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100th Anniversary of Macromolecular Science Viewpoint: Re-Engineering Cellular Interfaces with Synthetic Macromolecules Using Metabolic Glycan Labeling

Ruben M. F. Tomás and Matthew I. Gibson*

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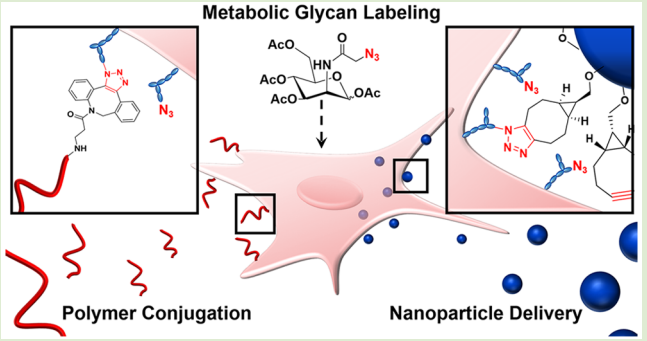
ABSTRACT: Cell-surface functionality is largely programmed by genetically encoded information through modulation of protein expression levels, including glycosylation enzymes. Genetic tools enable control over protein-based functionality, but are not easily adapted to recruit non-native functionality such as synthetic polymers and nanomaterials to tune biological responses and attach therapeutic or imaging payloads. Similar to how polymer−protein conjugation evolved from nonspecific PEGylation to site-selective bioconjugates, the same evolution is now occurring for polymer−cell conjugation. This Viewpoint discusses the potential of using metabolic glycan labeling to install bio-orthogonal reactive cell-surface anchors for the recruitment of synthetic polymers and nanomaterials to cell surfaces, exploring the expanding therapeutic and diagnostic potential. Comparisons to conventional approaches that target endogenous membrane components, such as hydrophobic, protein coupling and electrostatic conjugation, as well as enzymatic and genetic tools, have been made to highlight the huge potential of this approach in the emerging cellular engineering field.



Cell surface re-engineering with small molecules, nanoparticles, and polymers has expanded the repertoire of tools used in biological sciences and modern medicine, increasing our understanding of fundamental biological processes and expanding the arsenal of future cell-based therapies. Recruitment of natural and synthetic polymers offers an attractive opportunity to install non-native functionality directly to the cell membrane, enabling modulation of cell−cell and cell−microenvironment interactions along with targeted delivery of therapeutic agents.[1−5] Recently, receptor-engineering of cell surfaces using multiplex genome editing has emerged as a potent treatment in oncology, such as chimeric antigen receptors (CAR),[6,7] reaching the clinic, despite their challenging manufacturing and transport processes.[8,9] For example, lentiviral and γ-retroviral transduction delivery of transgenes can lead to a variable copy number, semirandom integration, heterogeneous expression, and insertional mutagenesis.[10−13] Re-engineering cellular interfaces with synthetic polymers provides an alternative platform for potential advancement of fields, including cell-based therapies to alter cellular signaling pathways, mask surface antigens, and install unnatural functionality through recruitment of bioactive macromolecules,[14−16] drug cargoes,[5,17] and imaging agents.[4,18]

Polymer conjugation to cell surfaces has so far focused on targeting endogenous membrane components using non-specific approaches including covalent conjugation to amino acid residues and electrostatic interactions with the negatively charged cell membrane.[19−21] Such nonspecific conjugation approaches are straightforward but possess caveats for the production of polymer−cell hybrids with functional importance, including lack of compatibility with cell culture conditions, inadaptability for in vivo labeling, inhomogeneous labeling of cell populations, and cell death. Alternatively, membrane insertion of lipidated glycopolymers is particularly appealing for noninvasive remodeling of the glycocalyx to regulate its structural, metabolic, and recognition roles, but the short cell surface retention capabilities may limit its potential applications.[22−24]

Metabolic oligosaccharide engineering (MOE) has emerged as an alternative approach to re-engineer the glycocalyx, allowing the installation of exogenous chemical receptors to glycan residues by "hijacking" the glycan biosynthetic pathway. Addition of biorthogonal, cell surface bound reactive units in

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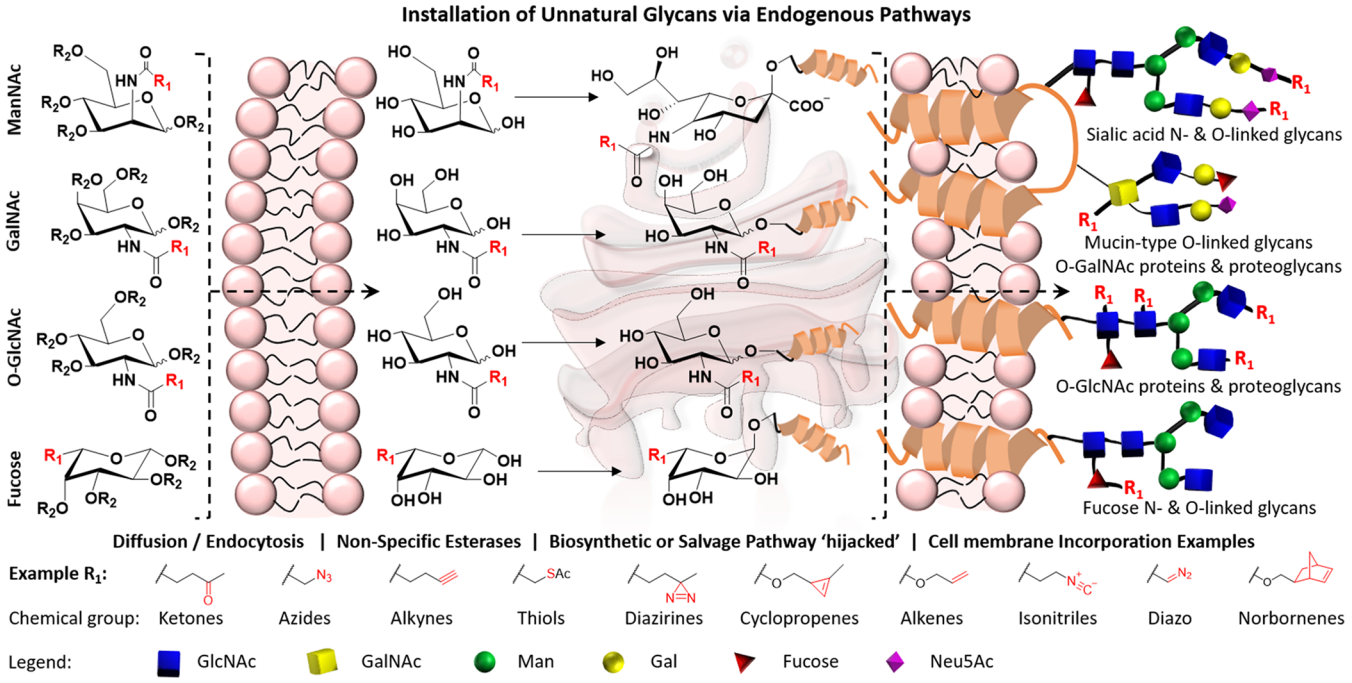

