# Supporting Information

## A versatile biomaterial ink platform for the melt electrowriting of chemically-crosslinked hydrogels

Daniel Nahm[1,2], Franziska Weigl[2], Natascha Schaefer[3], Ana Sancho[2], Andreas Frank[4], Jürgen Groll[2], Carmen Villmann[3], Hans-Werner Schmidt[4], Paul D. Dalton[2,*] and Robert Luxenhofer[1,5,*]

[1] Functional Polymer Materials, Chair for Advanced Materials Synthesis, Department of Chemistry and Pharmacy, and Bavarian Polymer Institute, University of Würzburg, Röntgenring 11, 97070 Germany.

[2] Department of Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University Hospital of Würzburg, Pleicherwall 2, 97070, Würzburg, Germany

[3] Institute for Clinical Neurobiology, University Hospital, Julius-Maximilians-University Würzburg, Versbacherstr. 5, 97078 Würzburg, Germany.

[4] Macromolecular Chemistry I and Bavarian Polymer Institute, University of Bayreuth, Universitätsstraße 30, 95440, Bayreuth, Germany

[5] Soft Matter Chemistry, Department of Chemistry, Helsinki University, 00014 Helsinki, Finland

# Table of content

**Experimental section:**

*Materials and Methods*

All substances for the material synthesis were purchased from Sigma-Aldrich (Steinheim, Germany) or Acros (Geel, Belgium) and were used as received unless otherwise stated. 2-ethyl-2-oxazine (EtOzi), benzonitrile (BN) and methyl trifluoromethyl sulfonate (MeOTf) were dried by refluxing over $CaH_2$ under inert atmosphere followed by distillation prior use. The maleimide functionalized fluorescent dye DY-647P1-Maleimide was purchased from DYOMICS (Jena, Germany). The Peptide ($NH_2$-CGGGRGDS-COOH) was purchased from GeneCust (Boynes, France)

*NMR*

Nuclear magnetic resonance spectroscopy (NMR) measurements were performed on a Bruker Fourier 300 ($^1$H 300.12 MHz) at 298 K, Bruker BioSpin (Rheinstetten, Germany). Spectra were referenced on the residual solvent signals (MeOD 3.31 ppm; $CD_3CN$ 1.94 ppm)

*GPC*

Gel permeation chromatography (GPC) was performed of a Polymer Standard Service (PSS, Mainz, Germany) system (pump mod. 1260 infinity, RI-detector mod. 1260 infinity, precolumn GRAM 10 µm (50 × 8 mm), 30 Å PSS GRAM 10 µm (300 × 8 mm) and 1000 Å PSS GRAM 10 µm (300 × 8 mm)). The device was calibrated against PEG standards and hexafluoroisopropanol (HFIP) (3 g/L potassium trifluoroacetate (KTFA), 313 K, 1 mL/min) was used as eluent.

*IR*

IR-Spectra were recorded on a Jasco (Groß-Umstadt, Germany) FT/IR-4100 equipped with an ATR-unit. A scan count of 64 with a resolution of 4 $cm^{-1}$ was used. All spectra were post-processed by baseline correction using the software Spektra Manager Version 2.

*DSC*

Differential Scanning Calorimetry (DSC) measurements were performed on a SC 204 F1 Phoenix system from NETZSCH (Selb, Germany) equipped with a CC200 F1 controller unit. For the reversibility studies of the DA material a temperature ramp from -50 °C to 200 °C with a heating rate of 10 K/min was performed under nitrogen atmosphere. Between each liquefaction cycle the material was cured at 60 °C for 1 h. Using the determined reaction heat of 4.3 J/g ($2^{nd}$ cycle) and 2.6 J/g ($7^{th}$ cycle) along with the mean molecular weight of the two polymeric species of 11.8 kg/mol and 5 DA units per polymer chain it is possible to calculate a mean reaction enthalpy of 10.1 kJ/mol and 6.1 kJ/mol respectively.

*TGA*

Thermogravimetric analysis (TGA) was performed on a TG 209 F1 Iris from NETZSCH (Selb, Germany) with a heating rate of 10 K/min in synthetic air. Relative mass loss was calculated from the absolute mass loss at 900 °C.

*Rheology*

Rheology experiments were performed on a Anton Paar (Ostfilden, Germany) Physica MCR 301 utilizing a plate-plate geometry (diameter 25 mm) and a measurement gap of 0.5 mm. All samples were added in powder state and then liquefied by heating to 140 °C followed by 2 min constant shear (10 1/s) segment to remove air bubbles. Strain sweeps from 0.01 rad/s to 10 rad/s were conducted at different temperatures to verify linear viscoelastic behaviour of the material (Figure S10). Frequency sweeps were performed from 0.1 to 100 rad/s with an amplitude of 1 %. For the reversibility studies were a strain of 1 % and a frequency of 10 rad/s was used.

*Processing via MEW*

A custom-made MEW-printer was used as shown in Figure S11, similar to that to presented in detail elsewhere.[1] Briefly, the MEW head is electrically-heated and has a nitrogen line to pressurize a glass syringe. A high voltage is applied to the nozzle while the collector plate is earthed and moves freely underneath the MEW head on an x-y motion stage, with the collector distance (z-axis) able to be manually adjusted.

To ensure the best processing experience it is important to ensure a homogeneous mixture of the two polymeric components as well as to remove all volatile substances from the mixture. Therefore, the two polymers were grinded in a liquid nitrogen filled mortar. This was done in an argon filled portable glove bag to prevent absorption of air moisture. The resulting powder was then dried at 40 °C and 300 mbar for at least one day. After this step, the 2-component biomaterial ink is crosslinked yet ready for liquefaction and MEW processing. For printing a custom-made MEW device described elsewhere was used.[1] To ensure maximum processing time the material was liquefied for only 10 min before the printing experiments were started. For optimal lifetime while allowing a continuous fibre deposition a reservoir temperature of 130 °C and a nozzle temperature of 150 °C were used. The collector distance was kept constant at 3.3 mm. While, the acceleration voltage was varied between 2 kV and

4 kV and the feeding pressure was varied between 1.5 and 2 bar to allow a homogenous fibre deposition. A collector speed of 1300-1800 mm/min was used. For better handling the scaffolds were printed on glass microscope slides. Box like structures with a fibre distance of 500 µm on an area of 2.3 $mm^2$ as well as 3.2 $mm^2$ were printed.

*Hydrogel formation*

After processing the structures were allowed to rest for at least 16 h. After that the scaffolds were incubated in Millipore water and swelling occurred immediately. The overall height of a swollen construct consisting out of 10 layers is approximately 500 µm.

*Dye functionalisation*

The dry scaffold was incubated overnight in 2 mL of a DY-647P1-Maleimide solution in Millipore water (0.05 mg/mL). The functionalized scaffold was then washed several times with Millipore water to ensure full removal of non-bound dye.

