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Core and Corona Modifications for the Design of Polymeric Micelle Drug-Delivery Systems

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Congratulations to Robert Langer, Koch Professor, MIT, on winning the Wolf Prize in Chemistry, 2013

Abstract: Polymeric nanoparticle micelles are formed from amphiphilic polymers with a hydrophobic core and a hydrophilic corona. Often comprised of a biodegradable, biocompatible polymer core and a poly(ethylene glycol) corona, these nanoparticle micelles encapsulate a hydrophobic drug and enable surface modification with targeting ligands. Strategies to enhance hydrophobic drug encapsulation are described as chemistries that facilitate covalent modification with antibodies using water-based click chemistry.

Keywords: click chemistry · drug delivery · micelles · nanoparticles · polymers

1 Introduction

The effective treatment of human diseases using small-molecule and macromolecular therapeutics continues to present significant challenges to the biomedical community. In part, this stems from the physical properties of individual drug candidates, which are typically hydrophobic and poorly aqueous soluble. This necessitates the use of excipients to solubilize these compounds for delivery, which often have their own associated toxicity. The poor selectivity of many small-molecule therapeutics also often results in dose-limiting side effects when delivered systemically.[1] Alternative macromolecular therapeutics under development that use RNA, DNA and proteins display good solubility in aqueous conditions, but can suffer from degradation and short circulation times in vivo.[2]

Nanoparticles have been studied as an alternative strategy to circumvent the broad distribution profile of small-molecule therapeutics, to protect sensitive biomacromolecules and deliver them more selectively to a required site of action.[3] A key feature of these composite nanoparticles is that they demonstrate 'value added' properties, that is, they can serve as delivery and diagnostic vehicles and/or deliver multiple molecules simultaneously. Moreover, such materials can now accurately interface with both small molecules and biological macromolecules in a manner that does not significantly disrupt their innate function.[4] Continuing efforts are now focused on developing these materials to meet the stringent requirements for benign in vivo circulation and improved pharmacokinetic properties. The synergistic properties intrinsic to numerous nanoparticle platforms continue to make them attractive as potential drug-delivery systems for cancer, viral infections, cardiovascular disease, pulmonary and urinary tract infections.[5]

Despite the range of current nanoscale drug-delivery systems in development, such as colloidal metals, liposomes and polymeric nanoparticles, few have successfully reached the clinic.[4,6] Creating particles that contain a clinically relevant drug dose that can release this payload within the diseased cell remains a challenge.[7] Keeping the nanoparticle diameter below 200 nm and including poly(ethylene glycol) (PEG) on the surface have been shown to partially decrease mononuclear phagocytic system (MPS) clearance.[5] Additionally, nanoparticle shape plays a significant role in delivery-system efficacy, specifically in relation to circulation time and the mechanism of cellular internalization.[8] Most of the current successes in nanoparticle drug delivery have been with cancer chemotherapeutics, where nanoparticles accumulate in tumours due to the enhanced permeability and retention (EPR) effect. However, variations in tumour/vasculature physiology and the many biological transport mechanisms involved in biodistribution still require better control of nanoparticle surface functionalization to achieve directed delivery.[9] Towards this goal, optimizing ligand density has become an important factor to balance the degree of targeting and clearance.[6a] Specifically, the degree of avidity and multi-valency for a given nanoparticle platform has been shown to significantly impact internalization rates and biodistribution based on the in vitro

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or in vivo model under study.[10] Continued advancements in the understanding of disease pathologies are thus inspiring a new set of bioengineering criteria to tailor the specific physiochemical properties of nanoscale drug-delivery systems.[9,11] To maintain this convergent interplay between biology and materials science, the introduction of functional groups capable of promoting high drug loading and selective biomacromolecule conjugation is an important design consideration for the engineering of multifunctional drug-delivery systems.

A number of excellent reviews have outlined the different types of nanoparticles currently being developed for drug delivery and the numerous physiological barriers that will affect in vivo pharmacological profiles.[3,4,9] We focus our attention herein on polymeric nanoparticle micelles and recent strategies to bioengineer them for use as more efficient drug-delivery systems. Specifically, we look at how chemical modifications of the core-forming polymer chains can be used to increase drug loading and in vivo stability. We also review how functional groups can be incorporated into the micelle corona for selective coupling to active targeting motifs using orthogonal click-based reactions.

