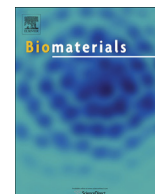


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Controlled release from a mechanically-stimulated thermosensitive self-heating composite hydrogel



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ABSTRACT

Temperature has been extensively explored as a trigger to control the delivery of a payload from environment-sensitive polymers. The need for an external heat source only allows limited spatiotemporal control over the delivery process. We propose a new approach by using the dissipative properties of a hydrogel matrix as an internal heat source when the material is mechanically loaded. The system is comprised of a highly dissipative hydrogel matrix and thermo-sensitive nanoparticles that shrink upon an increase in temperature. Exposing the hydrogel to a cyclic mechanical loading for a period of 5 min leads to an increase of temperature of the nanoparticles. The concomitant decrease in the volume of the nanoparticles increases the permeability of the hydrogel network facilitating the release of its payload. As a proof-of-concept, we showed that the payload of the hydrogel is released after 5–8 min following the initiation of the mechanical loading. This delivery method would be particularly suited for the release of growth factor as it has been shown that cell receptor to growth factor is activated 5–20 min following a mechanical loading.

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1. Introduction

Most of the efforts in the field of drug delivery systems has focused on the development of environment-sensitive polymers. Temperature and pH are commonly used environmental variables [1–4]. While pH can be coupled to variations within the body, temperature sensitive polymers are designed to be altered either externally [5–7] or are in off/on mode almost immediately after being injected in the body [8–10].

Temperature-responsive drug delivery systems are usually based on polymer hydrogels with a lower critical solution temperature (LCST) of around 38 °C. The drug is released when the tissue surrounding the hydrogel reaches a temperature slightly above normal body temperature [4,11]. While these systems work well for a number of applications, they also have some limitations. The need for an external means to cool or heat in many applications only allows limited spatiotemporal control over the delivery process.

As hydrogels have dissipative properties, their temperature may also be altered internally by viscous dissipation during cyclic loading, a process generally referred to as self-heating [12–14]. There are potentially several advantages of using the self-heating property of materials for drug delivery.

First, the drug release is coupled to a mechanical loading. As mechanical loading has been demonstrated to activate some growth factor cell receptors involved in the healing process of different tissues such as cartilage [15–18], the coupling of mechanical loading and drug release could induce some positive synergetic effects. Drug delivery system coupled to mechanical loading has already been developed, the drug being simultaneously released during the mechanical loading [19–21].

However, it is important to realize that cell receptors are not immediately activated following a mechanical loading. A delay of 5–20 min has been observed between the initiation of the mechanical loading and the activation of the cell receptor [22]. To induce a maximum potency, the release of a drug following a mechanical loading should then also be delayed by several minutes.

As the self-heating property induces a local temperature increase, which is related to the dissipative properties of the material and to the number of loading cycles, a delay can be obtained between the initiation of the mechanical loading and the temperature

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increase triggering the drug release. The use of dissipative energy generated in hydrogel could then offer a second advantage, which is the unprecedented spatiotemporal control over the delivery process. In particular, the use of self-heating property would be particularly suited for the delivery of growth factors to induce healing in a cartilage defect where the cells are naturally subjected to mechanical loading.

In this study, we establish the proof-of-concept that dissipative properties can be used as a new environmental variable to spatiotemporally control the release of a drug.

2. Materials and methods

2.1. Principle of dissipation used as an environmental variable

To explore the feasibility of using dissipative properties to control delivery from thermosensitive polymer based systems, we developed a unique hydrogel system consisting of two components: i) poly(2-hydroxyethyl methacrylate) (PHEMA)-based hydrogel matrix with highly dissipative properties and ii) poly(N-isopropyl acrylamide) (PNIPAM)-based thermosensitive nanoparticles, which are entrapped in the matrix hydrogel and shrink at temperatures above their LCST.

The principle used to temporally control the delivery of a payload following a mechanical loading is shown in Fig. 1A. Upon applying a mechanical load, a part of the mechanical energy is transformed into heat due to the dissipative properties of the hydrogel matrix. The heat produced by the hydrogel increases the temperature of the nanoparticles above their LCST and induces their collapse, which subsequently facilitates diffusion of the payload outside the hydrogel. The dissipative properties of the hydrogel can be modulated to link the duration of the cyclic loading with a targeted increase in temperature. Thus, a specific delay between the initiation of the mechanical stimulation and the release of a payload can be obtained.

