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Engineering the Surface of Therapeutic "Living" Cells

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Abstract

Biological cells are complex living machines that have garnered significant attention for their potential to serve as a new generation of therapeutic and delivery agents. Because of their secretion, differentiation, and homing activities, therapeutic cells have tremendous potential to treat or even cure various diseases and injuries that have defied conventional therapeutic strategies. Therapeutic cells can be systemically or locally transplanted. In addition, with their ability to express receptors that bind specific tissue markers, cells are being studied as nano- or microsized drug carriers capable of targeted transport. Depending on the therapeutic targets, these cells may be clustered to promote intercellular adhesion. Despite some impressive results with preclinical studies, there remain several obstacles to their broader development, such as a limited ability to control their transport, engraftment, secretion and to track them in vivo. Additionally, creating a particular spatial organization of therapeutic cells remains difficult. Efforts have recently emerged to resolve these challenges by engineering cell surfaces with a myriad of bioactive molecules, nanoparticles, and microparticles that, in turn, improve the therapeutic efficacy of cells. This review article assesses the various technologies developed to engineer the cell surfaces. The review ends with future considerations that should be taken into account to further advance the quality of cell surface engineering.

Graphical Abstract

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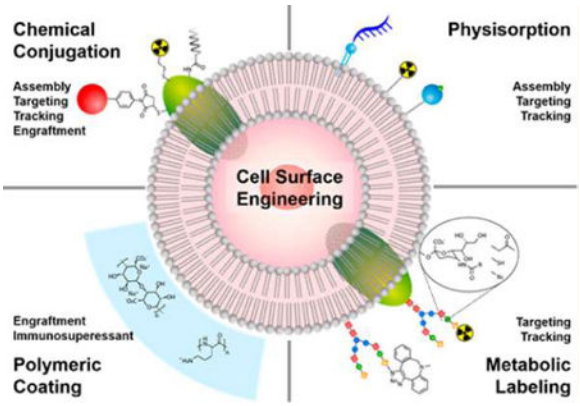

1. INTRODUCTION

Living cells are invaluable biomedical tools for the treatment and study of several incurable diseases, including cancer,[1] metabolic disorders,[2,3] and tissue defects.[4] These diseases are particularly well-suited to cell-based therapies, because they are difficult to treat with available drugs and surgical techniques.[5] For instance, patients with type I diabetes have been successfully treated with islet cells isolated from the pancreas, which are able to secrete insulin endogenously.[6] Mesenchymal stem cells derived from bone marrow and adipose tissue are able to sustainably secrete a series of therapeutic cytokines and growth factors[7,8] and, thus, have the potential to treat nonhealing wounds,[9–11] cardiovascular diseases,[12–15] and neurological disorders.[16–18] Mesenchymal stem cells are also able to repair and regenerate various tissues and organs, including cartilage,[19] muscle,[20] bone,[21] nerve,[22] and blood vessels.[23] Encouragingly, studies of patients treated with human leukocyte antigen-mismatched mesenchymal stem cells show no observable long-term side effects.[24,25]

Bone-marrow-derived hematopoietic stem cells and their lineages, such as macrophages and T-cells, are able to treat malignant diseases, including cancer, and are able to restore immune function following conventional cancer treatments.[26] Red blood cells are able to treat vascular diseases, such as sickle cell anemia.[27] Some cells in this lineage, namely, mesenchymal stem cells, red blood cells, and macrophages, can act as drug delivery systems by transporting drug-loaded nanoparticles. When injected into the circulation, these cells locate and engraft to target tissue.

Despite the impressive examples described above, cell therapies have multiple hurdles to overcome before they can be widely adopted for clinical use. In this regard, one of the most important challenges is controlling transport, particularly for cells delivered systemically that are intended to treat multiple pathological sites in a noninvasive manner. Often the percentage of cells reaching the target tissue is limited to ≤5%.[28] Because of the low and variable degree of target engagement, it is important to determine dosing, and thus, it is desirable to measure the circulation time and biodistribution of transplanted cells.

Another key issue is that therapeutic cells delivered to injured or diseased tissues often lose viability and their therapeutic activity as a result of the hostile extracellular microenvironment. For instance, cells expanded in vitro or obtained from donors may

encounter undesired host immune responses.[29] If they successfully reach the site of injury or the diseased tissue, an additional obstacle is reactive oxygen species that are overproduced and negatively impact cellular adhesion at the target site.[30] Even though cells retain viability and manage to engraft to target tissue in these hostile environments, the question of whether cells remain therapeutically active is often difficult to answer. Additionally, cell therapies require controlling the cadherin-mediated cell–cell adhesion, which plays a major role in the cellular differentiation.

What these challenges mean practically is the need to use a large number of cells during transplantation. This leads to increased medical costs and ultimately to a less practical process.[31,32] Toward this end, considerable effort has focused on modifying the cell surface to help regulate all aspects of the activity of therapeutic cells. To date, many studies have reported positive results; however, there is still a need to carefully examine the merits and weakness of these approaches and the development of new strategies to enhance the controllability of therapeutic cells.

Here we review chemical technologies developed to engineer the surface of therapeutic cells to control or enhance transport (section 2), tracking (section 3), engraftment (section 4), secretion (section 5), and intercellular adhesion (section 6). The evolution of these modifications provides a roadmap for future advances in cellular therapies. In turn, new advances will further expedite the clinical use of cells, improving the quality of disease treatments and the repair of tissue defects. Because this review article is focused on surface modification of “living” cells, it will not cover studies that aim to engineer the surface of artificial cells, which only partly recapitulate the structure and functionality of living cells.[33–37]

2. CONTROLLING CELL TRANSPORT

Controlling the transport of therapeutic cells following systemic transplantation is critical to improving the therapeutic efficacy, lowering the number of transplanted cells needed, and minimizing any undesirable side effects. One approach to achieving this aim is by mimicking the leukocyte recruitment or hematopoietic stem cell homing process (Figure 1).[38] Damaged, inflamed, infected, or diseased tissue often presents defective vasculature that overexpresses surface receptor proteins, such as selectin, vascular cell adhesion molecules (VCAM), or intercellular cell adhesion molecules (ICAM).[39,40] Leukocytes then bind to these endothelial receptors, initiating a cascade of events including rolling, adhesion, and extravasation processes that, in turn, lead to transmigration into the diseased tissue

HSCs home in on various organs because the CXCR4 receptors of the cells bind the stromal-derived factor-1 that is overexpressed on damaged or inflamed endothelium.[41] The interaction between CXCR4 and stromal-derived factor-1 polarizes the cells on the endothelium and, in turn, facilitates transendothelial migration. However, some of the therapeutic cells, such as mesenchymal stem cells, do not respond to the inflamed endothelial ligands or endogenous SDF-1.[42] For this reason, one focus of cell surface engineering has been developing methods for the surface presentation of various bioactive

molecules and nanoparticles that target endothelium. Progress in this area is described below.

2.1. Chemical Conjugation of Biomolecules

Primary amine residues are abundant on cell surface proteins and readily available for the chemical ligation with targeting ligands (Table 1) that are known to associate with endothelial receptors.[43] For instance, a controlled number of sialyl Lewis X molecules, a tetrasaccharide carbohydrate that can promote the cell rolling on P-selectin, were conjugated to the surface of mesenchymal stem cells via amide coupling with *N*-hydroxysuccinimide-activated biotin (Figure 2).[44] The biotinylated mesenchymal stem cell surface was sequentially incubated with streptavidin and a biotinylated sialyl Lewis X moiety, forming a biotin–streptavidin–biotin complex that results in a sialyl Lewis X-coated cell surface.[44]

Using a P-selectin-coated substrate as a model of an inflamed vasculature, this study demonstrated a considerable decrease of the velocity of cells flowing over the substrate when coated with sialyl Lewis X in comparison to untreated cells and biotinylated cells.[45] Specifically, sialyl Lewis X-conjugated mesenchymal stem cells showed a velocity of only ~2 μm s^{-1} at a wall shear stress of 0.36 dyn cm^{-2} due to the interaction between sialyl Lewis X and P-selectin, whereas unmodified stem cells showed ~65 μm s^{-1} in the same condition (Figure 2b).

In a mouse model with an injured ear, intravenous injection of sialyl Lewis X-conjugated mesenchymal stem cells displayed enhanced extravasation compared with untreated cells.[46] Thus, sialyl Lewis X-conjugated stem cells localized to the inflamed ear with 56% increased efficiency compared to that of the unmodified stem cells (Figure 2b). On the other hand, there was no significant difference between the numbers of sialyl Lewis X-conjugated mesenchymal stem cells and unmodified stem cells within the noninflamed ear.

To affirm the enhanced targeting ability, a study of the biodistribution of these cells would be useful. In addition, the injection method (i.e., intravenous and intra-arterial) affects the transport of therapeutic cells and consequently homing efficiency.[47] The biotin conjugated to the cell surface did not affect the viability of cells significantly[44] (Figure 2c). The mesenchymal stem cells conjugated with biotin groups did display a slightly slower cell growth rate during the initial phase (i.e., days 2–4) of an 8-day period where the cell culture was monitored (Figure 2d).[44]

On the other hand, synthetic polymers, such as polyglycerol, can be used as a macromolecular factor that controls cellular transport due to their biocompatibility and the variety of their derivatives.[48–50] Dendritic polyglycerol substituted with sulfate groups exerted high-affinity binding to mediators of inflammation and therapeutic effects on endothelium in vivo, using an acute contact dermatitis mouse model.[50] Due to the high local concentration of sulfate groups, polyglycerol bound to L-selectin of leukocytes and P-selectin of inflamed endothelium and, in turn, shielded leukocyte adhesion to endothelium and subsequent extravasation.[50]

2.2. Modification by Physisorption

A series of noncovalent bioconjugation methods have been developed to regulate cell transport. These methods can be sorted into two groups: (1) the intercalation of functionalized lipids or alkyl chains into the phospholipid layers of the cell membrane and (2) the fusion of biofunctionalized liposomes with the cell membrane.

2.2.1. Hydrophobic Association.—The surface of cells can be functionalized with targeting groups by using synthetic lipids or alkyl chains. The interaction is driven by the hydrophobic effect, where these groups intercalate between phospholipids in the cell membrane, thereby anchoring the attached group. Simple alkyl chains were used to immobilize vasculature-binding molecules on mesenchymal stem cells.[51] Thus, a polyvalent hyperbranched polyglycerol core was functionalized with oligopeptides that bind vascular cell adhesion molecule and with octadecyl anchoring chains. The resulting hyperbranched polyglycerol conjugated with vascular binding peptide (VBP–HPG) spontaneously anchored on the stem cell surface in a thermodynamically favorable manner upon mixing with cells suspended in media (Figure 3a).

In an in vitro experiment conducted with endothelium induced to overexpress vascular cell adhesion molecules using the tumor necrosis factor-alpha, mesenchymal stem cells coated with VBP–HPG adhered to the endothelium more favorably than unmodified cells. In particular, a kinetic analysis showed that coating of cells with VBP–HPG significantly increased the binding affinity of cells with the endothelium that overexpressed vascular cell adhesion molecules (Figure 3b,c).[51] A likely explanation for this observation is that the HPG-functionalized cell surface allows the oligopeptides to bind synergistically with cell surface receptors and to increase the number of cells immobilized to the targeted endothelium.

Analogously, long-chain alkyl groups were reported to immobilize proteins such as protein A and protein G on cell surfaces, which, in turn, allowed immobilization of tissue-binding antibodies. These proteins have been widely used in immunoassays because of their strong affinity toward Fc regions of various immunoglobulin G (IgG) molecules.[52,53] Therefore, the physical immobilization of these proteins on the cell membrane can facilitate the coating of cells with antibodies of interest. A systematic study to examine the extent to which these hydrophobic surface modifications affect cell viability and phenotypic activities would be useful.

Using the *N*-hydroxysuccinimidyl ester of palmitic acid, free amines on the surface of recombinant protein A were conjugated with palmitate (Figure 4).[54] The resulting palmitate-conjugated protein A rapidly associated with the cell membrane via phospholipid intercalation. To enable the targeting of protein A-coated cells, they were exposed to rabbit anti-mouse IgG. These antibody-coated cells exhibited a 4–5-fold increase in adhesion to B lymphoma cells. This result indicates that the rabbit anti-mouse IgG on the cells can act as a receptor for B lymphoma cells.[54] Although more rigorous in vivo testing is needed, this encouraging result suggests that cells modified with anti-mouse IgG can serve as a homing system to improve the efficiency of chemotherapy against B lymphoma.

Protein G was also used to immobilize antibodies on the cell surface and has the advantage of a higher IgG-binding affinity relative to protein A.[55] In a similar fashion to the protein A modification described above, protein G was coupled to palmitic acid groups via *N*-hydroxysuccinimidyl ester-mediated amidation of the amine residues on protein G. Upon simple mixing, the palmitate-conjugated protein G intercalated into the membrane of chondrogenic progenitor cells.[56–58] Subsequent incubation of the cells with antibodies to cartilage matrix (e.g., antibodies against collagen type II and keratin sulfate) resulted in enhanced adhesion at a cartilage injury site when compared to untreated cells. The antibody coating on the transplanted cells minimally affected viability, cell growth, and chondrogenic differentiation.[56]

Using the palmitate-conjugated protein G, mesenchymal stem cells were also coated with antibodies to target defective vasculature. The recombinant antibodies to intercellular adhesion molecule-1 were bound to protein G on the surface of stem cells via a 1 h incubation of cells in ice-cold media dissolved with the antibodies. The in vitro study determined that the immobilized antibodies to intercellular adhesion molecule-1 served to increase the number of mesenchymal stem cells adhering to an endothelium treated with tumor necrosis factor-alpha to induce intercellular adhesion molecule-1 overexpression.[58] Again, a systematic study to examine the biodistribution of these cells in vivo would be useful.

Heparin is a glycosaminoglycan that can act as a cell transport mediator by interacting with various extracellular matrix proteins and growth factors.[59] When heparin is used as a cell surface coating agent, it can modulate the adhesion of stem cells, including adipose-derived stem cells, by regulating the cellular expression of integrin β1 and selectin.[60,61] Heparin can be anchored to the cell membrane by lipid conjugation. Thus, a series of carboxylate groups in heparin were coupled with the free amine of 1,2-dipalmitosyl-*sn*-glycero-3-phosphoethanolamine using an in situ activation with hydroxybenzotriazole and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (Figure 5a).[61] This synthetic route is superior to the more traditional oxidation-induced ring-opening reaction of heparin because heparin loses bioactivity when the saccharide backbone is opened.[62,63]

The resulting 1,2-dipalmitosyl-*sn*-glycero-3-phosphoethanolamine-conjugated heparin anchors to the cell membrane when incubated, because the lipid can intercalate into the cell membrane phospholipid groups.[61] In vitro, the 1,2-dipalmitosyl-*sn*-glycero-3-phosphoethanolamine-conjugated heparin remained stable for at least 24 h on the cell surface in physiological media. Following intravenous injection, the heparin-coated adipose-derived stem cells accumulated more in the liver and spleen than untreated cells (Figure 5b).[61]

Additionally, cells coated with heparin were less likely to become entrapped in the lung compared to the untreated cells. This higher level of liver uptake is consistent with the tendency of heparin to accumulate in the liver.[64] Using heparin as a cell transport mediator may minimize unwanted cell accumulation in the lung. A potentially powerful strategy would involve incorporation of both heparin and vasculature binding peptide or antibody

coatings to amplify and increase the homing efficiency of transplanted cells, particularly to injured liver and spleen tissue.

2.2.2. Liposomal Fusion.—As we discussed in section 2.1, biotin can be used to display receptor-binding molecules on the surface of cells to regulate cell transport. To circumvent the need for covalently linking biotin to the cell surface, an alternative is to synthesize a biotin-conjugated lipid molecule that can intercalate into the cell membrane. This process can be achieved by incubating the cells with the vesicles of biotinylated-lipids, wherein a lipid exchange process occurs with native and vesicle lipids slowly exchanging to give a biotin-coated cell surface. As previously described, mesenchymal stem cells coated with sialyl Lewis X mediated a biotin–streptavidin–biotin complex.

