Journal of
Materials Chemistry B
Materials for biology and medicine

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View Article Online
DOI: 10.1039/D5TB01457C

Reversible photo-induced formation of iron alginate hydrogels

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Sodium alginate is well-known to be crosslinked by various polyvalent metal ions. While calcium ions (Ca^{2+}) are being the most used, the crosslinking of alginate with other metal ions such are under much less attention as seen in the literature. For instance, Fe^{2+} and Fe^{3+} ions can also crosslink sodium alginate, though with varying strengths. A change in the charge of the iron ion can significantly affect the hydrogel's crosslinking density, potentially leading to full dissolution. This study demonstrates a novel approach to reversibly control of alginate hydrogel formation and dissolution using visible light as an external stimulus. Visible light irradiation (450 nm) leads to the decomposition of the iron-containing sandwich complex (ISC). Liberated Fe^{2+} ions undergo quick oxidation by potassium peroxydisulfate and the resulting Fe^{3+} ions crosslink alginate chains to form a hydrogel. Conversely, treatment with 405 nm visible light induces a redox reaction between lactic acid and Fe^{3+} ions. The recovery of Fe^{3+} into Fe^{2+} leads to the hydrogel's full de-crosslinking and reversion to a solution. Notably, this process can be performed in a single step via visible light irradiation. The photochemical processes are rapidly leading to gelation and re-gelation occurring within minutes. Envisaged applications of reversible photo-induced gelation are under discussion.

Introduction

Alginate is a polysaccharide-based polymer widely used in various industries, including medicine and biotechnology. It consists of a linear anionic biopolymer composed of β-(1,4)-linked D-mannuronic acid and α-(1,4)-linked L-guluronic acid residues and is typically extracted from the cell walls of brown seaweed.[1] Its structural similarity to living tissues[2] makes it a promising biocompatible[3] material for various biomedical applications[4] as drug delivery[5], wound healing[6], tissue engineering,[7] and cell therapy.[8] Moreover, recent advantages in hydrogel technologies have unlocked novel applications in environmental science,[9] forensic science,[10] and nanotechnology.[11]

There are two primary methods for preparing alginate-based hydrogels: chemical crosslinking, which results in more stable but irreversible gels, and physical crosslinking, which is reversible but generally less stable. Chemical crosslinking often involves modifying the alginate molecule with acrylic, methacrylic, or click reagents to initiate crosslinking reactions between them.[12] In contrast, physical crosslinking can be achieved through ionic crosslinking, in which metal cations bind to the alginate chains. Divalent and trivalent ions are commonly used for gel formation, with Ca^{2+} and Ba^{2+} being the most frequently employed divalent ions, and Fe^{3+} and Al^{3+} being the most common trivalent cations. Typically, ionic crosslinking of alginate hydrogels occurs by mixing sodium alginate with an inorganic salt solution, leading to rapid but uncontrollable gelation.[13,14] The use of iron ions as crosslinkers has gained attention because Fe^{3+} ions can induce strong crosslinking, whereas Fe^{2+} ions do not form hydrogels. The ability to switch the charge of iron ions allows for reversible control of the hydrogel's density.

Photopolymerization has also become an attractive alternative for hydrogel preparation, as it enables localized crosslinking. By varying the intensity or duration of irradiation, different crosslinking densities can be achieved. Photochemical crosslinking enables the production of hydrogels with predefined mechanical properties or gradient-based crosslinked structures.[15] Furthermore, the ability to control iron ion charge with light allows for the reversible adjustment of hydrogel density without the addition of external reagents.

While UV light (200-400 nm) is commonly used in photo-polymerization, it has significant limitations due to its shallow penetration depth and potential cytotoxicity, which restrict its use in biomedical applications. Additionally, many UV photoinitiators have poor solubility in water, further limiting their effectiveness.[16] Visible light (400-800 nm), on the other hand, offers several advantages, including lower toxicity to living organisms and deeper tissue penetration. This makes it an appealing option for photopolymerization, particularly in photodynamic therapy. Despite these advantages, there is limited research on visible and red-light-induced photogels.[12] Thus, the development of hydrogel systems that can be controlled by visible light may offer a non-toxic alternative for biomedical applications.

