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Engineering the Mammalian Cell Surface with Synthetic Polymers: Strategies and Applications

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Manipulating the surface of living cells represents a powerful tool by which to control cell behavior and provides a unique strategy to modulate cellular function and cell–cell interactions. Recent progress in this area has seen the development of robust and elegant approaches to selectively decorate the cell surface, leading to unprecedented advances in cellular manipulation and cell-based therapies. Despite some impressive in vitro results, several obstacles remain to the broader application of some of these strategies, including their limited translation in vivo. In this review, the leading techniques used to introduce polymers at the plasma membrane of mammalian cells are discussed, focusing on strategies that generate a stable and homogeneous distribution of polymeric chains at the cell surface. Application of these strategies to control cell behavior and deliver cell-based therapies to targeted tissues are highlighted.

1. Introduction

The cell membrane is an incredibly sophisticated environment, the complex composition of which regulates the variety of chemical and physical processes that occur inside and outside the cell.[1] A wide range of reactive functional groups, including amines, thiols, and carbonyls, offer great opportunities for conjugation of exogenous molecules.[2] Introducing functionalities such as small molecules, proteins, peptides, polymers, and nanoparticles in such a diverse environment provides an excellent platform to manipulate cell behavior.[3–5] In the past few decades, cell engineering has endowed scientists with new routes for the development of drug delivery systems, cell-based therapies, and biomaterials for tissue engineering.[6–8] Recent developments in polymer conjugation to the cell membrane have allowed modification of cell–cell and cell–extracellular matrix (ECM) interactions, as well as targeted delivery of therapeutic agents.[9–12] Common methods used to achieve polymer cell surface engineering have employed covalent strategies, via conjugation to amines or thiols, physical approaches, including electrostatic interactions and insertion through the phospholipid structure, or metabolic labeling.[2,5] The potential advances enabled by cell engineering approaches include the development of cell-based therapies, targeted delivery of drugs and imaging agents, recruitment of bioactive molecules, masking of cell surface agents, and control over cell–environment interactions.[5,13]

Although cell engineering with polymers is undoubtedly a promising field, cell surface manipulation is a challenging technique that requires careful consideration of cell engineering chemistries to achieve a successful and stable conjugation of materials. Herein, the chemistries adopted for the attachment of polymeric materials at the cell surface will be evaluated, focusing on both single polymer chain attachment and delivery of polymeric nanoparticles. Importantly, cell response to the materials and therapeutic potential of the cell engineering approaches will be highlighted and future directions in the field discussed.

2. Noncovalent Strategies

Noncovalent conjugation of macromolecules offers a versatile and facile approach to introduce modification at the cell membrane without perturbing cell physiology, and with minimal interference with cell behavior. The design of cytocompatible methodologies for cell engineering through physical conjugation of polymer chains has significantly evolved over the past few decades, providing a noninvasive strategy to decorate the cell membrane with synthetic polymers. However, several challenges, including achievement of stability over polymer conjugation and high grafting density, still need to be addressed.

2.1. Electrostatic Interactions

Physical conjugation of polymers by electrostatic interactions is an attractive strategy that takes advantage of the negative charge present at the cell surface, as a result of sialic acid residues at the cell membrane.[14] Multiple layer-by-layer

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approaches have been used to coat the cell membrane with positively charged polymers, including poly(L-lysine) (PLL), poly(styrene sulfonate) (PSS), poly(allylamine hydrochloride) (PAH), and poly(ethylenimine) (PEI).[15] The layer-by-layer deposition method, introduced over 20 years ago, consists in the alternate adsorption of polyelectrolytes that self-organize on the cell surface, leading to the formation of polyelectrolyte multilayer (PEM) films.[15] Layer-by-layer assembly of PEM films has emerged as a versatile surface engineering approach, representing a powerful tool to increase the plethora of available cell surface modifications. The feasibility of multi-layer-by-layer approaches has been proved in rodent and pig islets, with encouraging in vitro and in vivo results.[16] Kozlovskaya et al. used hydrogen-bonded interactions of a natural polyphenol (tannic acid) with poly(*N*-vinylpyrrolidone) deposited on the human islet surface by nonionic layer-by-layer assembly, showing that in vitro cell viability and function could be maintained for a few days.[17] In another study, PAH and PSS sodium salts were alternatively deposited on human islets forming a nanocoating that ensured in vitro preservation of β-cell structure and function. Moreover, protection against anti-glutamic acid decarboxylase (anti-GAD) antibody recognition was achieved, demonstrating this approach can be used to create a multifunctional capsule for immune protection of pancreatic islets.[18] Alternatively, a combination of cationic polymers and poly(ethylene glycol) (PEG) spacers has also been used to engineer the cell membrane through electrostatic interactions. The use of PEG offers the advantage to introduce a polymer spacer between the cell surface and the positively charged polymers, hence reducing the risk of cytotoxicity.[19–22] In an exemplary study, Wilson et al. reported the first example of in vivo transplantation of PEG-*g*-PLL coated pancreatic islets (**Figure 1**).[23,24] In order to build multiple layers, alginate, a natural and biocompatible polysaccharide, was used as the polyanionic species that inserts in between the PLL layers, enabling a film growth around pancreatic islets that does not affect biocompatibility and insulin production.

Layer-by-layer PEM deposition was also used to induce immunocamouflage in red blood cells (RBCs).[24,25] In this case, the polyelectrolyte sets were made of three nontoxic and biodegradable polymers: alginate, chitosan-graft-phosphorylcholine, and PLL–PEG. RBCs functionalized with this methodology exhibited high longevity in vivo and did not provoke immunogenic responses.[25]

Polyelectrolyte multilayer coatings have also been used to encapsulate mouse mesenchymal stem cells (MSCs) within polymeric shells consisting of hyaluronic acid and PLL. This strategy was investigated to provide new means for harvesting MSCs and successfully achieving their transplantation as intact, cohesive sheets into diseased tissues.[26] More broadly, this approach opens the possibility to broaden the scope and application of PEM film coating to develop new opportunities in cell-based therapeutics for tissue engineering applications.

