Mammalian cells are different from plant and microbial cells, having no exterior cell walls for protection. Environmental assaults can easily damage or destroy mammalian cells. Thus, the ability to develop a biomimetic cell wall (BCW) on their plasma membrane as a shield can advance various applications. Here we demonstrate the synthesis of BCW with a framing template and a crosslinked matrix for shielding live mammalian cells. The framing template is a supramolecular DNA structure. The crosslinked matrix is a polyelectrolyte complex made of alginate and polylysine. As the entire procedure of BCW synthesis is strictly operated under physiological conditions, BCW-covered mammalian cells can maintain high bioactivity. More importantly, the data show that BCW can shield live mammalian cells from not only physical assaults but also biological assaults. Thus, this study has successfully demonstrated the synthesis of BCW on live mammalian cells with great potential of shielding them from environmental assaults.
D\textit{uring} evolution, most plant and microbial cells have developed an exterior cell wall that has a framing structure and a crosslinked matrix\textsuperscript{4,5}. While this cell wall is an ultrathin nanomaterial, it can function as an effective shield to protect the cells from environmental assaults. Thus, plant and microbial cells can survive and recover even after their living environment is significantly changed from benign to harsh conditions\textsuperscript{1–3}. Mammalian cells, however, do not have a cell wall. The outermost layer of a mammalian cell is only a delicate, twolayered structure of phospholipids with proteins embedded\textsuperscript{6}. While the internal cytoskeleton mechanically helps mammalian cells maintain their shapes, mammalian cells can be easily damaged or destroyed when exposed to physical or biological assaults. Therefore, to advance the applications of mammalian cells, it is necessary to develop methods and materials to shield them externally.

Great effort has been made to deposit materials on the plasma membrane of mammalian cells for their protection\textsuperscript{7–13}. The most common strategy is to drive the mixture of cells and polymers into another phase for gelation or solidification using fluidic devices\textsuperscript{14,15}. For instance, the He group has applied this strategy to develop core-shell microcapsules for the differentiation and delivery of pluripotent stem cells for the treatment of myocardial infarction\textsuperscript{16}. However, the cell flow to the nozzle of a device is an intrinsically random process determined by Poisson statistics, which leads to heterogeneous polymer deposition and poor quality control\textsuperscript{17,18}. The deposited polymer usually has a very high volume, which can be several orders of magnitude higher than the cell. It causes poor molecular transport and cell survival\textsuperscript{19–21}. Moreover, if an application requires in vivo cell transplantation, it is challenging for target tissues or organs to accommodate a high number of transplanted cells owing to the large volume of polymers\textsuperscript{19–21}. To reduce the volume of depositing materials, methods have been developed to convert metal ions into a metallic nanomaterial with a redox reaction on the plasma membrane\textsuperscript{22,23}. However, the reaction conditions are too harsh to maintain the viability of mammalian cells that have a very fragile cell membrane\textsuperscript{9}. As the cell surface is negatively charged, studies have also been carried out to deposit cationic polymers or nanoparticles on the plasma membrane via electrostatic interactions\textsuperscript{24–26}. However, positively charged polymers or nanoparticles are intrinsically cytotoxic\textsuperscript{27–29}. Moreover, direct contact between the negatively charged cell membrane and positively charged molecules may turn on random endocytosis pathways for these materials to cross the cell membrane\textsuperscript{29,30}. Thus, while the amount of depositing materials on the plasma membrane can be reduced, it remains challenging to apply these elegant methods to cover and shield live mammalian cells. Notably, all existing methods depend on the direct deposition of covering materials on mammalian cells. No method has been studied to generate a structure mimicking the exterior wall of plant and microbial cells.