This study aims to develop a visible light-responsive reversible crosslinking hydrogel, using alginate as the base polymer and iron ions as the photo-reversible crosslinker. The gelation of alginate was induced by the photodecomposition of the iron sandwich complex (ISC), as in our previous studies.[17] Using the distinct crosslinking properties of iron ions, we induced a redox reaction between Fe^{3+} ions and sodium lactate, resulting in the reduction of Fe^{3+} to Fe^{2+}

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Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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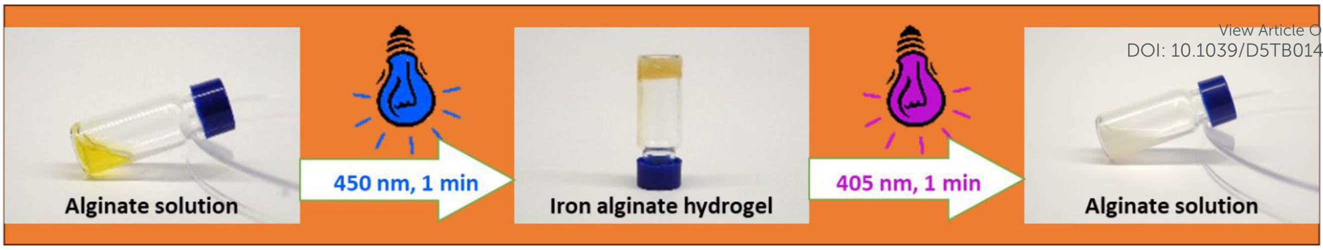

Figure 1 Reversible photo-induced gelation of alginate

ions, which subsequently led to the dissolution of the hydrogel. [18,19] Both processes were triggered by visible light (450 nm for gelation and 405 nm for dissolution), without requiring any additional chemical reagents between gelation and re-gelation steps **(Figure 1).** Inducing both chemical reactions separately, we achieved controllable formation and dissolution of the hydrogel.

Materials & Methods

Iron Sandwich Complex Decomposition Kinetics

0.1 ml of the iron sandwich complex solution (10 mg/mL, 0.033 mmol/mL) and citric acid (10 mg/mL, 0.052 mmol/mL) was irradiated in 96-well plate with an LED lamp (λ = 460 nm, 5 mW/cm²). After equal time intervals, the light absorption spectra were recorded, and the absorption at λ = 450 nm was measured.

Gelation Point Determination

A 30 mg sample of the iron sandwich complex $[C_5H_5Fe^+C_6H_5CH_3]BF_4^-$ (0.1 mmol) was mixed with 27 mg of $K_2S_2O_8$ (0.1 mmol) and dissolved in 2 mL of a 3% (w/w) sodium alginate solution (0.3 mmol). To this mixture, 1 mL of water and 220 µL of a 5% (w/w) sodium lactate solution (0.1 mmol) were added. 0.1 ml of the mixture was irradiated with an LED lamp (λ = 460 nm, 5 mW/cm²). Every 10 seconds, the lamp was switched off, and the reservoir was flipped. After 80 seconds of irradiation, hydrogel formation was occurred.

Preparation of Photo-Switchable Hydrogel

A 30 mg sample of the iron sandwich complex (ISC) $[C_5H_5Fe{+}C_6H_5CH_3]BF_4^-$ (0.1 mmol) was mixed with 27 mg of potassium persulfate ($K_2S_2O_8$) (0.1 mmol) and dissolved in 2 mL of a 3% (w/w) sodium alginate solution (0.3 mmol). To this mixture, 1 mL of water and 220 µL of a 5% (w/w) sodium lactate solution (0.1 mmol) were added. A small portion of the mixture (0.3 mL) was irradiated with an LED lamp (λ = 460 nm, 20 mW/cm^2) for 1 minute. The freshly formed hydrogel was stored under room conditions for 1 hour before being irradiated with an LED lamp (λ = 405 nm, 50 mW/cm^2) until complete dissolution.

