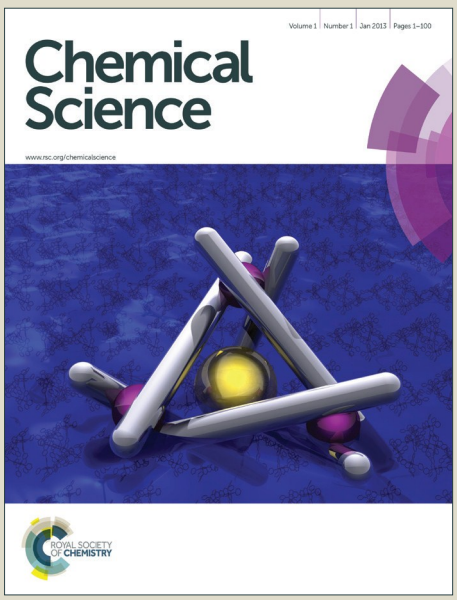
Chemical Science

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemicalscience

Journal Name

ARTICLE

Endogenous Signalling Control of Cell Adhesion by Using Aptamer Functionalized Biocompatible Hydrogel

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Wen Li,[a,b] Jiasi Wang,[a,b] Jinsong Ren,[a] and Xiaogang Qu*[a]

Design of biological signal-responsive biomaterials is essential for controlling cell-cell and cell-matrices interactions. Herein, we developed a dynamic hydrogel to control cell adhesion with biological signal in cellular microenvironment. The basic principle was based on using nucleic acid aptamer to recognize cell signalling and control the display of bioligands on hydrogel. Not only the exogenous signalling but the endogenous signalling secreted by surrounding cells could activate the dynamic scaffold and tune the cell adhesion state. Since diverse aptamers have been developed, our design can be extended to multiple biological inputs. The biochemical signal-responsive system will greatly enhance the understanding of complex biological process as well as the ability to manipulate cellular behaviours.

Introduction

The fate of cell is not only regulated by cell itself but also highly related to its interactions with microenvironment.[1] As the local surrounding of cells, cellular microenvironment is beyond an inert structural support to an active component of biochemical signals. [2] It plays an essential role in regulating cell behaviour, and even determines the cell fate. [3,4] Recently, great efforts are underway towards the design of scaffolds to mimic the dynamic features of native cell environment. [5,6] To this end, some switchable cell scaffolds have been fabricated, [7] which can controllably tune the components to regulate cell activities. Although interesting, most of the switchable substrates were only able to recognize simple physical or chemical stimuli. [8,9] In contrast, the cellular microenvironment uses complex biochemical signals to communicate with cells and guide the proper cell functions. [10] For example, in vascular regeneration process, neighbouring endothelial cells can secrete certain kind of growth factor to control the differentiation of stem cells. [11,12] And bacteria cells deploy a range of quorum-sensing regulators to communicate with each other and then regulate group behaviour. [13] Therefore, the synthetic cell scaffolds with the ability to recognize specific biological input are highly desirable.

Engineering materials to control cell adhesion is a fundamental issue for cell biology and medical application. [14-16] Cell adhesion not only provides cell an anchor to environment, but also transduces mechanochemical signal into cells [17,18] It regulates diverse cell behaviours and physiological events.[19] In addition, for many biomedical applications, such as tissue engineering, and cell-based therapy, it always requires the precise control over when and where cells adhere, migrate, or release.[20-21] Over the past decade, material science has provided a variety of artificial substrates to control cell adhesion by external stimuli. [22-24] Most of them are designed to respond to pH, [25] temperature, [26,27] magnetic field, [28] light [29-31] or electric field. [32,33] It should be pointed out that these physicochemical stimuli-activated substrates often could not effectively communicate with the real biological systems. Design of biological signal-responsive surface to control cell adhesion is still challenging. This is mainly ascribed to the fact that the biological signals are too complex for the synthetic scaffolds to selectively recognize and respond to them. As the current biological recognition systems mainly focus on antigen-antibody interactions, the lack of appropriate screening methods has limited their effectiveness. What's more, the success in designing one signal-responsive system could not be readily extended to others, making the developing process very slow.

Herein we fabricated a dynamic hydrogel substrate to regulate cell adhesion with biological signal. The overall concept was illustrated in Fig. 1. Aptamer is a kind of single-stranded oligonucleotide that can selectively recognize targets ranging from small compounds, proteins, to whole cells. [34-39] In comparison with antibody, aptamer is relatively easy to synthesize, flexible to modification, stable to denaturation. Recently, aptamer has been successfully used as affinity site for specific cell capture, cell detection, and targeted therapy. [40-44] However, it has not been reported for the fabrication of cell microenvironment-mimic scaffolds, which could sense intracellular signalling and then guide cell fate.