DNA, polysaccharides and polypeptides are three essential polymers in nature. They and their synthetic counterparts have been widely used to build up materials for biological and biomedical applications\textsuperscript{31–37}. For instance, two DNA hairpins can be used to synthesize long DNA polymers for biosensing through hybridization chain reaction (HCR)\textsuperscript{38,39}. Alginate and polylsine, a polysaccharide and a polypeptide, can be crosslinked to form microcapsules for islet delivery through polyelectrolyte complexation (PC)\textsuperscript{24,40,41}. We apply DNA, alginate and polylsine to synthesize a covering material to mimic the plant cell wall (i.e., BCW) on live mammalian cells.

BCW has a framing template and a crosslinked matrix that are synthesized with HCR and PC, respectively (Fig. 1a). The framing template is a supramolecular DNA structure. It functions to direct molecular assembly and crosslinking of polysaccharides and polypeptides. Notably, as alginate is initially conjugated with one DNA hairpin to form an alginate-DNA macromer, the formation of the supramolecular template leads to automatic alginate assembly on the cell membrane (Fig. 1b) for the next-step polyelectrolyte complexation (Fig. 1c). Moreover, the entire procedure of BCW construction is conducted under physiological conditions. Thus, this method is promising for the synthesis of BCW in shielding live mammalian cells.

**Results**

**DNA template-directed polymer assembly and crosslinking.** We conjugated alginate and DNA to synthesize the alginate-DNA macromer using copper-free click chemistry (Fig. 2a). After conjugation, we characterized this macromer using UV spectroscopy and gel electrophoresis. The results demonstrate that these two polymers could be conjugated to form a hybrid macromer (Fig. 2b and Supplementary Fig. 1). However, as alginate and DM2 are both negatively charged, the presence of alginate in the macromer might repel intermolecular DM1–DM2 hybridization during HCR. Moreover, as alginate was conjugated with DM2, alginate might affect DM1–DM2 hybridization due to steric hindrance. Thus, it is important to examine whether two DNA hairpins can maintain the ability of hybridization for polymerization. The gel image (Supplementary Fig. 2) shows that DM1 and alginate-DM2 could polymerize, suggesting that steric hindrance and charge interactions did not significantly affect HCR. Notably, as alginate was conjugated to DM2, the result also suggests that the polymerization of the two DNA hairpins led to alginate assembly during the polymerization.

We further examined the polymerization of DM1 and alginate-DM2 on the DNA initiator-coated particles. Specifically, streptavidin-coated particles were sequentially treated with biotinylated DNA initiators and the solution of DM1 and alginate-DM2. The analysis of dynamic light scattering (DLS) showed that the radius of the particles was increased by ~140 nm (Fig. 2c). As the calculated length of one pair of DM1 and DM2 is approximately 16 nm, the increase of the particle size demonstrated that the polymerization of DM1 and alginate-DM2 led to the formation of the supramolecular DNA polymer, and more importantly that this supramolecular polymer functioned as a template to direct alginate assembly. Consistent with the DLS characterization, the zeta potential of the particles was decreased by ~70% owing to the increased density of negative charges on the particle surface (Fig. 2c).

As alginate assembled during the formation of the supramolecular DNA template, we anticipated that the template could further function as a framing structure to direct alginate-polylsine crosslinking. Indeed the treatment of the template-covered particles with polylsine led to an increase of the diameter and the reversal of the zeta potential (Fig. 2c). As alginate and polylsine were fluorescently labeled, we also imaged the particle suspension. The imaging analysis of the particle suspension is consistent with the measurements of size and zeta potential (Fig. 2d). These results further demonstrate that the supramolecular DNA template can form on the particle surface and also importantly this template can direct molecular assembly and crosslinking of alginate and polylsine through polyelectrolyte complexation to form BCW.