Water retention control via light irradiation

Photo-switchable mixture was prepared as described above. 1g of the mixture was placed into pre-weighed plastic Petri dish and spread across its surface. The amount of the mixture was controlled via weighting because of high viscosity of alginate solutions, which prevents precise volume measurement. Samples were divided into 3 groups and irradiated by 460nm light (10 mW/cm^2) 3, 5 and 7 minutes. After formation of hydrogels, 10 ml of distilled water was added into each Petri dish. All dishes were stored for 20 hours in darkness. After storage all water was eliminated, Petri dishes were weighed with hydrogels inside, and hydrogel mass changes were calculated.

Photopatterning of the hydrogel via laser irradiation

Photo-switchable mixture for laser-induced gelation was prepared as described above, but 1ml of $K_4[Fe(CN)_6]$ solution (3g/L) was taken instead of 1 ml of water. Ferricyanide was used as a specific dye, which produces blue color upon interaction with Fe^{3+} ions (Prussian blue). A small amount of the mixture was placed between two microscopic glasses. Gelation was induced using 450 nm laser. Laser beam was focused on the sample using objective lens (NA 0.2). Using beam expansion optics, laser beam was enlarged to fill the rear lens of the objective, thus ensuring diffraction limited performances. Theoretical spot size was calculated to be ~30 um, 65 um distance between spots was set. Beam intensity was measured to be 19 mW. 3 ms espousers were used to cure the alginate locally.

Results & Discussion

Iron Sandwich Complex Decomposition Kinetics

Photochemical decomposition of the ISC was determined by monitoring the decrease in the light absorption of the iron sandwich complex (ISC) solution during light irradiation.

During light treatment, the formation of precipitates was observed. To ensure complete dissolution, the solution was treated in short steps (10 seconds) with the precipitate being suspended after each interval. Citric acid likely interacts with the released iron ions at a slower rate, but the solution becomes transparent again after suspension. The absorption spectra of the iron sandwich complex solution revealed two peaks **(Figure 2)**.

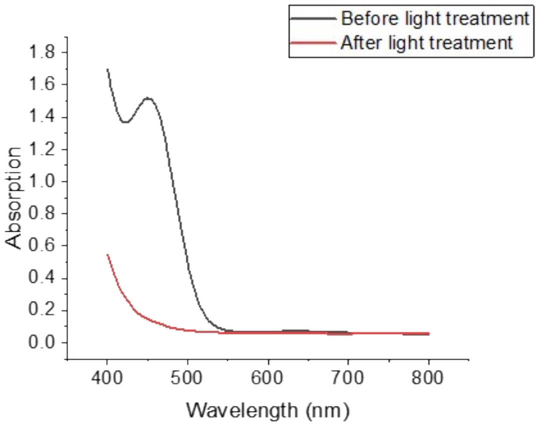

Figure 2 Light absorption spectra of ISC before and after light treatment

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The first peak (λ = 450 nm) decreases with increasing light exposure, indicating the presence of the complex. The second peak in the UV region (< 400 nm) is likely due to π-π conjugation in the arene molecule,[20] both before and after complex decomposition.

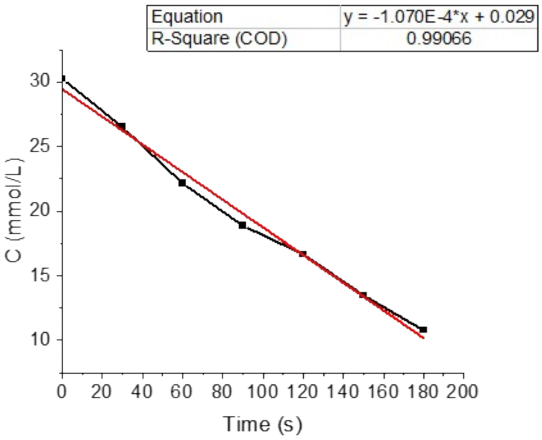

Figure 3 Linear approximation of the ISC decomposition

Kinetics of ISC decomposition is presented on **Figure 3**.

