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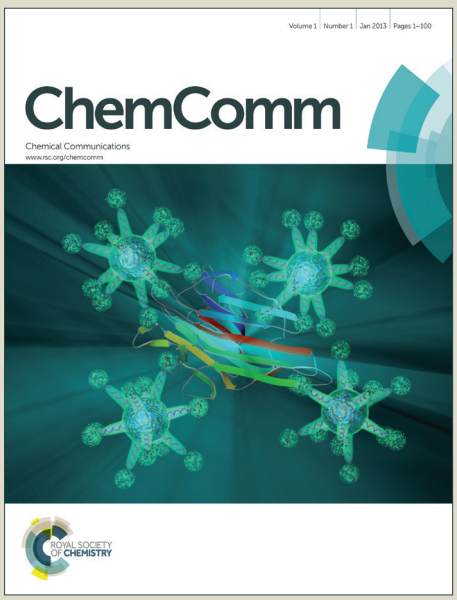

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ARTICLE TYPE

Towards Intelligent Bioreactor Systems: Triggering the Release and Mixing of Compounds Based on DNA-Functionalized Hybrid Hydrogel

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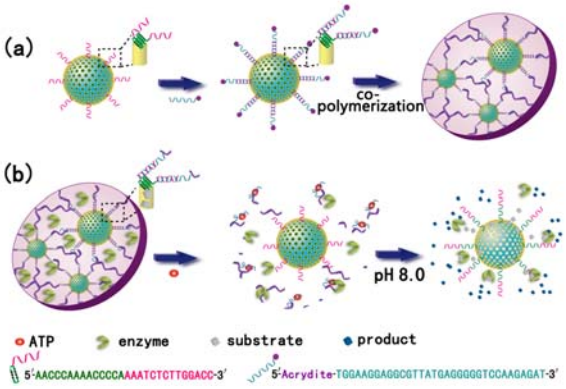
Herein we designed and synthesized an intelligent mesoporous silica nanoparticles-DNA hydrogel bioreactor system whose function could be controlled by external stimulus. The system allowed the simultaneous incorporation of multicomponent and the separation between the components could be destroyed by the structure change of DNA to enable the start of a reaction.

The development of efficient systems for the protection and sustained release of encapsulated molecules have garnered considerable attention in recent years for their potentials to improve how we treat disease and study complex biochemical processes.[1] Owing to the unique features such as three-dimensional, elastic networks and fast phase transitions upon external stimuli, multifunctional responsive polymer hydrogels have received particular interest and have been recognized as new and promising materials for encapsulating a variety of biomacromolecules.[2] For instance, the hydrogel can act as a "cage" and hide enzyme molecules to prevent them from interacting with the substrate until desired.[2b] Despite the burgeoning achievements, challenges still remain in their complexity as well as efficacy. Recently, the ever growing nanotechnology has inspired researchers to incorporate nanomaterials into the hydrogels to marry the nanoscale world with that of materials of macro dimensions.[3] The three-dimensional network structures of hydrogels can be used as scaffolds to incorporate various nanomaterials (metallic, inorganic, bioactive, etc.), resulting in hybrid hydrogels with enhanced properties.[3b-f] For example, by encapsulating mesoporous silica nanoparticles (MSP) into hydrogel scaffolds, a hybrid system that allowed the sustained separation of anionic dyes and enzyme within a single capsule for selective polyanions detection had been demonstrated.[3d] Although the heterostructured hybrid hydrogels hold great promise in biomaterials and microdevices, how to make these novel 3D hybrid materials to perform further functions still remains a big challenge in this field.

Performing biochemical reactions entails the controlled mixing of reactants. Various strategies for the fabrication of bioreactors with the ability of releasing and mixing of biologically or chemically relevant substances have been developed.[4] However, examples of synthetic DNA-incorporated controllable bioreactors with properties of accommodating multiple compartments are still scant. As a well-known biopolymer, DNA has been increasingly employed for material purposes.[5] It is well suited to the task of producing "smart" system with sensitivities toward various non-invasive stimulus.[5c-e, 6] For example, intelligent DNA-based logic gate systems that were responsive to various targets and could function in vitro had been developed.[5c] DNA cross-linked hydrogel which could realize sol-gel transition through DNA-strand displacement had recently been demonstrated.[5d] Inspired by the unique properties of DNA molecules, we expected that a combining of DNA with hydrogel could provide a novel strategy for accommodating multiple compartments, for example, an enzyme and its substrate, in the same capsule. Such multi-compartment capsules could exhibit exciting potential: the separation between the compartments could be selectively destroyed, thus enabling a mixing and, hence, the start of a reaction. In the present study we show the feasibility of conducting bioreactions by triggering release and mixing of compounds stored individually in the reactor system. This proof of concept of an externally triggerable intelligent bioreactor may be a step forward in equipping artificial biosystem with complex functionalities for technical as well as biomedical applications. The working principle of this hybrid system is shown in Scheme 1. In the hybrid system, two distinct microdomains, namely the inner nanopores of MSP and aqueous gel bulk phase, were produced in semi-wet condition. Two pieces of DNA, DNA1 and DNA2 were used in this work. The single strand DNA1 containing two functional domains: a 14-mer interlocking i-motif domain with cytosine-rich stretches (marked in green in Scheme 1) and a 14-mer domain that could hybridize with DNA2 (marked in red) was first anchored to the openings of the MSPs (MSPs-DNA1). At low pH, DNA1 folded into the interlocking i-motif structure, the pores were capped by the quadruplex and the release of the substrate was strongly inhibited. Meanwhile, DNA aptamer (DNA2) (marked in green-blue) labeled with acrydite was attached to the MSPs through DNA complementarity to form MSPs-DNA1/DNA2. When mixed with acrylamide, the solution was in transparent liquid form. The addition of initiator finally resulted in the transformation of the solution into hydrogel (Scheme 1b). The feasibility of conducting bioreactions in our hybrid hydrogel system could be demonstrated by embedding enzyme inside the 3D network of gel matrix and its substrate within the MSPs (Scheme 1b). When a target (ATP) was



