Communications

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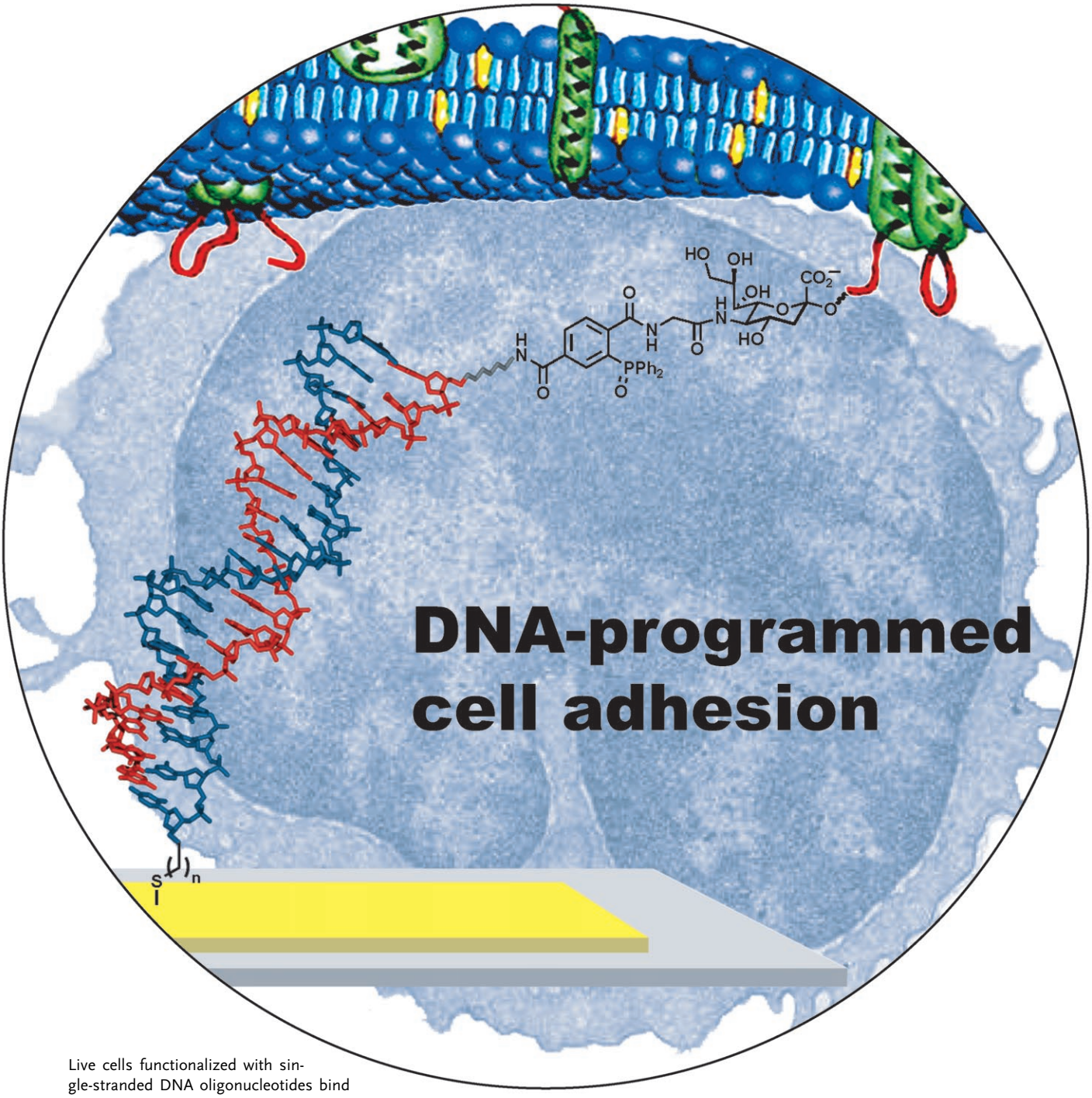

Live cells functionalized with single-stranded DNA oligonucleotides bind to substrates that bear complementary DNA strands in a sequence-specific manner. This binding is independent of the native adhesion mechanisms. The strategy has been successfully utilized with several common mammalian cell lines and device platforms. For more information see the Communication by M. B. Francis and co-workers on the following pages.