Similarly, using vesicles of a biotin-conjugated lipid, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(biotinyl), a series of sialyl Lewis X groups were anchored to the surface of mesenchymal stem cells (Figure 6).[65] As previously demonstrated, sialyl Lewis X increases the number of cells that rolled on a P-selectin-coated surface, which is a reported model for an inflamed vascular wall.[65] Sialyl Lewis X-modified mesenchymal stem cells exhibited significantly improved rolling with a velocity of 8 μm/s as compared to 61 μm/s for unmodified mesenchymal stem cells at a shear stress of 0.5 dyn/cm^2. On the other hand, the cell surface modification did not affect the viability and multilineage differentiation potential of mesenchymal stem cells.[48] These results suggest that using vesicular fusion to immobilize other molecules responsible for cell adhesion and extravasation (e.g., vascular cell adhesion molecule, intercellular adhesion molecule binding peptides, appropriate antibodies, and CXCR4-binding stromal derived factor-1) will similarly augment cell homing efficiency.

3. CELLULAR TRACKING

Tracking the transport and destination of cells systemically or locally injected into a body is necessary to ascertain their homing and engraftment. As such, efforts are being made to image transplanted cells using noninvasive bioimaging approaches, including magnetic resonance imaging, positron emission tomography, optoacoustic tomography, and luminescence/fluorescence-based in vivo imaging systems. To increase imaging sensitivity, cells are labeled with a series of molecules or nanoparticles to generate positive or negative contrast in clinical images captured with the appropriate bioimaging instrument.

In most cell-labeling methods, contrast agents are introduced into the cell. Use of these methods has raised concerns about the possible negative impact on cellular viability, biomolecular secretion, and phenotypic activities. Furthermore, the intracellular environment can negatively impact the stability and function of contrast agents. As a result of these considerations, there has been a greater effort to immobilize contrast agents on the cell membrane. Discussed in the following sections are some promising developments in cell surface labeling with magnetic resonance contrast agents (e.g., gadolinium, superparamagnetic iron oxide nanoparticles), positron emission tomography contrast agents (e.g., ^{89}Zr, ^{19}F), optoacoustic contrast agents (e.g., gold nanoparticles), and fluorescent molecules, each allowing for in vivo tracking.

3.1. Magnetic Resonance Imaging Tracking and Contrast Agents

Magnetic resonance imaging is a noninvasive method to diagnose disease and tissue defects in the clinic by converting the relaxation rates of protons on water in the body into 3D images of organs and tissue.[66,67] Despite the multiple merits of magnetic resonance imaging, such as high-resolution imaging, magnetic resonance imaging has low sensitivity in differentiating between transplanted cells and healthy tissue. Contrast agents that can modulate the relaxation rate of water protons are being engineered to label transplanted cells, thereby highlighting in magnetic resonance images the tissue where cells are accumulated.[68–72] Magnetic resonance imaging contrast agents currently used in clinical settings, with approval by the FDA, include gadolinium (a positive contrast agent) and superparamagnetic iron oxide nanoparticles (a negative contrast agent).

3.1.1. Gadolinium Contrast Agents.—Labeling cells for tracking was achieved by improving the intracellular entry of gadolinium-chelating agent complex with transfection agents or gadolinium-loaded nanoparticles.[73] However, concerns about the reduced molar relaxivity of gadolinium within cells, cellular retention of gadolinium, and possible cytotoxicity if gadolinium is released from the chelating agents led to the development of alternative labeling methods.[74,75] Thus, cell surface immobilization of gadolinium was used as an alternative labeling method. When 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetic acid, a cyclen gadolinium(III) chelating agent, is coupled to hydrophobic alkyl chains, they can intercalate into the phospholipid membrane of cells (Figure 7). Using 1,4,6-tribromophenol, a long-chained alkyl group was appended to the phenolic group via the Mitsunobu reaction.[76] The alkylated 1,4,6-tribromo-phenol ether was coupled with alkyne groups through a Sonagashira coupling with trimethylsilylacetylene followed by deprotection with potassium fluoride. The resulting molecule was reacted with an azide–gadolinium complex using click chemistry.[76] Cells labeled with these molecules displayed enhanced labeling and retention of gadolinium.[55,77]

The 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetracetic acid–gadolinium(III) complex can also be linked to the cell surface through a disulfide exchange reaction with the cysteine thiol groups of extracellular proteins. The thiolated gadolinium(III) complex gadolinium(III)–DO3AS–Act (DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) (Figure 8) has a 2-pyridyl-dithio group that readily exchanges with exofacial protein thiols and has been used to label human myeloid leukemia K562 cells.[57]

The labeled cells displayed higher positive contrast than unlabeled cells. However, some of the disulfide-linked gadolinium(III) complexes were internalized by the cells.[78]

An alternative potential method to label cells with gadolinium uses a surface-coating material. For instance, chitosan functionalized with ethylenediaminetetraacetic acid–gadolinium(III) complexes and alkyl groups was reported to coat liposomes. Liposomes are often used as a model cell membrane and coating them gave good contrast in comparison to encapsulated ethylenediaminetetraacetic acid–gadolinium(III) complexes.[79]

Liposomes decorated with gadolinium(III) complexes and fluorescent dyes can be used to label cells and perform dual imaging.[80] The liposomes are prepared with a combination of

lipids, including 1,2-dioleoyl-3-trimethylammonium-propane, a phosphocholine lipid with a diacetylene tail, rhodamine B sulfonyl-conjugated lissamine, and a gadolinium(III) complex lipid construct. In the absence of light, variable concentrations of the designed lipids are dried in vacuo and resuspended in solution. The solution is sonicated and treated with 254 nm light to effect polymerization of the diacetylene units. The resulting nanoparticles have surface and encapsulated gadolinium(III) complexes and rhodamine B dyes. Cancer cells labeled with these nanoparticles were highlighted in fluorescence and T_1-weighted MR images in vivo following local subcutaneous injection. Site-specific labeling in systemically transplanted cells could be possible in the future if target specific ligands are added to the surface.

Exploiting the tolerance of glycosylation enzymes for unnatural substrates, a peracetylated *N*-levulinoylmannosamine was used to metabolically incorporate *N*-levulinoyl sialic acids onto cell surface glycoproteins. The resulting *N*-levulinoyl sialic acid moiety has a bioorthogonal ketone group that can react with amine or hydrazide groups. As such, the cells could be readily coated with an aminooxy-terminated diethylenetriaminepentaacetic acid, which, in turn, complexed europium(III) (Eu^{3+}). The resulting engineered cells were used as a dual fluorescence and magnetic resonance imaging agent (Figure 9). One possible advantage to this conjugation method is the ability to highlight tumor cells that intrinsically overexpress sialic acid.[81]

3.1.2. Superparamagnetic Iron Oxide Nanoparticles.—Cells can be labeled with SPIONs by controlling their intracellular entry with transfection agents and optimizing external fluid flow.[82] The drawbacks to intracellular labeling are the reduced molar relaxivity, cellular retention, cytotoxicity, and impaired cell differentiation caused by internalized SPIONs.[83] To date, only limited effort has focused on localizing cell tracking SPIONs on cell surfaces. However, a strategy developed to target and label pathologic cells for diagnosis could be used to immobilize SPIONs on the surface of cells.[84,85] For instance, SPIONs functionalized with antibodies may bind with antigens intrinsically expressed by cells.[86] Modulation of the antigen expression of cells would improve the efficacy of the antibody-based immobilization of superparamagnetic iron oxide nanoparticles.[86]

3.2. Positron Emission Tomography Imaging

Positron emission tomography (PET) is a whole-body-imaging method used to monitor the function of organs and tissues. The PET instrument detects γ-rays emitted from radionuclides such as ^{89}Zr and ^{19}F.[87,88] Therefore, a radiotracker is often coupled with bioactive molecules and injected into a body. One radiotracker extensively used in imaging is ^{18}F-fluorodeoxy-D-glucose. This radiotracker has been used to monitor cellular glucose uptake, which is more rapid in cancer.[89] In another example, the ^{18}F-based molecule was used to evaluate the effects of warm ischemic stress on islets in the early stage of transplantation to the liver.[90]

The ^{18}F-fluorodeoxy-D-glucose tracker was also used to monitor the discharge of cardiac progenitor cells from a collagen matrix implanted in an ischemic site.[91]

However, according to the study conducted with neural stem cells labeled with ^{18}F-fluorodeoxy-D-glucose for cell transport into the brain, the cell tracker is quickly cleared from the cells.[92] Longer radiotracker retention can be achieved with hexadecyl 4-[^{18}F]fluorobenzoate (Figure 10), which was used to track human circulating progenitor cells.[93] The hexadecyl 4-[^{18}F]fluorobenzoate exhibited a higher binding efficiency and stability than ^{18}F-fluorodeoxy-D-glucose, thus leading to the stronger signal from cells transplanted into a rat with myocardial infarction. With hexadecyl 4-[^{18}F]fluorobenzoate, the dynamic positron emission tomography imaging demonstrated a significant decrease in the number of cells at the engrafted site (<40% of the initial cell dose) 4 h post-transplantation. Chemical reaction of trackers with extracellular proteins or physical association of trackers with phospholipids of the cell membrane may lead to more reliable results.

Another radionuclide used in cell transport studies is ^{89}Zr–oxalate. This tracker was synthesized by forming a $^{89}ZrCl_4$–oxine complex.[94] Using the ^{89}Zr–oxine tracker, bone-marrow-derived cells were labeled via incubation. Following systemic cell transplantation into wild-type mice, it was possible to monitor homing and mobilization of the cells. In vitro studies indicated that there is no significant impact on the viability or phenotypic activities of ^{89}Zr–oxine-labeled cells.

3.3. Optoacoustic Imaging

On the basis of the photoacoustic effect, optoacoustic imaging generates images by detecting ultrasonic waves produced by the interaction of a nonionizing laser pulse with irradiated tissue.[95] The energy absorbed into tissue is transformed into heat, which results in wideband ultrasonic emission. The degree of energy absorption is associated with physiological conditions, including the local concentration of hemoglobin and oxygen.[96]

Gold nanoparticles with controlled sizes and shapes (e.g., spheres, rods) have been used as exogenous contrast agents in optoacoustic imaging because their optical absorption can be tuned by the surface plasmon resonance effect.[94] Gold nanoparticles can be functionalized to target specific tissue; for instance, antibody conjugation allowed selective binding of epithelial growth factor receptors and the imaging of human epithelial cancer cells. The epithelial growth factor receptor was selected because it is highly expressed in certain cancerous cells, such as A431 cells (human epidermoid carcinoma cell line), and not in others, such as MDA-MB-435 cells (breast cancer cell line).

Gold nanoparticles were prepared by reduction of chloroauric acid with citric acid followed by anti-epithelial growth factor receptor monoclonal antibody conjugation using a dithio/adipic hydrazide heterofunctional polyethylene glycol linker. The in vitro optoacoustic imaging of the A431 cells treated with gold functionalized with epithelial growth factor receptor antibody in a model tissue shows a promising ability to detect cells that express an epithelial growth factor receptor with high sensitivity and selectivity. The gold construct can be attached to the cell surface via antibody–ligand interactions, allowing for target-specific optoacoustic imaging.[97]

The surface modification of gold nanoparticles with dithiol heterolinkers, the reactive functional groups (e.g., *N*-hydroxysuccinimimdyl ester, aldehyde, maleimide) of which

allow them to readily link to extracellular proteins, increases the stability of the gold nanoparticles–cell surface conjugation and reduces the number of nanoparticles necessary for labeling.[98–100] This approach would minimally affect cellular viability and function compared with intracellular loading of nanoparticles.[101–103]

3.4. In Vivo Imaging with Fluorescent Labeling

Commercially available in vivo imaging systems generate 3D bioimages with spectra from fluorescent or luminescent probes coupled with molecules or cells of interest.[104] Many preclinical studies have utilized this imaging system to analyze the biodistribution of therapeutic cells post-transplantation.

One well-established way to functionalize the surface of cells is through the use of "function–spacer–lipid constructs."[105] Function–spacer–lipid constructs are lipid-based cell-coating molecules that have three components: functional, spacer, and lipid domains. The lipid domain acts as an anchor to keep the construct on the cell surface. The lipids used are typically 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, cholesterol, or ceramide.[106] The spacer controls the distance of the functional domain from the cell surface. The spacer is composed of adipate or carboxymethylated oligoglycine with lengths in the range from 2 to 7 nm. The functional domain has featured a range of moieties, including polysaccharides, blood group determinants, sialic acids, radiolabels, peptides, biotin, and fluorophores.[105,107–109]

In the transplantation of red blood cells, fluorescein-conjugated function–spacer–lipid constructs were used to label cells ex vivo by incubating at 28 °C for 2 h in a suspension of red blood cells. This construct enabled imaging of cells transplanted into a zebrafish embryo.[110] Furthermore, there were no apparent effects on the transport or the function of the transplanted red blood cells.[110]

Fluorophores can also be covalently attached noninvasively to the cell surface through metabolic labeling. By treating cells with *N*-azidoacetyl-D-mannosamine, cells will metabolically incorporate an azide-containing saccharide to the surface of glycoproteins, as previously discussed (section 3.1.1).[111] Using dibenzylcyclooctyne-conjugated Cy5, a copper-free click-reactive moiety, the Cy5 dye can be attached to cell surface proteins (Figure 11). Using this method, adipose-derived mesenchymal stem cells were fluorescently labeled and injected into the outer thigh muscle of a mouse with an ischemic injury in the inner thigh muscle. The migration and fate of transplanted cells were monitored with the imaging system.

The Cy5 cell-labeling strategy was also utilized to monitor chondrocytes with near-infrared fluorescent imaging up to 4 weeks after transplantation.[112] Because cells that are fluorescently labeled via a covalent linkage have such longevity, this labeling method can be useful for tracking cells that target certain biomarkers. For instance, human breast cancer cells that overexpress human epidermal growth factor receptor 2 were labeled with quantum dots conjugated with antibodies specific for human epidermal growth factor receptor 2.[113]

4. PROMOTING CELLULAR ENGRAFTMENT

Cell engraftment is another important factor to consider for the successful application of cell therapy. To support the engraftment of transplanted cells to target tissue, reducing the immune response and neutralizing the oxidative environment are crucial. For instance, pancreatic islet transplantation often causes thrombosis, which, in turn, causes inflammation and is detrimental to transplanted cells.[114] Islets are clusters of various cell types, including insulin-producing beta cells, and are commonly transplanted into patients with type I diabetes.[6] Clinically, ca. 5×10^5 donor islets are inserted into a patient's liver through a catheter. Impressively, these transplanted islets eliminated hyperglycemia unawareness in patients, a condition wherein a potentially dangerous blood glucose drop goes unrecognized. However, without constant administration of immunosuppressive drugs, these allogeneic cells rapidly lose viability and their therapeutic activity.

In sickle cell anemia, patients are frequently transfused with red blood cells from a healthy donor. Blood transfusions are necessary to alleviate the detrimental effects caused by sickle-shaped red blood cell, which characterizes this genetically inherited incurable disease. The abnormal red blood cell shape is caused by a mutation in the β-globin gene, which leads to the polymerization of hemoglobin, reducing the number of healthy red blood cells.[115,116] Most commonly, patients suffer from pain, severe anemia, and organ damage caused by blockages in blood flow and reduced amounts of oxygen transported through the bloodstream. The transplanted red blood cells work by decreasing the number of hemoglobin S cells and recovering the level of oxygen transported through the bloodstream. However, a major challenge to this therapy is transplantation failure, resulting from the host immune response.

In ischemic heart disease and injury, stem cells, and especially mesenchymal stem cells that secrete paracrine factors, are useful in repairing damaged tissue via revascularization and tissue regeneration.[117,118] These paracrine factors include the vascular endothelial growth factor, fibroblast growth factor, and hepatocyte growth factor.[119–121] However, reperfusions of blood flow after infarction or ischemia result in the overproduction of reactive oxygen species, including hydrogen peroxide and oxygen radicals.[122,123] These reactive oxygen species hinder the adhesion of transplanted mesenchymal stem cells to damaged tissues and cause cell apoptosis, a process called anoikis.[124] These hurdles prompted a series of cell surface engineering techniques that can extend the period in which transplanted cells remain therapeutically active.[125,126] In the next section, we will examine the strategies that can prevent thrombosis, reduce immune response, and neutralize oxidative stress to transplanted cells.

