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Macromolecular Amplification of Binding Response in Superaptamer Hydrogels

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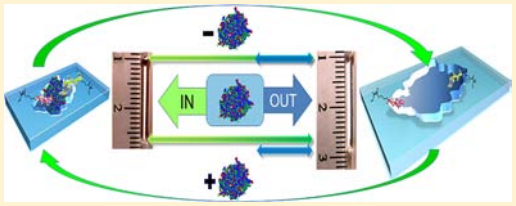
Supporting Information

ABSTRACT: It is becoming more important to detect ultralow concentrations of analytes for biomedical, environmental, and national security applications. Equally important is that new methods should be easy to use, inexpensive, portable, and if possible allow detection by the naked eye. By and large, detection of low concentrations of analytes cannot be achieved directly but requires signal amplification by catalysts, macromolecules, metal surfaces, or supramolecular aggregates. The rapidly progressing field of macromolecular signal amplification has been advanced using conjugated polymers, chirality in polymers, solvating polymers, and polymerization/depolymerization strategies. A new type of aptamer-based hydrogel with specific response to target proteins presented in this report demonstrates an additional category of macromolecular signal amplification. This superaptamer assembly provides the first example of using protein-specific aptamers to create volume-changing hydrogels with amplified response to the target protein. A remarkable aspect of these superaptamer hydrogels is that volume shrinking is visible to the naked eye down to femtomolar concentrations of protein. This extraordinary macromolecular amplification is attributed to a complex interplay between protein−aptamer supramolecular cross-links and the consequential reduction of excluded volume in the hydrogel. Specific recognition is even maintained in biological matrices such as urine and tears. Furthermore, the gels can be dried for long-term storage and regenerated for use without loss of activity. In practice, the ease of this biomarker detection method offers an alternative to traditional analytical techniques that require sophisticated instrumentation and highly trained personnel.



INTRODUCTION

The development of methodology that permits the naked eye detection of a target molecule in a complex mixture without the need for sophisticated instrumentation is an important challenge for improving sensors and assays.[1] An additional challenge is detection of ultralow concentrations of the target analyte that can ultimately be used to detect biomarkers for medical diagnostics, environmental toxins, or compounds of interest for national security. Methods of detection for low levels of analytes rely heavily on signal amplification, which can be achieved in a variety of ways including the use of catalysts, macromolecules, metal surfaces, and supramolecular aggregates.[1] Focusing on macromolecular amplification, one of the most successful methods is the "molecular-wire" approach used for fluorescent chemosensors.[2,3] A related approach is the use of polymer aggregation or folding in the presence of a target molecule to affect visible or fluorescent probes.[4−7] The "sergeants-and-soldiers principle" makes use of molecular chirality to influence macromolecular chirality such as helix formation[8−10] or orientation of liquid crystals.[9] Last, polymerization[11] or depolymerization[12,13] responses have been used as macromolecular detectors of target molecules. This study presented herein investigates an original aptamer-based hydrogel system with volume response to ultralow levels of protein analytes, with a different mechanism behind the signal amplification than the four known types listed above.

Responsive hydrogels are an important component of the field of "smart materials", characterized by change in morphology, volume, or color in response to external stimuli such as temperature, pH, analytes, and electric or magnetic fields.[14−20] Hydrogels are insoluble polymer networks that are extensively hydrated and provide elastic, semiwet, 3D environments suitable for material-to-biology communication.[21,22] Of all possible communication pathways, interaction at the molecular level is the most specific for triggering material response to a biological target. A nontemplated hydrogel has recently demonstrated that biological molecules, that is, DNA hybrids, can be used as reversible cross-linkers that exhibit a volume change response in the material.[23] Hydrogels with response mechanisms more closely related to those in the study presented here have been demonstrated by Miyata et al.[17,24,25] These examples utilized the method of biomolecular imprinting, where template proteins are precomplexed to polymerizable bioreceptors that are subsequently copolymerized with hydrogel monomers. Removal of the imprinted protein reversibly dissolves cross-links, resulting in a swelling response of the hydrogel; subsequent addition of the protein back into the hydrogel gave a shrinking response in the narrow range of approximately $(2.0−6.0) \times 10^{-7}$ M protein concentration.[24] A surprising result for the differently