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2 Polymeric Micelles as Drug-Delivery Systems

2.1 Basic Design and Self-Assembly

Polymeric nanoparticle micelles have been engineered in a number of ways for use as drug-delivery platforms.[12] Typically, these structures are comprised of amphiphilic polymers that self-assemble under specific conditions to yield micellar nanostructures that can be easily manipulated and handled in aqueous solutions.[13] Micelle properties can be tuned chemically to alter size, shape and composition.[14] Selective modification is a key facet in the engineering of polymeric micelles suitable for drug-delivery applications where one needs to ultimately administer clinically relevant doses of a therapeutic to the disease site in a manner that limits toxic exposure. Some of the required features for a drug-delivery system are biocompatibility, sustained stability under relevant in vivo conditions and selective delivery to diseased cells or tissue. These requisite features rely on specific physicochemical properties and can be implemented into the polymeric micelle design using recent advances in polymer and functional group chemistries.[15]

As shown in Figure 1, polymeric micelle formation is dependent on the chemical nature of each hydrophilic (blue) and hydrophobic (grey) block, solvent composition, and polymer concentration.[13] In addition, the amphiphilic polymer can be comprised of either a linear or graft copolymer. Micellization is an entropically driven process based on an equilibrium between attractive and repulsive forces.[16] These forces are mainly hydrophobic in nature, with non-polar segments of the polymer backbone decreasing contact with water. Typical materials used in the hydrophobic block include polyesters (e.g., poly(lactic acid), poly(lactic-*co*-glycolic acid), poly(caprolactone)), polyimines (e.g., polyethyleneimine) and poly(-amino acids) (e.g., poly(aspartic acid)).[17] These well-studied polymers are generally considered biocompatible, which makes them versatile components for drug-delivery systems. Most amphiphilic polymers use PEG as their hydrophilic block. PEG has been approved by the United States Food and Drug Administration (US FDA) for clinical use in a wide range of applications.[18] Alternative hydrophilic polymers to PEG include *N*-(2-hydroxylpropyl)-methacrylamide (HPMA) and poly(acrylic acid).[12a]

The micelles generated from these amphiphilic polymers are thus comprised of a hydrophobic interior

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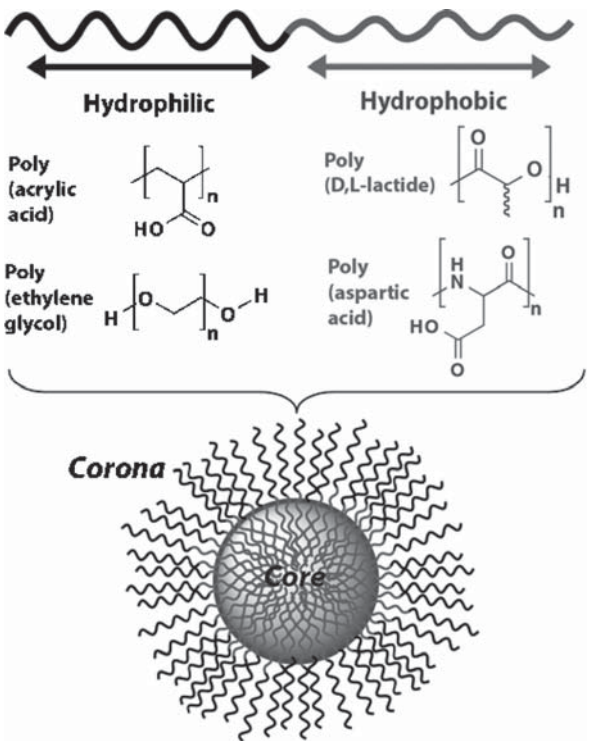

Figure 1. Amphiphilic polymers can be synthesized by using a variety of chemistries, and subsequently, self-assembled into well-defined micelles. Each fully assembled micelle thus consists of a hydrophobic core and hydrophilic corona.

('core') and hydrophilic periphery ('corona'). The functional capacity for these nanosized structures stems from the unique properties inherent to these domains. The core can be used to selectively encapsulate hydrophobic drugs, while the hydrophilic corona ensures solubility and stability under the aqueous conditions required for in vivo administration. Overall, strategic chemical transformation of the core and/or corona can be used to better engineer drug loading, biodistribution and cellular targeting properties of polymeric micelle drug-delivery systems.

