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Injectable oligomer-cross-linked gelatin hydrogels via anhydride-amine-conjugation

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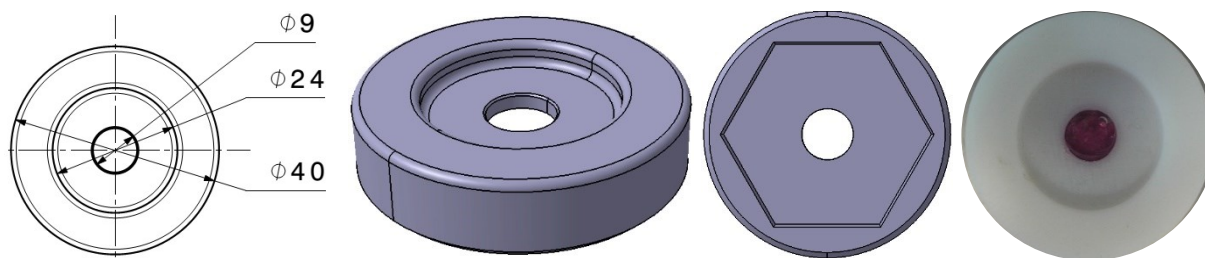
Figure S1: (A) Graphical representation of size and shape of gel confinement that was fixed to the base plate of rheometer (B) Photograph of confinement with hydrogel in the center.
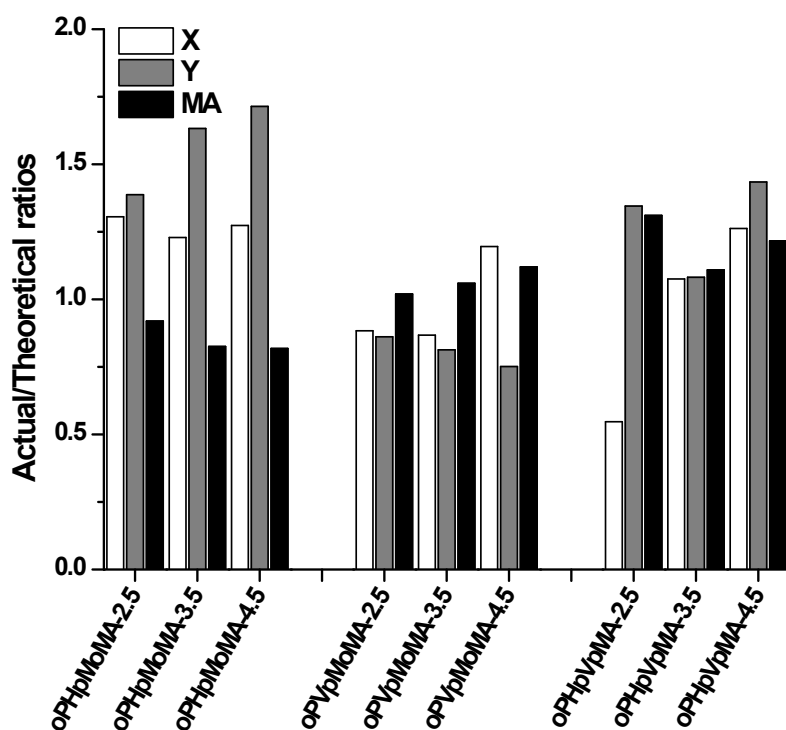

Figure S2: Ratios (actual content as determined by 1H-NMR over theoretical content) of comonomer incorporation of different oPHpMoMA- (X: HPA, Y: AMO), oPVpMoMA- (X: NVP, Y: AMO) and oPHpVpMA-type (X: HPA, Y: NVP) quarter-oligomers.

Table S1: Hansen solubility parameters estimated for hydrophilic comonomers in polymerized form used for quarter-oligomer synthesis. The difference to the corresponding parameter to water (last row) is shown for each monomer in parentheses.