HEMA is a hydrogel-forming material that is widely used in the biomaterials field [23,24]. Water content and crosslink density are the key parameters to control the mechanical and dissipative properties of these hydrogels [25,26]. The hydrogels can be obtained using different crosslinkers in a one-step photo-polymerization process [27]. The PHEMA hydrogels investigated in this study were crosslinked with ethylene glycol dimethacrylate (EGDMA) and contained 40% water.

2.2. Materials

2,2-dimethoxy-2-phenylacetophenone (Irgacure 651, 97%), ethylene glycol dimethacrylate (EGDMA, 98%), TWEEN® 80, Span® 80, N,N,N',N'-Tetramethylethylenediamine (TMEDA, 99%), Ammonium persulfate ($\geq 98\%$) and Xylene Cyanol FF were purchased from Aldrich and used as received. 2-Hydroxyethyl methacrylate (HEMA, 97%) and poly(ethylene glycol) dimethacrylate (PEGDMA, average M_n 550) were purchased from Aldrich and purified by basic aluminum oxide column chromatography to remove inhibitor. N-isopropylacrylamide (Aldrich, 97%) was purified

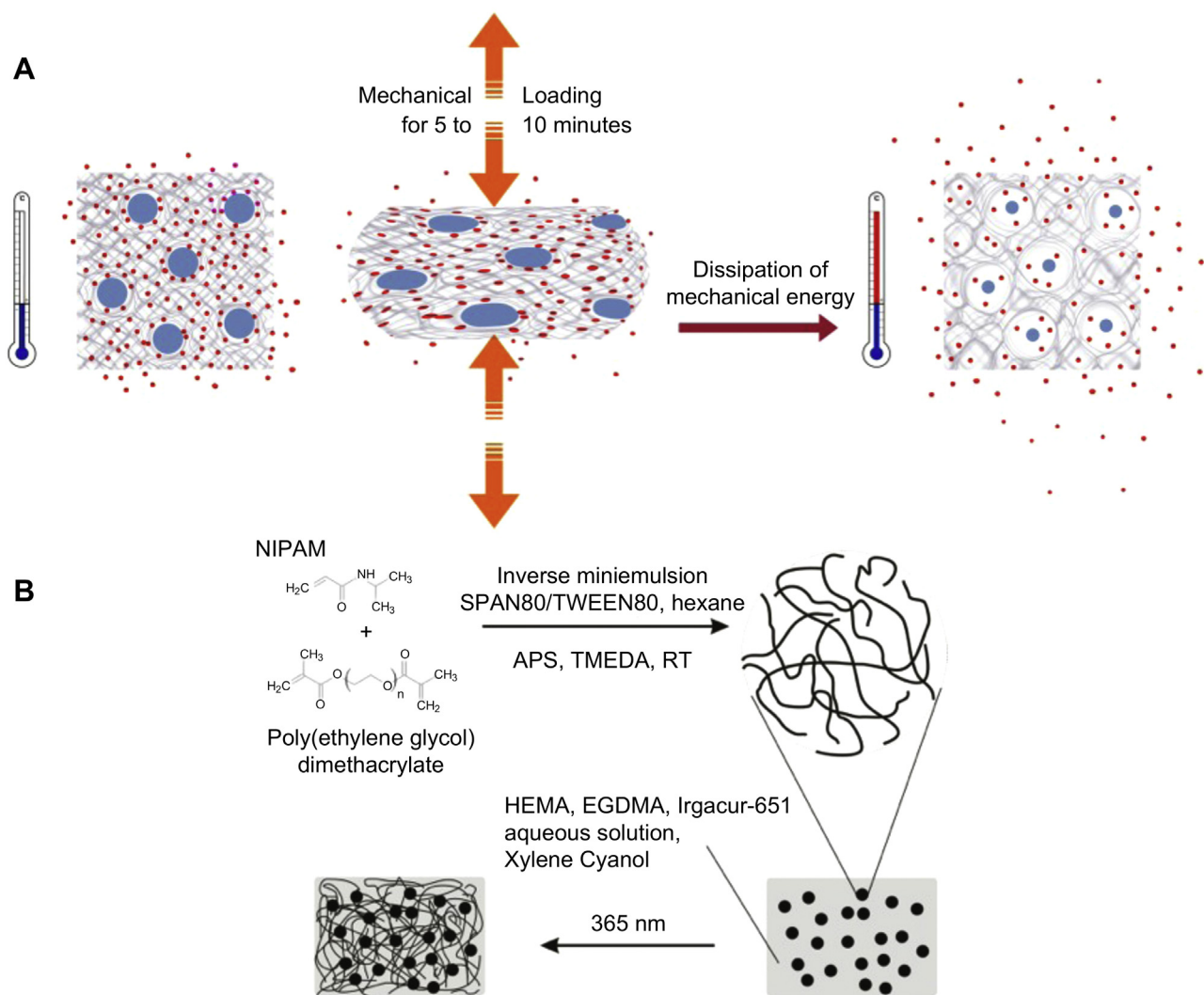


Fig. 1. A) Release of a payload from a thermosensitive delivery system using dissipation as an internal heat source. Thermosensitive nanoparticles (blue dots) and payload (red dots) are distributed randomly inside the hydrogel (gray). At temperatures below the LCST of the nanoparticles, only limited amount of payload can be released from the hydrogel. When the hydrogel is under a mechanical loading of 5–10 min duration, its dissipative properties induce a temperature increase, a process called self-heating. If the temperature rises above the LCST of the nanoparticles, they shrink, which increases the permeability of the hydrogel and facilitates the release of the payload. B) Synthesis of the composite hydrogel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by recrystallization from hexane. Spectra/Por 6 dialysis tubing, 10K MWCO was purchased from Spectrum Labs Europe.

2.3. Preparation of the nanoparticles

