Aptamer-functionalized superporous hydrogels for sequestration and release of growth factors regulated via molecular recognition

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A R T I C L E   I N F O

Article history:
Received 10 May 2014
Accepted 1 June 2014
Available online 18 June 2014

Keywords:
Porosity
Hydrogel
Affinity
Controlled drug release
Growth factors
Protein adsorption

A B S T R A C T

While the discovery of highly potent biologics has led to the development of promising therapies for various human diseases, biologics can cause severe toxicity if delivered inappropriately. Thus, great efforts have been made to synthesize polymeric systems for safe and efficient delivery of biologics. However, the application of polymeric delivery systems is often limited by problems such as harsh reaction conditions, low drug sequestration efficiency, and difficult drug release regulation. This study was aimed at developing a superporous material system with a hydrogel and an aptamer to overcome these challenges. The results have shown that the superporous hydrogel is capable of instantaneously and fully sequestering a large amount of growth factors, owing to the presence of superporous architectures and aptamers. Moreover, the sequestering and loading procedure does not involve any harsh conditions. The release kinetics of growth factors can be molecularly modulated by either changing the binding affinity of the aptamer or by using a triggering effector. Therefore, this study presents a promising superporous material for the delivery of highly potent biologics such as growth factors for clinical applications.

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1. Introduction

Advances in life science and biotechnology over the past few decades have led to the discovery and development of highly potent biologics such as growth factors, cytokines, peptides, and vaccines [1–3]. While biologics hold great potential for treating various diseases such as cancer, cardiovascular diseases, and ocular disorders, their safe and effective delivery in the human body remains a long-standing challenge in a clinical setting [4]. Traditional delivery methods rely on subcutaneous, intramuscular, or intravenous injections of liquid formulations. These methods are technically simple, fast, and inexpensive. However, biologics usually have short half-lives in vivo and can be quickly cleared before reaching target sites at a sufficient concentration [5–7]. As a result, multiple administrations and substantial dosages are often necessary to produce therapeutic effects. These necessities not only raise treatment costs, but also lead to a wide biodistribution of biologics and generate systemic side effects [8–10]. Therefore, great efforts have been made to develop polymeric systems for the localized delivery of biologics [11,12].

Hydrogels are commonly studied for the development of drug delivery systems since hydrogels contain a large amount of water and have structural and viscoelastic similarities to human tissues [13]. However, a significant percentage of loaded biologics can be released from hydrogels within a few days or even hours [14]. Thus, the incorporation of affinity ligands such as heparin or metal ions has been widely used for the functionalization of hydrogels to overcome this problem [15–19]. While these affinity molecules can function as binding effectors of the loaded biologics, they have problems such as low affinity, low specificity, and high toxicity. Recently, nucleic acid aptamers were applied to approach this challenge [20,21]. Aptamers are short oligonucleotides that can, in principle, be selected against any biologic in an in vitro environment [22,23]. Because of their rigorous selection procedure, aptamers bind to their target molecules with high affinities and specificities [24]. While aptamers were shown to reduce the rapid release of growth factors from hydrogels, the initial burst release remained significant, which indicates the difficulty of retaining growth factors within the hydrogels synthesized via the methods reported previously [20,21]. Moreover, the involvement of harsh conditions such as free radicals can cause the denaturation of a large amount of growth factors [20]. Finally, the loading amount of growth factors in a large hydrogel was at the level of picograms [20,21], which is far from the requirement of real applications that need at least several hundred nanograms or even several...
the sensor chip was activated by carboxyl group-functionalized sensor chip (Reichert Analytical Instrument; Depew, NY). A HeNe red laser (633 nm), respectively, using a Coulter FC500 (Beckman Coulter, Brea, California). Ten thousand events were collected.

2.1. Materials

Sodium bicarbonate, acetic acid, N,N,N',N'-tetramethylethylene-1,2-diamine (TEMED), ammonium persulfate (APS), Tween 20, phosphate buffer solution (PBS), and sodium azide were purchased from Fisher Scientific (Pittsburgh, PA). Poly(ethylene glycol) diacylate (PEG700DA, Mₙ = 700) and Pluronic F-127 were obtained from Sigma–Aldrich (St. Louis, MO). All oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) and are listed in Table 1. Platelet-derived growth factor BB (PDGF-BB, MW = 24,310 Da) was purchased from Peprotech (Rocky Hill, NJ). Bovine serum albumin (BSA) and 2.2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Invitrogen. Streptavidin-coated polystyrene microparticles (5.2 µm diameter) were purchased from Spherotech (Lake Forest, IL).