4.1. Controlling Thrombosis

One of the major factors that negatively impacts transplanted pancreatic islets is blood coagulation. This thrombotic reaction results from tissue factors secreted by the transplanted cells.[114] Heparin, which acts as an anticoagulant and an immune-suppressive agent, is systemically administered to prevent this problem.[127–129] However, this approach increases the risk of uncontrolled bleeding in healthy tissue.

To this end, efforts were made to coat red blood cells with heparin to localize the anticoagulation effect.[130,131] To attain heparin-coated red blood cells, a biotin–avidin binding assembly process was used. Biotin coating of cells was performed by conjugating surface amino groups with sulfosuccinimidyl-6-[biotin-amido]hexanoate. The biotin-coated surface was incubated with avidin to yield an avidin-coated surface, which was bound with macromolecular conjugates of heparin.[131] The heparin-coated islet cells inhibited thrombosis, decreased the risk of bleeding, and exhibited no cytotoxicity to islet cells.[131]

In addition to heparin, thrombomodulin is another anticoagulant that has been used to improve the performance and viability of implanted cells via cell surface conjugation.[132,133] Covalent attachment of recombinant thrombomodulin was performed using Staudinger ligation with a triarylphosphine–poly(ethylene glycol) linker (Figure 12a). The Staudinger ligation reaction proceeds through an aza-ylide intermediate that reacts with a neighboring ester group to form an amide bond. This reaction is useful in bioconjugation applications because it is fast, high-yielding, and works under physiological conditions.[134]

The sequential two-step reaction with both poly(ethylene glycol) linker and azido-thrombomodulin led to significant islet labeling, which was not observed on islets exposed to azido-thrombomodulin or poly(ethylene glycol) linker alone (Figure 12b–e).[133] An assay performed with the anti-human thrombomodulin antibody demonstrated a 10-fold increase in antibody binding to remodeled islet surfaces when compared to untreated islets or to those treated with the linker alone (Figure 12f).[133] The masked islets coated with thrombomodulin displayed significantly reduced thrombogenicity.[96]

4.2. Modulating Immune Response

4.2.1. Modification of Pancreatic Islets.—In allogeneic transplantation of pancreatic islets, the innate immune system is triggered and can lead to early cell death.[135] Using immunosuppressant drugs can lessen the response, but it limits the clinical potential of islet transplantation because the drugs also suppress the regeneration of beta cells in the transplanted islets.[125] Therefore, to resolve this challenge, poly(ethylene glycol) polymer coatings were used to mask transplanted cells from host immune cells.[136] These initial efforts to coat islets with poly(ethylene glycol) involved covalent amidation of the collagen matrix. Another study involved simply adding poly(ethylene glycol)s of various molecular weights to the preservation solution used for the islets.[137] According to a systematic study, poly(ethylene glycol) with an average molecular weight of 20 kDa led to the enhanced engraftment of islets without negatively impacting the secretion function of islets.

As noted above, one common method of conjugating poly(ethylene glycol) to cells is amide bond formation using an *N*-hydroxysuccinimide ester activated monomethoxy-poly(ethylene glycol) propionate and reacting with cell surface amino groups.[138] Covalent linkage of poly(ethylene glycol) to the islet surface showed a significant enhancement in engraftment compared to unmodified islets and did not affect glucose recognition or insulin secretion activity of islet cells.[138] Moreover, the poly(ethylene glycol)-conjugated islet cells survived for more than a year when coadministered with a widely used immunosuppressant, cyclosporine A. In contrast, unmodified islets were completely rejected within 2 weeks even with the administration of cyclosporine A.[136] In the future, it will be ideal to find a cell

coating method that does not require the coadministration of the immune-suppressive drugs or, alternatively, the identification of an immunosuppressive drug that minimally affects beta cells in both transplanted and host islets.

4.2.2. Red Blood Cell Modification.—As noted above, red blood cell transfusion is extensively used to treat sickle cell disease.[27,139] However, a major challenge is alloimmunization, an immune response to foreign antigens after exposure to genetically disparate cells or tissues. A study examined the alloimmunization in sickle cell disease patients who received 1–56 units of blood in the span of six years. The study reported that 30% of the patients had an immune response to transfused red blood cells, as characterized by the overproduction of autoantibodies.[140,141] As a consequence, the patients faced an abnormal level of hemoglobin in the blood, fever, and jaundice.[142]

To circumvent alloimmunization, the surface of red blood cells was engineered to block antigenic determinants (Figure 13). One approach to mask red blood cells, analogous to that used above with pancreatic islets, is to coat the cell surface with poly(ethylene glycol). There are several chemical strategies to covalently link poly(ethylene glycol) to the surface of red blood cells, including some analogous to the amidation methods described above. Cyanuric chloride conjugated to methoxy-poly(ethylene glycol) can readily react with surface amine groups of the cell membrane. The molecular weight of poly(ethylene glycol) was 5 kDa (Figure 13a). The resulting poly(ethylene glycol)-coated red blood cells resisted agglutination, the clumping of cells, and exhibited a reduction of phagocytosis of poly(ethylene glycol)-coated sheep red blood cells by human peripheral blood monocytes compared with untreated cells.[143] Further, the poly(ethylene glycol)-coated sheep red blood cells survived in mice longer than the uncoated cells. Tuning the molecular weight of poly(ethylene glycol) may further increase the survival rate and period of transplanted red blood cells.

Amide coupling reactions can also be used to surface coat red blood cells with poly(ethylene glycol) via surface amine groups. Poly(ethylene glycol) with either a succinimidyl propionate (Figure 13b) or a benzotriazole carbonate end group (Figure 13c) has been used to conjugate poly(ethylene glycol) to the surface amine groups of red blood cells.[144,145] Studies of covalently attached poly(ethylene glycol) show that the molecular weight and density of the polymer units played a role in enhancing cell survival in vivo. In particular, the poly(ethylene glycol) with an average molecular weight of 20 kDa maximized the cell survival rate. It is also possible to coat red blood cells with poly(ethylene glycol) by using 2-iminothiolane, also known as the Pierce Traut's reagent (Figure 13d). This reagent reacts with surface amine to ring open the cyclic thiol imidate and to generate an amidinium linker with a terminal thiol group. The terminal sulfhydryl can undergo the thiol-Michael addition reaction with poly(ethylene glycol) polymers tagged with a maleimidophenyl group.[146]

In a similar fashion, poly(ethylene glycol) conjugation methods can be used to reduce immune response caused by passenger T lymphocytes, immune cells that bind with red blood cells. It is common to irradiate blood samples with γ-rays before transplantation to eliminate the passenger lymphocyte cells; however, the irradiation reduces the therapeutic function of red blood cells transfused into patients. To circumvent the irradiation,[147]

methoxy-poly(ethylene glycol) was used to coat T lymphocytes. The methoxy-poly(ethylene glycol) effectively blocked the antigen-specific, memory-cell-dependent, and the major histocompatibility complex class II mediated T-cell activation. Blocking these three distinct activation pathways showed promise for minimizing the passenger T-cell-induced alloimmunization during the transfusion of red blood cells. In particular, the poly(ethylene glycol) molecules on the cell surface may inhibit the donor T-cell attack on host cells and prevent the graft-versus-host disease.[147]

4.3. Immunosuppressant Barriers

4.3.1. Polymeric Coating of Cells.—In pancreatic islet transplantation, the immune response is also of great concern. One of the early approaches to diminish the immune response was to encapsulate islets in submicron-sized hydrogel beads through in situ polymer cross-linking.[148–151] Several studies demonstrated that hydrogels prepared by cross-linking alginate with calcium and reinforcing the surface with a polyelectrolyte complex using poly-L-lysine or poly(ethylenimine) allowed diffusion of cell-secreted insulin and glucose while limiting transport of larger molecules such as immunoglobulins.[148] Further optimization of this encapsulation method, accomplished by lowering the viscosity of the alginate, enhanced the viability of encapsulated cells. The enhancement in viability was attributed to the reduction of shear stress on the cell membrane during capsule preparation.[152] The resulting microencapsulated xenograft islet reversed the diabetic state for up to 1 year compared to unencapsulated islets that were only effective for 2 weeks or less.[152]

Alternatively, islets were encapsulated in the hollow fibers formed from the extrusion of poly(acrylonitrile-*co*-vinyl chloride) into water and coated in alginate. These encapsulated islets maintained normoglycemia up to 60 d when the fibrous constructs were implanted in streptozotocin-induced diabetic mice subcutaneously or intraperitoneally.[153]

Agarose is another gel-forming polymer used for encapsulation of islets. Hamster islets encapsulated in 5% (w/w) agarose microbeads with an average diameter of 520 ± 180 μm via emulsification retained the normogly cemia over 100 d in streptozotocin-induced diabetic mice.[154] However, with mice presensitized by free hamster islets, the period during which islets retain the normoglycemic function decreased to 32 d. Also, the coating layer allowed diffusion of antibodies and ultimately led to cell death.[155] Further increasing the concentration of agarose of the microbeads to more than 7.5% (w/w) enhanced the structural integrity of the microbeads and prolonged the period during which islet grafts remained viable.[155] However, this approach was not able to extend the survival of xenograft islets in nonobese diabetic mice. In this mouse model, leukocytes infiltrated the pancreatic islets and subsequently caused type I diabetes.[156]

In an effort to improve upon this approach, polystyrene-sulfonic acid sodium salt was mixed with agarose to form denser microbeads. The large diameter of the beads, ranging from 600 to 800 μm, negatively affected the oxygen and nutrient transport into islets. Therefore, efforts were made to develop a process to produce smaller microbeads.[156] One such process is using spray-gelling. In spray-gelling a microdroplet generator is used to control the air flow, temperature, and relative velocity of islets to the pregel to control the encapsulation

process.[157] By using a blend of sodium alginate and poly-L-ornithine, this process could generate islet-encapsulating microbeads with an average diameter of 340–400 μm (Figure 14).[157]

Smaller microencapsulated islets, with an average diameter of 200 μm, were prepared by coating individual islets with emulsified sodium alginate and poly(ethylene glycol) and overlaying the coating layer with poly-L-ornithine and sodium alginate. This process resulted in a thin hydrogel film on single islet surface.[157] The resulting microencapsulated islets facilitated noninvasive transplantation but stimulated the inflammatory response.[157]

A multilayer coating of islets has been proposed to improve the structural integrity of the coating layer and to control the molecular cutoff of the coting layer.[158,159] One traditional method is to form a polyelectrolyte complex layer on microencapsulated islets through the sequential layer-by-layer deposition.[160,161] The polyelectrolyte coatings have opposite charges at physiological pH and are used to assemble a reinforced layer around islets.[160] For example, poly(allylamine hydrochloride) or poly(diallyl dimethylammonium chloride) was used to form the polycation layer, and poly-(styrenesulfonate) was used to form the polyanion layer. The resulting polyelectrolyte coating protected the islet cells from the host immune response, which was examined by the antibody recognition of encapsulated islets using fluorescently labeled antiglutamic acid decarboxylase (+) antibodies. The coated islets also retained their viability and insulin secretion properties, depending on the surface charge of the cell coating layer.[162] In particular, the polycationic layer induced adhesion and angiogenesis, as opposed to the externally polyanionic coated cells.[163] The thickness and coverage of the coating layer also influenced the viability and therapeutic activities of the coated cells due to the changes of permeability.[163]

Using polyelectrolyte polymers, the surface of islets was further engineered with a series of functional moieties that allowed coupling of other coating materials. For example, graft copolymers of poly-L-lysine and poly(ethylene glycol) were used to coat pancreatic islets.[164] The poly(ethylene glycol) block end group was biotin, hydrazide, or azide, thus allowing subsequent bioorthogonal ligation. To demonstrate the presence of the functional groups, the biotin, hydrazide, or azide groups were treated with, respectively, Cy3-labeled streptavidin, fluorescein-labeled alginate-aldehyde, and a cyclooctyne poly(ethylene glycol)–biotin conjugate. Notably, grafting shorter poly(ethylene glycol) blocks to the poly-L-lysine lowered the cytotoxicity.[164] Although the poly(ethylene glycol) layer showed better biocompatibility than poly-L-lysine with the encapsulated islet cells, the coating layer could not prevent fibrotic overgrowth.[165]

To improve the efficacy of islets transplanted to treat diabetes, the effect of the diameter of islet-encapsulating hydrogel spheres on the host immune response was studied. This study used the diabetic mice model induced by streptozotocin.[149] The effect of sphere diameter on biocompatibility was investigated by fabricating different sizes of Ba^{+}-cross-linked alginate hydrogel spheres.[149] Islets loaded in 1500 μm diameter alginate spheres could restore the normal glucose level in blood for up to 180 days. This period is 5 times longer than that obtained with islets encapsulated within 500 μm diameter alginate spheres.[149]

4.3.2. Cell Coating by Physisorption.—Coating islets with multiple layers of polymeric films increases the total volume of the transplanted cells. This limits the locations where cells can be transplanted. Therefore, a method that minimizes the total volume, but maintains the islet stealth properties, was developed. Protecting islets from immune response by using a hydrophobically modified poly(ethylene glycol) as a spacer and a primer for layer-by-layer deposition is one alternative approach that generates an ultrathin coating. In this approach, one end of the poly(ethylene glycol) is linked to a lipid or an alkyl chain.[166] The resulting amphiphilic poly(ethylene glycol) unit can readily intercalate into the phospholipid layer of the islets, as previously described. Using this method, the poly(ethylene glycol)-coated islet surface can be further functionalized with a series of molecules to control the immunogenic response (Figure 15). For instance, an amine-terminated poly(ethylene glycol)-conjugated phospholipid associates with the lipid bilayer of the cell membrane and produces an overall cationically charged surface. The charged surface, afforded by the terminal amine group, facilitates the formation of polyion complex on the islet surface via the layer-by-layer deposition process.[166] Sodium alginate and poly-L-lysine were the polyion polymers used to build the coating layer on the islet surface. The overall process limited the volumetric increase of the cells and did not impede the release of insulin.[166]

In a similar fashion, islets were also covered with poly(vinyl alcohol). In this approach, a maleimide-terminated poly(ethylene glycol)-conjugated phospholipid construct was used to anchor the template polymer coating. A thiol pendant group was attached to the poly(vinyl alcohol) backbone by using a diisocyanate disulfide linker to cross-link the poly(vinyl alcohol) via urethane bond formation and reducing the disulfide bond to yield a polymercapto-poly(vinyl alcohol). The polymercapto-poly(vinyl alcohol) was covalently attached to the terminal maleimide groups of the anchored poly(ethylene glycol).[167] The remaining free thiol groups could be used to repeat the coating process via disulfide bond exchange. The resulting multiple layers of polymercapto-poly(vinyl alcohol) activated with pyridyl disulfide generated an ultrathin coating with minimal effects on islet function.

In addition to reducing innate immune response, this islet-coating method can be used to stop coagulation and subsequent tissue inflammation. Coating the surface of islets with the fibrinolytic enzyme urokinase or heparin was used to minimize inflammation or coagulation, respectively. Thus, biotin-terminated poly(ethylene glycol)-conjugated phospholipids were immobilized on the cell membrane. Streptavidin was then used to layer multiple biotin-conjugated bovine serum albumin coatings.[168] On top of this coating layer, a heparin or a fibrinolytic enzyme urokinase coating layer was prepared via two distinct procedures. To prepare the heparin-coated islets, a polyion with protamine, an arginine-rich cationic peptide, was built upon the bovine serum albumin coating layer to yield an external coating of heparin. To prepare the urokinase-coated islets, oxidized dextran with aldehyde groups was placed on the bovine serum albuimin layer. The surface aldehyde groups undergo Schiff base formation with the surface amine residues of urokinase to yield the enzyme-coated islets.