As BCW will be exposed to biological fluids in potential applications, we incubated template-coated or BCW-coated particles in serum to examine the stability of the template and BCW with flow cytometry. Based on the examination of the fluorescence signal of Cy5-conjugated alginate, the template-covered particles lost over 80% of fluorescence intensity within
the first two days (Fig. 2e). By contrast, the BCW-covered ones maintained high fluorescence intensity during the two-week incubation after the initial decrease by 5–10% (Fig. 2e). The signal of FAM-labeled DM1 in the template also quickly decreased (Supplementary Fig. 3). However, while the signal of DM1 in BCW decreased, this decrease was much slower than that in the template. This observation is reasonable as the template is an uncrosslinked open system whereas BCW has the crosslinked alginate-polylysine complexes. The data also show that while the signal of DM1 in BCW was close to the background level in one week (Supplementary Fig. 3), the signal of alginate in BCW could be maintained over 90% (Fig. 2e). These data suggest that while the DNA template by itself or in BCW is degradable in biological fluids, the crosslinked alginate-polylysine cover once formed has high stability. The degradation of the DNA template after the construction of BCW may be beneficial to reduce potential inflammatory response caused by the interaction of DNA and toll-like receptors if BCW-covered cells will be used for in vivo cell transplantation.

**Synthesis of BCW on mammalian cells.** We next studied whether this method would be effective in synthesizing BCW on the plasma membrane of live mammalian cells using the CCRF-CEM cell line as the primary cell model. Cholesterol-conjugated DNA initiators were immobilized on the cell membrane through the insertion of cholesterol into the lipid bilayer. This insertion would be sufficient for the immobilization of DNA initiators and the initiation of HCR since membrane lipids are the major components in the plasma membrane. To examine the formation of the framing template on the cell surface (Fig. 3a), we used flow cytometry to analyze the signals of DM1 and alginate-DM2. Both of these signals exhibited a sharp shift (Fig. 3b). Importantly, the comparison between the one-unit and template groups demonstrates the success of DNA polymerization for the formation of the framing template on live cells (Fig. 3b). We further studied the effect of time on the formation of the framing template. The results suggest that within 3 h, one DNA template could grow to the level of displaying approximately 10 alginate molecules (Fig. 3c and Supplementary Fig. 4). The fluorescence imaging was consistent with the flow cytometry analysis, showing the strong signals of FAM and Cy5 localized on the cell membrane (Fig. 3d).

After demonstrating the formation of the template, we examined whether this template could direct the formation of BCW on live cells. The template-covered cells were incubated in the solution of Cy3-labeled polylysine for 1 min before centrifugation. The fluorescence images clearly show the strong Cy3 signals on the cell surface (Fig. 3e). Moreover, the same location on the cell membrane displayed the signals of three fluorescent labels for the DNA template, alginate and polylysine concomitantly (Fig. 3e). It is also important to note that the fluorescence intensity inside the cells was minimal compared to that on the cell membrane (Fig. 3e and Supplementary Fig. 5). These data clearly demonstrate that the template could direct alginate-polylysine crosslinking to form BCW on the cells, and that the cellular uptake of any molecule used for BCW synthesis was virtually negligible. We further quantified the yield of single cells after the formation of BCW. The percentage of single cells was over 90% (Supplementary Fig. 6). It suggests that the majority of BCW-covered cells were separate with minimal cell aggregation.

BCW was also examined using zeta potential and transmission electron microscopy (TEM). The negative zeta potential of the cells was decreased by 72% after the formation of the framing template (Fig. 3f). This negative zeta potential was reversed to 9 mV after template-directed alginate-polylysine complexation and a further treatment with alginate led to the reversal of the zeta potential (Fig. 3f). The TEM images suggest that the DNA-templated alginate-polylysine complexation led to the formation of BCW on the cell membrane with a thickness of approximately
70–150 nm (Fig. 3g and Supplementary Fig. 7). The ultrathin BCW ensures an ultralow polymer-to-cell ratio. It makes this method fundamentally different from other methods that need hydrogels of several hundred microns to cover cells\textsuperscript{10}. Such a thick hydrogel cover is associated with problems such as poor molecular transport and high occupancy volume.

