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A multifunctional multimaterial system for on-demand protein release

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ABSTRACT

In order to provide best control of the regeneration process for each individual patient, the release of protein drugs administered during surgery may need to be timely adapted and/or delayed according to the progress of healing/regeneration. This study aims to establish a multifunctional implant system for a local on-demand release, which is applicable for various types of proteins. It was hypothesized that a tubular multimaterial container kit, which hosts the protein of interest as a solution or gel formulation, would enable on-demand release if equipped with the capacity of diameter reduction upon external stimulation. Using devices from poly(ε-caprolactone) networks, it could be demonstrated that a shape-memory effect activated by heat or NIR light enabled on-demand tube shrinkage. The decrease of diameter of these shape-memory tubes (SMT) allowed expelling the payload as demonstrated for several proteins including SDF-1α, a therapeutically relevant chemotactic protein, to achieve e.g. continuous release with a triggered add-on dosing (open tube) or an on-demand onset of bolus or sustained release (sealed tube). Considering the clinical relevance of protein factors in (stem) cell attraction to lesions and

the progress in monitoring biomarkers in body fluids, such on-demand release systems may be further explored e.g. in heart, nerve, or bone regeneration in the future.

Keywords: Shape-memory polymer, On-demand release, Proteins, Poly(ε-caprolactone) networks, Near infrared light triggered shape-recovery

1. Introduction

The controlled release of therapeutic proteins holds great promise for treatment of a variety of diseases and syndromes. However, short *in-vivo* half-lives of circulating proteins [1] and their easy degradation before reaching the targeted sites are still strongly limiting their clinical application. Additionally, a local delivery is typically advantageous to promote tissue formation in Regenerative Therapies, e.g. in heart muscle regeneration, nerve regeneration, or the treatment of critically sized bone defects, where relevant growth factors are known but may be of limited efficacy when administered systemically [2] [3]. Accordingly, container-like drug delivery systems may be useful, which could be placed in the diseased tissue during medical intervention and would provide a local factor release. Furthermore, in order to provide best control over the regeneration process for each individual patient, the release of predefined and mandatorily required factors may need to be timely adapted to the progress of healing/regeneration, i.e. delayed and/or pulsed as recently emphasized [4]. In some cases also blood markers are known, based on which the preferred time point of a bolus release may be determined [5].

So far, a number of responsive polymeric delivery systems have been suggested in order to control the rate of drug release on-demand. Exemplarily, physical changes of a polymer can be

applied. For instance, a polymer composite membrane filled with poly(*N*-isopropylacrylamide)-based nanogels opens its pores upon inductive heating in a magnetic field by nanogel collapse [6]. Additionally, the heating of polymeric drug carriers might be applied to interfere with protein-polymer interaction in hydrogels [7] or to enable enhanced diffusivity above the polymers glass transition temperature [8]. Furthermore, chemical alterations of matrix materials such as UV or NIR light-induced polymer network degradation were reported to enhance the release of entrapped molecules from particulate carriers [9] [10]. Finally, modular microchip based devices with internal battery and wireless transmission have been clinically evaluated, which provide a multitude of release opportunities but have a complex design, do not fit all application sites, and may require an additional intervention for removal after exhaustion [5].

This study aims to establish a multifunctional carrier system for a local on-demand release, which should be suitable for loading with different types of proteins in an easy and flexible manner and does not demand complex electronic interfaces. The system should give the opportunity for initially either continuous or no release, thus providing the capability of a trigger-induced add-on dosing or a delayed initiation of protein release, respectively. Thus, this system would provide a technology for those cases, where local factor delivery is mandatory while the optimal time point can only be defined during the course of regeneration.

It was hypothesized that a sealed tubular container kit, which hosts the protein of interest as a solution or gel formulation, would enable an on-demand release if equipped with the capacity of diameter reduction upon external stimulation. This shrinkage should facilitate to drain and expose the payload of the shape-memory tubes (SMT) to its environment (Fig. 1). The concept was based on a multimaterial system consisting of (1) a tube-like container made from a shape-

memory polymer to provide the shrinkage function, (2) a thermosensitive hydrogel filling to serve as diffusion barrier for incorporated proteins and to support the translation of SMT switching forces into coating breakage, and (3) a coating to seal the openings of the tube for payload protection, prevent premature leakage, and allow realizing the delayed release option. Optionally, (4) NIR dyes may be incorporated in the shape-memory polymer tube (SMT) to enable contact free activation. In this multifunctional device, a sequential coupling of functions will be realized, i.e. NIR light absorption resulting in local heating, shape switching of SMT and viscosity reduction of the gel, and eventually drug release.

Generally, tube-shaped reservoirs allow realizing a sustained diffusion of compounds from their lumen, with diffusion rates being determined by the orifice size of the tube [11, 12]. Poly(ε-caprolactone) (PCL) networks synthesized by photocrosslinking of oligo(ε-caprolactone) (oCL) precursors were selected as matrix material for SMT, as they can be programmed to exhibit a spatially-directed mechanical actuation by the shape-memory effect (SME) and typically exhibit high recovery rates [13] [14]. To seal the orifices of the SMT, a single-dip coating should be employed. By using a PCL melt without photoirradiation and a minimum exposure time at the orifices only, potential detrimental effects on protein integrity may be excluded. As the gel filling, a thermosensitive degradable triblock copolymer, poly[(*D,L*-lactic acid-*co*-glycolic acid)-*b*-polyethylene glycol-*b*-poly(*D,L*-lactic acid-*co*-glycolic acid)] (PLGA–PEG–PLGA), was selected as it may be filled into the SMT in the sol state below body temperature, gels at physiological conditions, and acts as a diffusion barrier for drug release [15] [16]. In addition to direct heat exposure, the capacity for NIR light-induced shape switching should be explored by incorporating a NIR absorber dye in the SMT wall.

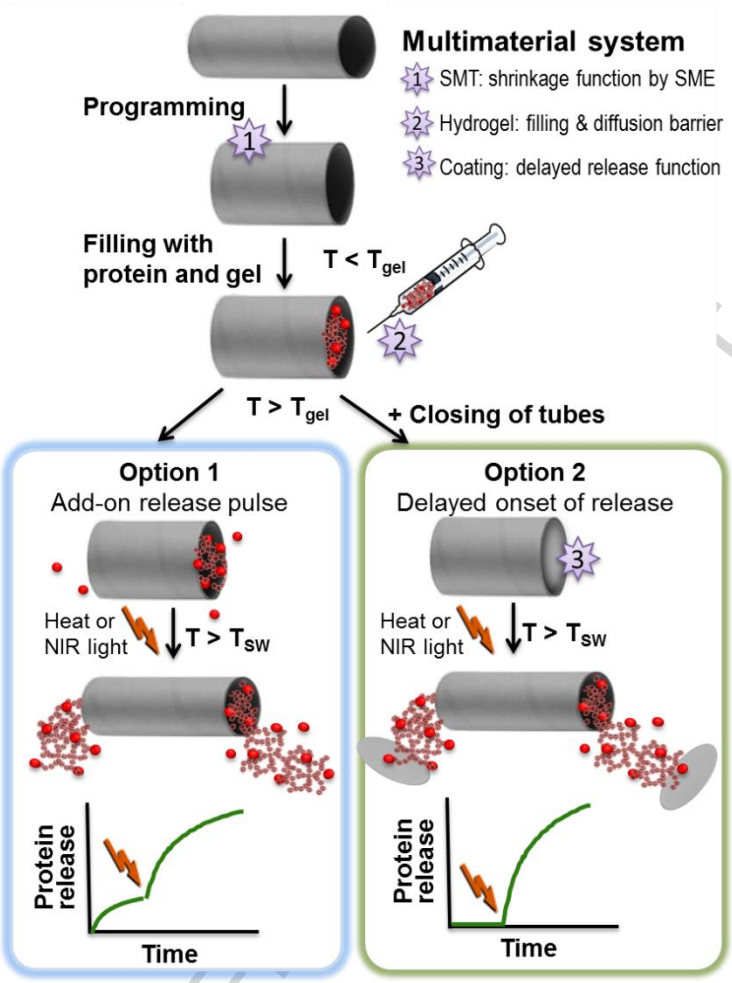

Fig. 1: Schematic illustration of the principle of multimaterial implants for on-demand protein release by shape switch of the container system. Left: Option 1 for continuous release with a SME-induced add-on release pulse. Right: Option 2 with a delayed onset of release initiated on-demand.