The organic phase consisted of n-hexane (15 mL) and a mixture of surfactants TWEEN80 (75 mg) and SPAN80 (225 mg). The oxygen dissolved in the organic phase was removed by evacuating the surfactant mixture for 5 min followed by purging with nitrogen for short period (repeated 3 times). Hexane was purged with nitrogen for 10 min. The aqueous phase was prepared as follows: 150 mg of NIPAM were added to the flask along with 10 mg of poly(ethylene glycol)dimethacrylate (PEGDMA). Solid monomer and cross-linker were dissolved in 0.4 mL PBS pH 7.4 and sonicated. Organic phase was added to aqueous phase and the mixture was emulsified by sonicating 30 s. Polymerization was initiated by the addition of 2 drops of tetramethylethylenediamine and 25 mg of ammonium persulfate in 0.1 mL PBS pH 7.4 during sonication. Additional drops of TMEDA were added 10 min after; the miniemulsion was stirred for 4 h at room temperature. The reaction was stopped by exposure to air. Hexane was removed on rotary evaporator at 30 °C and 5 mbar, wax-like substance was redispersed in ethyl ether and precipitated. Supernatant was removed, and particles were redispersed in ether two more times. After that, the particles were redispersed in water, dialyzed against water for 3 days (MWCO 10,000 membrane, water was changed at least twice a day) and freeze-dried. Purified particles were redispersed in water, and the diameter was analyzed by DLS as the average of 10 measurements.

2.4. Preparation of the composite hydrogel

2-Hydroxyethyl methacrylate (HEMA) (880 µL), ethylene glycol dimethacrylate (EGDMA) (84 µL, 6%mol), and Xylene Cyanol FF aqueous solution (1 mg/mL, 590 µL) were mixed in a 5 mL Eppendorf tube. The Xylene Cyanol FF was used as drug model. For hydrogels containing nanoparticles, the aqueous phase also contained 15 mg/mL nanoparticles, which is equal to 5.5 mg/mL nanoparticles in total reaction mixture. A solution of Irgacur-651 (photo initiator) in ethanol (36 µL of 57 mg/mL solution) was then added to the polymer mixture. The mixture was stirred and sonicated for 1 min, then transferred to the cylindrical wells (8 mm diameter and 4 mm depth), placed under UV lamp (365 nm, 8 Watt) and irradiated for 15 min, whilst maintaining the temperature below 25 °C (using a flow of compressed air). The samples were washed and kept in 1 mL water for two days before mechanical tests (Fig. 1B).