As the positively charged polylysine was directed to crosslink with the negatively charged alginate, one may suggest to directly cover the positively charged polylysine on the negatively charged cell surface. To address this potential concern and illustrate the advantage of our method, we incubated the cells in the solution of FITC-labeled anti-CD4 antibody that can recognize and bind CCRF-CEM cells. The whole cell surface would be adequately covered by the negatively charged supermolecular template that is able to direct complexation between polylysine and alginate. To do this, we quantified the amount of DNA initiators on the cell surface. This amount increased with the incubation time and the concentration (Supplementary Fig. 9). When the cells were incubated in the solution with 1 \textmu M of DNA initiator, the surface of one cell displayed approximately 4 × 10\textsuperscript{6} DNA initiators through the streptavidin-biotin interaction.

We also characterized molecular transport across BCW and the stability of BCW on the cell surface. Molecular transport was examined by incubating cells in the solution of FITC-labeled anti-CD4 antibody that can recognize and bind CCRF-CEM cells. The
data show that while BCW was permeable to the antibody, it significantly blocked antibody transport in comparison to the template or the naked cell membrane (Supplementary Fig. 12). The stability was examined in two situations. One group of BCW-covered cells were cultured in a normal culture medium (10% FBS), and the other group of BCW-covered cells were cultured in a reduced-serum medium (1% FBS). With the treatment of 10% FBS, the fluorescence intensity of BCW on the cell surface decreased after 24 and 48 h (Supplementary Fig. 13). With the treatment of 1% FBS, the fluorescence intensity of BCW on the cell surface barely changed during the 48 h culture (Supplementary Fig. 13). As CCRF-CEM cells are divided faster in 10% FBS than in 1% FBS, these data
suggest that BCW had high stability against enzymatic degradation, and that BCW-covered cells could maintain their normal capability of cell proliferation. Moreover, as the signal of alginate-Cy5 primarily came from the surface rather than the inside of the cells, the data suggest that BCW was not internalized during the procedures of BCW construction and cell culture.

After the synthesis and characterization of BCW on CCRF-CEM cells, we further synthesized BCW on the membrane of additional four types of live mammalian cells using the exactly same protocol. BCW successfully formed on the cell membrane in all cases (Fig. 4 and Supplementary Fig. 14), although we did not optimize reaction conditions for each different cell type. Together, the data demonstrate that DNA-templated molecular assembly and crosslinking is an effective, potentially universal method for the synthesis of BCW on the plasma membrane of live mammalian cells.

**Evaluation of shielding effectiveness.** After demonstrating the formation of BCW on mammalian cells, we studied the effect of BCW on cell viability. The inability to maintain high cell viability is a major reason for the failure of many traditional methods for cell surface engineering and/or shielding. For instance, the direct deposition of cationic polymers on the naked cell membrane is well-known to cause high cytotoxicity. In our method, a framing template was synthesized on the cell membrane before the treatment of cells with polylysine. Polylysine would mainly react with DNA-templated alginate, sparing the plasma membrane of cells. Moreover, the entire procedure of BCW formation did not involve any harsh conditions. Indeed BCW-covered cells could maintain much higher viability than those naked cells directly exposed to polylysine (Fig. 5a and Supplementary Fig. 15).

As BCW was developed for cell protection, we examined the effectiveness of BCW in shielding mammalian cells from physical...
and biological assaults (Fig. 5b). Reiterative centrifugation and osmotic imbalance were used as physical assaults. The results show that naked cells were highly sensitive to centrifugation (Supplementary Fig. 16). For instance, when the centrifugal force was as low as 110 g, over 30% of the cells lost cell viability during the procedure of cyclic washing and centrifugation. By contrast, even though the centrifugal force was increased over 6000 × g, approximately 85% of the cells could maintain viability (Supplementary Fig. 16). Thus, these data clearly demonstrate that BCW can shield live cells from the damage of centrifugation (Fig. 5c). The shielding enhancement increased linearly with the centrifugal force (Fig. 5c), suggesting that live cells can be...
protected more effectively in a harsher centrifugation situation. We also examined the effect of osmotic imbalance on the cell viability. The shielding enhancement-osmotic imbalance relationship exhibits a bell curve (Fig. 5d). This relationship suggests that when the osmotic imbalance changed from 0.1 to 0.6, BCW was strong enough to protect the covered cells. With a further increase of the osmotic imbalance, BCW could still play the role of shielding the cells while this shielding effect started to weaken (Fig. 5d and Supplementary Table 2).