Figure 1. Metabolic oligosaccharide engineering with unnatural derivatives of glycan's allows "hijacking" of biosynthetic pathways of endogenous glycan analogues to install biorthogonal handles (R_1) for chemoselective ligation. R_2 = OH or Ac.

this manner supplies "anchor" sites for targeted in vitro and in vivo delivery of abiotic therapeutic components to the cell surface.[25,26] Due to the expanding potential therapeutic benefits of cell−polymer hybrids, polymer cell surface re-engineering can be considered the next evolution from polymer−protein conjugation; a field that progressed from nonspecific conjugation techniques (e.g., targeting lysine and cysteine side chain groups) to site-selective modification of non-natural amino acids.[27,28] As with proteins, this advancement is underpinned by improvements in regio- and chemoselective "bio-orthogonal" coupling reactions.[29]

Considering the above, this Viewpoint will highlight advances in using MOE as a versatile tool for the recruitment of polymeric nanoscale materials such as synthetic polymers, oligonucleotides, and nanoparticles to the cellular interface; exploring the expanding therapeutic and diagnostic potential in biomolecule capture, drug delivery, microfabrication, and immune therapy. Alternative methods to modify glycans (e.g boronic acids) are not included here, which have previously been reviewed.[30,31] We highlight the opportunities in synthetic polymer/materials chemistry in the context of taking the next steps from polymer−protein to polymer−cell engineering.

Metabolic oligosaccharide engineering (MOE), a technique pioneered by Bertozzi and co-workers,[26] allows the installation of exogenous glycans into the cellular glycocalyx through chemically modified versions of native sugars. These unnatural sugars "hijack" the promiscuous biosynthetic or salvage pathways of endogenous glycans, allowing the installation of biorthogonal functional groups onto the cell surface, Figure 1, and hence recruitment of additional functionality. MOE has enabled the installation of sugars modified with ketone,[26] azide,[25] alkyne,[32] thiol,[33] diazirine,[34] cyclopropene,[35] alkene,[36] isonitrile,[37] diazo,[38] and norbornene[36] functional groups into plants,[39] bacteria,[40−42] and yeast,[43] along with mice,[44] rats,[45] zebrafish,[46] *Caenorhabditis elegans*,[47] and *Drosophila melanogaster*.[48] Unnatural *N*-acetyl mannosamine (ManNAc) derivatives hijack the promiscuous sialic acid biosynthetic pathway, providing the highest abundance of cell surface coverage with biorthogonal functional groups compared to all other unnatural glycan analogues in multiple cell types, including hMSCs, hippocampal, CHO, and MDA-MB-231.[49−52] Cell surface labeling is often achieved following 72 h of incubation with low concentrations of unnatural sugar (50 μM). Alternative unnatural sugar analogues include *N*-acetyl glucosamine (GlcNAc), *N*-acetyl galactosamine (GalNAc), and fucose that, in addition to sialic acids, are overexpressed in certain diseases and may be suitable alternatives to ManNAc for cell surface labeling, depending on glycan overexpression levels and the desirability to label, or avoid labeling, the range of glycans mentioned in Figure 1.[53] Glycans modified with an azide group, such as *N*-azidoacetylmannosamine-tetraacetate (Ac_4ManNAz), are the most widely used glycan for targeted in vitro and in vivo delivery of nanoscale materials due to their synthetic simplicity, commercial availability, and advances in copper-free "click" azide−alkyne reactions, providing a chemoselective and cytocompatible conjugation approach that can occur rapidly under physiological conditions.[50] As glycosylation is a post-translational modification procedure, exogenous target receptors can be introduced to multiple cell types with MOE without resorting to gene editing approaches. Targeted in vivo delivery of Ac_4ManNAz can be accomplished intratumorally,[54] intraperitoneally,[44,55] or even intravenously using caged Ac_4ManNAz derivatives requiring endogenous enzyme cleavage for intracellular uptake, such as histone deacetylase and cathepsin L overexpressed in cancerous cells[56] or caspase-3/-7 in live apoptotic cells,[57] or ligand-targeted liposomes to target expressed or up-regulated cell surface receptors.[54,58] In addition, Xie et al. demonstrated successful incorporation and probing of exogenic azide receptors on mice brain sialoglycans via liposomal delivery of 9-azido sialic acid (9AzSia), highlighting the true in vivo labeling potential of MOE, even surpassing the blood−brain barrier.[56]

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In the following paragraphs, we survey the literature and show the current progress and potential of MOE for recruitment of synthetic and natural polymers, as well as nanoparticles to living cell surfaces, with comparisons to current strategies for polymer/nanomaterial conjugation.

Nonmetabolic polymer−cell hybrid examples: Polymer conjugation to endogenous cell membrane components has shown potential in the masking of cell surface antigens and modulation of biological functions. For example, "stealthy" erythrocytes have been produced by passive installation of polyethylene glycol (PEG) into the cell membrane or by covalent conjugation to membrane proteins to evade immune recognition and reduce malaria parasite binding.[59−63] Similarly, islet cell encapsulation can prevent xenogenic and human embryonic stem cell (hESC)-derived allogenic transplant rejection through modulation of the immediate blood inflammatory response and blocking of host immune cells by natural (heparin,[64,65] thrombomodulin,[66] and urokinase)[67,68] or synthetic (PEG,[69−72] poly-L-lysine,[19,73,74] or polyacrylates)[75−77] polymer conjugation to the cell membrane, through covalent attachment to amino acid residues or polycation electrostatic interactions, while permitting glucose responsive secretion for treatment of type 1 diabetes.

However, the full potential of recruiting polymers to the cell membrane is not merely to provide physical isolation from the immune system, but also to modulate biological processes and functions. Bertozzi and co-workers pioneered glycocalyx remodeling with lipid-terminated mucin mimetic glycopolymers to passively insert glycan epitopes, with no loss of membrane function or mobility.[78] For example, the installation of sialylated glycopolymers onto Jurkat cells, CD34+ hematopoietic stem cells (HSCs), and pig aortic epithelial cells enables recruitment of sialic acid-binding immunoglobulin-like lectin 7 (Siglec-7), a cell surface receptor containing a cytosolic immunoreceptor tyrosine-based inhibitory motif able to attenuate a natural killer (NK) cell response.[24] Godula and co-workers revealed that lipidated synthetic neoproteoglycans, mimetics of native sulfated glycosaminoglycans (HS GAGs), can recruit fibroblast growth factor 2 (FGF2) to induce neural specification downstream signaling pathways in embryonic stem cells (ESCs) deficient in exostosin, a key glycotransferase enzyme required for native HS GAGs assembly.[79] Similarly, rat cortical neurons engineered with chondroitin HS GAG conjugated liposomes, for membrane fusion, have been used to enhance nerve growth factor-mediated signaling and promote neural outgrowth in rat cortical neurons by activating neurotrophin-mediated signaling pathways.[23]