DNA Recognition

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Programmable Cell Adhesion Encoded by DNA Hybridization**

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Microfabrication and patterning techniques from the semiconductor industry have yielded promising new tools for the study of cell biology,[1] foreshadowing future opportunities for cell-based devices, including biosensors,[2] drug-screening platforms,[3] artificial tissues,[4] and designed networks of neurons.[5] To date, the attachment of cells to surfaces has been achieved by exploiting the cell's natural repertoire of adhesion receptors, with a particular emphasis on the integrins.[6] To achieve cell adhesion in these systems, cognate surfaces have typically been patterned with integrin-binding ligands, such as laminin or fibronectin, or synthetic RGD-containing peptides (RGD = arginine, glycine, and aspartic acid) that are derived from them.[1,6] A particular advantage of this approach is its generality as most adherent cell types use integrins as their primary adhesion receptors.[7] However, the commonality of this mechanism makes it difficult to form patterns in which multiple types of cells are arrayed with precision on a single surface. As a result, considerable attention has been directed toward the development of new technologies for the programmable attachment of cells to material surfaces.[8,9]

Herein we show that cell adhesion events can be programmed through the attachment of synthetic single-stranded DNA (ssDNA) strands to the surfaces of living cells. These strands are then used to anchor cells to specified locations on surfaces in a sequence-dependent fashion. This provides an attractive means by which to control the adhesion properties of living cells without a dependence on the

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

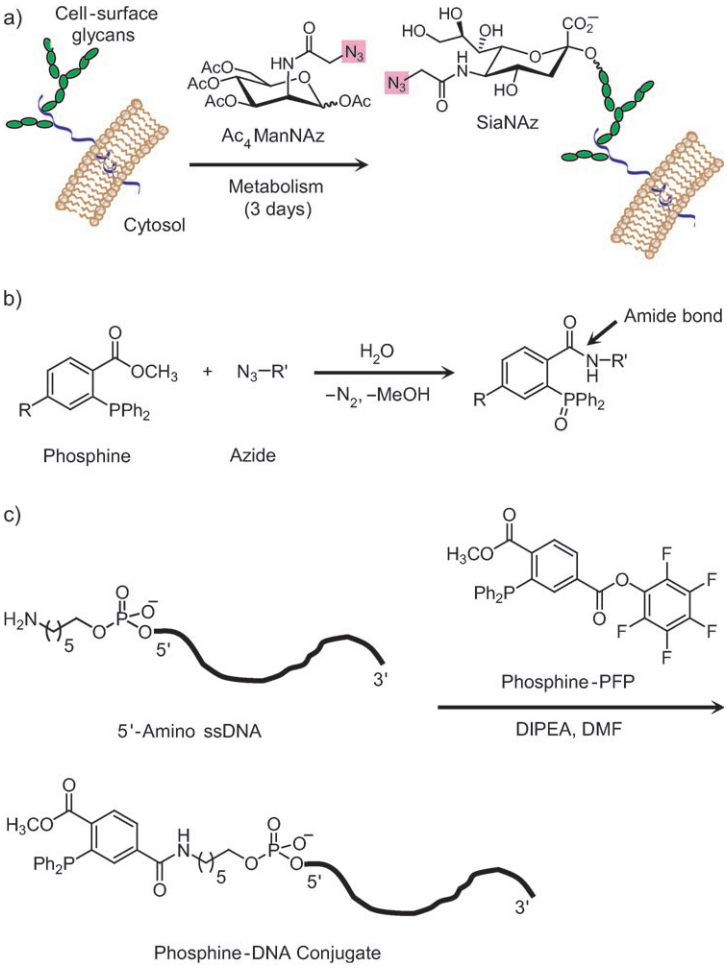

Figure 1. Covalent attachment of ssDNA to cell surfaces. a) Azides can be delivered to cell-surface glycans thorough metabolism of Ac_4ManNAz to SiaNAz. b) The Staudinger ligation of a functionalized phosphine and an azide results in the formation of an amide bond. c) Phosphine-bearing ssDNA strands are synthesized from amino ssDNA and phosphine–PFPs. Ac_4ManNAz = acetylated-*N*-α-azidoacetylmannosamine, SiaNAz = *N*-α-azidoacetyl sialic acid, PFP = pentafluorophenyl.