2.5. Quantification of Xylene Cyanole FF release

After each test the supernatant water was collected from the test chamber and the amount of released Xylene Cyanole FF was quantified spectrophotometrically at 590 nm. The release amount $c_d(t)$ was reported as µg/mL using the normalized curves we prepared by spectrophotometric analysis of solutions at different concentrations of Xylene Cyanol FF in water.

2.6. Mechanical loading

Mechanical compressive test was performed with an Instron E3000 linear mechanical testing machine (Norwood, MA, USA). To provide a heat-isolated environment, controlling the initial temperature and monitoring the temperature during the test, a specifically designed thermally isolated system was installed on the mechanical testing machine (Fig. 1S, Supplementary data). The temperature of the test chamber was maintained by water circulation around the chamber either at 34 °C or at 36 °C for different experiments. The hydrogel immersed in 600 µL fresh water was placed in the center of the chamber. The hydrogel temperature was monitored with a thermistor positioned in the center of test chamber (Fig. 1S, Supplementary data). Cyclic compression was applied at 1.5 Hz on displacement control mode. Deformations of 15% amplitude following a 5% prestrain were applied for 5 and 8 min. The temperature was recorded every 10 s during the test.

2.7. Effective diffusion coefficient measurement

To calculate the effective diffusion coefficient of the composite hydrogel, we prepared samples in the form of microfilms by injecting the polymer mixture between two glass slides having a 150 µm gap and polymerized them under UV light. After polymerization, films were punched with 8 mm punch and immersed in 600 µL water. Hydrogels with or without nanoparticles were placed either at 34 °C or at 39 °C (6 samples per group). Xylene Cyanole FF release ($c_d(t)$) was measured every 2 h for 14 h from each of the 4 different hydrogel groups (with or without nanoparticles, at 34 °C and 39 °C). The transient distribution of Xylene Cyanole FF in a cylindrical sample is governed by the following equation [28]:

$$\frac{\partial c}{\partial t} = \frac{D_{\text{eff}}}{\phi} \left(\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial c}{\partial r} \right) + \frac{\partial^2 c}{\partial z^2} \right) \quad (1)$$

where c is the Xylene Cyanole FF concentration within the sample, D_{eff} is the effective diffusivity and ϕ is the hydrogel water content equal to 40% in our case.

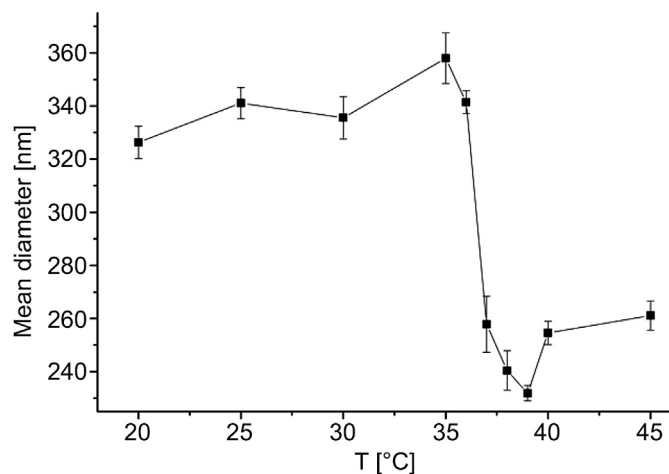


Fig. 2. The effect of temperature on the size of the PNIPAM nanoparticles. The temperature leading to decrease in nanoparticle size is referred to as LCST and is comprised between 36 and 37 °C. The diameter of the nanoparticles was analyzed by DLS as the average of 10 measurements.

