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Programmable Hydrogels for Controlled Cell Catch and Release Using Hybridized Aptamers and Complementary Sequences

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Supporting Information

ABSTRACT: The ability to regulate cell–material interactions is important in various applications such as regenerative medicine and cell separation. This study successfully demonstrates that the binding states of cells on a hydrogel surface can be programmed by using hybridized aptamers and triggering complementary sequences (CSs). In the absence of the triggering CSs, the aptamers exhibit a stable, hybridized state in the hydrogel for cell-type-specific catch. In the presence of the triggering CSs, the aptamers are transformed into a new hybridized state that leads to the rapid dissociation of the aptamers from the hydrogel. As a result, the cells are released from the hydrogel. The entire procedure of cell catch and release during the transformation of the aptamers is biocompatible and does not involve any factor destructive to either the cells or the hydrogel. Thus, the programmable hydrogel is regenerable and can be applied to a new round of cell catch and release when needed.

Materials are usually functionalized with affinity ligands to achieve specific cell–material interactions through polyvalent ligand–receptor recognition.[1] Polyvalent interactions are strong and beneficial in a number of applications, such as targeted drug delivery.[2] However, strong cell binding poses a challenge to the subsequent release of the cell from the bound material. The ability to convert strong cell binding to a state of cell release is critical in various applications such as regenerative medicine and biological separation.[3] Smart materials that are responsive to light, electricity, compression, temperature, and enzymes[4] have been extensively studied to tackle this challenge.[5] However, it is still challenging to realize highly efficient and specific cell release without sacrificing the structural and functional integrity of the materials and cells. Herein we demonstrate a programmable DNA-responsive hydrogel for cell-type-specific catch and release controlled by nucleic acid hybridization. Importantly, the entire procedure of regulating the cell–hydrogel interactions does not involve factors destructive to either the cells or the hydrogel.

The overall concept is illustrated in Figure 1 and Figure S1 in the Supporting Information (SI). Three single-stranded oligonucleotides are used in this programmable hydrogel-based system, including a primary complementary sequence (CS), a nucleic acid aptamer, and a secondary CS. The primary CS is initially conjugated to the supporting hydrogel through free radical polymerization. It is able to hybridize with the tail of

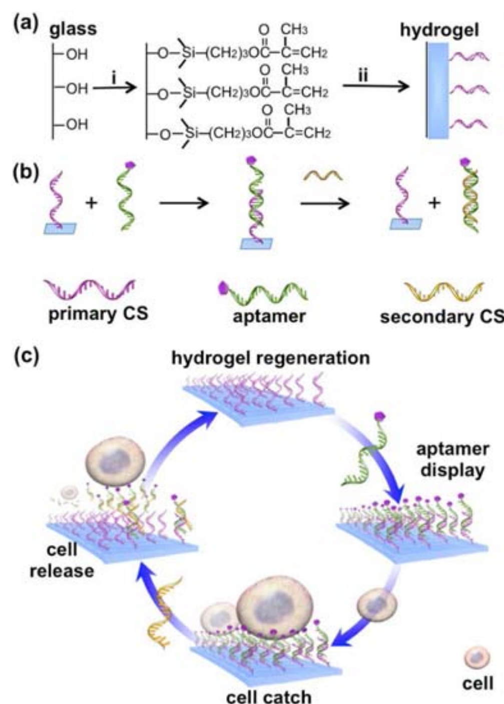

Figure 1. Schematic illustration of the use of a programmable hydrogel for cell catch and release. (a) Synthesis of the hydrogel on glass: (i) silanization; (ii) polymerization. (b) Transformation of the aptamer. (c) Cell catch and release during the transformation of the aptamer. The hybridization with the primary CS enables the display of the aptamer for cell catch. The secondary CS competes against the primary CS to hybridize and release the aptamer from the hydrogel, resulting in cell release.

the nucleic acid aptamer and therefore plays the role of a mediator between the hydrogel and the aptamer. The nucleic acid aptamer is a single-stranded oligonucleotide selected from a synthetic nucleic acid library.[6] Because nucleic acid aptamers have high binding affinities and specificities that are comparable to those of antibodies,[7] they have been studied in a variety of applications at the levels of small molecules, large biomolecules, and whole cells.[8] In this concept, the aptamer is hybridized with the primary CS tethered to the hydrogel and induces cell-type-specific binding via polyvalent aptamer–receptor interactions. When the secondary CS is applied to trigger the hydrogel, the

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aptamer dissociates from the primary CS and hybridizes with the secondary CS. As a result, the polyvalent interactions between the cells and the hydrogel are weakened under physiological conditions without the need to use factors that potentially damage the cells or the hydrogel. Thus, the state of strong cell binding can be nondestructively converted to a state of cell release simply by using a secondary CS. Notably, because the supporting hydrogel is regenerated during cell release, it can be repeatedly used for additional rounds of cell catch and release.