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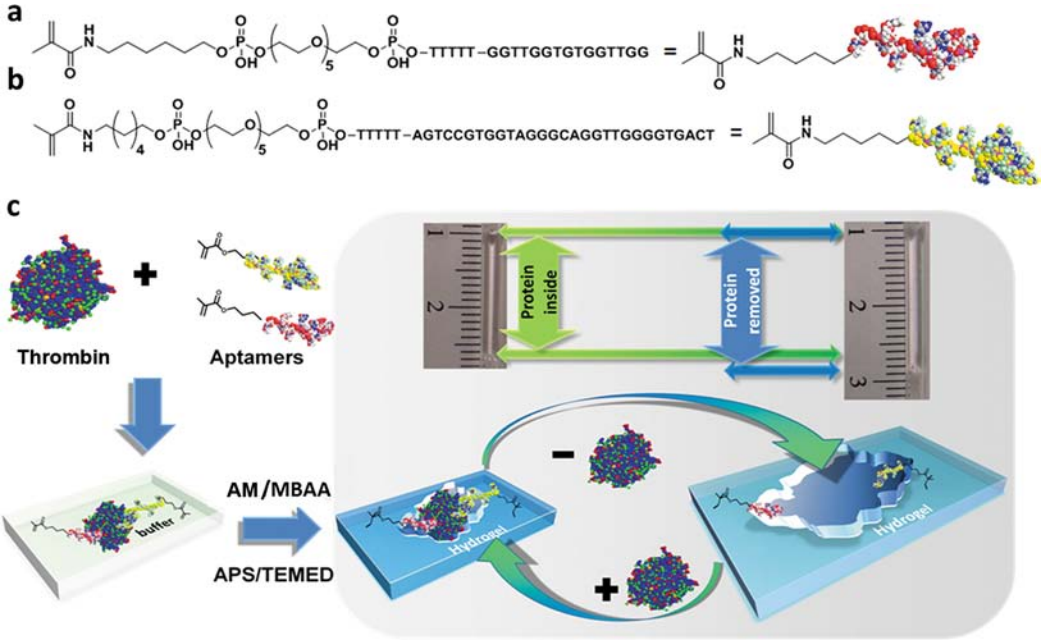
Table 1. Formulation of Thrombin-Bioimprinted Aptamer Hydrogels[a]

reagent	MW (Da)	mass of reagent (mg)	reagent concn (M)	molar equiv of reagents
thrombin	36 700	6.07×10^{-2}	1.65×10^{-5}	1.0
aptamer 1[b] (A1)	6838	1.13×10^{-2}	1.65×10^{-5}	1.0
aptamer 2[c] (A2)	11 198	1.86×10^{-2}	1.65×10^{-5}	1.0
acrylamide (AM)	71	11.3	1.59	9.64×10^{4}
methylenebisacrylamide (MBAA)	154	0.123	7.97×10^{-3}	4.83×10^{2}

[a]PDGF-$\beta\beta$ hydrogels were formulated with the same relative molar equivalents of reagents; however, the concentration of each of the reagents was exactly half those used for thrombin reported here. [b]This aptamer has the sequence GGTTGGTGTGGTTGG[32] attached to the linker shown in Scheme 1a. [c]This aptamer has the sequence AGTCCGTGGTAGGGCAGGTTGGGGTGACT[33] attached to the linker shown in Scheme1b.

Scheme 1. Outline of the Biomolecular Imprinting Scheme Used to Create Superaptamer Thrombin-Responsive Hydrogels[a,]



[a](a) Aptamer 1 modified with a polymerizable methacrylamide terminus linked to the aptamer via a linker incorporating five thymidine units, six ethylene oxide units, and six methylene units. (b) Aptamer 2 with the same modification for incorporating the polymerizable methacrylamide moiety. (c) Visualization of the volume change response by the hydrogels is shown in the upper right-hand corner, where length measurements of the gels within the capillary were made from meniscus to meniscus with a reticulated magnifying glass. The thrombin-bioimprinted aptamer hydrogels were prepared as described in the Supporting Information.

formulated hydrogel materials presented herein is that the shrinking response to the target protein is found at concentrations ranging over several orders of magnitude; for example, in the 10^{-14} to 10^{-6} M or 10^{-17} to 10^{-12} M ranges. The large response to low concentrations of target proteins for the superaptamer hydrogels described here indicates the response is significantly amplified compared to other molecular responsive hydrogels.

The term "superaptamer" used here describes the cooperativity of the aptamers in creating the binding response and illustrates a general approach to developing responsive materials toward any biomarker. Aptamers are an exciting new class of synthetic bioreceptors evolved in vitro by the systematic evolution of ligands by exponential enrichment (SELEX) method to give artificial receptors that can often rival the binding of antibodies.[26−28] In comparison to antibodies, aptamers often exhibit better stability, do not come from animal sources, are relatively inexpensive to synthesize once the sequence is known, and can be reversibly denatured for multiple cycles of capture and release of target proteins.[28] The high level of macroscopic amplification by these superaptamer hydrogels makes them competitive with other analytical methods of detection for ultralow concentrations of target analytes. The ability to see the response of the superaptamer hydrogels by the naked eye makes this a useful assay for low-cost, easy-to-use, and portable detection of molecules of interest for biomedical, environmental, and warfare analysis.

