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Endonuclease-responsive aptamer-functionalized hydrogel coating for sequential catch and release of cancer cells

Shihui Li [a,1], Niancao Chen [a,1], Zhaoyang Zhang [b,c], Yong Wang [a,b,*]

[a] Program of Biomedical Engineering, School of Engineering, University of Connecticut, Storrs, CT 06269-3222, USA
[b] Department of Chemical, Materials & Biomolecular Engineering, University of Connecticut, Storrs, CT 06269-3222, USA
[c] National Institutes for Food and Drug Control, Beijing 100050, China

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ABSTRACT

Rare circulating tumor cells are a promising biomarker for the detection, diagnosis, and monitoring of cancer. However, it remains a challenge to develop biomedical devices for specific catch and nondestructive release of circulating tumor cells. The purpose of this study was to explore a unique system for cell catch and release by using aptamer-functionalized hydrogels and restriction endonucleases. The results show that the hydrogel coating was highly resistant to nonspecific cell binding with ~5–15 cells/mm^2 on the hydrogel surface. In contrast, under the same condition, the aptamer-functionalized hydrogel coating could catch target cancer cells with a density over 1000 cells/mm^2. When the hydrogel coating was further treated with the restriction endonucleases, the bound cells were released from the hydrogel coating because of the endonuclease-mediated sequence-specific hydrolysis of the aptamer sequences. The release efficiency reached ~99%. Importantly, ~98% of the released cells maintained viability. Taken together, this study demonstrates that it is promising to apply endonuclease-responsive aptamer-functionalized hydrogels as a coating material to develop medical devices for specific catch and nondestructive release of rare circulating tumor cells.

1. Introduction

Circulating tumor cells in blood are a promising biomarker to determine the stage of a tumor and to guide the design of an appropriate therapeutic protocol [1–5]. However, these cells have a very small population. In general, there are less than 10 circulating tumor cells in 1 mL blood of a cancer patient whereas the same volume of blood contains approximately 5×10^9 normal cells [3]. Therefore, a variety of methods and materials have been recently investigated for sensitive detection and accurate characterization of rare circulating tumor cells.

Tumor cells in a blood sample can in principle be detected by the quantification of specific messenger RNAs with polymerase chain reaction (PCR) [6–9]. However, presumably because of the instability of RNAs and the complex amplification procedure, the PCR-based cell analysis often leads to results with considerable variations or even false-positive results [3]. Alternatively, circulating tumor cells can be examined directly through cytometric analysis. Because circulating tumor cells have a very small number in comparison to the entire cell population, specific cell labeling and immunomagnetic enrichment are often applied to pretreat a blood sample [10,11]. After the pretreatment, the cell mixture can be analyzed with numerous methods [3]. A commonly used method is flow cytometry [11,12]. Some clinical studies have demonstrated that flow cytometry is very sensitive and can detect a single tumor cell per 10^7 cells [11]. However, circulating tumor cells have been found to form clusters or aggregates that have different scatter characteristics from individual cells [3]. Thus, the need of sample pretreatment and the heterogeneous geometry of tumor cells might compromise the sensitivity of cytometric analysis.

Recently, affinity-based microfluidic systems have been extensively studied for selective separation and detection of viable circulating tumor cells from the whole blood without the need of pre-labeling or processing samples [13,14]. The examination of temporal changes in the number of circulating tumor cells has showed a reasonable correlation with the stage of disease determined by standard radiographic methods. Despite great promise, there are still two critical issues that need to be addressed. One is the specificity of cell catch because the identified circulating tumor cells exhibited approximately 50% purity [13]. The other is the

* Corresponding author. Program of Biomedical Engineering, School of Engineering, University of Connecticut, 191 Auditorium Road, Storrs, CT 06269-3222, USA. Tel.: +1 860 486 4072; fax: +1 860 486 2959.
E-mail address: yongwang@engr.uconn.edu (Y. Wang).
[1] Equally contributed.

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capability to release tumor cells nondestructively after cell catch. Strong cell binding can trigger intracellular signaling cascades or even cell death [15–17], which will undoubtedly produce negative effects on the critical analysis of circulating tumor cells. Therefore, significant efforts are needed to address these two issues.

The development of an appropriate device for cell separation mainly involves the upstream design of biomolecules and coatings and the downstream optimization of device geometry and flow conditions [18–23]. This study focused on the former one, aiming to develop a system for specific catch and nondestructive release of target cells using aptamer-functionalized hydrogels as a coating material and restriction endonucleases as a cell release reagent (Fig. 1). Aptamer-functionalized hydrogels are an emerging biomaterial and have recently attracted great attention in the fields of drug delivery [24], biomimetic engineering [25], and molecular biosensing [26]. However, no attempt has been made to explore their potential for the development of medical devices for cell separation. In this study, aptamer-functionalized hydrogels were coated on a glass surface for cell type-specific catch. Numerous control surfaces were also investigated to evaluate the specificity of aptamer-functionalized hydrogels in catching target cancer cells. After the examination of cell catch, two types of restriction endonucleases were used to release cells from the hydrogel surface. Both the specificity and kinetics of cell release were studied. A cell-staining assay was also carried out to determine whether the entire procedure of cell catch and release would cause the decrease of cell viability.

2. Materials and methods

2.1. Chemical reagents

The acrylamide/bis-acrylamide solution (40% w/v; 29:1), ammonium persulfate (APS), N,N,N′,N′-tetramethylenediamine (TEMED), phosphate buffered saline (PBS), and sodium hydroxide were obtained from Fisher Scientific (Suwanee, GA). 3-(Trimethoxysilyl) propyl methacrylate (TMSPM), the magnesium chloride solution (1.0 M), and the glucose solution (45% w/v) were purchased from Sigma–Aldrich (Louis, MO). Dulbecco's phosphate buffered saline (DPBS), bovine serum albumin (BSA), and LIVE/DEAD staining kit were all purchased from Invitrogen (Carlsbad, CA). Nucleic acid oligonucleotides (Table 1) were produced by Integrated DNA Technologies (Coralville, IA) and used directly without further purification. Restriction endonucleases *Bam*HI-HF (100,000 units/mL) and *Kpn*I-HF (100,000 units/mL) were purchased from New England Biolabs (Ipswich, MA). RPMI medium 1640 was obtained from ATCC (Manassas, VA). Fetal bovine serum (FBS, 10%) and the penicillin–streptomycin solution (100 units/mL) were purchased from Hyclone (Logan, UT). The trypsin solution (0.05% w/v) was purchased from Mediatech (Manassas, VA).