An alternative method to coat islets with urokinase and bypass the need for the poly(ethylene glycol) primer group was developed. Thus, thiolated poly(vinyl alcohol)

derivatives substituted with alkyl chains were synthesized. The pendant alkyl chains facilitated the spontaneous anchoring of the poly(vinyl alcohol) derivative on the surface of the islets. The urokinase was conjugated to the sulfhydryl groups of poly(vinyl alcohol) via a thiol-Michael reaction with maleimide groups coupled to urokinase. The urokinase-coated islets exhibited reduced blood coagulation and suppressed inflammatory response (Figure 16).[168,169] The fibrin plate at 13 h after spotting with the urokinase–islets showed a significant dissolution around the urokinase–islets. In contrast, the untreated islets resulted in a minimally dissolved area (Figure 16).

4.3.3. Cell Coating with Living Cells.—In an effort to eliminate the host response and ultimately make transplanted islets bioinert, xenograft islets were encapsulated by the cells of the recipient.[170] Both islets and human embryonic kidney (HEK-293) cells were coated with biotin-terminated poly(ethylene glycol)-conjugated 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine. Clusters were assembled via biotin–streptavidin complexation at the interface of human embryonic kidney HEK-293 cells and islets.[170] The islets covered with HEK-293 cells did not exhibit any necrosis, but there was a slight reduction in insulin secretion compared with unencapsulated islets. The diminished insulin secretion was attributed to low insulin permeability through human embryonic kidney cell layer.[170]

4.4. Protecting against Reactive Oxygen Species

Coating mesenchymal stem cells transplanted to tissue damaged by cardiac infarction with graphene oxide flakes is an approach that has emerged to support cell adhesion in oxidative environments. Extracellular matrix proteins, physisorbed on the surface of graphene oxide flakes, bind cellular integrins and facilitate cell coating.[171] The nanoflakes reduce the number of dead cells and support the cellular engraftment to cardiac tissue by reducing anoikis of the transplanted cells mediated by reactive oxygen species.[172] This approach resulted in a 5-fold increase in the number of cells engrafted to defective tissue compared with uncoated cells.[172]

Reduced graphene oxide also adsorbs to mesenchymal stem cells and improves their therapeutic efficacy when treating myocardial infarction. Reduced graphene oxide works by stimulating paracrine factor secretion and gap junction protein expression of the cells.[121] The reduced graphene oxide anchors to cells similar to graphene oxide flakes. This anchoring interaction is mediated by electrostatic adsorption of extracellular matrix proteins that bind integrins of stem cells. The cells coated with reduced graphene oxide triggered a series of signaling cascades that are responsible for the secretion of paracrine factors.[173] In addition, the higher electrical conductivity of reduced graphene oxide compared to graphene oxide promotes cellular expression of gap junction proteins, including connexin 43.[174] As a result, mesenchymal stem cells modified with reduced graphene oxide attenuated cardiac remodeling and improved cardiac function.[121] Mesenchymal stem cells coated with these reduced graphene oxide nanoflakes could be repurposed to treat ischemic cutaneous wounds and limbs.

Recently, a microfluidic system has been developed to coat individual mesenchymal stem cells and preadipocyte cells with a thin (6 μm thickness) alginate gel layer. By doing so,

individual cells can be encapsulated in a thin gel capsule. This microfluidic process significantly improves the coating efficiency compared to conventional bulk-cell-coating techniques.[175] In this process, cells are first exposed to calcium carbonate nanoparticles, which are passively adsorbed onto the cell surface. Cells are then covered in alginate solution by passing through a cross-junction of the microfluidic device and submerged in acetic acid, which triggers the release of calcium from nanoparticles. The single coating layer significantly increased the residence time and secretion activities of the mesenchymal stem cells transplanted via intravenous injection. This process can also potentially improve cellular engraftment by mediating the effects of ROS on transplanted cells.

5. CONTROLLING CELLULAR SECRETION ACTIVITIES

Multiple cells, including mesenchymal stem cells, hematopoietic stem cells, macrophages, and T-cells, have tumoritropic properties defined as a tendency to home in on tumor sites following systemic cell injection.[176] Tumor-secreted stromal derived factor-1α stimulates the expression of cell surface chemokine receptor (CXCR)-4.[177–179] The resulting interaction between the stromal derived factor-1α and CXCR-4 activates extravasation of cells from the vasculature into the tissue. Therefore, cells have been employed as a carrier of drugs that can treat malignant diseases by the following three strategies: (1) attaching drug-encapsulating particles on the cell surface, termed “cellular hitchhiking”; (2) attaching particles loaded with coadjuvants that can regulate endogenous cellular secretion activities onto cells; and (3) genetic engineering of cells to secrete exogenous protein molecules. This section discusses chemical technologies that modify the cell surface with particles that release therapeutic drugs and coadjuvant drugs or with synthetic receptors.

5.1. Nanoparticles with Drug Release

Developing methods for the attachment of nanosized particles on the surface of cells can provide a way to prolong the circulation and target the delivery of therapeutics. Although red blood cells have been developed as a delivery system for drug-releasing polymeric nanoparticles because of their bioavailability and biocompatibility, over time shear force and cell–cell interaction eventually separate particles from the cells and then the particles are cleared through the liver and spleen. Also, unlike other cells, red blood cells are not tumoritropic nor do they possess other homing properties.[180,181] In addition, the incubation of cells attached to a substrate with nanoparticles often results in the uncontrolled internalization of the nanoparticles.[182]

On the basis of the issues described above, one approach for surface localization is to first couple biotin to the cell membrane chemically via an amide coupling reaction with sulfosuccinimidyl 6-(biotinamido)hexanoate. Polystyrene nanoparticles with 40 nm diameter prepared with a NeutrAvidin coating, deglycosylated native avidin from egg whites, adhere to biotin-coated cells (Figure 17).[183] According to the in vitro studies, the nanoparticles attached to the mesenchymal stem cell membrane did not significantly affect the cellular tumoritrophic function.[183]

To control the transport of polymeric nanoparticles, noncovalent attachment to red blood cells was performed for “cellular hitchhiking” applications.[181,184] Carboxylated polystyrene

particles associate with the red blood cell surface noncovalently through hydrophobic and weak electrostatic interactions. Multiple improvements can be envisioned for the traditional cellular hitchhiking method. One approach is to use nonspherical polystyrene particles. Another is to coat polystyrene particles with intercellular adhesion molecule-1 antibodies. Changing the shape of the particle enhances stealth properties, whereas coating the particles with antibodies improves their cell adhesion and targeting properties. Control spherical noncoated particles were attached to the red blood cell surface via simple incubation. As a result of the weak noncovalent attachment, nanoparticles were physically separated from red blood cells when the cells pass through capillaries with a diameter of 5 μm. The nonspherical and intercellular nanoparticles conjugated to cell adhesion molecule-1 exhibited up to a 9-fold higher level of accumulation in the lung than unmodified polystyrene nanoparticles. Also, the number of nanoparticles accumulated in liver and spleen through the reticuloendothelium was reduced more than 2-fold. The results of this proof of concept study can be applied to clinically relevant disease using biocompatible nanoparticles that can discharge encapsulated drug molecules at controlled rates, instead of polystyrene nanoparticles.

5.2. Combining Adjuvants with Nanoparticle Drug Release

Adjuvant-releasing nanoparticles chemically coupled to T-cells and hematopoietic stem cells can regulate cellular phenotypic and therapeutic activities. For example, liposomes or multilamellar lipid-coated poly(lactide-*co*-glycolic acid) nanoparticles with an average diameter of 100–300 nm were loaded with interleukin-15SA and interleukin-21 via in situ film rehydration and subsequent extrusion. The lipid layer of the liposome was decorated with maleimide groups. Liposomes with maleimide groups were immobilized on T-cells via thiol-maleimide coupling with thiol groups on the cell surface (Figure 18a). According to an in vivo study conducted with a mouse cancer model, the resulting T-cells proliferated and disappeared gradually. As such, T-cells suppressed the tumor growth successfully and, in turn, increased survival rates (Figure 18b).[185] Unmodified T-cells disappeared quickly. In particular, IL-15SA- and IL-21-releasing liposomes elicited 81-fold higher proliferation of T-cells than unmodified ones. During the subsequent contraction period, T-cells coupled with IL-15Sa- and IL-21-laden nanoparticles showed 15-fold higher persistence than unmodified T-cells after infusion.

Separately, the glycogen synthase kinase (GSK)-3β inhibitor-releasing nanoparticles were immobilized on the surface of hematopoietic stem cell via a similar thiol-Michael coupling method to enhance the repopulation kinetics of HSCs.[185] These effects were insignificant when adjuvant drugs were delivered systemically.[185]

Incorporating thiol groups to the glycocalyx of cells can be accomplished by metabolic oligosaccharide engineering. As previously discussed (section 3.1.1), ManNAc analogs are well-tolerated by the sialic acid biosynthetic pathway and allow an array of functionalities to be displayed. Although thiol groups are found on the surface of cells and can be targeted as seen in the previous example, most cell surface protein thiol groups are inaccessible. Incubation of cells with 1,3,4,6-tetra-*O*-acetyl-2-acetylthioacetamido-2-deoxy-α-D-mannopyranose stimulated cells to express *N*-thioglycolylneuraminic acid (Figure 19a).[186]

Thiols on the cell surface significantly stimulated cells to self-aggregate into clusters.[186] Having accessible thiol groups provides another reactive nucleophile that can be exploited for cell surface modification.

The mesenchymal stem cell surface can also be modified with polyelectrolyte complex layers consisting of poly-L-lysine and hyaluronic acid, giving surface groups that are bioactive and able to stimulate cellular secretion activity. In the layer-by-layer deposition process, the coating layer can be increased up to 6 nm after 10 layers. The thin layer can maintain permeability sufficient for nutrient and cellular waste transport.[158] Depending on whether the outermost layer is poly-L-lysine or hyaluronic acid, the surface charge could be tuned to regulate the interaction between cells and extracellular matrix proteins. Moreover, the alternating positively and negatively charged layers would allow the inclusion of biomolecules and drugs that can potentially control cellular secretion activities between the layers, although such applications have not been reported yet.[158]

5.3. Using Synthetic Receptors

Viral-mediated gene transfer has been widely used to control exogenous gene expression and subsequent biomolecular secretion of mammalian cells. Viruses are “living” vehicles that can carry foreign genes.[187] Viruses enter cells following the specific binding to cellular receptors, such as the low-density lipoprotein receptor and dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin.[188] However, the lack of these cell surface receptors limits the range of viral-mediated gene transfer. To resolve this challenge, a controlled number of biotin groups that can function as adenovirus receptors were added to the cell surface. In this process, cells were first metabolically functionalized with ketone-bearing sialic acid analogs using *N*-levulinoylmannosamine, a modified mannosamine (Figure 19b). By doing so, the unnatural ketone-bearing sialic acids were conjugated with a biotin-hydrazine reagent via hydrazone formation to the cell surface (Figure 19c).[189] The construction of this artificial adenovirus receptor on the cell membrane enhanced the transfer and expression of the genes by approximately 50-fold.[189] This method will also be useful in improving endocytosis of artificial viruses carrying the nonviral genes.[190]

Efforts were also made to facilitate endocytosis of proteins for fundamental and applied biological studies. Toward this end, artificial cell surface receptors conjugated to *N*-alkyl-3β-cholesterylamine were incorporated into cell membranes to facilitate cellular internalization of molecules that do not ordinarily penetrate the cell membrane. The *N*-alkyl-3β-cholesterylamine was coupled to a nitrilotriacetic acid moiety that can chelate metals. The construct was immobilized on the surface of Jurkat lymphocytes by the cholesterol units inserting into the phospholipids of the cell membrane. The Ni^{2+} nitrilotriacetic acid complex was reported to act as a synthetic cell surface receptor, binding oligohistidines proteins on the cell surface and facilitated their endocytosis.[191,192]

Similarly, efforts were made to present synthetic Fc receptors on the cell surface and, in turn, facilitate the entry of ligands. Leukocytes and epithelial cells expressed Fc receptors on their surfaces. The Fc receptors bound and internalized antibodies through receptor-mediated endocytosis. The synthetic Fc receptor was synthesized by coupling *N*-alkyl-3β-amino-5α-cholestane to a disulfide-constrained cyclic peptide.[193] This process enabled the

incorporation of 6.2×10^5 synthetic Fc receptors on the surface of cells that lacked Fc receptors, such as Jurkat lymphocytes. In turn, these receptors significantly enhanced the cellular uptake of human immunoglobulin G.[193] This method can allow cells to uptake and destroy the immunoglobulin G overproduced in the autoimmune diseases through the late endosome and lysosome. Moreover, the artificial Fc receptor may serve to deliver impermeable therapeutic molecules to intracellular targets.

6. IN SITU FORMATION OF CELL CLUSTERS

Cells are often transplanted in a clustered form, for example, as a sheet and spheroids.[121] These clustered cells can promote cadherin-mediated cell–cell adhesion, which plays an important role in regulating growth and phenotypic activities of cells.[194,195] Inducing aggregation between different cell populations allows formation and cotransplantation of mixed cell clusters. Following transplantation, these cell clusters can modulate therapeutic activities of host cells by secreting paracrine-signaling factors and also develop new tissue with a morphological and physiological resemblance to the native cells. Toward this end, this section will discuss some emerging efforts designed to control cell cluster formation by immobilizing cell-assembly biomolecules, such as single-stranded DNAs (DNAs) and growth-factor-releasing particles. To date, the resulting cell clusters have been mostly analyzed in vitro, but these approaches present promising techniques for rapid use in clinical transplantation.

6.1. Chemically Induced Cell Clustering

It is possible to form cell clusters by chemically altering the surface of cells.[196] One approach is to chemically oxidize native sialic acid residues to yield non-native aldehyde groups using a mild sodium periodate solution (Figure 20).[197–199] Generating aldehyde groups enables a series of bioorthogonal chemical reactions for cell surface conjugation.[197] In particular, hydrazide linkers were used to cluster hepatocarcinoma cells chemically treated to present aldehyde groups. By flowing the linkers and the cells in microfluidic channels, cells were cross-linked to form clusters.[196] Compared to cells cultured in a monolayer, the resulting cell clusters displayed enhanced albumin production and uridine 5′-diphospho-glucuronyltransferase activity, each of which represents the synthetic and metabolic activity of the cells, respectively.[196] One drawback to this method is the possible uncontrolled reaction of the generated aldehydes with amines on the cell surface.[200]

This concern was addressed by using metabolic labeling to generate ketone groups on the cell surface. The cells were treated with *N*-leveulinoylmannosamine, a ketone-bearing analog of *N*-acetylmannosamine (section 3.1.1). The *N*-leveulinoylmannosamine is metabolized to *N*-levulinoylsialic acid.[201] The terminal ketone group in *N*-levulinoylsialic acid undergoes condensation reactions with aminooxy and hydrazide groups to form oxime and hydrazine products, respectively.[202]

As indicated, ketone groups on the cell surface enabled selective conjugation of peptides, oligosaccharides, and organic molecules that can further regulate the cadherin-mediated cell–cell interactions as well as cell–matrix interactions.[201] Therefore, this cell surface engineering may be useful to organize different cell populations in a cluster. However, the

N-leveulinoylmannosamine-induced metabolic engineering of cells for ketone ligation reactions may lead to competition with endogenously expressed keto-metabolites.[203]

6.2. DNA-Directed Cell Assembly

The hybridization of two complementary strands of DNA is a selective and high-affinity process. Functionalizing the surface of cells with DNA strands can afford highly selective cellular self-assembly. Multiple methodologies exist to modify the cell surface with DNA strands. One such approach employs metabolic oligosaccharide engineering to express azidosialic acid (SiaNAz) on cells using a *N*-azidoacetylmannosamine (ManNAz) analog. Staudinger ligation, a highly selective and bioorthogonal reaction described earlier, was used to covalently tether DNA strands to the surface of cells (Figure 21).[203] The single-stranded DNA with a terminal amine reacted with the triarylphosphine-pentafluorophenyl to synthesize triarylphosphine-bearing single-stranded DNA.[204] The azidosialic acid on the cells readily reacted with the triarylphosphine to form amide-bonded DNA-coated cells under physiological conditions.