receptors that they possess. An advantage of DNA-based systems is the virtually unlimited number of possible coding specificities as was demonstrated in previous studies that have directed synthetic lipid vesicles to supported lipid bilayers with complementary sequences.[10] It is envisioned that, when combined with DNA-patterning techniques developed for microarray platforms,[11] this strategy will allow increasingly complex networks of living cells to be created through self-assembly.

Although there are well-established methods for the functionalization of devices with ssDNA,[11,12] the attachment of ssDNA to the surface of living cells has not yet been reported. To do this,[13] we have developed a synthetic method based on the introduction of specific chemical handles onto cell surfaces by using metabolic oligosaccharide engineering. Briefly, this was achieved though the incorporation of azides into cell surface glycoconjugates by the administration of peracetylated *N*-α-azidoacetylmannosamine (Ac_4ManNAz). Previous studies have shown that this unnatural sugar is metabolized to the corresponding *N*-α-azidoacetyl sialic acid (SiaNAz) within membrane-associated glycans (Figure 1a).[14]

We have reported previously the use of the Staudinger ligation (Figure 1b) as a bioorthogonal reaction for the modification of cell-surface azides with phosphine reagents, yielding stable amide linkages.[15] For these studies, we have expanded this technique to include oligonucleotide attachment.[16] We prepared modified ssDNA with a phosphine group through the reaction of a 5′-amine-modified ssDNA with a phosphine pentafluorophenyl (PFP) ester, as shown in Figure 1c. Model studies conducted with simple azides confirmed that the phosphine–ssDNA conjugate undergoes the Staudinger ligation to form the expected amide-linked product (see Supporting Information). Little or no oxidation of the phosphine–DNA conjugates was observed during handling or storage.

To verify the ability of the phosphine–DNA conjugates to undergo ligation to cell surfaces, human embryonic kidney (HEK) cells were first treated with Ac_4ManNAz in culture media for three days to introduce the azide groups. The cells were then reacted with phosphine–DNA conjugates that were prehybridized with complementary strands that bore a biotin reporter tag for a period of 1–2 h (Figure 2a). After the unreacted phosphine–DNA was removed through rinsing, the cells were treated with fluorescein isothiocyanate-labeled avidin (avidin-FITC). Flow cytometry analysis established the presence of DNA bound to the cell surface (Figure 2b). From these data, we estimate the number of surface DNA conjugates to be approximately 270000 per cell under the conditions used. This value represents a lower limit as it is unlikely that each step of the detection assay (prehybridization, cell-surface conjugation, and avidin-FITC labeling) proceeded with a quantitative yield.[15] Similar results were obtained for Jurkat (human T-cell line) cells. Control experiments, carried out by using DNA strands that lack reactive phosphines, showed no detectable binding above background levels. Similarly, experiments conducted using azide-free cells, but with

