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Enzymatically-Triggered Jack-in-the-box-Like Hydrogels

Maria Balk1,2, Marc Behl1,2, Ulrich Nöchel1, and Andreas Lendlein1,2,3*

1 Institute of Active Polymers, Helmholtz-Zentrum Geesthacht, Kantstr. 55, 14513 Teltow, Germany

2 Tianjin University–Helmholtz-Zentrum Geesthacht, Joint Laboratory for Biomaterials and Regenerative Medicine, Kantstr. 55, 14513 Teltow, Germany

3 Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Straße 24-25, 14476 Potsdam, Germany

* Prof. Dr. A. Lendlein Corresponding author: andreas.lendlein@hzg.de

Abstract

Enzymes can support the synthesis or degradation of biomacromolecules in natural processes. Here, we demonstrate that enzymes can induce a macroscopic directed movement of microstructured hydrogels following a mechanism that we call a “Jack-in-the-box” effect. The material’s design is based on the formation of internal stresses induced by deformation load on an architectured microscale, which are kinetically frozen by the generation of polyester locking domains, similar to a “Jack-in-the-box” toy (i.e. a compressed spring stabilized by a closed box lid). In order to induce the controlled macroscopic movement, the locking domains are equipped with enzyme-specific cleavable
bonds (i.e. a box with a lock and key system). As a result of enzymatic reaction, a transformed shape is achieved by the release of internal stresses. There is an increase in entropy in combination with a swelling-supported stretching of polymer chains within the microarchitected hydrogel (i.e. the encased clown pops-up with a pre-stressed movement when the box is unlocked). This utilization of an enzyme as physiological stimulus may offer new approaches to create interactive and enzyme-specific materials for different applications such as an optical indicator of enzyme’s presence, or actuators and sensors in biotechnology and in fermentation processes.

Keywords: enzyme, hydrogels, stimuli-sensitive materials, shape-change, poly(ε-caprolactone), switch, microporous

INTRODUCTION

The integration of multiple functions into materials is of technological relevance for enabling and accelerating the development and manufacture of future products.¹ Often these multifunctional systems are inspired by a biological analogue but are designed to perform various technical tasks through prudent combinations of their basic functional capabilities. Various combinations of almost independent functions, such as shape-memory capability or actuation, self-healing, controlled drug-release, degradability or even fluorescence, could be successfully implemented in polymers and hydrogels including.²⁻³ A certain kind of material-based intelligence can be achieved in such systems by sequentially coupling meaning that the response signal of a first function serves as trigger of a second material function. Recent examples are the sequential coupling of pH-sensitivity and actuation or of magnetic heating and actuation.⁴⁻⁵ While the purpose of material degradability is typically related to the removal of a device, which is only temporarily required, we questioned our self, whether degradation or partial degradation could be implemented as a function initiating a
sequence in hydrogels. Such an arrangement of functions could be visualized by a Jack-in-the-box toy, in which the spring-driven pop-up of the pre-stressed clown out of the box (function 2) is controlled by unlocking a lock (function 1). As here the function 1 would act as sensory function whereas the function 2 would be an actuator, we would have transferred the electronic principle of an Sens-Actor into a material.6

Solvent based degradation of polymers demands functional groups, which can be cleaved upon reaction with the solvent and can be catalysed by pH or by enzymes. With the raised interest in preventing environmental pollution by plastics, research in degradable polymers has been intensified.7 However, these functional groups to be cleaved later on need to be integrated into the polymer before. A classical approach to integrate hydrolysable groups in polymers is the ring-opening polymerization (ROP) of lactones and cyclic diesters, which is well known from degradable biomaterials like suture materials. The development of the substitution of organometallic catalysts like tin-octanoate needed for the ROP of lactones with enzymes,8 vice versa also stimulated the investigation of the backward reaction of this equilibrium reaction, the enzymatic degradation of polyesters.9-10 The degradation capability of polyesters has been investigated for various lipases. For poly(ɛ-caprolactone) three kinds of lipase were found to significantly accelerate the degradation: *Rhizopus delemar* lipase,11 *Rhizopus arrhizus* lipase, and *Pseudomonas* lipase.12-13 Especially *Pseudomonas* became a versatile tools as it able to degrade amorphous and crystalline PCL.10 In hydrogels, enzymatic degradation was demonstrated impressively by the development of hydrogels with weak links sensitive to matrix metalloproteinase (MMP).14 However, here the weak links are a part of the polymer network, which cause upon degradation a reduction of covalent netpoints and results in swelling. Because this increase in volume would impede with a coupled function of a shape-shift, this strategy was not considered.