RESULTS AND DISCUSSION

Thrombin-Imprinted Hydrogels. The first aptamer pair to be investigated was composed of two different aptamers that bind different sites on the protein thrombin. Thrombin is a multifunctional serine protease[29,30] that has recently been reported to be a biomarker found in urine for the detection of glomerulonephritis (inflammation of the kidney's filtering systems), which can occur as a symptom of certain diseases such as hepatitis, lupus, diabetes, or cancers.[31] Initial investigation of the shrinking response in the thrombin-bioimprinted aptamer hydrogels used the formulation of components shown in Table 1. Details of polymer preparation and measurement of the hydrogel rebinding responses are reported in the Supporting Information. The prepolymerization

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complex of the thrombin template with aptamers 1 and 2 (expressed as A1−thrombin−A2) was formed from stoichiometric amounts of each of these components. Only a stoichiometric amount of each aptamer was used due to the high binding constants of the aptamers,[32,33] avoiding nonspecific interactions arising from uncomplexed aptamer. Polymerization of the polymer formulation incorporating the A1−thrombin−A2 complex resulted in gels illustrated in the upper right image of Scheme 1c. Subsequent removal of the template resulted in a hydrogel with visibly increased length (top right of panel c in Scheme 1), but this effect is reversible if the thrombin template is reintroduced to the gel sample. It was determined that 92% of the original aptamer concentration remains in the hydrogel by UV analysis for DNA in the washing supernatant (details provided in Supporting Information). The volume response is attributed to formation of supramolecular cross-links provided by the thrombin template;[24] however, the origins of the amplified volume response of these hydrogels can likely be traced back to macromolecular excluded-volume effects (vide infra).[23−25] Because the gels are measured in capillaries, the volume change of the gel is limited to only one dimension and the percent change in that dimension is proportional to the overall volume change:

$$\text{percent shrinkage} = \frac{d_0 - d}{d_0} \times 100 \quad (1)$$

where d = length of the gel in the presence of protein and d_0 = length of the gel in the absence of protein. Previous examples by Miyata et al.[34,35] have used similar measurements to determine cross-link density from compression modulus.

The isotherm in Figure 1 shows the percent shrinking as a function of the amount of thrombin bound to the hydrogel,

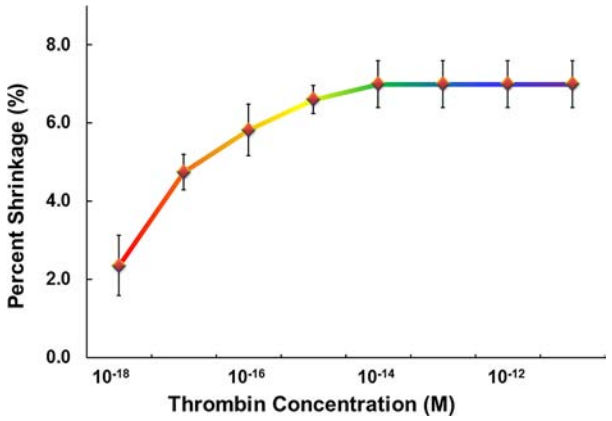

Figure 1. Binding isotherm of thrombin-imprinted hydrogels: the volume response of the hydrogel increased with increasing concentration of protein in solution.

which is directly proportional to the increase in noncovalent cross-links resulting from the aptamer−thrombin complex. In the isotherm, the binding response in terms of percent shrinking (eq 1) can be seen down to the femtomolar range of thrombin for the limit of detection, becoming saturated at picomolar concentrations, giving the dynamic range. Figure 2 compares the response of the imprinted hydrogel versus control hydrogels with different combinations of aptamers and thrombin. The volume shrinking response for the imprinted hydrogel in column T1 is signficantly larger than an identically prepared nonimprinted hydrogel shown in column T2 that was

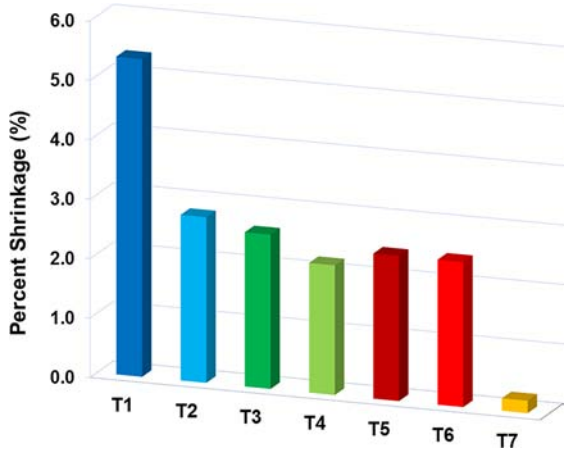

Figure 2. Imprinting effect resulting in volume shrinking for the bioimprinted hydrogel T1, validated versus a series of control hydrogels, with a thrombin concentration of 1.0×10^{-6} M. T1 gel incorporates the entire A1−thrombin−A2 complex; T2 gel contains A1 + A2 but no thrombin during polymerization. T4 and T3 gels contain only A1 + thrombin and A2 + thrombin, respectively, during complex formation and polymerization. T5 and T6 gels contain only A1 and A2, respectively, during polymerization, and T7 contains no components of the A1−thrombin−A2 complex.

made with both aptamers but lacking thrombin. The data are reproducible within 5% as shown by Figure S2 in the Supporting Information. The lower response of the nonimprinted hydrogel can be attributed to randomly distributed aptamers A1 and A2 throughout the gel that cannot simultaneously bind thrombin. *This emphasizes that random placement of aptamers cannot compete with the preorganized aptamers in the bioimprinted hydrogel.*