Comonomer	Hansen solubility parameter [MPa$^{1/2}$]		
	δ_d (Δ_{water})	δ_p (Δ_{water})	δ_h (Δ_{water})
HPA	15.4 (0.1)	13.1 (2.9)	10.3 (32.0)
AMO	16.5 (1.0)	5.0 (11.0)	7.5 (34.8)
NVP	18.8 (3.3)	13.4 (2.6)	7.5 (34.8)
Water	15.5	16.0	42.3

Solubility parameters as proposed by Hildebrand and Scott have been used to predict absorption or penetration of blood components on biomaterials and to envisage solubility/miscibility of polymer or co-polymer matrix in composite materials to be applied in biological systems.[1,2] Here we estimated Hansen partial solubility parameters via the group contribution method.[3] Hansen parameters have divided Hildebrand solubility parameters in three components i.e. dispersion force component (δ_d), hydrogen bonding component (δ_h) and polar component (δ_p). The largest differences were found for the energy parameter of hydrogen bonding (δ_h). The energy parameter describing dipolar intermolecular force (δ_p) of HPA and NVP were found more proximal to the corresponding parameter of water than AMO. However, δ_h of AMO and NVP were far from δ_h of water but found very close to one another and could account for strong inter-oligomer hydrogen bonding between these co-monomers. Comparing the parameter describing disperse molecular interactions (δ_d), which is often considered most decisive for solubility and miscibility estimations, it was found that HPA has the strongest affinity to water followed by AMO and NVP.
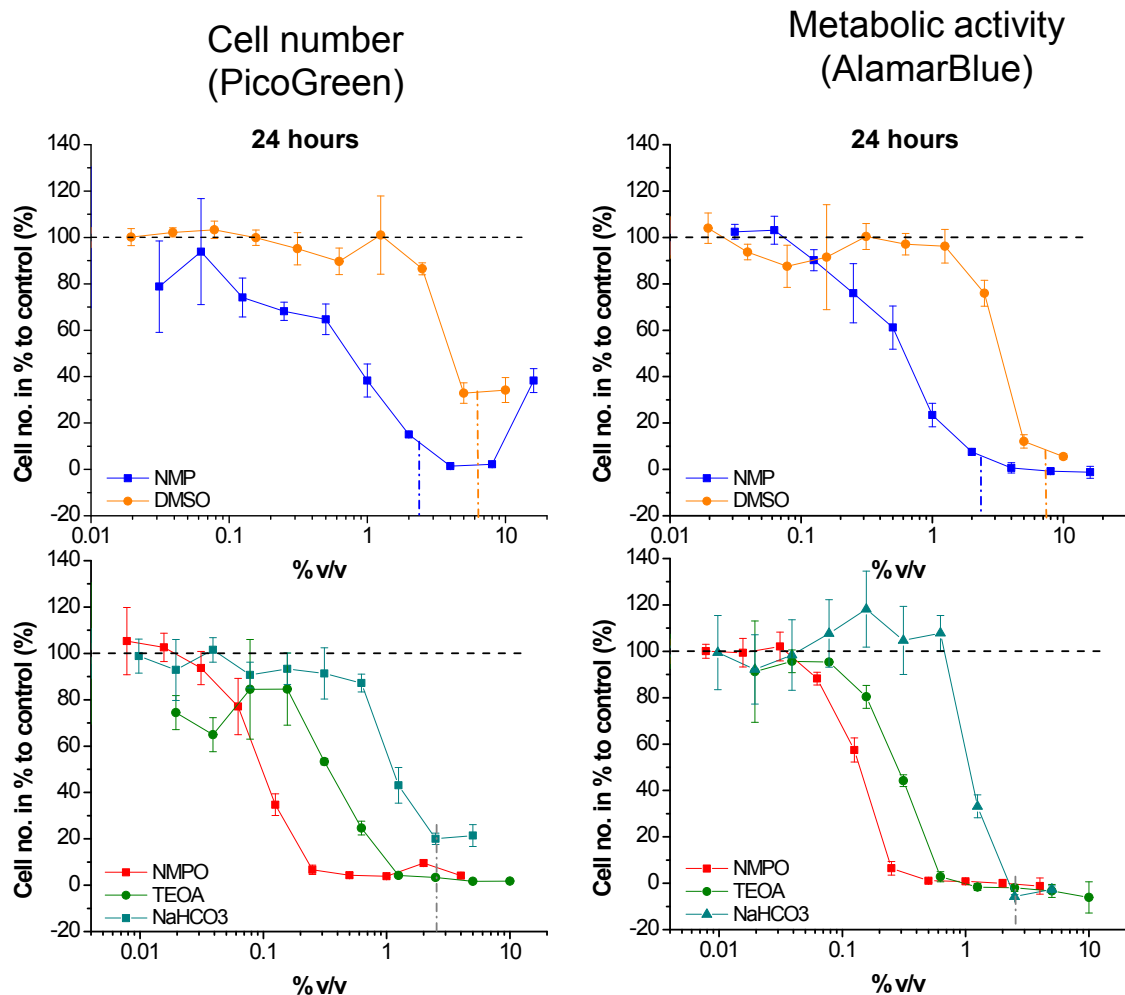