Metabolic recruitment of synthetic polymers: Although re-engineering cellular interfaces has huge potential for cell-based therapies, targeting endogenous cell membrane components presents challenges limiting its translational application including cytotoxicity, short membrane retention time, and lack of specificity.[20] As previously discussed, the use of MOE has various advantages including cytocompatibility, a panel of biorthogonal functional groups to select from and universal applicability across a range of cell types, providing the ideal platform for polymer conjugation. Tomás et al. demonstrated that MOE on adenocarcinomic human alveolar basal epithelial (A549) cells with Ac_4ManNAz allows chemoselective recruitment of strained alkyne-terminated poly(*N*-hydroethyl acrylamide) (pHEA) polymers, Figure 2.[80,81] Altering Ac_4ManNAz dosage enabled modulation of azido glycan incorporation to the cell surface for immobilization of reversible addition−fragmentation transfer (RAFT) synthesized telechelic polymers bearing an azide-targeting [dibenzocyclooctyne (DBCO)] unit and a model cargo, fluorescein. Therefore, Ac_4ManNAz dosage can be varied to prevent polymer oversaturation of target exogenous receptor sites or reduced to restrict polymer conjugation; compared to targeting endogenous membrane components such as amino acid residues. Crucially, cell surface reengineering with synthetic materials was completed in a highly controllable, dose- and molecular weight-dependent manner, with flow cytometry revealing homogeneous labeling of over 95% of cell populations; evident by narrow Gaussian distributed cell populations. Thus, polymer grafting densities using MOE can be fine-tuned to achieve optimum coverage for specific applications where it, and molecular weight dependence, has functional importance (i.e., linkers to prevent cytotoxicity of charged species,[82] enhancing the activity of enzymes,[83] attachment of large biomolecules).[84] The robust covalent linkages developed between azido labeled sialic acid residues and pHEA polymers remained stable for over 72 h, surviving multiple mitotic divisions. Loss of cell surface bound polymer was attributed to polymer passing to daughter cells during mitosis and potentially membrane turnover processes; however, commenting on such complex processes is difficult as the time scale of glycocalyx recycling is highly variable.[85,86] A549 cells untreated with Ac_4ManNAz demonstrated minimal nonspecific binding of pHEA; thus, MOE cells re-engineered

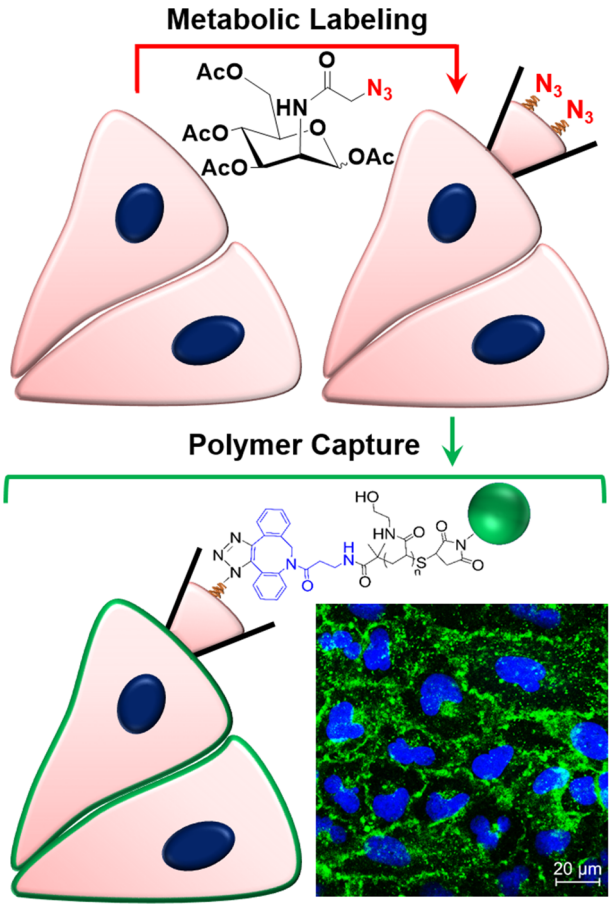

Figure 2. Metabolic glycoengineering with Ac_4ManNAz provides bioorthogonal azide handles for cell-surface recruitment of pHEA polymers bearing DBCO and abiotic fluorescent cargos (green). Adapted from ref 81 and reproduced with permission. Copyright 2019 American Chemical Society.

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with synthetic polymers do not require purification to obtain homogeneously labeled cells due to high labeling efficiencies and selectivity, providing a powerful tool to introduce cell-surface receptors and capture abiotic components for the development of polymer−cell hybrids.

Conventional versus metabolic approaches for polymer recruitment: To demonstrate the potential of MOE for synthetic polymer re-engineering of cell surfaces, comparisons to nonspecific polymer grafting approaches, especially those already discussed as having potential therapeutic value as nonmetabolic polymer-cell hybrid examples, must be considered, Figure 3. For example, one of the most widely exploited moieties for polymer grafting to mask cell surface antigens are the nucleophilic (primarily amine or thiol) side chains of amino acids with, for example, *N*-hydroxyl-succinimidyl ester (NHS) functionalized polymers, Figure 3C.[60,87,88] However, labeling heterogeneity (i.e., production of homogeneously polymer-labeled cell populations) and perturbation of vital protein activity remain drawbacks, along with variable surface retention.[89] Specificity and homogeneity can be improved through site-specific introduction of non-natural amino acids to avoid targeting amino acids with vital functional importance; however, such approaches rely on genetic alterations[90] or metal catalysts to chemically modify endogenous amino acid residues.[91−94] Hawker and co-workers attempted an alternative grafting-from approach whereby Jurkat T cells were modified with NHS-functional RAFT agents for photoinduced electron transfer-reversible addition−fragmentation chain-transfer polymerization directly from the cell surface.[95] Poor cell viability was observed due to mammalian cell sensitivity to mechanical and chemical environmental changes, so lipid insertion of RAFT agents was a necessary alternative to preserve cytocompatibility. While successful, polymer conversions and heterogeneous labeling remained an issue. Grafting-from approaches are also limited due to unwanted side reactions with protein functional groups, protein denaturing, oxygen radical formation, and cytotoxic catalyst requirements, restricting the capability to maintain normal cell culture conditions.[95,96]