2. Material and Methods

A detailed description of materials and methods can be found in the Supporting Information. Briefly, four-armed star-shaped oligo(ε-caprolactone) (^{4}oCL-OH) was prepared by ring opening polymerization of CL in dry argon atmosphere using pentaerythritol as initiator. The ^{4}oCL-OH

was subsequently functionalized with 2-isocyanatoethylmethacrylate to obtain ^{4}oCL-IEMA network precursors (number average molecular weight $M_{n,\,NMR}$ = 12 kDa, T_m = 53 °C, degree of IEMA functionalization 100%; see Supp. Info. Table S1). A PLGA-PEG-PLGA triblock copolymer (as hydrogel matrix) was synthesized as described before [17] (see Supp. Info.).

In model studies conducted to define preparation conditions, films were synthesized by crosslinking 20 or 40 wt.% solutions of ^{4}oCL-IEMA in either ethyl acetate (EA) or chloroform (TCM). The reaction was performed without photoinitiator by UV irradiation (XeCl Excimer lamp, 308 nm; 4 cm distance with 26 mW·cm^{-2}) of precursor solutions in a rectangular mold with quartz glass cover for up to 20 min. Residual stress in dried network samples (N-^{4}oCL) was removed by melting and cooling.

SMT were synthesized from 40 wt.% ^{4}oCL in EA by 10 min irradiation in a mold consisting of glass capillaries (3.0 mm outer diameter (OD), 2.4 mm inner diameter (ID); Hilgenberg, Germany) centered around a needle (1.0 mm OD). The SMT were purified by extraction in excess EA and dried, yielding dimensions of OD 1.65 mm, ID 0.75 mm, and a length typically cut to 2.5 cm. The SMT were programmed to a temporary shape with expanded ID by introducing a needle (OD = 1.2 mm) in SMT at 50 °C in a water bath with subsequent cooling to 5 °C for shape fixation. In some cases, the SMT were swollen in a solution of IR-26 dye (Radiant Dyes Laser & Accessories GmbH, Germany) in dichloromethane (DCM) before programming to enable NIR-light absorption for a contact-free heating.

The $M_{n,GPC}$ and polydispersity *PD* of the synthesized materials were determined in TCM on two linear M-columns 300 x 8.0 mm (Polymer Standards GmbH, Germany) by multidetector gel

permeation chromatography (GPC) with universal calibration. The hydroxyl-number was determined by titration and used to calculate $M_{n,OH}$. The degree of functionalization (Methacrylation, D_f) and $M_{n,NMR}$ was determined by ^{1}H-NMR analysis (Avance 500 MHz, Bruker, Germany) in $CDCl_3$. Fourier transform infrared spectroscopy (FTIR, Nicolet 6700 spectrometer, Thermo Scientific, USA) and swelling studies in DCM with discs (diameter 1.24 ± 0.01 cm) punched from polymer network films provided information on the crosslinked polymer network structure. Thermal properties were determined by differential scanning calorimetry (DSC, Netzsch DSC 204 F1, Netzsch, Germany) in the 2nd heating run at a heating rate of 10 K·min^{-1}. PLGA-PEG-PLGA gelation was characterized by both a tube tilting test after heating in a water bath and a plate-plate rheometer setup (Physica MCR 301 Anton Paar, Germany; plate diameter 25 mm; initial distance 500 µm) in oscillatory mode in the linear regime of deformation.

The shape-switching function was investigated for full tubes in warm water as well as for cross-sections on a heating plate using digital microscopy (VW 6000E, Keyence, Germany) or an infrared camera (VarioCAM HiRes, InfraTec GmbH, Germany). A custom-made microscopy-NIR illumination instrument (Bias GmbH, Germany) containing a pulsed NIR-laser (Minilite I Nd: YAG, Continuum, USA; 1064 nm, 50 mJ, 15 Hz, pulse width 3-7 ns), a microscope (Olympus BX53, Japan) and a heating stage at 37 °C was used to explore shape switching for IR-26 loaded samples.

For protein loading, SMT were filled with cold solutions of 30 wt.% of PLGA-PEG-PLGA and typically 1% (w/v) of the protein of interest in Dulbecco's phosphate buffered saline (DPBS, Invitrogen). To enable an on-demand onset of release, the ends of the SMT were occluded by

shortly dipping the orifice in ^{4}oCL-OH molten on a 60 °C heating plate. Release studies were conducted in Eppendorf tubes with 2 mL DPBS at 37 °C in a horizontal shaker with a sampling volume of 1 ml (Certomat IS, Sartorius BBI Systems GmbH, Melsungen, Germany). To apply a temperature stimulus, SMTs were transferred into new Eppendorf tubes immersed in a water bath adjusted to 49 °C. Proteins were quantified by the Micro BCA assay (Pierce, Rockford, IL) or an ELISA for SDF-1α (R&D Systems).

3. Results and Discussions

3.1. Design of multifunctional multimaterial system

In order to construct a PCL container system with temperature-induced shape-switching functionality, a number of requirements had to be fulfilled. This includes a material with a network structure, a thermal transition temperature T_{trans} above body temperature, and the opportunity for NIR dye incorporation without interference with the shape-switching capability. Furthermore, an experimental procedure for network synthesis in the shape of tubes and a thermomechanical programming process to implement the SME were needed. As the wall thickness of the tube is on the one hand directly correlating with recovery forces demanded to expel the hydrogel, but on the other hand is inversely proportional to the internal volume available for protein loading, the ratio of matrix material volume and internal lumen should here be fixed at ~1.5:1.

PCL networks were synthesized from 12 kDa star-shaped methacrylate functionalized precursors (^{4}oCL-IEMA) by photo-induced (308 nm) initiator-free radical polymerization of the methacrylate moieties. This resulted in a material with covalent crosslinks that serve as permanent netpoints defining the permanent shape of the switchable device. The existence of a

covalent network structure also enabled subsequent swelling in organic solutions, as reported below for incorporating a dye as NIR absorber. Additionally, by its semi-crystalline morphology, crystalline domains with a melting temperature T_m above body temperature (Table 1) were present as needed to fix the temporary shape and enable the SME, which will be reported in the subsequent sections. Other than SMP with a homogeneous morphology and amorphous switching domains, which may be prone to plasticization, crystalline domains of PCL are typically not affected by aqueous environment in the time until delayed release should be triggered.

To define synthesis conditions, model reactions with films were conducted to study the impact of process parameters on network properties. The irradiation time (t_{UV}, 10 or 20 min), the mass fractions of the precursor in solution (ω_{wt}, 20 or 40 wt.%), and the effect of the solvent (ethyl acetate (EA) or chloroform (TCM)) were explored. Both solvents provided materials with high gel contents G in swelling studies in dichloromethane (DCM), i.e. a quantitative incorporation of oligomeric telechels in the network structure, when 40 wt.% precursor and 10 min of photoirradiation was used (Table 1). Therefore, ethyl acetate was eventually selected for SMT synthesis as it is rated as less toxic according to ICH regulations. The covalent crosslinking had no detrimental effect on the formation of the crystalline domains, which exhibited a T_m above body temperature as desired. Compared to films, the synthesized SMT showed slightly reduced G possibly due to a larger extent of light scattering at the curved mold and thus less UV light intensity during the crosslinking reaction. An extraction of SMT in ethyl acetate was applied for purification before further use. The SMT showed a homogeneous wall thickness, a smooth surface without macropores and dimensions with 1.68 ± 0.02 mm outer and 0.76 ± 0.02 mm inner diameters (non-programmed state; Fig. 2).