[a] Laboratory of Chemical Biology and State Key laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China. Email: xqu@ciac.ac.cn; Fax: (+86) 431-85262656.
[b] University of Chinese Academy of Sciences, Beijing, 100039, P. R. China.
† Electronic Supplementary Information (ESI) available: Reagents and materials, apparatus and characterization, experimental details and additional data. See DOI: 10.1039/x0xx00000x

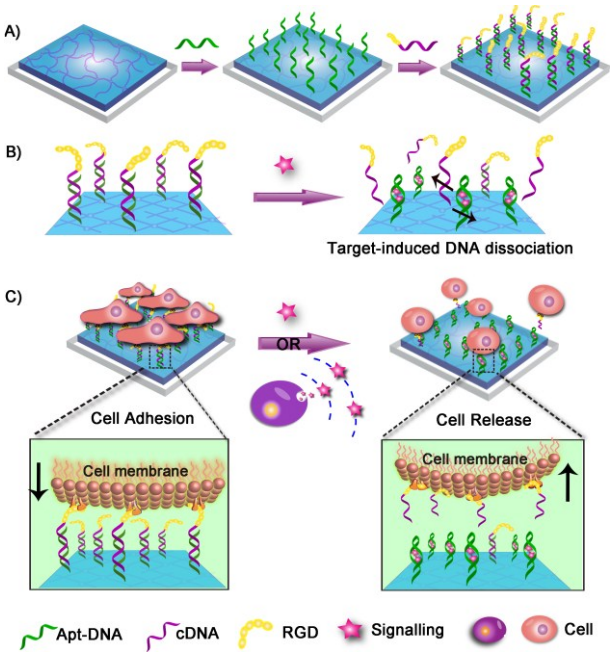


Fig. 1 Dynamic control of cell adhesion by using biological signal-responsive hydrogel. A) The fabrication of aptamer-programmed hydrogel. B) Illustration of the structure change of DNA scaffold during the target recognition. In the presence of target, aptamer would bind it and thereby induce the dissociation of dsDNA. C) The controlled cell adhesion and release. Both the exogenous and the surrounding cell-secreted signalling could induce the structure change of DNA linker and cause the cell detachment.

As a proof-of-concept, adenosine triphosphate (ATP) was demonstrated as signalling molecule here to illustrate the versatility of our strategy. ATP is mainly concentrated in the intracellular cytosol and its extracellular concentration is very low. While, under certain stimulation, ATP will be released from cells to participate in cellular communication and function regulation. [45-47] The extracellular ATP is therefore regarded as an important signal molecule in biological processes. In our strategy, hydrogel was chosen as the supporting substrate due to its excellent abilities to simulate natural extracellular matrix. The ATP aptamer (Apt-DNA) [48-50] and its complementary DNA (cDNA) were firstly conjugated onto the hydrogel. They worked as the intelligent mediators to immobilize the cell-adhesive RGD ligand.[51] The DNA-mediated RGD presentation promoted the adhesion of target cells to hydrogel surface. However, in the presence of ATP, Apt-DNA would bind it and form stable tertiary structure, which promoted the dissociation of DNA duplex. Subsequently, the original adherent cells would be released form the hydrogel surface. Based on this, the artificial cell scaffold could selectively recognize signalling molecules and then tune surface properties to regulate cell behaviour, which worked in a manner similar to cell microenvironment. This work will greatly enhance our ability to actively manipulate cellular behaviours in biomedical applications.

Results and discussions

The fabrication of DNA scaffold-modified hydrogel substrate.

The dynamic scaffold was fabricated as shown in Scheme S1 (ESI†). Alginate hydrogel was chosen as the supporting substrate because of its biocompatibility and resistance to nonspecific cell binding. The hydrogel coatings were constructed on glass surface via in-suit photo-polymerization. Their formation was monitored with Fourier infrared (FT-IR) spectra (Fig. S1, ESI†). The morphology of hydrogel film was studied by scanning electron micrographs (SEM). As shown in Fig. S2 (ESI†), a uniform film was formed and the surface appeared smooth with no appreciable defects. Fluorescence image of Rhodamine B-encapsulated alginate substrate also confirmed the presence and confluency of hydrogel layer (Fig. S3, ESI†). After the synthesis of hydrogel coatings, the Apt-DNA bearing amine groups were covalently conjugated on the surface through reacting with carboxyl group of alginate polymer. The substrate was then incubated with the cDNA to realize hybridization. In previous works, the adenosine-induced aptamer conformational switch and DNA duplex dissociation have been used for design of ATP-responsive sensor, the disassembly of nanoparticles, and the controlled drug release.[48-50] Here, to investigate whether the surface property of hydrogel could be programmed with ATP molecules, we labelled the cDNA with a fluorescent dye, fluorescein isothiocyanate (FITC) (Fig. 2a). After the immobilization of FITC, a bright green fluorescence was observed from the substrate. However, in the presence of ATP molecules, the component change of hydrogel substrate was visualized by the obvious fluorescence decrease. To confirm that the dimming of fluorescence was caused by ATP-induced aptamer conformational switch, the aptamer-contained double strand DNA (dsDNA) was replaced with a random dsDNA. The ATP treatment had little influence on the fluorescence intensity of the control substrate (Fig. S4, ESI†).