Columns T5 and T6 in Figure 2represent hydrogels composed of only A1 or A2, respectively, and these important controls show that both aptamers are needed to provide cross-links in the hydrogel that ultimately lead to volume-change responses. Similarly, columns T3 and T4 show that the addition of thrombin to each of the single aptamers alone still does not enhance any molecular recognition response, similar to the single-aptamer gels T5 and T6. The lowest response is shown in column T7, which is the gel without aptamers or thrombin, and thus little binding to thrombin is expected. The fact that the thrombin protein is needed with both aptamers for a large shrinking response by the hydrogel implies that a supramolecular cross-linking effect is necessary for the response. Furthermore, the hydrogel is still active toward thrombin spiked in an artificial urine solution,[36] giving virtually the same specific shrinking response after pre-equilibration in urine solution without thrombin (details provided in Supporting Information). Last, specificity was evaluated by use of an imprinted hydrogel where thrombin was removed after several cycles of thrombin rebinding. Incubation with a bovine serum albumin (BSA) solution (1.0×10^{-6} M) actually caused the gel to swell 0.78% at equilibrium rather than shrink as it would in the presence of thrombin. It was subsequently shown that the hydrogel was still active after the BSA treatment by directly adding the gel to a thrombin solution, which immediately displayed shrinking on the same order that is normally found for the thrombin-imprinted gels.

Optimization of Hydrogel Formulation Parameters. A study to determine the optimum amount of methylenebisacrylamide (MBAA) cross-linker in order to obtain the largest

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shrinking response of the imprinted hydrogels was carried out. Initial tests were done on thrombin-bioimprinted hydrogels with a variable amount of MBAA ranging from 0.25 to 1.0 mol %, relative to the total monomer concentration (entries 1−3 in Table 2). Gels with 1.0 mol % MBAA did not show measurable volume changes in the presence of thrombin. On the other hand, gels incorporating 0.25 mol % MBAA formed hydrogels that lost integrity by segmenting apart; and below 0.1 mol % MBAA, the polymers exhibited solution behavior. Thus, the formulation shown in entry 1 of Table 2 gives the optimum amount of cross-linker for maximum volume response of the bioimprinted hydrogel. With this MBAA concentration, two other imprinted gels were synthesized; one with half the amount of A1−thrombin−A2 complex (entry 4 in Table 2), and the other with 3 times the original concentration of the A1−thrombin−A2 complex (entry 5 in Table 2). As anticipated, decreasing the number of thrombin-based cross-links does not enhance the hydrogel response. However, increasing the number of thrombin-based cross-links surprisingly showed a slight decrease in the response of the hydrogel as shown in entry 5 (compared to entry 1). One last observation was that reducing the concentrations shown in Table 1 by 50%, by adding an equal volume of plain buffer to the imprinting formula, did not change the performance of the hydrogels (entry 6 in Table 2). This is an advantage for rare or expensive bioconjugation components or proteins, because less can be used.

Table 2. Optimization of Hydrogel Performance via Crosslinker Content and Concentration of Prepolymer Complex for Maximum Volume Shrinking[a]

entry	MBAA (mg)	A1−thrombin−A2 prepolymer complex (M)	% change in volume
1	0.123	1.65×10^{-5}	5.46 ± 0.95
2	0.0613	1.65×10^{-5}	4.37 ± 2.3
3	0.246	1.65×10^{-5}	2.59 ± 2.2
4	0.123	0.83×10^{-5}	2.93 ± 1.45
5	0.123	4.95×10^{-5}	4.83 ± 0.05
6[b]	0.0613	0.83×10^{-5}	5.25 ± 1.45

[a]Cross-linker content is defined as the mass of cross-linker added to the formulation. Prepolymer complex concentration is inferred from stoichiometric addition of aptamer 1, aptamer 2, and thrombin in solution prior to addition of other components. Hydrogels were incubated with 1.0×10^{-6} M thrombin for shrinking measurements. [b]The acrylamide monomer concentration is half that used in entries 1−5.

Bioimprinted PDGF-ββ Hydrogels. A second protein, PDGF-ββ, was targeted for development of aptamer-based responsive polymers to determine the universality of the hydrogel imprinting process. PDGF-ββ is a dimeric protein that is released during blood clotting, and elevated levels of PDGF-ββ could be an important biomarker for angiogenesis and arteriosclerosis.[37] In addition, PDGF-ββ has been shown to be a biomarker in tears for corneal healing in concentrations as low as 3 pM.[38] An aptamer that binds PDGF-ββ (referred to as A3) has been developed to specifically recognize each of the identical sites on each of the dimer subunits.[39] The symmetry of the dimeric PDGF-ββ was taken advantage of by using 2 equiv of A3 along with PDGF-ββ to form the A3−PDGF−A3 prepolymerization complex. Hydrogels were formed as shown in Scheme 2 with 50% of the formulation concentrations shown in Table 1 as a cost-saving practice; however, all the relative equivalents of PDGF-ββ protein and aptamer remained the same. Rebinding of PDGF-ββ was determined at different concentrations, resulting in the isotherm shown in Figure 3.