Table 1: Model studies exploring the effect of crosslinking conditions on network properties.

Crosslinking conditions					Swelling studies			Flory-Rehner		DSC	
Shape	**Solvent**	t_{UV} (min)	ω_{wt} (wt%)	ω_{vol} (vol%)	Z (µm)	G (%)	Q^* (%)	v_c (mmol·ml^{-1})	M_c (kDa)	T_m (^oC)	ΔH_m (J·g^{-1})
Films	EA	10	20	16.4	490	69$^{\#}$	2600$^{\#}$	0.06	20.8$^{\#}$	51	63
			40	34.4	720	99	790	0.46	2.4	48	61
		20	20	16.4	565	98	1500	0.16	7.4	51	67
			40	34.4	725	99	720	0.54$^{\#}$	2.1	47	58
Films	TCM	10	20	24.5	630	77	1700	0.12	9.0	51	62
			40	46.4	795	99	600	0.77	1.5	46	55
		20	20	24.5	635	98	1000	0.28	4.0	51	63
			40	46.4	790	99	590	0.81	1.4	44	57
Tubes	EA	10	40	34.4	n.a.	91	780	0.47	2.6	49	62

n.a. = not applicable; $^+$ Shapes and solvents (EA = ethyl acetate, TCM = chloroform) as applied during UV-crosslinking; t_{UV} irradiation time; precursor concentrations given as weight fractions (ω_{wt}) and volume fraction (ω_{vol}; calculated with ρ_{oCL} = 1.14 g·mL^{-1}); z thickness of unextracted dried film samples (relaxed state after melting); Gel content G and degree of swelling Q from swelling studies in DCM; Crosslink density v_c and average segment chain length M_c calculated by the Flory-Rehner theory (n=3) using the polymer solvent interaction parameter χ = -0.26 (PCL/DCM) [18], the molar volume of DCM V_1 = 63.8 ml·mol^{-1}, and individual sample densities as determined by volume and weight. DSC data from extracted samples, second heating run (10 K·min^{-1}). Standard deviations determined from n = 3 samples (maximum deviation $^{\#}$/ typical mean standard deviation): z (10 µm$^{\#}$/ 5 µm), G (3.5%$^{\#}$/ 0.2%), Q (80$^{\#}$/ 20), v_c (0.02$^{\#}$/ 0.01), M_c (1.8 kDa$^{\#}$/ 0.4 kDa). Details for calculations are provided in the supporting information.

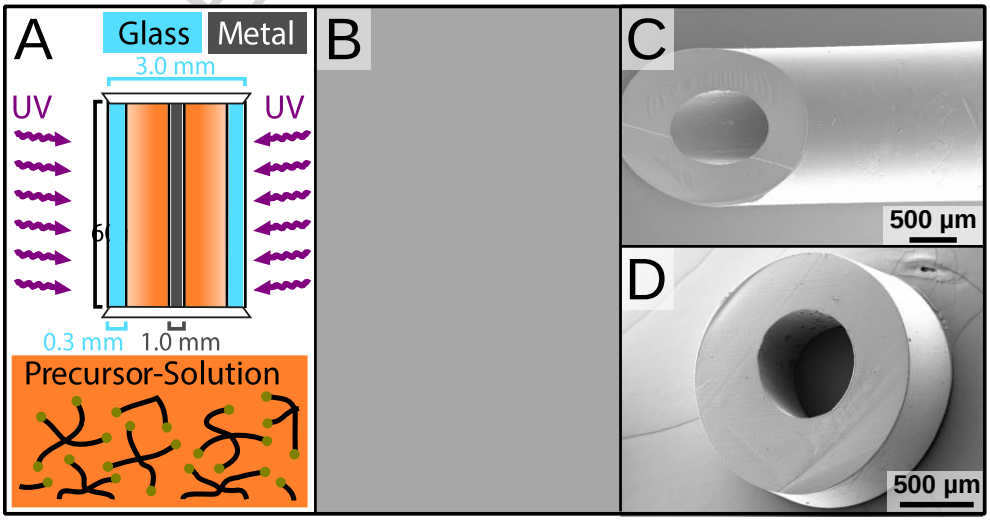

Fig. 2: Synthesis of SMT. (A) Scheme of setup. (B) Photograph of SMT (2.5-3 cm length). (C-D) SEM analysis of SMT with angular (C) and vertical (D) cross-section.

For the hydrogel filling, a PLGA-PEG-PLGA triblock copolymer with a $M_{n,GPC}$ = 5.0 kDa (PD = 1.6) containing a 1.5 kDa PEG central block was synthesized according to literature [17]. In order to identify a formulation with sufficiently strong physical interactions, which stabilize the gel structure and define the gelation temperature, the concentration of the polymer in phosphate buffer solution (DPBS) was systematically altered in the range of 5 – 40 wt.%. For 30 wt.% PLGA-PEG-PLGA, tube tilting demonstrated a gelation at 33 °C, i.e. below body temperature as desired. Rheology confirmed the sol-gel transition temperature (31 °C). Furthermore, *G'* and *G"* were determined to be 1040 and 790 Pa, respectively, and remained relatively stable upon constant shearing (Supp. Info. Fig. S1).

In order to occlude the SMT orifices as basis for an on-demand onset of release, a process should be selected that leads to sufficiently tight occlusion to prevent premature leakage. This process should not contain a chemical reaction that may potentially affect the payload and that might possibly create a disadvantageously tight connection to the SMT, which does not break upon SMT contraction. The coating should be based on a hydrophobic material to provide a sufficient diffusion barrier. Compared to previously reported in situ synthesized coatings based on brittle cyanoacrylates [19], here a more elastic material should be used that prevent a potential premature coating rupture und drug leakage e.g. due to mechanical forces applied during administration. These requirements were fulfilled by a non-crosslinked PCL coating, which was

shown to provide a leak-proof capping of SMT by single-dipping in ^{4}oCL-OH melt of 60 °C with an exposure time of the orifices only for less than 5 seconds.

3.2. Switching function upon direct heating

To introduce switching capabilities, the SMT were subjected to a programming procedure at 50 °C, where the tubes were in a state characterized by entropy elasticity. For radial expansion of SMT (inner diameter 0.76 mm) to an expanded temporary shape, a needle (outer diameter 1.2 mm) was introduced, followed by cooling to fix the expanded temporary shape by crystallization of the PCL switching domains. The lumen of tubes that later would host a payload was successfully increased as illustrated by cross-sections (Fig 3A-B), resulting in an average wall thickness of ~0.35 mm and a diameter of the tube lumen of ~1.3 mm. Accordingly, tube wall and tube lumen contribute at a ratio of approximately 1.36 : 1 to the overall volume of the container system. Other ratios may be obtained e.g. by alteration of the mold during synthesis and/or using thicker or thinner needles during programming, which was beyond the scope of this study.