Scheme 1 (a) Schematic presentation of the formation of mesoporous silica entrapped DNA-functionalized hydrogels. (b) Operation principle of the DNA-functionalized gel used for intelligent bioreactor by encapsulating the enzyme in the gel and the substrate in the mesoporous silica.

introduced, the aptamer would bind with it, and the gel would then be dissolved as a result of reducing the cross-linking density by competitive target-aptamer binding. When the pH was increased to basic, DNA1 would unfold to a single-stranded form as the optimal i-motif assembly occurred at about pH 5.5, a value close to the pKa for free cytosines.[7] A cascade of events could thus set in motion, whereby ATP binding triggered an enzyme release, changing of the pH allowed the substrate to release, which, finally, resulted in the on-demand mixing of substrate and enzyme for carrying out an enzymatic reaction.

To perform the experiment, MSP was synthesized according to a previously reported method.[8] The resulting particles (100 nm diameter) that contained hexagonally arranged pores were confirmed by TEM, SEM and X-ray diffraction (Fig. S1 and S2). The surface of MSP was first functionalized with amine groups through reacting with 3-aminopropyltriethoxysilane to afford MSP-NH_2. N_2 adsorption-desorption isotherms of MSP-NH_2 revealed a typical Type IV curve with a specific surface area of 995 m^2 g^{-1} and average pore diameter of 3.2 nm (Fig. S3 and Table S1). The as-prepared MSP-NH_2 was then functionalized with carboxylic groups by reacting with succinic anhydride to afford MSP-COOH, which was further reacted with 5-amino-modified DNA1 to yield MSP-DNA1. The successfully anchoring of DNA was demonstrated by FTIR and ^{13}C CP-MAS NMR spectroscopy (Fig.S4, S5) and the immobilization amount was about 8.72 μmolg^{-1} SiO_2 (Fig. S6).

To study the secondary structure of the DNA1 and DNA1/DNA2 under different pH values, circular dichroism (CD) and UV-Vis melting experiments were carried out. As shown in Fig. S7A, the CD spectra of DNA1 at pH 8.0 exhibited a positive peak at 276 nm and a negative peak at 245 nm respectively. At a lower pH of 5.5, a shift and increasing of the positive peak near 276 nm was observed, showing that the cytosine-rich oligonucleotide DNA1 folded into an i-motif structure.[7] We further examined the structural conversion of 1:1 mixture of DNA1 and DNA2. The positive peak at 270 nm showed that the dominant structure of the mixture was B-form duplex at pH 8.0. However, the CD intensities at 270 nm decreased and its maximal position was shifted from 270 to 280 nm through a pH change from 8.0 to 5.5, which indicated the formation of i-motif structure. Compared to DNA1, the two transitions in the melting profile of DNA1/DNA2 further confirmed the formation of both duplex and i-motif structure (Fig. S8).

The above observations were consistent with the nature and properties of the DNA used in the hydrogel. To study the principle of the bioreactor, we chose β-D-galactosidase (β-gal) and its substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) as a model system. β-gal is a well-known enzyme and can catalyze hydrolytic of X-gal into indigo derivative (product), which will induce a color change from transparent to dark blue.[9] To investigate the proton-responsive gating behavior of the MSPs-DNA1 system, X-gal was first loaded and the pore was subsequently capped by i-motif quadruplex by adjusting the pH of the solution to 5.5. As could be seen in Fig. S9, a very clear and highly effective pH-operable gating effect was demonstrated by monitoring the absorbance of the enzymatic product (401 nm) as a function of time. When the pH value was adjusted to 8.0, 91% release was obtained after 80 min. However, only negligible release occurred at pH 5.5 under the same condition. These data clearly demonstrated that we were able to close the pore system of MSP with i-motif quadruplex DNA, and to release the loaded substrate by pH stimulus.