2.2. Methods

2.2.1. Examination of the functionality of Apt-H in binding to PDGF-BB using a microparticle assay

Apt-H-functionalized microparticles were prepared via biotin-streptavidin binding. Fifteen microliters of streptavidin-coated microparticles (0.5% w/v) were incubated with 11 pmol of biotinylated aptamer for 1 h at room temperature. The aptamer solution was prepared by dissolving the aptamer in PBS containing 0.1% BSA and 0.05% Tween 20. After incubation, the microparticles were washed to remove free aptamers with centrifugation at 10,000 rpm for 10 min. The microparticles were resuspended by adding 0.3 ng PDGF-BB. After 2 h of incubation, the suspension was treated with 11 pmol of complementary sequence (CS) for 1 h. The CS-treated suspension centrifuged and the supernatant was collected. The collected supernatants were stored at -20 °C until PDGF-BB quantification.

2.2.2. Flow cytometry

Microparticles were prepared with the method presented in the previous section. To examine the capability of the aptamer in binding CS, 6-FAM-labeled CS-2 and CS-3 (ex. = 495 nm; em. = 520 nm) and TYLE-665-labeled CS-1 (ex. = 645 nm; em. = 665 nm) were used to treat the microparticles. The FAM and TYLE fluorophores were excited with an argon ion blue laser (488 nm) and a HeNe red laser (633 nm), respectively, using a Coulter FC500 (Beckman Coulter, Brea, California). Ten thousand events were collected.

2.2.3. Surface plasmon resonance spectroscopy

The molecular interaction between the PDGF-BB and its aptamers was studied using SPR spectroscopy (SR7500DA; Reichert Analytical Instrument; Depew, NY). A carboxyl group-functionalized sensor chip (Reichert Analytical Instrument; Depew, NY) was used as the substrate for PDGF-BB immobilization. To immobilize PDGF-BB, the sensor chip was activated by flowing 0.0040 g/mL EDC and 0.012 g/mL NHS for 7 min at 20 μL/min. After the activation of the sensor chip, 10 μg/mL PDGF-BB in 10 mM sodium acetate (pH = 5.2) was injected for 10 min at 20 μL/min. The running buffer was PBS containing 0.05% Tween 20. To test aptamer binding to PDGF-BB, 100 nM of aptamers were flowed over the sensor chip at 25 μL/min. The sensor chip was regenerated by washing the sensor chip with 40 mM NaOH for 1 min at 100 μL/min.

2.2.4. Hydrogel synthesis

Hydrols were synthesized using a free radical polymerization reaction coupled with a gas foaming reaction [25]. In brief, Pluronic F-127, acetic acid, TEMED, APS, and PEG700DA were mixed with or without aptamers. The prepolymer solution (25 μL) was then added to sodium bicarbonate in a cylindrical mold to initiate the formation of the gas foam and the hydrogel. After the formation, the hydrogels were thoroughly washed in deionized water for three days to remove any unreacted molecules.

2.2.5. PDGF-BB loading

Lyophilized PDGF-BB was reconstituted by dissolving the powder in PBS with 0.1% BSA. Prior to loading the superporous hydrogel with PDGF-BB, the hydrogel was gently blotted with tissue paper to dehydrate it. PDGF-BB solution of 120 μL was then added to the hydrogel and the loaded hydrogel was incubated at 4 °C for 24 h. The loaded hydrogel was directly used for the examination of PDGF-BB retention and release without washing.

2.2.6. Examination of PDGF-BB release

Hydrols were incubated in 1 mL release medium (PBS supplemented to a final concentration of 0.1% BSA, 0.05% Tween 20, and 0.05% sodium azide) at 37 °C. At determined time points, the supernatant was totally collected and replaced with 1 mL of fresh release medium. To regulate PDGF-BB release, the solution of the complementary sequence of the anti-PDGF-BB aptamer was added to the release medium. The collected supernatant was stored at -20 °C until PDGF-BB quantification.