*Confocal microscopy of the dye-functionalized scaffolds*

Scaffolds functionalized with DY-647P1-Maleimide were visualized using a TCS SP8 SMD inverted confocal microscope from Leica. A illumination wavelength of 633 nm a detection wavelength range from 650-694 nm was used. The image dimensions are 1162.5 µm x 1162.5 µm x 434.7 µm with a z-step number of 146.

*Optical analysis*

Optical analysis was performed on a Discovery V.20 stereomicroscope from Carl Zeiss AG (Oberkochen, Germany)

*Equilibrium water content*

The bulk material was prepared as described in the Processing via MEW section above. For determining, the swelling degree 3 samples were incubated in Millipore water for 48 h (weight equilibrium was reached after 24 h). During this time, the incubation medium was exchanged 3 times. The wet samples mass (MW) was then determined followed by drying of the samples for 16 h at 40 °C and 300 mbar. Finally, the dry mass (MD) was determined. The following equation was used for calculation of the EWC.

$$EWC = \frac{MW - MD}{MW} * 100$$

*Environmental scanning electron microscopy*

The water swollen scaffold was placed in the sample chamber of the scanning electron microscope (FEI Quanta FEG 250, Thermo Fisher Scientific). The measurement was conducted in the environmental scanning electron microscopy (ESEM) mode. The sample holder was cooled to 2 °C and the chamber was purged twice with water vapour. Subsequently, the chamber was stabilized for 30 minutes at a pressure of 730 Pa, which corresponds to a relative humidity of about 100%. Under these conditions, the swollen state of the scaffold was maintained. By changing the pressure to 320 Pa, the relative moisture within the chamber is reduced and the fibres start to dry.

*Thermoresponsive Swelling*

Five MEW printed PEtOzi hydrogel scaffolds are placed in water-filled petri dishes. The scaffolds are incubated in a temperature control chamber (3-40 °C) or heated in a water bath (50-80 °C). The temperature inside the petri dishes is monitored using a thermometer. Incubated is prolonged for at least 15 min once the respective temperature inside the petri dishes is

reached. A picture of the scaffolds is taken quickly. Using the software ImageJ the width and height of the scaffolds is determined. The size at 3 °C is set to 100 %.

*Fibre stiffness*

The stiffness of the fibres was assessed as described before.[2] Briefly, the measurements were based on Single Cell Force Spectroscopy (SCFS) performed with an Atomic Force Microscope of the model Flex FPM (Nanosurf GmbH, Germany) combined with the FluidFM® technology (Cytosurge AG, Switzerland). The system was mounted on an inverted Axio Observer Z1 microscope (Carl Zeiss, Germany). Micropipette cantilevers with an aperture of 4 µm and a nominal spring constant of 0.3 N/m were used (Cytosurge AG, Switzerland). Polyethylene glycol coated polystyrene beads of 10 µm in diameter (Micromer® 01-54-104, Micromod Partikeltechnologie GmbH, Germany) were used as indenter and immobilized at the aperture of the cantilever by applying underpressure. Before every experiment, the deflection sensitivity of the cantilever linked to a polystyrene bead was calibrated by using the Cytosurge software procedure. For the determination of the fibre elasticity three independent two-layered scaffolds were investigated, generating 25 to 30 force-distance curves per scaffold. Indentations were performed on random spots of the fibres, omitting the intersections. The approach of the cantilever was done at 500 nm/s until a setpoint of 3 nN was reached. The data acquisition frequency was set to 6 kHz. The generated indentation curves were analyzed by a custom program written in Matlab 2017b (Mathworks, USA). After several steps of data processing[2] the curve is plotted in the form of force (nN) versus indentation (nm) and fitted according to the Hertz Model, to obtain the apparent Young's modulus. The model is based on the following equation[3]

$$F = \frac{4 \cdot E \cdot R^{1/2}}{3 \cdot (1 - \nu^2)} \cdot \delta^{3/2}$$

where F is the force, E the Young's modulus, R the radius of the spherical indenter, ν Poisson's ratio which was set as 0.5 for these experiments and δ represents the indentation.

*Cytotoxicity test*

Cytotoxicity was evaluated according to DIN-ISO 10993-5. Briefly, the polymer sample was sterilized in 70 % ethanol for 1 h, followed by drying for 3 days at room temperature. The sample was incubated in medium (10 mL/mg) at 37 °C for 48 h. Next, the supernatant was transferred into a sterile vessel and the eluate medium was prepared by dilution to 100 % (no dilution), 50 % and 25 % with medium. L 929 CC1 mouse fibroblasts were seeded in medium in a 48-well plate (25,000 cells per well) and incubated for 24 h at 37 °C / 5 % $CO_2$. The cell culture medium was exchanged by the polymer eluate and the cells were again incubated at 37 °C / 5 % $CO_2$ for 48 h. The cell medium was removed and the cells were incubated with WST-1 reagent for 30 min at 37 °C / 5 % $CO_2$. Cell count was determined using a Casy Cell Counter (OLS OMNI Life Science GmbH & Co KG).

*Longterm stability tests*

For the stability test two scaffolds with 10 layers and 500 µm fibre spacing were printed as described above. The scaffolds were then incubated in Millipore water and phosphate-buffered saline (PBS), in the dark, on a tilting table at 5 rpm. Scaffolds were visualized using stereomicroscopy and inspected for structural damage and the incubation was continued (Figure S6).

*Cell line and transfection*

Human embryonic kidney (HEK293) cells (ATCC®CRL-1573TM, Wesel, Germany) cells were grown in Minimum Essential Media (Life Technologies, Darmstadt, Germany). The medium was supplemented with 10 % fetal calf serum (FCS), glutaMAX (200 mM) and sodium pyruvate (100 mM), penicillin (10,000 U/ml) / streptomycin (10,000 µg/ml) at 37 °C and 5 % $CO_2$. Cells were seeded in 10 cm plastic dishes with a density of 3 x 106 cells/dish. At 75 % confluency cells were trypsinized, centrifuged, and seeded onto coated hydrogel scaffold. Cells

were imaged with the Leica S40/0.45 microscope, 20x/0.3 Objective and Leica 104550630 10x/23 Ocular.

*Immunocytochemical staining*

HEK293 cells together with hydrogel scaffolds – Scaffolds and cells were fixed with 4 % paraformaldehyde (PFA) and 4 % sucrose in phosphate-buffered saline (PBS) for 10 min at 21 ºC. Scaffolds and cells were washed two times in PBS to remove the PFA. Following fixation, cells attached to scaffold were blocked and permeabilized (0.1 % Triton-X 100, 5 % NGS in PBS) for 30 min at 21 ºC. The primary mouse-monoclonal β-actin antibody (EnCor Biotechnology Inc., Gainesville, FL, USA) was supplied in a dilution of 1:500 for 45 min at 21 ºC. Following, three washing steps with PBS, cells were incubated for 30 min with the secondary antibody goat-anti-mouse Cy3 (dilution1:500; Dianova, Hamburg, Germany). After washing with PBS cells were incubated with DAPI (1:5,000) for 10 min and mounted on glass cover slips with mowiol.