In order to explore the shape switching function by the SME, programmed SMT were heated to 49 °C, resulting in the recovery of the permanent shape (Fig. 3C), which is driven by the recoiling of chain segments that were stretched during programming. The shape recovery ratio of the internal diameter R_r was calculated to be 89 ± 4% (n = 6) according to equation 1, while the error was calculated according to error propagation by equation 2 (inner diameters of initial (D_o), programmed (D_{prog}) and recovered samples (D_{rec}); corresponding standard deviations ΔD).

$$R_r = \frac{D_{prog} - D_{rec}}{D_{prog} - D_0} \quad (1)$$

$$\Delta R_r = \sqrt{\left(R'_{r,D_{prog}}\Delta D_{prog}\right)^2 \cdot \left(R'_{r,D_{rec}}\Delta D_{rec}\right)^2 \cdot \left(R'_{r,D_0}\Delta D_0\right)^2} \quad (2)$$

Cyclic thermomechanical analysis in tensile testers are typically used to characterize the switching temperature T_{sw} of SMP. However, since this technique cannot analyze the switching of internal diameters of SMT, an infrared (IR) thermography technique was applied for real time measurements of surface temperature of the SMT cross-sections when heated on a hot plate from 42 to 50 °C. Shape recovery transition took place in seconds starting from 46 to 49 °C (Fig. 3D), which is, again, well corresponding to the T_m of crystalline domains. When SMT in the programmed state were filled with PLGA-PEG-PLGA hydrogel, the shape switching was proven to provide suitable forces to eject the gel (Fig. 3E). This is an important precondition for the planned on-demand protein delivery.

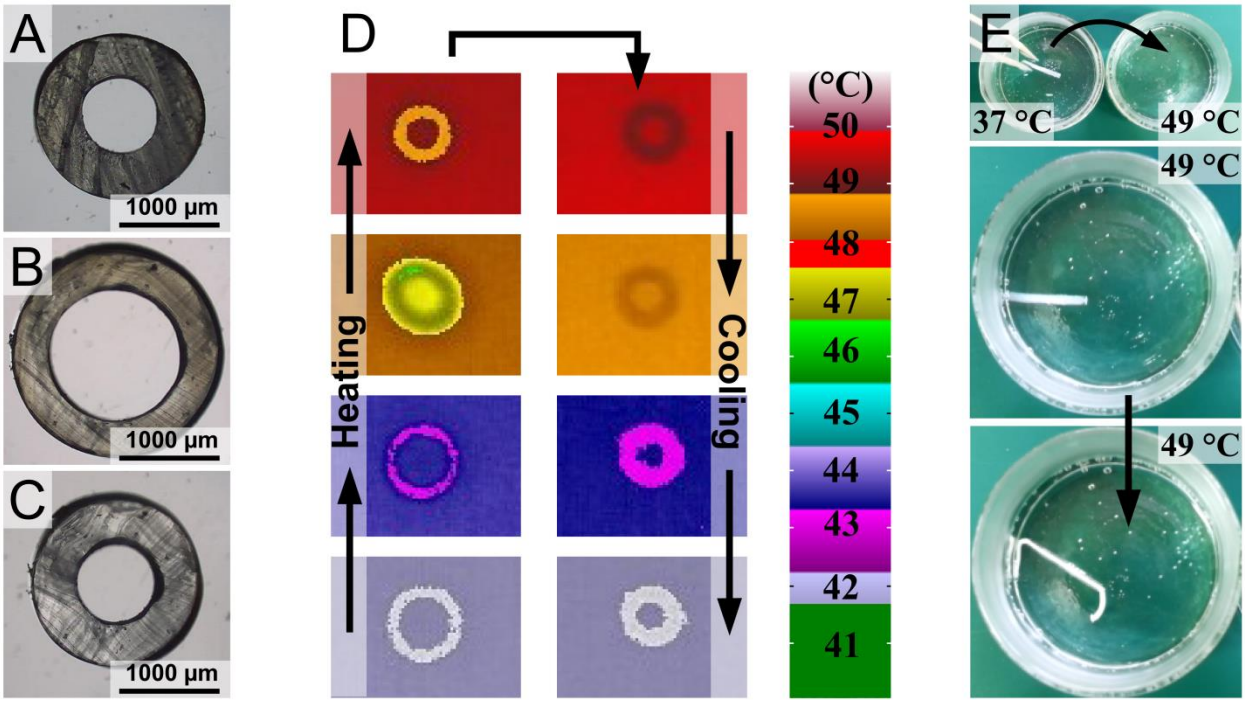

Fig. 3: Temperature-induced shape switching of SMT. (A-C) Light microscopy images of SMT cross-section (A) as synthesized, (B) after programming and (C) after shape recovery by

direct heat exposure. (D) Analysis of switching temperature by an IR camera. (E) Ejection of gel from SMT upon temperature triggering.

3.3. Switching function upon non-contact heating by NIR

Besides the direct exposure to temperature as SME trigger, the capability for non-contact stimulation was explored using NIR light, which is known for its capability to penetrate into tissue [20] [21]. A pulsed 1064 nm NIR laser was employed to indirectly trigger the temperature-induced shape recovery by means of the photo-thermal effect of photochromic dye molecules embedded in SMT. IR-26 dye was loaded into the PCL network by swelling in dye solution and subsequent drying (picture: Supp. Fig. S2 A), which resulted in a payload of 0.8 ±0.2 wt.% and a loading efficiency of 80 ± 16% without detrimental effects on tube properties during handling.

In order to explore a NIR light-induced shape switching, a specialized instrumental setup with an integrated pulsed NIR laser and a microscope was employed (Supp. Fig. S2 B-E). SMT cross-sections of 0.5 mm thickness were pre-heated to 37 °C in dry or aqueous environment to mimic physiological conditions. Within seconds of irradiation (50 mJ, 15 Hz), a fast shape recovery process could be observed. This holds true for air environment (Fig. 4) and, with a slightly longer irradiation due to larger heat loss, also for aqueous environment, but not for dye-free controls (Supp. Info. Fig. S3). By attenuation of the laser intensity from 50 to 25 mJ, the irradiation time needed for shape switching increased from 2 to 5 s and 10 to 40 s for dry and water-embedded samples, respectively. In summary, this series of experiments confirms that besides direct heating also a remote activation of SMT containers may be possible in principle.

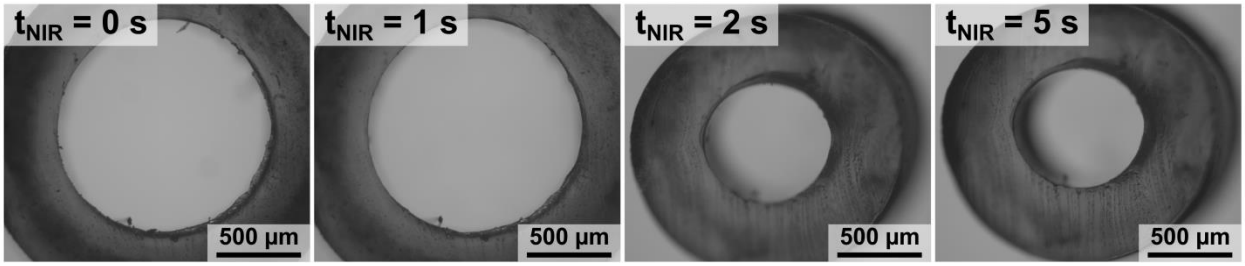

Fig. 4 Non-contact triggering of the shape switching by NIR laser light (1064 nm, 50 mJ, and 15 Hz). Images of SMT loaded with IR-26 dye in air atmosphere at an environmental temperature of 37 °C. The indicated time points from left to right correspond to the exposure time added to the previous stage with intermediate convective cooling of samples while taking the images. Control experiments with continuous NIR exposure without intermediate imaging suggest that switching is completed after 2 s.