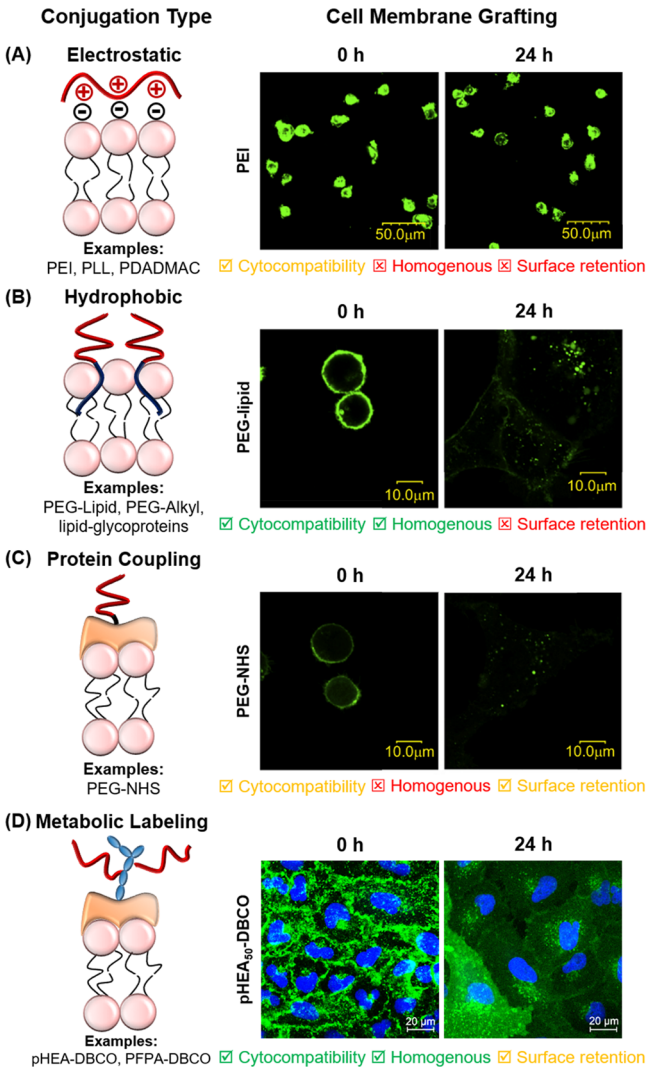

Figure 3. Comparisons between nonspecific conjugation and metabolic cell labeling approaches. Confocal images of live HEK293 cells nonspecifically labeled with (A) poly(ethylene imine), (B) PEG-lipid, and (C) PEG-NHS (from ref 88); and (D) live A549 cells metabolically labeled with Ac_4ManNAz and pHEA-DBCO (from ref 81). All images were taken both immediately and 24 h following polymer grafting to assess surface retention. Confocal images: green = fluorescent polymer; blue = nuclear DAPI stain. Criteria: green tick = positive outcome; amber tick = results vary; red cross = negative outcome. (A)−(C) are adapated from ref 88 with permission. Copyright 2008 Elsevier. (D) is adapted with permission from ref 81. Copyright 2019 American Chemical Society.

Alternative noncovalent approaches include electrostatic deposition of polyelectrolytes to the intrinsically negatively charged peripheral cellular membrane using layer-by-layer (LbL) assembly, Figure 3A.[74] However, direct deposition of polycations remains a huge challenge due to rapid and extensive membrane damage via polycation pores[97−99] or acid-catalyzed hydrolysis of cell membrane lipidic phosphoester bonds.[100] Controlling polycation cytotoxicity remains problematic as various factors can influence cell death, including polyions' functional groups, intracellular polymer uptake,[101,102] and polymer surface charge density.[103,104] In comparison to polymer grafting to proteins and electrostatic deposition of polymers, the metabolic glycan polymer labeling approach (Figure 3D) demonstrates that MOE allows rapid, homogeneous, and efficient polymer conjugation to occur under native biological conditions, with cells retaining ∼90% of viability.[81] Furthermore, the use of exogenous azide receptor sites allows selective installation of polymeric materials with no cellular function deterioration and is universally applicable across a range of cell types.

As previously discussed, hydrophobic insertion of polymers provides a noninvasive, simple, and cytocompatible approach to introduce functionality and has been used to probe and modulate the functional importance of the glycocalyx with synthetic polymers.[23,79] However, passive insertion of lipid-based polymers is limited by rapid dissociation times, with lipid-glycoconjugates possessing a surface half-life of 4−8 h[79] and the complete dissociation of the synthetic PEG-lipid occurring within 3 h due to intrinsic membrane turnover processes, Figure 3B.[89,105] Synthetic polymers installed onto exogenous azide receptors produce robust covalent linkages that survive multiple mitotic divisions, with the cell-surface bound polymer remaining beyond 3 days, a similar time scale compared to polymer−protein conjugation approaches,[20] and the cell surface bound polymer is passed onto daughter cells.[81] In addition, lipid-based approaches lack selectivity, only allowing labeling of the cell membrane, whereas careful

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selection of unnatural sugars can allow (or avoid) labeling of a specific range of glycans (e.g., mucin-type O-glycans);[106] however, it should be noted that MOE is uneasily adaptable to label individual glycoproteins. Thus, metabolic glycoengineering offers clear advantages over conventional polymer conjugation approaches in cytocompatibility, specificity, robustness, and stability.

Enzyme-mediated cell surface engineering provides an alternative approach for more selective candidate protein/molecular conjugation.[107] Enzymes developed to recognize and selectively cleave cell membrane peptide sequences include transglutaminases,[108,109] glycotransferases and hydrolases allowing subsequent site specific conjugation of abiotic materials.[110−112] However, enzymes, such as Transpeptidase Sortase A ("sortagging"),[113] suffer from reaction reversibility and self-competition.[114] In contrast, MOE with unnatural sugars provide the advantage of universal applicability across multiple cell types, with a plethora of functional groups for material capture.

Biomedical and biotechnological applications of MOE with polymers: The versatility of exploiting cell surface metabolic labels offers opportunities in a range of biotechnological and biomedical fields, where precision conjugation can introduce functionality to cells. Tomás and Gibson recruited pHEA functionalized with DBCO and biotin to capture streptavidin-cyanine5 (Cy5) as a simple demonstration of how polymers can be easily adapted to capture biomolecules.[81] Biomolecule capture utilizing MOE has also been extended to antibody immobilization, presenting potential for immunomodulation of the innate immune system. Uvyn et al. installed antibody recruiting polymers (ARPs) consisting of RAFT-synthesized pentafluorophenyl acrylate (PFPA) polymers functionalized with DBCO, a fluorescent marker and DNP onto metabolically glycoengineered Jurkat T cells to successfully capture anti-DNP monoclonal antibodies.[115] This approach was translatable to metabolically labeled mouse 4T1 spheroids, allowing anti-DNP antibodies to be captured with good penetration depth. Natural killer (NK) cell and macrophage activation rely on antibody Fc-domain recognition by types I and II Fc receptors; therefore, capturing antibodies in this manner demonstrates potential to induce innate immune cell responses.[116]

