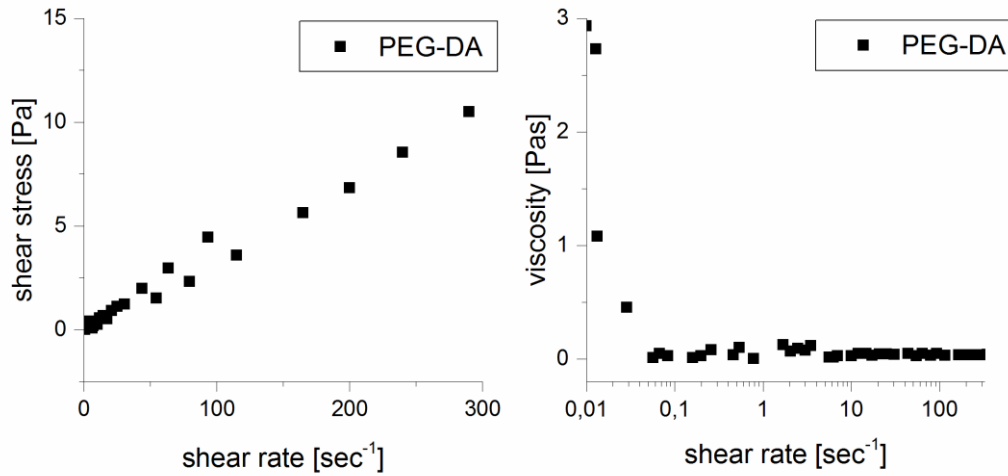


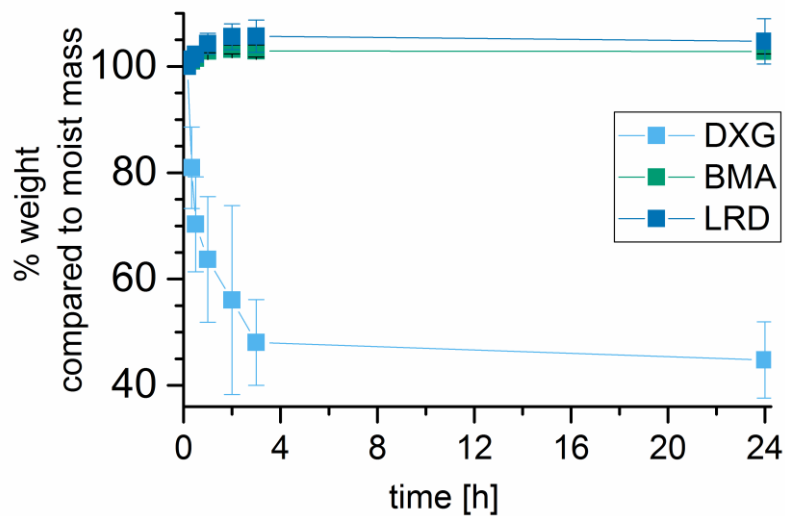
Supporting Information

S 1 Rheology of pure PEG-DA



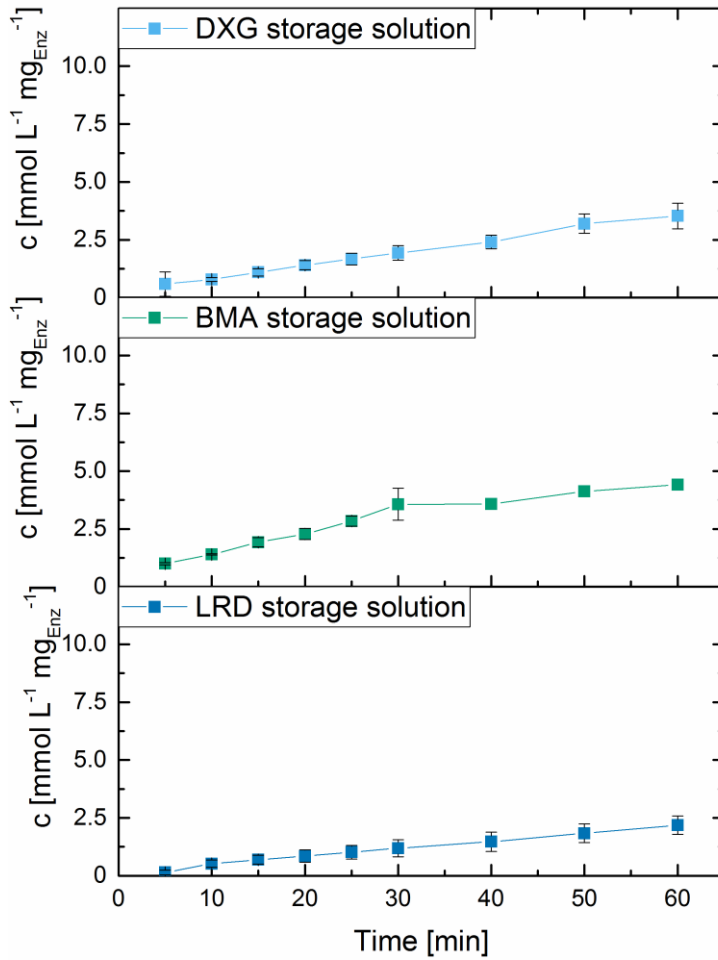
Flow curves of pure PEG-DA were obtained analogous to the flow curves of the hydrogel mixtures as described in section 2.3. Without additives, the average viscosity of PEG-DA is 35 mPa s at shear rates between 150 and 300 s⁻¹ compared to the viscosity range of 1.2 Pa s to 1.9 Pa s of the hydrogel mixtures containing a viscosity enhancing additive.

S 2 Hydrogel swelling in water



Hydrogel lattices were printed ($n = 5$ for each additive) and immersed into 50 mL of water. For the determination of the moist mass the hydrogel lattices were taken out of the water and residual moisture was removed with cleaning wipes (Kimberly-Clark Europe Limited, Rigate, United Kingdom). To have comparable starting conditions, the moist mass after 10 min of immersion in water was equated with 100%. For LRD hydrogels, moist mass is stable after 1 h at 105% of initial moist mass. For BMA hydrogels, a low additive content at the lower limit of printability was chosen to be able to crosslink the material via UV light. The measured mass increase of these BMA hydrogels is about 3%. If a BMA content for optimal printability is used, the crosslinking of PEG-DA is inhibited by the BMA particles. If these structures are immersed into water, the dissolution of the structure will take place within a few minutes. Water uptake and resulting dissolution of filigrane DXG hydrogel stands leads to mass loss of about 50% after 3 h. Part of the loss is due to the high hydration of the material, which leads to slimy properties. When the lattices are dried with cleaning wipes, part of the material is removed.