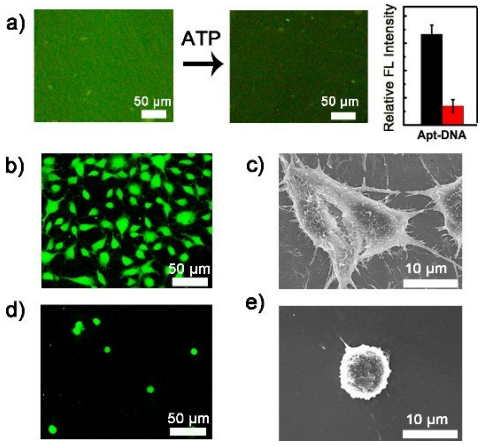


Fig. 2 a) The fluorescence images and the relative fluorescence intensity of FITC-labelled substrates before (left) and after (right) with ATP treatment. The fluorescence image (b) and SEM image (c) of cells on the RGD-immobilized hydrogel surface. The fluorescence image (d) and SEM image (e) of cells incubated on the hydrogel without RGD.

Cell adhesion on the hydrogel substrate.

After fabricating the substrate and confirming its ATP-responsive property, we intended to regulate cell adhesion on

the designed substrate. To this end, RGD ligand was modified on the terminus of cDNA to endow the surface with cell adhesive affinity. The hydrogel substrate was then seeded with NIH 3T3 fibroblast cells, to test its cell binding performance. After three hours of incubation, the cells were stained and characterized with fluorescence microscopy. As illustrated Fig. 2b, about 1.0×10^5 cells/cm^2 efficiently attached and spread on the hydrogel surface. The SEM image further showed that the adhered cells displayed fully extended shapes with pseudopodia attached to surface (Fig. 2c). In contrast, only 1.0×10^4 cells/cm^2 were observed on the control surface that did not present RGD (Fig. 2d). And cells on the control substrate remained relatively round (Fig. 2e). The differences suggested that alginate hydrogel provided a non-adhesive background and the cells could specially adhere on hydrogel through RGD ligand. After cultured for 24 hours, the cells could grow well on hydrogel with high viability (Fig. S5, ESI†). This implied the excellent biocompatibility of hydrogel substrate.

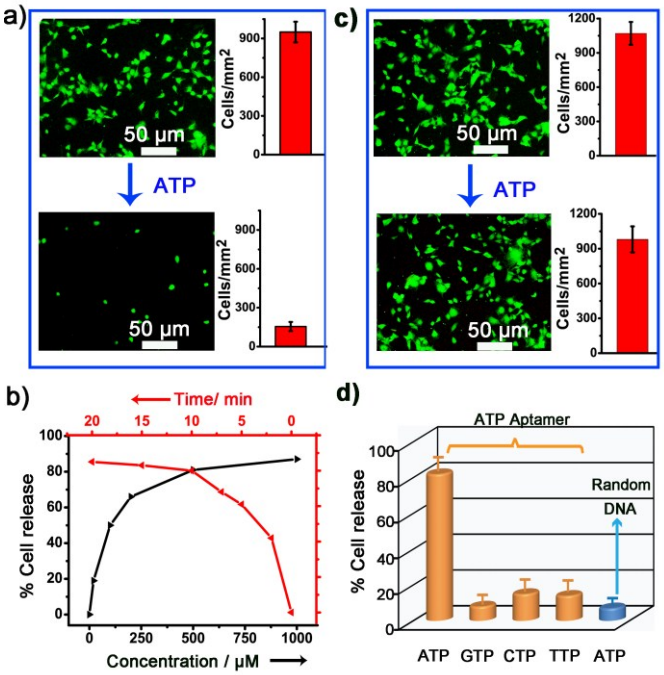


Fig. 3 a) The fluorescence images of ATP-induced cell release on the Apt-DNA based hydrogel substrate. The histograms showed the cell number on each surface. b) The percent of cell release after exposure to the different concentrations of ATP for 10 min (black line) or exposure to 0.5 mM ATP for different time (red line). c) The fluorescence images and the number of cells on the control substrate before and after ATP treatments. d) The percent of cell release on the ATP-, GTP-, CTP-, or TTP-treated Apt-DNA hydrogel or on the ATP-treated random DNA hydrogel. The error bars represented the standard deviation of three experiments.

Controlled cell detachment with exogenous ATP.