In addition to capturing biomolecules, Shi et al. demonstrated that metabolic glycoengineering of cellular interfaces allows spatiotemporal control over cell−cell interactions through installation of photoswitchable polymers, Figure 4.[117] MCF-7 cells treated with $Ac_4GalNAz$, to label mucin-type O-linked glycoproteins, allowed conjugation of alkynyl-PEG-β-Cyclodextrin (β-CD) using copper-catalyzed azide alkyne cycloaddition (CuAAC), Figure 4a. Upon addition of azobenzene-PEG-azobenzene (azo-PEG-azo) cell aggregation was observed due to the high binding affinity between β-CD and *trans*-azobenzene. This homobifunctional cross-linking agent mediated aggregation was reversed by conversion of trans-azobenzene to its cis form and vice versa using UV and visible light sources. Using a similar strategy, HeLa cells and peripheral blood mononuclear cells (PBMCs) treated with $Ac_4GalNAz$ and alkynyl-PEG-β-CD were used to capture azo-MUC1 aptamers for controllable targeting of mucin 1 protein expressed on epithelial cancer cells (MCF-7; MUC 1+) inducing cell−cell adhesion and enhancing the cytotoxic effects of PBMCs toward MCF-7 cells (Figure 4b). This clearly shows the potential of metabolic glycoengineering for immune

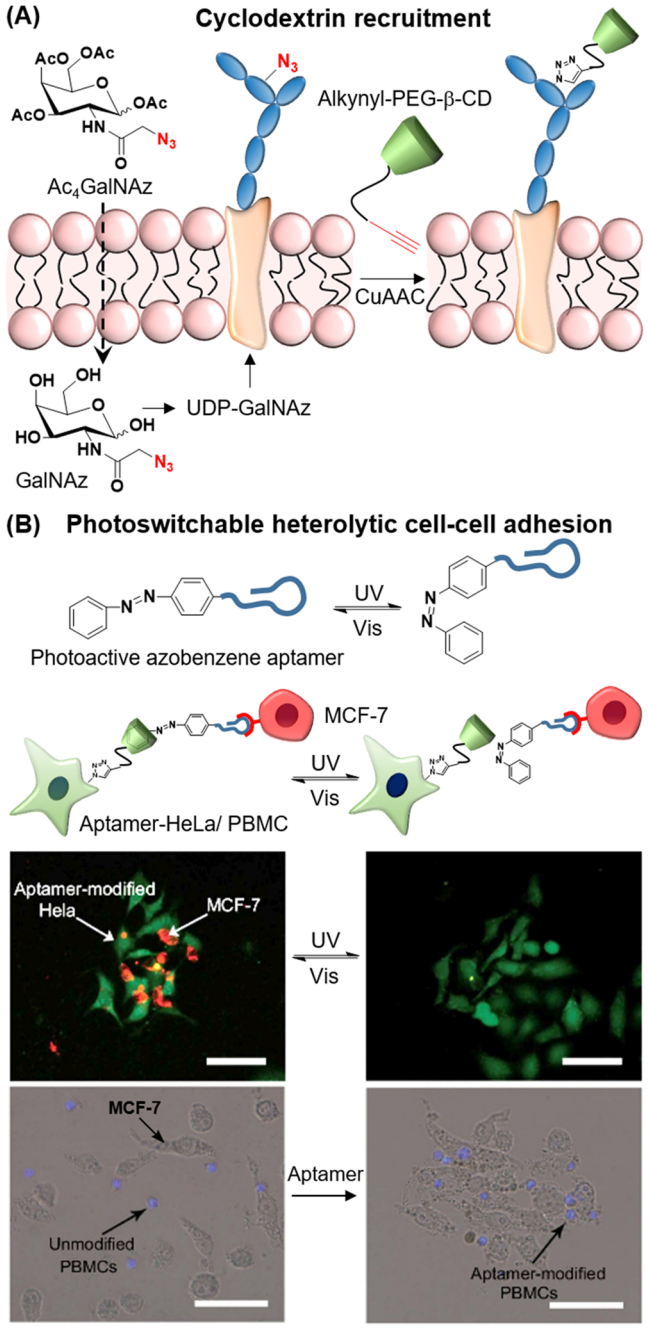

Figure 4. Heterolytic cell adhesion with MOE. (A) Cyclodextrin conjugation to cell surfaces using MOE; (B) recruitment of photoactive azobenzene MUC1 aptamers to Hela (green) and PBMC (blue) cell surfaces for subsequent photoswitchable adhesion to MCF-7 cells (red) expressing mucin 1. Heterolytic adhesion enhances cytotoxicity of PBMCs toward MCF-7 cells. Scale bar = 50 μm. Figure is adapted with permission from ref 117. Copyright 2016 SpringerNature.

therapy and to promote the future understanding of contact-dependent cell communication. MOE can also control cellular interactions to capture cells onto biomaterials including native extracellular matrices,[118] polymer nanofibrous scaffolds,[119] and hydrogels[120] for tissue regeneration and repair or controlling cell adhesion for diagnostic applications; however, this is beyond the scope of this review.

Polymeric nanoparticle delivery via metabolic glycoengineering: Nanoparticles have emerged as delivery carrier tools

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for imaging and drug delivery for both diagnostics and therapy. However, targeted delivery to organs, tissues, and cellular locations remains a challenge, especially with the efficacy of the enhanced permeation and retention (EPR) effect being questioned.[121] Attempts to enhance nanoparticle accumulation at disease sites, including altering physical and surface properties (size or shape), as well as introducing targeting biomolecules (antibodies, aptamers, or peptides), have had insufficient success for clinical use.[122,123] Endogenous receptor sites are limited; thus, the use of specific targeting moieties results in nanoparticle saturation, preventing successful accumulation. Metabolic glycoengineering allows a controlled installation of exogeneous biorthogonal receptors and, as has already been discussed, in vivo labeling is possible to install such receptor targets, removing the previously discussed caveats of conventional approaches. Here, we discuss the imaging, drug delivery, and therapeutic opportunities provided by using MOE for the recruitment of polymeric nanoparticles.

In vivo nanoparticle imaging: Initially, in vivo nanoparticle delivery using MOE relied on intratumoral injection of Ac_4ManAz into xenograft mice models bearing A549 tumors. Exogenous azide receptors allowed selective accumulation of intravenously injected DBCO functionalized PEGylated liposomes (DBCO-PEG-Lipo) with incorporated Cy5-lipid to tumor target sites with minimal nonspecific binding to azido untreated tumors.[124] However, intratumoral pretreatment with Ac_4ManAz is impractical for clinical translation as the exact location/dimensions of tumor is often unknown, thus Kim and co-workers proposed a two-step tumor targeting strategy.[125] First, intravenous delivery of Ac_4ManNAz was achieved by loading into glycol chitosan nanoparticles (Ac_4ManNAz-CNPs), synthesized by conjugation of 5β-cholanic acid groups to a glycol chitosan backbone. In vitro, Ac_4ManNAz-CNPs demonstrated cytocompatibility and universal applicability on multiple cell lines, regardless of surface heterogeneity, and revealed similar labeling capabilities to free Ac_4ManNAz; thus, nanoparticle encapsulation of Ac_4ManNAz allows successful delivery without limiting uptake. Intravenous administration of Ac_4ManNAz-CNP in A549 tumor-bearing mice demonstrated high accumulation of azides at tumor sites by EPR effect of nanosized carriers. Introducing exogenous receptors removes limitations arising from receptor-binding molecules that target limited endogenous receptors and are influenced by tumor heterogeneity. Second, folate bicyclo[6.1.0]nonyne (BCN)-modified and chlorin e6 (Ce6)-loaded CNPs (BCN-Ce6-CNPs), labeled with fluorescein isothiocyanate, were intravenously injected into tumor-bearing mice pretreated with Ac_4ManNAz-CNP for copper-free "click" reaction. Targeted accumulation of BCN-Ce6-CNPs in the tumor tissue of Ac_4ManNAz-CNP treated mice increased over time, demonstrating tumor targeting capabilities, long blood circulation time, and ease of tumor vessel penetration. Laser irradiation of mice treated with Ac_4ManNAz-CNP and BCN-Ce6-CNP induced the photodynamic therapeutic properties of Ce6 resulting in tumor growth suppression, even 21 days after irradiation, showing that this two-step metabolic glycoengineering and nanoparticle accumulation strategy could be successfully used for tumor therapy.

