Emerging Frontiers in Drug Delivery

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ABSTRACT: Medicine relies on the use of pharmacologically active agents (drugs) to manage and treat disease. However, drugs are not inherently effective; the benefit of a drug is directly related to the manner by which it is administered or delivered. Drug delivery can affect drug pharmacokinetics, absorption, distribution, metabolism, duration of therapeutic effect, excretion, and toxicity. As new therapeutics (e.g., biologics) are being developed, there is an accompanying need for improved chemistries and materials to deliver them to the target site in the body, at a therapeutic concentration, and for the required period of time. In this Perspective, we provide an historical overview of drug delivery and controlled release followed by highlights of four emerging areas in the field of drug delivery: systemic RNA delivery, drug delivery for localized therapy, oral drug delivery systems, and biologic drug delivery systems. In each case, we present the barriers to effective drug delivery as well as chemical and materials advances that are enabling the field to overcome these hurdles for clinical impact.

1. INTRODUCTION

Medicine relies on the use of pharmacologically active agents (therapeutics or drugs) to manage or reverse the course of disease. The current global pharmaceutical market is valued at $980 billion annually, and, in the U.S., nearly 50% of the population has used at least one prescription medication in the past 30 days.[1,2] Notably, pharmacologically active agents are not inherently effective; their benefit is directly coupled to the manner by which they are administered. Administration affects drug pharmacokinetics (PK), absorption, distribution, metabolism, duration of therapeutic effect, excretion, and toxicity.[3] As new therapeutic molecules are discovered, there is an accompanying need for improved modes of delivery, and a clearer scientific understanding of how drug administration affects safety and efficacy.

In the ideal case, drugs would be applied *in vivo* at exactly the therapeutic concentration and would precisely target cells that cause disease. However, drug delivery is not easily controlled. Drug release rates, cell- and tissue-specific targeting, and drug stability are difficult to predict. To address these limitations, drug delivery systems (DDS) have been designed using a wide array of materials and chemical strategies. Here, we define DDS as technologies that are designed to improve the specificity of therapeutics by stabilizing them *in vivo*, controlling their release, and localizing their effect. Many materials have released therapeutics for prolonged periods of time and at targeted locations within the body; the properties of DDS are tailored to the physicochemical attributes of the drug and the intended route of administration (Figure 1). DDS have been propelled
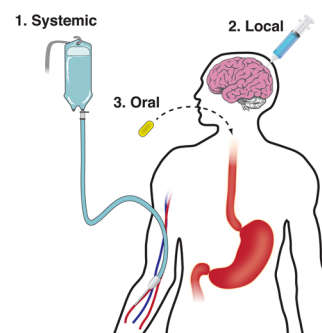
Figure 1. Hurdles to delivery and DDS design criteria vary with route of administration. Drugs can be administered in a variety of ways, and their successful delivery requires different design criteria. For example, systemic delivery requires the drug to avoid clearance by the reticuloendothelial system, and enter the correct tissue. DDS for local delivery must avoid damage to the surrounding tissue, and must control release to prevent dose "dumping". Oral delivery systems must overcome extreme changes in pH as well as accommodate changes in biomolecule concentrations that vary with food intake.

by advances in synthetic chemistry, materials science, medical chemistry, and conjugate chemistry, and are growing increasingly common in the clinic. However, the field of medicine is in active transformation as therapies based on nucleic acids, antibodies, proteins, and drug conjugates emerge. The translation of these therapeutic molecules, which can be orders of magnitude larger than therapeutic small molecules, and significantly more sensitive to environmental effects, will require adequate protection, bioavailability, and specificity. As a result, DDS will need to evolve. In this Perspective, we first

outline guiding principles for effective DDS and provide a historical overview of controlled release. We then illustrate how these guiding principles are informing DDS design for the administration of emerging drugs, focusing on nucleic acid drug delivery systems, injectable drug delivery systems, oral drug delivery systems, and cell-based drug delivery systems.