2.2 Micelle Stability

Micelles are not static structures, and changes in the environment, such as the presence of a hydrophobic drug, can dramatically change their characteristics. The thermodynamic stability is indicative of how the micelles form and reach equilibrium, while the kinetic stability describes the details of polymer exchange and micelle disassembly.[19] While the thermodynamic stability gives information about the critical micelle concentration (CMC), the kinetic stability is indicative of the rate of dissociation below the CMC; an important parameter for the use of micelles in vivo. Fast dissociation means that, upon dilution below the CMC in the bloodstream, the nanoparticles will quickly fall apart. If the dissociation is slow, a nanoparticle may stay intact long enough for it to reach its target within the body. Thus, kinetic stability has significant implications for the efficacy of a polymeric micelle delivery system.[19]

Efforts have been made to improve the long-term stability of polymeric micelles by cross-linking the core post-micellization. Although this approach has shown some success, the drug loading of these micelles is generally low, drug release is slowed, and cross-linking reduces the mobility of the hydrophilic segments to make the particles larger.[20] Thermodynamic modelling of drug loading in the micelle core has shown that it is limited by three parameters: the size of the block copolymers, the interaction parameter between the drug and the hydrophobic core, and the interfacial tension between the core and the corona.[21]

3 Core Chemical Modifications to Improve Drug Loading and Stability

Polymeric micelles have shown good biocompatibility and their ability to encapsulate hydrophobic chemotherapeutics within their core makes them promising drug-delivery vehicles.[22] Despite their promise, drug delivery by polymeric micelles has been challenging due to limited in vivo stability and insufficient therapeutic loading. Many of these challenges stem from a lack of understanding of the dynamics of the micelle system and poor characterization of the drug encapsulation mechanism.[23] Research on polymeric micelles for drug delivery has been focused on the characterization of the micelle itself and not necessarily on the interaction between the carrier and cargo. Specifically, polymers are developed that have a very low CMC, a narrow polydispersity index (PDI) and a uniform spherical morphology upon micellization.[19] After polymer optimization, small hydrophobic drugs are encapsulated within the polymeric micelle for delivery to cells.

When a drug is introduced into the system, it will have a certain affinity for the core that dictates the magnitude of its incorporation. This is described by the Flory–Huggins interaction parameter, which evaluates the solubilization of a drug in the polymeric micelle [Eq. (1)]:

$$\chi_{sc} = (\delta_s\text{-}\delta_c)2\,V_s/RT \quad (1)$$

in which δ_s and δ_c are Schatchard–Hildebrand solubility parameters of the drug and core-forming polymer block, respectively, and V_s is the molar volume of the drug. A lower value indicates greater compatibility between the drug and the core. This parameter suggests that there is no universal polymer that can be used for every drug.[24] Although hydrophobic interactions have shown moderate drug loading ($\approx$10% w/w), facilitating drug–polymer in-

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teractions based on the drug's chemical structure allows for higher drug loading and greater micelle stability. Ideally, delivery systems will have a high percentage weight of drug, while maintaining the pharmacokinetic profile of the micelle.

Introducing chemical functionalities into the core that can participate in non-covalent interactions with the drug is an alternative strategy to improve both the drug loading and kinetic stability of the micelle. A summary of these interactions is shown in Figure 2, some of which we describe in more detail below.

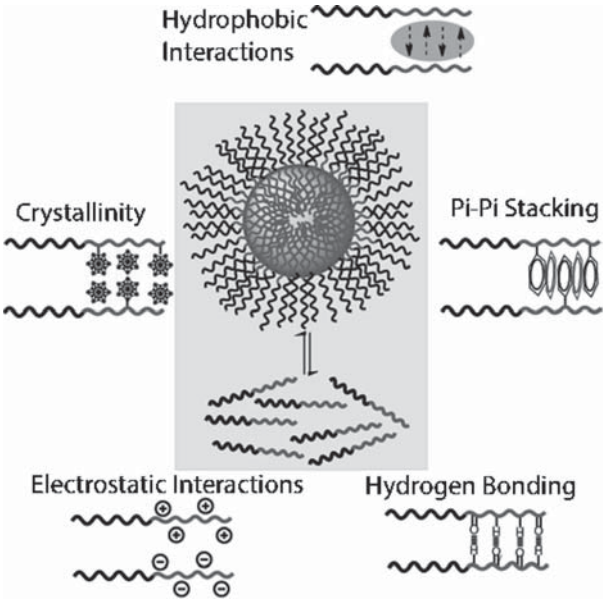

Figure 2. Schematic representation of various core interactions that can be incorporated to both stabilize micelles and increase drug loading.