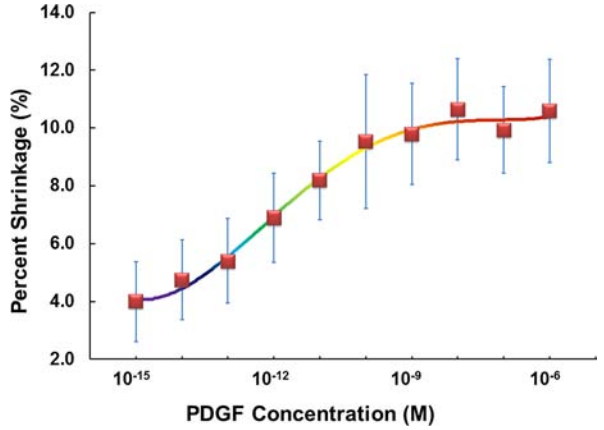
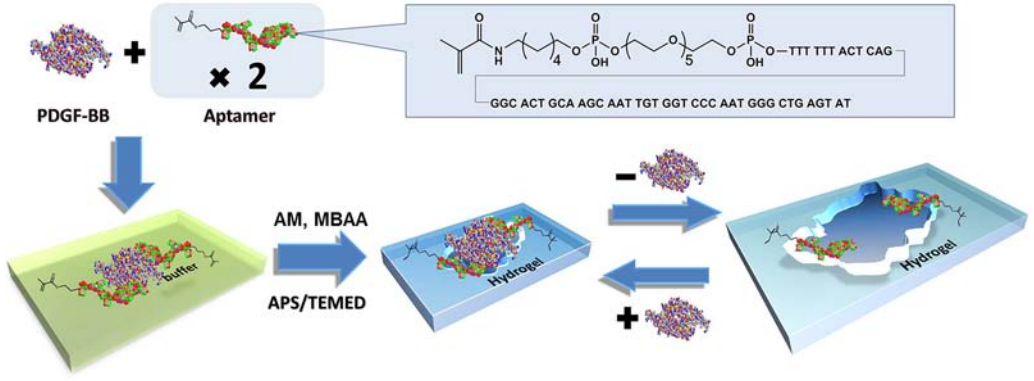


Figure 3. Binding isotherm of PDGF-ββ for imprinted hydrogels, showing response in the 10^{-12} to 10^{-9} M concentration range of analyte.

Scheme 2. Aptamer Design and Preparation Steps for PDGF-ββ Superaptamer-Responsive Hydrogels[a]



[a]The PDGF-ββ-bioimprinted aptamer hydrogels were prepared by adding A3−PDGF−A3 complex to a solution of APS, MBAA, and Am; polymerization was carried out in identical fashion as the thrombin hydrogels. PDGF-ββ was removed with a 5 wt % solution of sodium dodecyl sulfate (SDS).

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The maximum response was found to level off in the 10^{-8} to 10^{-6} M concentration range, and remaining studies were carried out at a PDGF-$\beta\beta$ concentration of 10^{-6} M. The sensitivity of this system was also demonstrated by the detectable volume change (approximately 4.0%) down in the femtomolar concentration range, which gives a detection limit in the picomolar range due to the error in measurement. The larger error in measurements reflects the use of small-volume hydrogels (due to the cost of PDGF-$\beta\beta$), resulting in lower signal-to-noise data versus the thrombin-responsive gels in Figure 2. Thus, the overall dynamic range for detection is between 10^{-12} and 10^{-6} M PDGF-$\beta\beta$. Greater accuracy in the quantitative measurement of the hydrogel response can be obtained by use of larger-volume gels, which also requires significantly longer incubation periods, as in the case with thrombin.

To verify that imprinting took place, control hydrogels that differ in the presence or absence of PDGF-$\beta\beta$ template and aptamers were compared to PDGF-$\beta\beta$-imprinted hydrogels. Column 1 (P1) in Figure 4 shows the volume change averaged

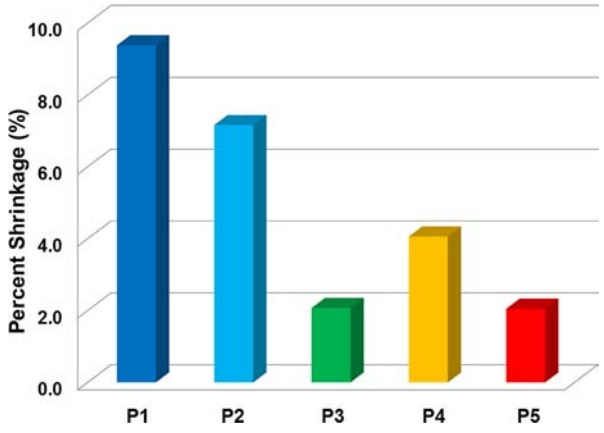

Figure 4. Volume shrinking response for PDGF-$\beta\beta$-bioimprinted hydrogel versus control hydrogels incubated with 1.0×10^{-6} M PDGF-$\beta\beta$. P1 shows the shrinking response of the bioimprinted hydrogel in PBS (pH = 7.4); P2 is the response in artificial tear solution formulated from ref 40. P3 is a control synthesized with only template but no aptamer; P4 is a control made with aptamer but no template; and P5 is a control made without any A3−PDGF−A3 complex.