2.2. Preparation of silanized glass surface

Glass slides (Fisher Scientific, Suwanee, GA) were cut into small squares with a dimension of 4 × 4 mm^2. The glass squares were sonicated in NaOH (1.0 M) for 10 min. After washed thoroughly with deionized water, the slides were treated for 5 min in a silanization solution that was prepared by diluting TMSPM (0.5 mL) in the mixture of ethanol (50 mL) and diluted glacial acetic acid (1.5 mL, 10% v/v). The silanized glass squares were washed with ethanol, dried in the air and stored in a vacuum desiccator before use.

2.3. Preparation of polyacrylamide hydrogel coating

Polyacrylamide hydrogels were synthesized on the silanized glass surface to produce hydrogel coatings. The pregel solution was prepared by adding TEMED (0.15 μL, 5% v/v) into the mixture of 10% acrylamide solution (1 μL) containing the sequence A_{1A} (100 μM) and APS (0.08 μL, 10% w/v). Immediately after the preparation of the pregel solution, it was transferred to a supporting glass slide and covered by the silanized glass square. After 1-h polymerization, the glass square was carefully flipped off the large glass slide and rinsed thoroughly with PBS.

2.4. Gel electrophoresis

Complementary DNA oligonucleotides were mixed together at a molar ratio of 1:1 in PBS containing $MgCl_2$ (10 mM) and incubated at 37 °C for 1 h. Restriction enzyme (5 units) was added to cleave 1 pmol of DNA double helix at 37 °C for 0.5 h. The DNA solutions were loaded into polyacrylamide gel (10% w/v) for running electrophoresis in a Bio-Rad Mini-PROTEAN tetra cell (Hercules, CA). After electrophoresis, the polyacrylamide gel was stained with ethidium bromide and then imaged with a Bio-Rad GelDoc XR system (Hercules, CA).

2.5. Imaging of hydrogel coatings

Both SEM and fluorescence imaging were used to characterize hydrogel coatings. For SEM imaging, glass slides coated with affinity hydrogels were dried by lyophilization. The slides were imaged under a JEOL 6335F field emission scanning electron microscope (FESEM). For fluorescence imaging, glass slides were incubated in B_{1T} solution (20 μL, 5 μM in DPBS) at 37 °C for 1 h. After thoroughly washed with DPBS, the slides were imaged under an inverted fluorescence microscope (Axiovert 40CFL, Carl Zeiss).

2.6. Cell culture

CCRF-CEM cells (CCL-119, human T lymphocytic leukemia cell line) and Ramos cells (Human B lymphoma cell line) were obtained from ATCC (Manassas, VA). CCRF-CEM cells were cultured in RPMI medium 1640 supplemented with 10% FBS and the 100 IU/mL penicillin–streptomycin solution. Ramos cells were cultured and maintained in RPMI medium 1640 supplemented with inactivated FBS and the penicillin–streptomycin solution. Both cells were cultured in an incubator at 37 °C in a 5% CO_2 atmosphere.

2.7. Cell catch and release

Glass squares coated with hydrogels were incubated in an aptamer solution (5 μM) at 37 °C to immobilize nucleic acid aptamers. After 1 h incubation, the glass squares were thoroughly washed with the binding buffer that was DPBS containing glucose (4.5 g/L), $MgCl_2$ (10 mM), and BSA (0.1% w/v). For cell catch, the glass squares were incubated in cell suspension (800 μL, 5 × 10^5 cells/well) in a 24-well plate at 37 °C for 30 min. The unbound cells were gently removed from the coatings by shaking the plate at 90 rpm for 1 min. For cell release, the glass squares were incubated in restriction enzyme solution (80 μL, 5 units/μL) at 37 °C for 30 min. The released cells were gently rinsed off the surface by shaking the plate at 90 rpm for 10 min. The glass slides were imaged using an inverted microscope (Axiovert 40CFL,

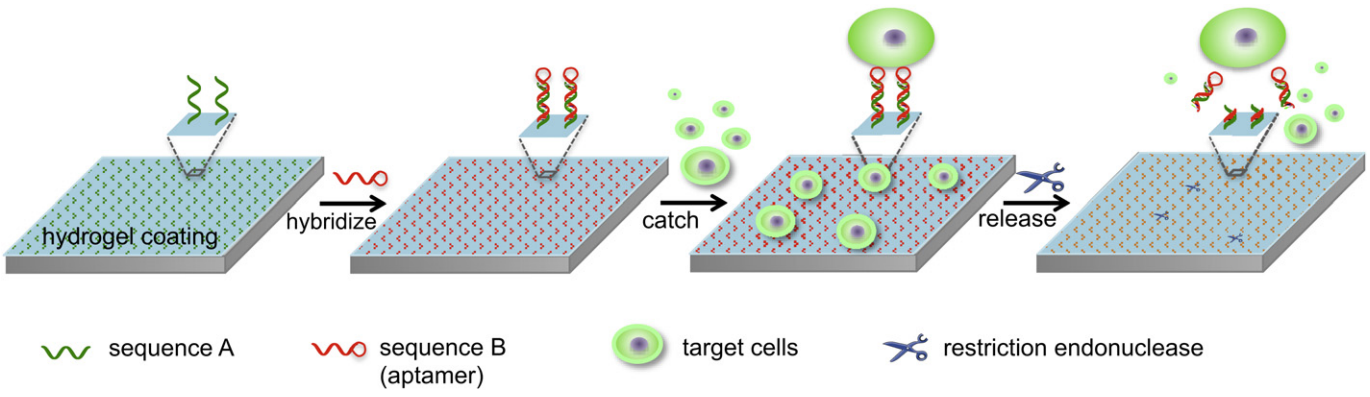

Fig. 1. Schematic of sequential cell catch and release using aptamer-functionalized hydrogel coating and restriction endonuclease.

Carl Zeiss). The cells on hydrogels were quantified using ImageJ. Three images were randomly selected for each sample. A total of three samples were used in each group.