*Confocal microscopy, image acquisition and analysis of the scaffolds seeded with HEK293 cells*

All images shown were acquired using an inverted IX81 microscope equipped with an Olympus FV1000 confocal laser scanning system, a FVD10 SPD spectral detector and diode lasers of 405 nm (DAPI), and 550 nm (Cy3). Images were acquired with an Olympus UPLSAPO 40x (numerical aperture: 1.35) objective. The images were further organized by Adobe Photoshop and Illustrator software (Adobe). Video and surface composition (Fig. 4F,G) were obtained using Imaris 7.6 Software (Imaris v 7.6, Bitplane AG, software available at http://bitplane.com.)

*Synthesis of 3-(2-furyl)propionic acid*

2.5 g (2.38 mL, 14.9 mmol) Ethyl-3-(furan-2yl)propionate was dissolved in 10 mL THF and 20 mL of a solution of NaOH in 1:1 (by volume) $H_2O$:MeOH was added. The mixture was heated to 80 °C for 1.5 h. After cooling to RT 20 mL diethylether and 1 M aq. NaOH are added. The water phase was extracted 3 times with 20 mL diethylether and the combined org. phase were washed 3 times with 10 mL 1 M aq. NaOH. The combined water phase was acidified to pH=1 (1 M aq. HCl) and extracted 4 times with 20 mL of diethylether. The organic phase was combined and washed with 10 mL water, dried over $MgSO_4$, filtered and the solvent is removed in vacuo.

Yield: 95 % (1.98 g, 14.2 mmol)

$^1$H-NMR (MeOD-$d^4$, 300MHz) δ (ppm): 7.34 (d, 1H), 6.29-6.27 (q, 1H), 6.06-6.04 (d,1H), 2.94-2.89 (t, 2H), 2.64-2.59 (t, 2H)

*Synthesis of 2-ethyl-2-oxazine*

160 g of propionitrile (0.97 eq, 222 mL, 2.9 mol) and 16.5 g zinc acetate dihydrate (0.25 eq, 0.75 mol) are mixed in a nitrogen flushed flask and heated to 100 °C. 225 g aminopropanol (1 eq, 227 mL, 3 mol) was slowly added within 30 min. The mixture was stirred for 7 days. The raw product purified by distillation under reduced pressure, dried over $CaH_2$ and finally distilled again.

Yield: 110 g (33 %)

$^1$H-NMR: ($CDCl_3$-d3, 300MHz) δ (ppm): 4.14 (t, 2H), 3.35 (t, 2H), 2.14 (q, 2H), 1.84 (q, 2H), 1.08 (t, 3H)

*Synthesis of Poly(2-ethyl-2-oxazine)*

The polymer synthesis was carried out via an adapted procedure of Witte and Seeliger.[4] Briefly, 1 eq of initiator methyltrifluormethylsulfonat (MeOTf) was dissolved in the respective amount of dry benzonitrile (4 mol/L). 100 eq of the monomer 2-ethyl-2-oxazine (EtOzi) was added and the mixture was heated to 130 °C. Reaction progress was followed via $^1$H-NMR. As soon as full conversion was reached, the polymerisation was terminated at 50 °C for 16 h using a 3-fold excess of 1 M KOH in MeOH. The solvent was removed at 60 °C and under reduced pressure. Finally, the product was purified via dialysis against deionized water using a 1 kDa cut off cellulose membrane. Dialysis was performed for at least 24 h with regular water exchange. The product was recovered via lyophilisation. Two homopolymer master batches were synthesized and used within this publication. No significant difference in the general characteristics as well as in the processing behaviour were noticed. Therefore, no differentiation between the batches is done in the main paper.

Batch 1:

Yield: 27.4 g (91 %)

GPC (HFIP): $M_n$: 5.0x10$^3$ g/mol; D: 1.09

$^1$H-NMR: ($CD_3CN$, 300MHz) δ (ppm): 3.43-3.08 (br, 400H), 2.97-2.81 (br, 3H), 2.44-2.21 (br, 202H), 1.88-1.58 (br, 202H), 1.16-0.91 (br, 300H)

Batch 2:

Yield: 72.9 g (97 %)

GPC (HFIP): $M_n$: 4.2x10$^3$ g/mol; D: 1.09

$^1$H-NMR: ($CD_3CN$, 300MHz) δ (ppm): 3.43-3.08 (br, 400H), 2.97-2.81 (br, 3H), 2.44-2.21 (br, 210H), 1.88-1.58 (br, 202H), 1.16-0.91 (br, 300H)

*Hydrolysis of Poly(2-ethyl-2-oxazine)*

For the hydrolysis a kinetic screening of the reaction was performed to identify the required reaction time for the specific hydrolysis degree of 5 %. The hydrolysis reaction was carried out via an adapted procedure reported by Van Kuringen *et al.*[5] Briefly poly(2-ethyl-2-oxazine) was dissolved in the respective amount of deionized water (0.96 mol/L amide concentration). The solution was heated to 75 °C and the temperature within the flask was monitored and kept constantly at 75 °C. 10 min after the inner temperature was reached the respective amount of 37 % HCl was added to the mixture. Samples were taken at regular time intervals, immediately transferred into an ice bath and quenched with 20 m% aq. NaOH until pH 10 was reached. Hydrolysis degree was determined via $^1$H-NMR (Figure S1) with the following equation:

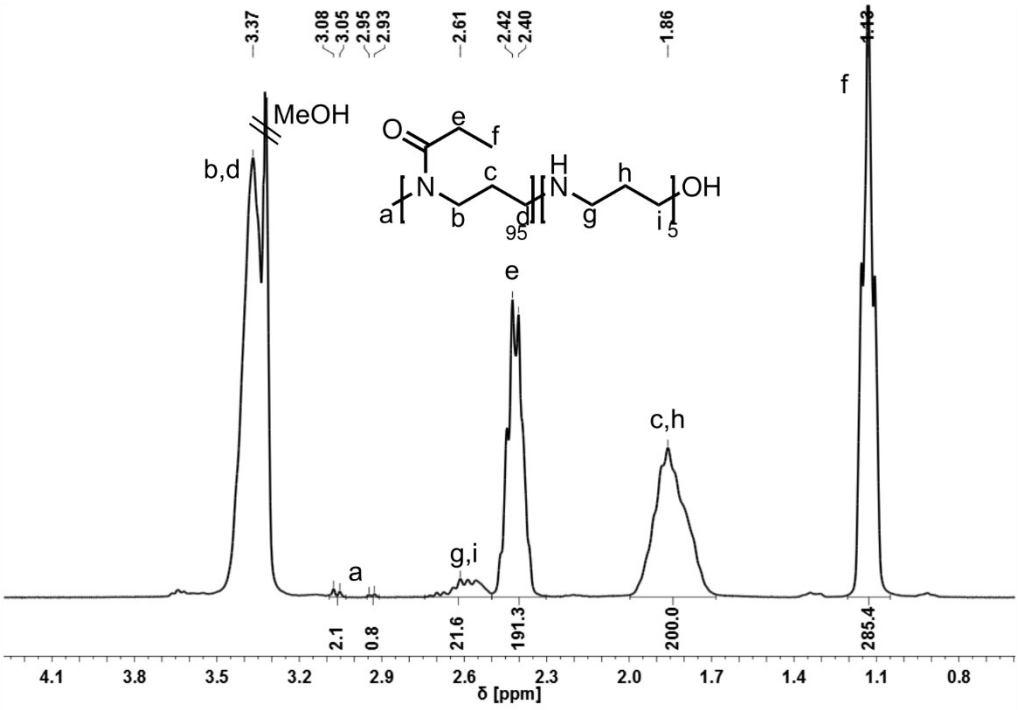
$$D(\%) = \frac{\frac{Int(g,i)}{4}}{\frac{Int(e)}{2} + \frac{Int(g,i)}{4}} * 100$$

The specific reaction time for the synthesis of 5 % hydrolysed PEtOzi was identified by a linear fit of the hydrolysis degree against reaction time. The synthesis procedure was identical.

Batch 1:
Yield: 8.4 g (95 %)
GPC (HFIP): $M_n$: 9.0x10$^3$ g/mol; D: 1.09
$^1$H-NMR: (MeOD-d4, 300MHz) δ (ppm): 3.5-3.25 (br, 360H), 3.09-2.90 (br, 2.9H), 2.74-2.50 (br,22H), 2.50-2.30 (br, 191H), 2.00-1.68 (br, 200H), 1.21-1.05 (br, 285H)
Degree of hydrolysis (NMR): 5.4 %

Batch 2:
Yield: 7.1 g (95 %)
GPC (HFIP): $M_n$: 8.3x10$^3$ g/mol; D: 1.1
$^1$H-NMR: (MeOD-d4, 300MHz) δ (ppm): 3.5-3.25 (br, 360H), 3.09-2.90 (br, 2.9H), 2.74-2.50 (br,19H), 2.50-2.30 (br, 190H), 2.00-1.68 (br, 200H), 1.21-1.05 (br, 285H)
Degree of hydrolysis (NMR): 4.8 %

Batch 3:
Yield: 19.14 g (96 %)
GPC (HFIP): $M_n$: 7.8x$10^3$ g/mol; D: 1.1
$^1$H-NMR: (MeOD-d4, 300MHz) δ (ppm): 3.5-3.25 (br, 360H), 3.09-2.90 (br, 2.9H), 2.74-2.50 (br,19H), 2.50-2.30 (br, 190H), 2.00-1.68 (br, 200H), 1.21-1.05 (br, 285H)
Degree of hydrolysis (NMR): 4.8 %




Supporting Figure S1. $^1$H-NMR spectrum of 5 % hydrolysed PEtOzi (Batch1). Important Peaks are assigned. The calculated degree of hydrolysis is 5 %.

*Synthesis of furan and maleimide functionalized Poly(2-ethyl-2-oxazine)*

The synthesis procedure is adapted from a previous report by Chujo *et al.*[6] The specific amount of hydrolysed polymer was dissolved in acetonitrile (0.03 mmol/mL). The solution was chilled in an ice bath. A 4-fold excess (referring to the respective amine concentration) of N,N'-dicyclohexyl carbodiimide (DCC) and either 6-maleimidohexanoic acid or 3-(2-furyl)propionic acid were added. The mixture was stirred for 16 h and a white solid precipitated. The precipitate was removed via centrifugation and decantation. The solvent of the supernatant was removed under reduced pressure and the remaining viscous liquid was dissolved in methanol (approx. 150 mg/ml with respect to the expected polymer mass). The polymer solution was 3-times precipitated in ice cold diethyl ether. The precipitate was dried under reduced pressure dissolved in a mixture of methanol and water and recovered via lyophilisation. Three different batches of the biomaterial ink were used for this work. Both showed similar characteristics and performed equal during processing. The data shown here is representative for both batches (Figure S2, S3, S4).

Batch 1 (Furan functionalized):
Yield: 2.5 g (92 %)
GPC $M_n$: 4.2x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 7.35 (br, 5H), 6.30 (br, 5H), 6.05 (br, 5H), 3.36-3.13 (br, 395H), 2.96-2.79 (br, 15H), 2.72-2.54 (br, 11H), 2.40-2.20 (br, 190H), 1.87-1-59 (br, 200H), 1.09-0.94 (br, 282H)
Degree of functionalisation (NMR): 5.0 %