In addition to the two physical assaults, we also studied cell viability after the cells were exposed to biological assaults using both in vitro and in vivo assays. In the in vitro assay, a natural killer cell line (NK-92MI) was used to attack BCW-covered K562 cells. The results show that BCW enhanced the survival rate of the target cells from the assault of NK-92MI virtually linearly (Fig. 5e and Supplementary Fig. 17). For instance, when the effector/target ratio was 5:1, ~50% of target cells lost viability whereas ~85% of BCW-covered cells could maintain viability (Supplementary Fig. 17). In the in vivo assay, we subcutaneously transplanted human umbilical cord mesenchymal stem cells (MSCs) expressing red fluorescence protein (RFP) into BALB/c mice. As foreign cells, human MSCs would be quickly destroyed in mice through the host immune response. As indicated by the signal of RFP, we could initially detect MSCs covered with (+) or without (−) BCW. However, the RFP signal could be barely detected in the mice transplanted with naked MSCs (i.e., no BCW) by day 7. In contrast, the RFP intensity maintained 30% in the mice transplanted with BCW-covered MSCs (Fig. 5f and Supplementary Fig. 18). So the sharp difference strongly demonstrates that BCW can shield human MSCs from biological assaults in the in vivo environment. To better understand this shielding effect, we also conducted two in vitro experiments. In the first one, we incubated BCW-covered MSCs on the cell culture plate. The results demonstrate that adherent MSCs could maintain viability, break BCW and attach to the cell culture plate for proliferation (Supplementary Fig. 19). In the second one, we transferred the fibrin hydrogel with MSCs onto the cell culture plate. The results show that MSCs covered with or without BCW did not migrate from the fibrin hydrogel to the outside (Supplementary Fig. 20), suggesting that the disappearance of MSCs from the implantation site in mice did not result from MSC migration. As it is known that MSCs can induce the growth of blood vessels because of their ability of releasing angiogenic factors43,44, in another in vivo assay, we transplanted MSCs into mice for examining angiogenesis. The tissues at the transplantation sites were collected and stained with a mouse-specific anti-CD31 antibody as CD31 is a typical endothelial cell biomarker. The data show that BCW-covered MSCs could stimulate more angiogenesis than the naked cells (Fig. 5g and Supplementary Fig. 21). Taken together, both in vitro and in vivo data demonstrate that BCW holds great potential to cover and shield mammalian cells from environmental assaults.

**Discussion**

A cell wall is not a simple ultrathin polymer layer but a structured polymer matrix crosslinked by microfibrils1–3. Because of this organized structure, while the cell wall is thin, it is mechanically tough with the ability to shield the cell. For instance, a major function of the cell wall is to prevent the over-swelling of the cell when water enters under osmotic imbalance4,5. We mimicked the key features of the natural cell wall to develop BCW on mammalian cells. BCW has a supramolecular DNA frame that guides the formation of the polysaccharide-polypeptide matrix. Notably, while we used alginate and polysyline to illustrate the formation of the matrix, in principle, they can be replaced with any other biocompatible polymers that can undergo crosslinking reactions including but not limited to polyelectrolyte complexation.