2. DRUG DELIVERY SYSTEMS FOR CONTROLLED RELEASE

One important class of DDS is controlled release systems (CRS), which are engineered to deliver drugs for days to years with a predetermined release profile. An ideal CRS confers several advantages.[4−6] In order to avoid the "peaks and valleys" of standard administration, it should maintain drug concentration within the therapeutic window. It should localize the therapeutic to the desired site of action in order to limit off-target side effects and increase potency. A CRS should also seek to improve adherence by decreasing the number of required doses. This provides the additional benefit of reducing the total amount of drug needed for therapeutic effect. Finally, an ideal CRS should enable the delivery of drugs that are rapidly cleared or degraded when administered on their own. CRS engineering is challenging; it requires a material that can house a sufficient quantity of therapeutic, protect the therapeutic from breakdown during the lifetime of release, and predictably release the therapeutic over the course of days to years. During CRS design, the system should be engineered to avoid potential drawbacks.[5] For example, the design must account for potential toxicity of the CRS material, its degradation products, or leachants. The CRS should not succumb to unintended rapid release of the therapeutics, which may cause acute tissue damage or medical complications. In order for broad adoption, the CRS should avoid discomfort during and after administration. Finally, the design of the CRS should mitigate the additive cost of the device.

Given these design constraints, it was unknown whether materials could control the release of drugs in the body. However, in the 1960s, it was observed that hydrophobic, lipophilic small molecules diffused through silicone tubing.[7] This inspired the use of silicone rubbers for the controlled release of biologically active agents, including antimalarial and antischistosomal drugs, as well as atropine, histamine, and steroid hormones.[8−13] Notably, these materials released molecules over the course of days to months. These findings demonstrated that materials could control the release of biologically active agents in the body, and led to the development of an early approved DDS, Norplant, an implantable contraceptive composed of silicone rubber capsules that release levonorgestrel for up to 5 years.[14] From these early findings the field of drug delivery and controlled release evolved rapidly (Figure 2). Osmotic pumps were employed as oral CRS,[15−17] drug-loaded hydrogels were applied as ophthalmic DDS,[18−21] microsphere encapsulation was used for sustained release,[22−26] researchers developed mathematical models to quantify drug release from CRS,[27−32] and the ALZA corporation was founded to commercialize CRS.[7] A comprehensive history of the fields of drug delivery and controlled release are beyond the scope of this Perspective, and we direct the reader to additional reviews.[7,33−36]

As molecular biologists improved their ability to generate and characterize proteins and other biomolecules, an emergent need to control the release of these large molecules arose.[37] However, it was believed that large molecules could not be
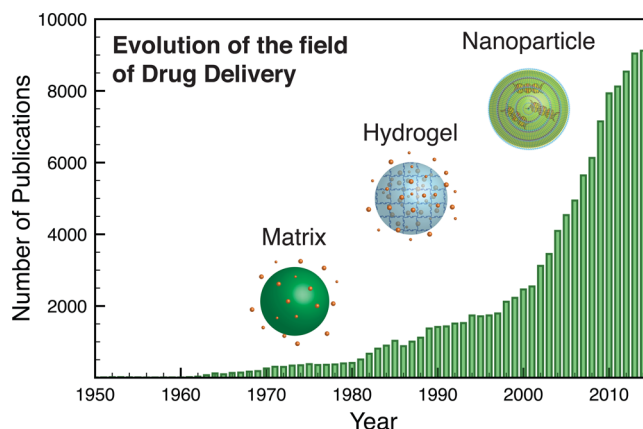

Figure 2. Evolution of the field of drug delivery. The fields of drug delivery and controlled release have evolved significantly from the early days, and now include the fabrication and application of macroscale devices and targeted nanoparticles. Here, we present the number or scientific articles published each year (1950−2014) on PubMed with the phrase "drug delivery systems".

entrapped and released in a controlled manner from an implanted polymeric material.[38] This view changed with the demonstration that proteins diffused out of polymeric implants over the course of 100 days.[39] Hydrophobic polymers, e.g., poly(ethylene-co-vinyl acetate) (EVA), were solubilized and mixed with lyophilized protein, before phase separation of protein from polymer during solvent evaporation introduced a tortuous network of interconnected pores within the otherwise impermeable polymer matrix. Macromolecules up to millions of daltons in molecular weight diffused through the pores as aqueous fluid entered, while narrow constrictions slowed protein release so that it occurred over several months. This method was used to release angiogenic factors as well as angiogenesis inhibitors, and contributed to the understanding of vascular growth and pruning.[40,41]