3.4. Protein release studies

According to the hypothesis of this study, on-demand control of protein release should be enabled by a switching of diameters of a tubular container system. The different release opportunities that arise from the SMT containers as proposed in Fig. 1 include on the one hand a continuous release with an opportunity for an add-on dosing pulse for open tubes (option 1) and on the other hand a negligible initial release from coated tubes with an on-demand onset of protein delivery (option 2).

In a first set of experiments, the release pattern of bovine serum albumin (BSA) as a model protein was explored from PLGA-PEG-PLGA hydrogel in a vial in comparison to the gel formulation in the container system (open tube). PLGA-PEG-PLGA solution doped with BSA

was filled in SMT and pre-incubated for 1 h at 37 °C for hydrogel gelation before adding release buffer to start the experiment (Fig. 5A). Compared to the experiment in vials, which released about 50% of the protein in 24 h, the use of the SMT container enabled a linear release over ~12 days with 50% of protein being released after 6 days. This release pattern can be assigned to the SMT device geometry with defined orifices for nearly constant-rate diffusion processes. In order to explore the capacity for add-on dosing from tubes with open orifices by the SMT switching function, a temperature trigger was applied after 2 d of incubation. By this experiment, it was shown that the ejection of protein loaded hydrogel with an increase of accessible surface area for diffusion processes indeed resulted in a pulse in the release curve of BSA (Fig. 5B). In order to demonstrate that the concept is applicable to various proteins of different sizes, lysozyme (Lys; 14 kDa), ovalbumin (OVA; 43 kDa), BSA (66 kDa) and immunoglobulin G (IgG; 150 kDa) were exemplarily evaluated in direct comparison. With exception of the smallest molecule, Lys, which had an initially faster release, all other studied proteins provided an on-demand release pulse in a virtually identical fashion irrespective of their molecular weights (Fig. 5B). This may be due to the relatively large dimensions of diffusion pathways and high mobility of vesicles in the micelle-based PLGA-PEG-PLGA hydrogels, which do not exclude larger molecules from diffusion. It may be concluded that a wide applicability of the presented release option 1 to various proteins appears to be possible.

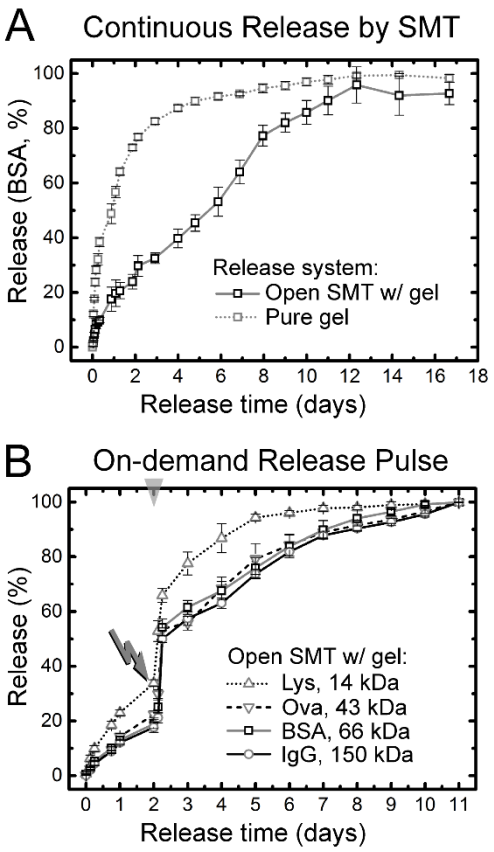

Fig. 5: Continuous and on-demand add-on pulse for protein release from open SMT tubes. (A) Continuous release from hydrogel gel-filled SMT with open orifices vs. pure gel in a vial. (B) On-demand pulse of protein release by SMT switching by a temperature trigger after 2 d (49 °C, 1 min). Applicability to various proteins of different sizes released from gel-filled SMT with open orifices. Data represent mean ± SD for n = 3-4; samples w/ gel use 30 wt.% pre-gelled PLGA-PEG-PLGA.

In the next step, the capacity for on-demand initiation of delayed release should be demonstrated using SMT tubes with orifices sealed with a coating. As demonstrated for BSA as model protein, a very low initial release of less than 10% in 2 d confirming that the coating

strategy for SMT sealing has been successful (Fig. 6A). Furthermore, the on-demand protein release could be successfully triggered by a thermal stimulus (Fig. 6A). When pure protein solution instead of protein plus hydrogel was filled in SMT with closed orifices, again very low initial release was found, followed by triggered delivery of a much higher dose compared to gel-filled orifices due to rapid protein leakage from the entire SMT lumen. This illustrates the role of the PLGA-PEG-PLGA hydrogel as an optional parameter for dosage rate control. In comparison to open tubes used as a control in this experiment, the desired on-demand initiation of a delayed release from SMT with closed orifices could be confirmed (Fig. 6A).

Finally, the capability to recall the SMT shape-switch and thus the delivery of the protein from SMT of identical composition at different time points should be demonstrated. Here, SDF-1α as a therapeutically relevant chemotactic protein often proposed to attract stem cells e.g. for heart tissue [22] and neuronal regeneration [4] was exemplarily employed. Using tubes with occluded orifices, a protein leakage of less than 10% over a time as long as 7 days confirmed that the SDF-1α as a relatively small protein can be retained in SMT containers (Fig. 6B). Subsequently, SDF-1 α could be released on-demand at the desired time-point, here triggered either after 2 d, 4 d, or 7 d.

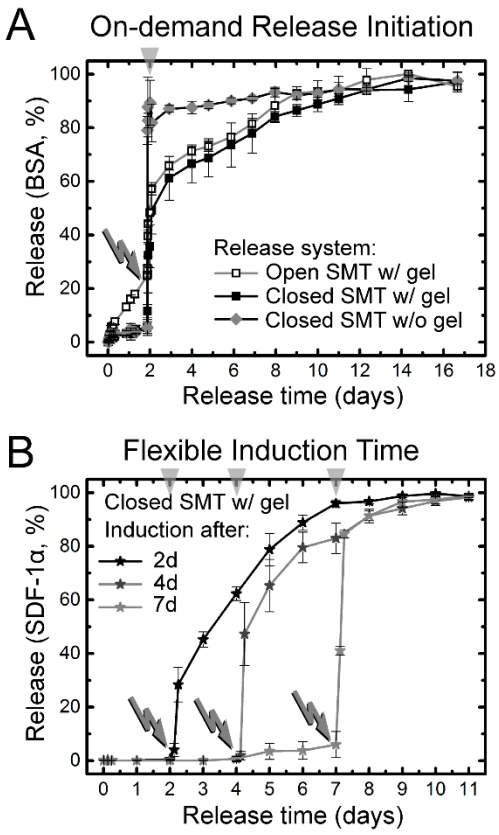

Fig. 6: On-demand initiation of delayed protein release from coated SMT containers. (A) Release of BSA from closed tubes filled with hydrogel in comparison to closed tubes with protein solution (effect hydrogel) and open hydrogel-filled tubes (effect coating). The temperature trigger (49 °C, 1 min) was applied after 2d. (B) SDF-1α release induced at different time points from SMT with occluded orifices. Data represent mean ± SD for n = 3-4; samples w/ gel use 30 wt.% pre-gelled PLGA-PEG-PLGA.

4. Conclusion

In this study, it could be successfully demonstrated that the hypothesized on-demand release from a multifunctional multimaterial device can be realized when container tubes are equipped with a shape-switching capacity for diameter reduction. So far, SMP have been proposed e.g. for anchoring of continuous release implants, while a direct link of spatially directed mechanical

actuation of SMP to an on-demand release of bioactive molecules from container systems has, to the knowledge of the authors, here been demonstrated for the first time. By illustrating the applicability to different proteins, realizing different time-points of on-demand release, and enabling either negligible or continuous initial release plus an optional boost dosage, a wide variety of release options may be provided by the presented approach. It may be explored in the future if this concept is also applicable to other types of bioactive molecules including e.g. heparin as a polysaccharide or small molecule drugs.