2.2. Hydrophobic Insertion into the Cell Membrane

Originally pioneered by Chung et al. while investigating the effect of lipid–PEG concentration and lipid chain length on the stability of polymer conjugation at the cell membrane, lipid–lipid interactions have since been used as a powerful tool to manipulate the cell membrane using a less invasive approach, by simply inserting polymers and therapeutics through the phospholipid layer.[27,28] PEG conjugation at the erythrocyte membrane was more durable with a longer lipid linker and when more than one lipid chain was conjugated to the polymer. Compared to covalent modification or electrostatic interactions, this approach is considered less harmful and more cytocompatible.[28]

Teramura et al. reported the microencapsulation of pancreatic islets using poly(vinylalcohol) (PVA) anchored to a PEG-phospholipid conjugate bearing a maleimide group (Mal–PEG–lipid).[29–31] The advantage of using thiol–maleimide chemistry for polymer conjugation relies on the relatively mild reaction conditions that allow effective and rapid functionalization at

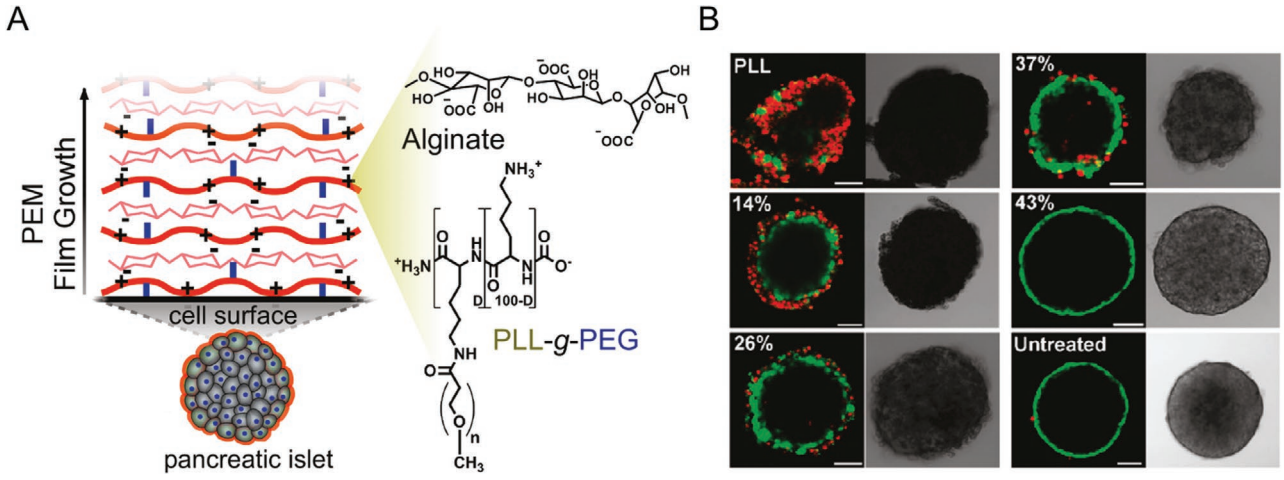

Figure 1. Cell-surface-supported PEM films. A) Scheme illustrating that PLL-*g*-PEG copolymers can be used for layer-by-layer self-assembly of PEM films directly on the surface of cells comprising a pancreatic islet. Alginate, a natural and biocompatible polysaccharide, was chosen as the polyanionic species. B) Confocal and bright field microscopic images of pancreatic islets stained with calcein AM (green, viable) and ethidium homodimer (red, nonviable) after incubation with PLL and PLL-*g*-PEG copolymers with variable degrees of PEG grafting. Reproduced with permission.[24] Copyright 2011, American Chemical Society.

physiological pH.[32,33] The Mal–PEG–lipid analog was first used to coat the cell surface, where the lipid is used to immobilize PEG chains through the hydrophobic interaction between the lipid bilayer of the plasma membrane and the PEG–lipid conjugate. A PVA derivative functionalized with thiol groups was then used to create a first PVA layer on top of the PEG, and subsequent PVA layers were added by forming disulfide bonds between PVA chains. This strategy allowed for the encapsulation of pancreatic islets by forming an ultrathin layer-by-layer PVA membrane, and thus represents a simple method to reduce immune response in islet transplantation. Notably, when single cells were treated with this methodology, cell viability was heavily compromised. However, when islets were treated using the two polymers conjugates (both PEG and PVA), no difference in cell viability or cell function was observed. In a following report, hydrophobic insertion was also used to immobilize Mal–PEG–lipid analogs on islet surfaces followed by urokinase and thrombomodulin binding through thiol–maleimide chemistry. No impair in insulin release in response to glucose stimulation was recorded after islet surface modification. Moreover, the activity of urokinase and thrombomodulin was maintained, hence helping in preventing thrombus formation on the surface of pancreatic islets (**Figure 2**A).[34]

Hydrophobic insertion as a means to engineer the cell surface has not only been used for immunocamouflage. Bertozzi and co-workers developed a strategy, glycocalyx, to introduce synthetic glycopolymers at the cell surface, designed to mimic native cell surface mucin glycoproteins.[35] Once in the cell surface environment, these polymers exhibit behaviors similar to native mucins, such as specific protein binding and internalization through endocytic pathways. This same strategy was also employed to provide a mechanistic understanding of the correlation between hypersialylation and immunoprotection, which is a characteristic of many tumor types.[36] The introduction of sialylated glycopolymers onto Jurkat cells, $CD34^+$ hematopoietic stem cells (HSCs), and pig aortic epithelial cells enabled recruitment of a tyrosine-based motif able to attenuate natural killer cell response.