We used gel electrophoresis to examine the competitive hybridization between the three single-stranded oligonucleotides (Figure 2a and Figure S2; see Table S1 in the SI for oligonucleotide sequences). The A and B sequences hybridized together through 20 base pairs with a melting temperature of ~65 °C. Thus, the AB complex is stable at room and body temperature. Although sequence C_{20} could also hybridize with sequence B, C_{20} did not effectively induce the dissociation of the AB complex. In contrast, sequence C_{25} effectively hybridized with sequence B and induced the dissociation of the AB complex. The difference between C_{20} and C_{25} lies in the hybridization length. Sequences B and C_{20} form 20 base pairs, which is the same as the number of base pairs in the AB complex, whereas sequence C_{25} forms 25 base pairs with the B sequence. Increasing the number of base pairs usually leads to more stable hybridization.[9] Thus, C_{25} is more competitive than C_{20} in hybridization with B. The surface plasmon resonance (SPR) sensorgrams confirmed the gel electrophoresis results. The SPR data showed that A and B formed a stable AB complex that did not dissociate in the presence of C_{20} (Figure 2b). In contrast, C_{25} induced the dissociation of the AB complex (Figure 2b).

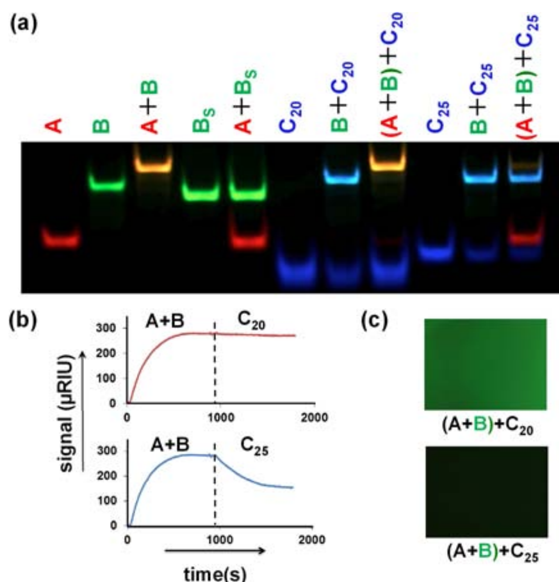

Figure 2. Characterization of nucleic acid hybridization. (a) Electrophoretic gel image. The colors of the letters indicate the fluorophores used for sequence labeling. The sequences A, B, and C are the primary CS labeled with TAMRA, the aptamer labeled with FAM, and the secondary CS labeled with TYE 665, respectively. The subscript after C indicates the hybridizing length. (b) SPR sensorgrams. The solutions of B and C_{20} or C_{25} were sequentially run on an A-coated biochip surface. (c) Fluorescence images. Hydrogels functionalized with sequence A were treated with fluorophore-labeled B and then incubated in the solution of C_{20} or C_{25}.

After analyzing competitive hybridization between multiple oligonucleotides, we chemically incorporated sequence A to a polyacrylamide hydrogel formed as a coating on a glass surface. Free radical polymerization was used to incorporate A, as our previous study showed that free radical polymerization is a simple and effective method for chemical incorporation of oligonucleotides bearing Acrydite into a hydrogel network.[8e] We chose a hydrogel as the cell-binding material in this study because hydrogels usually do not have affinity sites that induce specific cell binding. In addition, hydrogels have been extensively studied for a variety of biological and biomedical applications because of their biocompatibility and structural similarities to extracellular matrices.[10] After the synthesis of the hydrogel coating, the hydrogel was incubated in a solution of FAM-labeled sequence B, washed, and examined under a fluorescence microscope. The hydrogel exhibited strong green fluorescence (Figure S3), showing that sequence B successfully hybridized with sequence A in the hydrogel. When the hydrogel was further treated with sequence C_{25}, the strong fluorescence dramatically diminished (Figure 2c). In contrast, the fluorescence intensity did not change when the hydrogels were treated with either C_{20} (Figure 2c) or C_{25S} (Figure S3). These results are consistent with the electrophoretic gel image (Figure 2a) and SPR sensorgram (Figure 2b), which showed that C_{25} successfully induced the dissociation of B from A in the hydrogel.