In the following decades, many controlled release technologies were developed for the controlled release of macromolecules, including those based on diffusion-controlled matrices and reservoirs, chemically regulated biodegradable and bioerodible materials, and solvent-activated hydrogels and osmotic pumps (Figures 3 and 4).[42] Additional advances introduced "intelligent" materials that release drugs in response to environmental stimuli.[43−48] Pharmaceutical nanotechnology was established[49] and has expanded to include liposomes, dendrimers, polymeric nanospheres, and polymeric micelles.[50−52] Controlled release technologies have even incorporated microelectronics, to engineer remotely triggered and pulsatile therapeutic release.[53,54] Indeed, the field of drug delivery has grown substantially; over 9000 articles on "drug delivery systems" were published in 2014 alone (Figure 2). Chemists, chemical engineers, materials scientists, and biomedical engineers are developing DDS with increasing control and sophistication. Many drug delivery products are on the market and helping patients; the estimated sales of DDS was $150 billion in 2013.[55] For example, Doxil, a PEGylated liposomal doxorubicin, is indicated for several types of cancer, while Lupron Depot, PLGA microspheres releasing leuprolide acetate, is used to treat prostate cancer and endometriosis.

Data accumulated over the past 40 years has revealed a few concepts that are fundamental to DDS. First, DDS efficacy is intimately related to the chemical structure of the material. For
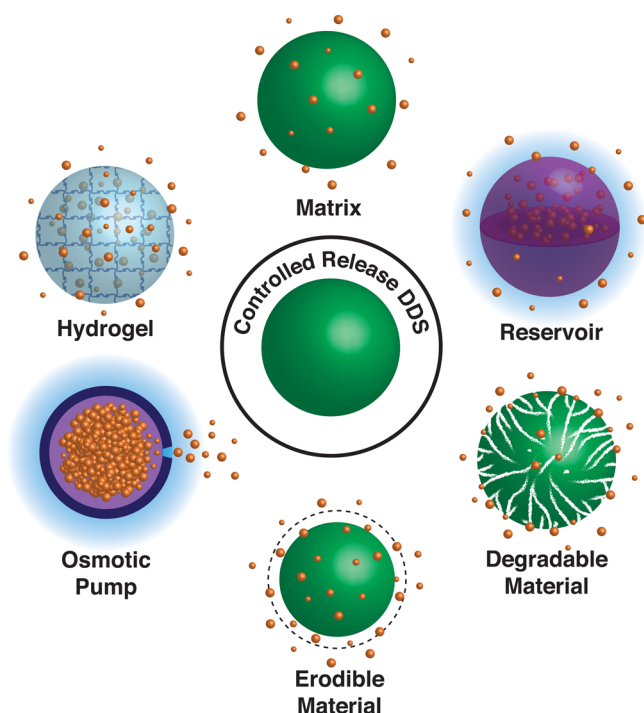
Figure 3. Controlled release systems. Drug delivery systems have been engineered to control release using different material strategies. For example, in a matrix-based system, the drug diffuses through a tortuous network of interconnected pores. In a reservoir, the drug passes through a semipermeable membrane. In a degradable DDS, the drug is released when pores are created as the material degrades throughout. Similarly, in an erodible DDS, the drug is released as the material dissolves at the surface. Osmotic pumps release drugs actively through one or more small pore(s) in an impermeable membrane in response to osmotic gradients. Finally, hydrogel-based DDS release drug through a constrained network whose mesh size depends on hydration and polymer architecture. Notably, controlled release systems often operate through a combination of two or more of these mechanisms.

example, minor chemical modifications to polymer structure can drastically affect material degradation, safety, and targeting. Second, the physical shape and size of DDS matters; this can affect material properties and even interactions with the

immune system. Third, DDS actively engage with the body, even when they are not designed to.

3. SYSTEMIC RNA DELIVERY

RNAs can manipulate gene expression through several biological mechanisms. For example, siRNAs and miRNAs can inhibit protein production; long, non-coding RNAs (lncRNAs) can affect epigenetic signaling; mRNA can produce functional protein; and sgRNAs, along with the Cas9 enzyme, can induce permanent changes to genomic DNA.[56−58] However, regardless of their biological mechanism of action, all systemically administered RNAs must overcome the same physiological hurdles that impede delivery: they must avoid clearance by the reticuloendothelial and immune systems, exit the bloodstream, access the right cell in a complex tissue, and enter the cytoplasm or nucleus, all without eliciting an unwanted immune response.[59,60] Each step in this process is inefficient. For example, between 95 and 98% of the siRNA that enters the endosome *in vivo* is degraded in lysosomes or expunged through exocytosis.[61,62] Despite these obstacles to effective systemic delivery, the clinical impact of nucleic acids has been demonstrated already by siRNAs targeted to melanomas and hepatocytes in humans.[63−66] Importantly, the delivery of modified siRNAs does not change appreciably with RNA sequence, and, therefore, a vehicle that effectively delivers one siRNA will likely deliver others as well as miRNAs, which have similar chemical and physical characteristics.