3.1 Hydrophobic Interactions

Most of the interest in improving the affinity of the drug for the core is focused on hydrophobic interactions within the core of polymeric micelles. One of the most fundamental ways to improve the drug loading is to change the ratio of hydrophobic to hydrophilic polymer block length.[24] While this modification increases the cargo space, it also causes a reduction in stability due to less shielding, causing the micelle to dissociate rapidly.[25] To evade a reduction in stability, higher drug loading can be achieved by increasing the hydrophobicity of the core without changing the ratio between the hydrophilic and hydrophobic blocks. This can be done by using alternative core-forming blocks or by chemically modifying polymers with hydrophobic functionalities.

Several groups have studied the differences in drug loading between poly(lactide) (PLA) and the more hydrophobic poly(ε-caprolactone) (PCL) cores with PEG coronas. Dormidontova et al. investigated the difference in loading of either doxorubicin or β-lapachone.[25] The core influenced the release kinetics of both drugs both experimentally and through modelling. The release rate of both drugs was significantly slower from the more hydrophobic PCL core, while the drug loading was significantly higher. A detailed explanation of this effect was recently described by Inoue et al., who showed using differential scanning calorimetry (DSC), wide-angle X-ray diffraction (WAXD) and UV analyses, the variations in interaction between a hydrophobic drug, quercetin, and these polymers.[26] These analyses showed that interactions were limited to the hydrophobic core in PCL–PEG polymers, while the drug interacted with both the core and the corona in the more hydrophilic PLA–PEG formulation.

Hydrophobic effects can be further exploited by either post-functionalizing polymers or by incorporating a modified monomer into the hydrophobic polymer block. Hedrick et al. used a PEG-poly(trimethylene carbonate) (PTMC) with several cholesteryl 2-(5-methyl-2-oxo-1,3-dioxane-5-carboxyloyloxy)ethyl carbamate groups incorporated to improve micelle formulations of paclitaxel.[27] Polymers with incorporated cholesterol groups gave micelles with high paclitaxel loadings of around 15% and exceptional kinetic stability. An alternative strategy to improve formulations of paclitaxel came from Hammond et al., who post-functionalized a poly(propargyl-L-glutamate) with a variety of six different hydrophobic side groups.[28] They found that these modifications improved drug loading, but more importantly had a dramatic impact on the particle stability in blood. Polar side chains led to higher CMC values, but also showed enhanced kinetic stability in the presence of serum proteins.

3.2 Core Crystallinity and π–π Stacking

While increasing the hydrophobicity of the core is a general strategy that will increase the loading of a wide range of hydrophobic drugs, recent trends have focused on facilitating specific interactions between a drug and the core based on the chemical groups in the small molecule. One of the most explored interactions is π–π stacking to establish core crystallinity. The earliest example came from Kataoka et al., who conjugated doxorubicin to a polyaspartic acid-PEG to form the polymeric micelle *NK911*.[29] Although the conjugated doxorubicin showed no antitumour activity, free doxorubicin was entrapped within the hydrophobic core due to stacking interactions with the conjugated drug. These interactions give a gradual release of free drug over a 24 h period. Drug–drug stacking approaches have been applied to other polymers and drugs[30], including docetaxel[31] and paclitaxel,[30,32] with some success. The balance of conjugated drug to hydrophobic polymer is delicate. When taxanes are conjugated directly to polymers, the CMC increases to cause rapid dissociation upon dilution.[33]

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In an effort to enable specific π–π stacking interactions, polymers with aromatic groups can be used. Hennink et al. have synthesized analogues of poly(2-hydroxypropyl)methacrylamide (P(HPMA))–PEG with either benzoyl or naphthoyl groups to form polymeric micelles with the drugs paclitaxel and docetaxel.[33] The micelles formed showed very high drug loading (>30 wt%) and increased stability. Using solid-state NMR spectroscopy, these improved features were attributed to π–π stacking between the aromatic rings on the drugs and those appended to the polymers. A similar strategy was used by Zhang et al. to improve doxorubicin loading in a PEG–polyamide amine micelle. Incorporating phenyl groups onto the hydrophobic segment of the copolymer enabled π–π stacking and increased drug loading up to 25 wt%.[34]

3.3 Electrostatic Interactions and Hydrogen Bonding

Additional intermolecular interactions can increase the compatibility of a drug for the core, as well as improve the core stability without covalent modifications that may influence the particle properties.

Electrostatic interactions within the core provide a sustained release profile of a specific drug and improve structural stability.[35,36] By incorporating an opposing charge on the hydrophobic polymer, weak charges on small molecules are stabilized. Borsali et al. showed specific interactions between a poly([2-dialkylamino)ethyl methacrylate] core and several drugs with weak carboxylic acid groups, including ibuprofen and indomethacin.[36] ^{1}H NMR spectroscopy measurements confirmed acid–base interactions and improved loading capacities.