Huang et al. used hydrophobic insertion to anchor synthetic neoproteoglycans (neoPGs) on the surface of mouse embryonic stem cells (ESCs), resulting in an enhanced cell affinity for the fibroblast growth factor 2 (FGF2). The binding of ESC to FGF2 directed ESC differentiation toward a neural phenotype. This represents an important example where cell engineering was used to direct cell differentiation, and the versatility of this technology makes it widely applicable to other types of differentiation.[37] Similarly, rat neural cells were modified with chondroitin sulfate (CS) glycosaminoglycans (CS GAGs), using liposomes functionalized with a ketone handle for appending the glycans via oxime chemistry. Liposomes fused with the lipidic membrane were able to enhance nerve growth-factor-mediated signaling and promote neural growth. Moreover, glycan surface density as well as specific structure could be precisely controlled (Figure 2B).[38] In another attempt to engineer stem cells with polymers, bioactive hyperbranched polyglycerol (HPG) conjugated to vasculature binding peptides was covalently modified with octadecyl chains for hydrophobic insertion in MSCs.[39] Tuning the number of octadecyl chains linked to the HPG allowed for control over the binding affinity of HPG to cells. HPG could noninvasively associate with stem cells to yield a cell-based therapy targeted toward defective vascular endothelium. The bioactive polymer coating significantly enhanced the cellular affinity for the vascular endothelial adhesion molecule, a protein overexpressed in inflamed blood vessels.

3. Nonspecific Covalent Binding

While physical interaction might offer noninvasive approaches to coat the cell membrane with polymers, covalent binding has the potential to provide a more stable and durable conjugation. The cell surface is a heterogeneous and dynamic environment that presents different functional groups available for the conjugation of macromolecules. Among these, primary amine groups from lysine residues are the most used for direct covalent attachment of polymers, proteins, peptides, and even small molecules.[3] While cysteine residues are also available at the cell surface, their thiol side chains are normally oxidized to disulfides, hence limiting their availability for polymer conjugation.[40] Covalent conjugation of polymers is often preferred as it offers a simple, straightforward solution to engineer the cell membrane compared to genetic manipulation. However, its lack of specificity can potentially affect cell morphology and functionality and induce cell apoptosis, hence limiting the use of this approach for some applications.

In order to react with amines present at the cell surface, macromolecules can be activated with a cyanuric chloride group or a *N*-hydroxysuccinimide (NHS) functionality. One of the first examples of cell engineering with polymers was reported by Chen and Scott, where RBCs were coated with methoxy-PEG (mPEG) activated with a cyanuric chloride reactive group.[41,42] This strategy was used to physically shield RBCs from recognition from the immune system by reducing antibody binding to receptors at the cell surface.[43,44] The immunocamouflage provided by the presence of PEG chains around RBCs was proved to be effective not only in vitro but also in vivo.[45,46] Sheep RBCs modified with mPEG were transfused in mice and demonstrated normal in vivo survival with no immunogenicity associated with the mPEG-modified RBCs. This strategy was envisaged to be useful in reducing the incidence and severity of transfusion reactions.[47] While these are not very frequent (1 in 6000) when transfusions are required occasionally, 20–30% of chronically transfused individuals will eventually show evidence of alloimmunization against minor blood group antigens, making subsequent transfusions more problematic.[48] Moreover, engineering RBCs with PEG chains can help to prevent immune reactions in patients with autoimmune hemolytic disease or in those suffering severe trauma where their matching blood type is not available. Interestingly, the same group investigated the effect of changing the chemistry of the linker used to attach mPEG to RBCs and the effect of changing the size of the polymer graft. Cyanuric chloride was determined to be the most efficient functionality to achieve a high level of functionalization in the shortest reaction time compared to benzotriazole carbonate and NHS ester. Moreover, it was found that a higher-molecular-weight PEG (20 kDa compared to 2 or 5 kDa) could achieve a higher grafting density

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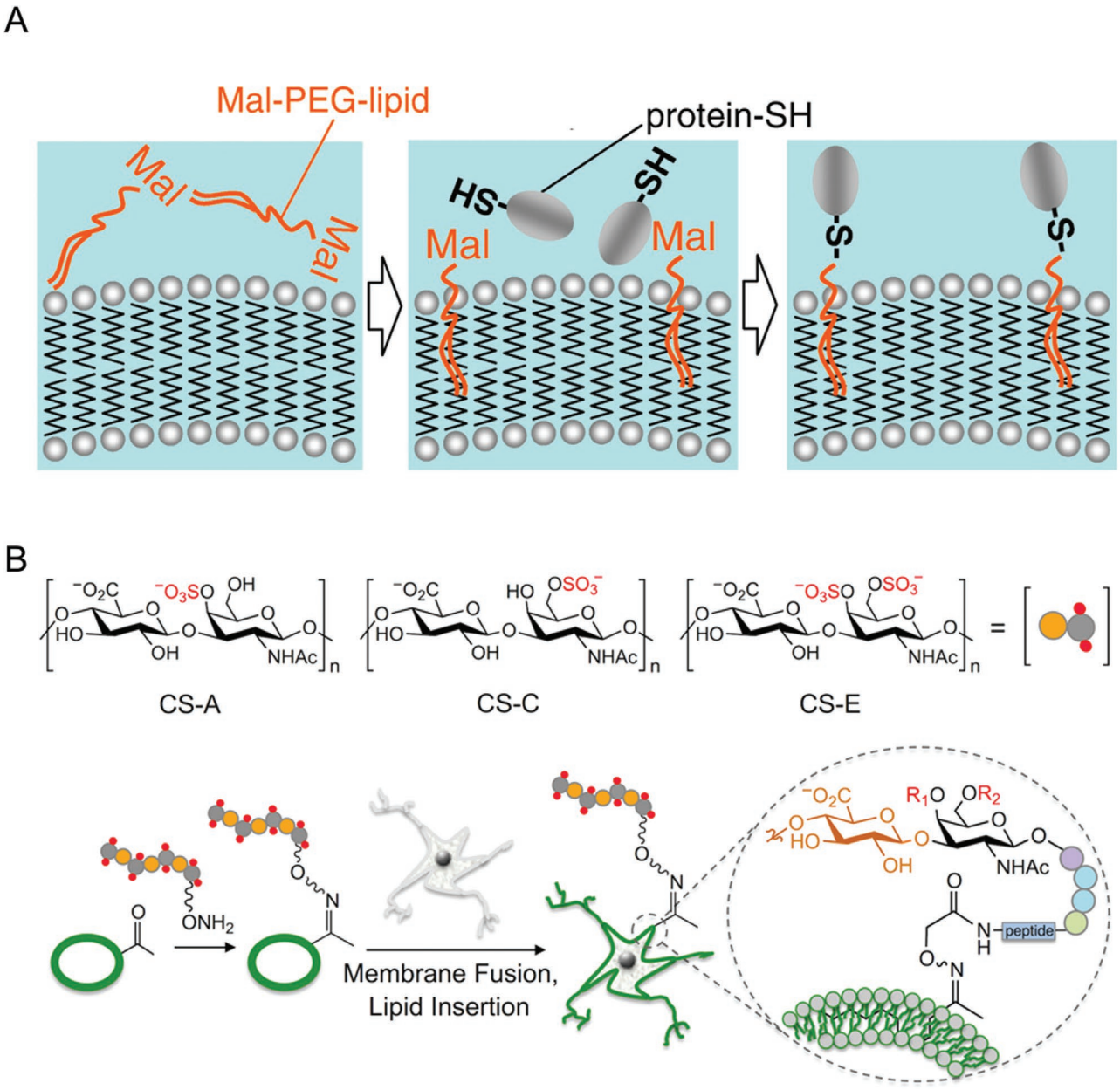

