineering, regenerative medicine and biofabrication. We investigated a small library of ABA-type triblock copolymers comprising poly(2-methyl-2-oxazoline) as the hydrophilic shell A and different aromatic poly(2-oxazoline)s and poly(2-oxazine)s cores B in aqueous solution at different concentrations and temperatures. Interestingly, aqueous solutions of poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazine)-*block*-poly(2-methyl-2-oxazoline) (PMeOx-*b*-PPheOzi-*b*-PMeOx) undergo inverse thermogelation below a critical temperature by forming a reversible nanoscale worm-like network. The viscoelastic properties of the resulting gel can be conveniently tailored by the concentration and the polymer composition. Storage moduli of up to 110 kPa could be obtained while the material remains shear-thinning and retains rapid self-healing properties. We demonstrate 3D-printing of excellently defined and shape persistent 24-layered scaffolds at different aqueous concentrations to highlight its application potential e.g. in the research area of biofabrication. A mesoporous microstructure, which is stable throughout the printing process, could be confirmed via cryo-SEM analysis. The absence of cytotoxicity even at very high concentrations opens wide range of different applications for this first-in-class material in the field of biomaterials.

Introduction:

Stimuli-responsive polymers, also referred to as smart polymers undergo changes with respect to their physico-chemical properties or morphology when they are exposed to external stimuli such as temperature, pH and/or light.^{1, 2} Thermoresponsive polymers can be primarily categorized into those that exhibit a lower critical solution temperature (LCST), upper critical solution temperature (UCST) or physical gelation.³ Polymers, which undergo physical gelation have gained great interest because of their potential applications in drug delivery and release, tissue engineering and regenerative medicine, biofabrication, cosmetics and smart coatings.⁴ The gelation process is mainly defined by the (reversible) formation and growth of supramolecular structures yielding three-dimensionally, physically crosslinked networks. The molecular interactions defining the network formation may be coulomb interactions, hydrogen bonding or hydrophobic/aromatic interactions. Polymers with UCST behavior reversibly phase separate upon cooling, typically leading to precipitation. In some cases, precipitation does not occur, instead a gel is formed. UCST-type behavior can be observed in solvent mixtures and, though much more rarely, in water.⁵⁻⁸ Already in 1979, Wellinghoff *et al.* described a poly(styrene) and poly(dimethylphenylene oxide) system which forms gels in organic solvents upon rapid cooling.⁹ In 2015 a thixotropic poly(glycerol sebacate)-based supramolecular structure with UCST behavior was reported to form a hydrogel at rather low concentrations (5.2 %) with self-healing ability with a storage modulus of 100 kPa. The clear liquid turned into a turbid gel upon cooling.¹⁰ Theato and co-workers described polymers based on vinylcyclopropane and investigated their UCST and physical gelation behavior in ethanol and ethanol-water mixtures at different concentrations.¹¹ The transition temperature hysteresis between the heating and the cooling phase was very large (about 45 K). Another class of thermoreversible physically crosslinked hydrogels formed from ABA linear triblock copolymers was reported recently.¹² The polymers feature a hydrophilic poly(poly(ethylene glycol) methyl ether methacrylate) (PPEGMMA) middle block B and flanking UCST-type

thermosensitive poly(acrylamide-co-acrylonitrile) (P(AAm-co-AN)) A blocks. At distinct block lengths, chemical compositions, and polymer concentration, aqueous solutions of these polymers showed cooling-induced, reversible sol–gel transitions in water. Polymers of cyclic imino ethers, in particular poly(2-substituted-2-oxazoline)s (POx) and poly(2-substituted-5,6-dihydro-4H-1,3-oxazine)s (poly(2-oxazine)s; POzi) are important classes of thermoresponsive polymers^{13, 14} and can be synthesized via living cationic ring-opening polymerization to yield different polymeric architectures^{15, 16} and compositions.^{17, 18} Over the last decades POx were intensively studied as thermoresponsive materials¹⁹, surface coatings²⁰⁻²², but also in biomedical applications²³⁻²⁵ and drug delivery.²⁶⁻²⁹ More recently, Bloksma et al. investigated the thermoresponsive behavior of some POzi homopolymers.³⁰ Interestingly though, only a few reports can be found that show thermogelation in water for pure POx/POzi based systems. In fact, Zahoranova et al. investigated different triblock copolymers mimicking Pluronic-type polymers based on the hydrophilic poly(2-methyl-2-oxazoline) (PMeOx) and the thermoresponsive poly(2-*n*-propyl-2-oxazoline) (PnPrOx), but no thermogelling could be observed.³¹ In contrast, Lorson *et al.* established a thermogelling ($G' \sim 4$ kPa), cytocompatible and printable poly(2-oxazoline)/poly(2-oxazine) (POx/POzi) supramolecular hydrogel.³² These diblock copolymers, comprising a hydrophilic poly(2-methyl-2-oxazoline) block and a thermoresponsive poly(2-*n*-propyl-2-oxazine) block of equal or similar block length, undergo thermogelation above a defined sol/gel-temperature. Very recently, Hoogenboom and Monnery reported thermogelling ABA triblock copolymers bearing thermoresponsive poly(2-*n*-propyl-2-oxazoline) and hydrophilic poly(2-ethyl-2-oxazoline) blocks, but block lengths necessary for thermogelation were extremely high.³³ More recently, Lübtow et al. reported on a different ABA triblock copolymer, again with hydrophilic poly(2-methyl-2-oxazoline) A blocks and a hydrophobic poly(2-*iso*-butyl-2-oxazoline) B block which forms thermogels, albeit rather weak ones that are barely printable.³⁴ To the best of our knowledge, there has been no reported case of UCST-type POx/POzi in water, nor inverse gelation of POx/POzi based polymers.

Hoogenboom and co-workers described a UCST-based thermoresponsive micellization of random copolymers comprising poly(2-nonyl-oxazoline) and poly(2-phenyl-2-oxazoline) blocks.³⁵ Upon cooling these structures form micelles in different water-ethanol mixtures.

In our ongoing endeavor for an enhanced understanding of the interaction between drugs and polymeric drug delivery systems^{28, 36-40}, we recently studied ABA triblocks featuring aromatic hydrophobic B blocks.²⁷ For the B blocks we used the well-known aromatic polymers poly(2-phenyl-2-oxazoline) (PPheOx) and poly(2-benzyl-2-oxazoline) (PBzOx). Here, we report on first-of-their-kind ABA triblock copolymers featuring the aromatic poly(2-oxazine)s, specifically poly(2-phenyl-2-oxazine) (PheOzi) and poly(2-benzyl-2-oxazine) (BzOzi) (Scheme 1). Together with their POx-based homologues (PheOx/BzOx), the rheological properties of aqueous solutions were investigated in dependence of concentration and temperature. Interestingly, only one polymer, poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazine)-*block*-poly(2-methyl-2-oxazoline) (PMeOx-*b*-PPheOzi-*b*-PMeOx = A-PPheOx-A) undergoes inverse thermogelation at around room temperature at a concentration of 5 wt.% and above. The unusual gelation behavior was examined in detail.