S 3 Bioreaction kinetics of storage solutions



3D-printed hydrogel lattices were stored overnight in 3 mL of deionized water. To monitor the enzyme release into solution, samples of 1 mL storage solution were mixed with citrate buffer and ONPG. The activity test of leached enzymes with an ONPG concentration of 2.2 mmol/L was done analogue to section 2.6.

S4 Calculation of catalytic efficiency, Thiele modulus and effective diffusion coefficient

For the approximation of the mass transfer limitations occurring during biocatalytic reactions within the hydrogel, the kinetic data of free enzyme in solution (see Figure 4) and enzyme entrapped in hydrogel fragments (small cubes of around 1mm edge length, see Figure 5) are compared for the three hydrogel variants applied. In all cases the educt concentration $c_{0,substrate}$ of ONPG is 2.2 mmol/L. During the initial linear period of the biocatalytic reactions the effectiveness factor (catalytic efficiency) is directly related to the ratio of the specific product formation (amount of product produced per mg enzyme) [1]:

$$\eta_{cat} = \frac{r_{hydrogel\ fragment}}{r_{free\ enzyme}} = \frac{\frac{m_{product,hydrogel\ fragment}}{\text{amount of enzyme within hydrogel}}}{\frac{m_{product,free\ enzyme}}{\text{amount of free enzyme}}}$$

If the batch volume of substrate solution used is the same for the hydrogel and free enzyme experiments, specific product amounts can also be replaced by the respective specific product concentrations shown in Figure 4 and 5.