To verify whether the developed surface was applicable to regulate cell adhesion, exogenous ATP was used to stimulate the substrate. Upon incubation with 0.5 mM ATP, most of the original adherent cells were rounded up and released from the hydrogel substrate (Fig. 3a). And clearly, the cell release was ATP concentration-dependent (Fig. 3b). The kinetic study of cell detachment process was also performed. For the random dsDNA-modified control surface, only small amounts of cells were released under ATP stimulus (Fig. 3c). The result was consistent with the fluorescein model assay, which verified that aptamer played a key role in ATP-responsive cell release. It was also noteworthy that cells on the hydrogel surface were not affected by other nucleoside triphosphates including guanosine triphosphate (GTP), cytidine triphosphate (CTP), and thymidine triphosphate (TTP) (Fig. 3d and Fig. S6, ESI†). Due to the complexity of cell environment, the specific recognition was essential for the on-demand control of cell adhesion accurately. We then determined the viability of released cells with live/dead assay, which implied that 92% of cells were still viable (Fig. 4a). When re-incubated on the glass side, the released cells could grow and proliferate into a confluent layer (Fig. 4b). Their proliferation potential was similar to that of the control population (Fig. 4c and S7, ESI†). These results clearly indicated that the aptamer-based control process did not affect cell viability and the released cells could be used for further biological applications.

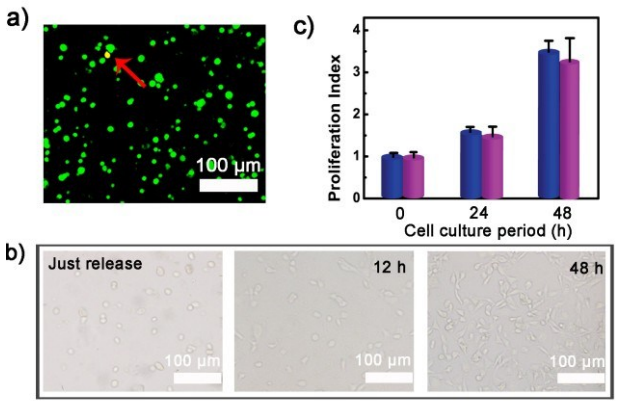


Fig. 4 a) The viability of released cells assessed with a live (green) / dead (red) assay; b) Spreading and proliferation of the released cells after seeding on glass slide for 0 h, 12 h, and 48 h; c) The proliferation ability of the normal control cells (blue bar) and the released cells (purple bar).

Controlled cell release with ATP secreted from surrounding sender cells.

We next examined whether the adhesion state of cells could respond to ATP molecules secreted from neighboring cells. In cellular microenvironments, cells communicate with each other and cellular matrix by processing various biological signals, which can further guide cell behaviour. ATP is an important signalling, and it can be released from cells to regulate neuronal network, tissue blood flow, tumourigenesis, and host-pathogen interactions. Here, the platelets were used as the model senders of endogenous ATP since their ATP release process has been clearly demonstrated (Fig. 5a). When the platelets were activated by triggers, such as thrombin, the adenosine molecules copackaged in the platelet dense granules would be secreted into extracellular environment rapidly. According to previous works, adenosine molecules elicited from the thrombin-activated platelets (10^8 platelets/ml) could reach 3-10 μM. [52-53] To indicate whether the secreted molecules were effective to activate the hydrogel substrate and control the adhesion of cells, the response of NIH 3T3 cells

to ATP-producing platelets was examined. NIH 3T3 cells firstly adhered on the aptamer-modified hydrogel surface, and then different amounts of platelets were co-cultured with them. To increase the response sensitivity, the incubation time of NIH 3T3 cells on substrate was decreased to 2 hours to avoid strong adhesion. As shown in the fluorescence image, without the addition of thrombin, the platelets have little influence on the adhesion of NIH 3T3 cells, and the NIH 3T3 cells spread well on substrate (Fig. 5b). While, after the platelets were activated by thrombin, about 57% of NIH 3T3 cells were released from surface within 20 min. As expected, the release rate of NIH 3T3 cells was correlated directly with the number of platelets co-cultured in the same substrate and the amount of thrombin added (Fig. 5c). In addition, we observed that the thrombin itself without the platelets could not induce the obvious cell release (Fig. S8, ESI†). This result showed that thrombin was not the directed factor for cell release but the inducer of endogenous ATP molecule. The DNA scaffold on hydrogel could recognize the platelet-secreted ATP in real time and then go on the "duplex-to aptamer" conformational switch. Subsequently, the NIH 3T3 cells adhered on surface could be released.