Considering the boundary condition $c = 0$ at $r = R$, $z = 0$ and $z = h$ (R and h : sample diameter and height) and the initial condition $c = c_0$ (initial Xylene Cyanole FF concentration inside the hydrogel), we obtained the analytical solution for c . By integrating c over the sample fluid volume and subtracting the result from the total Xylene Cyanole FF release gives the following solution for the Xylene release in the water bath $c_d(t)$:

$$c_d(t) = c_{\text{max}} \left[1 - \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{16[1 - (-1)^n]}{q_m^2 n^2 \pi^2} \exp\left(-\frac{D_{\text{eff}} \lambda_{nm}^2 t}{\phi}\right) \right] \quad (2)$$

$c_d(t)$ is the Xylene Cyanole FF release, c_{max} is the total Xylene Cyanole FF release from the sample in water if we assume all Xylene in the sample is released in 600 µL (for microfilm samples $c_{\text{max}} = 2.83$ µg/mL), q_m is the m^{th} zero of zero-order Bessel function (J_0) and

$$\lambda_{nm}^2 = \frac{q_m^2}{R^2} + \frac{n^2 \pi^2}{h^2} \quad (3)$$

All parameters in Equation (2) except D_{eff} were known or measured independently. D_{eff} was calculated by fitting Equation (2) to the experimental data from desorption bath (Fig. 2S, Supplementary data).

3. Results

3.1. Temperature response of nanoparticles

The nanoparticles had a diameter of 340 nm as determined by dynamic light scattering (DLS) at room temperature and showed a lower critical solution temperature of 37 °C (Fig. 2). At 37 °C, the PNIPAM nanoparticles collapsed and had an average diameter of 255 nm. The resulting decrease in volume corresponds to about 50%.

3.2. Composite hydrogels

The final formulation of the composite hydrogel consists of an EGDMA crosslinked PHEMA-based hydrogel containing 40% aqueous phase with dispersed nanoparticles and Xylene Cyanole FF, which was used as model drug. Fig. 3 shows the dispersion of nanoparticles in hydrogels with different concentrations of nanoparticles. For the final formulation we had 5.5 mg nanoparticles per 1000 µL of reaction mixture.

3.3. Self-heating of hydrogels due to dissipation

The self-heating property of the composite hydrogels were investigated using a custom-designed thermally isolated system, which was coupled to a mechanical testing machine (Fig. 1S). To

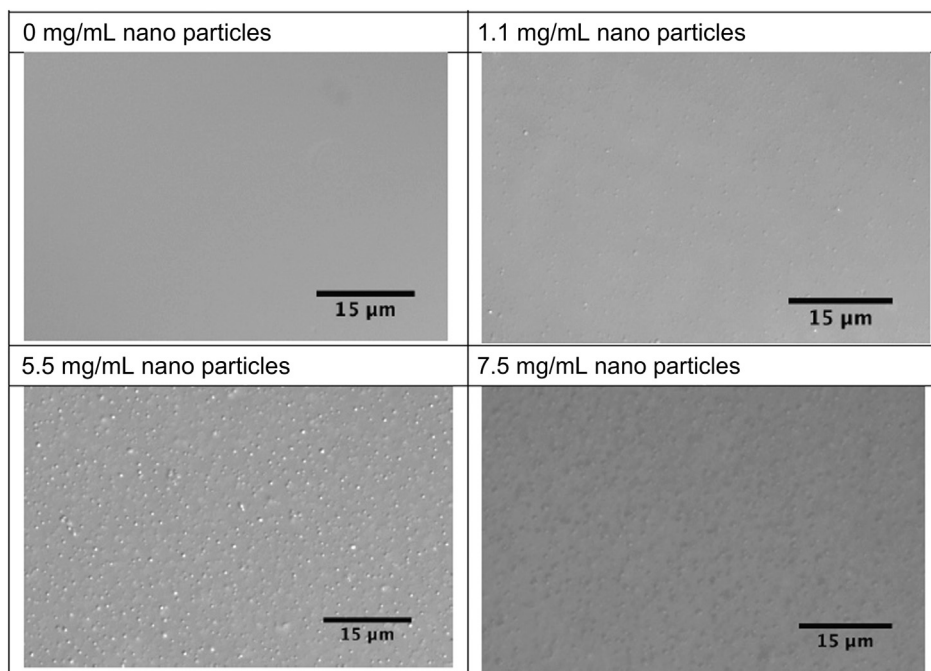


Fig. 3. Light microscopy images of PHEMA hydrogels with different concentrations of nanoparticles. The distribution of nanoparticles is shown for 0 mg/mL, 1.1 mg/mL, 5.5 mg/mL, and 7.5 mg/mL of nanoparticles concentration in the reaction mixture before polymerization. The 5.5 mg/mL nanoparticles concentration was used for the experiments.