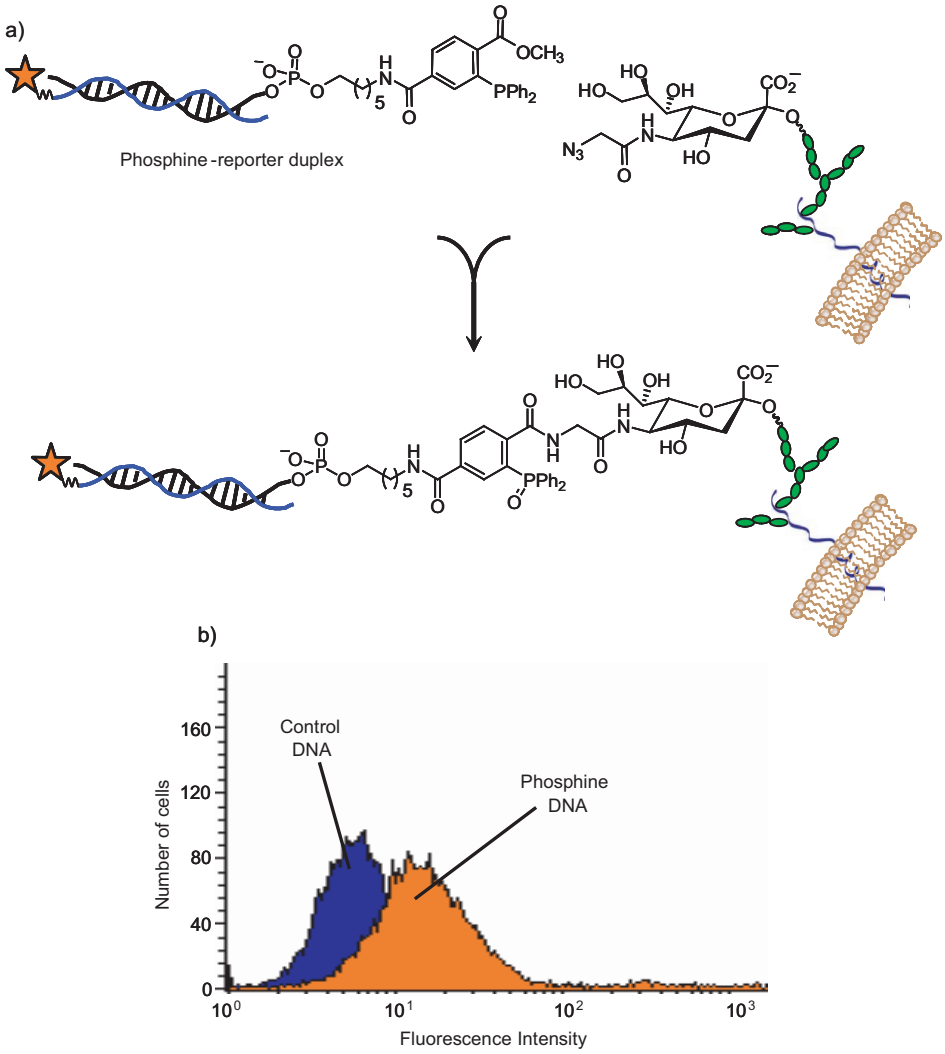

Figure 2. dsDNA complexes are covalently attached to the surface of live cells. a) Experimental overview for the quantification of exogenous DNA on the cell surface. Complementary biotinylated reporter strands are hybridized to the phosphine-bearing DNA. Biotinylated DNA strands are detected after binding to avidin-FITC. b) Flow cytometry analysis of human embryonic kidney (HEK) cells treated with biotinylated DNA strands that lack (blue) or bear (orange) reactive phosphine groups.

phosphine–DNA, showed detectable but greatly reduced labeling efficiency.

In parallel, experiments were carried out by using phosphine–ssDNA conjugates. Although these species are likely to react with surface azides with rates similar to those observed for double stranded DNA (dsDNA), attempts to quantify these groups through subsequent hybridization to biotinylated DNA were unsuccessful. This is likely due to the slow hybridization kinetics associated with the low concentrations of DNA on the plasma membrane, resulting in incomplete labeling. As noted by others,[17] the kinetics of DNA hybridization are substantially different at material surfaces owing to the dramatically higher local DNA concentration. Thus, we chose instead to confirm the presence of ssDNA on the cell membranes through surface attachment.