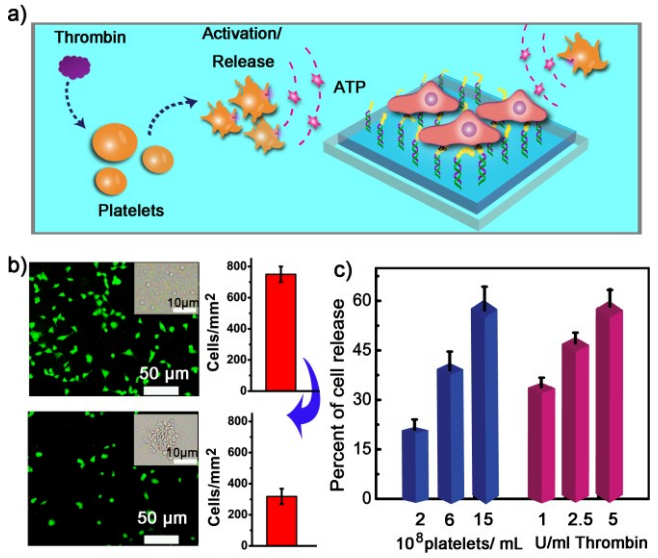


Fig. 5 a) The control of cell adhesion with adenosine molecules elicited from the thrombin-activated platelets. b) The fluorescence images and the number of NIH 3T3 before (up) and after (below) the activation of platelets by thrombin. Insets showed bright field images of platelets. After the addition of thrombin, platelets were activated and formed aggregation. c) The percent of NIH 3T3 cell release with the number of platelets co-cultured and the amount of thrombin added for platelet activation.

Discussion

Although ATP was used as the model signalling molecule here, this system can be programmed as a universal platform of cell microenvironment-responsive substrate. Since a wide range of aptamers have been developed, the stimulating factor could be extended to growth factors, metal ions, biomarker proteins, and even some pathogen and cancer cells. In addition, two different aptamers could be simultaneously modified on the surface to immobilize two kinds of cells. Then one signalling molecule could just activate its corresponding aptamer and stimulate the release of specific cells. This can therefore control the sequential release of multiple cells in distinct stages according to the signalling molecules. What's more, due to the flexibility of DNA in design and synthesis, two different aptamers could be integrated into one DNA scaffold to control the adhesion of cells in a highly organized fashion. Specially, the target cells could be designed to detach only when the two biological inputs were simultaneously present, and this mechanism was thus equivalent to a logical AND gate. Alternatively, the dynamic substrate could perform an "OR" logic gate operation, for which either of biological molecules could activate the release of target cells. This allowed the accurate control over the cell-matrix interaction. In the previous works, the controlled cell adhesion systems mainly focused on using physicochemical stimuli, such as pH, temperature, magnetic field, light, or electric field. In contrast, the hydrogel designed here could respond to the specific biological signal within cellular microenvironment and then made a change to control cell adhesion behaviour. As the signal molecules play an essential role in various biological regulation, the biologically responsive hydrogels will provide a more effective communication with the real biological system. They may ultimately enable us to mimic the dynamic features in complex cellular process. This design can be used to manipulate cell behaviors for special applications, such as stem cell differentiation, tissue development, and cell-based therapy.

In the present work, aptamer was modified on the surface of hydrogel to modulate cells cultured on the top of hydrogel. Further exploitation of this approach for 3D cell culture and manipulation will hold great promise for biological applications. Recently, the 3D aptamer-hydrogels have been reported by using the aptamer-contained DNA structure as crosslinker to assemble polymer networks.[54-55] The aptamer domains in hydrogel could bind the target, causing the dissociation of crosslinker and disassembly of hydrogel. If cells are encapsulated inside the aptamer-crosslinked hydrogel, the hydrogel can recognize the special signaling molecule in cellular microenvironment, and then make a controlled phase transition to regulate the embedded cells. This provides the potential for biological signal-responsive control of cellular microenvironment and cell behaviors in a 3D manner. For practical applications, one problem may be raised with the stability of DNA scaffold modified on hydrogel. Nucleic acid (especially RNA) was susceptible to nucleases in biological media, particularly in blood. In our present in vitro study, to avoid such potential question, the incubation time of DNA scaffold with cells was short and no serum was added in the incubation buffer. For further practical applications, this issue can also be solved by several solutions. With the developments of nuclear acid synthetic technology, a lot of interesting researches have been reported to protect nuclear acid against nuclease degradation.[56-58] For example, the use of modified nucleosides, such as 2'-amino pyrimidine nucleosides, 2'-fluoropyrimidine nucleosides, 2'-O-methyl purine, and 2'-O-methyl pyrimidine nucleosides, significantly

increased the resistance to nuclease. Some of these modified-nucleic acids have been successfully adopted for in vivo study. Therefore, it is possible that the dynamic hydrogel will provide potential for cell-based fundamental study and biomedical applications.