Gelation Point Determination

At 80 seconds of light irradiation hydrogel formation had occurred. The concentration of ISC in the gelation point is calculated to be 0.020 mol/L (**Figure 3**). This implies that the residual concentration of ISC at the gelation point was calculated at approximately 66%. ISC was taken in 1:3 molar ratio to alginate carbohydrate monomers, but liberation of 33% Fe^{3+} ions was enough for inducing of hydrogel formation.

Preparation of Photo-Switchable Hydrogel

The proposedmechanism of reversible photo-induced gelation of alginate is presented on **Figure 4**. During the first step of light treatment (λ = 450 nm) photochemical decomposition of ISC leads to release of Fe^{2+} ion, which are rapidly oxidized by potassium peroxydisulfate. The resulting Fe^{3+} ions interact with alginate chains, forming a cross-linked hydrogel. This process was described in our previous work.[17] During the second step of light treatment (λ = 405 nm) Fe^{3+} ions in iron alginate hydrogel are reduced by lactic acid to Fe^{2+} ions, which crosslink alginate chains only weakly. As a result, crosslinking density of the hydrogel decreases, which leads to complete dissolution of the hydrogel. This process and its kinetics were described in other studies.[18,19] Briefly, 405 nm light induces redox reaction between Fe^{3+} and lactate, what leads to production of Fe^{2+} ion and aldehyde formation (**Figure 4**).

In our experiments, 30 mg of the iron sandwich complex produced 3 mL of hydrogel mixture. It is preferable to prepare small batches (1-3 mL) to avoid spontaneous gelation, which can occur after prolonged storage, even under refrigeration (+4°C) and in the absence of light. This is likely due to the slow decomposition of the iron sandwich complex by potassium persulfate. The interaction between $K_2S_2O_8$ and the complex in the presence of potassium hexacyanoferrate (IV) results in the formation of a blue color, indicating the presence of free Fe^{3+} ions. Additionally, we observed that the iron sandwich complex also produces a blue color with potassium hexacyanoferrate(III) in solution, though to a lesser extent, suggesting a small presence of Fe^{2+} ions in the dry complex crystals. This could be due to accidental light exposure during synthesis, $K_2S_2O_8$ and potassium hexacyanoferrate(II) indicates rapid oxidation of these pre-liberated ions and slow experiments, or storage. The more intense blue color observed with decomposition of the sandwich complex without light exposure.

A mixture of sodium lactate with $FeCl_3$ in the presence of potassium hexacyanoferrate(III) also turns blue after several hours of dark storage. This suggests that the recovery of Fe^{3+} ions occurs slowly even without light exposure. Notably, no decomposition of the freshly formed iron-alginate hydrogel was visually observed during long-term storage.

In a closed vial, the photo-induced gelation-dissolution cycle occurs only once. Initially, the iron sandwich complex decomposes under blue light (λ = 450 nm), releasing Fe^{2+} ions and initiating the photochemical reaction. Fe^{2+} ions are rapidly oxidized by potassium persulfate, and the resulting Fe^{3+} ions crosslink the sodium alginate. Upon treatment with violet light (λ = 405 nm), sodium lactate reduces Fe^{3+} ions back to Fe^{2+}.

Although the measured absorption peak of the ISC is at 450 nm, a light source with 460 nm peak wavelength was used. The absorption band of the ISC is sufficiently broad to enable its photodecomposition under 460 nm irradiation and even at slightly higher wavelengths **(Figure 2)**. According to measured data, absorbance at 460 nm is slightly lower than at 450 nm, corresponding to 96.8% of the maximum. Moreover, most commercially available LED sources have wide bands of irradiation. For example, LED diodes used in this experiment (TDS-P001/3L4) have 20 nm band pass.