Scheme 1. a) Schematic illustration of investigated ABA-triblock copolymers comprising the hydrophilic shell poly(2-methyl-2-oxazoline) and different aromatic hydrophobic cores based on poly(2-oxazoline)s or poly(2-oxazine)s. In case of A-pPheOzi-A, also b) different chain length of the hydrophobic blocks were synthesized and for A-pPheOzi₁₅-A also various batches with varying terminating agents Ω were prepared.

Experimental Section:

Materials and Methods: All substances and reagents for the monomer synthesis and polymerization were purchased from Sigma-Aldrich (Steinheim, Germany) or TCI-chemicals (Eschborn, Germany) and were used as received unless otherwise stated. Deuterated solvents for NMR analysis were obtained from Deutero GmbH (Kastellaun, Germany). All substances used for polymerization, specifically methyl trifluoromethylsulfonate (MeOTf), MeOx, PheOzi and BuOzi were refluxed over CaH_2 for several hours and distilled prior usage. The solvent benzonitrile (PhCN) was dried over phosphorus pentoxide. All dried reagents were stored under dry and inert conditions.

Refractive index of synthesized monomers was performed on a RFM 870 refractometer from Bellingham+Stanley at 20 °C (Farnborough, England). The monomers were analyzed via *mass-spectrometry* using an Agilent 5977B MDS system coupled with a gas-chromatography system Agilent 7820A. The GC-system was equipped with an Agilent 19091S-433UI HP-5ms ultra inert column (30 m x 250 μm x 0.25 μm). Temperature gradient was set from 40 °C to 300 °C with constant heat rate of 15 °C/min and a constant flow of 1 mL/min.

Nuclear magnetic resonance (NMR) was performed on a Bruker Fourier 300 (^1H : 300.12 MHz) spectrometer at 298 K from Bruker BioSpin (Rheinstetten, Germany) and calibrated using the solvent signals.

Gel permeation chromatography (GPC) was performed on a Polymer Standard Service PSS (Mainz, Germany) system with following specifications: pump mod. 1260 infinity, MDS RI-detector mod. 1260 infinity (Agilent Technologies, Santa Clara, California, USA), precolumn: 50 x 8 mm PSS PFG linear M; 2 columns: 300 x 8 mm PSS PFG linear M (particle size 7 μm ; pore size 0.1 – 1.000 kg/mol) with hexafluoroisopropanol (HFIP, containing 3 g/L potassium trifluoroacetate (KTFA)) as eluent calibrated against PEG standards with molar masses from 0.1 g/mol to 1000 kg/mol. The columns were held at 40 °C and the flow rate was set to 0.7

mL/min. Prior to each measurement, samples were dissolved in eluent and filtered through 0.2 µm PTFE filters (Rotilabo, Karlsruhe, Germany) to remove particles, if any.

Dynamic light scattering (DLS) experiments were performed on an ALV SP125 (Langen, Germany) equipped with a He-Ne-laser (22 mW, $\lambda = 632.8$ nm) and a single photodiode-detector. Scattering angles from $30 \rightarrow 150^\circ$ were measured with a 2.5° angle interval ($T = 293.15$ K, correlation time 60s, average of 3-5 runs). Prior to each measurement, samples were dissolved in methanol (10 g/L) and filtered through 0.02 µm Anotop membrane filters from Whatman GE Healthcare followed by IC Millex-LG 0.2 µm. After evaporation of methanol the samples were dissolved in prefiltered (Millex-LG 0.2 µm) Millipore water containing 2 mM NaNO_3 . 1 g/L samples were filtered (Millex-LG 0.2 µm) into dust free cuvettes under laminar flow. The decay of the electric field-time autocorrelation function (ACF) was fitted using biexponential fit functions (equation 1) with respect to polydispersities, like used previously²⁷:

$$g_1(t) = a_1 \cdot e^{\left(-\frac{t}{\tau_1}\right)} + a_2 \cdot e^{\left(-\frac{t}{\tau_2}\right)} \quad (1)$$

With the amplitudes a_i and the decay times $\tau_i = \frac{1}{q^2 \cdot D_i}$, with q being the absolute value of the scattering vector and D the Brownian diffusion coefficient which is indirect proportional to the hydrodynamic radius R_h (Stokes-Einstein equation 2):

$$R_h = \frac{k_B \cdot T}{6 \cdot \pi \cdot \eta \cdot D} \quad (2)$$

With Boltzmann constant k_B and the viscosity of the solvent η .

In case of polydispersity the hydrodynamic radius is obtained by extrapolation to zero angle and in the limit of high dilution given by:

$$\langle 1/R_h \rangle_z^{-1} = \frac{\sum_i a_i}{\sum_i a_i \cdot R_i^{-1}}$$

For *transmission electron microscopy (TEM)* investigations the polymer was dissolved in ultra pure water to a final concentration of 20 g/L. For the negative stain method, 400 mesh copper-rhodium grids (maxtaform) with a homemade carbon layer were glow-discharged in air for 1.5 min at medium power in a Harrick PDC-002 plasma cleaner. The 20 g/L sample was diluted (1/625) and 8 μ L were incubated on the grids for one minute before blotting (Whatman filter paper No 50). Then the grids were washed with water (3x) and with 2 % (w/v) uranyl acetate (3x). For *cryo* sample preparation Quantifoil copper grids (400 mesh, R1/2) were used. 3.5 μ L of undiluted sample was applied on the grids and plunge-frozen in liquid ethane with a Vitrobot IV (FEI). The humidity was set to 100 % and the temperature was set to 5 °C, 25 °C or 40 °C. For imaging either a single-tilt room temperature holder (negatively stained samples) or a Gatan 626 *cryo*-transfer holder (vitrified samples) in an FEI Tecnai T12 Spirit transmission electron microscope equipped with a LaB₆ emitter at 120 kV were used. Images were recorded with an Eagle CCD camera under low-dose conditions. The micrographs were binned 2 times resulting in a pixel size of 4.4 Å/pix at specimen level.