2.8. Flow cytometry

Three flow cytometry experiments were run to determine the binding functionality of the hybridized aptamer, to demonstrate the endonuclease-mediated hydrolysis of the hybridized aptamer, and to test the influence of enzymatic hydrolysis on cell properties. In the first experiment, 5×10^5 cells were incubated in mixture of A_{1F} and B_1 (100 μL) for 30 min at 4 °C. The mixture was prepared with A_{1F} (0.2 μM) and B_1 (0.1 μM) in DPBS. After the incubation, the cells were washed with 1 mL of cold washing buffer (DPBS containing 4.5 g/L glucose and 10 mM $MgCl_2$). The washed cells were immediately analyzed by the flow cytometer (BD FACSCalibur, San Jose, CA). A total of 10,000 events were counted. B_{1S} was used as control. In the second experiment, *Bam*HI (1 μL) was added to 100 μL of the mixture of A_{1F} and B_1 and the mixture was incubated at 37 °C for 0.5 h. Afterwards, a total of 5×10^5 CCRF-CEM cells were incubated in the *Bam*HI-treated mixture for 30 min at 4 °C, washed with cold washing buffer (1 mL), and analyzed by the flow cytometer. A total of 10,000 events were counted. In the third experiment, the cells bound to the hydrogels were treated with *Bam*HI (40 units) in an 80 μL of binding buffer or 80 μL of trypsin solution (0.05% w/v). FBS were added to the trypsin solution to stop cell trypsinization at the end of the release step. The released cells were labeled with the hybridized aptamer using the same protocol as described in the first flow cytometry experiment. A total of 5000 events were counted.

2.9. Live/dead cell staining

The released cells were stained with a mixture of calcein AM (1 μM) and ethidium homodimer-1 (1 μM) using the LIVE/DEAD cell staining kit. Fluorescence cell images were captured using the inverted fluorescence microscope (Axiovert 40CFL, Carl Zeiss).

3. Results and discussion

3.1. Synthesis of hydrogel coating on glass surface

Numerous methods have been studied to coat a solid surface [27]. For instance, a material can be incubated in a solution to allow for molecules to be physically adsorbed onto its surface. However, the adsorbed molecules may easily desorb under a dynamic shaking or flow condition because physical adsorption usually depends on weak hydrophobic or charge–charge interactions [27]. To stabilize a coating on a surface, the coating materials need to be conjugated to a surface via covalent bonds. In this study, we used simple, single-step free radical polymerization to synthesize a cross-linked hydrogel coating that is chemically conjugated to the glass surface. Fig. 2A shows the schematic of the synthesis of the hydrogel coating using a sandwich method. The small glass square was silanized with 3-(trimethoxysilyl)propyl methacrylate to carry methacrylate groups. When the mixture of acrylamide, bis-acrylamide, and DNA with acrydite was initiated to polymerize by APS and TEMED (Fig. 2B), the small glass square was immediately put on the liquid surface. Thus, after polymerization, the formed hydrogel was chemically conjugated to the glass square. The SEM images show that the hydrogel coating is very smooth with a thickness of ~10 μm (Fig. 2C). Importantly, when the glass square was washed and shaken in aqueous solutions, the hydrogel coating was stable on the glass surface.

3.2. Examination of hydrogel coating for resisting nonspecific cell binding

When a device is applied to cell separation and detection, it is important that cells do not have strong nonspecific interactions with the surface of this device. It is particularly important to separate rare circulating tumor cells from the human blood because of the overwhelmingly large number of normal cells with a diverse array of surface properties. A number of microfluidic devices have been developed for the capture of target cells based on the direct conjugation of affinity ligands to glass slides or silicon wafers [13,20–23]. Many of these devices did not exhibit the problem of nonspecific cell binding. However, other studies show that a specific coating is often necessary to avoid nonspecific binding [28–31]. This difference in literature may be attributed to the use of different techniques of surface treatment or operating conditions.

In this study, we examined nonspecific cell binding in a pseudo-static condition, in which cells were allowed to gradually precipitate to the material surface from the cell suspension. Different surfaces were studied and compared, including untreated glass surface, NaOH treated glass surface, silanized glass surface, and the hydrogel coating. The cell images show that the density of CCRF-CEM cells on the untreated glass surface, NaOH treated glass surface, and silanized glass surface were ~1100, 1200, and 1400 cells/mm^2, respectively (Fig. 3). In contrast, the cell density on the hydrogel coating was ~5 cells/mm^2 (Fig. 3). These results indicate that it would be important to prepare a coating to prevent nonspecific cell binding to the surface of a device, and that the hydrogel coating would be suitable for solving this non-specific binding problem. Although the polyacrylamide hydrogel was studied herein, other polymeric hydrogels (*e.g.,* poly(ethylene glycol) (PEG), poly(vinyl alcohol), and poly(2-hydroxyethyl methacrylate)) may provide similar or better effectiveness in resisting nonspecific cell binding. In addition to the hydrogels, other materials such as PEG brush [32,33] and zwitterionic polymers [34,35] may also be used to reduce nonspecific cell binding. Moreover, the variation of numerous reaction conditions may further improve the capability of the hydrogel coating in resisting nonspecific cell binding.

3.3. Examination of aptamer-functionalized hydrogel coating for catching cells

After demonstrating the functionality of the polyacrylamide hydrogel in resisting nonspecific cell binding, we studied whether aptamers were capable of inducing cell type-specific binding to the polyacrylamide hydrogel. The model aptamer used in this study was originally selected from a DNA library to bind CCRF-CEM cells with Ramos cells as a negative control [36]. Its functionality has been well studied. Therefore, CCRF-CEM cells and Ramos cells were used as positive and negative cells to illustrate the concept. The aptamer (*i.e.,* sequence B) was rationally designed to present three functional regions (Fig. 4A). The first region is the binding motif that is the

Table 1
List of DNA oligonucleotides.

Name	Sequence (5′–3′)
A_1	ATATTGTTTGTTACACGGGATCCCGATTTT
A_{1A}	*Acrydite*-ATATTGTTTGTTACACGGGATCCCGATTTT
A_{1F}	ATATTGTTTGTTACACGGGATCCCGATTTT-*FAM*
A_{2A}	*Acrydite*-ATATTGTTTGTTACAGGGGTACCCCATTTT
B_1	TCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATAGTAAAAATCGGGATCCCGTGTAA
B_{1S}	CAATGGCGTTGGGAGGACTCCGGTTACTGATTACGTCAATCACAAAAAATCGGGATCCCGTGTAA
B_{1T}	TCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATAGTAAAAATCGGGATCCCGTGTAA-*TAMRA*
B_2	TCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATAGTAAAAATGGGGTACCCCTGTAA