Early work focused on targeting siRNA to the liver because DDS are often cleared by it, and because its dysfunction can lead to diseases including cancer, cardiovascular dysfunction, and metabolic disorders, among others.[67−69] The dose required for effective siRNA delivery to hepatocytes *in vivo* has decreased by more than 10 000-fold in the past 10 years; target protein production can now be reduced after a systemic injection of 0.001 mg/kg siRNA.[70,71] Low dose liver delivery has led to promising results in clinical trials, and enabled scientists to turn off genes for weeks after a single injection or deliver several siRNAs concurrently for multigene therapies.[64,65,71−73] Advances in liver delivery can be attributed in part to physiology, since the liver naturally absorbs lipids from the bloodstream, and regions of the liver are covered by blood vessels with 100−150 nm pores.[74] However, these advances have largely been
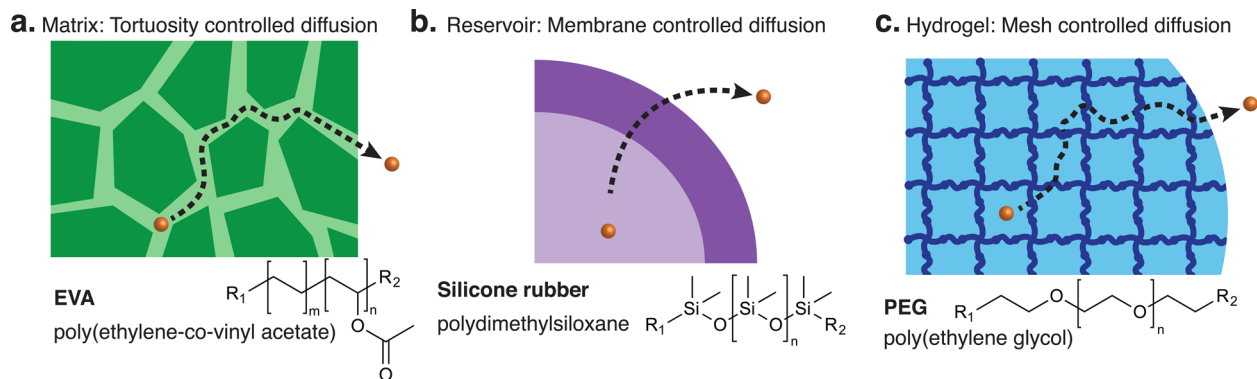

Figure 4. Mechanisms of controlled release. (a) Solid matrix-based controlled release systems (CRS) release drugs over time as the therapeutic molecules diffuse through a tortuous network of interconnected pores that form during phase separation of drug/excipient from polymer. One example polymer used in the fabrication of matrix-based CRS is poly(ethylene-*co*-vinyl acetate) (EVA). (b) Reservoir CRS limit release of entrapped therapeutics via membranes that regulate the rate of therapeutic diffusion out of the reservoir. These membranes have been made from polymeric materials, including silicone rubber (chemically cross-linked polydimethylsiloxane). (c) The rate of release of drugs from hydrogel CRS is controlled by the mesh size of the swollen polymer network. Hydrogels are fabricated from water-soluble polymers, such as poly(ethylene glycol) (PEG).

driven by improvements in nanoparticle chemistry and formulation. Thousands of effective cationic lipids, ionizable lipids, defined polymers, and lipid-like molecules called lipidoids can now be synthesized.[71−73,75−79] Once synthesized, these compounds are formulated into stable nanoparticles with poly(ethylene glycol) (PEG), cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-*sn*-glycero-phosphoethanolamine (DOPE), or other helper molecules. Helper molecules play a critical role in nanoparticle behavior; their presence or absence, as well as their relative molar ratios, can alter particle size, charge, and, ultimately, efficacy.[80] Particle behavior also varies with the way the nanoparticle is formulated; the same materials formulated into nanoparticles with microfluidic devices outperformed those formulated with extrusion.[81−84]