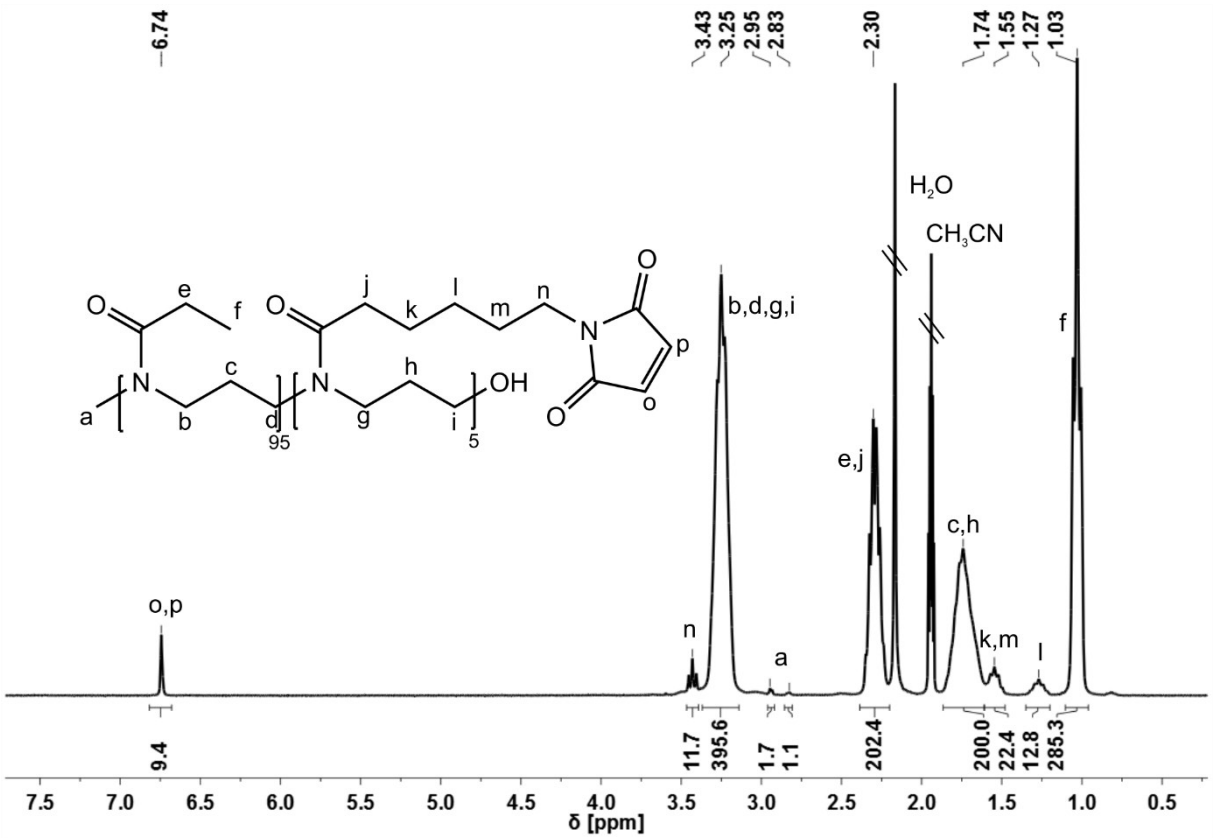
Batch 1 (Maleimide functionalized):
Yield: 2.6 g (96 %)
GPC $M_n$: 4.1x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 6.74 (br, 10H), 3.37-3.14 (br, 392H), 2.96-2.81 (br, 3H), 2.39-2.20 (br, 198H), 1.89-1.60 (br, 200H), 1.60-1.48 (br, 23H), 1.35-1.20 (br, 15H), 1.09-0.96 (br, 279H)
Degree of functionalisation: (NMR): 5.0 %

Batch 2 (Furan functionalized):
Yield: 2.7 g (91 %)
GPC $M_n$: 4.4x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 7.35 (br, 5H), 6.30 (br, 5H), 6.05 (br, 5H), 3.38-3.10 (br, 398H), 2.96-2.81 (br, 13H), 2.71-2.54 (br, 10H), 2.40-2.21 (br, 192H), 1.88-1-57 (br, 200H), 1.15-0.92 (br, 287H)
Degree of functionalisation (NMR): 4.6 %

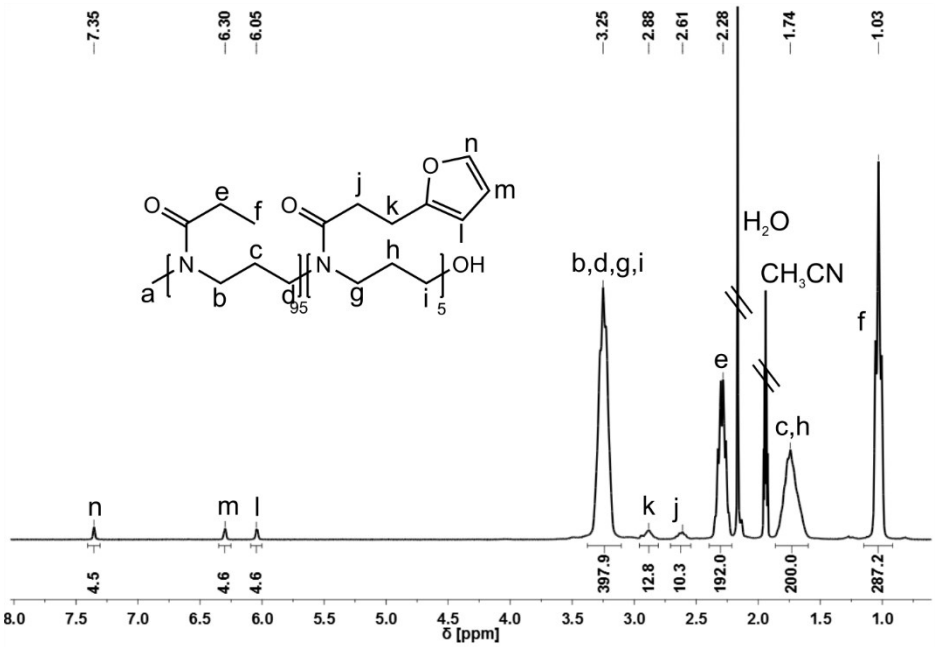
Batch 2 (Maleimide functionalized):
Yield: 2.8 g (92 %)
GPC $M_n$: 4.6x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 6.74 (br, 9H), 3.67-3.39 (tr, 12H), 3.37-3.14 (br, 397H), 2.96-2.81 (br, 3H), 2.39-2.20 (br, 202H), 1.89-1.60 (br, 200H), 1.60-1.48 (br, 22H), 1.35-1.20 (br, 13H), 1.1-0.96 (br, 285H)
Degree of functionalisation: (NMR): 4.7 %

Batch 3 (Furan functionalized):
Yield: 7.7 g (97 %)
GPC $M_n$: 4.2x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 7.35 (br, 5H), 6.30 (br, 5H), 6.05 (br, 5H), 3.38-3.10 (br, 398H), 2.96-2.81 (br, 13H), 2.71-2.54 (br, 10H), 2.40-2.21 (br, 192H), 1.88-1-57 (br, 200H), 1.15-0.92 (br, 287H)
Degree of functionalisation (NMR): 4.3 %