Similarly, metabolic glycoengineering allows in vivo targeted delivery of small molecules[56] and also metal-based nanoparticles possessing both imaging and photodynamic properties for photothermal therapy, allowing accurate and targeted thermal ablation of solid tumors.[126,127] Although another prime example of the therapeutic efficacy of metabolic glycoengineering for nanoparticle delivery, these examples are beyond the scope of this viewpoint.

Nanoparticle tracking is also fundamental for the advancement of stem cell-based therapies, aiding in the understanding of their biodistribution and local microenvironment. However, conventional tracking technologies are not effective for heterogeneous stem cells with low endocytic capacity. Lee et al. demonstrated that MOE of human mesenchymal stem cells (hMSCs) with Ac_4ManNAz (10−20 μM) allows the introduction of azide receptors with no alterations to function, viability, surface markers, or oncogenic gene expression levels, for subsequent controllable, efficient (almost 100%), and homogeneous delivery of imaging agents (Cy5.5, iron and gold) using BCN-CNPs (Figure 5).[49] Particle uptake occurred rapidly (6 h) and remained internalized for up to 5 days in vitro, revealing the successful distribution into daughter cells through cytokinesis (Figure 5B). Dorsal subcutaneous

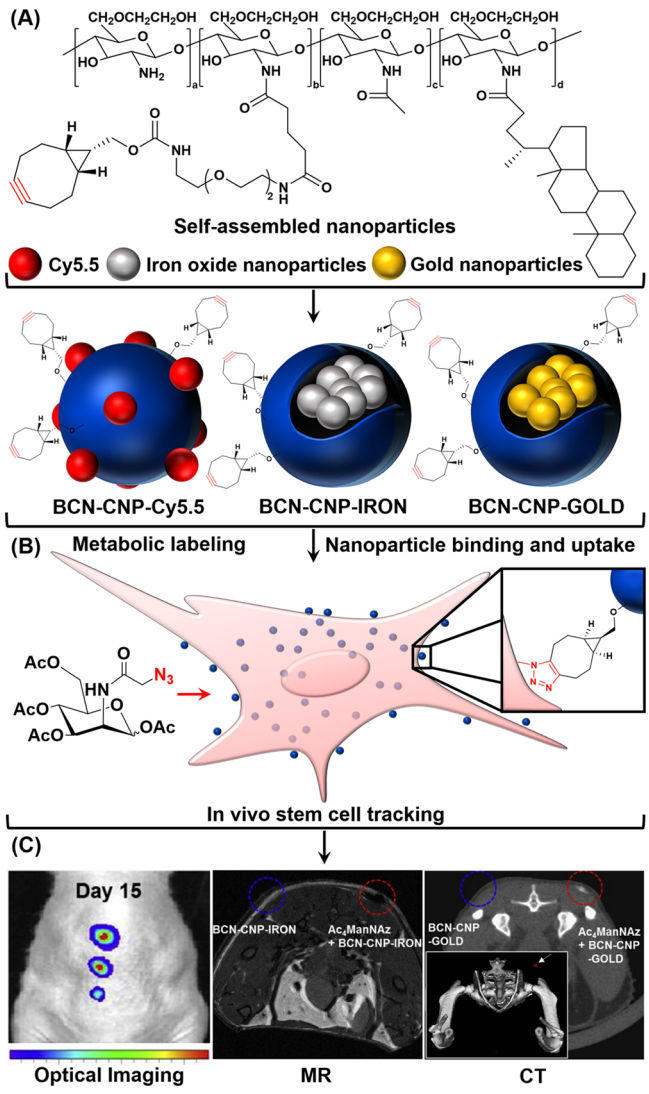

Figure 5. (A) BCN-CNPs are self-assembled under aqueous conditions possessing imaging agents (Cy5.5, iron and gold) and a strained alkyne for (B) cell surface binding and internalization by hMSCs. (C) Subcutaneous implantation into mice allowed non-invasive optical, MR, and CT stem cell tracking. Figure adapted with permission from ref 49. Copyright 2017 Elsevier.

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implantation of BCN-CNP-Cy5.5-labeled hMSCs pretreated with Ac_4ManNAz into nude mice allowed noninvasive in vivo stem cell tracking of as little as 1000 cells for up to 15 days, with a direct proportionality between fluorescence intensity observed at the implanted site and hMSC cell number administered (Figure 5C). The long-retention capabilities of BCN-CNP-Cy5.5 surpasses conventional nanoparticle-based probes in minimizing the risks of in vivo false imaging caused by exocytosis of imaging agents, followed by nonspecific uptake of nearby normal cells or macrophages. Similarly, cellular uptake of BCN-CNP-IRON and BCN-CNP-GOLD particles into Ac_4ManNAz labeled hMSCs were subcutaneously implanted into dorsal regions of mice, demonstrating potential usage in deep tissue tracking of stem cells using MRI or micro CT scanners (Figure 5C). This noninvasive stem cell imaging technology demonstrates that metabolic glycoengineering allows prolonged in vivo stem cell tracking with diverse imageable nanoparticles to obtain high spatial resolution for future stem cell therapy applications.

Nanoparticle drug delivery: Kim and co-workers have shown the potential that metabolic glycoengineering holds for in vivo capturing of nanoparticles as imaging agents, but also possible opportunities as drug delivery systems. Initial studies conducted by Iwasaki et al. confirmed that recruitment of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer nanoparticles, functionalized with hydrazide groups (PMBH), could be used to deliver immobilized anticancer drugs (Doxorubicin (DOX) and paclitaxel (PAX)) in vitro to human uterine cervical cancer (HeLa) cells treated with *N*-levulinoylmannosamine (ManLev) to install cell surface bound ketone glycans.[128] Nanoparticle uptake via endosomal pathways provided controlled release of DOX over 48 h with approximately 60% of cells died with 3 days of cultivation. Similarly, PAX-loaded nanoparticles reduced ManLev-treated Hela cell viability by 50% within 3 days of initial culture. Direct addition of free DOX, DOX-PMBH, free PAX, or PAX-PMBH lead to minimal decreases in cell viability; thus, confirming metabolic glycoengineering possesses delivery capabilities for cell impermeable chemotherapeutic small molecules.