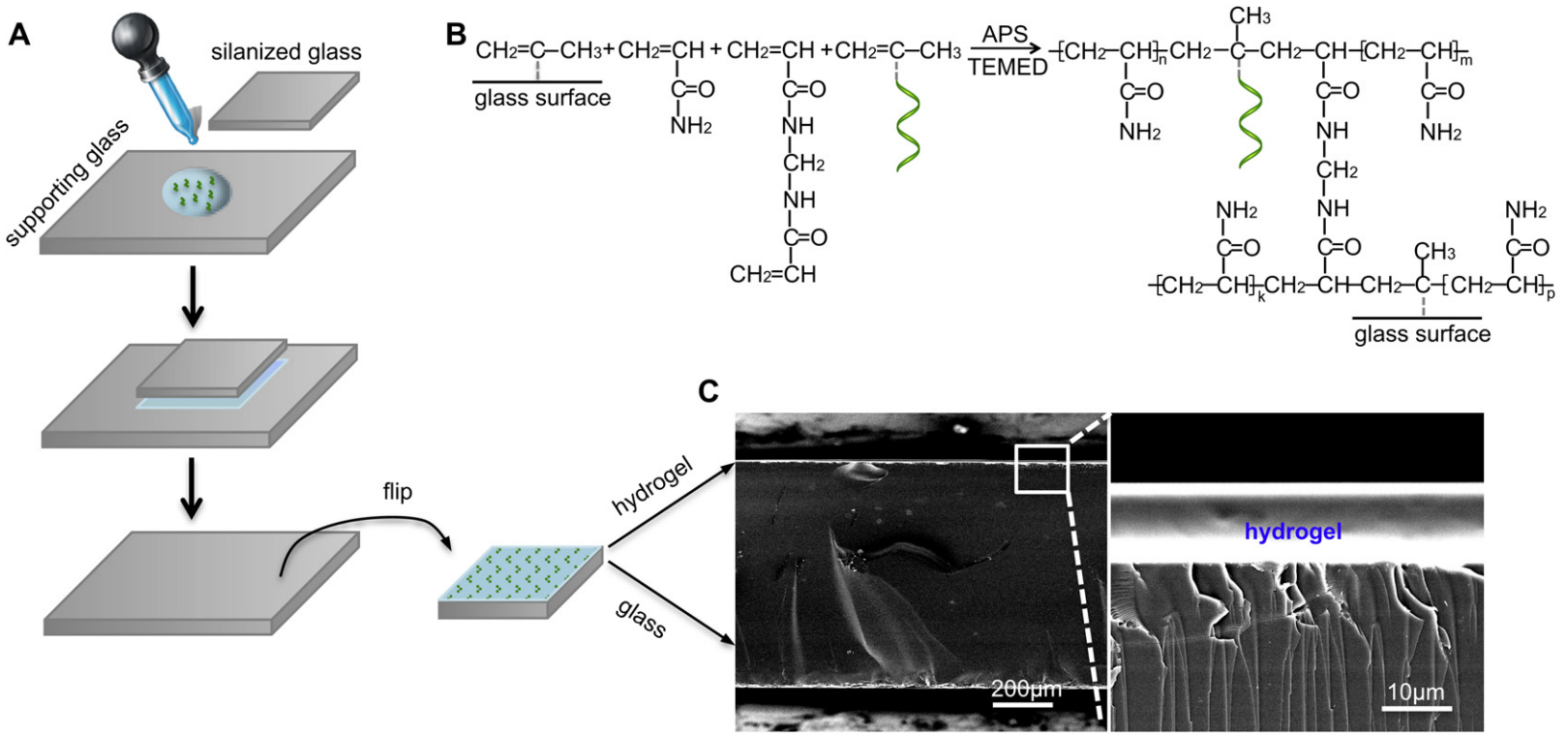

Fig. 2. Preparation of hydrogel coating. (A) Schematic of the sandwich method for coating a polyacrylamide hydrogel on the glass square. (B) Chemical structures and the principle of chemical reaction. (C) SEM images.

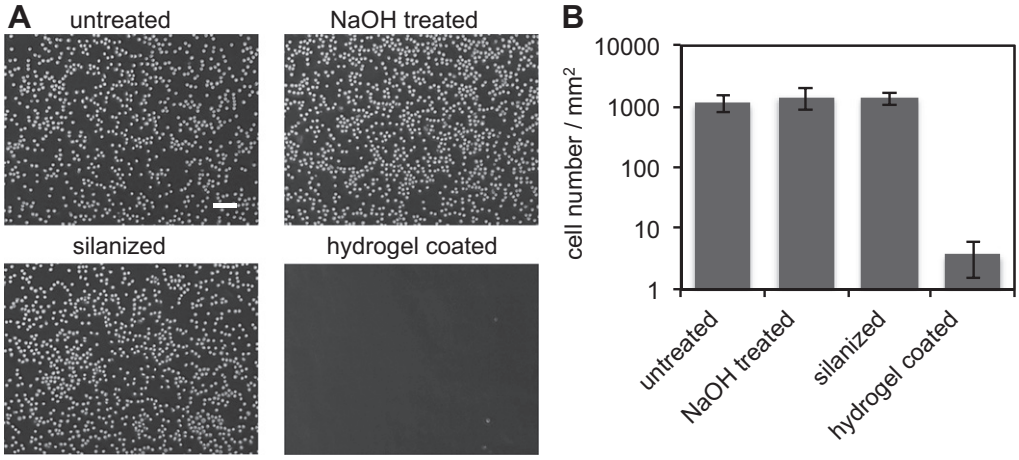

Fig. 3. Characterization of the functionality of the polyacrylamide hydrogel coating in resisting nonspecific cell binding. (A) Representative microscopy images of cells on different surfaces. (B) Comparison of the density of cells on different surfaces. The cell numbers were quantified with ImageJ. Scale bar: 10 μm.

same as that of the parent aptamer. It contains a total of 40 nucleotides. The second region is a five-nucleotide linker used to increase molecular flexibility and reduce steric hindrance. The third region is a twenty-nucleotide tail used to hybridize with sequence A immobilized in the hydrogel. Importantly, this tail was specially designed with a restriction endonuclease cleavage site in the middle.

The sequences A_1 and B_1 can hybridize through 20 base pairs with a melting temperature higher than 60 °C. The control sequence B_{1S} can also form the same 20 base pairs with the sequence A_1. The gel electrophoretogram showed that these pairs stably hybridized in aqueous solutions (Fig. 4B). In addition to the examination of intermolecular hybridization in aqueous solutions, we also investigated the feasibility of hybridizing these sequences in the hydrogel coatings (Fig. 4C). A total of three hydrogels were synthesized. The first one was a native polyacrylamide hydrogel. The second one was a polyacrylamide hydrogel that was prepared with a pregel solution containing sequence A_1 without acrydite. Because sequence A_1 did not have acrydite, it would not be able to participate in free radical polymerization. In contrary, the third one was prepared with the pregel solution containing sequence A with acrydite (*i.e.*, A_{1A}). Thus, during the free radical polymerization, acrydite enabled the chemical incorporation of sequence A into the hydrogel network. All three hydrogel coatings were treated with sequence B_{1T} and then subjected to thorough washing. TAMRA was used to label sequence B_{1T} for clear legibility of the hybridization. The fluorescence image shows that the A_{1A} hydrogel exhibited stronger fluorescence intensity than the other two hydrogels (Fig. 4C). It demonstrates that A_1 was successfully incorporated into the hydrogel, and that A_1 and B_{1T} hybridized successfully in the hydrogel.