As an experimental platform, microfluidic devices were constructed to create controlled environments for the introduction of cells and rinsing solutions (Figure 3a).[18] First, a series of Au pads was patterned on glass surfaces by using standard photolithographic techniques (see Supporting Information). After treatment of the pads with commercially obtained 5′-thiolated ssDNA strands, a prefabricated channel layer of (poly)-dimethylsiloxane (PDMS) was affixed on top to form enclosed chambers. The resultant channels were 6 cm long, 600 μm wide, and 32 μm deep, which corresponds to a total volume of 1.5 μL. An external syringe pump was attached to control solution delivery at rates between 1–100 $\mu L\,min^{-1}$, and a mounted epifluorescence microscope with a digital camera was used for analysis. Two complementary 20-mer DNA-sequence pairs (**A**/**A**′ and **B**/**B**′) were chosen such that they possessed an identical overall base composition, but no appreciable affinity for each other. Strands **A**′ and **B**′ were bound to the Au surfaces within the channels, and strands **A** and **B** were attached to the cells by using the Staudinger ligation method described above. The presence and hybridization ability of the surface-immobilized DNA strands in the devices were determined through incubation with fluorescently labeled probe strands (Figure 3b).

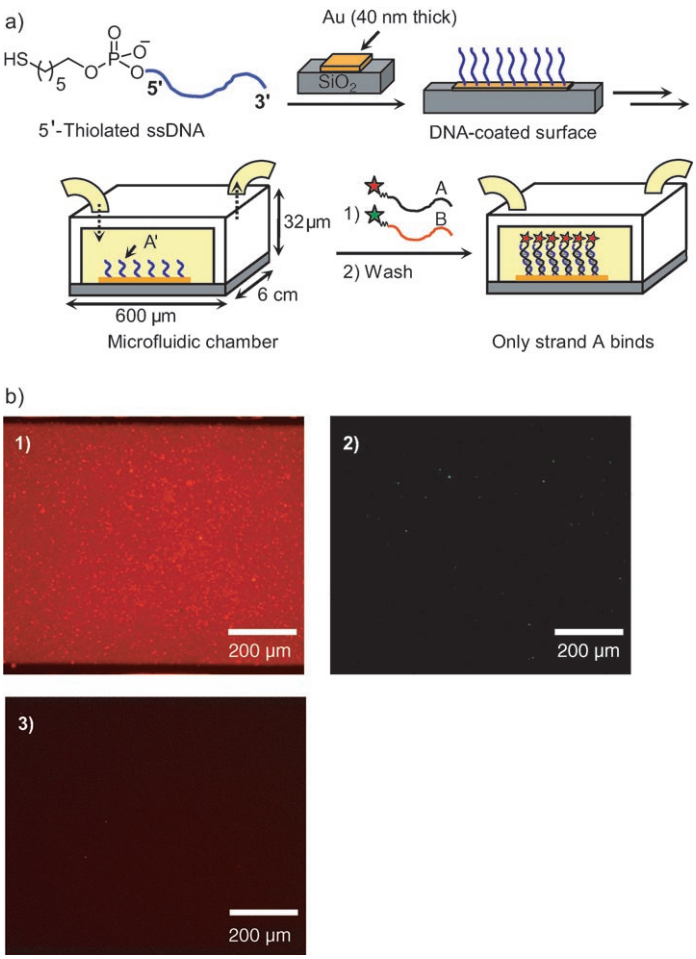

Figure 3. Construction of a microfluidic device for DNA display. a) Thiolated ssDNA strands are bound to patterned Au pads located in microfluidic chambers, solution delivery and washing can be controlled. Coating DNA strands were **A**′: 5′-AGT GAC AGC TGG ATC GTT AC-3′ and **B**′: 5′-CCC TAG AGT GAG TCG TAT GA-3′. The presence of DNA was detected by using matched (**A**, red) and mismatched (**B**, green) probe strands. b) Fluorescence images of 1) Probe **A** incubated with **A**′-coated surface; 2) Probe **B** incubated with **A**′-coated surface; 3) Probe **A** incubated with **B**′-coated surface.