The challenge for our function coupled Jack-in-the-box hydrogels was the incorporation of enzyme-sensitive moieties in a polymer network (enabling a shape transformation in a predefined manner once a specific enzyme is present) and the macroscopic retention of the structure after the directed
movement. Furthermore, this hydrophilic polymer network should be designed in a way to enable simultaneous deformability e.g. by compression and the capability of a directed predetermined movement.

The required mechanical stability can be addressed by a microarchitected system. A minimalistic design consists of Y-shaped profiles interconnecting tridentate star-shaped junctions. In this way, the externally applied forces would be transferred to the inner tridentate junctions resulting in an optimized dissipation of the load. In addition, the microarchitected design would act as a spring to provide sufficient tension as required for the macroscopic movement and would prevent a collapse of the entire system once the enzymatic reaction has occurred. The maximized surface to volume ratio of the microstructured hydrogel facilitates the interaction with the enzyme at the interface. Hydrophobic domains capable of storing the deformation energy should be introduced into the hydrophilic polymer network as enzyme-sensitive moieties able to react with the enzyme, resulting in a directed movement into a transformed shape. Semi-crystalline polyester can act as such enzyme-selective shape-locking elements but need to be introduced as grafted side chains into the microarchitected hydrogel in order to ensure the integrity of the network structure after reaction with enzymes.
Figure 1: Enzymatically-induced shape shifting of Jack gels (swollen state) on different hierarchical levels. Upper part: Schematic representation of the enzymatically-induced shape shifting process from the macroscopic to the molecular mechanism on the nano scale from top to bottom. From left to right: Original shape, locked deformed shape after programming, and transformed shape of Jack-in-the-box gels after addition of the enzyme. Lower part: Potential application as an enzyme-sensitive sensor. Upon presence of enzymes the degradation process starts, which causes actuation and shifts
a magnet in proximity of a Reed-relay. Upon closure of the relay, an electrical circuit is closed and a
light switched on (Supporting Information, Figure S15).

The mechanism of the enzymatically-triggered “Jack-in-the-box” effect of the microarchitected
hydrogels on the micro and nano scale is visualized in Figure 1. In the original shape, the Jack gels
consist of hydrophilic main chain segments and hydrophobic side chains, which, in an aqueous
environment, are capable of aggregating to form crystalline fractions acting as locking domains. On
the nano-scale the polymer chains of the Jack gels exhibit a random coil formation, which is slightly
stretched by swelling in water. The elasticity of the polymer chain segments at a temperature, at
which the locking domains are dissociated, enables deformation of the hydrogel by compression or
bending. This deformation results in orientation of the polymer chain segments in the direction of
deforation. On the microlevel, the deformation of the system will cause an elastic buckling and
folding of polymer walls (Figure 1) and differently directed tensile forces will be transmitted
dependent on the geometric locus, which would control the orientation of main and side chains. This
state of lower entropy can be kinetically frozen by decreasing the temperature to induce the
formation of locking domains through recrystallization of side chains. Once the enzyme is added, this
frozen state is unlocked by cleavage or fragmentation of the locking domains and the enzymatically-
induced directed movement can be initiated. An interconnected microarchitecture is required to
enable the diffusion of the enzyme through the porous matrix. However, the enzyme’s access to the
crystalline locking domains is directed by the polymer network from the surface towards the inner
part of the polymer braces.13, 15-18 Once a sufficient number of locking domains are cleaved the
directed movement to the enzymatically-transformed shape is initiated by the rearrangement of
polymer main chains towards their initial random coil formation. This reduction of locking domains
acting as additional physical net-points increases the mesh size of the polymer network and enables
additional local water uptake in the braces. The local increase in swelling capacity may facilitate the
access of the lipase to polyester segments by the soaking effect as well as by the increase in mesh size, and accelerates the directed movement by supporting the re-positioning of deformed polymer walls. The continuous procedure of enzymatic ester bond cleavage, increase of mesh size, and local increase in swelling capacity results in the transformed shape. The overall integrity of the polymer network architecture is ensured by integrating the polyester segments forming the locking domains as grafted side chains onto the hydrophilic polymer network, the outer dimension shall be kept by the microarchitectured design.