Therefore, the 10 nm difference between the ISC absorption maximum (450 nm) and the LED emission peak (460 nm) does not significantly affect the experiment.

Photo-Induced Gelation in Microfluidic Devices

In contrast to a closed vial a flow-through microfluidic setup allows for repeated gelation and re-gelation cycles. A T-shaped microfluidic channel was used in an experiment where solution was pumped through the system **(Figure 5)**. Eppendorf tube A collected fractions, while Eppendorf B served as a waste tube. The system was kept in darkness during the experiment.

Initially, the solution flowed through both tubes. After 90 seconds, Eppendorf tube A was replaced with an empty one, and a portion of the channel was irradiated with blue light (λ = 460 nm, 1 min) via a photomask. After the second pumping cycle, the solution no longer flowed into tube A, but the flow into tube B increased. After another 90 seconds, tube A was replaced again, and the same channel area was irradiated with violet light (λ = 405 nm, 1 min). The flow resumed into tube A, demonstrating reversible gelation. This process was repeated five times, confirming the ability to alternate gelation and re-gelation in the microfluidic setup.

Water retention control via light irradiation

As the hydrogel layer in the Petri dish was thicker than in previous experiments, a higher light intensity and longer time of irradiation were chosen. Prolonged light exposure led to the formation of dense hydrogels in all samples, which did not mix with water and retained

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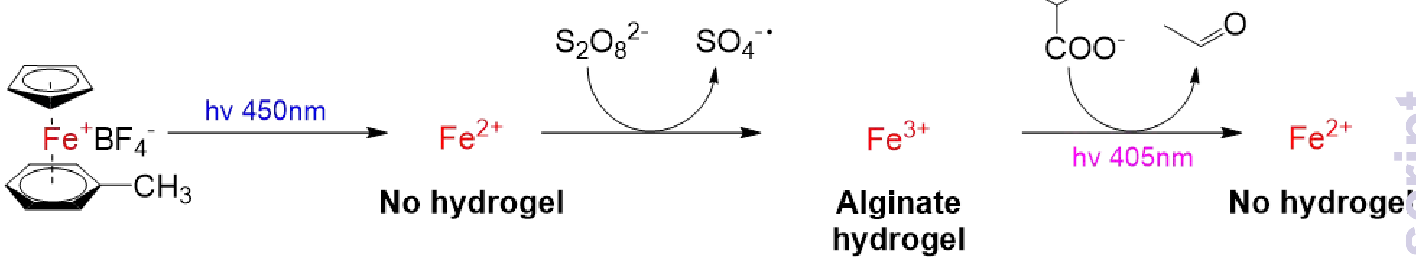

Figure 4 Proposal mechanism of reversible photo-induced alginate gelation

their shape throughout the experiment. By the end of the experiment, the water in Petri dishes acquired slight yellow color, which can be explained by free diffusion of non-decomposed ISC molecules. Changes in hydrogels mass are presented in **Figure 6.** It is clearly seen that water retention decreases with increasing light exposure time, as expected. Hydrogels with the shortest time of light treatment (3 min) demonstrated highest mass increase up to 74%, whereas those irradiated for 7 min exhibited a much lower mass gain, not exceeding 12%. This effect can be explained as follows: longer light exposure generates a higher concentration of Fe^{3+} ions, what leads to higher crosslinking density, which increases the crosslinking density of the hydrogel and consequently reduces its swelling capacity. Therefore, by varying the irradiation time, it is possible to control the physical properties of the hydrogel.