2.2.7. Protein quantification

PDGF-BB was quantified using a recombinant human PDGF-BB enzyme-linked immunosorbent assay (ELISA) following the instructions provided by the manufacturer (Peprotech, Rocky Hill, NJ). Prior to analysis, supernatant samples were diluted with the diluent to ensure that the PDGF-BB concentration fell within the detectable range of the assay. The absorbance of each sample was measured using a BioTek Synergy™ HT Multi-Mode Microplate Reader (BioTek, Winooski, VT) at 405 nm and doubly referenced by subtracting the absorbance at the reference wavelength (650 nm) and the absorbance of the zero PDGF-BB concentration.

2.2.8. Scanning electron microscopy

Superporous hydrogels were prepared by completely drying the hydrogels at 37 °C for several days in a convective air incubator and sputter coated with gold. Hydrogels were imaged using a JEOL JSM6335F Field Emission Scanning Electron Microscope. The dimensions of the pore in the hydrogel were calculated using the software ImageJ (http://rsb.info.nih.gov/ij/). The average of the longest and the shortest diameter of the orifice of the pore was calculated and presented as the pore diameter.

2.2.9. Ethidium bromide staining

Superporous hydrogels were stained with ethidium bromide to assess aptamer incorporation into the hydrogel network. Hydrogels were incubated in an ethidium bromide bath for 1 h and washed for 2 h to remove unbound ethidium bromide. Hydrogels were then imaged under UV light with a Bio-Rad GelDoc XR system (Hercules, CA).

2.2.10. Confocal microscopy

Aptamer-functionalized superporous hydrogels were incubated in the release medium containing a 6-FAM-labeled complementary sequence for 1 h. After thorough washing, the hydrogels were subjected to observation under a Nikon A1R Spectral Confocal Microscope (Nikon Instruments Inc., Melville, NY) to image the porous network at various depths into the hydrogel. The fluorescences were excited at 488 nm with an argon laser. X-Y scans were made every 10 μm from the surface of the hydrogel to a depth of 400 μm in the z-direction at an optical resolution of 0.47 μm. Captured images represent a 1280 μm × 1280 μm field of view.

2.2.11. Fluorescence imaging

Aptamer-functionalized hydrogels were labeled with FAM-labeled complementary oligonucleotides and washed to remove unbound oligonucleotides. Fluorescence imaging of bulk hydrogels was performed with a Maestro Multi-Mode Imaging System (Woburn, MA).

2.2.12. Prediction of secondary structures

The secondary structures of the DNA sequences and their hybridized structures were predicted using the software RNAstructure, v5.3, which is used to predict both RNA and DNA structures [26]. The structure presented with the lowest free energy was presumed to be the predominant structure. Three-dimensional conformations were generated using NUPACK [27].
3. Results and discussion

3.1. Synthesis and characterization of the superporous hydrogel

Many methods have been developed for the synthesis of superporous materials, including gas foaming [28], particulate leaching [29], particle sintering [30], phase separation [31], freeze drying [32], and three-dimensional printing [33]. The method used in this study was based on free radical polymerization coupled with gas foaming. The free radical polymerization enabled poly(ethylene glycol) diacrylate (PEGDA) and Acrydite-modified aptamers to crosslink and form a polymer network because these precursors have unsaturated carbon–carbon double bonds for the reaction with radicals and the propagation of polymer chains. While free radical polymerization can be used to synthesize the hydrogel, the polymerization itself does not lead to the formation of large pores within the hydrogel. To produce large pores in the hydrogels, the prepolymer solution with acetic acid was reacted with sodium bicarbonate, where carbon dioxide was evolved during the reaction (Fig. 1A). Resultantly, the gas foam created the porous channels as the hydrogel cured. Since the concentration of monomers is critical to the synthesis of polymer networks, the effect of PEGDA concentration on the synthesis of the superporous hydrogels was first studied. The concentration of PEGDA was varied from 5 to 20% (v/v) (Fig. 1B). When the concentration was 5%, a hydrogel with large pores did not form. At 10% PEGDA, a porous hydrogel could form. However, the SEM micrographs show that many pores were collapsed, indicating that the formed pores were not stable at this concentration. In contrast, when the concentration was increased to 15 or 20%, large and deep pores formed in the hydrogels. This indicates that a concentration of at least 15% is needed to synthesize the superporous PEG hydrogel without the collapse of the pores. Because the SEM micrographs showed no significant difference in the porous structures or the morphology between the 15% and 20% hydrogels, 15% PEGDA was used to synthesize the superporous hydrogels in the following experiments.