A potential application for these Jack hydrogels could be a sensor in a laundry setup as lipases are a common component of laundry detergents. Assuming similar reactivity between the Jack hydrogels and the cleavable groups of the dirty laundry, the sensor would give a signal once a certain number of hydrolysable groups has been cleaved. In this way, the laundry process would not be controlled by time of the laundry automat but would be adaptive to the dirtiness of the laundry. Of course the sensor would be of one time usage, but that is the detergent too. The potential leakage of cleaved locking domains could be neglected, as these would be rinsed away with the laundry solvent.

Our concept for these so called “Jack-in-the-box-like hydrogels” (Jack gels) with a microarchitectured structure is the application of a template technique using condensed spheres resulting in constructions with Y-shaped braces and tridentate star-shaped junctions.\(^{19,20}\) The polymer network is based on hydrophilic main chains acting as swelling segments, as well as hydrophobic oligo(ɛ-caprolactone) (OCL) side chains which generate locking domains\(^ {21,22} \) and provide enzyme-sensitivity.\(^ {12,23} \) The original shape could be deformed on the macroscopic and microscopic level by the application of heat. Shape shifting of Jack gels was initiated by LPF, enabling an optical detection of the enzyme’s presence and resulted in the macroscopic original shape (denominated as the ‘transformed shape’) via modification of the molecular structure.

**RESULTS AND DISCUSSION**
The microarchitected design of the hydrogels was realized by a template from hexagonal close packed and condensed polyethylene microspheres (PE, \(d = 50 \pm 5 \, \mu m\)) (Figure 2a). The free volume of the template was filled by a polymer network based on \(N\)-vinyl pyrrolidone (NVP) as hydrophilic main chain forming component,\(^{24}\) oligo(ethylene glycol)divinyl ether (OEGDVE, \(M_n = 250 \, g \cdot mol^{-1}\)) as crosslinker, and monomethacrylated OCL (OCL-MA, \(M_n = 4100 \, g \cdot mol^{-1}\)) as side chains, which provided locking domains.

**Figure 2:** Design of microarchitected hydrogels. Light microscopy measurements of a) fused microsphere template and b) hydrogels with 15 wt% OCL in the swelling equilibrium in water. c) Scanning electron microscope images of Jack gels (freeze-dried state) with 15 wt% OCL.