Figure 2. Cell engineering through hydrophobic insertion. A) Schematic illustration of immobilization of protein onto the cell surface through Mal–PEG–lipid. Thiol groups on the protein can react with maleimide groups at the end of PEG chains on the cell membrane. Reproduced with permission.[34] Copyright 2011, Elsevier. B) Neural cells modified with chondroitin sulfate (CS) glycosaminoglycans using liposomes that fuse with the cell membrane. CS GAGs were used to control nerve growth-factor-mediated signaling and promote neural growth. Reproduced with permission.[38] Copyright 2014, American Chemical Society.

at the cell surface, hence enhancing the immunocamouflage effect.[46] Overall, the grafting efficiency, linker chemistry, and length, as well as molecular weight and density of the mPEG conjugation are essential parameters to consider in cell engineering for immunocamouflage purposes.[46] Further studies explored the ability of the immunocamouflage to be extended beyond the application to blood transfusions to include, more broadly, any guest–host immune response.[47,49,50] Immune-mediated response to tissue scaffolds or donor organs typically triggers the activation of T lymphocytes. By modifying the amine groups at the surface of T cells with mPEG conjugated through cyanuric chloride, T-cell activation could be minimized in order to significantly limit the host recognition and trigger a cascade immune response while compared to unmodified T cells.[49]

While cyanuric chloride was first widely used to engineer mammalian cells owing to its efficiency of conjugation and rapid reaction, its toxicity effects soon prompted for other mild and cytocompatible chemistries to be investigated. NHS was introduced as an alternative linker that readily reacts with

primary amine sites at the cell membrane. While this offered a more cytocompatible approach, a major drawback of cell coating with PEG was caused by poor association of the hydrophilic macromolecules with cell surfaces. In response to this, Rossi et al. developed a highly efficient, universal strategy in which nonreactive "additive" macromolecules, such as dextran or HPG, were used to modulate the grafting efficiency of cell surface reactive macromolecules. Unprecedented enhanced cell surface modifications by up to tenfold were observed when various concentrations of a suitable "additive" polymer were added with a constant and low concentration of a "reactive" macromolecule (**Figure 3**).[51]

In an attempt to provide an alternative to PEG, previously shown to trigger the production of anti-PEG antibodies,[52–54] hyperbranched polyglycerol was used to engineer the RBCs' surface, providing an effective method for immunocamouflage. Similarly to PEG, HPG coating was successfully able to shield antigens at the surface of RBCs, providing a significant reduction in the level of antibody binding to cells. Moreover, the level of immunocamouflage offered by HPG was significantly higher compared to PEG chains of similar molecular weight.[51,55,56]

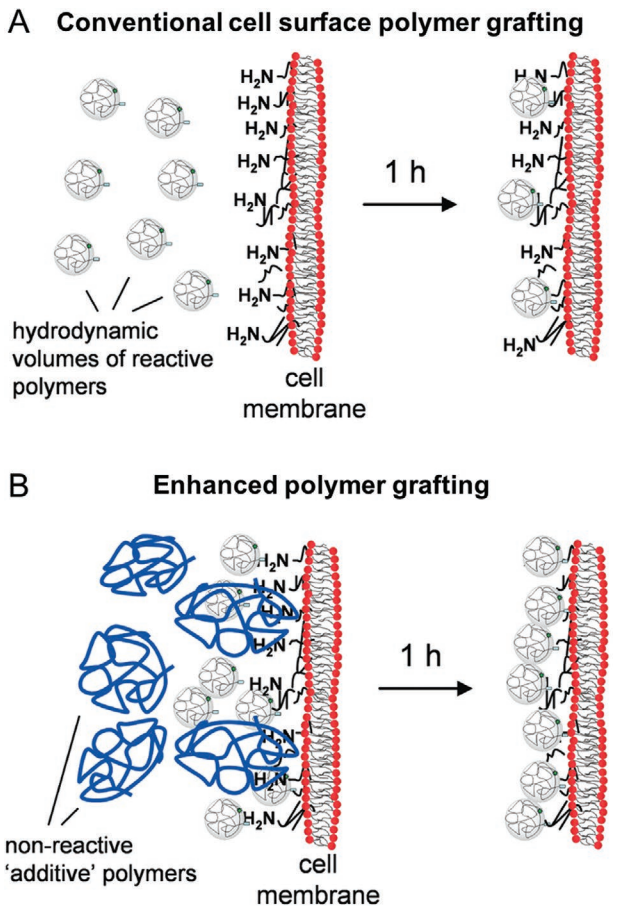

Figure 3. Enhanced cell surface polymer grafting in the presence of a nonreactive polymer additive. A) Representation of cell surface modification using primary amine reactive polymers. B) Cell compatible surface grafting process in the presence of a nonreactive polymer additive. Reproduced with permission.[51] Copyright 2010, American Chemical Society.