MSCs naturally traffic toward primary tumors and metastases in response to inflammatory signals, making them ideal candidates for therapeutic and diagnostic tools. Layek et al. devised an approach to utilize metabolic glycoengineering of hMSCs to express exogeneous azide receptors for nanoparticle capture, internalization, and subsequent use as an in vivo drug delivery release system (Figure 6).[129] Initially, mice bearing subcutaneous A549-Luc lung tumors were dosed intravenously with Ac_4ManNAz treated MSCs (MSC-Az) labeled with a DBCO-Cy5.5 dye. Tumor-selective accumulation of MSC-Az was detectable for 10 days, whereas in tumor-free animals, fluorescence detection was primarily in the clearance organs such as liver and spleen. Tumor tropism of glycoengineered MSCs-Cy5.5 was also observed after intraperitoneal injection into MA148-Luc ovarian tumor bearing mice for 4 weeks with no fluorescence in brain, lungs, spleen, kidneys, and heart, Figure 6A. Intraperitoneal delivery of PAX encapsulated within DBCO-functionalized poly(lactide-*co*-glycolide) (PLGA) nanoparticles (DBCO-PLGA-PAX) was subsequently demonstrated, targeting orthotopic ovarian tumors pretreated with intraperitoneal injection of MSC-Az (Figure 6B). DBCO-PLGA-PAX nanoparticles were detected within 15 min and demonstrated enhanced tumor targeting and growth inhibition compared to control saline treated tumors (Figure 6B).

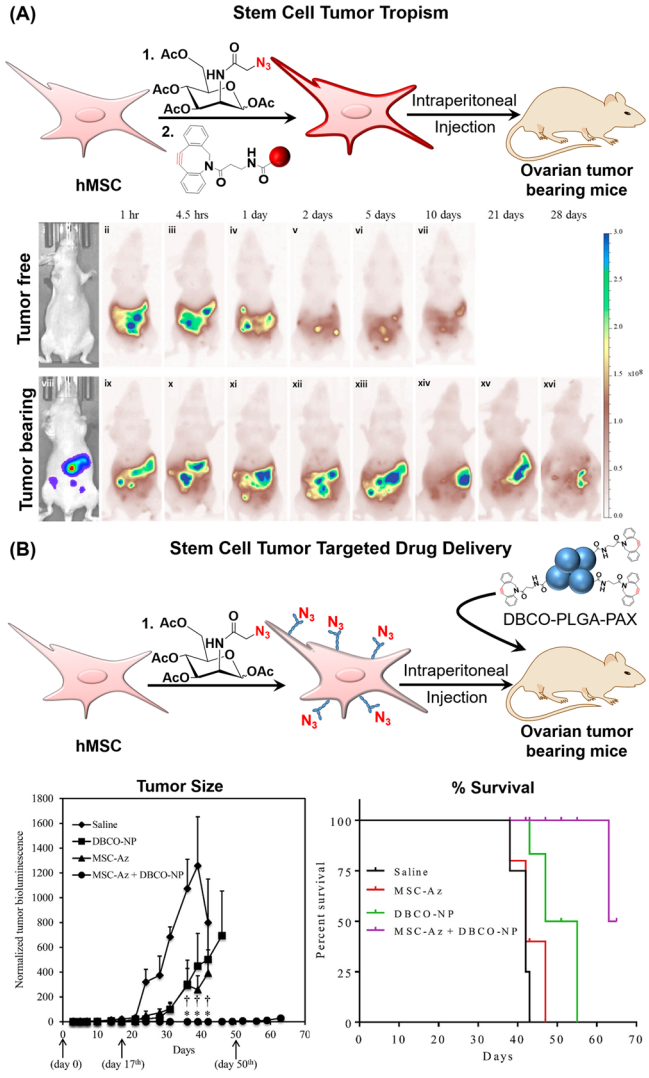

Figure 6. (A) Metabolically glycoengineered stem cells were labeled with DBCO-Cy5.5 and injected intraperitoneally into MA148-Luc ovarian tumor bearing mice to determine tumor tropism capabilities. Tumor-free mice are shown as controls. (B) Intraperitoneal injection of metabolically labeled stem cells was completed again, but followed by intraperitoneal injection of DBCO-PLGA-PAX nanoparticles with all relevant controls. Tumor growth and mice survival were measured over 65 days (n = 4). Figure adapted with permission from ref 129. Copyright 2016 Elsevier.

Although delivery using MSCs can be completed through gene alterations for protein/peptide-based therapeutics, small molecules cannot be easily adapted, demonstrating how metabolic glycoengineering of cell surface receptors provide a simple and practical approach compared to gene therapies.[130]

Microfabrication: Briefly, we summarize the current status of MOE's application for the recruitment of DNA aptamers within microfabrication and for controlling 3D microtissue interactions. Microfabrication, a technique used to generate patterns of cells on surfaces, conventionally relies on universal endogenous cell adhesion receptors, such as integrins, preventing simultaneous adhesion of multiple cell types with high specificity and pattern reproducibility on a single surface.[131] Previously, DNA−polymer hybrids have been grafted to the cell surface to encapsulate multiple cellular organisms,[132] alter intercellular adhesion and interac-

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tions,[133,134] and capture primary cells[135] in an attempt to mitigate caveats of microfabrication with universal cell adhesion moieties. However, DNA aptamer conjugation often relies on NHS coupling to membrane proteins which, as previously discussed, can perturb vital protein function.

Chandra et al. demonstrated that "DNA barcoding" could be achieved using MOE to attach phosphine-ssDNA onto Ac_4ManNAz treated Jurkat cells, enabling patterning onto Au pads with complementary ssDNA strands.[136] Microfabrication in this manner allowed subpopulations of Jurkat cells with different ssDNA and cytosolic dyes to be selectively patterned onto Au pads with two complementary ssDNA for over 25 h, demonstrating specificity, even within the same cell type. Similar results were obtained when applied to adherent cell line Chinese Hamster Ovary (CHO) and HEK cells ensuring the potential widespread use of this approach, for microfabrication of both suspension and adherent lines, providing an attractive means to control adhesion properties of living cells. Douglas et al. further expanded on this work, demonstrating that DNA barcoding allows capturing of cell cocultures within microfluidic systems, consisting of ssDNA-functionalized glass,[137] in desired microscale patterns with robust linkages withstanding lateral shear forces over 5-fold that of physiological levels for any application requiring intact cells in flow systems.[138,139] Thus, microfabrication advancements using MOE provide a universal approach for patterning cell cocultures, with the potential to develop cellular array microfluidic devices with integrated microelectrodes for functional studies.