In the swollen state in water (Figure 2b) the Jack gels provided homogeneous and isotropic round pores with pore diameters (\(d_{\text{pore}}\)) ranging from 73 \(\mu m\) to 81 \(\mu m\) and polymer walls with thicknesses (\(\sigma_{\text{wall}}\)) around 6 \(\pm 4 \, \mu m\) (Table S1). Once freeze-dried, the microarchitected structure consisting of the tridentate star-shaped junctions created during crosslinking of the polymer matrix in the
tetrahedral and octahedral sites of the hexagonally close-packed PE spheres and thin polymer walls of the Y-profile braces linking the junctions became apparent. The resulting hexagonal pore geometry was repeated throughout the whole polymer network. In addition, the walls featured voids ($d = 27 \pm 3 \mu m$) interconnecting the pores of the microarchitected design (Figure 2c). The deformation capability of the Jack gels in strain is with ~23 ± 4%, e.g., OCL(15)NVP is rather low (Figure S2, Supporting Information). Based on the bulk materials elasticity and the morphology, the number of the locking domains in a 1 cm³ cube was estimated as $5.1188 \times 10^{18}$ (see Supporting Information section 3, Figure S1). At a temperature when the locking domains are solidified, the samples are rather brittle and the deformation capability cannot be determined properly. However, the hydrogels could be deformed by compression at 60 °C. Similar to a hammer beam truss, the polymer skeleton was capable of distributing the applied deformation load uniformly by concentration of the external forces on the tridentate star-shaped junctions.

On the nano-scale, the polymer chains are stretched into random coil conformation, whereas on the microscale a remarkable increase of wall thicknesses $\sigma_{\text{wall}}$ from 6 ± 4 $\mu m$ (original shape) to thicknesses between 13 ± 6 $\mu m$ and 19 ± 9 $\mu m$ (transformed shape, dependent on OCL wt%) (Table S1) occurs. However, the macroscopic shape is maintained by the microarchitecture design. In orienting pre-experiments the suitability of the OCL chain segments to act as locking domains, which can be degraded by enzymes was explored. Degradation experiments with four different enzymes (lipase from Pseudomonas fluorescens, lipoprotein lipase from Pseudomonas fluorescens, lipoprotein lipase from bovine milk, and porcine pancreas lipase) at three different concentrations (30 U·mL⁻¹, 10 U·mL⁻¹, and 0.2 U·mL⁻¹) were performed in order to determine optimum conditions for the OCL cleavage. The highest degradation rate was obtained with the lipase from Pseudomonas fluorescens in a concentration of 30 U·mL⁻¹, which was selected for all subsequent degradation experiments. In an the nitrophenyl palmitate assay²⁵ the activity of the enzyme was studied. Within 7 days the calculated relative enzyme activity of the lipase from Pseudomonas fluorescens decreased to 81 ± 2%. Accordingly, in the enzymatic experiments of OCL based hydrogels the medium was
exchanged after 3 days to ensure 90% of the initial enzyme activity (Supporting Information Figure S3).

**Figure 3:** Enzymatically-triggered shape shifting of a Jack gel with 15 wt% OCL on the macro scale (swollen state) and on the micro scale (scanning electron microscope images of the freeze-dried state). a) Original shape of a Jack gel. b) This locked deformed shape was created by heating to 60 °C, folding into a face-like shape, and cooling to 5 °C. c) Transformed shape of the hydrogel after 24 h of treatment with LPF enzyme.

The interplay between the microarchitectured structure and the enzymatically-induced shape shifting process of the Jack gels on the macro scale is presented in Figure 3. The characteristic microarchitecture of the Jack gels enables macroscopic deformation of the original shape to the
deformed shape, which is subsequently locked (Figure 3b). In our demonstration, a deformed shape similar to a smiley face was created from the original shape. On the micro scale, the mechanically supported stability required was achieved by the isotropic geometry of the system as well as the tridentate star-shaped junctions and braces of the polymer skeleton. The deformation energy was dissipated into the inner structure and was stored in compressed pores, resulting in elastic buckling and folding of polymer walls. When the enzyme LPF was added to the deformed shape in its locked state, the enzymatically-induced cleavage of the locking domains initiated a shape transformation, similar to an optical indicator (Figure 3c). On the macroscopic scale, the transformed shape was comparable in shape and volume with the original Jack gel. The increase in thickness of the polymer braces reduced the influence of swelling on the material volume. In addition, the transformed shape is translucent whereas the original shape is opaque, indicating a change of light scattering resulting from the disappearance of hydrophobic domains. The mechanical stability at the macro scale was maintained, even after this enzymatically-induced shape shift, by the unique architecture on the microscopic scale, which prevented the collapse of the Jack gels. Hence, the function of the microarchitectured structure is indispensable in obtaining the enzymatically-induced directed movement in hydrogels. A reduction in size of the polymer braces was observed in the freeze-dried state and was accompanied by a decrease in the overall sample mass attributed to the cleavage and extraction of locking domains. Furthermore, the sizes of the interconnecting voids increased from 27 ± 3 μm (original shape) to 50 ± 4 μm (transformed shape) and the roughness of the Y-shaped braces increased. (Figure S4). By 1H-NMR analysis it could be noted that almost all PCL moieties were cleaved (Supporting Information, Figures S5-S9). This was confirmed by the analysis of the leached compounds, where also PCL and the slight amount of NVP was found (Figure S10).