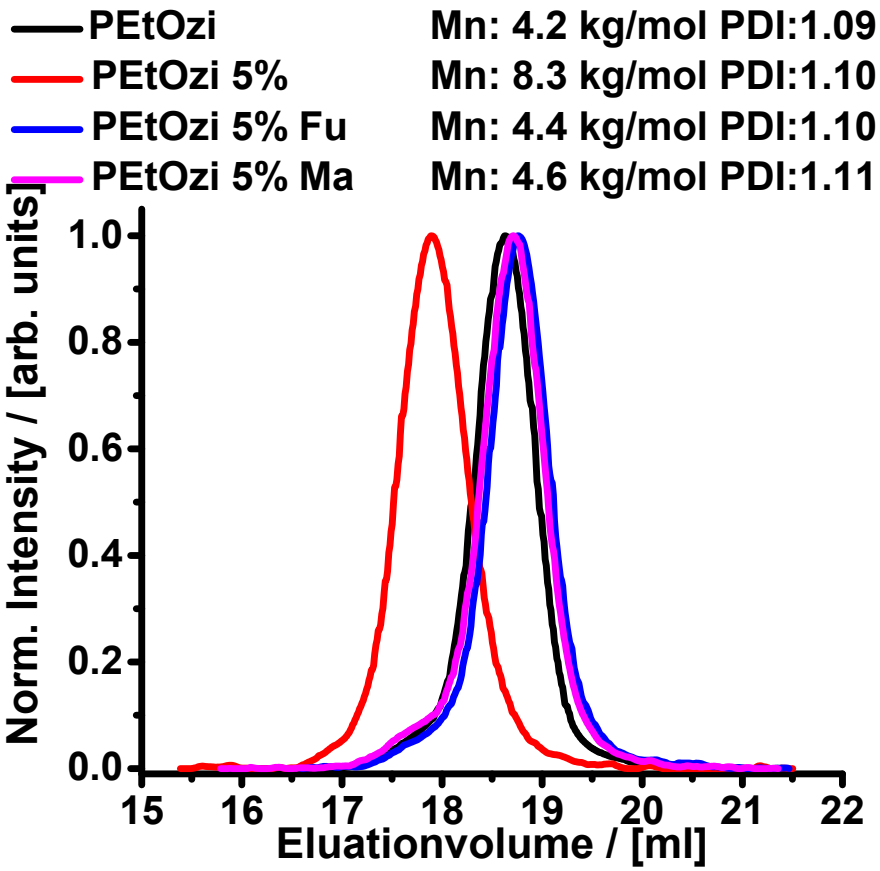
Batch 3 (Maleimide functionalized):
Yield: 7.8 g (98 %)
GPC $M_n$: 4.4x$10^3$ g/mol; D: 1.1
$^1$H-NMR: ($CD_3CN$-d3, 300MHz) δ (ppm): 6.74 (br, 9H), 3.67-3.39 (tr, 12H), 3.37-3.14 (br, 397H), 2.96-2.81 (br, 3H), 2.39-2.20 (br, 202H), 1.89-1.60 (br, 200H), 1.60-1.48 (br, 22H), 1.35-1.20 (br, 13H), 1.1-0.96 (br, 285H)
Degree of functionalisation: (NMR): 4.1 %



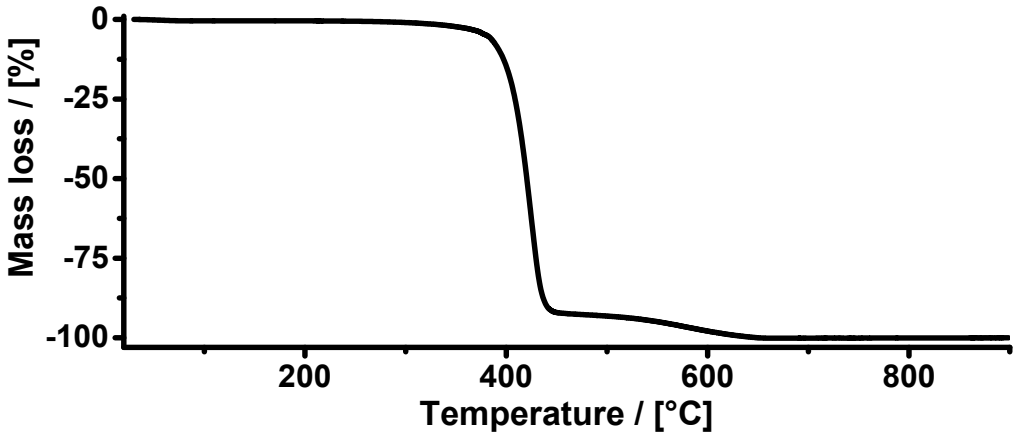

Supporting Figure S2. $^{1}$H-NMR spectrum of maleimide functionalized PEtOzi ($CH_3CN$; 300 MHz, 298 K) with all characterisitc peaks assigned.



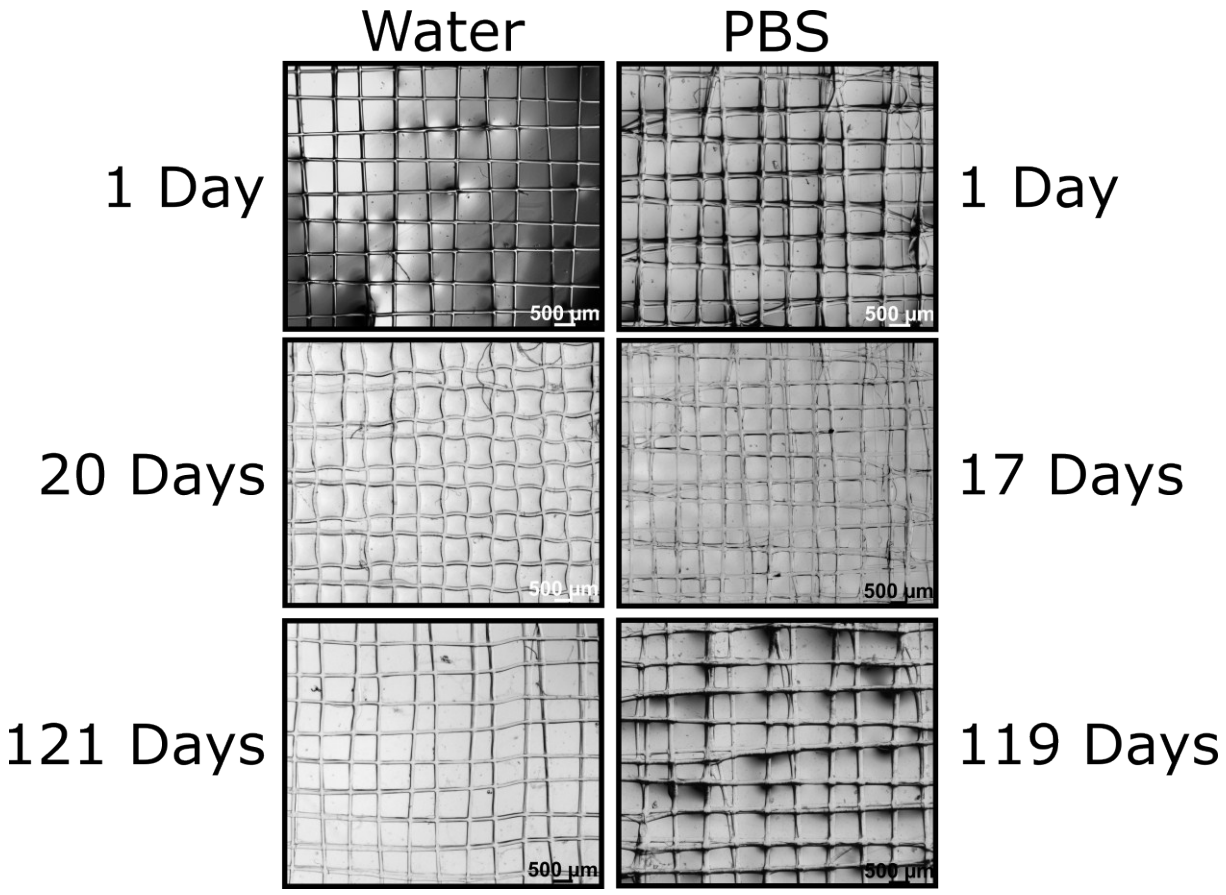

Supporting Figure S3. $^{1}$H-NMR spectrum of furan functionalized PEtOzi (Batch 2) ($CH_3CN$; 300 MHz, 298 K) with all characterisitc peaks assigned.