over three separate PDGF-$\beta\beta$ imprinted hydrogels that were submitted to three cycles of PDGF-$\beta\beta$ rebinding and removal in phosphate-buffered saline (PBS; 2.0 mM phosphate, pH = 7.4). The results are relatively constant over each cycle, which demonstrated again that the behavior of the hydrogels is reversible for target binding. The imprinted hydrogel response was then tested in a real-life application in column 2 (P2) with an artificial tear solution[40] spiked with PDGF-$\beta\beta$, simulating the bioassay for assessing corneal healing.[38] The imprinted hydrogel P2 shows that nearly the same response is seen for detection of PDGF-$\beta\beta$ in the tear solution as in buffer, indicating low interference by salt content.

Possible interference of the artificial tear solution was also assessed by incubation of the imprinted hydrogel in tear solution without any PDGF-$\beta\beta$ added, which showed a negligible volume shrinkage of 0.66% ± 1.31% from nonspecific effects of the salts in the solution mixture. The following three columns (P3−P5) are control gels without any A3−PDGF−A3 complex, with only PDGF-$\beta\beta$, and with only aptamer, respectively. The hydrogels prepared without aptamer and the blank hydrogel (P3 and P5, respectively) exhibit small volume changes when exposed to PDGF-$\beta\beta$, roughly on the order of one-fifth the volume change seen for the imprinted hydrogel (P1). P4 shows an interesting result where a hydrogel randomly incorporating aptamer alone shows a significant volume change (nearly half that of the imprinted hydrogel P1) that is attributed to adventitious positioning of two A3 aptamers at a proper distance to promote cooperative binding. Selectivity by the PDGF-$\beta\beta$-imprinted hydrogel was also tested against BSA, which only shrinks the gel 0.98%, giving a rough estimate of nonselective effects in the gel.

Nontemplated Hydrogels with Random Distribution of PDGF-$\beta\beta$ Aptamers. Further verification that cooperative effects by random distribution of A3 within the hydrogel is not as effective as the bioimprinted hydrogels was achieved by preparing a set of nonimprinted hydrogels (i.e., without PDGF-$\beta\beta$ template protein) with different A3 concentrations. The results plotted in the graph of Figure 5 indicate that recognition

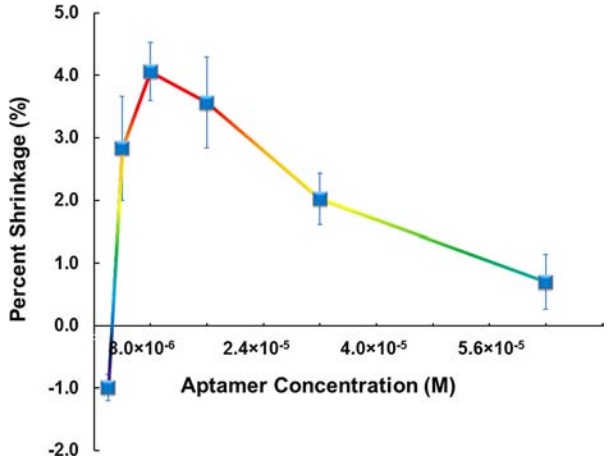

Figure 5. Volume shrinking response of nonimprinted hydrogels as a function of aptamer concentration. The maximum at 1.65×10^{-5} M aptamer A3 indicates some cooperativity; however, the response is significantly lower than that of the bioimprinted hydrogels.

of PDGF-$\beta\beta$ by the hydrogels with random aptamer distributions is affected by aptamer concentration. At lower concentrations of aptamer, it is postulated that the aptamers are immobilized too far apart for cooperative interactions with PDGF-$\beta\beta$. Conversely, if the concentration of aptamer is too high in the polymer mixture, the aptamer binding units will be too close in proximity to allow simultaneous interaction with both binding sites of PDGF-$\beta\beta$. However, it was anticipated that, at an optimum concentration of the aptamer, the proximity between aptamers would match the distance between binding sites on PDGF-$\beta\beta$ and lead to the highest change in volume response. In Figure 5 this optimum appears to be at an aptamer concentration of 1.65×10^{-5} M, which showed a volume change of approximately 4.0%. This is the same concentration of aptamer used to observe the best imprinting effect; however, an important observation is that the response by the PDGF-$\beta\beta$-imprinted hydrogel showed a 2.4-fold higher response (9.4% versus 4.0%) than the best random hydrogel. *This demonstrates that the imprinting process provides significantly better molecular recognition than is possible by merely immobilizing the aptamers in random fashion.*