Rheology experiments were performed using an Anton Paar (Ostfildern, Germany) Physica MCR 301 system utilizing a plate-plate geometry (25 mm diameter) equipped with a solvent trap and Peltier element for temperature adjustment. All aqueous samples were stored after dissolution at 5 °C for 48 h. The temperature-sweep was performed in oscillation mode from 5-40 °C (heating rate: 0.05 °C/s), holding the temperature for 60 s followed by a cooling phase from 40-5 °C (cooling rate: 0.05 °C/s) using a fixed amplitude of 0.1 % and angular frequency of 10 rad/s. The long-time gelation experiment was performed at an amplitude of 0.1 % and an angular frequency of 1 rad/s for several hours. To investigate the viscoelastic properties, the linear viscoelastic region (LVR) was determined by performing amplitude sweeps at different concentrations (5-40 wt.%) from 0.01 % to 500 % strain deformation and a fixed angular frequency of 10 rad/s at 5 °C. Subsequently, frequency sweeps from 0.1 to 100 and 500 rad/s, respectively, were conducted at 0.1 % strain deformation and 5 °C. To investigate structure

recovery properties, the deformations were alternated from 0.1 % to 150 % in seven consecutive cycles at a fixed angular frequency of 10 rad/s and 5 °C and the percentage of structure integrity after deformation was determined after the first and last cycle recovery sequence. For steady shear experiments, the control shear rate mode was used from 0.001 to 100 1/s at 5 °C. The obtained viscosity η decrease was fitted using the power-law expression established by Ostwald-de Waele (eq. 3).

$$\eta = K \cdot (\dot{\gamma})^{n-1} \quad (3)$$

Where K is the consistency index, n the flow index and $\dot{\gamma}$ the applied shear rate.

To evaluate the yield-stress/yield-point τ_0 of a hydrogel system the steady stress sweep- and the dynamic oscillatory stress sweep (amplitude sweep) were performed. Using the steady stress sweep from 5 - 1500 Pa shear stress the viscosity starts decreasing several orders of magnitude at a certain shear stress. The onset-value of this decrease is referred as yield point τ_0 . Also, the data of the dynamic oscillatory stress sweep were evaluated using onset-determination. The flow-point τ_{flow} is determined as the crossing of storage and loss modulus ($G' = G''$) by definition. These values were taken and compared and discussed with the steady stress sweep data.

Mechanical compression was tested using an electro force 5500 system (BOSE electro force systems group, Friedrichsdorf, Germany). The experiments were performed in static mode using a 250 g load cell. Cylindrical samples (4 diameter x 4 mm height) were prepared and analyzed. The crosslinking of samples was performed using a 40 mM aqueous CaCl_2 solution. For the hybrid system the 17 wt.% samples (2 wt.% alginate, 15 wt.% A-PPheOzi15-A) were stored for 24 h at 5 °C prior to the crosslinking step. All samples were incubated in 4 mL deionized water after crosslinking for 24 h at 37 °C. Six individual samples of the hybrid system and pure alginate hydrogels were analyzed and compared. Young's modulus was determined

using a linear fit function in the linear material response region, typically in the range of 0.1→0.2 strain deformation.

Dispense plotting (3D printing) of hydrogel scaffolds was conducted at room temperature using a compact bench-top 3D bioprinter (Inkredible, Cellink, Sweden) working on the principle of an extrusion-based printer following the preparation described in Scheme S2. The printing speed was set to 600 mm/min and different pressures were applied for different concentrations of hydrogel using a conical nozzle with 0.25 mm inner diameter. In general 24 orthogonal stacks (10x10 mm, 0.25 mm layer height and 1 mm strand-center to strand-center distance) were printed and analyzed with a stereomicroscope SteREO Discovery.V20 (Carl Zeiss Microscopy, Jena, Germany) equipped with a Zeiss icc 5 color camera (5 MP, 12 bit), two lenses (0.63x and 1.5x Plan Apo) and a zoom range up to 20:1. For alginate-A-PPheOzi₁₅-A hybrid ink system 30 wt.% of A-PPheOzi₁₅-A solution was mixed at 37 °C with a 4 wt.% alginate solution and stored for several hours at 5 °C. The crosslinking of the 24-layered construct was conducted using 10 mM CaCl₂ solution. The crosslinked hybrid scaffold was then incubated in water (Scheme S3) and analyzed via stereomicroscope and rheology.

Cell culture experiments of human embryonic kidney HEK293 cells (HEK-Blue™ IFN- α/β , Invivogen) were cultured in growth medium (DMEM 10 % FCS, 100 U/mL penicillin G and 100 μ g/mL streptomycin, San Diego, USA) on 25 cm² culture flasks at 37 °C and 5 % CO₂. Calu-3 cells (human lung adenocarcinoma, ATCC HTB-55) were maintained in growth medium (MEM 10% FCS, 100 U/mL penicillin G and 100 μ g/mL streptomycin, 1% non-essential amino acids (NEA), 1 mM pyruvate, 2 mM glutamine and 2.88 g/L glucose) at 37 °C and 5 % CO₂. The experiments were performed as described previously.³⁴ For the measurement of the cytotoxicity of the polymer, 5000 cells/well of both Calu-3 and HEK cells were seeded in 96-well plates in growth medium and incubated at 37 °C and 5 % CO₂ for 24 h and 48 h,

respectively. Final polymer concentrations of 10, 5, 1 and 0.1 wt.% were prepared from a stock solution (20 wt.%) in growth medium on ice and added to the cells. After 24 h of cell growth, the medium was removed and replaced by fresh cell culture medium. Cells were incubated with WST-1 reagent for 1-4 h at 37 °C according to manufacturer's manual. The formation of formazan was monitored at 450 and 630 nm using a Spectramax 250 microplate reader from Molecular Devices (Sunnyvale, CA, US).

Scanning electron microscopy of the polymer solutions were frozen with liquid nitrogen in the gel state and lyophilized afterwards. The dried powder was mounted on aluminium sample holders with conductive carbon tape and sputtered with a 4 nm layer of platinum in a sputter coater (Leica Microsystems ACE 400, Wetzlar, Germany). The morphology of the samples was subsequently analyzed using a Crossbeam 340 field emission scanning electron microscope (Carl Zeiss Microscopy, Oberkochen, Germany) by setting the acceleration voltage (ETH) to 2 kV and detection of secondary electron (SE) with an Everhart-Thornley detector. To visualize the native hydrogel structure, we also investigated a cryogenic sample preparation procedure. For this, samples were placed between two aluminium holders (d=3mm), both containing a notch with a diameter of 2 mm, inclosing the sample and rapidly frozen in slush nitrogen (SN) at -210 °C. The samples were then transferred into the sputter coater with a Leica EM VCT100 cryo-shuttle at -140°C (Leica Microsystems ACE 400, Wetzlar, Germany). Here, the upper half of the sample was knocked off to create a fresh fractured surface and freeze-etched at -85 °C for 15 minutes under vacuum ($< 1 \cdot 10^{-3}$ mbar). The samples were finally sputtered with 3 nm platinum and transferred with the cryo-shuttle into the SEM chamber. The morphology of the fractured surfaces was imaged at -140 °C, by detecting SE using acceleration voltages of 2 kV or 8 kV.