Conclusions

The aptamer-based DNA scaffold could recognize biological signal in real time, and then dynamically control the presentation of bioligands on hydrogel. This provided a flexible strategy to regulate the surface property of hydrogels as well as the behaviour of cells on them. Both the exogenous and the surrounding cell-secreted ATP could activate the scaffold and tune the cell-substrate interactions. To the best of our knowledge, this is the first example of dynamic hydrogel which could recognize the intercellular signalling to control cell adhesion. With the wide developments of aptamers, the hydrogel could be readily designed to respond to multiple biological inputs. In addition, by combining different kinds of aptamers, this system could be further programmed to control multiple cells in a highly organized manner. The advantage of flexible design, convenient operation, and ready engineering makes aptamer very suitable for design of complex signal-responsive scaffold. Compared with the traditional substrate, the biochemically responsive hydrogel rewired the communication between biological event, hydrogel property, and cell behaviors. Future works may be developed to engineer hydrogels to elicit targeted cellular behavior in special biomedical applications, such as the control release of therapeutic stem cells with the biomarkers in inflammation or tumor tissues.

Experimental

Trigger cell release by ATP.

After the adherence of cells on the aptamer-functionalized substrate, the substrate was examined for the ATP-triggered cell release. A series of 300 μL HEPES buffer solution (5 mM HEPES, 10 mM $MgCl_2$, 137 mM NaCl, pH=7.4) containing the different concentrations of ATP (0, 20 μM, 100 μM, 200 μM, 500 μM, 1 mM) were added onto the substrates in 48-well plate. They were gently shaken at 37 ^{o}C with 20 rpm for 2.5 min, 5 min, 7.5 min, 10 min, 15 min, or 20 min. After that, the ATP solutions were placed with the washing buffer (pH=7.4). And the substrates were further shaken at 80 rpm for 2 min to allow the released cells to be removed from the hydrogel surface. The cells remained on the substrate were stained with AM dye and measured with fluorescence microscope to quantify the cell number. To determine whether the stimulus process was biocompatible, the viability of released cells was assessed with live/dead assay. For this, the released cells were collected from the washing buffer by centrifugation at 1000 rpm for 5 min, and then PI and AM dyes were added into the cell solution with a final concentration of 2 μM. After 15 min staining, the cells were rinsed with pH=7.4 PBS buffer for 3 times. The cell images were obtained by the fluorescence microscopy. The released cells were also re-incubated on the glass side for 0 h, 12 h, and 48 h and then monitored with microscopy. The proliferation potential of released cells was further evaluated by MTT (methylthiazoletetrazolium) assay. After incubated in cell culture plate for different time, the cells were treated with MTT solution for 4 h. Subsequently, the supernatant was discarded, followed by the additional of DMSO into each well. Then the optical density (OD) of resulted solution was read at a wavelength of 570 nm. Proliferation index $=N_H/N_{H=0}$, where N_H = the cell number after incubation for H hours, $N_{H=0}$ = the cell number at the beginning.

Trigger cell release by ATP secreted from platelets.

The platelets were isolated from the whole blood.[59-61] Approximately 20 ml of blood was collected into sterile heparinized vacutainer tubes. Then the whole blood was centrifuged immediately at 1200 rpm for 15 min at room temperature to obtain platelet-rich plasma (top fraction). Transfer the yellow platelet-rich plasma to a fresh conical tube carefully to avoid collecting any of the buffy coat. To obtain a platelet pellet, the platelet-rich plasma was centrifuged at 3500 rpm for 15 min at room temperature. The platelet pellet was resuspended in PBS buffer (pH 7.4). The number of platelets was counted using a hemacytometer. For control of cell release by ATP secreted from the platelets, the NIH 3T3 cells firstly adhered on the hydrogel surface in 96-well plate for 2 hours. Then different amounts of palates were loaded into the same well. After a brief incubation at 37 ^{o}C, the palates were activated by addition of 0.5 μl of thrombin.[52,62] After gently shaken at 37 ^{o}C for 20 min, the hydrogel was washed with PBS buffer (pH=7.4) and the cells on it were stained and imaged with microscope.

Acknowledgements

This work was supported by 973 Project (2011CB936004, 2012CB720602), and NSFC (21210002, 21431007, 91413111).

Notes and references

1 D. E. Discher, D. J. Mooney and P. W. Zandstra, *Science,* 2009, **324**, 1673.
2 C. Bonnans, J. Chou and Z. Werb, *Nat. Rev. Mol. Cell. Biol.*, 2014, **15**, 786.
3 N. Huebsch, P. R. Arany, A. S. Mao, D. Shvartsman, O. A. Ali, S. A. Bencherif, J. Rivera-Feliciano and D. J. Mooney, *Nat. Mater.,* 2010, **9**, 518.
4 S. Singer, *Science,* 1992, **255**, 1671.
5 T. Dvir, B. P. Timko, D. S. Kohane and R. Langer, *Nat. Nano.,* 2011, **6**, 13.
6 M. P. Lutolf, P. M. Gilbert and H. M. Blau, *Nature,* 2009, **462**, 433.
7 P. M. Kharkar, K. L. Kiick and A. M. Kloxin, *Chem. Soc. Rev.,* 2013, **42**, 7335.
8 J. A. Burdick and W. L. Murphy, *Nat. Commun.,* 2012, **3**, 1269.
9 E. V. Skorb and D. V. Andreeva, *Adv. Funct. Mater.,* 2013, **23** 4483.
10 Y. Cheng, C.-Y. Tsao, H.-C. Wu, X. Luo, J. L. Terrell, J. Betz, G. F Payne, W. E. Bentley and G. W. Rubloff, *Adv. Funct. Mater.*, 2012, **22**, 519.