Photopatterning of the hydrogel via laser irradiation

Ferricyanide was used as a Fe^{3+} ions indicator in this experiment. Free Fe^{3+} ions, released during photochemical reaction, interact with ferricyanide with Prussian blue formation:

$$Fe^{3+} + K4[Fe(CN)_6] = KFe[Fe(CN)_6]$$

Iron alginate microgel particles colored by Prussian blue were observed (**Figure 7**) this way. Using the same principles of point gelation, complicated patterns were formed. Initially, the alginate mixture was treated by the same 450 nm laser (**Figure 8a**). When gelation was performed and the word became visible, the sample was treated by an LED lamp (λ = 405 nm, 50 mW/cm²). One half of the word was covered by a non-transparent material, while the other half was irradiated. Finally, the irradiated part became almost invisible after 180 seconds of irradiation (**Figure 8b**).

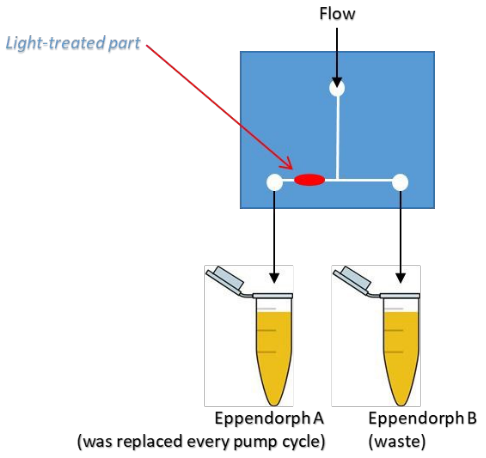

Figure 5 Illustration of T-shaped microfluidic channel blocking experiment

The photopatterning experiment represents the opportunity of formation of small hydrogel structures by laser beam. According to our calculations, height of capital letter is around 2,03 mm, while width of Prussian blue colored hydrogel line is around 100 µm. Thus, we present the opportunity of formation of microscopic-size hydrogel objects with complicated shapes.

It can be clearly seen that during the photo-dissolution experiment the right part of the pattern changed its color from yellow to transparent (**Figure 8**), what may witness decomposition of the ISC and reduction of Fe^{3+} ions to Fe^{2+}. Meanwhile, the left part of the pattern changed its color from transparent-yellow to blue, what witnesses about presence of Fe^{3+} ions in the mixture. This effect can be explained in the following way: as ISC can absorb wide spectral range of light (**Figure 2)**, it probably may be decomposed even at 405 nm irradiation. Probably, both chemical reactions (ISC decomposition and iron ions recovery) may be induced by 405 nm light. While ISC presences in the mixture, it releases iron ions, which immediately interact with the sodium lactate and recover themselves to Fe^{2+}. At current concentrations, which were taken in this experiment, Fe^{3+} ions were liberated faster than can be recovered to Fe^{2+}. Thus, blue coloring because of accidental 405 nm light treatment was observed.

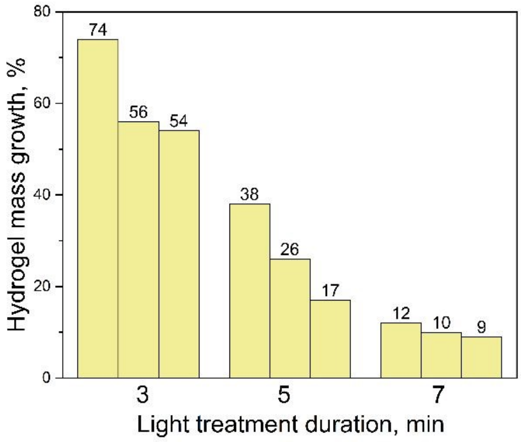

Figure 6 Swelling of different time light-treated hydrogels during storage in water

One can clearly see that the letters in the word are curved and wavy, while microgel particles seem to be strictly oriented in lines **(Figure 6).** This visual effect may relate to Prussian blue dye diffusion in thin layer of hydrogel mixture or small accidental shift of cover glass during experiment.