Figure S3: Relative cell numbers (hASCs), determined from DNA content (PicoGreen assay) and relative metabolic cell activity (AlamarBlue assay), after addition of DMEM low glucose augmented with different concentrations of organic solvent (NMP or DMSO) or base molecules (TEOA, NMPO and $NaHCO_3$) for 24 h. Data points represent mean ± standard deviation (n=4). Vertical lines indicate the concentration of NMP, DMSO and TEOA in the final formulation. The cytotoxicity of solvents and bases increased with extended exposure time. We consider the 30 min (as shown in the manuscript) most relevant for formulation development because the conditions will be changed after injection due to dilution and/or extraction of possible toxic substances in the surrounding media. Consequently, it is assumed that the direct contact to these substances in the tested concentration will last not longer than 30 min.
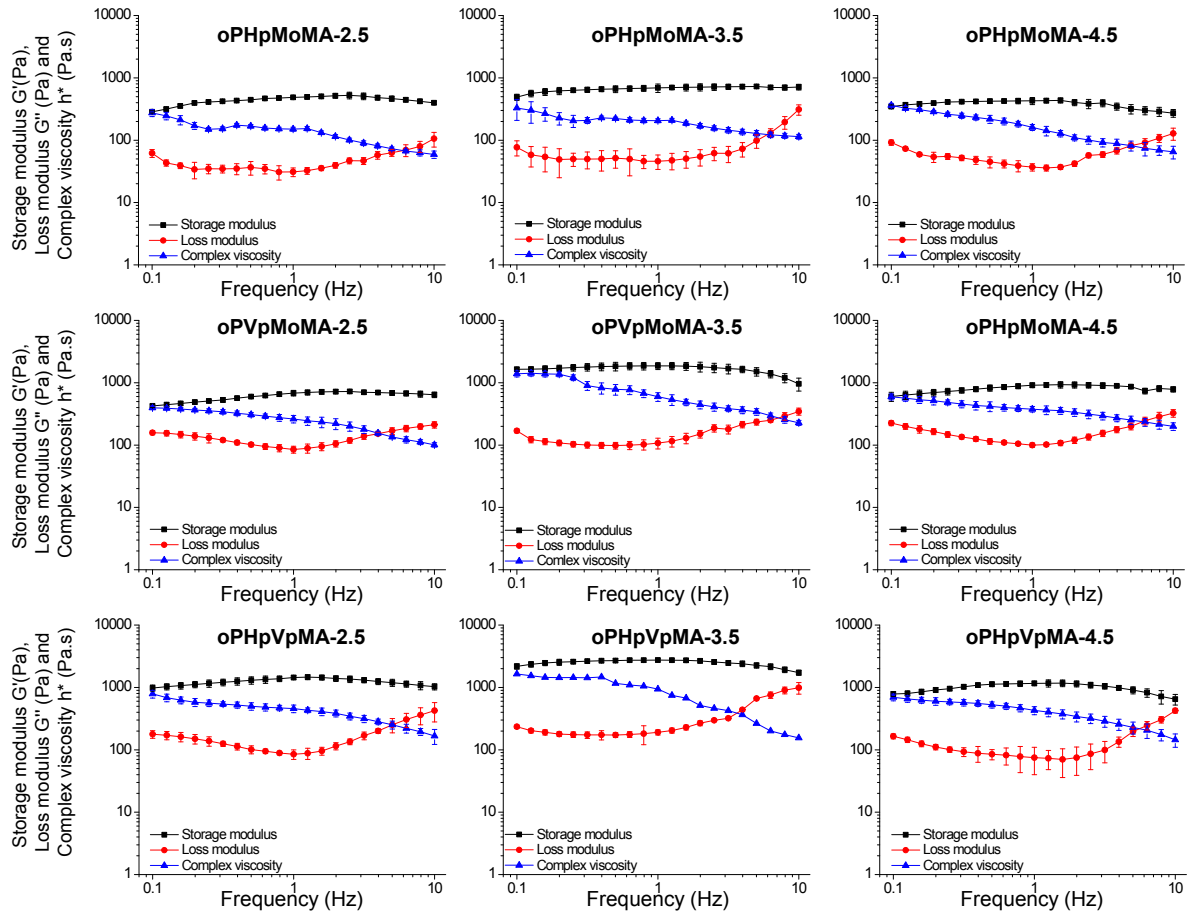
Figure S4: Oscillatory rheology (frequency sweeps) of injected hydrogels for three sets of hydrophilic oligomer (oPHpMoMA, oPVpMoMA and oPHpVpMA) with different MA feeds (2.5, 3.5 and 4.5). Storage moduli exceed loss moduli throughout the investigated frequency range illustrating covalently cross-linked hydrogel networks.
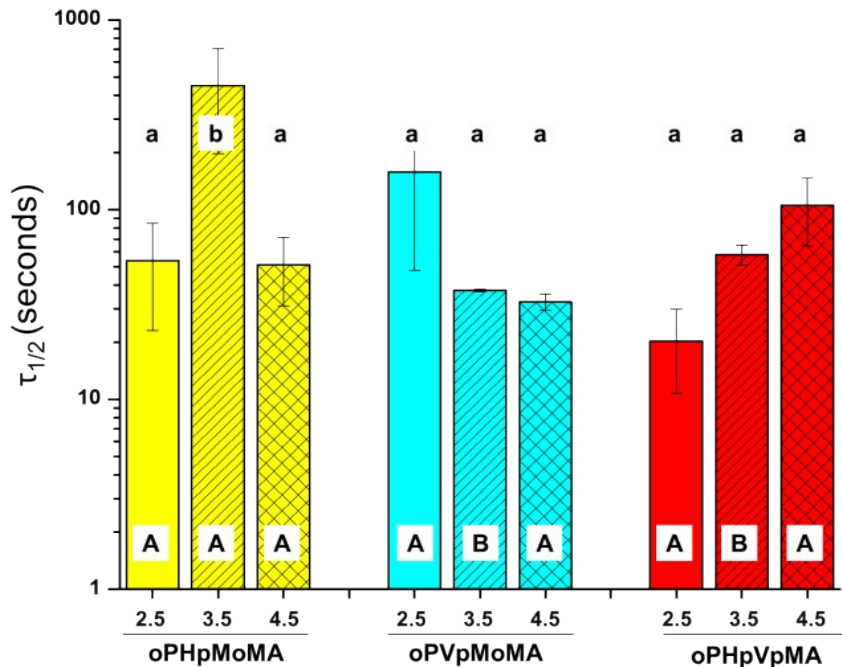
Figure S5: Stress relaxation speeds, expressed as time until stress relaxed to 50% of the initial value, of different two-component oligomer cross-linked gelatine hydrogels. Columns and error bars represent mean ± standard deviation (n=3). Means with different letters were statistically significantly different ($p<0.05$). (Small letters for differences within groups and capital letters for across the groups).