Subsequent the sol-gel transitions of the hybrid system were examined through the flow behavior. To help visualize the gelling transition, fluorescein isothiocyanate (FITC) was incorporated covalently into the silica walls by following a reported procedure.[1f] By mixing the assembled MSP-DNA1/DNA2 with the acrylamide solution, we obtain a fluid system. The system finally gelled after the copolymerization reaction, whereas system with MSP/DNA2 or MSP/DNA1 still kept fluid state under identical conditions (Fig. 1, Fig. S10, S11). Meanwhile, it was apparent that the acrylamide solution without MSP-DNA1/DNA2

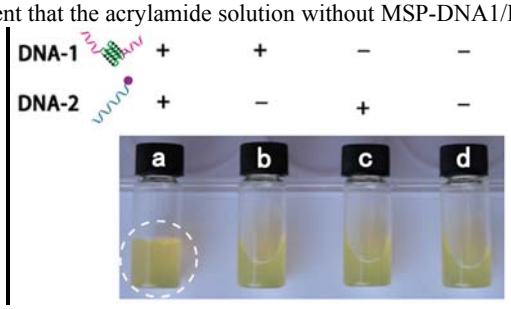


Fig. 1 Photograph of the sol transition switched by co-polylization. The vials were in a tilted position.

did not gell either due to the low cross-link density (Fig. 1d). Those results indicated that the hybridization between DNA1 and DNA2 played an important role for the observed sol-to-gel transition. By further addition of the ATP, the hydrogel reverted to the fluid state (Fig. S12, S13). Instead of the ATP, CTP, UTP, or GTP was not able to induce significant change of the DNA-functionalized hydrogel (Fig. S14), suggesting that the observed transition was indeed triggered by ATP/aptamer interactions.

To demonstrate the feasibility of conducting bioreactions in a controllable manner, we introduced β-gal and X-gal into the gel system. As shown in Fig. 2, after introduction of ATP to the hydrogel, a homogeneous fluid was obtained with evenly

dispersed enzyme and MSP-X-gal. No significant color change was observed for the sol mixture at pH 5.5 or the gel at pH 8.0 (Fig.2b, Fig. S15). When the pH value of the sol was gradually adjusted to 8.0, significant green color was observed due to the overlap of the yellow (FITC) and blue (enzymatic product) color (Fig. 2c, Fig. S16). The phenomena revealed the start of enzyme reaction after changing the pH to allow the delivery of X-gal from the pores to the solution. To further explore the result, we repeated the same experiment using a mutated linker (DNA3) which was shown to be incapable of forming i-motif structures. As shown in Fig. S16, green color was observed at pH 5.5 due to the lack of the i-motif quadruplex on the surface of MSPs. Moreover, when the experiment was carried out with a mutated linker (DNA4) that could not hybridize with DNA2, the solution remained sol after the polymerization (Fig. S17). Those results strongly suggested that the correct sequence of DNA played an important role in the operation of the bioreactors.

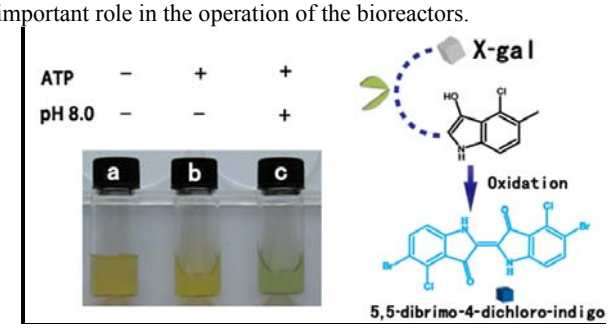


Fig. 2 Photograph of the on-demand enzymatic reactions carried out in the MSPs entrapped DNA-functionalized hydrogel. The figure shown in the right illustrated the enzymatic reaction of X-gal in presence of β-gal.

In summary, for the first time, we have designed and synthesized an intelligent and reliable MSP-DNA hydrogel bioreactor system whose function could be controlled by external stimulus. In the system, two distinct microdomains, namely the inner nanopores of MSP and aqueous gel bulk phase, were produced in semi-wet condition and were orthogonal to each other. This allowed the simultaneous incorporation of multiple components, for example, an enzyme and its substrate in the same system. The separation between the components could be selectively destroyed by the structure change of DNA molecules, thus enabling a mixing and, hence, the start of a reaction. Using MSPs with DNA of several different sequences, one can extend the process and realize a number of sequential reaction steps by programmed triggers. As the hydrogel can be easily manipulated and processed, we envision that our synthetic hybrid bioreactor system with an outstanding combination of properties may advance the field of artificial sophisticated biosystems and has great potential for biotechnical as well as biomedical applications.

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Notes and references

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