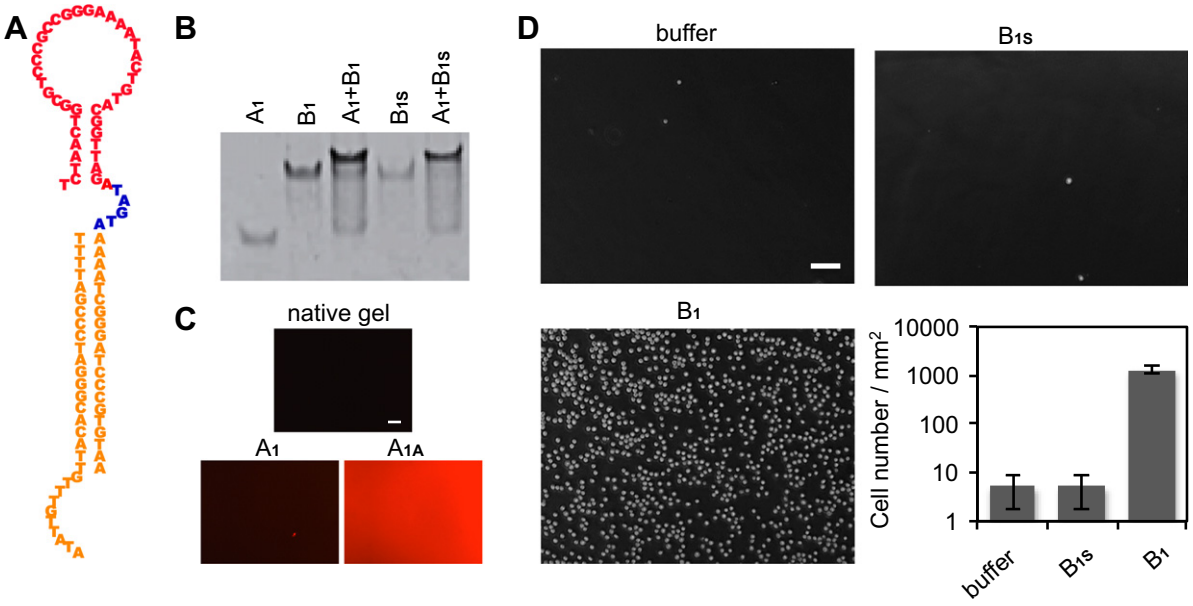

Fig. 4. Characterization of the functionality of the aptamer in catching CCRF-CEM cells. (A) Secondary structure of the hybridized aptamer. Red indicates the binding motif; blue indicates the linker and yellow indicates the hybridized segment. (B) Electrophoretogram of intermolecular hybridization. (C) Fluorescence images of hydrogel coatings treated with sequence B_{1T}. These hydrogels were thoroughly washed after B_{1T} treatment. Sequence B_{1T} carried TAMRA for clear legibility. Sequence A_1 in the A_1 hydrogel did not bear acrydite; sequence A_{1A} in the A_{1A} hydrogel was conjugated with acrydite. (D) Effect of different treatments on the capability of A-functionalized hydrogel in catching CCRF-CEM cells. Three A-functionalized hydrogel samples were treated with buffer, B_{1s}, and B_1, respectively. The cell images were captured under an inverted microscope. The cell numbers were quantified with ImageJ. Scale bar: 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

After the successful demonstration of intermolecular hybridization in the hydrogel, a cell catch experiment was run to examine whether the immobilized B_1 could induce cell binding to the hydrogel. The B_1 functionalized hydrogel could catch cells with the density over ~1000 cells/mm^2 (Fig. 4D). In contrast, only ~10 cells/mm^2 were observed on the other two control surfaces. These results demonstrate that the hybridized functional aptamers enabled the successful cell catch to the hydrogel coating.

The aptamer was purposely immobilized to the hydrogel using intermolecular hybridization rather than direct conjugation for an important concern. The aptamer is designed to carry an exogenous endonuclease-recognizing cleavage site comprised of nucleotides. These exogenous nucleotides may form intramolecular base pairs with the original nucleotides of the aptamer and therefore affect the binding affinity of the aptamer. The use of a hybridized aptamer can simply avoid this potential problem.

3.4. Determination of cell type-specific catch

The success of cell catch relies on not only the ability to catch target cells, but also the ability to resist the binding of non-target cells. Therefore, another cell catch experiment was run to compare the binding of CCRF-CEM and Ramos (*i.e.*, control) cells. The flow cytometry analysis shows that the aptamer specifically binds to CCRF-CEM cells rather than Ramos cells (Fig. 5A). Consistent with the flow cytometry analysis, the aptamer was able to catch CCRF-CEM cells rather than Ramos cells (Fig. 5B and C) to the hydrogel coating. The profile of binding kinetics shows that the density of Ramos cells on the hydrogel coating did not change throughout the experiment. Approximately 5 Ramos cells/mm^2 were observed on the hydrogel surface. In contrast, the cell density of CCRF-CEM cells rapidly increased during the first 30 min and then gradually reached plateau. These results show that the use of aptamers ensures cell type-specific catch to the hydrogel coating.

A number of other affinity ligands may also satisfy the need of cell type-specific catch. These ligands include but are not limited to antibodies, peptides, and certain small molecules (*e.g.*, folic acids). Although all of these affinity ligands can be in principle applied to catch target cells, we purposely used nucleic acid aptamers to catch cells for three main reasons. First, nucleic acid aptamers are synthetic oligonucleotides screened from DNA/RNA libraries with high binding affinities and specificities that are comparable to antibodies [37,38]. Second, aptamers are synthesized using standard phosphoramide chemistry [39]. Thus, aptamers exhibit little or no batch-to-batch variation, which is definitely beneficial to increase the reliability of cell catch. Third, our ultimate goal is to achieve not only specific cell catch but also nondestructive cell release based on endonuclease-mediated cleavage. It is easy to design and synthesize nucleic acid aptamers with an endonuclease-recognizing site rather than DNA-antibody or DNA-peptide chimeras.