Cells conjugated with single-stranded DNA molecules were further used to regulate spatial organization of cells through DNA hybridization.[204] In this process, mixing Jurkat lymphocytes engineered to express complementary single-stranded DNAs could readily form clusters, whereas cells without single-stranded DNA or noncomplementary DNAs showed limited cell–cell interactions (Figure 21).[205,206] This cell surface engineering technique provides a means for examining cross-talk between same or disparate cells in a 3D environment and subsequent cellular activities to develop new tissues and organs.[206]

The DNA molecules used to assemble cell clusters can also be covalently attached directly to the cell membrane by amide-coupling chemistry.[207] Using a heterobifunctional maleimidepoly(ethylene glycol)-*N*-hydroxysuccinimide linker, thiol-ended single-stranded DNAs were functionalized with a succinimidyl ester at their termini. The resulting *N*-hydroxysuccinimide-conjugated DNA was immobilized on the cell surface through amide bond formation with surface amine groups.[207] The cells modified by this method were used to direct cells to adhere to the predefined area of a substrate where the compliment DNAs were coupled.[207] This approach can be readily utilized for the spatial control of the same or different cell populations.[208] Furthermore, it would be interesting to examine how these DNA bridges between cells influence the cadherin-mediated cell–cell adhesion and intracellular signaling involved in secretion and differentiation of cells.

There are also noncovalent methods that have been developed to coat cells with DNA. DNAs conjugated with various types of lipids that can intercalate into the cell membrane have been prepared (Figure 22a).[209,210] These lipid-conjugated DNA derivatives were prepared by coupling solid-phase-synthesized hydroxyl 5′-ended DNA with dialkylglycerol phosphoramidites, cleaving from a solid support, and being deprotected. By placing the complementary single-stranded DNA in a desired microsized pattern, cells could be induced to form clusters on the substrate (Figure 22c).[209] The resulting cell clusters could then be detached from the substrate using deoxyribonuclease.[209] This method enables the formation of cell clusters with controlled size, shape, and spatial organization of different cell populations.[209]

6.3. Microparticle-Driven Cell Clustering

Coating stem cell surfaces with bioactive inorganic or polymeric microparticles during cell cluster formation has allowed cell differentiation and morphogenesis to be regulated.[211–214] For example, calcium phosphate-coated hydroxyapatite particles within pluripotent embryonic stem cell clusters enhanced differentiation of cells to cartilage-forming chondrocytes and bone-forming osteoblasts, termed osteochondrogenic differentiation. Gelatin methacrylate microparticles within embryonic stem cell clusters stimulated cellular expression of matrix metalloproteinase and promoted mesenchymal morphogenesis of the cell clusters.[215] Retinoic acid-releasing poly(lactic-*co*-glycolic acid) microparticles within embryonic stem cell clusters stimulated cavitation of the cell clusters. These results substantiate that the introduction of bioactive particles within cell clusters is advantageous for modulating phenotypic activities of cells in the cell cluster core by circumventing diffusion-limited conditions.

Similarly, gelatin microspheres loaded with growth factors could stimulate the differentiation of adipose-derived mesenchymal stem cells assembled in the form of sheets. Particles loaded with transforming growth factor-β1 increased the chondrogenic differentiation level of mesenchymal stem cells and, in turn, reproduced the cartilage-tissue-like constructs. Also, cell aggregates laden with gelatin microspheres releasing transforming growth factor-β1 and hydroxyapatite particles releasing bone morphogenic protein-2 enhanced both chondrogenesis and osteogenesis. These cell aggregates could promote vascularized bone regeneration in rat critical-sized calvarial defects via endochondral ossification.[216]

The role of these bioactive particles within cell clusters is mediated by the size. Therefore, it would be important to optimize the size and the mechanism by which particles stably anchor to the cell surface.

Also, microparticles derived from platelets can bind neutrophils and induce cell clustering.[217] In turn, these particles generated a higher percentage of CD41a-positive neutrophils than the platelets. The resulting neutrophil clusters presented an increased phagocytic activity.[217] This finding may serve to develop an advanced synthetic microparticle that can regulate activation of immune cells.

7. CONCLUSION AND OUTLOOK

In summary, cell surface engineering technologies have shown a strong potential to significantly improve the therapeutic efficacy of cells by controlling transport, tracking, engraftment, secretion, and cluster assembly of transplanted cells. These cell surface modifications were achieved by chemical or physical immobilization of a series of biomolecules and using nano- and microparticles. Additionally, a series of technologies developed to functionalize drug-carrying nanoparticles, bioimaging probes, or “artificial” cells can be translated into cell surface functionalization.[33–37,192,218–224] Although the efficacy of surface engineered cells was examined mostly by in vitro and in vivo preclinical studies, these studies serve to expedite the clinical use of cells for treatments of various diseases and tissue defects that are not possible with current therapeutic procedures.

In parallel, there should be additional studies to address multiple challenges. First, it is necessary to systematically study and reduce any negative impacts of cell-surface-loaded materials and underlying processes on cellular function involved in therapeutic activities. For instance, the abnormal osmotic pressure,[225] ionic strength,[226] pH,[227] serum concentration,[228] temperature,[229] and shear stress[230] are factors that can vary during the in situ cell surface engineering process and significantly influence cellular viability, secretion, and phenotypic activities. Second, cells may lose functional molecules, imaging probes, or particles during the intravascular transport, particularly through capillaries, because of the undulation of the cell membrane.[231] Conversely, cells used for exogenous drug delivery may have to discharge drug carriers immobilized on their surfaces at a desired location. It is vital to develop methods to ensure that limited cellular uptake of molecules and particles tethered to the cell surfaces occurs. Therefore, the strength, distance, and sensitivity of bonds between molecules or particles and the cell surface should be regulated in a more sophisticated manner. Finally, the coupling of cell surface engineering technologies with the proper biochemical technologies developed to regulate cellular antigen expression, secretion, and death would successfully take the quality of cell therapies to the next level.

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ABBREVIATIONS

18F-HFB	hexadecyl-4-[^{18}F]fluorobenzoate
3D	three-dimensional
ADSCs	adipose-derived stem cells
CD	cluster of differentiation
CXCR	cell surface chemokine receptor

DBCO-Cy5	dibenzylcyclooctyne-conjugated Cy5
DCM	dichloromethane
DHTR	delayed hemolytic transfusion reaction
DIAD	diisopropyl azodicarboxylate
DIPEA	*N,N*-diisopropylethylamine
DMF	dimethylformamide
DNAs	deoxyribonucleic acids
DPPE	1,2-dipalmitosyl-*sn*-glycero-3-phosphoethanol-amine
DTPA	diethylenetriaminepentaacetic acid
EDC	1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide
EGFR	epithelial growth factor receptor
ESC	embryonic stem cell
ETT	5-(ethylthio)-1*H*-tetrazole
FDG	fluorodeoxy-D-glucose
FSL	function–spacer–lipid
GSK	glycogen synthase kinase
HEK	human embryonic kidney
HER2	human epidermal growth factor receptor 2
HLA	human leukocyte antigen
HOBt	hydroxybenzotriazole
HPG	hyperbranched polyglycerol
HSC	hematopoietic stem cell
ICAM	intercellular cell adhesion molecules
IgG	immunoglobulins G
IL	interleukin
ManLev	*N*-levulinoylmannosamine
ManNAc	*N*-acetylmannosamine
ManNAz	*N*-azidoacetylmannosamine
MSCs	mesenchymal stem cells

Neu5TGc	*N*-thioglycolylneuraminic acid
NHS	*N*-hydroxysuccinimide
NOD	nonobese diabetic
PEG	poly(ethylene glycol)
PFP	pentafluorophenol
PLGA	poly(lactide-*co*-glycolic acid)
PLO	poly-L-ornithine
PPh_3	triphenylphosphine
PS	polystyrene
PVA	poly(vinyl alcohol)
RBCs	red blood cells
SDF	stromal derived factor
SiaLev	*N*-levulinoylsialic acid
SiaNAz	azidosialic acid
SLeX	sialyl Lewis X
SPIONs	superparamagnetic iron oxide nanoparticles
STZ	streptozotocin sulfo-NHS-LC-biotin sulfosuccini-midyl-6-[biotin-amido]hexanoate
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TEA	triethylamine
TGA	thioglycolic acid
TNF	tumor necrosis factor
UDP	uridine 5′-diphospho
UGT	glucuronyltransferase
VCAM	vascular cell adhesion molecules

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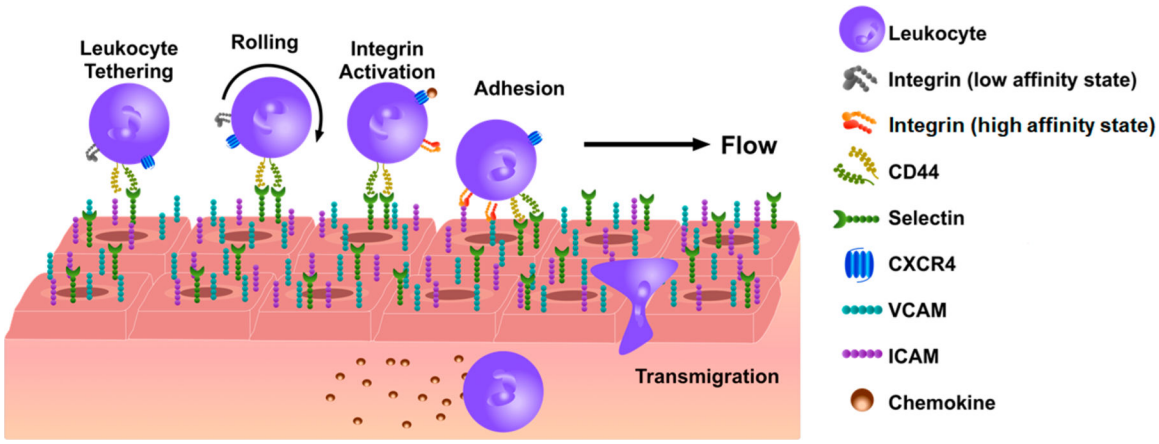


Figure 1.

Schematic illustration of the leukocyte transmigration process. In leukocyte transmigration, a series of surface receptor molecules work in concert to bind target tissue and signal adhesion and extravasation. The process involves multiple steps, including, tethering, rolling, integrin activation, adhesion, and transmigration. In leukocyte tethering, a cluster of differentiation (CD44) receptors bind selectin and induce rolling behavior. Chemokines bind C-X-C chemokine receptor type 4 (CXCR4) receptors of leukocytes to promote cellular adhesion. In the adhesion step, cellular receptors bind to various cellular adhesion molecules, including vascular cell adhesion molecules (VCAM) and intercellular cell adhesion molecules (ICAM), and finally lead to endothelial transmigration.

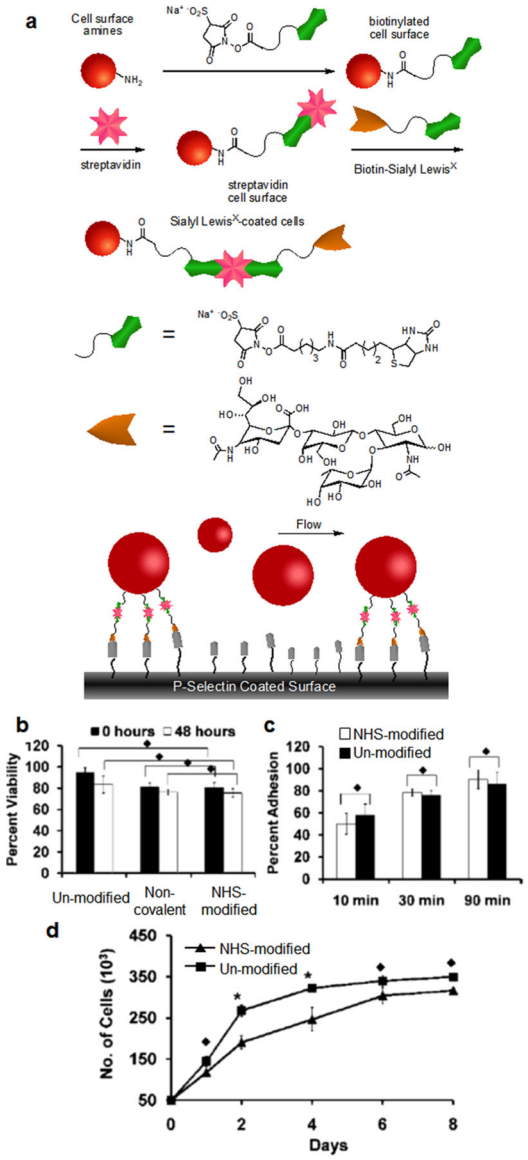


Figure 2.

(a) Schematic of SLeX cell surface coating process and coated cells interacting with P-selectin-coated substrate and noncoated cells flowing pass a functionalized surface. (b) Viability of the NHS-modified MSCs immediately after modification (0 h) and after 48 h. (c) Adherence of BNHS-modified cells measured at 10, 30, and 90 min compared to the PBS-treated cells. (d) Proliferation of the NHS-modified cells over an 8-day period compared to unmodified cells. Error bars denote the standard deviation from three separate experiments. Reprinted with permission from ref 44. Copyright 2008 American Chemical Society.

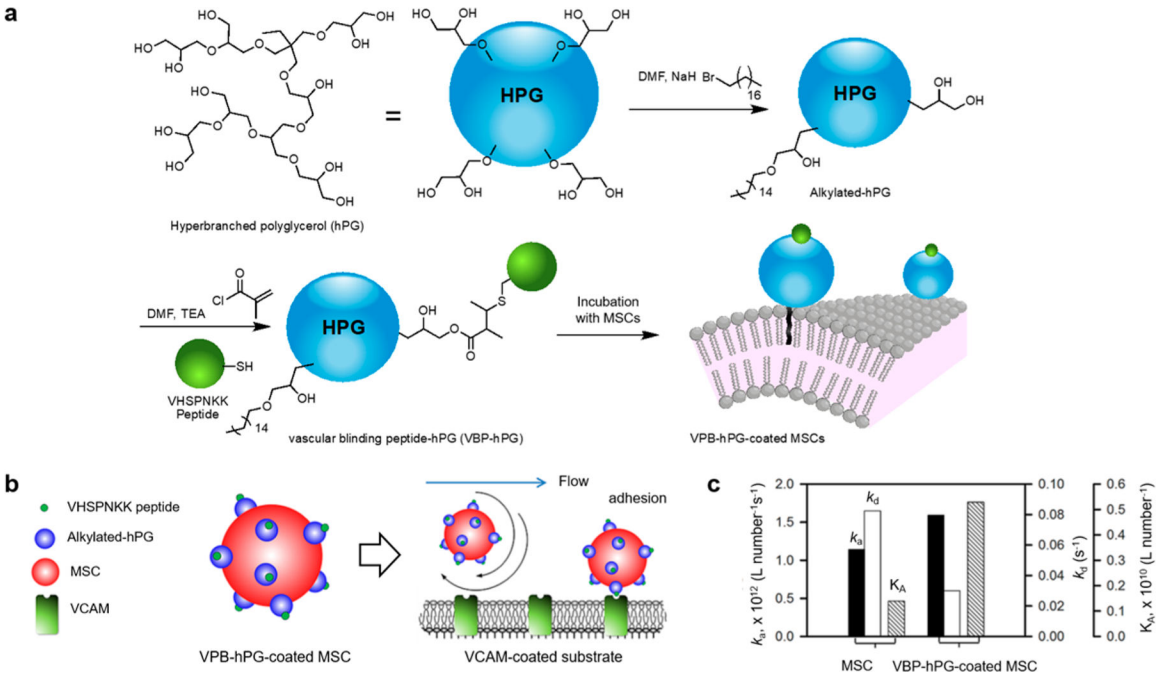


Figure 3.

(a) Schematic of the conjugation of vascular binding peptide (VBP) and hyperbranched polyglycerol (HPG) and the coating of mesenchymal stem cells (MSCs) with VBP–HPG. VBP has the sequence VHSPNKK. (b) Schematic of the surface plasmon resonance analysis to characterize adhesion of MSCs to a substrate coated with a target vascular cell adhesion molecule. (c) Association rate constant (k_a), dissociation rate constant (k_d), and affinity constant (K_A) of unmodified MSCs and VBP–HPG-coated MSCs, as determined from surface plasmon resonance response curves. Reprinted with permission from ref 51. Copyright 2013 American Chemical Society.