simulate human body environment, the initial temperature of the thermally isolated system was set to 36 °C. It was verified that after 5 min of loading (5% of prestrain followed by a 15% deformation amplitude at 1.5 Hz) the temperature of the hydrogel reached 37 °C (Fig. 4A). In another experiment, we set the environment temperature to 34 °C and after 5 min loading the temperature reached 34.7 °C (Fig. 4B).

3.4. Drug release due to dissipation

Under mechanical loading, when environmental temperature was fixed to 36 °C, a statistically significant increase (133%) in Xylene Cyanole FF release was observed between the 5 and 8 min loading demonstrating the delayed release of the dye following a mechanical loading (Fig. 5A). To rule out the possibility that the mechanical loading directly induced the expulsion of the Xylene Cyanole FF out of the hydrogel, we repeated the experiment but setting the initial temperature of the thermally isolated system to 34 °C. In this test, the temperature of the hydrogel following the mechanical stimulation stayed below the LCST of nanoparticles (Fig. 4B). It can be observed from Fig. 5A, that when the hydrogel temperature is below the LCST of nanoparticles, there was no significant difference in Xylene release between 5 or 8 min loading. Finally to confirm the role of the nanoparticles in the observed phenomenon and to exclude that the observed effects were due to a direct thermal effect on the hydrogel, the tests at 34 °C and 36 °C were repeated with hydrogels that did not contain the thermo-sensitive nanoparticles. Results in Fig. 5B showed that only the combination of nanoparticles in hydrogels and mechanical loading inducing a temperature increase of the hydrogel above 37 °C allowed a statistically significant increase of the release of Xylene Cyanole FF.

3.5. Effective diffusion coefficient

When nanoparticles are incorporated in the hydrogels and the hydrogel temperature is above the LCST of the nanoparticles, the

effective diffusivity of the hydrogel increases by a factor 3 (Fig. 6). When the hydrogel contained no nanoparticles, the effective diffusivity of the hydrogel was not affected by the temperature increase.

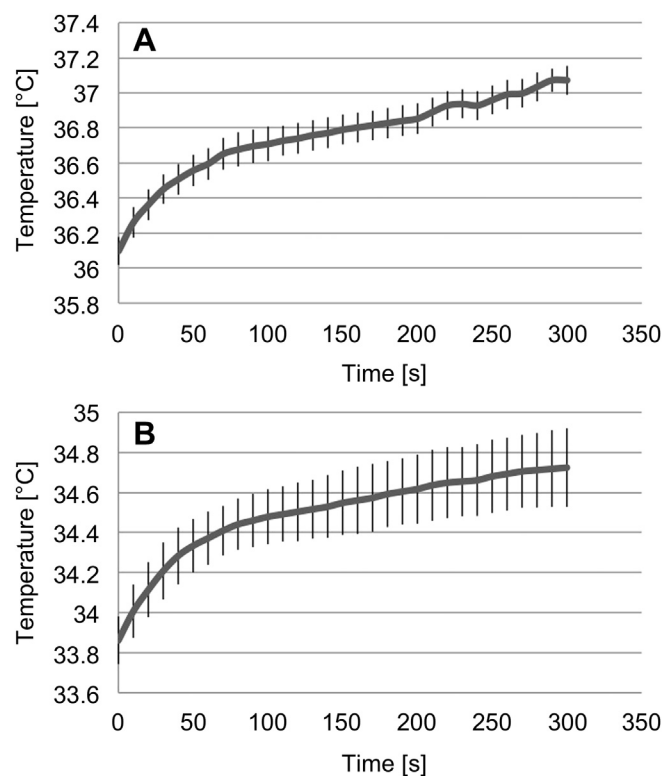


Fig. 4. Self-heating of the hydrogel following cyclic compressive load. The initial temperature was set in the test chamber (Fig. 1S) and increased due to the dissipated heat. A: The initial temperature was set to 36 °C and increased to 37 °C after 5 min of loading. B: The initial temperature was set to 34 °C and increased to 34.7 °C after 5 min of loading.