More recently, NHS–amine coupling was used as a strategy to coat pancreatic islets with a heparin-incorporated starPEG nanofilm.[57] Heparin, a highly sulfated glycosaminoglycan with anti-inflammatory and anticoagulant properties, was incorporated for its ability to facilitate islet vascularization by recruiting pro-angiogenic growth factors. This approach of living cell surface engineering demonstrated high cytocompatibility without negative consequences on cell viability and damage to cell morphology or function. More importantly, the heparin–PEG coating was also able to reduce instant blood-mediated inflammatory reaction, which typically occurs in intraportal islet transplantation, while facilitating islet survival in a pro-inflammatory environment.[57,58] In a second report, heparin was replaced with CS, which possesses a strong binding affinity toward multiple growth factors and cytokines, and hence represents a possible means for incorporation of desirable biologically active factors through live cell engineering. CS–PEG coating at the surface of pancreatic islets resulted in enhanced in situ revascularization that is protective against ECM disruption, while still alleviating immediate inflammatory reaction, blood coagulation, and inflammation-related islet cell death. Since then, thrombomodulin was also used to engineer pancreatic islets through NHS–amine chemistry, where its presence resulted in a significant increase in the production of activated protein C and a reduction in islet-mediated thrombogenicity.[59,60] Alginate- and PEG-based polymers, functionalized with azide and phosphine, respectively, which form spontaneous and chemoselective crosslinks via the bio-orthogonal Staudinger ligation, were conjugated to pancreatic cells through amine–NHS coupling.[61] The resulting coatings were nontoxic, with unaffected glucose-stimulated insulin secretion.

4. Covalent Conjugation Using Bio-Orthogonal Chemistry

The manipulation of functional groups already present at the cell surface has been for a long time a preferred strategy in cell engineering, owing to the easy conjugation and abundance of reactive groups. However, direct covalent modification of the proteins at the cell cytoskeleton can substantially affect cell viability and normal cell function, while hydrophobic insertion does not offer a permanent surface modification, with lipid–polymer conjugates rapidly dissociating from cells.[37,62,63] On the other end, introduction of bio-orthogonal functionalities at the cell membrane offers clear advantages over nonspecific polymer conjugation, including better cytocompatibility and stability of conjugation.[64–66] The cell surface repertoire can be expanded to include abiotic functionality through the biosynthetic introduction of unnatural sugars into cellular glycans, a process termed metabolic oligosaccharide engineering (MOE).[13,67,68] This method was pioneered by Bertozzi and coworkers and allows easy incorporation of bio-orthogonal handles in vitro and in vivo.[69–71]

Shi et al. engineered MCF-7 human breast cancer cells using *N*-azidoacetylgalactosamine ($Ac_4GalNAz$) to enrich cell surface glycoconjugates with an azide tag. Subsequent conjugation to a β-cyclodextrin (β-CD) through an azo–PEG–azo functional linker enabled controlled display of functional components

through cell–cell interactions. The azo–PEG–azo crosslinking agent was used to induce adhesion and aggregation of β-CD-modified cells and could be reversibly manipulated with UV light irradiation.[72]

Using *N*-azidoacetylmannosamine tetraacetate (Ac_4ManNAz), Gibson and co-workers introduced azide groups at the plasma membrane of adenocarcinomic human alveolar basal epithelial cells (A549). The bio-orthogonal functionality was then used to attach fluorescein-functionalized poly(*N*-hydroethyl acrylamide) (pHEAn) polymers through copper-free alkyne–azide cycloaddition. Polymers remained on the cell surface for over 24 h, and no reduction in cell viability was observed compared to the control group.[73] Moreover, by introducing polymers conjugated to biotin, streptavidin was selectively recruited at the cell surface, highlighting the ability to use polymers to recruit binding agents (**Figure 4**A,B). In a follow-up report, it was shown that synthetic polymers installed at the surface of azide-labeled A549 remained attached for over 72 h, even after multiple cell division cycles.[74] Grafting density was also evaluated in this study, with longer polymer chains (degree of polymerization higher than 50) showing lower grafting density as a consequence of steric hindrance.

The introduction of bio-orthogonal azide groups using metabolic glycan labeling has also been used to engineer live cells, through introduction of Ac_4ManNAz, with antibody-recruiting polymers (ARPs).[75] For this purpose, pentafluorophenyl acrylate (PFPA) polymers functionalized with an octyl alkyne were introduced in Jurkat cells and used to immobilize anti-2,4-dinitrophenyl (DNP) antibodies on the cell surface (Figure 4C,D).[76] This concept was further extended to mouse 4T1 spheroids, demonstrating good penetration into the azide-labeled spheroids and binding to the surface of individual cells.

Importantly, MOE using Ac_4ManNAz has been successfully achieved in vivo by injecting the modified oligosaccharides intraperitoneally[69,77] or intravenously using caged Ac_4ManNAz derivatives for cellular uptake.[78,79]

5. Grafting Polymers from the Cell Surface

Traditionally, modification of the cell surface with polymers has been achieved by physical or chemical conjugation of pre-formed materials.[71,80–83] However, grafting-to approaches are sometimes limited by low polymer grafting on the cell surface and the use of a large excess of reactive polymers for functionalization.[51] To overcome these challenges, grafting polymer chains directly from the cell surface was introduced as a methodology that could improve grafting density and site-specific polymer conjugation. Although this strategy is, in principle, promising, significant synthetic challenges, including the