To test specific cell binding to the hydrogel, we incubated the hydrogel with the immobilized AB complex in a suspension of CCRF-CEM cells. Sequence B consists of three regions: a 20 nucleotide (nt) region that can hybridize with sequence A, a 40 nt region that can recognize CCRF-CEM cells, and a 5 nt region used as a linker. The 40 nt region is the binding aptamer that was selected from a DNA library to bind to CCRF-CEM cells.[11] This model aptamer is composed of normal nucleotides and may be degraded in a cell culture medium, although chemically modified aptamers are highly resistant to nuclease degradation. Because the purpose of this study was to prove the concept, we used a binding buffer rather than a cell culture medium to avoid the potential problems such as nuclease degradation. The flow cytometry histogram confirmed that this aptamer binds to CCRF-CEM cells rather than the control cells (Figure S4). In the cell-binding assay, we observed 4 ± 2 cells/mm^2 on the native hydrogel coating (Figure S5). This result shows that the polyacrylamide hydrogel is resistant to nonspecific cell binding. Similarly, very few CCRF-CEM cells were observed on the A-functionalized hydrogel, the native hydrogel treated with B, and the A-functionalized hydrogel treated with the partially scrambled sequence B (B_{PS}). The cell densities on these three hydrogels were 7 ± 3, 4 ± 1, and 8 ± 3 cells/mm^2, respectively (Figure S5). In addition, the density of control cells on the hydrogel functionalized with the hybridized B was 6 ± 4 cells/mm^2 (Figure S5). In contrast, a total of 2519 ± 284 CCRF-CEM cells/mm^2 was observed on the hydrogel functionalized with the hybridized AB complex (Figure S5). These data clearly show that polyacrylamide hydrogels resist nonspecific cell binding and that hybridized nucleic acid aptamers can successfully induce cell-type-specific binding to the hydrogel surface.

After demonstrating the ability of the hybridized aptamers to bind CCRF-CEM cells to the hydrogel surface, we studied whether the state of cell binding could be transformed into a

state of cell release using the secondary CS. After treatment with C_{25}, the density of cells decreased to 19 ± 15 cells/mm^2 (Figure 3). The release efficiency was ~99%. In contrast, C_{25S} did not induce significant cell release. These results show that cell binding was successfully converted into cell release via sequence-specific nucleic acid hybridization. We also varied the length of the secondary CS to obtain further understanding of the ability of the secondary CS to induce cell release. The sequences C_{15} and C_{20} did not effectively induce cell release, whereas the sequences C_{25} and C_{30} both released cells successfully (Figure S6). This observation is consistent with the electrophoretic gel image and SPR analysis, which showed that the secondary CS needs to form more base pairs with the aptamer than the primary CS does. We also examined the effect of incubation time on cell release. The time duration was varied from 10 to 60 min, and the cell release kinetics showed that more than 95% of the cells were released within 10 min (Figure S7). After the successful demonstration of cell release, we used a live/dead cell assay to evaluate the viability of the released cells. The live/dead staining did not show a significant difference between the harvested cells and the released cells (Figure 3b). The percentage of viable cells in both groups was ~99%. Taken together, these results clearly demonstrate that it is effective to use a rationally designed secondary CS to trigger hybridized aptamers to release cells from a programmable hydrogel surface in a nondestructive manner.

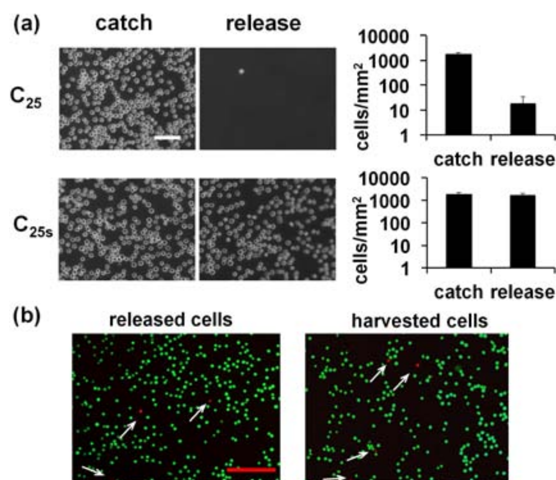

Figure 3. Sequential cell catch and release. (a) Representative images of cells on the hydrogel surface. Each group had three hydrogel samples. White scale bar: 20 μm. (b) Images of live and dead cells. The cells were treated with a mixture of calcein AM (green: live) and ethidium homodimer-1 (red: dead) using a live/dead cell staining kit. The cells in the right image were directly harvested from a cell culture flask. The arrows point to the dead cells (red). Red scale bar: 50 μm.