11 A. L. Ponte, E. Marais, N. Gallay, A. Langonné, B. Delorme, O. Hérault, P. Charbord and J. Domenech, *STEM CELLS,* 2007, **25**, 1737.
12 W. Zhao, S. Schafer, J. Choi, Y. J. Yamanaka, M. L. Lombardi, S. Bose, A. L. Carlson, J. A. Phillips, W. Teo, I. A. Droujinine, C. H. Cui, R. K. Jain, J. Lammerding, J. C. Love, C. P. Lin, D. Sarkar, R. Karnik and J. M. Karp, *Nat. Nano.,* 2011, **6**, 524.
13 M. Weitz, A. Mückl, K. Kapsner, R. Berg, A. Meyer and F. C. Simmel, *J. Am. Chem. Soc.,* 2013, **136**, 72.
14 X. Liu and S. Wang, *Chem. Soc. Rev.*, 2014, **43**, 2385.
15 W. Zheng, W. Zhang and X. Jiang, *Adv. Healthcare Mater.,* 2013, **2**, 95.
16 P. M. Mendes, *Chem. Soc. Rev.*, 2013, **42**, 9207.
17 O. Guillame-Gentil, O. Semenov, A. S. Roca, T. Groth, R. Zahn, J. Vörös and M. Zenobi-Wong, *Adv. Mater.,* 2010, **22**, 5443.
18 T. T. Lee, J. R. García, J. I. Paez, A. Singh, E. A. Phelps, S. Weis, Z. Shafiq, A. Shekaran, A. del Campo and A. J. García, *Nat. Mater.,* 2015, **14**, 352.
19 J. D. Humphrey, E. R. Dufresne and M. A. Schwartz, *Nat. Rev., Mol. Cell. Biol.* 2014, **15**, 802.
20 W. L. Murphy, T. C. McDevitt and A. J. Engler, *Nat. Mater.,* 2014, **13**, 547.
21 S. F. M. van Dongen, J. Janvore, S. S. van Berkel, E. Marie, M. Piel and C. Tribet, *Chem. Sci.*, 2012, **3**, 3000.
22 J. Robertus, W. R. Browne and B. L. Feringa, *Chem. Soc. Rev.,* 2010, **39**, 354.
23 M. P. Chien, C. A. Werley, S. L. Farhi and A. E. Cohen, *Chem. Sci.*, 2015, **6**, 1701.
24 A. Leal-Egaña, A. Díaz-Cuenca, A. R. Boccaccini, *Adv. Mater.* 2013, **25**, 4049.
25 Y.-H. Chen, Y.-C. Chung, I. J. Wang and T.-H. Young, *Biomaterials,* 2012, **33**, 1336.
26 H. Liu, X. Liu, J. Meng, P. Zhang, G. Yang, B. Su, K. Sun, L. Chen, D. Han, S. Wang and L. Jiang, *Adv. Mater.,* 2013, **25**, 922.
27 S. Iwanaga, Y. Akiyama, A. Kikuchi, M. Yamato, K. Sakai and T. Okano, *Biomaterials,* 2005, **26**, 5395.
28 A. Ito, K. Ino, T. Kobayashi and H. Honda, *Biomaterials,* 2005, **26**, 6185.
29 M. Wirkner, J. M. Alonso, V. Maus, M. Salierno, T. T. Lee, A. J. García and A. del Campo, *Adv. Mater.,* 2011, **23**, 3907.
30 W. Li, J. Wang, J. Ren and X. Qu, *J. Am. Chem. Soc.,* 2014, **136**, 2248.
31 D. Liu, Y. Xie, H. Shao and X. Jiang, *Angew. Chem. Int. Ed.,* 2009, **48**, 4406.
32 B. M. Lamb and M. N. Yousaf, *J. Am. Chem. Soc.,* 2011, **133**, 8870.
33 C. C. A. Ng, A. Magenau, S. H. Ngalim, S. Ciampi, M. Chockalingham, J. B. Harper, K. Gaus and J. J. Gooding, *Angew. Chem. Int. Ed.,* 2012, **51**, 7706.
34 L. Qiu, T. Zhang, J. Jiang, C. Wu, G. Zhu, M. You, X. Chen, L. Zhang, C. Cui, R. Yu and W. Tan, *J. Am. Chem. Soc.,* 2014, **136**, 13090.
35 K. Hwang, P. Wu, T. Kim, L. Lei, S. Tian, Y. Wang and Y. Lu, *Angew. Chem. Int. Ed.,* 2014, **53**, 13798.
36 Z. Zhang, N. Chen, S. Li, R. Battig and Y. Wang, *J. Am. Chem. Soc.,* 2012*,* **134***,* 15716.