Depending on the application and temperature sensitivity of the therapeutic protein, other degradable shape-memory polymers e.g. with lower switching temperatures may be explored in the future. Furthermore, in vivo studies may be of interest to demonstrate that the triggering option of SME by NIR light penetrating the tissue can be an alternative to externally applied hypothermia. In addition, this study may be the basis for future systems with the capacity of reversibly switching the release on and off by a cleverly matched device design, which allows the on-demand healing of the coating or shrinking to the level that the tube is closed. Shape-memory polymer actuators might play a key role in such future devices.

The concept presented in this study could be used to realize a kit-like system for controlled protein release, which allows physicians in an intraoperative scenario to load one or several therapeutic proteins specifically suited for the clinical setting of an individual patient and locally place the depot in an otherwise hardly accessible site of the body. Based on the subsequent clinical picture or, if suitable biomarkers are known, the monitoring of body fluids, the best time point may be selected to induce factor release

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Figure legends

Fig. 1: Schematic illustration of the principle of multimaterial implants for on-demand protein release by shape switch of the container system. Left: Option 1 for continuous release with a SME-induced add-on release pulse. Right: Option 2 with a delayed onset of release initiated on-demand.

Fig. 2: Synthesis of SMT. (A) Scheme of setup. (B) Photograph of SMT (2.5-3 cm length). (C-D) SEM analysis of SMT with angular (C) and vertical (D) cross-section.

Fig. 3: Temperature-induced shape switching of SMT. (A-C) Light microscopy images of SMT cross-section (A) as synthesized, (B) after programming and (C) after shape recovery by direct heat exposure. (D) Analysis of switching temperature by an IR camera. (E) Ejection of gel from SMT upon temperature triggering.

Fig. 4 Non-contact triggering of the shape switching by NIR laser light (1064 nm, 50 mJ, and 15 Hz). Images of SMT loaded with IR-26 dye in air atmosphere at an environmental temperature of 37 °C. The indicated time points from left to right correspond to the exposure time added to the previous stage with intermediate convective cooling of samples while taking the images. Control experiments with continuous NIR exposure without intermediate imaging suggest that switching is completed after 2 s.

Fig. 5: Continuous and on-demand add-on pulse for protein release from open SMT tubes. (A) Continuous release from hydrogel gel-filled SMT with open orifices vs. pure gel in a vial. (B) On-demand pulse of protein release by SMT switching by a temperature trigger after 2 d (49 °C, 1 min). Applicability to various proteins of different sizes released from gel-

filled SMT with open orifices. Data represent mean ± SD for n = 3-4; samples w/ gel use 30 wt.% pre-gelled PLGA-PEG-PLGA.

Fig. 6: On-demand initiation of delayed protein release from coated SMT containers. (A) Release of BSA from closed tubes filled with hydrogel in comparison to closed tubes with protein solution (effect hydrogel) and open hydrogel-filled tubes (effect coating). The temperature trigger (49 °C, 1 min) was applied after 2d. (B) SDF-1α release induced at different time points from SMT with occluded orifices. Data represent mean ± SD for n = 3-4; samples w/ gel use 30 wt.% pre-gelled PLGA-PEG-PLGA.

Tables

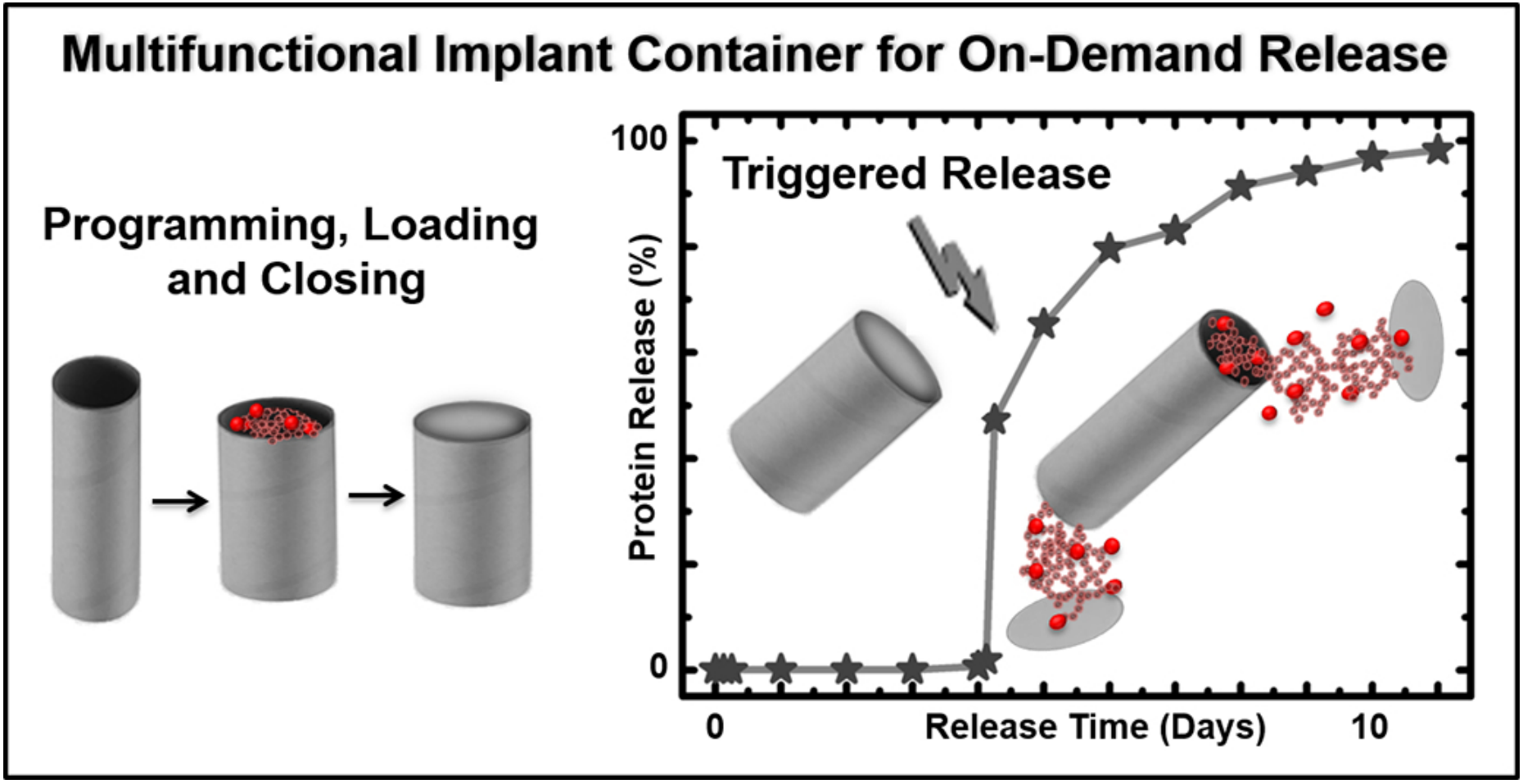
Table 1: Model studies exploring the effect of crosslinking conditions on network properties.