To test the system, we introduced azides onto the surface of Jurkat cells, a naturally non-adherent cell type. The labeled cells were then divided into two subsets and each of these was modified with either DNA sequence **A** or **B**. For tracking purposes, the two subsets of cells were also labeled with either blue (7-amino-4-chloromethylcoumarin) or green (5-chloromethylfluorescein diacetate) cytosolic dyes. Equal numbers of each labeled subpopulation were then mixed and introduced into the device through the syringe pump. The cells were incubated for 35 min under static conditions to allow hybridization, and then unbound cells were flushed from the system with excess phosphate-buffered saline (PBS) solution.

Representative fluorescence microscopy data for these experiments are shown in Figure 4. Only cells bearing ssDNA strands that were complementary to the immobilized DNA were observed to bind to the surface (Figure 4b), whereas otherwise identical cells bearing mismatched sequences were washed away. Quantitative binding data obtained from three device regions are shown in Figure 4c. We found that 95% and 91% of the bound cells had recognized the appropriate complementary sequence for the **A**′ and **B**′ pads, respectively. Control experiments with cells that lack surface DNA showed no binding.

As further confirmation of the DNA dependence of our immobilization method, DNA-coated pads were blocked with complementary DNA sequences prior to the introduction of labeled cells. Although many cells bearing complementary DNA strands were exposed to the pads, virtually no binding was observed following the standard rinsing procedure (Figure 4d).

A particular advantage of this method is the ability to immobilize naturally non-adherent cells as well as adherent ones. This is because metabolic engineering can be used to introduce azide functional groups on virtually any cell type that displays sialic acid residues on its surface.[14] We have explored the generality of this

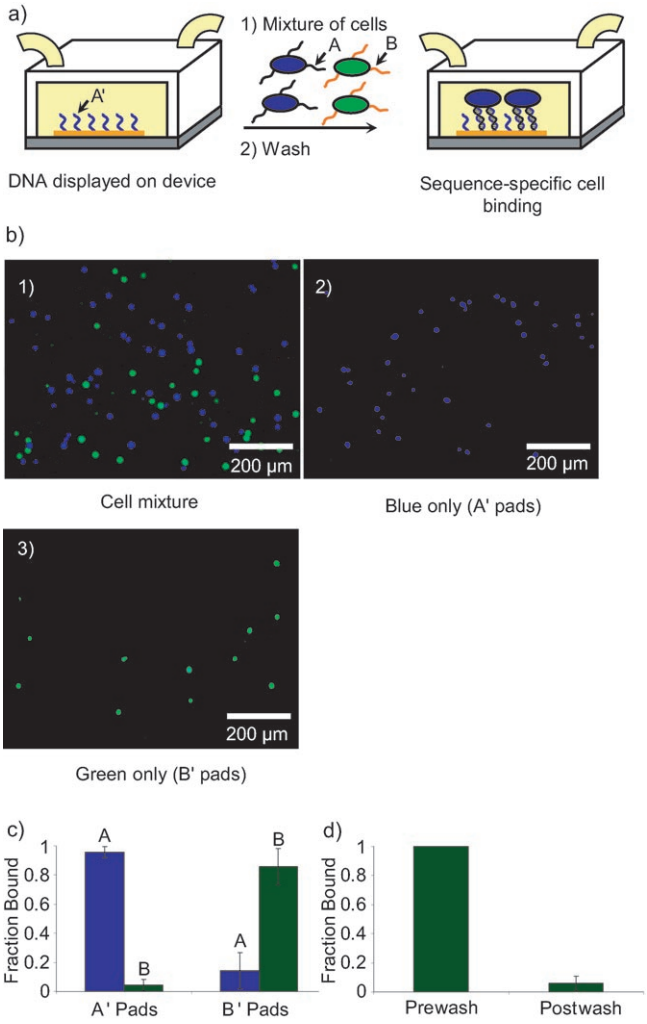

Figure 4. Immobilization of cells is DNA sequence-specific. a) Blue and green dye-labeled Jurkat cell populations were functionalized with DNA sequence **A** or **B**, respectively. An equal number of cells were combined and the mixture was introduced into the microfluidic device (as in Figure 3). b) Fluorescence images of: 1) the cell mixture prewash; 2) an **A**′-coated pad postwash; 3) a **B**′-coated pad postwash. c) Summary data for the fraction of the bound cells with either sequence **A** (blue), or sequence **B** (green) under each pad condition. d) **B**′-coated pads were pre-exposed to excess soluble strand **B**; these data summarize the fraction of green (sequence **B** bound) cells bound before and after washing. Error bars represent the standard deviation of three separate areas of the device.