The synthesis of BCW is fundamentally different from previous methods studied for the deposition of materials on a surface. Firstly, with previous methods, covering materials are directly deposited on the plasma membrane without a framing structure and a structured matrix. Secondly, the entire procedure of BCW synthesis is strictly operated under physiological conditions. It does not involve any harsh conditions like organic solvents, high pressure, high salts, chemical crosslinking, or direct cell-toxic polymer contact, which are often used in other methods. For this reason, the BCW-covered cells can maintain high bioactivity after BCW synthesis, which is critical to any live mammalian cell-based applications. Thirdly, the volume of BCW is several orders of magnitude lower than that of the covered cell. The ultralow polymer-to-cell ratio would be beneficial if an application (e.g., in vivo transplantation) requires a high number of cells but a small space of occupancy. Lastly, the synthesis of BCW is simple and straightforward as it only needs cell incubation in bio-compatible solutions. The formation of the template and the crosslinking of alginate and polysylane can both automatically happen in one single step. It does not need any specific devices or take much time or reiterative operations to accomplish. It is independent of cell type. Moreover, all cells are treated equally without an inherently theoretical difference. No empty BCW is generated. As a result, this method is suitable for the large-scale synthesis of BCW on a large number of cells. Therefore, we envision that BCW holds great potential as a shielding material for various live mammalian cell-based applications.

Cell shielding or encapsulation is needed in various areas45. Numerous elegant methods for cell shielding or encapsulation have been studied with great promise. Potential applications include cell delivery for tissue regeneration, cancer therapy, biosensing, bioprinting, etc.10,12,19,45,46. However, while all of these applications require cell shielding or encapsulation, they do not share the same requirements for the shielding methods or materials. For instance, when cells are delivered into a target tissue for tissue repair via the release of therapeutic growth factors, it is unnecessary and undesirable to shield those therapeutic cells permanently. Similarly, when cells are mechanically extruded for three-dimensional bioprinting, temporary cell shielding would be sufficient to protect them against shear stress or microenvironmental damage during the procedure of extrusion.

Our work shows that BCW can protect cells against various environmental assaults. Moreover, BCW-covered cells can survive and break BCW for proliferation during cell culture. It suggests that BCW would be a promising tool for applications such as cell delivery for tissue regeneration and bioprinting. It is also important to note that other applications may require “permanent” shielding or encapsulation. A typical example is islet delivery for diabetes treatment. Such an application requires the shielding materials to have long-term stability and integrity (i.e., no breakage) and meanwhile allow sufficient molecular transport to support cell survival. While this work is not focused on the optimization of BCW for those applications, it may be possible to tune the conditions of BCW synthesis to achieve high stability and to provide cells or islets with a “permanent” shielding cover. It will be one of the foci in our future work.

**Methods**

**Materials and instrumentation.** Dibenzocyclooctyne-PEG₇-NHS ester, Cy5-DBCO and Cy3 NHS Ester were purchased from Click Chemistry Tools (Scottsdale, AZ), Oligomers (Supplementary Table 3) were purchased from Integrated DNA Technologies (Coralville, IA). Sodium alginate (medium viscosity, 80–120 kDa) and O-[(2-Aminomethyl)-O'-(2-azidoethyl)pentamethylene glycol (N₃-PEG₇-N₃) were purchased from Sigma-Aldrich (St. Louis, MO). Streptavidin coated particles were purchased from Spherotech (Lake Forest, IL). PEGylated
polylysine (26 kDa) was purchased from Nanosoft Polymers (Lewisville, NC). Quantum™ FITC-5 MESF was purchased from Bangs Laboratories (Fishers, IN). Carboxyl-functionalized diethyl allylamine (CFAE) and aldehydes (Avanti Polar Lipids, Alabaster, AL) were used. Fatty acid-free Bovine Serum Albumin (BSA) was used from Sigma-Aldrich. DMSO was purchased from Fisher Scientific (Waltham, MA). DMSO was used as a solvent for DBCO-PEG4-NHS ester (DMSO, 50 mM NaHCO3). Then 25 µL of DBCO-PEG4-NHS ester (DMSO, 14 mg, 0.12 mmol), EDC (116 mg, 0.60 mmol) and NH2-PEG6-N3 (28 mg, 500 nm particles for the analyses of size and zeta potential. All of other experiments were conducted with 5 µm particles unless otherwise noticed. Streptavidin-coated particles (200 nm) (1 mg) were conjugated with biotinylated DNA initiator (DI, 2 nmol) in 500 µL of PBS buffer at room temperature for 1 h on a rotator. DI-modified particles were collected by centrifugation and further washed with PBS. To examine the polymerization of DM1 and alginate-DM2 on the particles, 0.1 mg of particles were incubated in 800 µL of PBS containing DM1 (1 µM) and alginate-DM2 (1 µM) for 3 h at room temperature. To examine poly-electrolyte complexation, the particles with the template were incubated with 0.01% (w/v) polylysine in PBS for 5 min. After centrifugation and washing with PBS twice, the particles were further incubated with 0.05% (w/v) alginate in PBS for 5 min. For each step, the size and zeta potential of particles were measured using Malvern Zetasizer Nano ZS.