In order to quantify the enzymatically-induced directed movement between the locked deformed shape and the transformed shape, the volumetric degree of swelling (Q), melting enthalpy (ΔHm), crystallinity (Xc), relative mass loss (mrel), as well as the efficiency of directed movement i.e. the transformation ratio (Rt), were investigated. As an example, Figure 4 shows a Jack gel with 15 wt%
OCL in the presence of the enzyme LPF and a control experiment without addition of enzyme. In the presence of LPF, the mass of the Jack gel decreased by about $3 \pm 2$ wt% within the first three hours of the shape shifting experiment and reached a plateau about $m_{re} = 13 \pm 4$ wt% within nine hours, which was attributed to complete cleavage and extraction of locking domains. Simultaneously, the changes in swelling capacity were confirmed by light microscopy measurements (increase in thickness of polymer braces), in which $Q$ increased from $1300 \pm 200$ vol% to $2400 \pm 700$ vol% ($t = 15$ hours). The loss of the crystalline locking domains was also reflected by the decrease in $\Delta H_m$ and $X_c$. The enzymatically-induced shape shift from the deformed shape to the transformed shape was almost completed within 21 h with $R_t = 98 \pm 2\%$. With respect to the number of estimated locking domains of $5.1188 \times 10^{18}$ or $3.8391 \times 10^{18}$ for the selected sample dimension and the enzyme activity of 30 U ml$^{-1}$ (1200 U) there is an excess of $\sim 188$ enzymes per locking domain. However, here it needs to be considered that the access of enzymes to the crystalline locking domains can be blocked by the hydrophilic polymer chain segments of the covalent polymer network and just can be released by ongoing degradation.
Figure 4: Investigation of enzymatically-induced shape shifting kinetics of Jack gels with 15 wt% OCL and changes of material properties during transformation. Number of samples per data point n = 3.
a) Transformation ratio (Rₜ), b) crystallinity (Xₜ), c) melting enthalpy (ΔHₘ), d) volumetric degree of swelling (Q), and e) relative mass loss (mₑ). Shape shifting in presence of enzyme (■) and control experiment in buffer solution without enzyme (□).

The initial content of the polyester, which was integrated into the Jack gels, affected the Rₜ of the enzymatically-induced shape shift (Figure S11). While hydrogels with a polyester content ≤ 26 wt% exhibited excellent Rₜ values above 96%, attributed to a complete cleavage of locking domains, a transformation < 50% was detected for the hydrogel with 31 wt% of polyester side chains. Here, the
incomplete cleavage and extraction of polyester segments was also reflected by determining $m_{re}$ (Table S2).