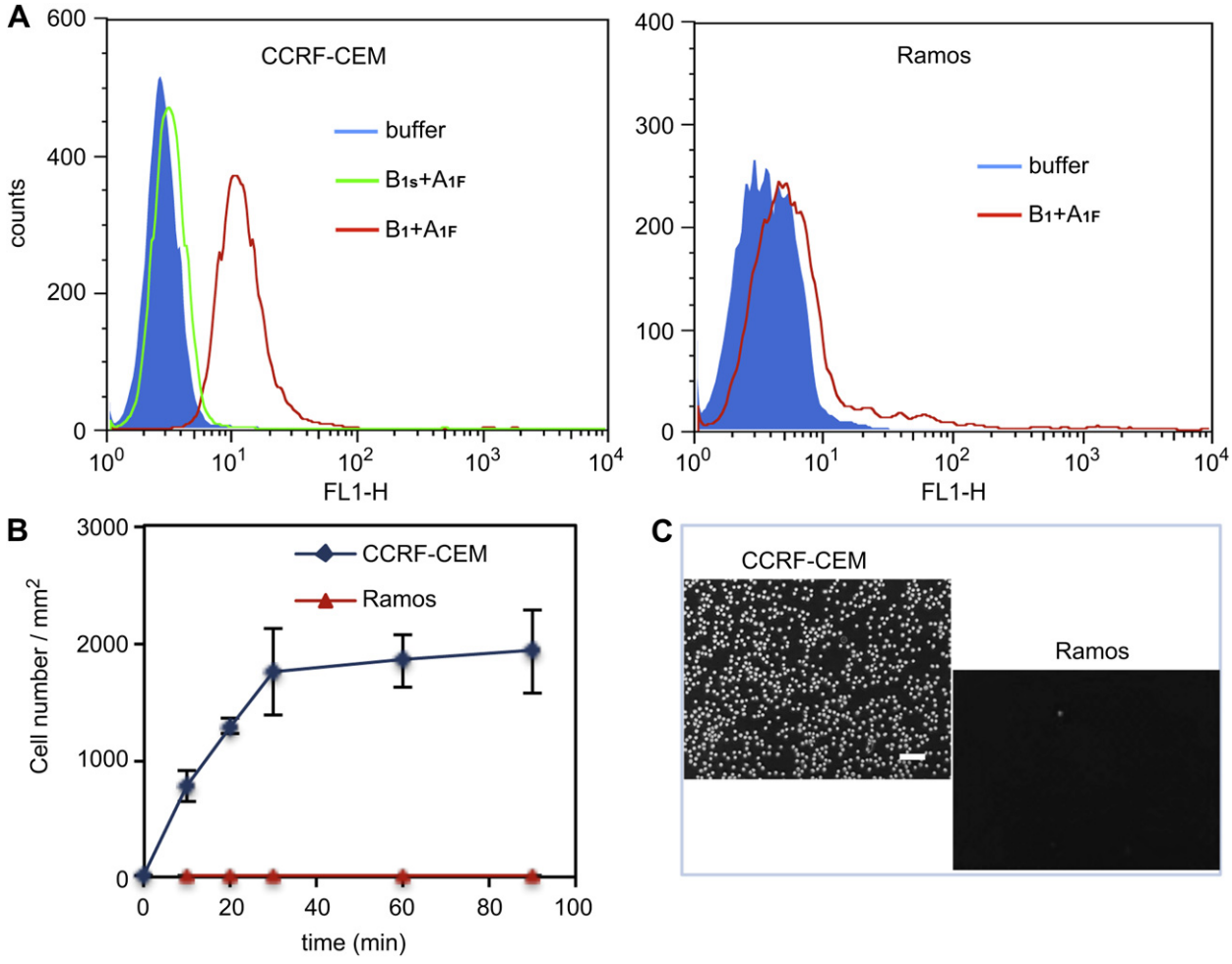

Fig. 5. Characterization of cell type-specific catch. (A) Flow cytometry histograms. (B) Kinetics of cell binding to the hydrogel coating. (C) Representative microscopy images of cells on the hydrogel coating. The images were captured at 30 min post cell seeding. Scale bar: 10 μm.

3.5. Endonuclease-mediated sequence-specific hydrolysis for cell release

After cell catch and separation, it is also important to release cells with minimized cell damage for downstream cell characterization. To release the cells, we used a restriction endonuclease (*i.e., Bam*HI) [40–42] to treat the aptamer-functionalized hydrogel coating. The cleavage sites of the aptamer duplex are shown in Fig. 6A. The gel electrophoretogram shows that the 30-min *Bam*HI treatment led to the degradation of the majority of A_1–B_1 duplexes (Fig. 6B). This result was confirmed by the flow cytometry analysis (Fig. 6C). After the demonstration of the effectiveness of using *Bam*HI to hydrolyze the A_1–B_1 duplexes, we performed a *Bam*HI-mediated cell release experiment. The cells and the hydrogel coatings were treated with *Bam*HI for 30 min. The result shows that the efficiency of cell release was ~99% and the cell density on the hydrogel coating was decreased to ~10 cells/mm^2 (Fig. 6D).

To confirm the observations in the *Bam*HI experiment and to illustrate the specificity of restriction endonucleases in releasing cells, we also examined the functionality of another restriction endonuclease, *i.e., Kpn*I. The recognition sequences of *Bam*HI (Fig. 6A) and *Kpn*I (Fig. 7A) have a high similarity with only the middle two nucleotides switched to the corresponding positions. Despite the high similarity of their recognition sequences, these two endonucleases exhibited high fidelity and accuracy of cutting the recognition sequences (Fig. 7B). *Bam*HI hydrolyzed the A_1–B_1 duplex rather than the A_2–B_2 duplex; *Kpn*I hydrolyzed the A_2–B_2 duplex rather than the A_1–B_1 duplex. The cell release data are consistent with the gel electrophoresis results. For the hydrogels functionalized with the A_1–B_1 duplex, the cells were released by *Bam*HI rather than *Kpn*I (Fig. 7C). For the hydrogel functionalized with the A_2–B_2 duplex, the cells were released by *Kpn*I rather than *Bam*HI (Fig. 7D). These results demonstrate that the restriction endonuclease-mediated cell release is sequence-specific.

Because tumor cells in the same tumor exhibit heterogeneous properties [43–45], it is reasonable that circulating tumor cells may have different characteristics. Thus, the ability to separate and detect the subgroups of circulating tumor cells may lead to a deep understanding of cancer development. In principle, multiple specific aptamers with different nuclease-recognizing sites can be rationally designed and immobilized into the hydrogel coating to catch the subgroups of tumor cells. Because our results have shown that *Bam*HI and *Kpn*I specifically hydrolyzed different recognition sequences (Fig. 7), it is promising that the subgroups of tumor cells would be specifically captured and released when sequence-specific aptamers and endonucleases were used.