**General cell culture conditions.** CCRF-CEM (CCL-119, human T lymphoblastic leukemia cell line) and K-562 (CCL-243, chronic myelogenous leukemia cell line) were purchased from ATCC (Manassas, VA) and maintained in RPMI-1640 supplemented with 10% FBS. NK-92MI (natural killer cell) was purchased from ATCC (Rockville, MD) and maintained in alpha-modified MEM supplemented with recommended supplements. Primary Aortic Smooth Muscle Cells were purchased from ATCC (Manassas, VA) and maintained in M231 containing Smooth Muscle Cell Growth Supplement. Human bone marrow CD34+ hematopoietic stem cells were purchased from StemCell Technologies (Tukwila, WA) and expanded with StemSpan-stem cell growth medium supplemented with NOG-1 conditioned media. hMSC (normal human bone marrow derived mesenchymal stem cells) was purchased from Lonza (Walkerville, MD) and maintained in recommended growth medium (Lonza). RFP-Tagged human bone marrow derived MSCs (RFP-MSCs) were purchased from Angio-Proteomix (Boston, MA) and maintained in stem cell growth medium (Lonza). Cells were maintained at 37 °C in an atmosphere of 5% CO2 and 95% relative humidity.

**Preparation of dibenzocyclooctyne-modified DNA (DNA–DBCO).** Hundred microliter of DNA-NH2 solution (1 mm) was added to 375 µL of modified buffer (DPBS, 50 mM NaHCO3). Then 25 µL of DBCO-PEG4-NHS ester (DMSO, 14 mg, 0.12 mmol), EDC (116 mg, 0.60 mmol) and NH2-PEG6-N3 (28 mg, 0.08 mmol) were added. After stirring for 30 min at room temperature, 55 µL of 6 M NaOH was added to adjust pH to 7.5–8.0. The reaction proceeded overnight at room temperature. Purification was achieved by 3 days of dialysis against water (10,000 MWCO membrane). To further remove any unreacted reagents, the alginate-N3 solution was precipitated in cold acetone, filtered and dried. The final product alginate-N3 was dissolved in d.-H2O, filtered through a 0.2 mm membrane, and lyophilized. Alginate-N3 was analyzed using NMR spectroscopy.

**Preparation of azide-modified alginate (alginate-N3).** Fifty milligram sodium alginate was dissolved in 5 mL of MES buffer (50 mM, pH = 5). To this solution, NHS (14 mg, 0.12 mmol), EDC (116 mg, 0.60 mmol) and NH2-PEG6-N3 (28 mg, 0.08 mmol) were added. After stirring for 30 min at room temperature, 55 µL of 6 M NaOH was added to adjust pH to 7.5–8.0. The reaction proceeded overnight at room temperature. Purification was achieved by 3 days of dialysis against water (10,000 MWCO membrane). To further remove any unreacted reagents, the alginate-N3 solution was precipitated in cold acetone, filtered and dried. The final product alginate-N3 was dissolved in d.-H2O, filtered through a 0.2 mm membrane, and lyophilized. Alginate-N3 was analyzed using NMR spectroscopy.