Synthetic Procedures: Monomer synthesis

The monomers 2-phenyl-2-oxazine (PheOzi) and 2-benzyl-2-oxazine (BzOzi) were synthesized following the procedure by Wittig and Seeliger (Scheme S1-S3).⁴¹ For the reaction 1 eq of respective nitrile, 1.2 eq. of 3-amino-propanol and catalytic amounts of zinc acetate dihydrate were added to a argon flushed flask and heated to 130 °C under reflux for several days until the reaction mixture turned brown. Reaction progress was controlled by ¹H-NMR-spectroscopy. After completion, the mixture was dissolved in dichloromethane and washed with H₂O (three times). The organic phase was dried with MgSO₄ and concentrated. The raw product was refluxed with CaH₂ and purified via vacuum distillation under argon atmosphere to yield the product as a colorless liquid. The resulting compounds PheOzi and BzOzi were characterized via refractive index, GC-ESI-MS analysis, and ¹H- and ¹³C-NMR spectroscopy (see supporting information).

Polymer synthesis

The polymers were synthesized following a general procedure based on previous reports.^{27, 28} Exemplarily, the preparation of methyl-PMeOx₃₅-*b*-PPheOzi₁₅-*b*-PMeOx₃₅-*N*-Boc-piperazine (A-PPheOzi-A, B1) was performed as follows. Under dry and inert conditions 131 mg (0.80 mmol, 1 eq.) MeOTf and 2.39 g (28.1 mmol, 35 eq.) of MeOx were added to 23 mL dry PhCN and stirred for 4 hours at 110 °C. Full monomer conversion was verified by ¹H-NMR-spectroscopy before adding the monomer for the second block. The mixture was cooled to room temperature, and 2.07 g (12.8 mmol, 16 eq.) of PheOzi was added. After stirring over night at 120 °C, 2.39 g (28.1 mmol, 35 eq.) of MeOx was added. After completion of the third block termination was carried out using 298 mg (1.6 mmol, 2 eq.) *N*-Boc-piperazine and stirring for several hours at 45 °C (termination with 3-(2-furyl)propionic acid was performed with 2.4 eq. of freshly sublimated carboxylic acid and 3 eq. of dried trimethylamine at 60 °C). After cooling to room temperature, 111 mg (0.80 mmol, 1 eq.) potassium carbonate was added and the mixture was stirred for 5 hours. The solvent was removed at reduced pressure and the flask was

placed in a vacuum drying oven at 40 °C and 20 mbar for two days to remove remaining benzonitrile. The residue was dissolved in deionized water, dialyzed overnight using a membrane with a MWCO of 1 kDa and freeze-dried (yield: 6.21 g, 88.7 %).

Results and Discussion:

We synthesized a small series of ABA-triblock copolymers PMeOx₃₅-*b*-PPheOzi₅-*b*-PMeOx₃₅, PMeOx₃₅-*b*-PPheOzi₁₅-*b*-PMeOx₃₅ (B1, B2, B3 and B4), PMeOx₃₅-*b*-PPheOzi₃₀-*b*-PMeOx₃₅, PMeOx₃₅-*b*-PBzOzi₁₅-*b*-PMeOx₃₅, PMeOx₃₅-*b*-PPheOx₁₅-*b*-PMeOx₃₅²⁷ and PMeOx₃₅-*b*-PBzOx₁₅-*b*-PMeOx₃₅²⁷ by living cationic ring opening polymerization (LCROP) and characterized the amphiphiles via ¹H-NMR spectroscopy and GPC (Table 1 and Figure S1-S14). The successful termination using different terminating agent could be demonstrated via specific signal assignment (See supporting information). Results from ¹H-NMR and GPC analysis were also compared with previously reported A-PPheOx-A and A-PBzOx-A.²⁷ The primary structural difference between the novel polymers is one additional methylene group in the backbone and a variation of the degree of polymerization of the hydrophobic core, which leads to different physico-chemical properties.

Table 1. Number average molar mass, chain length, dispersity Đ and yield of the synthesized triblock copolymers used in this study. The polymers A-PPheOx-A and A-PBzOx-A were previously described in ref. [27] and used herein.

Polymer	M _n ^a [kg/mol]	M _n ^b [kg/mol]	M _n ^c [kg/mol]	Đ ^d	yield [%]
A-PPheOx ₁₅ -A	8.5	8.5	5.3	1.10	88
A-PPheOzi ₁₆ -A (B1)	8.7	8.7	3.7	1.22	89
A-PPheOzi ₁₅ -A (B2)	8.6	8.3	3.8	1.28	83
A-PPheOzi ₁₆ -A (B3)	8.7	9.1	2.8	1.23	81
A-PPheOzi ₁₆ -A (B4)	8.7	8.9	2.3	1.30	89
A-PPheOzi ₅ -A	7.0	6.1	4.0	1.29	70
A-PPheOzi ₃₀ -A	10.9	9.6	4.0	1.26	79
A-PBzOx ₁₅ -A	8.7	10.3	5.2	1.25	77
A-PBzOzi ₁₅ -A	8.8	9.1	4.0	1.18	89

^a Theoretical values obtained from $[M]_0/[I]_0$. ^b Values calculated from $^1\text{H-NMR}$ end group analysis. ^c Obtained from gel permeation chromatography ($T = 40\text{ }^\circ\text{C}$, 0.7 mL/min (HFIP), poly(ethylene glycol) standards). ^d Obtained from GPC by using M_w/M_n .