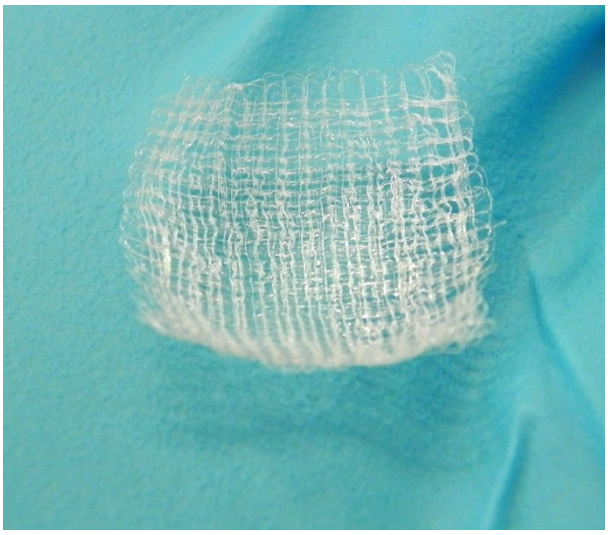

Supporting Figure S4. GPC elugrams of Batch 2. PEtOzi homopolymer (PEtOzi1), the hydrolysed PEtOzi (PEtOzi1 5%) as well as the furan (PEtOzi1 5% Fu) and maleimide (PEtOzi1 5% Ma) functionalized PEtOzi.



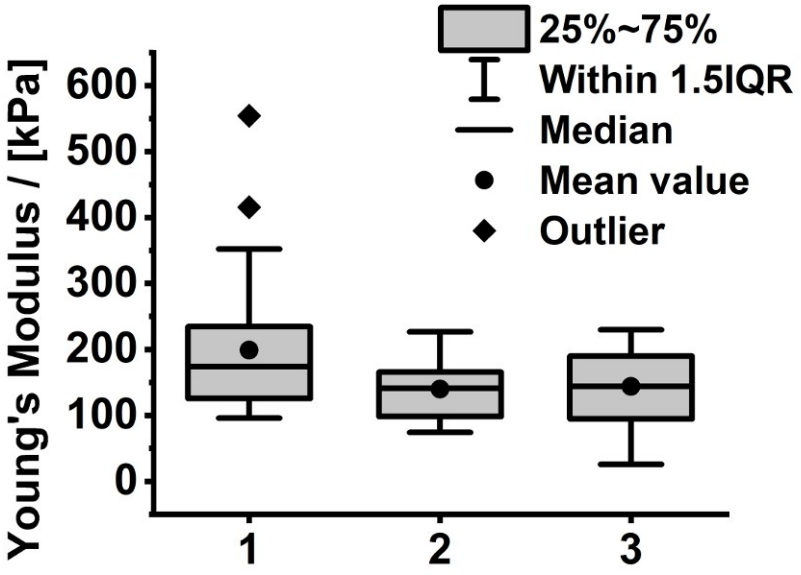

Supporting Figure S5. TGA thermogram of Diels-Alder crosslinked PEtOzi.



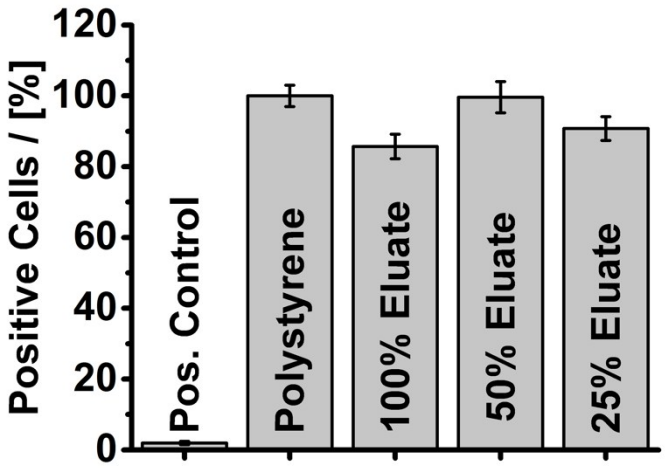

Supporting Figure S6. Stereomicroscope images of PEtOzi hydrogel scaffolds after incubation in water and PBS.



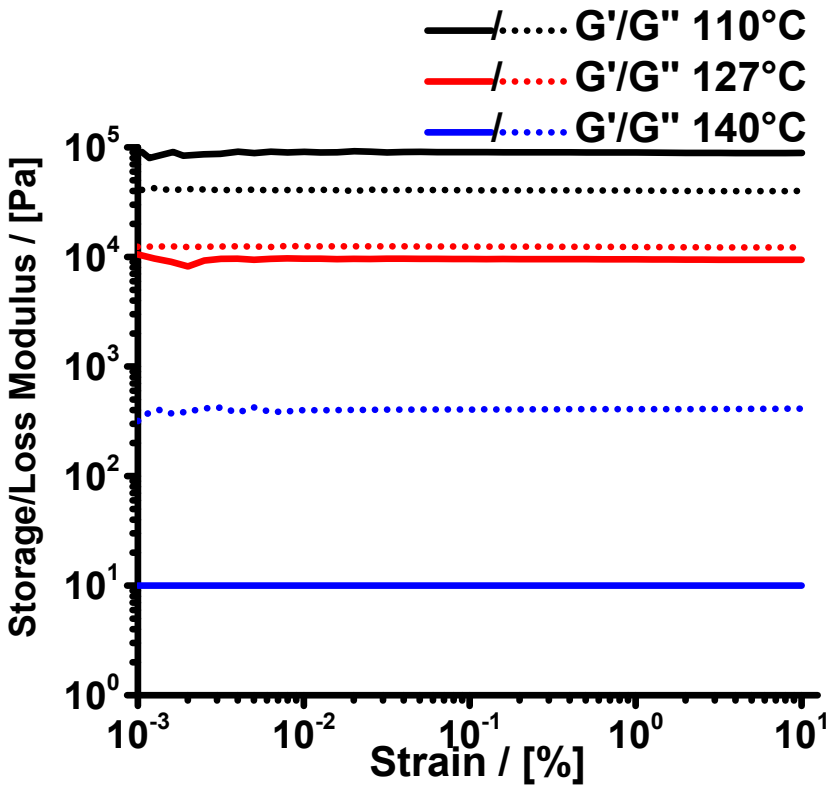
Supporting Figure S7. After drying, the scaffold structure is preserved.