Crosslinking conditions					Swelling studies			Flory-Rehner		DSC	
Shape	**Solvent**	t_{UV} (min)	ω_{wt} (wt%)	ω_{vol} (vol%)	Z (µm)	G (%)	Q* (%)	ν_c (mmol·ml^{-1})	M_c (kDa)	T_m (^oC)	ΔH_m (J·g^{-1})
Films	EA	10	20	16.4	490	69$^{\#}$	2600$^{\#}$	0.06	20.8$^{\#}$	51	63
			40	34.4	720	99	790	0.46	2.4	48	61
		20	20	16.4	565	98	1500	0.16	7.4	51	67
			40	34.4	725	99	720	0.54$^{\#}$	2.1	47	58
Films	TCM	10	20	24.5	630	77	1700	0.12	9.0	51	62
			40	46.4	795	99	600	0.77	1.5	46	55
		20	20	24.5	635	98	1000	0.28	4.0	51	63
			40	46.4	790	99	590	0.81	1.4	44	57
Tubes	EA	10	40	34.4	n.a.	91	780	0.47	2.6	49	62

n.a. = not applicable; $^+$ Shapes and solvents (EA = ethyl acetate, TCM = chloroform) as applied during UV-crosslinking; t_{UV} irradiation time; precursor concentrations given as weight fractions (ω_{wt}) and volume fraction (ω_{vol}; calculated with ρ_{oCL} = 1.14 g·mL^{-1}); z thickness of unextracted dried film samples (relaxed state after melting); Gel content G and degree of swelling Q from swelling studies in DCM; Crosslink density ν_c and average segment chain length M_c calculated by the Flory-Rehner theory (n=3) using the polymer solvent interaction parameter χ = -0.26 (PCL/DCM) [18], the molar volume of DCM V_1 = 63.8 ml·mol^{-1}, and individual sample densities as determined by volume and weight. DSC data from extracted samples, second heating run (10 K·min^{-1}). Standard deviations determined from n = 3 samples (maximum deviation $^{\#}$/ typical mean standard deviation): z (10 µm$^{\#}$/ 5 µm), G (3.5%$^{\#}$/ 0.2%), Q (80$^{\#}$/ 20), ν_c (0.02$^{\#}$/ 0.01), M_c (1.8 kDa$^{\#}$/ 0.4 kDa). Details for calculations are provided in the supporting information.

Highlights

- Shape-memory polymer tubes allow linking shape switching and on-demand drug release
- Switching to reduced diameters by heat or Near infrared light
- Expelling various types of protein payload from lumen of open or sealed tubes
- Triggered add-on dosing or delayed initiation of release demonstrated
- Potential as kit for intraoperative filling to be used in precision medicine



Graphics Abstract

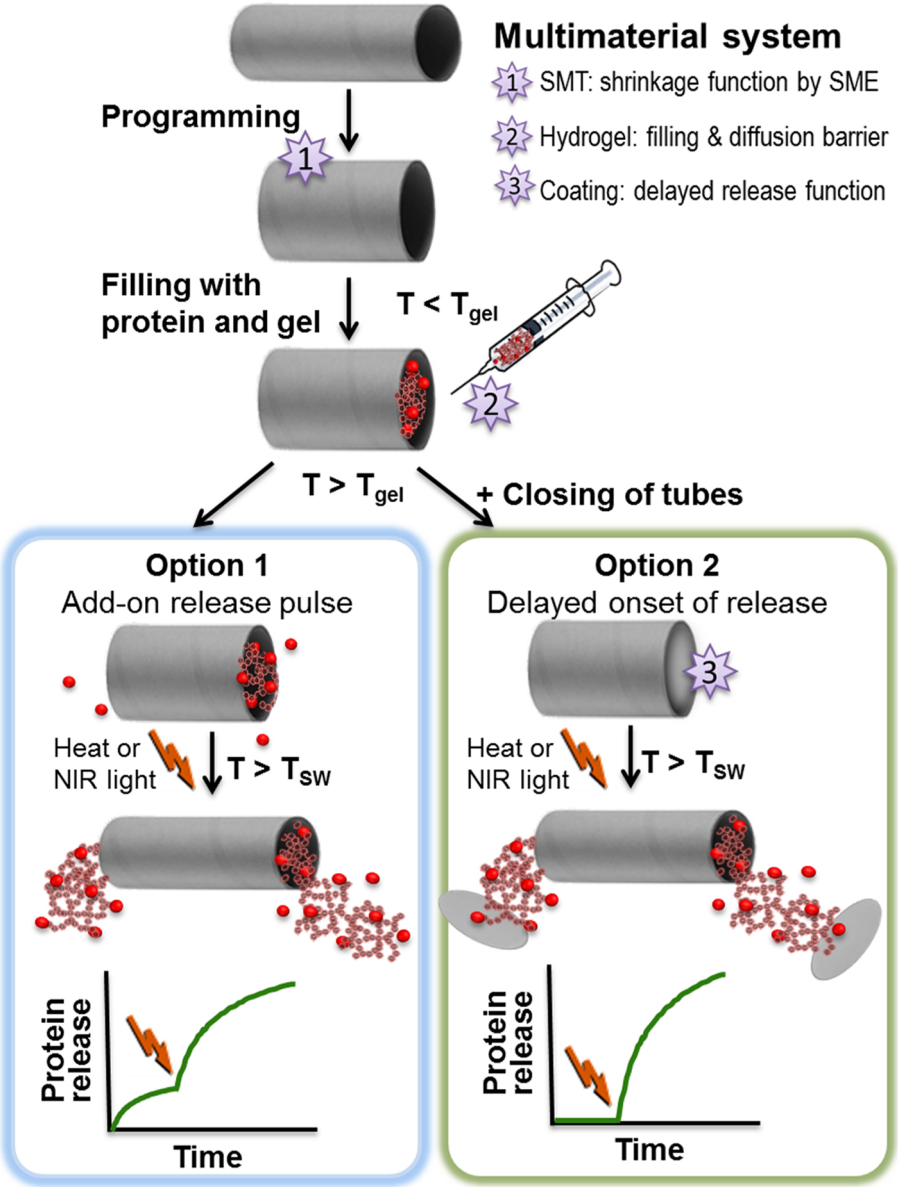

Multimaterial system
1 SMT: shrinkage function by SME
2 Hydrogel: filling & diffusion barrier
3 Coating: delayed release function
Programming
1
Filling with protein and gel
T < Tgel
2
T > Tgel
+ Closing of tubes
Option 1
Add-on release pulse
Heat or NIR light
T > Tsw
Protein release
Time
Option 2
Delayed onset of release
3
Heat or NIR light
T > Tsw
Protein release
Time

Figure 1

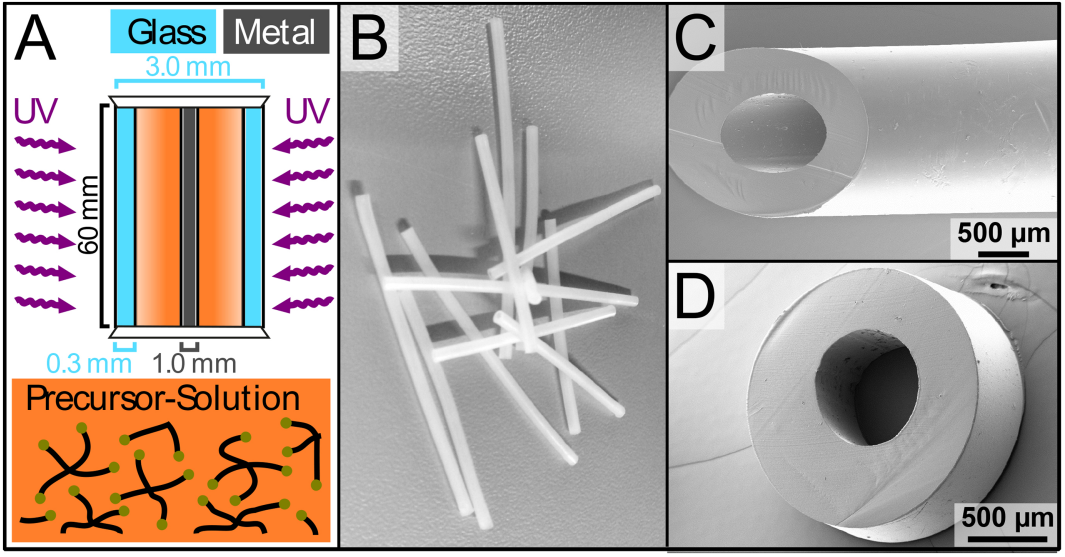

A
Glass
Metal
3.0 mm
UV
UV
60 mm
0.3 mm
1.0 mm
Precursor-Solution
B
C
500 µm
D
500 µm