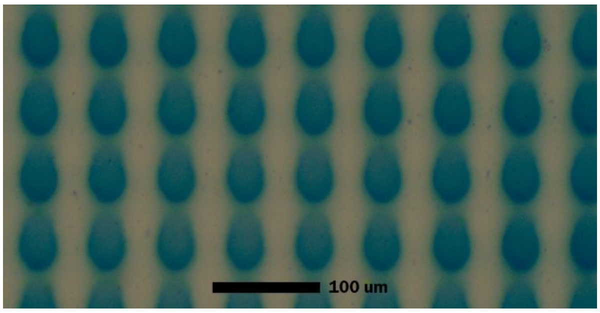

Figure7 Photochemically-formed iron alginate microgels, colored by Prussian blue

The successful photopatterning of iron alginate hydrogels demonstrates the feasibility of forming high-resolution structures, which makes this system suitable for various photolithography applications. Lasers can be focused on micrometer and even sub-micrometer spots, and this experiment witnesses that formation of iron alginate microgel particles is achievable.

Point-wise formation of microgels in flow-through microfluidic devices could find some new applications in drug delivery systems. For instance, drug-loaded iron alginate microgels with tunable crosslinking density could be produced using this approach. Moreover, the high spatial precision of laser irradiation enables the fabrication of small, complex hydrogel scaffolds that can mimic natural tissue structures [21]. Other photolithography techniques, such as digital light processing, could also be applied to produce heterogeneous, tissue-mimetic platforms for fundamental biological studies [22].

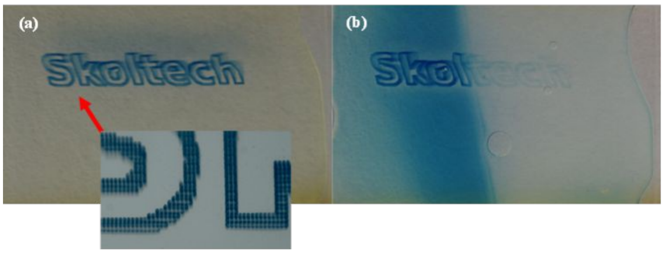

Figure 8 Photochemically-formed (a) and step-wised dissolved (b) hydrogel pattern

Conclusions

This study demonstrates that reversible photochemical gelation of alginate and its dissolution is feasible. This transition can be achieved once in a closed system but can be repeated multiple times in a flow system, with the process initiated solely by visible light. Photochemical formation of alginate hydrogel provides rapid and easy formation of homogenously crosslinked gel under exposed area, what is hard to achieve via other approaches such as direct mixing of sodium alginate with metal ions because of fast and inhomogeneous crosslinking. Light-triggered formation of hydrogel gives a way to produce gel particles of complex shape. These structures can be dissolved at later stages at subsequent light exposure at different wavelengths. Due to biocompatible nature of alginate, these hydrogels have potential applications in alginate-based biomaterials, media for cells proliferation and drug delivery systems where objects of interest can be caught, embedded in gel and later release solely by optical means. Investigation of compatibility of that photo-crosslinkable hydrogel with different biological cells are remaining a subject for further study as well as exploration of advantages and possible limitations of reversible iron alginate hydrogel application.

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Author contributions

A. Sokolov —investigation & writing (original draft), A.S. Abdurashitov — investigation (experiments with laser-induced formation of hydrogels) and writing (review & editing), P.I. Proshin —writing (review & editing), G.B. Sukhorukov — supervision and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgements

The authors acknowledge head of the laboratory for functionalized organoelement compounds INEOS RAS D.S. Perekalin for consultation and provision of facilities for the iron sandwich complex synthesis.

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DOI: 10.1039/D5TB01457C

Dear Editor,

We are pleased to submit the manuscript entitled "*Reversable photo-induced formation of iron alginate hydrogels*" by A.S. Sokolov, A.S. Abdurashitov, P.I. Proshin, G.B. Sukhorukov to be considered for publication as a research article in the *Chem Comm*.

The authors confirm that the data supporting the findings of this study are available within the article.

Sincerely,

Sokolov Alexey, corresponding author, on behalf of all authors,

Skolkovo Institute of Science and Technology