37 K.-R. Kim, T. Lee, B.-S. Kim and D.-R. Ahn, *Chem. Sci.*, 2014, **5**, 1533.
38 B. Zhao, J. Shen, S. Chen, D. Wang, F. Li, S. Mathur, S. Song and C. Fan, *Chem. Sci.*, 2014, **5**, 4460.
39 S. Li, N. Chen, Z. Zhang and Y. Wang, *Biomaterials*, 2013, **34**, 460-469.
40 L. Peng, C. S. Wu, M. You, D. Han, Y. Chen, T. Fu, M. Ye and W. Tan, *Chem. Sci.*, 2013, **4**, 1928.
41 L. Chen, X. Liu, B. Su, J. Li; L. Jiang, D. Han and S. Wang, *Adv. Mater.,* 2011, **23**, 4376
42 Q. Shen, L. Xu, L. Zhao, D. Wu, Y. Fan, Y. Zhou, W.-H. OuYang, X. Xu, Z. Zhang, M. Song, T. Lee, M. A. Garcia, B. Xiong, S. Hou, H.-R. Tseng and X. Fang, *Adv. Mater.,* 2013, **25**, 2368.
43 J. Li, W. Wang, D. Sun, J. Chen, P.-H. Zhang, J.-R. Zhang, Q. Min and J. Zhu, *Chem. Sci.*, 2013,**4**, 3514.
44 Y. Guo, J. Wu and H. Ju, *Chem. Sci.*, 2015, **6**, 4318.
45 Mo, T. Jiang, R. DiSanto, W. Tai and Z. Gu, *Nat. Commun.,* 2014, 5.
46 R. Corriden and P. A. Insel, *Sci. Signal.,* 2010, **3**, p. re1.
47 T. Tokunaga, S. Namiki, K. Yamada, T. Imaishi, H. Nonaka, K. Hirose and S. Sando, *J. Am. Chem. Soc.,* 2012, **134**, 9561.
48 X. Zuo, S. Song, J. Zhang, D. Pan, L. Wang and C. Fan, *J. Am. Chem. Soc.,* 2007, **129**, 1042.
49 J.-E. Jeong, B. Kim, S. Woo, S. Hwang, G. C. Bazan and H. Y. Woo, *Chem. Sci.*, 2015, **6**, 1887.
50 Y. Wang, Z. Li, D. Hu, C.-T. Lin, J. Li and Y. Lin, *J. Am. Chem. Soc.,* 2010, **132**, 9274.
51 M. D. Pierschbacher and E. Ruoslahti, *Nature,* 1984, **309**, 30.
52 R. Bergi, E. Kobatake, M. Aizawa and G. R. Dubyak, *Am. J. Physiol. Cell. Physiol.,* 1999, **276**, C267.
53 D. C. B. Mills, I. A. Robb and G. C. K. Roberts, *J. Physiol.,* 1968 **195**, 715.
54 H. Yang, H. Liu, H. Kang and W. Tan, *J. Am. Chem. Soc.,* 2008 **130**, 6320.
55 L. Yan, Z. Zhu, Y. Zou, Y. Huang, D. Liu, S. Jia, D. Xu, M. Wu, Y. Zhou, S. Zhou and C. J. Yang, *J. Am. Chem. Soc.,* 2013, **135**, 3748.
56 A.V. Lakhin, V.Z. Tarantul and L.V. Gening, *Acta. Nat.,* 2013, 5, 34.
57 K.S. Schmidt, S. Borkowski, J. Kurreck, A. W. Stephens, R. Bald, M. Hecht, M. Friebe, L. Dinkelborg and V. A. Erdmann, *Nucleic Acids Res.,* 2004, **32**, 5757.
58 P. E. Burmeister, S. D. Lewis, R. F. Silva, J. R. Preiss, L. R. Horwitz, P. S. Pendergrast, T. G. McCauley, J. C. Kurz, D. M. Epstein, C. Wilson and A. D. Keefe, *Chem. Biol.,* 2005, **12**, 25.
59 B. S. Beikmann, I. D.Tomlinson, S. J. Rosenthal and A. M. Andrews, *ACS Chem. Neurosci.,* 2012, **4**, 161.
60 O.-N. Bae, K.-M. Lim, J.-Y. Noh, S.-M. Chung, H. Kim, C.-R. Lee, J.-D. Park and J.-H. Chung, *Chem. Res. Toxicol.,* 2007, **20**, 1760.
61 K. N. Stevens, M. L. Knetsch, A. Sen, V. Sambhy and L. H. Koole, *ACS Appl. Mater. Interfaces,* 2009, **1**, 2049.
62 S. Ge, E. Woo, 54J. G. White and C. L. Haynes, *Anal. Chem.,* 2011, **83**, 2598.