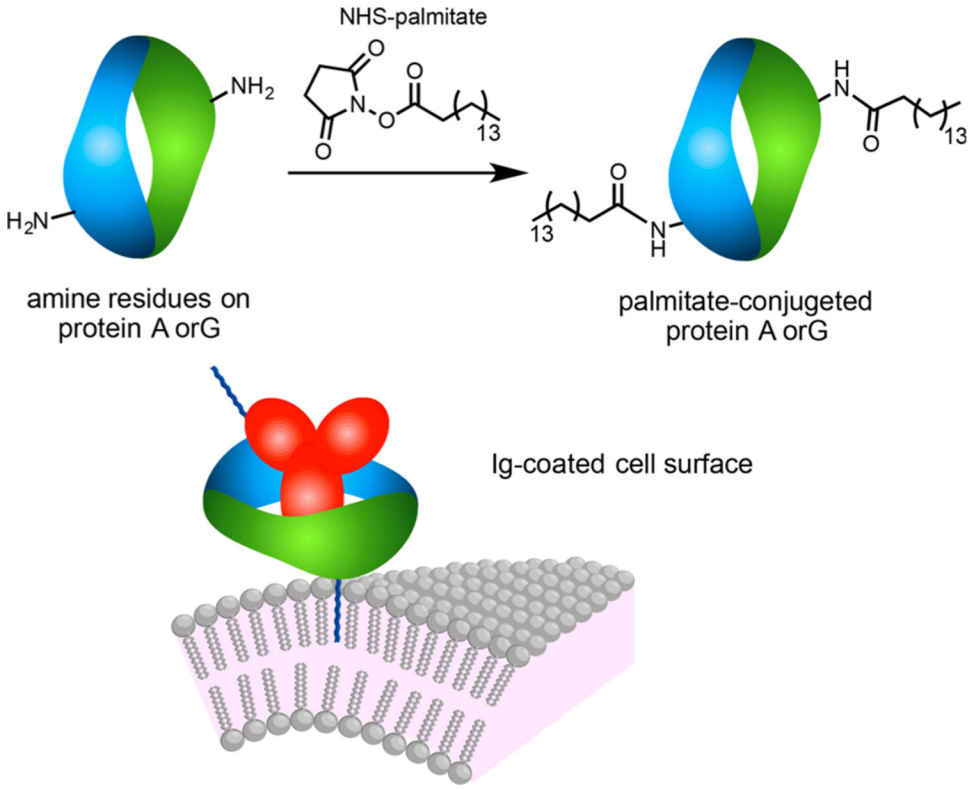


Figure 4.
Surface alkylation of protein A or G for membrane intercalation. Immobilization of immunoglobulin (Ig) by protein A or G binding.

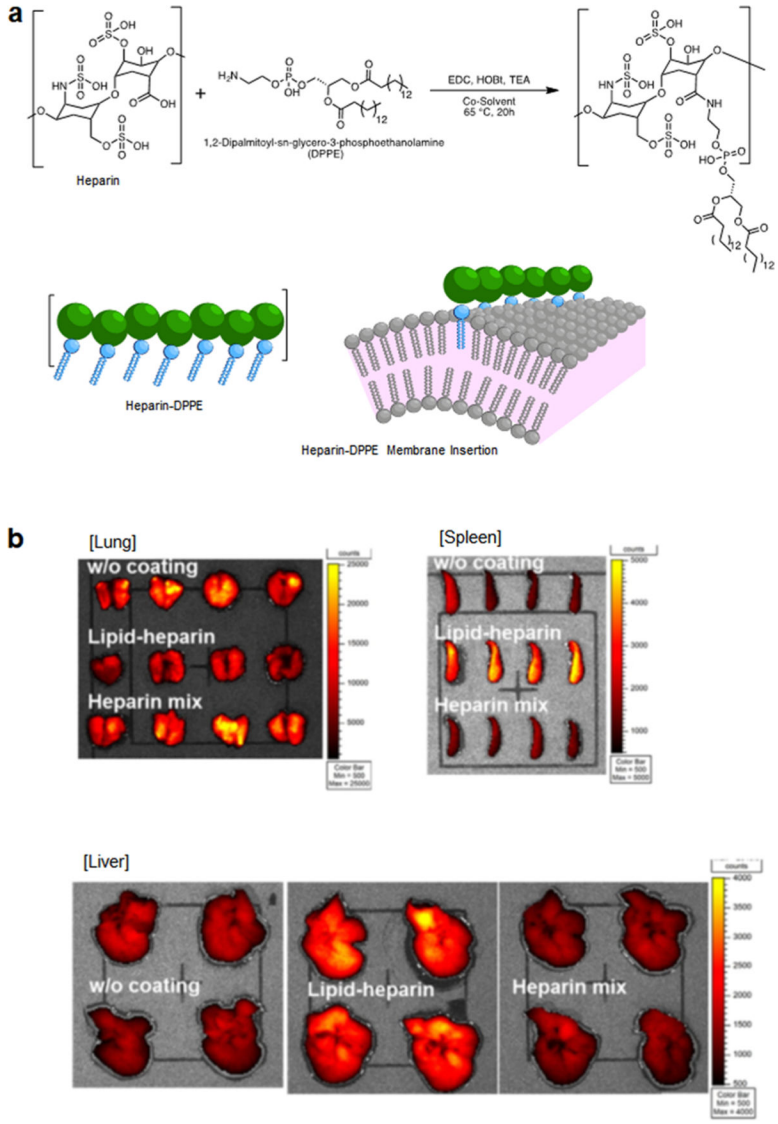


Figure 5.

(a) Amide-coupling conjugation of phospholipids to heparin for membrane anchoring and cell coating. (b) Biodistribution of adipose-derived stem cells in major organs of mice at 1 day after cell injection. Labeled ADSCs with or without heparin coating were injected intravenously in mice ($n = 4$). Fluorescence intensities of ADSCs in major organs were measured by an in vivo imaging system. Reprinted with permission from ref 61. Copyright 2015 Elsevier.

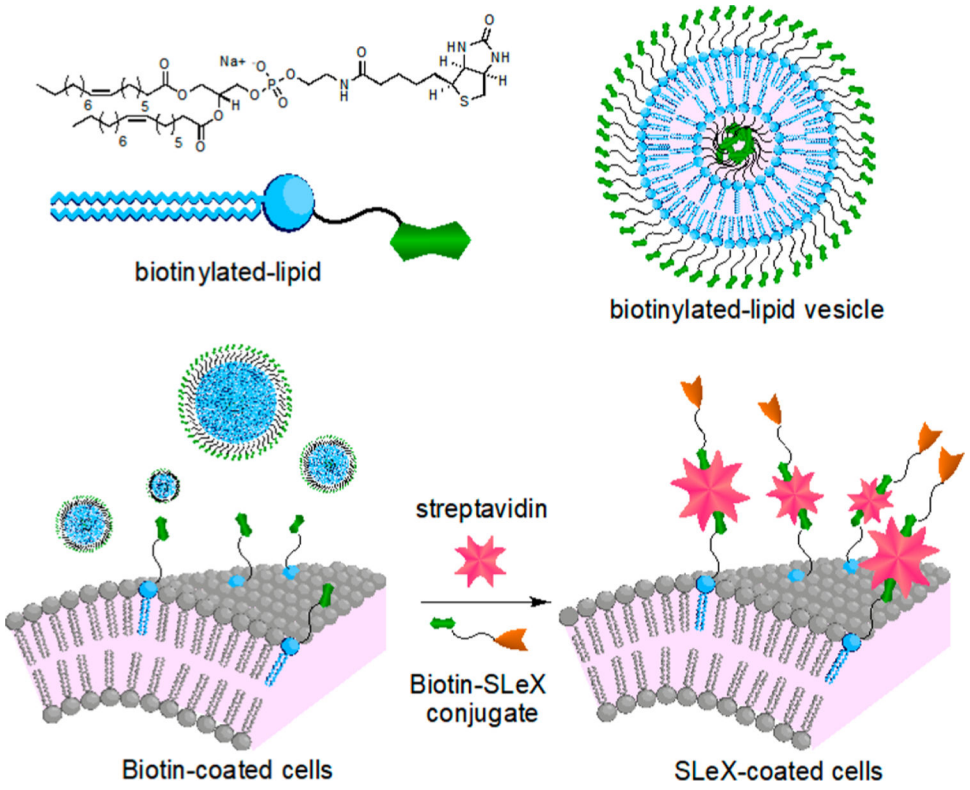


Figure 6.

Biotin-conjugated phospholipid vesicles used to biotin-coat cells for streptavidin-mediated sialyl Lewis X (SLeX) coating.

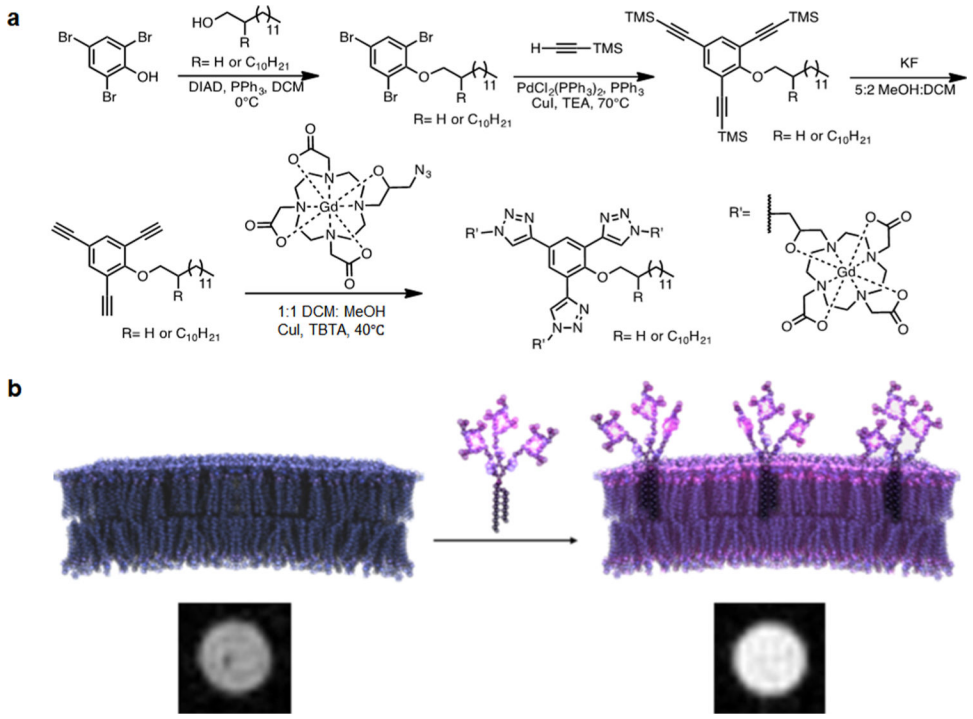


Figure 7.

(a) Synthesis of cell-coating trivalent 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)–gadolinium(III) complex tracking agent. (b) Schematic of trivalent DOTA–Gd(III) complex coating a cell surface and following enhancement of the T_1-weighted magnetic resonance imaging result. Reprinted with permission from ref 76. Copyright 2014 American Chemical Society.

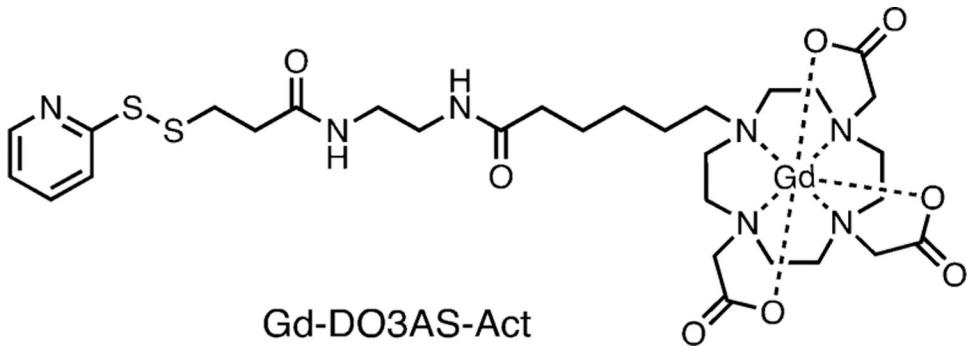


Figure 8.
Disulfide exchanging DOTA–gadolinium (GdIII) complex used for cell surface coating and tracking of implanted cells.

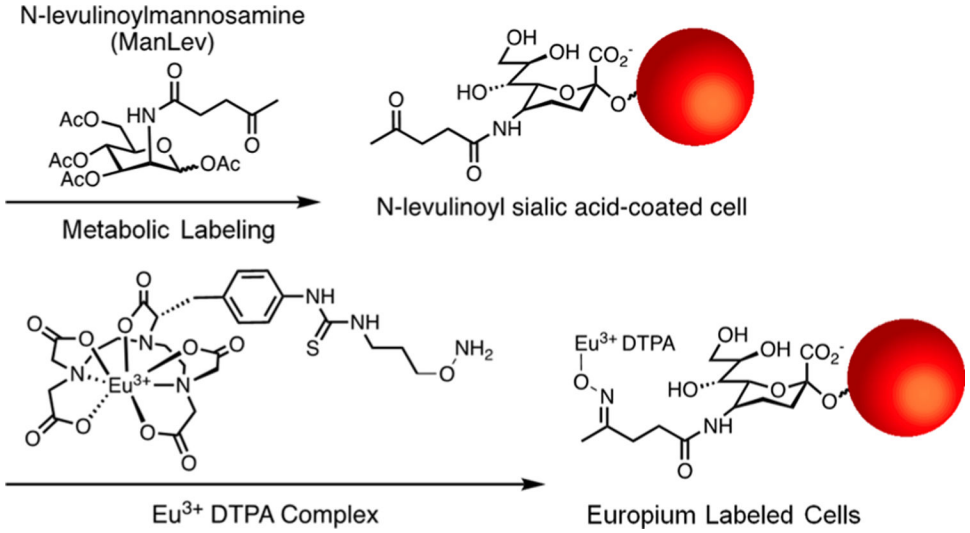
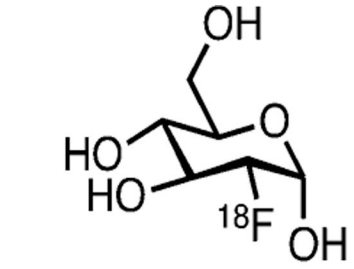
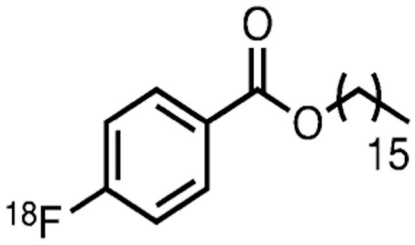


Figure 9.

Metabolic incorporation of *N*-levulinoylmannosamine (ManLev) ketone sialic acid analog and subsequent oximine linkage of a Eu(III) complex for cell labeling.



^{18}F-fluoro-deoxy-D-glucose (^{18}F-FDG)



hexadecyl-4-[^{18}F]fluorobenzoate (^{18}F-HFB)

Figure 10.

^{18}F-radionuclide-based radiolabeling agents, including ^{18}F-fluorodeoxy-D-glucose (^{18}F-FDG) and hexadecyl 4-[^{18}F]fluorobenzoate (^{18}F-HFB). ^{18}F-HFB is designed to intercalate into cell surface membrane.