3D microtissue interactions: Cell surface bound DNA oligonucleotides have also allowed the development of 3D microtissues to control native cell−cell interactions, a current challenge in the discovery of in vitro tissue models or materials for in vivo repair. Gartner and Bertozzi demonstrated that Jurkat cells, possessing different cytosolic dyes, could be metabolically labeled with *N*-azidoacetylmannosamine (ManNAz) and assembled through surface functionalization with complementary and noncomplementary phosphine-ssDNA or difluorinated cyclooctyne (DIFO)-conjugated ssDNA to form a large aggregate, Figure 7A.[140] Microenvironment architecture including size distribution, structure uniformity, and cell stoichiometry could be controlled by adjusting cell ratios and modulating cell−cell assembly rates through altering oligonucleotide sequence complexity and phosphine-DNA labeling concentrations. Purification of assembled 3D microtissues was completed with ease by FACS and removal of linkages from cell−cell junctions was accomplished by heating to 37 °C or using DNase without disrupting topology. This technology was adapted to synthesize a functional paracrine signaling network in 3D, Figure 7B. CHO cells engineered to secret interleukin-3 (IL-3) and untransformed hematopoietic progenitor cell line (FL5.12), which rely on IL-3 for survival and replication, were functionalized with complementary DNA to form a 3D tissue culture within a 3D agarose matrix. Structural growth was observed as IL-3 accumulated within the microtissue site, emulating cytokine-dependent immune expansion and tumor cell proliferation at inflammation sites. In control structures comprising CHO cells lacking the gene encoding IL-3, the FL5.12 cells displayed no growth and instead developed phenotypes consistent with apoptosis. This approach demonstrates how MOE for DNA aptamer attachment can be used to investigate cellular communication within microenvironments with potential to replicate stem cell niche tissue function,

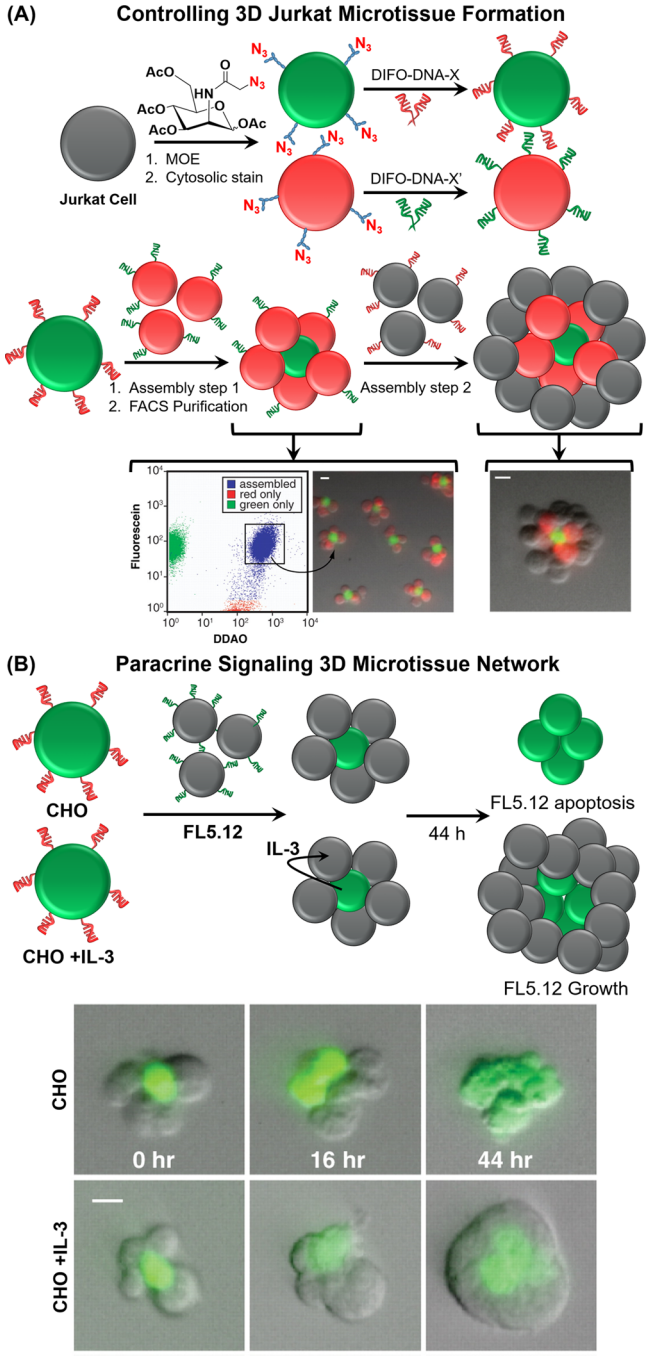

Figure 7. Microtissues by DNA conjugation. (A) Metabolic labeling of Jurkat cells allows cell surface recruitment of complementary ssDNA to form cell assemblies; (B) Construction of a microtissue possessing a paracrine signaling network using complementary DNA strands. CHO cells expressing murine IL-3 (and GFP) supply IL-3 for growth of murine pro-B cell line FL5.12, whereas in its absence apoptosis occurs. Figure adapted with permission from 140. Copyright 2009 NAS.

develop high-throughput screening platforms, and recapitulate human disease (tumor-like phenotypes or differentiation)[141,142] as in vitro models.

Concluding remarks: This Viewpoint has summarized the recent advances in metabolic glycoengineering for the recruitment of natural and synthetic polymer materials to cell surfaces. As was the case with polymer−protein conjugation for the past two decades, the field of cell engineering is moving

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from nonspecific methods (and associated challenges of characterization, heterogeneity, purification) to site-specific conjugation. Metabolic oligosaccharide engineering enables the selective introduction of a diverse range of functional handles to enable the capture of polymeric materials in a convenient and mild manner, taking advantage of advances in bioorthogonal chemistry. This Viewpoint has summarized how recruitment of synthetic macromolecules to cellular interfaces has allowed the advancement of (nonexclusively) cell-based therapies, in vivo cell tracking, biomolecule capture, photothermal therapy, drug delivery, 3D microtissue formation, and microfabrication. Clearly there is a significant opportunity to integrate synthetic polymer materials with living systems via site-selective conjugation methods. This is still an emerging field, and challenges still remain, including the following: (i) Balancing cell-surface labeling versus uptake, due to glycan recycling pathways, to ensure the correct lifetime of labels (or to promote update where delivery is desired); (ii) Selectively targeting individual glycans or locations of the glycocalyx; (iii) In vivo/vitro stability of linkages to ensure robustness over lifetime of experiments/investigations; (iv) Impact of conjugations on cellular function, to ensure they are passive where desired, but also active and responsive when needed.

AUTHOR INFORMATION

Corresponding Author

Matthew I. Gibson − *Department of Chemistry and Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom;* orcid.org/0000-0002-8297-1278; Email: m.i.gibson@warwick.ac.uk

Author

Ruben M. F. Tomás − *Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom;* orcid.org/0000-0002-7191-2539

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmacrolett.0c00317

Author Contributions

The manuscript was written through the contributions of both authors. All authors have given approval to the final version of the manuscript.

Notes

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