technique through the immobilization of several additional cell lines, including Chinese Hamster Ovary (CHO) and HEK cells. Both CHO and HEK cells are naturally adherent cell types and each was successfully immobilized through this approach. Furthermore, we have found that this attachment method can be utilized on micropatterned Au pads that possess the same spatial dimensions as the cells (Figure 5).

The strength of DNA-mediated binding was determined by subjecting immobilized cells to increasing lateral shear force. PBS solution was introduced at increasing flow rates (1–50 $\mu L\,min^{-1}$ over a period of 10 min) and the bound HEK cells were observed. In almost every instance, the bound cells were not washed away. Through the use of the Navier-Stokes

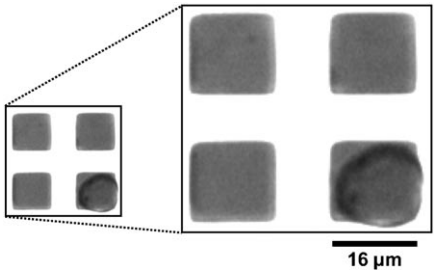

Figure 5. A single human embryonic kidney (HEK) cell immobilized on a 16-μm by 16-μm square Au pad. Cells bearing surface DNA strands were bound to Au pads decorated with complementary strands. Bright field microscopy was used to visualize the cells (magnified portion, 40×). Adhesion of cells lacking DNA or bearing noncomplementary DNA was not observed. Immobilized cells appeared morphologically similar to unbound cells.

method,[19] we calculated that bound cells are stable to shear forces that exceed 31 $dyn\,cm^{-2}$, which is far greater than that needed for most applications.[20] Similar results were noted for Jurkat and CHO cells.

For this method to be broadly useful, the longevity of cell surface–DNA complexes must extend beyond the time scale of the experiment.[1] Although it might be expected that there would be a loss of surface DNA owing to cell membrane recycling or secreted nuclease activity,[21] the cells have remained bound to the surfaces for periods exceeding 25 h despite the fact that we have taken no measures to avoid these degradation pathways.

Finally, we evaluated the prolonged loyalty of bound cells to the surface and their long-term viability though two additional studies conducted in parallel. In the first, Jurkat cells labeled with sequence **A** were immobilized as previously described, and a gentle flow of Jurkat culture medium was continuously applied to the device for 25 h. Cells were visualized by using light microscopy at several time points to determine their positions. Virtually all of the bound cells remained at their initial locations for the entire duration of the experiment. Secondly, we investigated cell viability and membrane integrity by using redoxsensor red CC-1, a fluorescent dye that is retained and activated in live cells. To more closely approximate typical culture conditions, the PDMS channel layer was removed from the device prior to introduction of the cells. After the cells had been allowed to bind for 35 min, Jurkat culture medium was added and the device was incubated at 37 °C for 25 h. The cells in the device were then treated with redoxsensor red CC-1 for 30 min, and the device was gently rinsed to identify the unbound cells. Analysis of both the bound and unbound cells indicated that 79 % of the DNA-bound cells remained viable after the 25 h period. This percentage was identical to that observed for the unbound Jurkat cells. We therefore believe that this method will be useful for applications that require extended cell immobilization.[1] More detailed studies of the physiological effects of DNA-based cell immobilization are currently underway.

In summary, we have described a new approach for engineering cell-adhesion events. This DNA-based strategy is sequence specific and applicable to a variety of devices and

cell types. Current work is focused on tuning the adhesive properties and studying the long-term behavior of DNA-immobilized cells, and ongoing efforts are capitalizing on the programmability of this method to form complex, multicellular patterns for sensing applications.

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