Acid functionalized polymers, such as poly(aspartic acid) or acid-functionalized polycarbonates, are stabilized with the addition of cations during the micellization process to increase drug loading and prevent burst releases.[37] Acid-functionalized polycarbonates have been used extensively in the Hedrick and Yang labs for the incorporation of amine-containing drugs through acid–base interactions.[37c] Specifically, the anti-cancer drugs daunorubicin, tamoxifen, imatinib and doxorubicin, all of which contain amines, have been incorporated with drug loadings of up to 35%. While the presence of the acids does increase the CMC due to a reduction in hydrophobicity, incorporating urea-containing polycarbonates to form mixed micelles provides enhanced stability within the core through hydrogen bonding.[38]

Incorporating hydrogen-bond donors and acceptors into the polymer can facilitate improved drug loading and stability, as shown through molecular dynamics simulation of cucurbitacin and polycaprolactone.[38] An increase in hydrogen bonds between the drug and the polymer decreases the Flory–Huggins interaction parameters, thereby increasing the drug solubility within the core.

4 Selective Chemical Group Modification of the Corona

4.1 The Importance of Surface Chemistry and Active Targeting Ligands

Although chemical modifications of the hydrophobic micelle core improves overall stability and drug-loading capacity, chemical modification on the hydrophilic corona with ligands that interact with receptors expressed on the cell surface of diseased cells enables active targeting.[17a] This is a crucial feature for effective polymeric micelle design, because a primary limitation of small-molecule drugs or unfunctionalized micelles is non-specific systemic interactions that effect both healthy and diseased cells. Although unmodified micelle carriers may display longer distribution times and passively enter specific areas (such as tumours through the EPR effect), cellular uptake may be minimal without the inclusion of groups that will actively mediate entry through one of the endocytotic pathways.[5] In some cases, these ligands also elicit a therapeutic activity themselves. These 'active' targeting groups are now being employed to help navigate through the various biological barriers en route to the intended site of action for a number of diseases.[9]

A wide range of ligands are currently being explored for receptor-based targeting of therapeutics.[39] These include vitamins (e.g., folic acid), sugars (e.g., *N*-acetylgalactosamine (GalNAc)), peptides (e.g., RGD), proteins (e.g., transferrin), aptamers (e.g., AS1411), antibodies (e.g., trastuzmab, huA33, brentuximab) and antibody fragments (e.g., trastuzmab Fab).[40] The common feature amongst these ligands is that they can be used to selectively recognize receptors over-expressed on either cells or tissues specific for a particular disease-related molecular pathology. Cell recognition of the targeting group modified nanoparticles can enhance uptake into the cell with subsequent release of a therapeutic payload.

4.2 Strategies to Conjugate Materials to the Micelle Corona: The Power of 'Click'

One of the primary challenges in designing micelle drug-delivery systems is to accurately interface the corona with small molecules or biomolecules to create multi-functional surfaces. Surfaces can either be modified using physical adsorption or through covalent linkages; the latter of which is desirable to maintain bioavailability and attachment of ligands in vivo. Modifying the shell can be achieved pre- or post-assembly of the amphiphilic polymers. This will depend mainly on the system being used and whether polymer modification alters self-assembly. In general, the post-self-assembly micelle modification strategy works well because the hydrophilic segments remain accessible at the aqueous interface, their bioactivity is not

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affected by organic solvents used in the processing step, and self-assembly is not hindered.

Tuning surface chemistry and controlling the degree of labelling both require the incorporation of functional groups that are capable of conjugating active targeting moieties. Coupling conditions are especially important for classes of biomacromolecules that derive activity based on their specific folding. These molecules are sensitive to harsh conditions and their structure can be destroyed by the most common organic solvents. Specifically, we focus herein on functional groups that allow for both facile and orthogonal reactivity under aqueous coupling conditions, where biomacromolecules should remain stable and optimally functional. As such, the use of bio-orthogonal chemistry can be of paramount importance to maintain activity for polymeric micelle delivery systems. Classical bioconjugation chemistry, which includes amidation reactions between amines and carboxyl groups, has been used extensively to modify the polymeric micelle shell.[41,42] Other chemistries include the highly specific biotin/avidin, hydrazone formation, and metal chelators (e.g., nitriloacetic acid (NTA)).[4] Although these approaches continue to find efficient use in micelle functionalization, they can be less selective and lead to cross-reactivity or by-product formation.