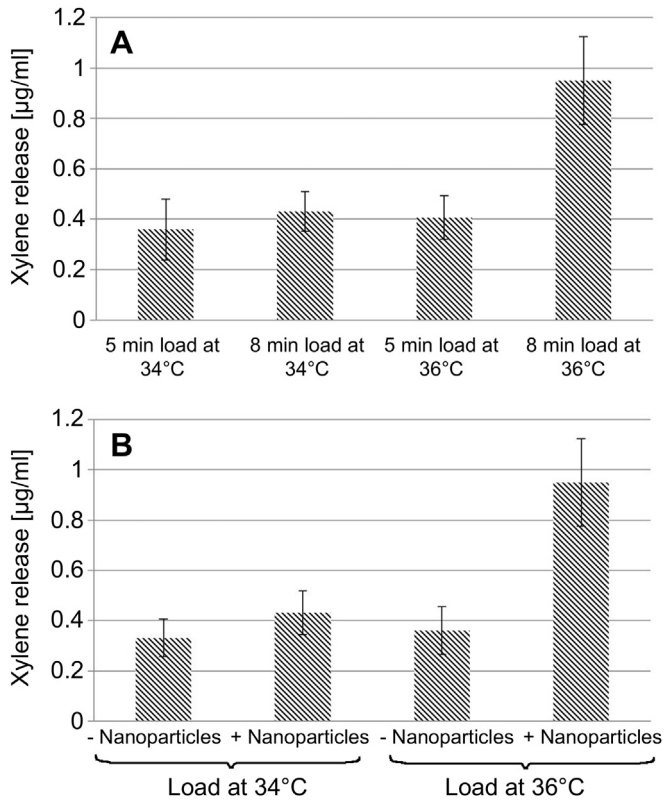


Fig. 5. A) Temporal control of Xylene Cyanole FF release triggered by dissipation properties of the composite hydrogel. The release of the Xylene Cyanole FF from hydrogels containing nanoparticles was quantified after 5 and 8 min of cyclic mechanical loading. When the initial temperature was set at 34 °C, no statistical difference was observed for the Xylene release between 5 and 8 min. When the initial temperature was set to 36 °C, a statistically significant increase (133%) in Xylene Cyanole FF release was measured between the 5 and 8 min loading ($p = 0.026$, T -test type 1, 1 tailed). B) Effect of nanoparticles on Xylene Cyanole FF release during mechanical loading. Mechanical load was applied on hydrogels with and without nanoparticles for 8 min at the initial temperature of 34 °C and 36 °C. No statistically significant increase in Xylene release was observed at 36 °C for hydrogels without nanoparticles. A statistically significant increase (125%) of dye release was measured when the load was applied on hydrogels containing nanoparticles and initially thermostated to 36 °C ($p = 0.031$, T -test type 1, 1 tailed).

4. Discussion

Mechanical loading has been demonstrated to activate growth factor receptors of cells involved in the healing process of different tissues [15,16,18]. However, a delay is necessary for the activation of

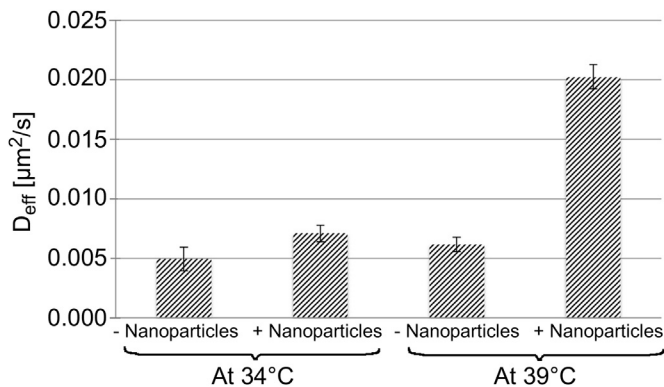


Fig. 6. Thin PHEMA hydrogel film effective diffusion coefficient (D_{eff}). D_{eff} was calculated by curve fitting of the mathematical model for Xylene Cyanole FF release from microfilm hydrogel with or without nanoparticles at temperature above and below the LCST of the nanoparticles.