Hepatocyte siRNA delivery has also improved by increasing our understanding of how DDS interact with the body (Figure 5). In one example, a lipid nanoparticle consisting of an ionizable lipid (DLin-KM2-DMA), DSPC, PEG-lipid, and cholesterol (Figure 5a,c) effectively delivered siRNA to hepatocytes in many animal models, but did not work in mice genetically engineered without the serum lipoprotein apolipoprotein E (ApoE).[85] The nanoparticle was bound by serum ApoE in normal mice; ApoE is naturally endocytosed by hepatocytes. In this way, the nanoparticle was "naturally targeted" to hepatocytes without antibodies, aptamers, or other targeting ligands. The relationship between the structure of this nanoparticle and ApoE binding remains unclear. However, the same ApoE dependence was not observed with lipid nanoparticles composed of cationic lipids. siRNA has also been conjugated to *N*-acetylgalactosamine (GalNAc), which binds the asialoglycoprotein receptor (ASPGR) expressed on hepatocytes (Figure 5b,c).[86] These GalNAc conjugates were quickly endocytosed by hepatocytes following intravenous or subcutaneous administration. Notably, subcutaneous injection of GalNAc conjugates were well tolerated in mice, rats, non-human primates, and have silenced genes for 140 days in human beings.[87] GalNAc conjugates have also delivered antisense oligonucleotides (AONs) effectively to the liver.[88] AONs are small, single stranded oligonucleotides that are chemically similar to siRNA, but function through distinct biological mechanisms.[89] AONs bind to mRNA and either direct mRNA cleavage or alter mRNA translation. Chemically modified AONs can be delivered to the liver without nanoparticle or conjugate delivery systems, and a systemically administered AON targeting apolipoprotein B (ApoB) has been clinically approved.[90] Endocytotic pathways can also be manipulated to increase delivery, and bioactive molecules can be administered alongside nanoparticles and conjugates to enhance delivery.[61,91]

While several advanced siRNA delivery systems target the liver, many patients would benefit from efficient delivery to non-liver tissues. siRNA delivery to non-liver tissues has remained challenging, but continues to improve.[92] siRNA silencing has been observed in human tumors after the administration of cyclodextrin nanoparticles and Atu027, a nanoparticle that homes to the lung and endothelial cells.[63,66,93−96] Pre-clinical data have also been generated with a growing number of DDS. Low dose delivery to endothelial cells was reported using the nanoparticle 7C1; this particle delivered up to five siRNAs concurrently *in vivo*, and was used to study gene regulation in pulmonary hypertension, primary tumor growth, and metastasis.[81,97,98] Notably, 7C1 did not
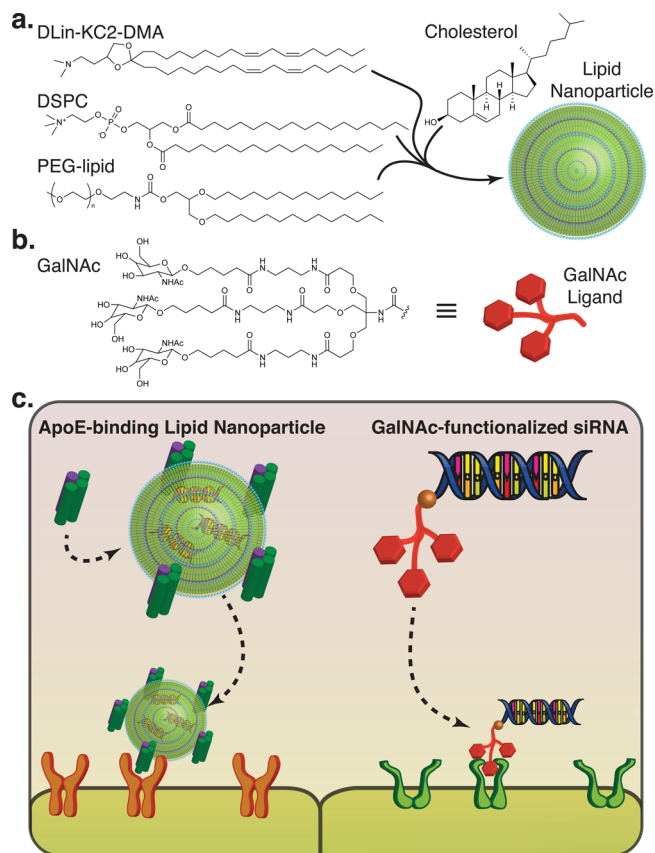
Figure 5. Biological interactions mediate RNA delivery. Adsorption of serum proteins and biochemical presentation can mediate receptor-mediated RNA uptake in hepatocytes. (a) Lipid nanoparticles for nucleic acid delivery have been formulated from combinations of ionizable lipids, PEG-lipids, and other helper molecules. For example, apolipoprotein E (ApoE)-dependent siRNA delivery was observed for lipid nanoparticles formed from the ionizable lipid DLin-KC2-DMA, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), PEG-lipid, and cholesterol. (b) *N*-Acetylgalactosamine (GalNAc) containing ligands have been used to functionalize nucleic acids to target them to surface receptors on hepatocytes. (c) The aforementioned lipid nanoparticles (yellow sphere) without antibodies, aptamers, peptides or other active targeting ligands conjugated to it, delivered siRNA to hepatocytes after the serum lipoprotein ApoE (green and purple rods) bound the particle in the blood. Hepatocytes naturally endocytose ApoE from the blood via the low density lipoprotein receptor (LDLR, orange receptor). Additionally, siRNA conjugated to GalNAc (red hexagons) was internalized by hepatocytes after GalNAc bound the asiologlyco-protein receptor (ASPGR; green receptors).