The effectiveness factor is closely related to the so-called Thiele modulus ϕ [1], describing the ratio between the reaction rate and mass transport due to diffusion in the enzyme carrier.

$$\eta_{cat} = \frac{3}{\phi} \left(\frac{1}{\tanh(\phi)} - \frac{1}{\phi} \right)$$

In case of a Thiele modulus $\phi > 3$ this relation simplifies in good approximation to:

$$\eta_{cat} = \frac{3}{\phi} \quad \text{which can be rearranged to} \quad \phi = \frac{3}{\eta_{cat}}$$

Substrate limited enzymatic reactions can be looked at as first order reactions with a reaction rate constant being linear dependent on the enzyme concentration. For first order reactions the Thiele modulus is defined as:

$$\phi = L \sqrt{\frac{k}{D_{eff}}}$$

If this equation is solved for the effective diffusion coefficient within the hydrogel one gets:

$$D_{eff} = k \cdot \left(\frac{L}{\phi} \right)^2$$

The reaction rate constant of a first order reaction within free solution can be calculated by the achieved degree of conversion X within a specific time t:

$$k = -\frac{\ln(1-X(t))}{t} \quad \text{with} \quad X(t) = \frac{c_{product}(t)}{c_{0,substrate}}$$

When transferring this parameter into the reaction rate constant within the hydrogel, it must be taken into account that the enzyme concentration applied within the hydrogel differs substantially from the one applied in free solution.

$$k_{hydrogel} = \frac{C_{E,hydrogel}}{C_{E,free\ solution}} \cdot k_{free\ solution}$$

In the investigated case, the local enzyme concentration entrapped in the hydrogel is 25 times higher.

The following table summarizes the effectiveness factor, Thiele modulus, reaction rates in free solution and within the hydrogel, and effective diffusion coefficient for all three types of hydrogel investigated.

	η_{cat} [-]	ϕ [-]	$k_{free\ solution}$ [s ⁻¹]	$k_{hydrogel}$ [s ⁻¹]	D_{eff} [m ² /s]
DXG	0.157	19.1	7.28E-5	1.82E-3	5.0E-12
BMA	0.132	22.7	6.08E-5	1.52E-3	2.9E-12
LRD	0.128	23.5	5.90E-5	1.47E-3	2.7E-12

Influence of leached enzyme onto hydrogel activity tests.

It can be assumed that leaching will be strongest for freshly printed hydrogel immersed in storage solution and will decay over time. Still, even if we assume a worst case scenario with a linear leaching rate during storage time of 18-24h, and that the same leaching rate holds after transferring the hydrogel structure into a new solution, the amount of enzyme released during 1h duration of the activity test is only around:

$$\frac{\text{Duration of activity test}}{\text{Duration of storage}} \cdot \text{fraction of enzyme leached during storage} \cdot \text{enzyme amount in hydrogel}$$

$$= \frac{1h}{20h} \cdot 0.077 \cdot 0.1\ mg = 0.00038\ mg$$

Activity of free enzyme is about 6 times higher than the effective activity of entrapped enzyme, however, the applied hydrogel structures contain 0.1 mg of enzyme and therefore much more than the amount leached (see section S3). Therefore, the percental influence of 0.00038 mg of leached enzyme onto the activity measured in LRD hydrogel experiments can be estimated to be:

$$\frac{0.00038\ mg \cdot 6}{0.1\ mg} = 0.023$$

S5 References

- [1] Vannice, M.A., Kinetics of catalytic reactions, Springer, New York, NY 2005.