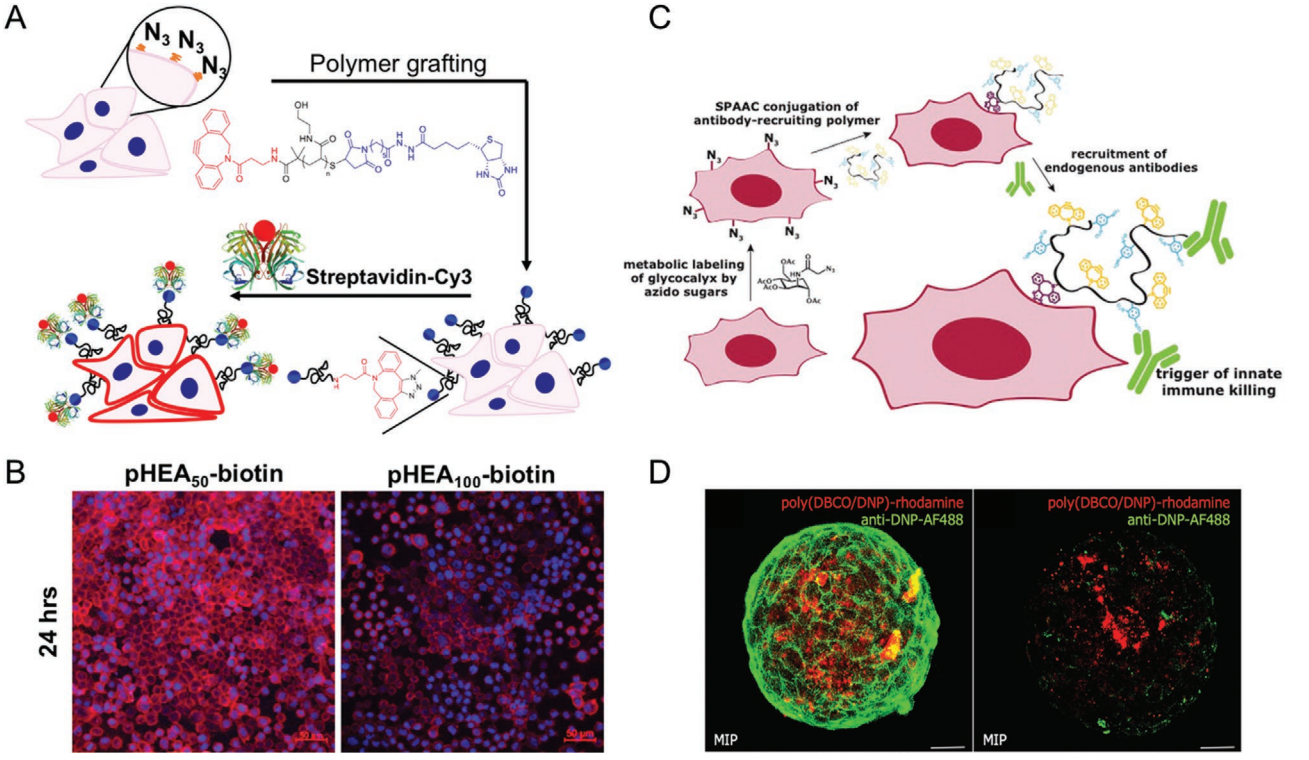

Figure 4. Metabolic engineering of mammalian cells with bio-orthogonal azide functional groups. A) Immobilization of dibenzocyclooctyne-functionalized pHEAn-biotin to azido-functionalized A549 cells, followed by recruitment of Strepavidin-Cy3 (red). B) Confocal images of cells after streptavidin recruitment. Reproduced with permission.[73] Copyright 2014, American Chemical Society. C) Antibody recruiting polymer (ARP) concept. The glycocalyx of a target cancer cell is engineered with azides by metabolic labeling with an azido sugar. ARPs are then conjugated to the cell surface by cooper-free azide–alkyne cycloaddition. D) Confocal microscopy images of 4T1 mouse breast cancer spheroids, cultured with (left) or without (right) azido sugar and treated with PFPA polymers (red fluorescence) and AF488-anti-DNP (green fluorescence). Reproduced with permission.[75] Copyright 2019, Royal Chemical Society.

cytotoxicity of most initiators, catalysts, and monomers, have limited progress in this area, with only two attempts of cell surface polymerization from mammalian cells reported so far. Hawker and co-workers reported the use of hydrophobic insertion to insert chain transfer agents (CTAs) at the plasma membrane of Jurkat cells.[84] Interestingly, this approach was selected to replace a covalent conjugation of CTAs to the primary amine groups at the cell surface which resulted in significant cell death. These CTAs were used to polymerize water-soluble cytocompatible monomers via visible-light-mediated photoinduced electron transfer reversible addition–fragmentation (PET-RAFT). In this study, molecular weights up to 10 kDa were observed, using methoxy-PEG acrylamide-1k as the monomer. The grafting-from approach was proved to achieve superior polymer grafting and increase chain density compared to grafting pre-synthesized polymers to the plasma membrane.

Following this, Qi et al. developed an approach for a cytocompatible polymerization at the cell surface to cluster cell surface receptors. An anti-CD20 aptamer-conjugated macromer was synthesized, which was then efficiently and stably introduced onto the Raji cell surface via ligand–receptor interactions. With the assistance of ammonium peroxysulfate as the initiator, the macromer bound onto the Raji cell surface polymerized, inducing the clustering of CD20 receptors, and thereby triggering cell apoptosis.[85] To a broader extent, this cell surface polymerization could be applied in modulating the fates and functions of other cells, especially those mediated by spatial distribution of cell surface receptors, such as T-cell activation.

6. Targeting Polymeric Nanoparticles at the Cell Surface

Polymeric nanoparticles have emerged as a promising tool in the biomedical field to deliver drugs and imaging agents to the targeted site, enhancing selectivity of therapeutics, increasing circulation lifetime and water solubility of drugs and imaging agents.[86–90] Targeted delivery technologies are essential to avoid side effects and decrease the administered dose of potentially toxic therapeutics. However, current delivery methods are far from perfect and present substantial challenges that nanomedicine is still facing.[91,92] Cell engineering has recently appeared as a potential strategy that can be used to direct polymeric nanoparticles to the surface of engineered cells. Despite the clear challenges of this approach, above all the selective engineering of cells directly in vivo, this area of research has attracted increasing interest, offering a promising new direction for the delivery of both small molecules and larger therapeutics.