In addition to PEG, another critical component of the superporous hydrogel is the nucleic acid aptamer that was used as the binding and sensing effector of the hydrogel. The anti-PDGF-BB aptamer was chemically functionalized with Acrydite at its 5'-end for polymerization (Fig. 1C). As shown in the images of the ethidium bromide-stained hydrogels, the hydrogel with the Acrydite-modified aptamer exhibited a much stronger fluorescence intensity than that of the control hydrogels without the aptamer or with the unmodified aptamer (Fig. 1C). Thus, the results show that the aptamer was successfully incorporated into the hydrogel network through the Acrydite end group during free radical polymerization and gas foaming. Scanning electron micrographs were captured to examine the pore structures of the aptamer-functionalized superporous hydrogel. The hydrogels with and

Fig. 1. Synthesis and characterization of superporous hydrogels. (A) Reaction scheme of hydrogel synthesis. The superporous hydrogel is synthesized by using free radical polymerization coupled with gas formation. Acrydite-functionalized aptamers are incorporated into the hydrogel network during the polymerization. (B) Comparison of superporous hydrogels with different PEG concentrations ranging from 5% to 20%. Scale bars: 100 µm. (C) Illustration of an Acrydite-modified aptamer (top). Images of ethidium bromide-stained hydrogels showing that aptamers were incorporated into the hydrogel through the Acrydite end group (bottom). (D) Comparison between hydrogels synthesized without (w/o) and with (w/) aptamers; PEG concentration: 15%; scale bars: 100 µm. (E) Composite of superimposed images captured in 10 µm intervals.
without the aptamer exhibited virtually no difference in the pore structures (Fig. 1D). This result indicates that the incorporation of the aptamer had little influence on the morphological properties of the hydrogel. In addition, the superporous hydrogel had large pores with a normal distribution of pore sizes ranging from 20 to 100 μm and an average pore size of 55 ± 16 μm. In addition to SEM, scanning laser confocal microscopy was used to visualize the three-dimensional architecture of the functionalized superporous hydrogel. The superimposed image assembled from images acquired at multiple depths shows that the gas foam created a highly tortuous and porous hydrogel network (Fig. 1E).

3.2. Evaluation of molecular recognition

The functionality of the superporous hydrogel in sequestering and regulating the release of biologics is determined primarily by the molecular recognition among three molecules: the biologic, the aptamer, and the triggering molecule. Thus, experiments were performed to examine their interactions. The capability of an anti-PDGF-BB aptamer in binding PDGF-BB was first characterized. The structures of the anti-PDGF-BB aptamer (i.e., Apt-H) are shown in Fig. 2A. Apt-H is a truncated form of an anti-PDGF-BB aptamer selected by Green et al. [34], with a linear oligonucleotide tail added.
to the 5’-end. To analyze the capability of Apt-H in binding PDGFBB, a microparticle-based assay was used. Quantification of PDGFBB showed that Apt-H was able to adsorb 98.3% of PDGF-BB onto the particles (Fig. 2B). This result demonstrates that Apt-H can bind its target with high efficiency. Since nucleic acid aptamers are single-stranded oligonucleotides, they can bind their complementary sequences (CSs) via intermolecular hybridization (Fig. 2C). To verify the capability of Apt-H in binding its CSs, aptamer-functionalized microparticles were treated with fluorophore-labeled CS-1 and CS-2 and analyzed with a flow cytometer. The rightward shifts of fluorescence intensity in the flow cytometry histograms show that both CS-1 and CS-2 bound to Apt-H (Fig. 2D). In contrast, the control CS (CS-C) with a randomized nucleotide sequence did not hybridize with Apt-H (Fig. 2D). Since one of our major goals is to use a CS to regulate the release of aptamer-bound PDGF-BB, we further examined whether CS could induce the dissociation of the complex of Apt-H and PDGF-BB. The results show that the CSs were able to dissociate the bound PDGF-BB from the aptamer-functionalized microparticles (Fig. 2E). Moreover, CS-2 exhibited a higher efficiency in inducing the dissociation of the complex because CS-2 is more competitive due to the formation of more base pairs with Apt-H in comparison to CS-1.