To gain insight into the differences with respect to aggregation, multi-angle dynamic light scattering experiments for A-PPheOzi₁₅-A and A-PBzOzi₁₅-A solutions were performed. A-PPheOx₁₅-A and A-PBzOx₁₅-A solutions have been previously analyzed similarly and polymer micelles with hydrodynamic radii of 8-10 nm were observed.²⁷ The measured autocorrelation functions were fitted by means of biexponential fit functions, the results of which is shown in Figure 1a for a scattering angle of 90° at $20\text{ }^\circ\text{C}$ and a polymer concentration of 1 g/L . By plotting the inverse hydrodynamic radius ($1/R_h$) as a function of the scattering vector, A-PPheOzi₁₅-A showed on average three time larger aggregates than A-PBzOzi₁₅-A and the respective poly(2-oxazoline) amphiphiles described previously (Figure 1b). Moreover, whereas the DLS of A-PBzOzi₁₅-A micelles with a hydrodynamic radius of 10 nm shows no angular dependency of $1/R_h$ (nearly monodisperse micelles) the average diffusion coefficient $1/R_h$ of A-PPheOzi₁₅-A sample exhibits a pronounced angular dependency. This can be attributed to a pronounced polydispersity typical for e.g. wormlike aggregates ('unlimited' 1D growth). The aggregation into spherical polymer micelles of polymer A-PBzOzi₁₅-A could be confirmed by transmission electron microscopy (Figure 1c). Rather homogeneous spherical micelles with hydrodynamic radii of ca. 10 nm were observed, corroborating DLS results. In contrast, for A-PPheOzi₁₅-A, a complete different and polymorph aggregation pattern was observed. This polymer aggregates at room temperature predominantly into worm-like structures with some residual spherical polymer micelles (Figure 1d), corroborating DLS data. It should be noted, that DLS and TEM experiments required different sample preparation. Therefore, different concentration and sample history could have affected polymer aggregation.

Figure 1| Comparison of A-PPheOzi₁₅-A and A-PBzOzi₁₅-A self-assembly via dynamic light scattering (a,b) and transmission electron microscopy (c,d). a) Autocorrelation function $g^1(t)$ in dependence of q^2t at polymer concentrations of 1 g/L (2 mM aqueous NaNO₃ solution) fitted by biexponential fit function. b) Inverse hydrodynamic radii R_h [nm^{-1}] as a function of the particular scattering vector q^2 . Obtained data were fitted using linear fit functions. c) TEM image of polymer micelles formed by A-PBzOzi₁₅-A (0.32 g/L). d) TEM image of polymorph aggregation of A-PPheOzi₁₅-A into wormlike structures and spherical micelles (0.32 g/L).

By dissolving the described amphiphiles in water (20 wt.%) and incubate the resulting solutions at 5 °C and 37 °C, we noticed that the A-PPheOzi-A polymers formed clear hydrogels at different concentrations (Figure 2a,c and Figure S15). Therefore, we investigated the temperature dependent rheology properties of all polymer amphiphiles with different

hydrophobic B blocks by performing temperature sweeps (5 – 40 °C) after storage for 24 h at 5 °C (Figure 2b).

Figure 2| Screening for sol/gel transition of POx/POzi based ABA type amphiphiles with varying aromatic core B. a) Images of 20 wt.% aqueous solutions of A-PPheOx-A (black triangle), A-PBzOx-A (red triangle), A-PPheOzi-A (green triangle) and A-PBzOzi-A (blue triangle) at 5 °C. Obviously, only A-PPheOzi-A forms a hydrogel (gel), all other samples stayed liquid (sol) b) Temperature-sweep (5→40 °C, heat-rate: 0.05 °C/s) of aqueous solutions (20 wt.%) of A-PPheOx-A (black), A-PBzOx-A (red), A-PPheOzi-A (green) and A-PBzOzi-A (blue) at a strain of 0.1 % and an angular frequency of 10 rad/s. Complex viscosity in dependence of the applied temperature is shown. c) Images of 20 wt.% aqueous solutions of A-PPheOx-A, A-PBzOx-A, A-PPheOzi-A and A-PBzOzi-A at 37 °C. All amphiphiles present as low viscous liquids (sol).

Starting as a gel, the aqueous solution of A-PPheOzi-A results in a clear liquid above a critical temperature. Conversely, by cooling the liquid samples, a clear hydrogel is obtained after some time. We also tested the behavior of the polymers A-PBzOx-A, A-PPheOx-A and the newly synthesized A-PBzOzi-A in aqueous solutions at 20 wt.%. Neither solution did undergo thermogelation in the temperature range of 5 °C to 40 °C. By visual inspection, we noticed that gelation does not occur immediately upon cooling. Therefore, the gelation kinetics were studied in more detail (Figure 3). The storage moduli G' at different concentrations were recorded at 5 °C and 10 °C for 8 hours (Figure 3a). At 5 °C, higher concentrations lead to faster gelation. The onset temperature of gelation for a 20 wt.% aqueous solution is ~ 90 minutes. For a 15 wt.% sample gelation started a few minutes later and end up with lower G' plateau values compared

to the 20 wt.% sample. A significant shift towards longer gelation time was observed for 10 wt.% samples. By increasing the temperature from 5 °C to 10 °C, the gelation was delayed significantly. A temperature sweep revealed that, starting from 5 °C, the complex viscosity of a 10 and 15 wt.% aqueous solution remains almost constant until about 30 °C, whereupon it decreases drastically over 3-4 orders of magnitude and the gel liquefies (Figure 3b, Figure S16). Once the gel is destroyed, no immediate, re-gelation is observed as the system is cooled again.

Figure 3| Gelation kinetics in dependency of concentration and temperature of A-PPheOzi-A hydrogels. a) Storage moduli of aqueous solutions of A-PPheOzi₁₅-A (B1) (10 wt.%: black, 15 wt.%: red, 20 wt.%: blue) at 5 °C (filled symbols) and 10 °C (open symbols) at constant strain deformation of 0.1 % and 1 rad/s angular frequency. The polymer solutions were placed on a pre-cooled rheometer plate and measured subsequently. b) Temperature-sweep of the complex viscosity (5→40→5 °C, heat/cooling-rate: 0.05 °C/s) of A-PPheOzi₁₅-A hydrogel (red: 15 wt.%, black: 10 wt.%). The stable pre-cooled hydrogels were mounted on a pre-cooled rheometer plate. c) *cryo*TEM analysis of A-PPheOzi₁₅-A temperature dependent aggregation in aqueous solutions (20 g/L). Samples were stored 24 h at respective temperature (5 °C, 25 °C, 40 °C).