**Preparation of alginic–DM2 macromer.** The alginic–DM2 was prepared using copper-free click reaction by mixing DM1 and alginate-N3. In brief, 100 µL of 1% w/v solution of alginate-N3 was incubated with 30 µL of 1 mM DNA-DBCO in DPBS for 4 h at 37 °C. After conjugation, the alginic–DM2 was collected and purified using a 3 kDa Amicon Ultra Centrifugal Filter. After washing with cacodylate buffer (0.1 M), the cells were dehydrated with a series of ethanol solutions (30, 50, 70, 90, and 100%). All of these treatments were performed at 4 °C. After dehydration, the cells were treated with propylene oxide, infiltrated and embedded in a liquid resin. The resin block was sectioned using an ultramicrotome and the slices were collected on grids. Imaging was performed under the FEI Talos F200X High-resolution transmission electron microscope using both TEM and STEM modes.

**Transmission electron microscopy (TEM).** For the preparation of TEM samples, naked or BCW-covered cells were fixed in the solution of glutaraldehyde (2.5%). The cell samples were then post-fixed with 1% osmium tetroxide. After washed with cacodylate buffer (0.1 M), the cells were dehydrated with a series of ethanol solutions (30, 50, 70, 90, and 100%). All of these treatments were performed at 4 °C. After dehydration, the cells were treated with propylene oxide, infiltrated and embedded in a liquid resin. The resin block was sectioned using an ultramicrotome and the slices were collected on grids. Imaging was performed under the FEI Talos F200X High-resolution transmission electron microscope using both TEM and STEM modes.

**Synthesis of BCW on live mammalian cells.** CCRF-CEM cells were centrifuged, washed twice and re-suspended in DPBS. 1 x 10^6 cells were incubated in 400 µL of Cholesterol·TEG-DI solution (1 µM, DPBS) for 30 min for the incorporation of DI into cell membrane through the spontaneous insertion of cholesterol into membrane lipids. Then DI-modified cells were collected, washed and subsequently mixed with DM1 (1 µM) and alginate-DM2 (1 µM) in DPBS for 3 h to form the supramolecular DNA template. To test the effect of reaction time on the formation of the template on the cells, the polymerization time was varied from 1 to 3 h. The cells were collected and the fluorescence intensity (Cy5 signal from alginate-DM2) was measured by flow cytometry. For the subsequent poly-electrolyte complexation, cells covered with the template were sequentially treated with 0.01% (w/v) poly-lysine for 1 min and 0.05% (w/v) alginate for 5 min. Finally, the cells covered with BCW were collected by centrifugation before any characterization.

**Evaluation of shielding enhancement.** Centrifugal force and osmotic imbalance were used to demonstrate the effects of physical assaults on cells. In the study with the centrifugal force, cells covered with or without BCW were suspended in the DPBS at a concentration of 1 x 10^6 cells/mL. The centrifugation was repeated by five times. Each centrifugation was performed for 5 min at 4 °C. The centrifugation force was varied from 110 to 6200 g. Cells were re-suspended in new DPBS after each centrifugation. After five cycles of centrifugation, the cells were incubated in the culture media for 24 h and their viability was measured by Live/Dead viability/ cytotoxicity kit. In brief, cells were incubated with calcine-AM and ethidium
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Acknowledgements
This work is supported by the U.S. National Science Foundation (1802953) and the National Institutes of Health (R01HL122311).

Author contributions
P.S. designed the study, performed the experiments, interpreted the data, and wrote the manuscript. N.Z. did animal studies and confocal microscopy imaging. J.C. did experiments. Y.W. conceived the concept, designed the study, interpreted the data, and wrote the manuscript.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-10231-y.

Competing interests: The authors declare no competing interests.

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Journal peer review information: Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work.

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