3.6. Comparison of BamHI and trypsin in releasing cells

In addition to restriction endonucleases, it is also possible to use proteases to induce cell release from the hydrogel coating. Thus, it is reasonable to question which type of enzyme will be more efficient to release cells from the hydrogel coating. To address this question, we compared the ability of *Bam*HI and trypsin in releasing cells. The reason for choosing trypsin for comparison is that trypsin is the most commonly used protease for detaching cells from a surface. As shown in Fig. 8A, *Bam*HI released 95 ± 4% cells within 10 min whereas trypsin released 80 ± 18% cells during the same

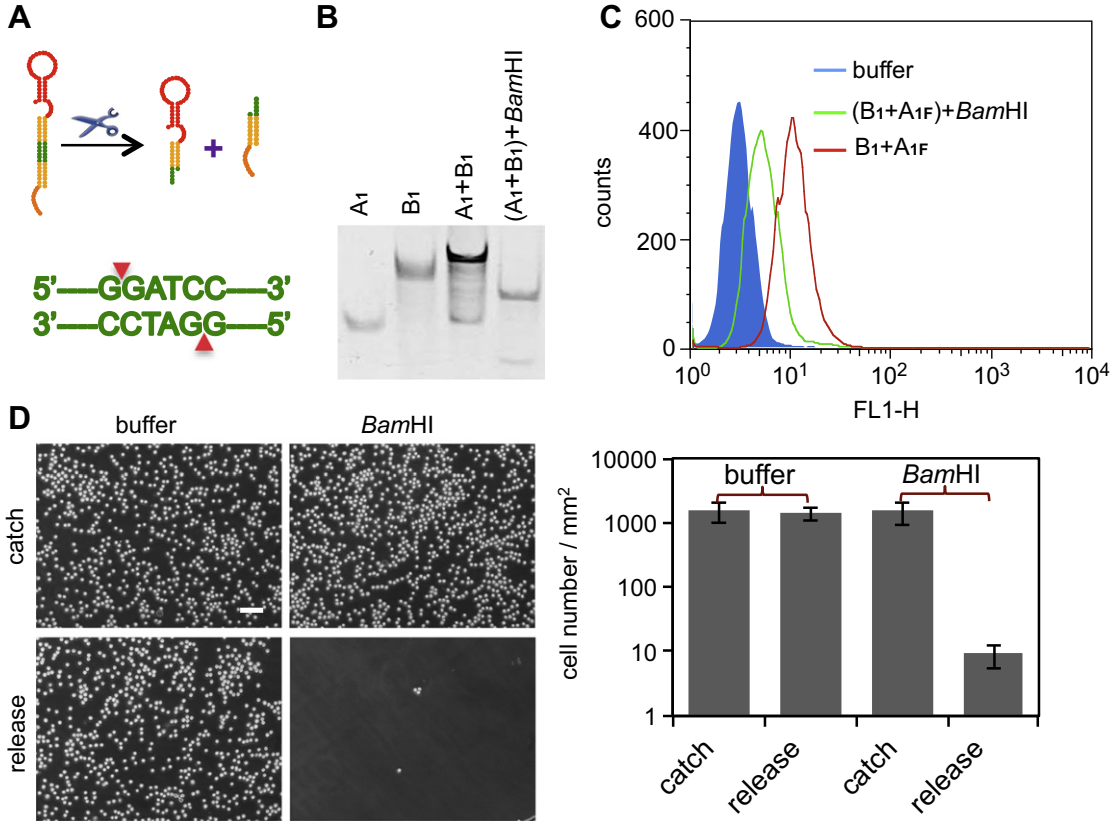

Fig. 6. *Bam*HI-mediated cell release from the hydrogel coating. (A) Schematic of *Bam*HI-mediated cleavage. The symbol of the scissor indicates the restriction endonuclease. The arrowheads point to the cleavage sites. (B) Gel electrophoretogram for analyzing the hydrolysis of the A_1–B_1 duplex. (C) Flow cytometry histogram for determining the binding capability of the hydrolyzed A_1–B_1 duplex. A_{1F}: sequence A_1 labeled with FAM. (D) Microscopy images of cells on the hydrogel coating before and after *Bam*HI treatment. Scale bar: 10 μm. The cell numbers were quantified using ImageJ.

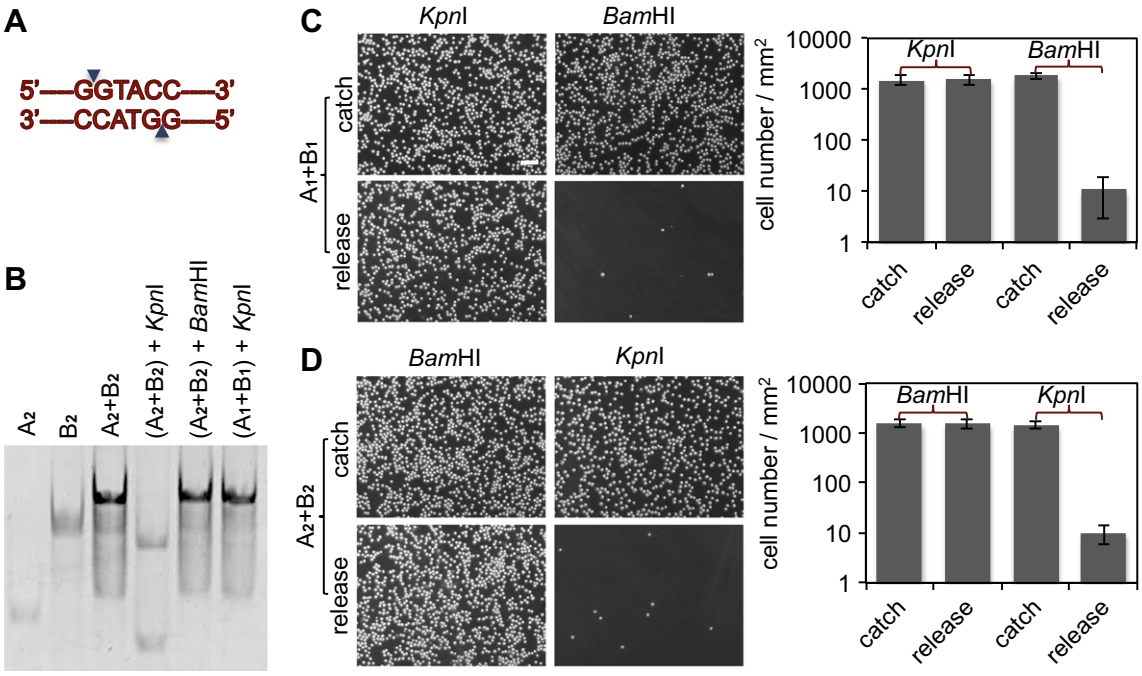

Fig. 7. Examination of sequence-specific DNA cleavage and cell release. (A) Recognition sequence of *Kpn*I. The arrowheads point to the cleavage sites. (B) Gel electrophoretogram for analyzing the hydrolysis of the aptamer duplexes. (C&D) microscopy images of cells on the hydrogel coatings before and after endonuclease treatment. The hydrogel coatings were functionalized with A_1–B_1 (C) and A_2–B_2 duplexes (D). Scale bar: 10 μm. The cell images were analyzed using ImageJ to provide a quantitative analysis.