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Origins of the Hydrogel Shrinking Response. The volume response of these bioimprinted hydrogels is not simply due to the change in cross-link density, since the supramolecular protein complex accounts for only a negligible 0.20 mol % of the total number of cross-links. Contrast this to the 50% decrease in total number of MBAA covalent cross-links shown in Table 2, entry 1 versus entry 3, required to show a significant increase in shrinking due to cross-linking density. However, change in cross-linking density has been the only underlying explanation put forth in the literature to account for the volume change in similar biomprinted hydrogel systems.[17,23,24] It is equally important to note that the volume changes cannot be attributed to mass increase or osmotic effects, because several of the control hydrogels have the same template loading as the corresponding bioimprinted hydrogels. In the case of thrombin, control polymers T2, T3, and T5 in Figure 2 have the same A1 loading as the bioimprinted hydrogel T1, and thus the same uptake of thrombin. This same reasoning can be applied more directly to the PDGF-$\beta\beta$ control hydrogel P3 reported in Figure 4 versus the imprinted gel P1, which has identical composition after removal of the PDGF-$\beta\beta$ template; and highlights the important role of the bioimprinting process to maximize the hydrogel response versus randomly polymerized materials.

Temperature studies on the volume-changing response revealed that there is a θ point reached, indicating a volume exclusion mechanism as a result of the supramolecular protein cross-links by classical polymer theory.[41−43] Effects of temperature (details provided in Supporting Information) on the PDGF-$\beta\beta$-imprinted hydrogel are shown in Figure 6, revealing

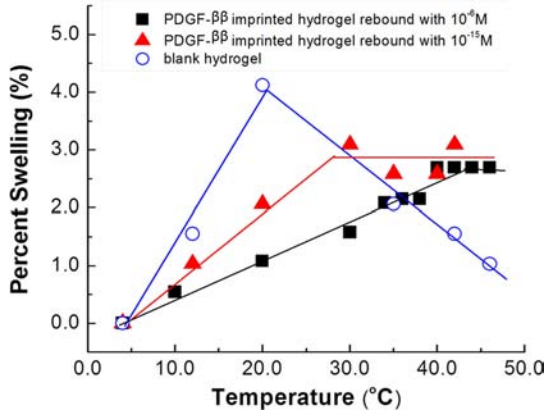

Figure 6. Temperature study of PDGF-$\beta\beta$-imprinted hydrogels, indicating an excluded-volume mechanism.

that the hydrogel first expands as temperature is increased and then contracts, or at least stops expanding, as the temperature is further increased. The temperature where the expansion stops is referred to as the θ condition, where hydrogel insolubility cancels the effects of excluded volume.[44] In the presence of low numbers of supramolecular protein cross-links (triangles in Figure 6), thermal swelling is counterbalanced, and therefore lower than that of blank hydrogel, by a decrease in volume exclusion occurring from restricted polymer chains. In addition, the θ temperature increased, indicating that the restricted polymer chains require more thermal energy (versus blank hydrogel) to be surrounded by solvent as expected for volume exclusion effects. As the concentration of protein is further increased (squares in Figure 6), the swelling decreases more, and the θ temperature continues to increase in response to increased supramolecular cross-links. Because the contribution of the protein−aptamer cross-links is small compared to the overall cross-linking density, the amplification is due to a more complex model of the hydrogel than can be described by classical polymer theory. For example, one possibility may be that the small amount of thrombin−aptamer cross-links can nucleate architectural changes that are scaled through hierarchical arrangement of components in the hydrogel structure. Thus, improvements to the amplified volume response of materials like these are anticipated to come from other considerations of volume exclusion (rather than simply cross-link density), arising from more general theories such as entropy theory or entanglement excluded volume put forth by Douglas and co-workers.[45,46]

Effects of Dehydration on Hydrogels. For stability, storage, and transport of the bioimprinted hydrogels, the dehydration/rehydration properties of these materials were investigated. Figure 7 shows a sequence of photographs of a

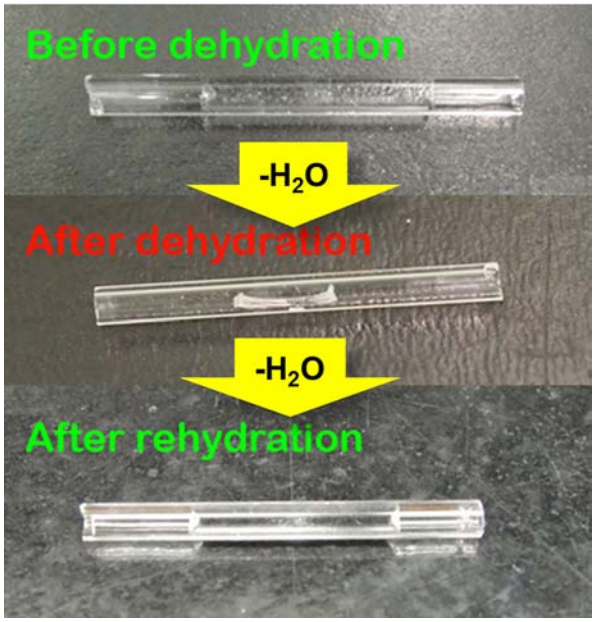

Figure 7. Sequential photographs logging the changes in appearance for dehydration and rehydration of the hydrogels. Dehydration was carried out on gels in the capillaries, and rehydration was carried out by addition of PBS (2.0 mM, pH = 7.4), replaced every 3−6 h until the size reached equilibration.