The shape shifting of Jack gels presented was induced by the enzyme LPS. In a control experiment in the absence of the enzyme, a directed movement of the locked deformed shape could be excluded as the hydrogel exhibited shape stability with $R_1 = 0\%$ for 24 hours (Video S1). Furthermore, in the control experiment constant material properties for $m_{re}$, $Q$, $\Delta H_m$, and $\chi_c$ were detected (Figure 4). A microarchitectured structure of hydrogels was a necessity for the enzymatically-induced transformation. Experiments on non-structured hydrogels exhibited an enzymatic cleavage of polyester domains only on the polymer surface (Figure S12). In addition, the enzymatically-triggered transformation, which was achieved with LPS, was not observed when enzymes such as porcine pancreas lipase or lipoprotein lipase from bovine milk (Figure S13) were used, reflecting the specificity of the designed Jack gels to a bacteria-derived enzyme. In another control experiment (Supporting Information Figure S14, Table S3), we used hydrogels with randomly shaped pores obtained by a combination of salt leaching and blowing agent. The hydrogels created by salt leaching only, did not show degradation with 21 days, the hydrogels created by the combination of salt leaching and blowing agent required 5 days until recovery reached equilibrium.

CONCLUSION

In conclusion, hydrophobic side chains, which are able to create locking domains integrated in microarchitectured hydrogels enabled an enzymatically-induced transformation of locked shapes in a swollen system for the first time. The shape shift was achieved by entropic elastic recovery as result of enzymatic cleavage of locking domains, swelling-induced re-positioning of buckled polymer walls, and the pronounced mechanical stability on the micro scale preventing collapse of the hydrogels.

This work provides a new direction to design directed movements in hydrogels acting as optical indicators for the presence of LPF and the applied strategy can be extended to generate a variety of
enzyme-sensitive hydrogels capable of undergoing shape transformations. The presence of enzymes, as a novel stimulus, can offer new approaches to meet the requirements of different applications such as actuators and sensors in biotechnology and in fermentation processes (Supporting Information Figure S15).

**EXPERIMENTAL PROCEDURES**

**Synthesis of Jack Gels:**

The microstructured template was obtained by hexagonal close packing of PE spheres in THF by ultrasonic vibration for 5 min, removal of THF, and subsequent fusion of the spheres by heating to 100 °C for 1 h. A mixture of the starting materials OCL-MA, NVP, the crosslinker OEGDVE (3 mol% in relation to co-monomers), and the thermal initiator AIBN were added to the PE template and crosslinked at 60 °C under vacuum for 24 h. The PE template was removed by extraction with toluene at 120 °C. Hydrogels were obtained by stepwise exchange of toluene with THF and afterwards THF with water.

**Enzymatically-Induced Shape Shifting Experiment of Jack Gels:**

For the deformation and locking of hydrogels: hydrogels (dimension of strip: 5 x 0.5 x 0.3 cm) were bent in water at 60 °C and cooled to 5 °C whereby the deformation was maintained. The locked deformed shape of the hydrogels was obtained after release of the deformation stress and transfer of the hydrogel to buffer solution (phosphate-buffer, 0.1 M, pH = 7.4) of 37 °C.

Enzymatic shape shifting experiment: stock solution of lipase from *Pseudomonas fluorescence* (1200 U·mL⁻¹) was added to the buffer solution containing the locked deformed hydrogel until a concentration of 30 U·mL⁻¹ was reached. The shape shifting was recorded with a video camera, from the video the angles between the ends of the strip were quantified. The transformation ratio $R_t$ as function of time was calculated as follows: $R_t = (\theta_\delta - \theta_t)/(\theta_\delta)$, where $\theta_\delta$ is the angle between the two segments of the folded strip of the locked deformed shape and $\theta_t$ is the angle between the strip ends.
of the transformed shape. As control, the locked deformed hydrogel was transferred to a buffer solution in absence of the enzyme.

ASSOCIATED CONTENT

Supporting Information. Materials and methods, the synthesis of OCL-MA, estimation of locking domains, and supporting figures and tables that include additional data (PDF). Video of the hydrogel exhibiting enzyme-induced transformation with $R_t = 98 \pm 2\%$ within 21 h (left) and shape stability with $R_t = 0\%$ for 24 h (right) when no enzyme was added. Increase of $T > T_{m,OCL}$ could also initiate shape transformation for the latter (MP4).

AUTHOR INFORMATION

Notes

A.L. and M.B. are co-inventors on patents in the field of polymer-based shape-memory materials.

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