In 2001, Sharpless and co-workers introduced the concept of the 'click' reaction, which generally refers to a reaction that is high yielding, produces few or no by-products, and contains functional groups that minimally cross-react (especially with other common biological functional groups).[43] Two of the most popular reactions are based on existing chemistries: the Diels–Alder (DA) and Huisgen 1,3-dipolar cycloadditions. Although thiol–ene and thiol–yne reactions do meet a number of the main click criteria, conditions must be more tightly controlled to avoid cross-product formation. However, the thiol–maleimide reaction continues to be a common means to attach peptides or small molecules in a number of systems.[44] Click functional groups provide chemoselective coupling routes that can be performed under relatively benign conditions and are invaluable for introducing targeting ligands onto the polymeric micelle surface. A number of excellent reviews have compiled the specific reaction conditions used for the above-mentioned coupling strategies and we focus herein on recent polymeric micelle corona transformations.[45]

4.2.1 Huisgen 1,3-Dipolar Cycloaddition

One of the most common click reactions that has found widespread use for biomolecule conjugation is the Huisgen 1,3-dipolar cycloaddition between an azide and alkyne to produce a stable 1,2,3,-triazole bond (Figure 3A). The reaction rate was found to be significantly catalyzed with copper and is referred to copper(I)-catalyzed alkyne and azide cycloaddition (CuAAC). However, the copper catalyst required for this reaction can be difficult to remove and has been identified as toxic in vivo.[45d] Although generally not limiting from a materials science perspective, researchers have also begun to favour the catalyst-free strain-promoted click reaction developed by Bertozzi and Jewett to circumvent any associated metal toxicity.[46] One example of this is shown in Figure 3B, where an azide is selectively reacted with a strained cyclooctyne derivative, dibenzylcyclooctyne (DBCO). The broad scope, high yields and bio-orthogonal coupling conditions have made this reaction the most popular click reaction used today across a number of materials and biomedical disciplines.

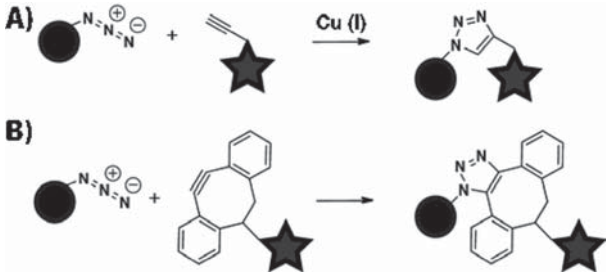

Figure 3. The Huisgen 1,3-dipolar coupling reaction to create a stable 1,2,3-triazole bond can be done under A) copper-catalyzed or B) metal-free conditions with a sterically hindered cycloalkyne.

Modified amphiphilic polymers can be self-assembled to form polymeric micelles that then display click functional groups (either alkyne or azide) distributed throughout the corona. Subsequent coupling can be done in aqueous conditions to selectively install a variety of targeting ligands onto the micelle surface. Alternatively, small molecules can be coupled prior to micelle self-assembly[47] or surface groups can be used to insert appropriate click ligands.[48] Shoichet and co-workers produced an early generation of clickable amphiphilic copolymers from hydrophobic poly(2-methyl-2-carboxytrimethylene carbonate-*co*-D,L-lactide) (poly(TMCC-*co*-LA)), which was derivatized with a hydrophilic PEG–azide.[49] Self-assembly generated a micelle with an azide shell that was selectively modified in an efficient manner by click chemistry with an RGD peptide for integrin–receptor-mediated cell attachment. These polymeric micelles could be modified with up to 400 peptides and showed selective interaction with integrin receptors on rabbit corneal epithelial cells. More importantly, this system can be used as a platform to couple a variety of targeting ligands and to carry therapeutic small-molecule drugs.