PDGF-$\beta\beta$-imprinted hydrogel before dehydration, after dehydration, and after a final rehydration of the same gel. The size of the rehydrated gel is the same as the original gel, and this behavior was found for all gels tested. Figure 8 presents the volume shrinking results comparing three PDGF-$\beta\beta$-imprinted hydrogels (PD1−PD3) versus a nonimprinted control hydrogel (PD4) made with PDGF-$\beta\beta$ but without any aptamers. PD1 was dehydrated immediately after polymerization, leaving the PDGF-$\beta\beta$ protein remaining in the gel. After rehydration of PD1 and removal of the protein, the shrinking response stayed virtually the same as P1 in Figure 4 which did not undergo any dehydration treatment, verifying stability of the imprinted sites in the dehydrated gel. The second hydrogel, PD2, was subjected to two iterations of PDGF-$\beta\beta$ removal/rebinding and then dehydrated after the last PDGF-$\beta\beta$ rebinding step

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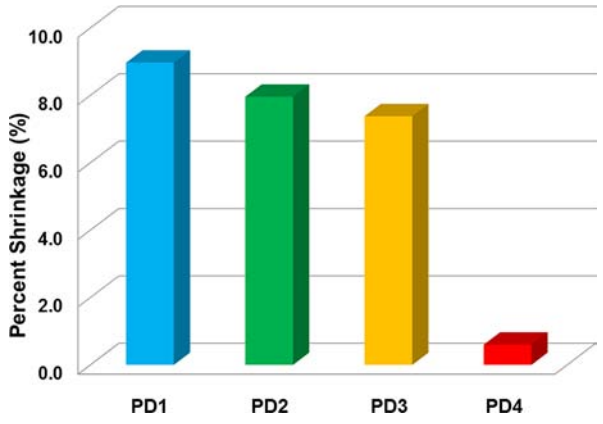

Figure 8. Comparison of volume shrinking response for dehydrated hydrogels that have undergone different predehydration treatments with a nonimprinted control. Hydrogels were incubated with 1.0×10^{-6} M PDGF-$\beta\beta$.

with the PDGF-$\beta\beta$ still in the hydrogel. The third entry is an imprinted hydrogel (PD3) that was treated similarly to PD2, but PDGF-$\beta\beta$ was not reintroduced in the final step before the gel was submitted to the drying process. While the response for PD1 is nearly unchanged from that seen for the typical imprinted gel P1 from Figure 4, PD2 and PD3 show only slightly lower responses, verifying that any of these procedures can be used to provide highly responsive rehydrated gels. The nonimprinted hydrogel PD4 prepared with PDGF-$\beta\beta$ but without aptamers shows negligible response to PDGF-$\beta\beta$, which demonstrates the irreplaceable role of the aptamers in fabricating these highly responsive hydrogels.

CONCLUSIONS

Bioimprinted hydrogels have been developed that use polymerizable aptamers that show specific response to target proteins. The reversible response of these superaptamer hydrogels provided an equal (approximately 5−6% volume change) or larger response (nearly 10% volume change) than reports of biomolecule-responsive hydrogels fabricated with antibodies, proteins, and glycoproteins.[24,25] Not only are the responses large but the sensitivity of the hydrogels is high, detecting protein biomarkers down to femtomolar concentrations, compared to micromolar or submicromolar concentrations by other bioimprinted hydrogels.[24,25] These detection limits are competitive with modern methods requiring sophisticated instrumentation and highly trained personnel such as surface plasmon resonance,[47,48] electrochemical devices,[49−52] microscopy,[53,54] microbalance technologies,[55,56] fluorescent methods,[57−59] and sandwich assays.[47,60,61] The macromolecular amplification seen cannot be attributed simply to a classical polymer theory of cross-link density, osmotic pressure, or the added mass of the analyte, which is minor compared to the volume change in the hydrogel. However, temperature studies reveal that excluded volume does change upon loss of protein-based supramolecular cross-links, providing insight into the large volume amplification that affords visual detection of biomolecules at concentrations lower than many analytical techniques and instrumentation. The greater understanding of the origins of macromolecular amplification in these hydrogels can be used to improve the magnitude of volume change and response time in the next generation of superaptamer hydrogels. In addition, the visibility of the hydrogel response eliminates the need for trained personnel, expensive equipment, or complex detectors, making them ideal for portable or point-of-care applications. Thus, these materials bring to light new avenues for the design of improved materials for molecule-specific responsive polymers that can ultimately be used for biosensors, drug delivery, and responsive microdevices.

ASSOCIATED CONTENT

Supporting Information

Additional text with experimental details for polymer preparation, removal of proteins, and hydrogel rebinding responses and temperature studies on PDGF-imprinted hydrogels, and two figures showing UV analysis of DNA removed from hydrogels and reproducibility of shrinking response. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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