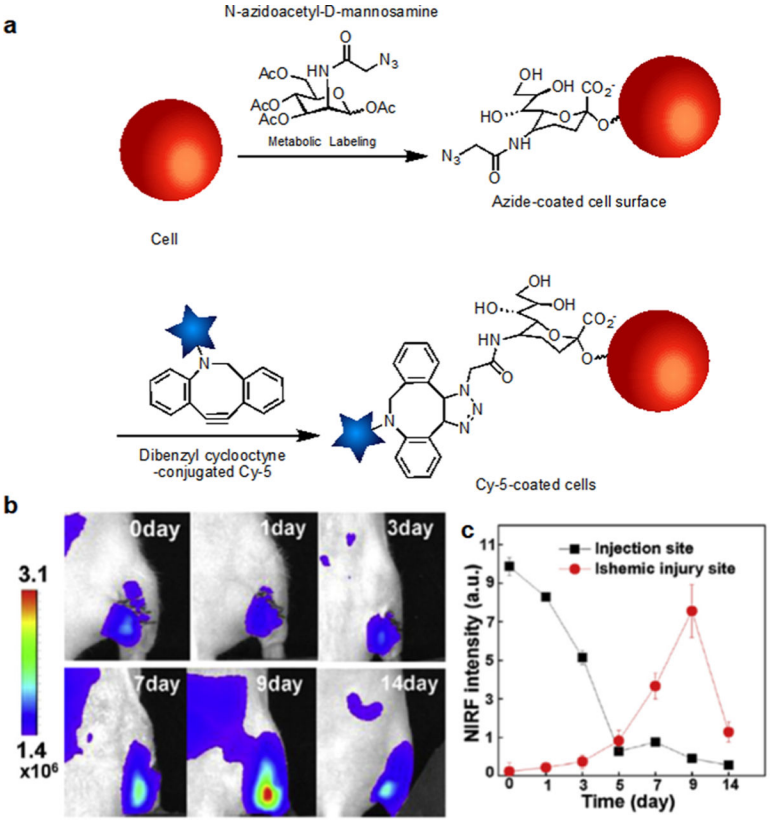


Figure 11.

(a) Fluorescent dye labeling of cell surface with metabolically engineered azido-sialic acid incorporation and strain-promoted click. (b) In vivo imaging system that captures near-infrared fluorescence (NIRF) of the Cy5-modified human adipose-derived mesenchymal stem cells in the ischemic hindlimb. (c) Quantitative analysis of the fluorescence from cells in the injection site and those in the ischemic injury site. Reprinted with permission from ref 111. Copyright 2016 Elsevier.

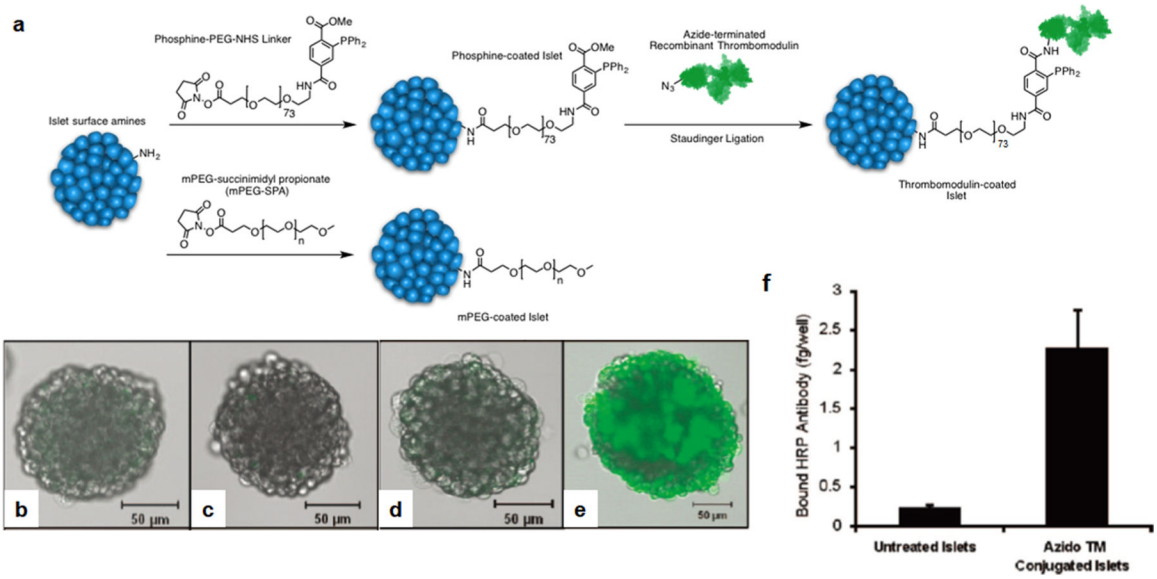


Figure 12.

(a) Schematic of islet coating techniques using surface amines. Thrombomodulin was conjugate to islet surface by *N*-hydroxysuccinimide (NHS) amide coupling followed by a Staudinger ligation (top) and poly(ethylene glycol) (PEG) coating (bottom). Confocal images (10×) after staining with the antibody of (b) native mouse islets, (c) PEG-linker-treated islet, (d) azidothrombomodulin-treated islet, and (e) both PEG-linker-and azido-thrombmodulin-treated islet. (f) Measurement of the islet-bound human thrombomodulin. Reproduced from ref 133. Copyright 2007 American Chemical Society.

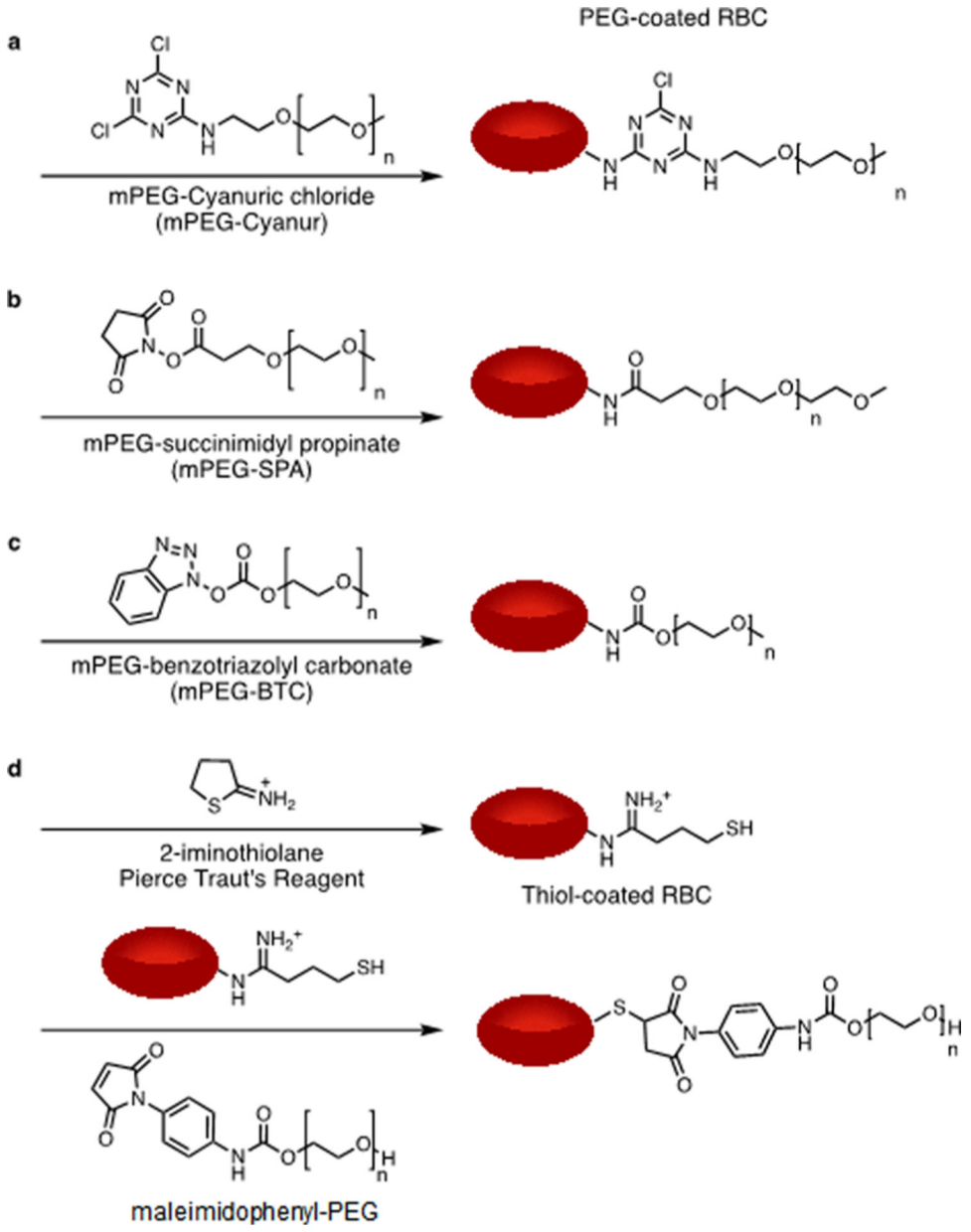


Figure 13.

Schematic of a series of techniques for poly(ethylene glycol) (PEG) coating of red blood cells (RBCs) by reacting with RBC surface amines: (a) cyanuric chloride activated PEGylation, (b) *N*-hydroxysuccinimide (NHS) amide coupling, (c) benzotriazolyl carbonate amide coupling, and (d) Pierce Traut's reagent thiol generation followed by a thiol-Michael reaction with maleimide.

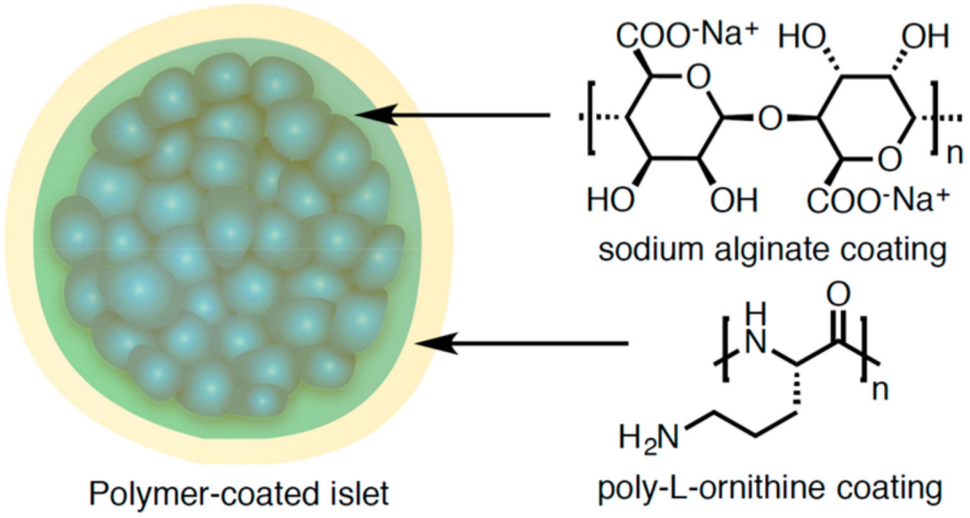


Figure 14.

Schematic of polymer-coated islets showing the inner sodium alginate layer (green) and exterior poly-L-ornithine layer (yellow).

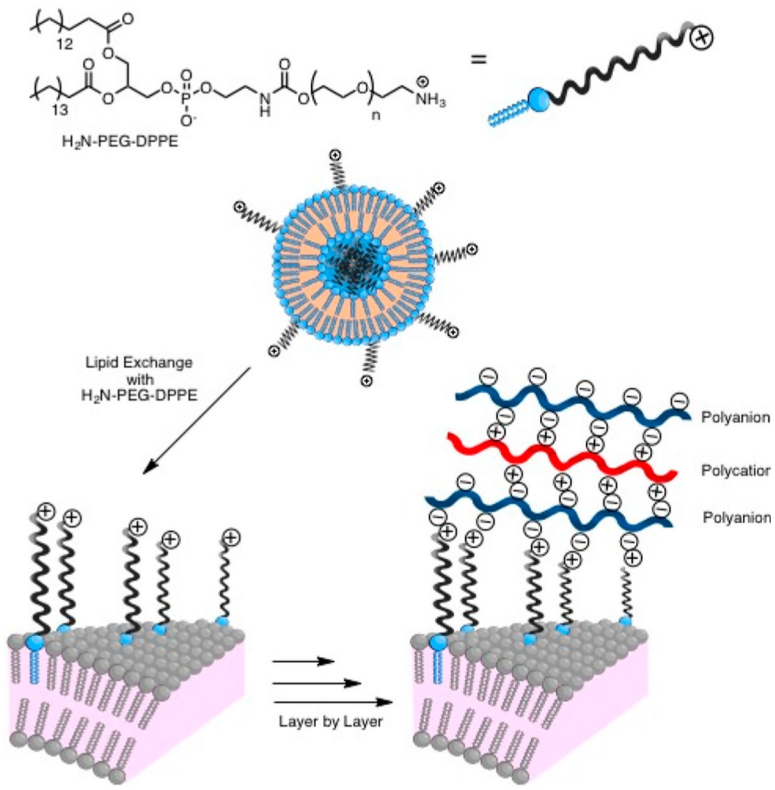


Figure 15.

Schematic of ultrathin layer-by-layer deposition of polyionic polymer coating template by amine-terminated amphiphilic poly(ethylene glycol) (PEG).

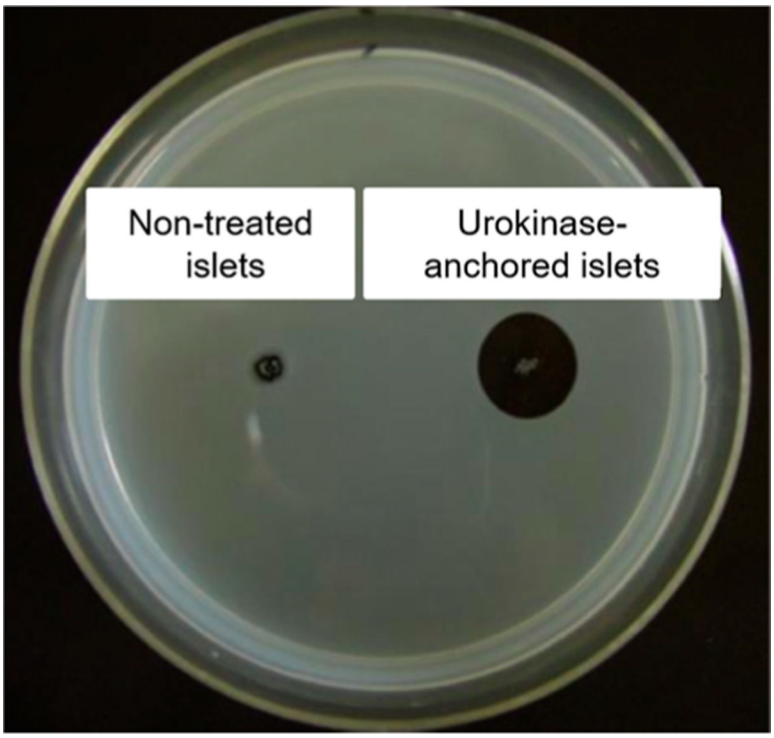


Figure 16.
Fibrin plate assay of nontreated (left) and urokinase-anchored (right) islets placed on a fibrin gel plate and incubated at 37 °C for 13 h. Reprinted with permission from ref 166. Copyright 2006 Elsevier.

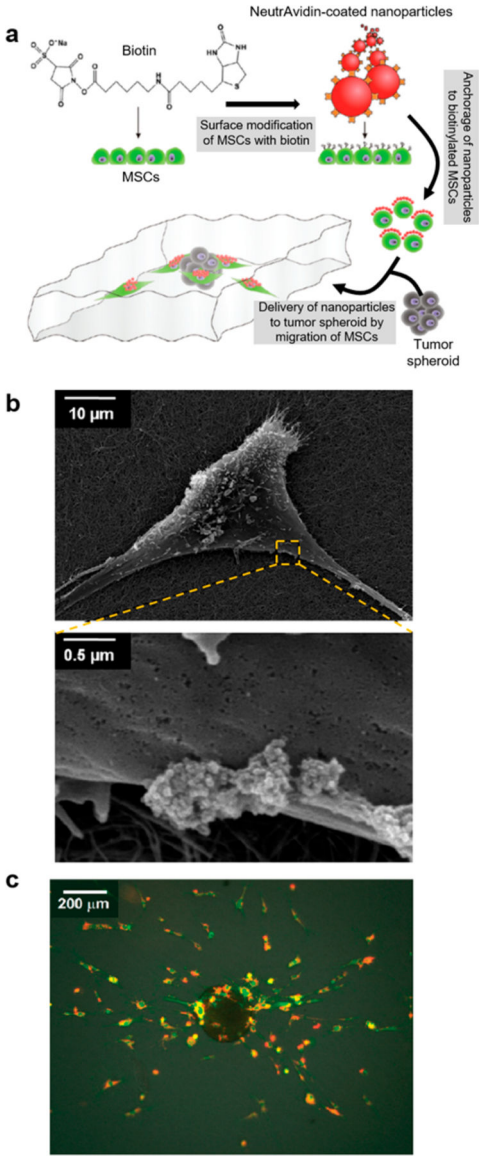


Figure 17.