6.1. Covalent Conjugation of Nanoparticles through Thiols at the Cell Surface

Stephan et al. used free thiol groups present at the surface of HSCs, as well as T and B cells, the only cell types with a sufficient free thiol content at the membrane, to conjugate drug-loaded nanoparticles via maleimide–thiol chemistry.[93] Nanoparticles in the size range of 100–300 nm were coated with phospholipids conjugated to maleimide and loaded with glycogen synthase kinase-3β, previously demonstrated to enhance HSC proliferation and cell viability following systemic injection. The drug-loaded nanoparticles released the drug into the HSCs over the course of 7 days and demonstrated increased cell proliferation post-engraftment compared to control experiments. Moreover, nanoparticle conjugation to cells was achieved without toxicity or interference with intrinsic cell functions. Prolonged retention of the particles on the surfaces of donor cells enabled sustained drug release without concerns of premature degradation of the particle carrier or cargo. In a follow-up study, the authors reported the conjugation of synthetic nanocarriers to the surface of T cells via maleimide–thiol chemistry. The surface-engineered lymphocytes delivered their therapeutic cargo, a small-molecule inhibitor of phosphatases that downregulate T-cell receptor activation in the synapse, to the tumor site in mice with advanced prostate cancer, leading to enhanced survival rate.[94] PEG–PLL protein-based nanogels were also synthesized to anchor the T-cell surface through thiol–maleimide chemistry. A disulfide-containing bis-*N*-hydroxy succinimide crosslinker (NHS–SS–NHS) was used to incorporate in the nanogel many copies of the same protein crosslinked to itself. The disulfide crosslinker was specifically designed to be cleaved in response to reducing conditions at the surface of T cells, releasing the protein cargo composed of interleukin (IL)–2Fc (a fusion protein between IL-2 and an antibody Fc fragment) and ALT-803, a human IL-15 superagonist complex. The nanogel delivery selectively enhanced T-cell response in vivo and allowed higher doses of cytokine to be administered without toxicity, compared to free cytokine administration.[95] This approach was then extended to the delivery of IL-2 in vivo, improving the efficacy of this therapy against melanoma metastases.[96]

6.2. Conjugation of Nanoparticles through Bio-Orthogonal Functionalities

Together with the bio-orthogonal strategies mentioned earlier, aldehydes have also been investigated for cell surface engineering, owing to their ability to react through Schiff base formation with primary amines at the cell membrane. This strategy has been used for drug delivery and cell surface manipulation through mild oxidation of sialic acid residues. To this end, Yang and co-workers reported a novel drug delivery vehicle by hybridizing macrophages with nanoparticles through cell surface modification. They generated aldehyde reactive sites at the surface of RAW264.7 macrophages through oxidation of the cell membrane by sodium periodate. These were then reacted with the nanoparticle surface composed of poly(amidoamine) dendrimers. Following this, a reducing agent, sodium cyanoborohydride, was applied to reduce Schiff bases to stable secondary amine linkages. The distribution of nanoparticles on the cell surface was confirmed by fluorescence imaging and was found to be dependent on the stability of the linkages conjugating the nanoparticles to the cell surface. Overall, this approach demonstrated that a higher drug concentration could be achieved using this strategy when compared with direct cell loading.[97]

The delivery of nanoparticles at the cell surface has also been investigated using biotin–avidin binding. Biotin is a

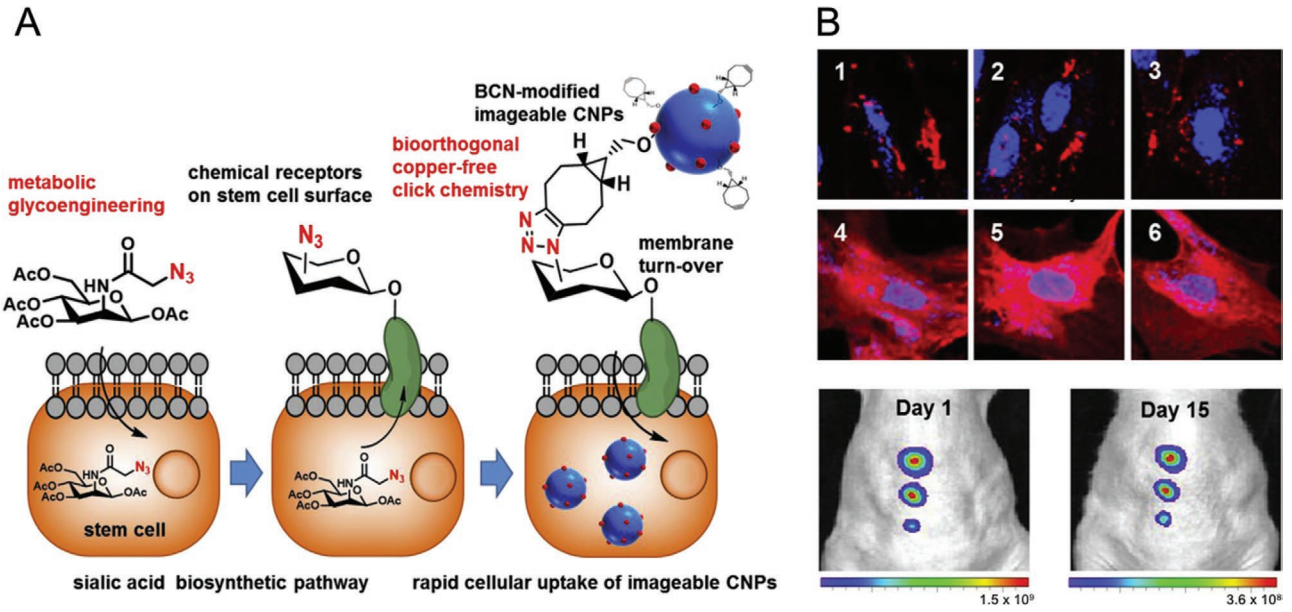

Figure 5. Conjugation of nanoparticles to the cell surface through bio-orthogonal chemistry. A) Schematic illustration of stem cell labeling and tracking method with nanoparticles via metabolic glycoengineering and bio-orthogonal copper-free click chemistry. B) Top: Comparison of cell labeling efficiency of BCN–CNP–Cy5.5 between 1–3) control stem cells and 4–6) Ac_4ManNAz-treated stem cells. Bottom: In vivo imaging of labeled hMSCs by optical imaging after subcutaneous transplantation with hMSCs prelabeled with Ac4ManNAz and BCN-CNP-Cy5.5. Reproduced with permission.[111] Copyright 2017), Elsevier.