To further corroborate that hydrogel formation is caused by reversible worm-spherical morphology changes, cryoTEM images were recorded on samples incubated at different temperatures. At 5 °C, the temperature of stable hydrogel formation, a network of interconnected worms could be confirmed (Figure 3c). At intermediate 25 °C, the majority of the polymer is still aggregated as worm-like micelles, but some spherical micelles are apparent. Above the liquefaction temperature of the hydrogel (at 40 °C), only spheres with sizes of ca. 10 nm are visible. After this initial assessment, we investigated the effect of polymer concentration, chain length and chain termini as well as the reproducibility of the rheological properties between different polymer batches. Amplitude sweeps showed that at 5 wt.% and 5 °C, the polymer solutions of A-PPheOzi₁₅-A (B1) represent a weak gel ($G' \sim 135$ Pa). However, both storage and loss modulus could be greatly modulated by changing the polymer concentration. Increasing the concentration to 40 wt.% the stiffness of the gel increased four orders of magnitude ($G' (40 \text{ wt.}\%) \sim 110$ kPa) whereas the regions of linear viscoelasticity decreased notably (Figure 4a). Frequency sweeps corroborated a strong viscoelastic-solid behavior over a large range of frequencies and concentrations (Figure 4b).

Figure 4| Viscoelasticity of A-PPheOzi₁₅-A (B1) inverse thermohydrogel at different concentrations. a) Amplitude sweeps at different concentrations (5→40 wt.% at 5 wt.% steps) at 5 °C and an angular frequency of 10 rad/s with storage moduli ($G' = \blacksquare$) and loss moduli ($G'' = \circ$) shown. b) Frequency sweeps at 5 °C and an amplitude of 0.1 %. Solutions were tempered at 5 °C for 48 h prior to measurements. c) Exponential increase (red

line) of storage modulus by increasing polymer concentration (G'_{LVE} : mean value of storage modulus in the plateau region of the respective amplitude sweep.)

Apart from changing the hydrophobic core with respect to the chemical structure of the repeat unit, the amphiphilic ratio was adjusted by varying the degree of polymerization within the hydrophobic core resulting in A-PPheOzi₅-A, A-PPheOzi₁₅-A and A-PPheOzi₃₀-A. In addition, several batches of A-PPheOzi₁₅-A were synthesized to assess batch-to-batch reproducibility⁴² of the viscoelastic properties of the polymers. The rheological properties of all four batches of A-PPheOzi₁₅-A are quite similar showing, a prominent gel-like character ($G' > G''$) at 15 wt.% aqueous solutions at 5 °C with storage moduli around 10 kPa (Figure 5a). Similar properties were also found in cell culture medium (Figure S17). Unexpectedly, the gel strength decreased significantly when increasing the degree of polymerization of the central hydrophobic block to 30 units with a concomitant increase of the linear viscoelastic region (Figure 5a, black ■, see also Figure S18). Decreasing the chain length to 5 units of the PPheOzi-block completely prevented gel formation in the investigated concentration range. At 10 wt.% the gel formed by A-PPheOzi₃₀-A was comparable to a 5 wt.% gel of A-PPheOzi₁₅-A.

Figure 5| Investigation of reproducibility and influence of aromatic block chain length of hydrogel formation. a) Storage ($G' = \blacksquare$) and loss moduli ($G'' = \circ$) in amplitude sweeps of different batches of A-PPheOzi₁₅-A (B1 (green), B2 (blue), B3 (violet) and B4 (cyan)), A-PPheOzi₃₀-A (black) and A-PPheOzi₅-A (grey) at 15 wt.% aqueous solutions, 5 °C and a constant angular frequency of 10 rad/s. b) Corresponding frequency sweeps of A-

PPheOzi₁₅-A (B1, B2, B3 and B4) at 5 °C and an amplitude of 0.1 % showing storage modulus ($G' = \blacksquare$) and loss modulus ($G'' = \circ$) as well as complex viscosity η^* (open triangles).

For solutions of A-PPheOzi₁₅-A at 5 °C and a 15 wt.%, the frequency had only minor effect on the viscoelastic properties of the gel. The gel character remained unchanged within the investigated frequency range up to 500 rad/s. This corroborates that the physical network is thoroughly developed and surprisingly stable in the entire frequency range investigated. On the other hand, the strongly decreasing complex viscosity values over several orders of magnitude show profound non-Newtonian fluid behavior. Well defined yield- and flow-points are beneficial properties for hydrogels when used in the field of biofabrication and 3D printing (Figure 6). Moreover, pronounced shear thinning (Figure 7a) and rapid structure recovery (Figure 7b) are desired and realized by the novel materials.⁴³ In literature, different experiments, namely the steady stress sweep (Figure 6a) and the dynamic oscillatory stress sweep (Figure 6b), are discussed to obtain the yield- and flow-point of a system direct from the plotted values. Here, these methods are compared using 10 and 15 wt.% A-PPheOzi₁₅-A (B1) hydrogels.

Figure 6| Yield- and flow-point determination using rotational and oscillation rheology approaches. a) Viscosity in dependency of the applied shear stress of 10 wt.% (black) and 15 wt.% (red) hydrogels at 5 °C. The intersection of dashed lines mark the onset of the viscosity decrease which is commonly referred to as the yield point τ_0 . **b)** Development of storage (G') (squares) and loss modulus (G'') (circles) with increasing shear stress of

10 wt.% (black) and 15 wt.% (red) A-PPheOzi₁₅-A hydrogels. The systems flow point τ_f is defined as the crossover of G' and G'' (arrows).

In the steady stress sweep the shear stress is increased steadily from low (5 Pa) to high (1000 Pa) values. At a certain stress the viscosity values decrease over several orders of magnitude. The onset, defined by the tangential method, is referred as the yield point τ_0 of the system (τ_0 (B1 (10 wt.%)) = 58 Pa, τ_0 (B2 (15 wt.%)) = 180 Pa, Figure 6a). Alternatively, an increase of G'' and decrease of G' during the dynamic oscillatory stress sweep indicates τ_0 (τ_0 (B1 (10 wt.%)) = 51 Pa, τ_0 (B2 (15 wt.%)) = 190 Pa, Figure 6b). In addition, the crossover of G' and G'' is defined as the flow point τ_f of the system (τ_f (B1 (10 wt.%)) = 305 Pa, τ_f (B2 (15 wt.%)) = 826 Pa, arrows Figure 6b). The corresponding viscosity values at the flow point can be obtained from the steady stress sweep experiment (η at τ_f (B1 (10 wt.%)) = 33 Pa·s, η at τ_f (B2 (15 wt.%)) = 74 Pa·s, arrows Figure 6a). Values for the other batches were obtained accordingly (Figure S19-S22) and are summarized in Table 2. The obtained values of the different batches do certainly vary, but are all in the same order of magnitude which indicates a good batch-to-batch reproducibility.