3.3. Examination of molecule sequestration

To load PDGF-BB, the hydrogel was first thoroughly washed to remove any unreacted molecule and then incubated in a PDGF-BB solution. This synthesis route decouples the loading of biologics from the preparation of the superporous hydrogel (Fig. 3A). It is different from most previous studies, including our own, in which the synthesis of polymeric materials and the loading of growth factors were integrated into one step [20,21,35–39]. It is important to note that the preparation of polymeric materials often involves the use of organic solvents or harsh conditions. In this study for instance, free radicals were produced to synthesize the hydrogel and acetic acid was used to generate gas bubbles. These reactive and harsh chemicals would easily denature or inactivate biologics if the biologics were in the prepolymer solution. In addition, the polymeric systems may contain unreacted monomers and reaction byproducts. Washing is often a necessary step to remove them and intensive washes may cause the denaturation or the loss of a large amount of loaded molecules. Moreover, not all affinity ligands will be incorporated into the hydrogel during the polymerization. The existence of free affinity ligands will neutralize or block the activity of the released growth factors and cause the significant initial burst

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**Fig. 3.** Examination of growth factor retention within the superporous hydrogels. (A) Illustration of growth factor loading. (B) Secondary structures and three-dimensional configurations of the Apt-M (top) and the Apt-L (bottom) aptamer. (C) Surface plasmon resonance sensorgram of Apt-H, Apt-M, Apt-L. Concentration of aptamers used was 100 nM. (D & E) Effects of the binding affinity and the mole ratio of Apt-H to PDGF-BB on growth factor retention. Left: The amount of free PDGF-BB in the washing solution after various times of incubation. Right: the amount of PDGF-BB retained in the hydrogels after 24-h incubation. The amount of loaded PDGF-BB was 50 ng. (F) Relationship between the amount of PDGF-BB loading and growth factor retention. The mole ratio of Apt-H to PDGF-BB was fixed at 10:1. Left: Apt-L; Right: Apt-H.
release. None of these potential issues will be problematic as the synthesis of the superporous hydrogel is decoupled from the molecule loading. It is also important to note that the aptamer-functionalized superporous hydrogel can be stored at typical room conditions since both aptamers and hydrogels are stable materials. Target molecules such as growth factors or other biologics can be loaded into the superporous hydrogel when needed in real applications, which will provide great flexibility and ease of use in clinical applications.

Three aptamers were used to examine the loading and retaining capability, including Apt-H, Apt-M, and Apt-L. Apt-M was created by introducing two nucleotide substitutions into Apt-H. While Apt-M retains 95% sequence identity of Apt-H, the predicted secondary structures are different (Fig. 3B). Apt-L was generated by changing the nucleotide sequence of Apt-H. Analysis of the binding affinity of these three aptamers through surface plasmon resonance spectroscopy reveals that Apt-H has the highest binding affinity, Apt-L has the lowest binding affinity, and Apt-M has a binding affinity between Apt-H and Apt-L (Fig. 3C).

The three aptamers were used to synthesize functionalized superporous hydrogels at a fixed mole ratio of ten aptamers to one PDGF-BB. After the PDGF-BB solution was transferred into the superporous hydrogels, the hydrogels were directly incubated in a washing solution and the amount of PDGF-BB in the washing solution was examined. After 5 min of incubation, 37 ng of PDGF-BB was detected in the washing solution of the nonfunctionalized superporous hydrogel. In contrast, 14, 4.5, and 0.5 ng of PDGF-BB were detected in the solutions of the Apt-L, Apt-M, and Apt-H groups, respectively (Fig. 3D). Because a high amount of PDGF-BB in the washing solution indicates a low retention capability, the results show that aptamers played an important role in retaining PDGF-BB within the superporous hydrogel. In addition, the capability of retaining PDGF-BB was affected by the binding affinity of the aptamer. An aptamer with a higher binding affinity for PDGF-BB was able to retain more PDGF-BB within the superporous material. Furthermore, as more PDGF-BB was retained, PDGF-BB could be released for a longer period (Fig. 3D). The percentages of PDGF-BB retained after the hydrogels were incubated in the washing solution for 24 h in the non-, the Apt-L-, the Apt-M-, and the Apt-H-functionalized superporous hydrogels, were 14%, 28%, 63%, and 90%, respectively (Fig. 3D).