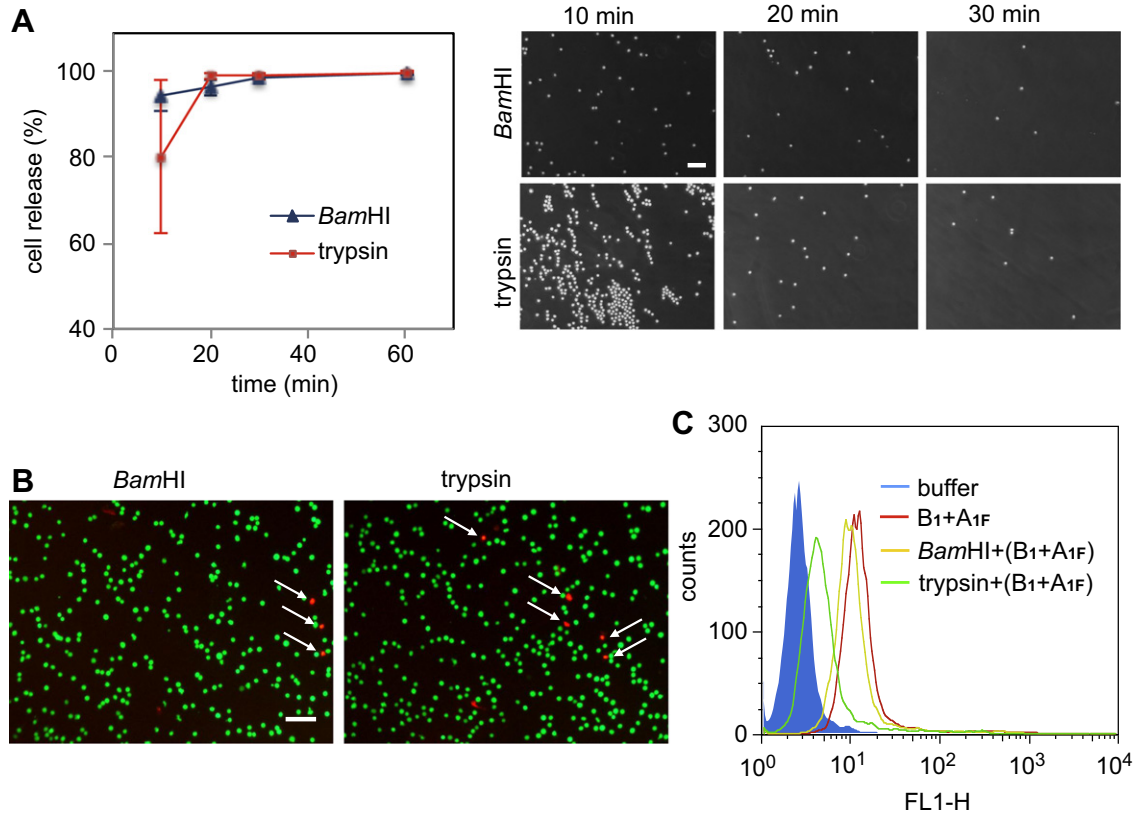

Fig. 8. Comparison of cell release mediated by *Bam*HI and trypsin. (A) Cell release kinetics. (B) Live/dead cell staining. The green and red colors indicate live and dead (pointed by the arrows) cells, respectively. (C) Flow cytometry histogram for qualitatively analyzing the presence of cell receptors. Four groups of cells were compared, including cells treated with buffer, normal cells labeled with the B_1–A_{1F} duplex, cells released by *Bam*HI and labeled with the B_1–A_{1F} duplex, and cells released by trypsin and labeled with the B_1–A_{1F} duplex. Scale bar: 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

period of time. In addition, the unreleased cells in the trypsin group were not evenly distributed on the hydrogel coating. These differences may be partly attributed to the steric hindrance. Cell receptors are directly attached to a compact cell membrane whereas the aptamers are immobilized on the porous hydrogel coating. In addition, the cleavage sites of endonucleases are located in the middle of double-stranded helixes. Resultantly, it would be easier for endonucleases to attack the cleavage sites than proteases to attack cell receptors. The results also show that both trypsin and *Bam*HI could release more than 99% cells after 30-min enzyme treatment (Fig. 8A).

Because the analysis of circulating tumor cells involve the characterization of not only signaling molecules and genes inside the cells but also their surface properties, it is critical to ensure minimal effects on cell properties both inside and on the surface during the procedure of cell release. This need is particularly important to the understanding of the properties of metastatic cancer cells that may rely on their surface receptors to find appropriate places to survive and grow into new tumors. Therefore, in addition to the cell release efficiency, we further compared the properties of released cells with two different assays: Live/dead cell staining and flow cytometry. The results show that the percentage of viable cells was approximately 98% in both groups, indicating that there is no difference between *Bam*HI and trypsin in affecting cell viability (Fig. 8B). However, the flow cytometry results show a significant difference (Fig. 8C). The *Bam*HI-released cells exhibited fluorescence intensity similar to that of normal cells whereas the trypsin-released ones exhibited fluorescence intensity close to that of the unlabeled cells. These results demonstrate that endonucleases barely affect cell receptors whereas proteinases cause a significant decrease of receptor density. Taken together, these cell release results indicate that endonuclease-mediated treatment is not only fast and efficient, but also nondestructive to cells.

4. Conclusions

A material system for cell catch and release was developed using aptamer-functionalized hydrogels and restriction endonucleases. The immobilized aptamers can specifically catch target cancer cells on the hydrogel surface that is highly resistant to nonspecific cell binding. In addition, sequence-specific restriction endonucleases can hydrolyze aptamers with rationally designed cleavage sites and rapidly release cells from the hydrogel without causing cell damage. Therefore, aptamer-functionalized hydrogels hold great potential as a coating material to functionalize medical devices (*e.g.*, microfluidic devices) for specific catch and nondestructive release of rare circulating tumor cells.

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