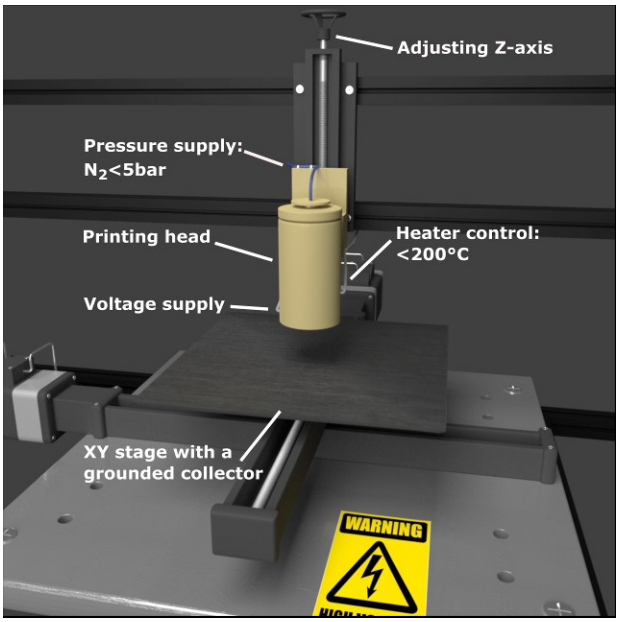

Supporting Figure S8. Young´s modulus of three different swollen PEtOzi scaffolds determined using Fluid-FM technology.




Supporting Figure S9. Results of the WST-1 cytotoxicity tests according to DIN-ISO 10993-5 showing the non-cytotoxic character of the material.




Supporting Figure S10. Rheology strain sweeps from 0.01 to 10 rad/s with a mixture of furan and maleimide PEtOzi.




Supporting Figure S11. Schematic illustration of the custom-made MEW setup.

*Thermal reversibility of the Diels Alder reaction*

Melt processing of polymers has high demands on the thermal inertness of the processed materials. However, the chemical crosslinking of polymers requires the introduction of reactive species within the material to allow crosslinking after processing. Above all, melt electrowriting is very sensitive to degradation of the processed polymer.[1,7] Therefore, we did a detailed investigation of the thermal reversibility of the novel biomaterial ink. While TGA confirmed no thermal degradation in the processing temperature range (Figure S5). DSC analysis (under $N_2$ atmosphere) showed a gradual decrease in the reaction heat in 7 cycles. This decrease can either be due to an incomplete recovery of the DA adduct between each heating cycle caused by insufficient speed of the crosslinking reaction. However, it could also be an evidence that the DA is not fully reversible under the DSC conditions. Very prominently we noticed thermally induced side reactions during the rheological analysis. When these experiments were performed at ambient conditions the material irreversibly solidified at the polymer/air interface while it still remained liquefiable underneath the rheology head of the device. This indicates that the DA functional groups undergo irreversible crosslinking when heated to high temperatures in the presence of oxygen. In our printing setup, this was avoided by using nitrogen to deliver the melt to the nozzle. Interestingly such oxygen induced side reactions are not well studied in literature. In contrast, the maleimide/furan pair is often reported to show good reversibility even in the presence of oxygen.[8-10] Reported side reactions of maleimides and furan are aromatization of the adduct,[11,12] ring opening of the furfuryl ring[13] and homopolymerization of the maleimide.[14,15] While none of these reactions are reported to occur preferably in the presence of oxygen, melt processing and melt-based analysis are highly demanding environments and especially the homopolymerisation of the maleimide might be initiated by oxygen radicals under these conditions. Yet, when performed under inert conditions, rheology indicates excellent reversibility of the DA reaction. All in all, considering the analytical results the reversibility is promising. Yet, it has to be addressed that the material in its current state allows

only a limited processing time window of 1-2 h. After this time, the material gradually irreversibly solidifies and allows no further processing. As two high molecular weight unimers are used, even small amounts of irreversible crosslinking of DA functions can induce drastic changes in the processability. Due to the low overall fraction of DA units within the material, these changes are challenging to analyse and quantify. Yet we are already investigating potential solutions for these issues and preliminary results show that the processing time window can be extended to over 5 h without changing the chemistry concept of the material.

## *References*


1. F. Chen, G. Hochleitner, T. Woodfield, J. Groll, P. D. Dalton and B. G. Amsden, *Biomacromolecules*, 2016, **17**, 208-214.
2. A. Sancho, I. Vandersmissen, S. Craps, L. Aernout and J. Groll, *Sci. Rep.*, 2017, **7**, 46152.
3. B. J. Briscoe, K. S. Sebastian and M. J. Adams, *J. Phys. D: Appl. Phys.*, 1994, **27**, 1156-1162.
4. H. Witte and W. Seeliger, *Justus Liebigs Ann. Chem.*, 1974, **1974**, 996-1009.
5. H. Van Kuringen, J. Lenoir, E. Adriaens, J. Bender, B. De Geest and R. Hoogenboom, *Macromol. Biosci.*, 2012, **12**, 1114-1123.
6. Y. Chujo, K. Sada and T. Saegusa, *Macromolecules*, 1990, **23**, 2636-2641.
7. G. Hochleitner, F. Chen, C. Blum, P. D. Dalton, B. Amsden and J. Groll, *Acta Biomater.*, 2018, **72**, 110-120.
8. T. Defize, J.-M. Thomassin, M. Alexandre, B. Gilbert, R. Riva and C. Jérôme, *Polymer*, 2016, **84**, 234 - 242.
9. S. Magana, A. Zerroukhi, C. Jegat and N. Mignard, *React. Funct. Polym.*, 2010, **70**, 442 - 448.
10. X. Chen, M. A. Dam, K. Ono, A. Mal, H. Shen, S. R. Nutt, K. Sheran and F. Wudl, *Science*, 2002, **295**, 1698-1702.
11. Y. S. Patel and H. S. Patel, *Int. J. Plast. Technol.*, 2013, **17**, 10-23.
12. J. Hao, W. Wang, B. Jiang, X. Cai and L. Jiang, *Polym. Int.*, 1999, **48**, 235-243.
13. A. Gandini and M. N. Belgacem, *Prog. Polym. Sci.*, 1997, **22**, 1203 - 1379.
14. B. J. Adzima, H. A. Aguirre, C. J. Kloxin, T. F. Scott and C. N. Bowman, *Macromolecules*, 2008, **41**, 9112-9117.
15. J. L. Hopewell, D. J. T. Hill and P. J. Pomery, *Polymer*, 1998, **39**, 5601 - 5607.