To understand further the ability of aptamers to retain PDGF-BB within the superporous hydrogel, the mole ratio of Apt-H to PDGF-BB was also varied. The loaded amount of PDGF-BB was fixed at 50 ng (i.e., 2 pmol). After the incubation of the hydrogels in the washing buffer for 5 min, the amount of PDGF-BB in the washing solution was 37 ng in the 0:1 group (i.e., the nonfunctionalized hydrogel), 23 ng in the 1:1 group, 0.5 ng in the 10:1 group, and 0.5 ng in the 100:1 group (Fig. 3E). The amount of PDGF-BB retained in these four groups after 24 h incubation was 14%, 53%, 90%, and

Fig. 4. CS-regulated PDGF-BB release from the superporous hydrogels. (A) Effect of the time of CS treatment on CS penetration (top) and PDGF-BB release (bottom). The mole ratio of CS to aptamer was fixed at 1:2; the amount of loaded PDGF-BB was 50 ng. (B) Effect of the mole ratio of CS-2 to aptamer on CS penetration (top) and PDGF-BB release (bottom). The time of CS treatment was fixed at 1 h; the amount of loaded PDGF-BB was 50 ng. Fluorescence images were captured with an imaging system. Right: The total release of PDGF-BB during the 24 h.
96%, respectively (Fig. 3E). When the mole ratio was increased, the probability for the growth factor to be bound to the aptamer increased in the hydrogel. Thus, the ability of the hydrogel to retain PDGF-BB was improved with the increase of the mole ratio of Apt-H to PDGF-BB.

The effect of the initial loading amount of PDGF-BB on the retaining efficiency was also studied with the mole ratio of Apt-H to PDGF-BB fixed at 10:1. The purpose of designing this experiment was two-fold. First, it was used to understand further the ability of the superporous hydrogel to retain loaded molecules. Second, the absolute amount of biologics directly affects the effectiveness of a delivery system in real applications. For instance, the total amount of growth factors loaded in an implant for in vivo applications usually needs to be at the level of several micrograms [40–47]. In contrast, the previous studies showed that the loaded amount of growth factors in a large piece of aptamer-functionalized nonporous hydrogels was at the level of picograms via a single-step approach [20,21]. Therefore, we examined two different loading levels while maintaining a mole ratio of aptamer to growth factor of 10:1 to validate its therapeutic potential. The Apt-L-functionalized hydrogels loaded with 50 ng and 2 μg of PDGF-BB retained 28% and 85% PDGF-BB after 24-h incubation, respectively (Fig. 3F). The Apt-H-functionalized hydrogels loaded with 50 ng and 2 μg of PDGF-BB retained 90% and 99.7% PDGF-BB after the 24-h incubation, respectively (Fig. 3F).

These results indicate that the density of the aptamer significantly affects the retention of the loaded molecules. At a fixed hydrogel volume, if the concentration of the aptamer is low, the likelihood for a released growth factor to bind to an aptamer downstream in its diffusion pathway is small. In contrast, if the aptamer concentration is high, the occurrence of rebinding events will be significantly increased as the protein travels the same distance from the interior of the hydrogel outwards. Taken together, the results show that not only the mole ratio of Apt-H to PDGF-BB, but also the overall density of the aptamer, affects the retention of PDGF-BB. In addition, to our knowledge, the ability to retain effectively such a high level of biomolecules in superporous hydrogels has never been achieved.

Fig. 5. Examination of the high retention and regulated release of PDGF-BB in a 15-day test. (A) Daily release (left) and cumulative release (right) of 2 μg PDGF-BB from the superporous hydrogel functionalized with Apt-H or Apt-L. (B) Daily release (left) and cumulative release (right) of 2 μg PDGF-BB from the Apt-H functionalized superporous hydrogel in the presence or absence of CS-2. The mole ratios of CS-2 to Apt-H were 1:2 on day 5 and 2:2 on day 10, respectively. (C) Amount of PDGF-BB released on days 5 (left) and 10 (right). The release during the 1-h triggering and the total release during the 24 h are presented.
3.4. Examination of CS sensing and hybridization in the superporous hydrogel

Nucleic acid aptamers in principle can be selected from oligonucleotide libraries to bind to any molecule with a high affinity and specificity [22–24]. Importantly, nucleic acid aptamers can be changed from an active state to an inactive state by hybridization with a CS [48,49]. Thus, in addition to its binding role, the aptamer can play the role of sensing CSs to change its binding functionality. As a result of hybridization, the aptamer can release the bound molecules out from the superporous hydrogel as needed in the presence of CSs. This unique function is difficult to realize using other affinity molecules.