Finally, we examined whether this nucleic acid-functionalized hydrogel is able to repeat the procedure of cell catch and release. FAM-labeled B and C_{25} were used to treat the hydrogel. Fluorescence micrographs showed that the hydrogel was able to repeatedly catch and release B under the control of C_{25} (Figure S8), indicating that cell catch and release could be repeated. Indeed, the micrographs showed that the cells did attach to the hydrogel and that the attached cells were released from the hydrogel after treatment with C_{25} in an additional round of cell catch and release (Figure 4). Taken together, these results clearly demonstrate that the entire procedure of intermolecular hybridization and transformation of the aptamer is non-destructive to not only the cells but also the hydrogel. Thus, the functionality of the programmable hydrogel is regenerable.

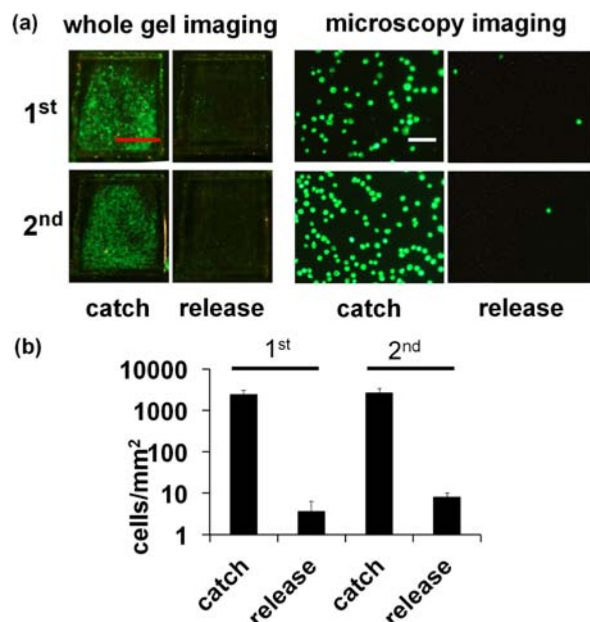

Figure 4. Repetition of cell catch and release. (a) Fluorescence images of cells in two successive rounds of cell catch and release. The cells were labeled with a Vybrant cell-labeling solution for clear observation. Red scale bar, 2 mm; white scale bar, 20 μm. (b) Quantitative analysis of cell catch and release using ImageJ.

The nondestructive cell catch and release controlled by nucleic acid hybridization under physiological conditions makes this platform fundamentally unique and suitable for numerous biological and biomedical applications that need temporal control of the cell−material interactions. For instance, studies have shown that affinity ligands with high density in a synthetic material are important to maintain cell attachment and viability in the early stages but may inhibit cell growth and differentiation in the late stages.[12] Thus, the temporal control of the cell−material interactions has been suggested for regulation of cell behavior and extracellular matrix deposition for regenerative medicine.[12d] Meanwhile, it is important to note that long-term matrix deposition would make complete cell release from the hydrogel difficult. This difficulty might be solved if this current affinity system can be integrated with thermosensitive polymers that have been shown to allow for cell detachment.[13] Another important example is cell separation. It is critical to be able to reverse specific and strong cell−material interactions for cell recovery during the purification of a specific cell population from a heterogeneous cell mixture.[3d−f] Otherwise, strong ligand−receptor interactions could turn on intracellular signaling cascades that would change the cell properties or even induce cell death,[14] which would directly affect the downstream analysis of the separated cells. Conventional methods for releasing cells from a bound material often involve enzyme treatment, high shear stress, or material hydrolysis. These conditions may cause changes in cell or material properties. The platform presented in this study does not involve any of these factors.

One question may be raised regarding the stability of sequence A in the hydrogel, since nucleic acids are susceptible to nuclease degradation. If sequence A were degraded, the

supporting hydrogel would lose its programmable and regenerable capability of immobilizing the aptamer sequence for a new round of cell catch and release. This potential issue may not be problematic because synthetic nucleic acids can be chemically modified using a diverse array of methods.[14] Modifications to the sugar, nucleobase, or phosphodiester bond of nucleotides have been demonstrated to provide significant improvements in the stability of nucleic acids.[15] For instance, an oligonucleotide composed entirely of 2′-*O*-methyl nucleotides has been shown to resist nuclease degradation completely in a 96 h test.[16] Thus, it is possible that the programmable hydrogel could maintain its integrity and be used repeatedly for sequential cell catch and release.

In summary, we have successfully developed a hydrogel-based platform for cell-type-specific catch and release by using nucleic acid oligonucleotides. Importantly, the entire procedure of intermolecular hybridization and transformation of hybridized aptamers does not involve any factor that is potentially destructive to either the cells or the hydrogel. Therefore, this programmable hydrogel-based platform holds great potential for numerous biological and biomedical applications such as regenerative medicine and cell separation. Our future work will test this concept using various cell types.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, table of oligonucleotide sequences, and additional experimental data. 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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