Figure 2

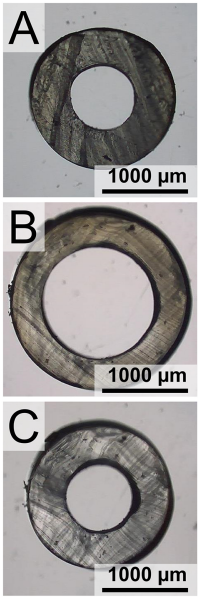

A
1000 µm
B
1000 µm
C
1000 µm
D
Heating
Cooling
(°C)
50
49
48
47
46
45
44
43
42
41
E
37 °C
49 °C
49 °C
49 °C

Figure 3

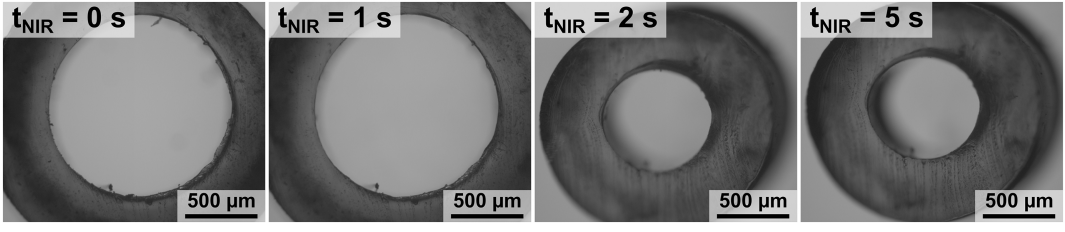


Figure 4

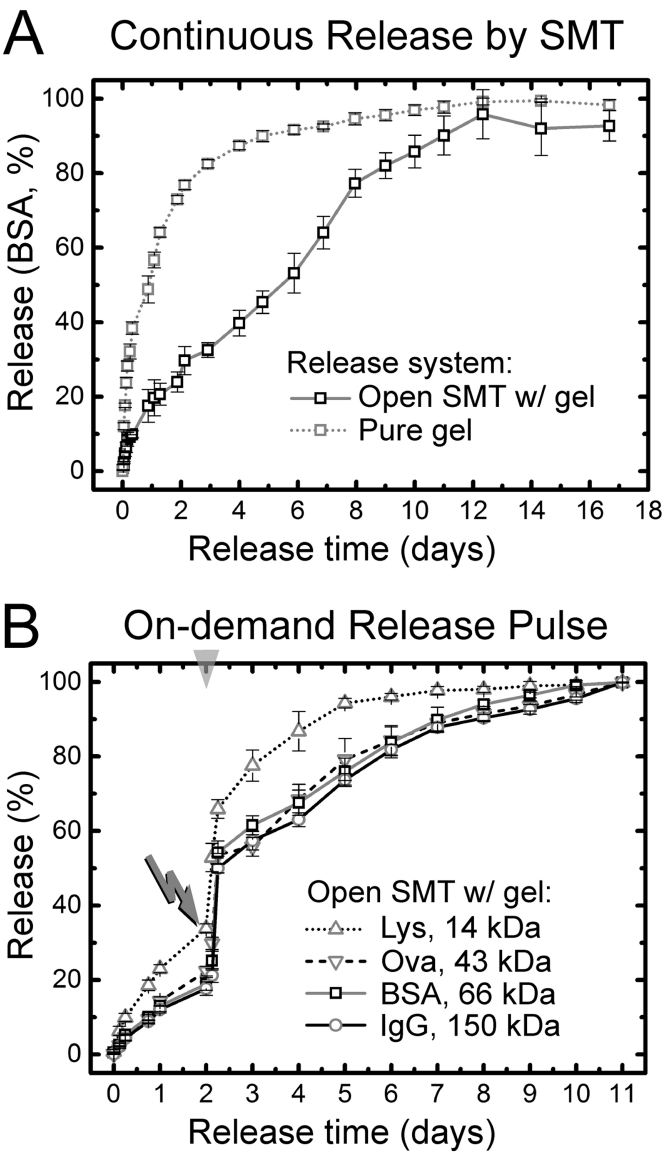

A
Continuous Release by SMT
Release (BSA, %)
Release time (days)
Release system:
Open SMT w/ gel
Pure gel
B
On-demand Release Pulse
Release (%)
Release time (days)
Open SMT w/ gel:
Lys, 14 kDa
Ova, 43 kDa
BSA, 66 kDa
IgG, 150 kDa

Figure 5

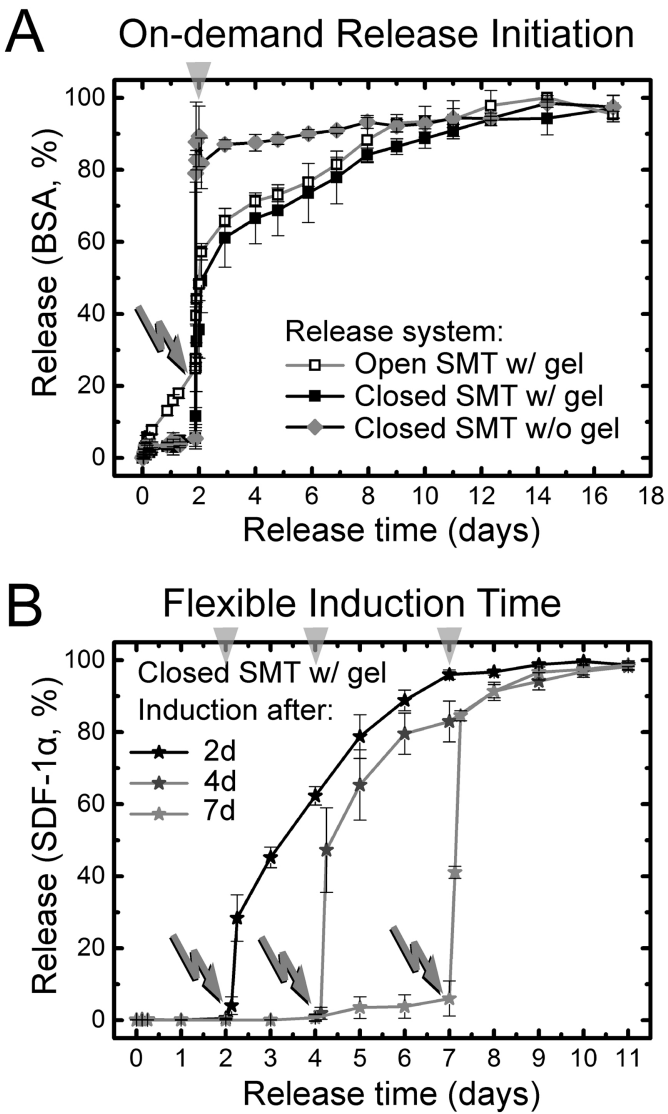

A
On-demand Release Initiation
Release (BSA, %)
Release time (days)
Release system:
Open SMT w/ gel
Closed SMT w/ gel
Closed SMT w/o gel
B
Flexible Induction Time
Release (SDF-1α, %)
Release time (days)
Closed SMT w/ gel
Induction after:
2d
4d
7d

Figure 6