To determine the ability of the aptamer-functionalized superporous hydrogel to sense and hybridize with the CS, the hydrogels were treated with FAM-labeled CS-2 for various times, CS-2 was chosen because it is more effective in hybridizing with Apt-H than CS-1. The imaging result shows that green fluorescence was detected on the superporous hydrogel, even within incubation times as short as 5 min (Fig. 4A). The result also shows that the intensity of green fluorescence increased with the treatment time. These observations demonstrate that CS-2 was able to penetrate rapidly most areas of the superporous hydrogel on the macroscopic scale, and that the superporous hydrogel exhibited high sensitivity to interact with the CS.

To understand the regulating effectiveness of the CS in the superporous hydrogel, both the time of CS treatment and the mole ratio of CS to aptamer were varied. The outcomes were examined through fluorescence imaging and PDGF-BB measurement. The PDGF-BB release in both cases was consistent with the fluorescence imaging analysis (Fig. 4B). The amount of PDGF-BB released from the hydrogel increased with the treatment time. Additionally, the amount of PDGF-BB released from the hydrogel increased when the mole ratio of CS-2 to Apt-H increased (Fig. 4B).

3.5. Evaluation of the high retention and regulated release of growth factors

After demonstrating that oligonucleotides could be used as the binding, sensing, and regulating effectors to control the properties of the superporous hydrogel, we carried out a systematic study to determine whether the superporous hydrogel could retain the loaded molecules for a long period and whether the release of PDGF-BB could be regulated at the molecular level through either the variation of the binding affinity of the aptamer or the use of a CS. The initial amount of PDGF-BB loaded into the hydrogels was 2 μg and the release was tested for 15 days.

The release profile shows that only 0.3% was released from the Apt-H-functionalized superporous hydrogel by day 1 and only 3% was released by day 15. The results also show that approximately 15% PDGF-BB was released from the Apt-L functionalized superporous hydrogel during the first day, 39% by day 5, 52% by day 10, and 60% was released by day 15 (Fig. 5A). Our results demonstrate for the first time that a superporous hydrogel could retain a large amount of loaded molecules effectively, despite the fact that the burst release of loaded molecules is a typical phenomenon to most polymeric materials, regardless of the presence or absence of large pores or channels. Moreover, the release kinetics of loaded biologics can be modulated by changing the binding affinity of the aptamer.

In addition to demonstrating that Apt-H could retain PDGF-BB, we applied CS-2 to treat the superporous hydrogel for 1 h at days 5 and 10 with the mole ratio of CS to aptamer at these two days being 1:2 and 2:2, respectively (Fig. 5B). The superporous hydrogel released approximately 0.2 and 0.4 μg PDGF-BB during the 1-h treatment at days 5 and 10, respectively. The total amount of the released PDGF-BB during days 5 and 10 were approximately 0.3 and 0.7 μg, respectively (Fig. 5C). In contrast, the superporous hydrogel without the CS-2 treatment released approximately 0.004 μg PDGF-BB during both days 5 and 10 (Fig. 5C). These results clearly demonstrate that the retained PDGF-BB could be released via CS regulation at determined time points and the release amount could be facilely controlled by varying the CS concentration.

4. Conclusions

A superporous material was developed with hydrogels and oligonucleotide aptamers via free radical polymerization coupled with gas foaming. The presence of superporous architectures and oligonucleotide aptamers allows for the fast absorption and high retention of a large amount of growth factors without the involvement of any harsh conditions. The release kinetics of growth factors can be modulated by using aptamers with different binding affinities. The release amount of growth factors can also be regulated by using the CS of an aptamer at predetermined time points. Therefore, the aptamer-functionalized superporous hydrogel presents a promising platform to overcome the problems such as harsh reaction conditions, low drug sequestration efficiency, and difficult drug release regulation and to expedite the clinical translation of various biologics such as growth factors, cytokines, peptides, and vaccines.

Acknowledgments

The authors thank Ruth Nissly and the Penn State Microscopy and Cytometry Facility—University Park, PA for technical support and use of the flow cytometer. We also greatly acknowledge financial support from the NSF (DMR 1332351) and the Penn State Start-up Fund.

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