A variety of elegant, click-functionalized polymer capsules have been produced by Caruso and co-workers.[45c] They generated a polymeric micelle system that displayed alkynes throughout the corona.[50] Subsequent click reactions were used to couple a humanized monoclonal antibody (huA33) that specifically targeted colorectal cancer cells. This functionalized polymeric micelle delivery

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system was able to selectively target colorectal cancer cells in mixed-cell populations where the target was as little as 0.1%. Additionally, a PEG spacer was used to help reduce antibody aggregation caused by the copper-catalyzed coupling conditions. Organomicelles have also been modified to contain DBCO on the corona.[51] A number of azido-containing molecules (fluorophore, biotinylated and peptide) could then be efficiently coupled to the micelle surface using the strain-promoted click reaction. More recently, click chemistry has been combined with other functional groups (such as disulfides) to tailor the degradability and molecular responsiveness of polymeric micelles for better drug-delivery release profiles.[52]

Variations on the Huisgen 1,3-dipolar cycloaddition reaction, either metal-catalyzed or using strained alkynes, will continue to allow researchers to selectively modify polymeric micelle surfaces for potential drug-delivery applications. Perhaps more importantly, the combination of this click reaction with other available functional groups will be an efficient route to create multi-functional micelle surfaces, as discussed below.

4.2.1 Diels–Alder Cycloaddition

Much like the 1,3-dipolar cycloaddition, the DA [4+2] cycloaddition reaction is an effective strategy for the selective modification of various materials[53] and is well suited for surface functionalization of polymeric micelles. The DA reaction couples an electron-rich conjugated diene and electron-poor alkene (commonly referred to as a dienophile) to generate a stable cyclohexene ring (Figure 4A).[54] This reaction is generally selective, with maleimide and furan functional groups being two of the more common reactive precursors.[54] The reaction is also thermally reversible (retro-DA), but typically requires temperatures greater than 100 °C. This cycloaddition is an orthogonal reaction and has been demonstrated to proceed rapidly and in high yield under mild aqueous reaction conditions.[53]. Additionally, no metal catalyst is required for the reaction to proceed, which makes this coupling strategy attractive for the design of in vivo drug-delivery systems.

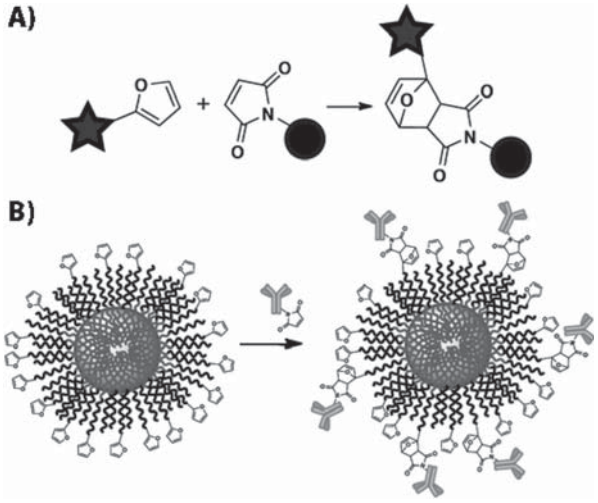

Figure 4. A) An example of transition-metal-free [4+2] DA cycloaddition between furan and maleimide functional groups. B) The high coupling efficiency of the DA reaction was used to selectively couple furan groups on the corona of a polymeric micelle with maleimide-modified antibodies.[55]

An early example of polymeric micelles functionalized with DA cycloaddition chemistry incorporated a furan diene into the polymeric corona (Figure 4B).[55] Coupling could be achieved between maleimide-modified antibodies (trastuzumab) and furan-functionalized PEG units on the micelle corona. Although antibody conjugation for this post-micelle modification scheme proceeds efficiently (MES buffer, pH 5.5, 37 °C), a large number of furans remain available for subsequent conjugation to small molecules. These immuno-polymeric micelles were shown to specifically target HER2-over-expressing cells. The selectivity and mild coupling conditions for the DA cycloaddition make it a unique reaction to engineer multi-functional polymeric micelle drug-delivery systems, of which the full potential has yet to be realized.

4.2.3 Multi-click Polymeric Nanoparticle Shell Functionalization

Among the current challenges for nanoparticle drug-delivery platforms is how to selectively incorporate different moieties onto the same micelle surface. To do so would allow for various multi-ligand relationships and possible 'theranostics' (containing both drug carrier and diagnostic capability) to be tested for better targeted delivery.[56] The orthogonal reactivity between Huisgen 1,3-dipolar and DA cycloaddition chemistries makes this an advantageous pairing for designing a multifunctional, polymeric micelle, drug-delivery platform.[42]

Chan et al. created polymeric micelles that displayed two orthogonal click functional groups.[57] As shown in Figure 5, two modified polymers that contain either a furan or an azide are mixed and subsequently self-assemble to generate the first dual, clickable polymeric micelles. The micelle surface can be sequentially labelled in a selective manner with maleimide and DBCO functional groups under aqueous conditions. To be most effective, the DA reaction should be done before the strain-promoted click reaction because there is some cross reactivity between DBCO and the furan functional groups. As a proof of concept, the micelle shell was coupled to both a trastuzumab–maleimide conjugate and FLAG–DBCO peptide. Treating the ovarian cancer cell line SKOV-3luc with these dual-functionalized micelles showed co-localization of the antibodies and peptides by confocal imaging. More recently, this same group has utilized the selective dual click reactivity to create and demonstrate the in