The decrease in shear stress was recorded over time (0-1000 seconds) and reported as time to reach half of the initial stress value ($\tau_{1/2}$). All of the hydrogels exhibit fast stress relaxation with an average $\tau_{1/2}$ below 100 seconds. The relaxation speed was independent of the initial stress values (data not shown). Within the groups, only oPHpMoMA-3.5 showed significant differences in comparison to other oligomer cross-linked hydrogels, while no differences could be found in oPVpMoMA and oPHpVpMA oligomer cross-linked hydrogels. Across the groups, oPHpMoMA-3.5 also showed significant different values as oPVpMoMA-3.5 and oPHpVpMA-3.5.

A video can be found as ESI that shows a reconstruction of a cell-laden oPVpMoMA-3.5-cross-linked gelatine hydrogel at day 7 based on CLSM data. The cells are stained with Calcein-AM. The tick marks on the frame represent a distance of 100 μm.

References

1   C. M. Hansen, Polymer additives and solubility parameters, *Prog. Org. Coat.*, 2004, **51**, 109–112.
2   C. M. Hansen, 50 Years with solubility parameters--past and future, *Prog. Org. Coat.*, 2004, **51**, 77–84.
3   D. W. van Krevelen, *Properties of Polymers: Their Correlation with Chemical Structure; Their Numerical Estimation and Prediction from Additive Group Contributions, Third Completely Revised Edition*, 1997.