Apart from a defined yield-point, shear thinning and fast structure recovery are very important for printing purposes. A-PPheOzi₁₅-A exhibited very pronounced shear thinning (Figure 7a), and excellently reproducible structure recovery properties of 93 % after the first recovery step and 87 % after 7 recovery cycles (Figure 7b). The steady rate sweep from 0.001 to 100 1/s was fitted using the power-law expression described by Ostwald-de Waele to obtain the flow-index n and consistency index K to characterize the hydrogel.

Figure 7| Shear-thinning and structure recovery are crucial requirements for dispense plotting. a) Viscosity in dependency of the applied shear rate for a 10 wt.% (black) and 15 wt.% (red) A-PPheOzi₁₅-A hydrogel at 5 °C. b) Strain-step experiment: Complex viscosity in dependency of the applied strain (0.1→150, 7 cycles) at 5 °C and an angular frequency 10 rad/s.

Clearly, the novel material is highly shear-thinning and follows the power-law expression exhibiting low flow indices n ($n \ll 1$) across all synthesized A-PPheOzi₁₅-A batches ($n < 0.15$) (Table 2). Notably, the gel structure of the system recovers very rapidly (Figure 7b), which is highly favorable for processing like 3D printing.

Table 2. Summary of rheological data and parameters of A-PPheOzi₁₅-A hydrogels (10 and 15 wt.%) obtained from steady stress sweep, dynamic oscillatory stress sweep and steady rate sweep experiments across different batches. The missing viscosity values could not be obtained, as in rotational steady shear stress experiments the limit was set to 1 kPa. The upper limit of the steady stress sweep was set to 1 kPa.

c	batch	τ_0 (osc)	τ_0 (rot)	τ_f (osc)	η at τ_f	K	n
[wt.%]		[Pa]	[Pa]	[Pa]	[Pa·s]	[Pa·sⁿ]	
10	B1	51	58	305	33	180	0.14
	B2	183	148	576	5	333	0.13
	B3	80	134	376	8	237	0.14
	B4	59	137	393	7	230	0.14
15	B1	190	180	826	74	538	0.12
	B2	650	560	1600	n.d.	856	0.09
	B3	300	410	1100	n.d.	625	0.10
	B4	350	420	1050	n.d.	642	0.12

To obtain first insights into the morphology and microstructure of the novel hydrogels, we obtained scanning electron microscopy (SEM) images after freeze-drying. At 20 wt.%, the hydrogel of A-PPheOzi₁₅-A exhibited a highly porous and rather homogeneously distributed microstructure with pores in the 5 to 10 μm range (Figure S23). However, as currently strongly discussed in the community, due to slow water evaporation during lyophilization or ice crystal formation during slow freezing, these features could in fact be artefacts of sample preparation. To avoid such, we also conducted cryogenic SEM (*cryo*-SEM) analysis. It has been shown that for very small hydrogel samples mounted on a TEM grid, liquid nitrogen (LN) as cooling agent provides sufficient cooling rates despite of the Leidenfrost effect⁴⁴, for bigger samples however, more efficient cooling agents like slush nitrogen (SN)⁴⁵, liquid ethane⁴⁶ or high pressure freezing in LN⁴⁷ are necessary. Here, we used SN to investigate whether the microporous structure observed by SEM was an artifact from freeze-drying or not (Figure 8). The result is rather obvious, clearly, the sample preparation affects the observed morphology. *Cryo*-SEM images obtained of the gel of A-PPheOzi₁₅-A shows a highly ordered porous structure with round pores in the sub-micrometer to low micrometer range, which is in agreement with the rheological data of a strong physical network (Figure 8a,b). In comparison, for aqueous solutions of A-PPheOzi₅-A, which does not form a hydrogel, rather lamellae-like structure are observed (Figure 8c,d). Therefore, it appears that even though a 3D network is formed, it is too weak to lead to macroscopic gelation, presumably due to a limited correlation length of the underlying physical network. In addition, we investigated the gel morphology of 15 wt.% A-PPheOzi₁₅-A before and after dispense plotting (see below) by *cryo*-SEM (Figure 8e,f). It appears that the microstructural morphology remains unaffected, which also corroborates the rheological data, which show rapid recovery of the gel structure after printing.

Figure 8| Microstructural analysis of the hydrogel structures. a,b) *Cryo*-SEM images of 5 wt.% A-PPheOzi₁₅-A hydrogel at (a) 1k x magnification and b) 5k x magnification). c,d) *Cryo*-SEM images of 5 wt.% A-PPheOzi₁₅-A sol at (c) 1k x magnification and d) 5k x magnification). *Cryo*-SEM images of 15 wt.% A-PPheOzi₁₅-A hydrogel prior (e) and after dispense plotting (f) (5k x magnification).

From a rheological and structural point of view, the hydrogels of A-PPheOzi₁₅-A appear well suited for (bio)printing. Obviously, cytocompatibility represents another critical feature for any hopeful bioink or biomaterial ink. For the present polymers, no dose dependent cytotoxicity

(WST-1 assay) was found for HEK and Calu3 cell lines at concentrations of up to 10 wt.% (Figure 9). In contrast, we found an increased apparent cell viability, which has been found similarly for other POx block copolymers.³²

Figure 9| Dose dependent cell viability of A-PPheOzi₁₅-A (B15) aqueous solutions using WST-1 assay. Cell viability was assessed for HEK (a) and Calu3 (b) cell lines (n=3) after 24 h of incubation with polymer solutions.

Finally, the printability of the novel material was assessed (Figure 10). We printed first scaffolds using 10, 15 and 20 wt.% of A-PPheOzi₁₅-A and 20 wt.% of A-PPheOzi₃₀-A hydrogels using a 3D bioprinter equipped with a conical nozzle with 0.25 mm inner diameter (Figure 10a, Figures S24-S26). To evaluate printing conditions such as speed and pressure, we first printed a four layer 10x10 line wood-pile structure (Figure S24a-c; S25b, S26a-c). The strength of a 10 wt.% of A-PPheOzi₁₅-A hydrogel is insufficient and strand fusion is observed, which is in line with the low yield point of the system. Nevertheless, a 3D cube could be printed (Figure S25c,d). Also, the 20 wt.% of A-PPheOzi₃₀-A hydrogel could be successfully printed in 24 layers, but again some strand fusion was observed and shape fidelity was not very good (Figure S24d,e). Using the 20 wt.% (Figure 10b) and 15 wt.% hydrogel of A-PPheOzi₁₅-A and optimized printing parameters, 24-layer constructs of 10x10 lines and 1 mm strand distance could be printed (Figure S26d,e). Excellent shape fidelity and layer integrity was clearly evident (Figure 10c,d; top view, Figure 10e,f; side view).