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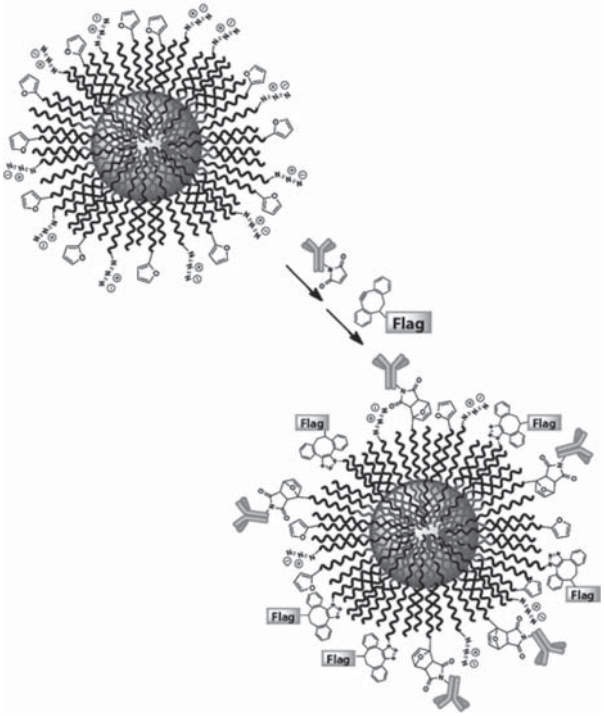

Figure 5. Polymers modified with either a furan or an azide functional group are mixed together and self-assembled into a polymeric micelle. Coupling of the corona functional groups in a sequential fashion, first with a maleimide-modified antibody then DBCO-modified peptide, generates a dual-functionality micelle platform.[57]

vitro therapeutic potential by gene knockdown of a polymeric micelle that contained both an oligonucleotide therapeutic (siRNA) and selective targeting ligand (trastuzumab).[58]

Combining click reactions can be efficiently performed under aqueous conditions where ligand bioactivity should be preserved. More importantly, modification of the preformed micelle helps to eliminate any potential coupling reactions post-micellization. Dual-click polymeric micelles are promising systems to further investigate how surface chemistry can be tuned to better understand biodistribution and targeting capabilities for engineered drug-delivery systems. Moreover, future work can be directed to further integrate emerging click reactions in an effort to create an array of multi-functional drug-delivery systems.

5 Conclusions and Future Outlook

Over the past few decades, polymeric micelle drug-delivery systems have been used for the administration of therapeutics in numerous in vitro and in vivo systems. With a greater understanding of the required physicochemical properties, the design of polymeric micelles for passive targeting (based on size and shape of the nanoparticle) and active targeting (requiring a targeting ligand on the corona) have improved. There are increasing numbers of clinical trials, which is promising for clinical translation; however, most, if not all of these, are based on passive targeting.

Building on the success of antibody–drug conjugates, the active-targeted polymeric micelle promises even greater selective cytotoxicity, with more chemotherapeutic per antibody and by taking advantage of the passive targeting achieved by the nanoparticles themselves. To deliver on the promise of the elegant delivery strategies described herein, we will require selective targeting ligands, stable encapsulation, and high loading of chemotherapeutic drugs. Moreover, these two parameters need to be combined into a formulation that is not just stable in vivo, but can be easily handled and stored by practitioners in a clinical setting.

The chemistry described herein also has application in the emerging area of theranostics, which addresses early detection and simultaneous treatment, yet probably requires more complicated clinical trials. Similarly, designing a triple threat, that is, one polymeric micelle that encapsulates a chemotherapeutic and delivers an siRNA or antisense oligonucleotide and targeting ligand, also holds promise for the future.

By integrating these delivery and formulation properties, breakthroughs in the understanding of other disease pathologies will drive the design of polymeric micelle drug-delivery platforms. Future translational research will thus endeavour to explore polymeric drug-delivery platforms applied to viral infections, cardiovascular disease, and pulmonary and urinary tract infections. Ultimately, clinical translation requires a continued strong partnership between academia and industry to bring some of these innovative strategies forward.

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