water-soluble B vitamin that binds tightly to the tetrameric protein avidin, with a dissociation constant, K_d, on the order of 10^{-15} M, one of the strongest known protein–ligand interactions.[98,99] The high biotin–avidin affinity has provided researchers with a powerful tool that can be applied in the fields of biotechnology and biomedicine. Biotin- and avidin-terminated linkers have been designed to provide a variety of small molecules, polymers, or proteins that can be used for functionalization.[100–102] It is important to note that streptavidin, a form of avidin synthesized by certain strains of *Streptomyces* bacteria, can generate immune response in humans, while avidin derived from egg whites exhibits nonspecific binding in some applications. Hence, deglycosylated, neutral forms of avidin, such as Extravidin,[103] NeutrAvidin,[104] and NeutraLite,[105] should be used to investigate the clinical translation. Deglau et al. modified primary amine groups on coronary artery endothelial cells using NHS–PEG–biotin.[106] Targeted delivery to the damaged arteries was achieved using NeutrAvidin-coated polystyrene microspheres, which were delivered over the endothelial monolayers under flow conditions that mimic arterial shear stress.[107]

The metabolic glycoengineering approach described earlier for the conjugation of single polymer chains has also been applied to the delivery of drug-loaded nanoparticles to cells both in vitro and in vivo. Through metabolic glycoengineering, unnatural glycans are introduced onto cells by feeding specific precursors on the basis of their intrinsic metabolism. Therefore, bio-orthogonal chemical reactions can occur specifically on target living cells with artificially introduced unnatural glycans containing particular chemical groups.[108] Koo et al. developed a strategy to introduce nanoparticles on the cell surface through bio-orthogonal copper-free click chemistry in vivo. Nanosized PEGylated liposomes modified with dibenzyl cyclooctyne (DABCO-lipo) were intravenously injected into tumor-bearing mice, prior to exposure in cancer cells of unnatural sialic acids containing azide groups through intratumoral injection of Ac_4ManNAz.[109] This in vivo targeting strategy could successfully enhance accumulation of nanoparticles at the tumor site, showing the feasibility of this approach for targeted intracellular drug delivery. In a second report from the same group, chitosan nanoparticles were used to directly accumulate Ac_4ManNAz at the tumor site, taking advantage of the enhanced permeation and retention (EPR) effect.[110] Bicyclo[6.1.0]nonyne-modified glycol chitosan nanoparticles (BCN-CNPs) were also used to deliver imaging agents to human mesenchymal stem cells (hMSCs) in vitro through metabolic engineering of glycans with an azide functionality (**Figure 5**A).[111] Nanoparticles were internalized by hMSCs and successfully distributed through cell division. In vivo injection of Cy5.5-, gold-, and iron-labeled hMSCs allowed noninvasive cell tracking for up to 15 days through fluorescence imaging and magnetic resonance imaging, demonstrating that Ac_4ManNAz labeling can be used for prolonged in vivo cell tracking (Figure 5B).

While azide groups have been primarily used to express functional groups at the cell membrane, as a consequence of their ability to react using bio-orthogonal, cytocompatible chemistry, *N*-levulinoylmannosamine (ManLev) was also used to introduce ketones at the cell surface.[112] 2-methacryloyloxyethyl phosphorylcholine (MPC) nanoparticles, functionalized with hydrazide groups, could be used to deliver anticancer agents to ManLev-engineered HeLa cells.[113] Release of doxorubicin or paclitaxel over 3 days of cell culture determined a reduction in cell viability of 60% and 50%, respectively. Nonspecific delivery

of these nanoparticles to cells was effectively reduced because of the presence of MPC units, demonstrating the advantage of cell engineering.

7. Conclusion and Outlook

The ability to rationally and precisely manipulate the plasma membrane of mammalian cells has great potential to overcome many of the challenges faced by conventional cellular therapies and provide innovative prospects in the field of biotechnology. Herein, strategies to conjugate linear polymeric chains and polymer-based nanoparticles at the cell membrane are discussed, covering both nonspecific and site-specific conjugation, and the advantages and disadvantages of each technique are highlighted. While noncovalent polymer conjugation has been widely explored in vitro and offers a noninvasive, cytocompatible approach to interface materials with cells, it has been limitedly applied in vivo to induce immunocamouflage in RBCs.[25] Covalent conjugation has been investigated as a better alternative to noncovalent binding, and has found application in reducing the immune response after transplants in vivo,[45–47] as well as in cancer therapy by directly targeting T cells and boosting the immune response toward tumors.[94–96] However, the lack of selectivity of covalent approaches that use functional groups already present at the plasma membrane, such as amines and thiols, limits their applications in targeted cell therapies and increases their side effects. On the other hand, bio-orthogonal chemistries offer unique and highly biocompatible routes to explore mammalian cell surface engineering. These strategies have been widely exploited in vivo and represent a powerful tool by which to introduce polymers at the cell surface homogeneously.[71,109–111] Future research should focus toward exploring the potential offered by these chemistries in the development of new cell-based therapies and in the manipulation of cell behavior through bio-orthogonal cell engineering approaches.

The emerging and evolving area of cell engineering offers significant opportunities to interface polymeric materials with biological components to manipulate cell behavior and direct cell function. However, important challenges need to be overcome, predominantly linked to the stability of the polymer conjugation, control over polymer grafting density, and translatability of the cell engineering approaches in vivo.

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Conflict of Interest

The author declares no conflict of interest.

Keywords

bioconjugation, bio-orthogonal chemistry, cell surface engineering, polymers, tissue targeting

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