cell receptors following the initiation of a mechanical stimulation [22]. A synergetic effect combining the delivery of a drug and the mechanically induced activation of the corresponding receptor could be maximized if drug release is delayed by several minutes following the initiation of the mechanical stimulation. In this study we proposed to use the dissipative property of hydrogel as a new environmental variable allowing to spatially and temporally control the release of a drug. The proposed delivery system could then not only provide unparalleled spatiotemporal control over the release process, but also allow the delivery to be synchronized with the mechanical stimulus favoring the healing of the damaged tissue. The dissipative properties of the hydrogel can be modulated to link the duration of the cyclic loading with a targeted increase in temperature. Thus, a specific delay between the initiation of the mechanical stimulation and the release of a payload can be obtained.

The most probable effect which can explain the results presented in Fig. 5A and B is to consider that the temperature-induced shrinkage of nanoparticles due to the dissipation properties of the hydrogel changed the permeability of the hydrogel. To verify this claim, the effective diffusion coefficient was calculated from Xylene Cyanole FF release experiments performed on thin layer hydrogel film. The obtained result confirmed that the augmentation of the hydrogel temperature affects its permeability. By itself, the observation that a cyclic mechanical loading can affect hydrogel permeability is an original result not previously reported. As the change in permeability is obtained following the temperature increase, which in turn is due to the mechanical loading, a time delay is obtained between the initiation of the mechanical loading and the release of the payload. The value of this time delay can be tuned by varying the LCST of nanoparticles [4,29] and/or the dissipative properties of the hydrogel as well as the loading amplitude and frequency.

As previously mentioned, different systems have been developed to couple mechanical loading and drug release [19–21]. However, most of these systems could be considered as a sponge containing a payload so that no specific correlation between mechanical loading and drug release could be controlled. Another advantage of the proposed system is that it considerably reduced undesirable passive drug release. If we measure the amount of passive release over days from the composite hydrogel (Fig. 2S, Supplementary data), we observe that after 5 days its value represents 9% of total loaded payload if the temperature is above the LCST and less than 4% of total loaded Xylene if the temperature is below the LCST. In comparison, the release due to mechanical load was about 1.2% of total Xylene after 8 min. The reluctance of the system to passive release is most probably due to the hydrophobic properties of PHEMA-based hydrogels. In spite of hydrophilic nature of HEMA monomers, it is postulated that PHEMA hydrogel has, in addition to its covalently linked network structure, a secondary structure stabilized by hydrophobic bonding [30]. It has been showed that for a hydrophobic porous material, because of an defiltration (drainage) and infiltration pressure, force is required to move liquid in and out of the material pores [31]. Without mechanical loading the structure is reluctant to exchange fluid and facilitate the payload release. Therefore the amount of passive release is highly decreased with the developed composite hydrogel.

High dissipation properties of PHEMA hydrogels also increase its toughness, which is important if the composite hydrogel is used at load-bearing sites like in cartilage. In general because hydrogels have poor mechanical properties and are very brittle, their use is limited for load-bearing applications. It has been shown that the poor mechanical performance and fragility of hydrogels mainly originate from their very low resistance to crack propagation due to the lack of an efficient energy dissipation mechanism in the gel network [32,33]. The high dissipation property of the developed

composite hydrogel is then a particular advantage also for its mechanical behavior.

5. Conclusion

Temporal control over the delivery process induced by a mechanical loading is proposed in this study and is an innovative way to deliver a payload. Dissipation properties of materials can be considered as a new environmental variable to trigger the release from thermosensitive, polymer-based delivery systems. A synergistic effect between loading and delayed payload delivery could be obtained with the use of dissipative phenomenon, which is not possible with other delivery systems.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.biomaterials.2013.09.065>.

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