Direct-Ink-Writing

Figure 10| Dispense-plotting using A-PPheOzi₁₅-A hydrogels. a) Extrusion based printing setup (Nozzle: Conical, 0.25 mm inner diameter; Speed: 600 mm/min; Dimensions: 10x10 mm; 1 mm strand distance, 0.25 mm layer height). b) Printed 24-layer scaffold of B1 (20 wt.% aqueous solution; b: Photographic image, c, d: Top view; e, f: side view).

To highlight the potential of the novel material as e.g. a sacrificial matrix material or component in a hybrid system, we increased the temperature on the previously printed scaffolds, upon which strand fusion and collapse of the hydrogel network (Figure S27) was observed and the gel liquefied rapidly. Accordingly, a mild temperature stimulus compatible with cell culture conditions can be used to remove scaffolds printed with A-PPheOzi₁₅-A. This could be very useful if used as a sacrificial support matrix to assist printing of materials which by themselves are not easily 3D-printed with good shape fidelity.

Alginate as a biopolymer is an ideal candidate to test this hypothesis, as it can be easily crosslinked by incubation with aqueous CaCl₂ and is often employed in biofabrication, but usually suffers from poor printability due to poor rheological properties.^{48, 49} To improve the printability of alginate often pre-crosslinking⁵⁰ or composite systems are used.⁵¹⁻⁵⁴ Here, we present an exceedingly simple alternative. We simply mixed the liquid A-PPheOzi₁₅-A polymer

with liquid alginate and cooled the mixture leading to gelation. We characterized this hybrid system via rheology prior to the printing process (Figure S28). A 17 wt.% aqueous hybrid system (A-PPheOzi₁₅-A/alginate 15:2 w/w) exhibited storage moduli up to 11 kPa (Figure S28a) and a pronounced shear thinning response favorable for extrusion based printing (Figure S28b).

Accordingly, these hybrid systems could also be successfully printed into 3D scaffolds with excellent shape fidelity (Figure 11a). Simple addition of CaCl₂(aq) leads to alginate crosslinking and subsequent incubation at 37 °C, results in the dissolution of the sacrificial component A-PPheOzi₁₅-A. The residual alginate scaffolds stay structurally intact thereafter for at least 4 days being incubated in a water bath without significant shrinkage, loss of shape or stiffness in the temperature range of 5 °C to 40 °C (Figure 11b-d). After crosslinking the storage modulus increases from 11 to 80 kPa which remained at similar levels (60 to 70 kPa) for several days during incubation in aqueous solution (Figure 11d). Important to note, due to the removal of the A-PPheOzi₁₅-A during curing and developing step, no thermoresponsive behavior was observed for the chemical cross-linked structure. However, the mechanical stability increases significantly. For the hybrid system, a more than 10-fold increase of Young's modulus (130 ± 12 kPa) compared to native alginate (8 ± 3 kPa) was obtained (Figure S29). We hypothesize that the alginate network could be formed more regular due to macromolecular crowding and microphase separation during casing and crosslinking. However, a detailed characterization of this observation is outside the scope of the current contribution.

Hybrid Biomaterial Ink

Figure 11| A-PPheOzi-A hydrogels as support material in dispense plotting of biomaterial inks. a) Workflow for the preparation of the hybrid material, its printing and development. b) Top and c) side view of printed 24-layer scaffolds of B1/alginate hybrid (17 wt.% aqueous solution (B1/alginate 15:2) (Extrusion based bioprinter; Nozzle: Conical, 0.25 mm inner diameter; Speed: 600 mm/min; Dimensions: 10 x10 mm; 1 mm strand distance, 0.25 mm layer height). d) Storage moduli as a function of temperature (T : 5 °C to 40 °C, heat rate: 0.05 °C/s, strain: 0.1 %, angular frequency: 1 rad/s) of B1/alginate hybrid prior to crosslinking (black) and after crosslinking (red) and incubation in aqueous solutions for 48 h (blue) and 72 h (green).

The described hydrogel platform offers highly beneficial properties for a variety of applications such as a sacrificial biomaterial, a component in hybrid systems as well as support material in biofabrication, namely temperature responsive physical gelation and liquefaction, tunability of storage modulus over several orders of magnitude, printability and cytocompatibility. In addition, the polymer platform of POx/POzi offers different modification strategies during synthesis and as well as additional post-polymerization modifications to further utilize and modify the unique aqueous solution properties of A-PPheOzi-A amphiphiles in different applications in the field of biofabrication, regenerative medicine and tissue engineering.

Conclusion

In this study, we investigated the influence of different aromatic moieties on the viscoelastic properties and thermogelation of aqueous solutions of poly(2-oxazoline)/poly(2-oxazine)-based ABA triblock copolymers. We introduced and characterized ABA triblock copolymers comprising poly(2-phenyl-2-oxazine) and poly(2-benzyl-2-oxazine) as hydrophobic core B. Most notably, the polymers bearing PPheOzi moieties undergo inverse and reversible thermogelation at a defined temperature and rather low concentrations of 5 wt.%. Presumably the polymer A-PheOzi₁₅-A exhibited the right balance of flexibility caused by poly(2-oxazine) based backbone and rigidity of the phenyl sidechain, as well as amphiphilicity, to form a reversible worm-like network at lower temperatures and a thermoresponsive transition into spherical polymer micelles upon heating. We established important properties for the hydrogels in the context of biofabrication, namely the printability, as defined by distinct yield- and flow points and favorable shear-thinning and structure recovery properties, as well as cytocompatibility. By means of *cryo*-SEM analysis, we obtained first insight into the structure of the hydrogels, which exhibit macroporous features. Also, we highlight the application as sacrificial support material by 3D printing of excellently resolved and shape-persistent scaffolds of alginate, which is otherwise not printable in 3D due to its poor rheological properties.

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Supporting Information Description

Detailed description of monomer and polymer synthesis and characterization. Tube inverting test of hydrogels as a function of concentration. Detailed rheological characterization of all synthesized polymer batches and the hybrid system. Schemes to visualize hydrogel and hybrid system preparation for dispense plotting. Microscopy investigations of different printed scaffolds using different batches concentrations and printing architectures. SEM analysis of lyophilized sample. Compression test of alginate hydrogels

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Table of Contents graphic

I. ABA-Type Amphiphile

A: Poly(2-methyl-2-oxazoline)

II. Inverse Thermogelation

III. 3D Printing

Cytocompatible Tunable Sacrificial Biomaterial Ink