(a) Schematic of MSC-mediated delivery of nanoparticles to tumor spheroids in an in vitro tumor model. (b) SEM images of nanoparticle clusters on a MSC cell membrane. (c) Optical image of MSCs coated with nanoparticles (red) and a tumor spheroid. Reprinted with permission from ref 183. Copyright 2010 American Chemical Society.

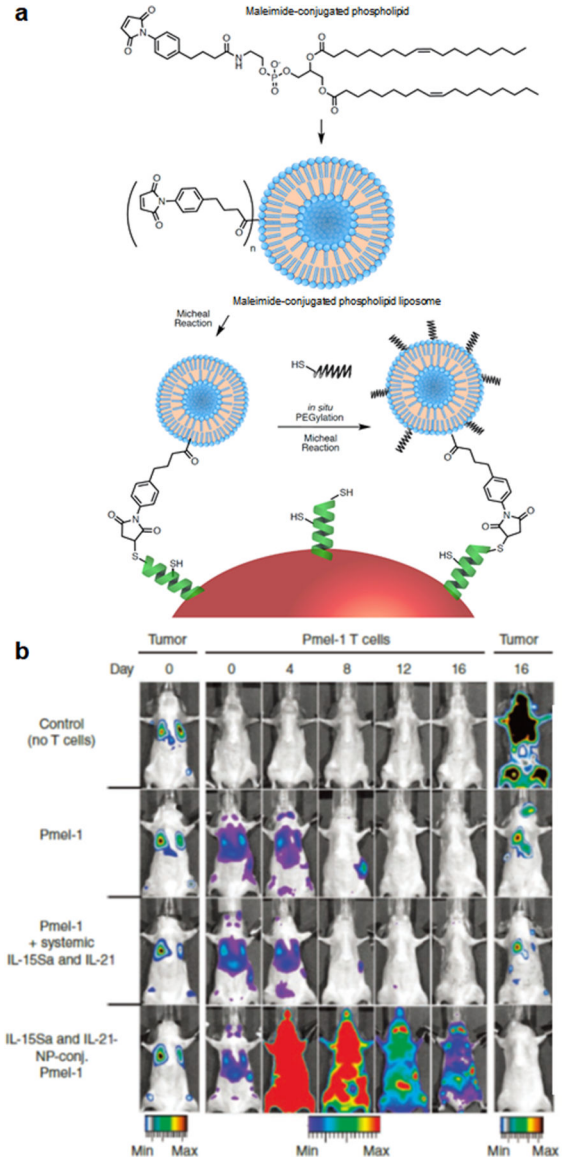


Figure 18.

(a) Schematic of the cell surface tethering process of poly(ethylene glycol)-coated liposomes using the maleimide-conjugated phospholipid. (b) Dual longitudinal in vivo bioluminescence imaging of tumors and Pmel-1 T-cells. T-cells conjugated with nanoparticles releasing IL-15Sa and IL-21 proliferate in vivo and remove tumors pre-established in lung and bone marrow. These tumors were established by tail vein injection. Reprinted with permission from ref 185. Copyright 2010 Nature Publishing Group.

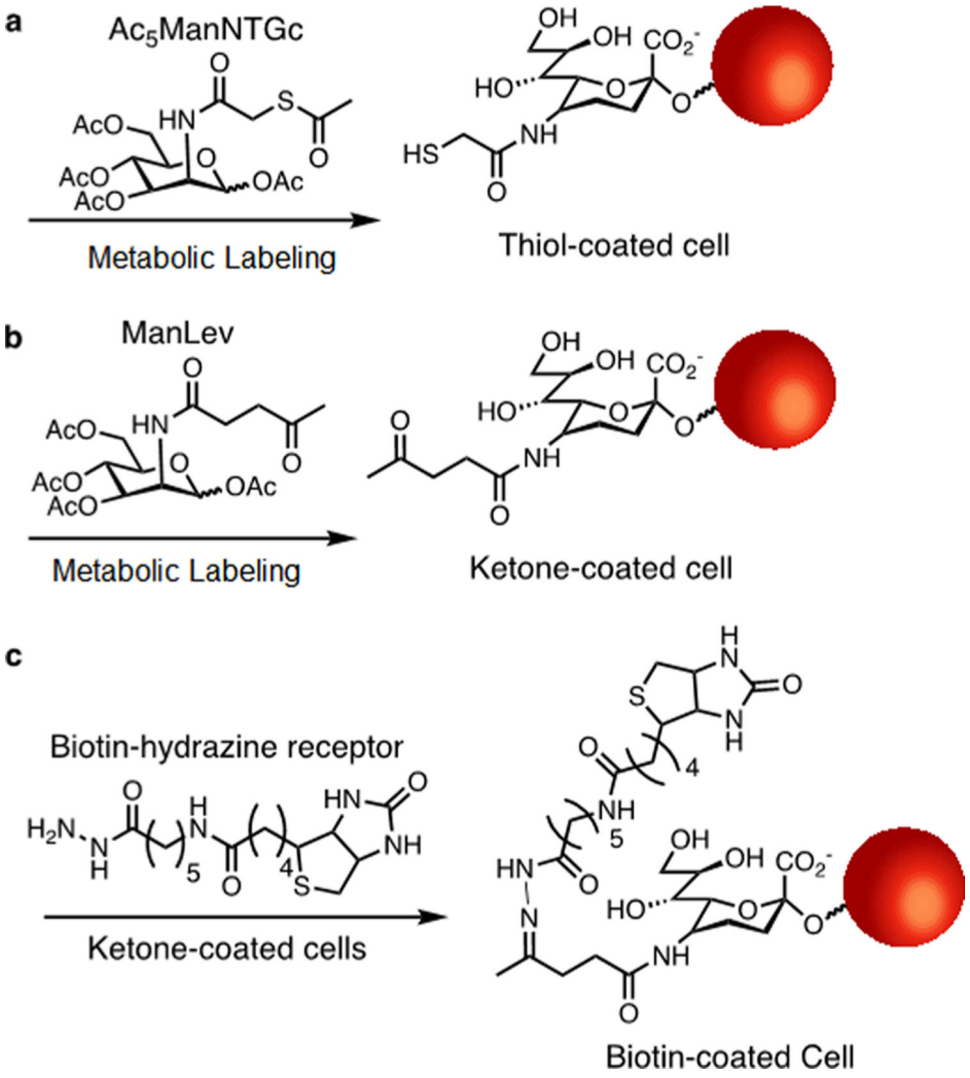


Figure 19.

Schematic of oligosaccharide metabolic incorporation of (a) thiol- and (b) ketone-bearing sialic acid analogs. (c) Covalent attachment of biotin to cells via biotin-conjugated hydrazide reaction with ketone via hydrazone formation.

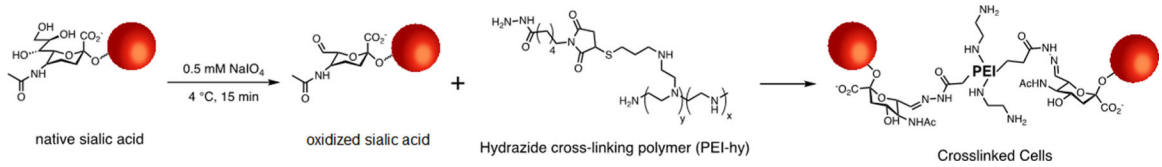


Figure 20.

Schematic of cell–cell cross-linking for cell clustering. In the first step, native sialic acid is oxidized with sodium periodate to generate an aldehyde moiety (top) and then it is cross-liked with tetravalent hydrazine.

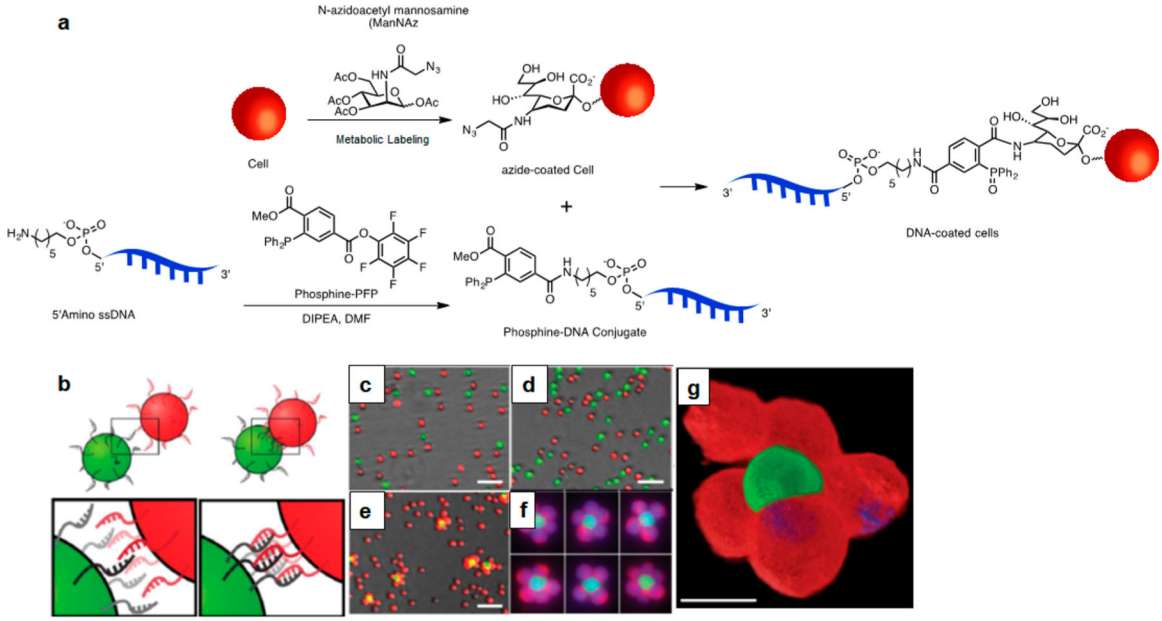


Figure 21.

(a) Schematic of the covalent attachment of ssDNAs onto the metabolically engineered cell surface. Cells are metabolically labeled via the sialic acid biosynthetic pathway with *N*-azidoacetyl mannosamine (top), ssDNA is coupled to triarylphosphine (bottom) for a Staudinger ligation to azido sialic acid. Oligonucleotides direct the synthesis of three-dimensional multicellular structures with defined patterns of connectivity. (b) Complementary oligonucleotides conjugated to cells associate to create the stable cell–cell contacts. (c) Nonadherent Jurkat cells labeled with green and red cytosolic stains. (d) Green- and red-colored Jurkat cells coupled with the mismatched oligonucleotides. (e) Green- and red-colored Jurkat cells conjugated with the complementary oligonucleotides. (f) Higher magnification of discrete structures from part e. (g) An image of a single multicellular structure developed by using the deconvolution fluorescence microscopy; red, Texas Red; green, fluorescein; blue, DAPI. (Scale bars: c–f, 50 μm; g, 10 μm.) Reprinted with permission from ref 206. Copyright 2009 National Academy of Sciences.

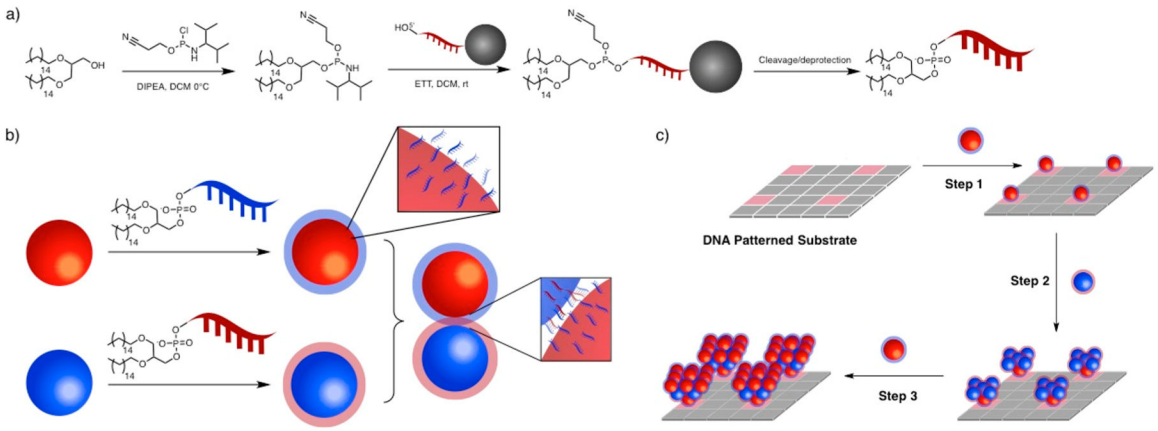
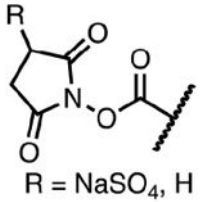
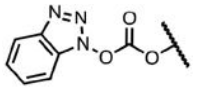
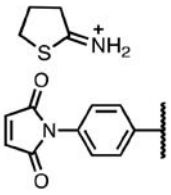
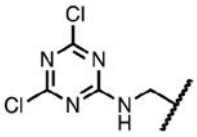
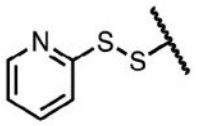


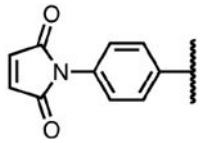
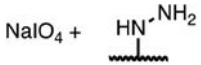
Figure 22.

Schematic of DNA-directed assembly of cells using lipid-conjugated ssDNAs. (a) Synthetic preparation of lipid-conjugated ssDNA, (b) cell coating process, and (c) multistep assembly on DNA-patterned substrate.

Table 1.

Summary of Covalent Reactions for Cell Surface Engineering

Cell Surface Functional Group	Cross-linker	Reaction Conditions	Benefits/Limitations	Ref.
Native Amines		Incubation with NHS ester in pH 8.0 for 20 min – 1 hr at room temperature to 37 °C	Most Common conjugation method Stable amide bond formation Slower reaction than cyanuric chloride	44, 131, 133, 136, 138, 144, 145, 183, 207
		Incubation with benzotriazole carbonate in pH 8.0 for 1 hr at room temperature	Stable amide bond formation Slower reaction than cyanuric chloride More reagent required	145
		Incubation with 2-iminothiolane and malimidophenyl in pH 7.4 for 2 hr at room temperature	Iminothiolane can reach buried amine groups Provides a 8 Å extension away from surface making attachment of bulky groups possible Will not alter net surface change of functionalized surface	146
		Incubation with cyanuric chloride in pH 8.7 for 20–30 min	Highly reactive, shorter reaction times Introduction of bulky triazine group Possible over functionalization	143 – 5
Native Thiols		Incubation with 2-pyridyl-dithio group for 4 hr at 37 °C	Close to quantitative conjugation High cell viability(~ 85%) Limited to the number of exofacial protein thiols (cell dependent) Possible internalization of conjugated group	78

Cell Surface Functional Group	Cross-linker	Reaction Conditions	Benefits/Limitations	Ref.
		Incubation with malimidophenyl group for 30 min at 37 °C	Cellular function maintained Limited to the number of exofacial protein thiols (cell dependent) Possible internalization of conjugated group	185
Glycans		Incubation with $NaIO_4$ for 15 min at 4 °C followed by Incubation with hydrazine linker for 30 min	Limited cytotoxicity ≤ 20% Maintained cell proliferation High incorporation of ligand	197–9