appreciably reduce target gene expression in hepatocytes. Endothelial cell silencing has also been reported using liposomes formulated to express VCAM-1, dendrimer-based nanoparticles, and cationic lipids.[99−102] Small RNAs are known to affect cancer signaling, and as such, a number of small RNA therapies have been designed to target primary tumors and metastasis.[103] For example, miRNAs are naturally produced small RNAs that reduce the production of several proteins concurrently.[58] Because these molecules are the same size and have the same charge as siRNAs, they can be packaged into the same nanoparticles to achieve rational combination therapies.[98,104] Other approaches have exploited tumor physiology to promote tumorigenic delivery.[105] An AON targeting the oncogenic miRNA miR-155 was designed with a modified backbone lacking anionic charge, and conjugated to a pH
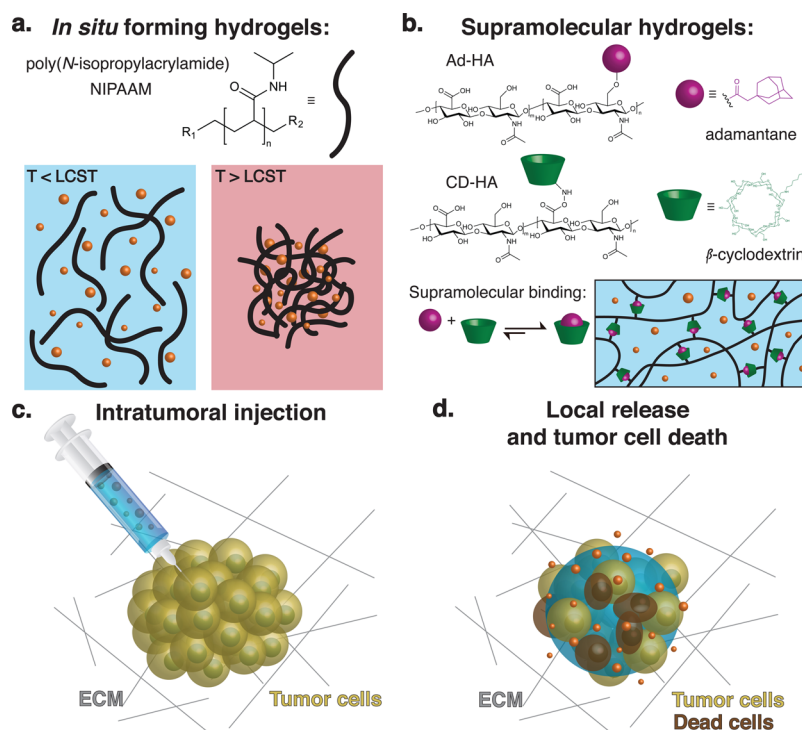
Figure 6. Injectable DDS localize therapeutics and reduce systemic off-target effects. Injectable drug delivery depots can be applied directly to the tissue of interest. (a) *In situ* forming injectable hydrogels have been fabricated from solutions of polymers that possess a lower critical solution temperature (LCST), e.g., poly(*N*-isopropylacrylamide) (NIPAAM). Below the LCST, a liquid solution of drug and polymer can be injected into the body and above the LCST (near 37 °C for biomedical applications) the polymer will condense, trapping the drug and forming a controlled release depot at the site of administration. (b) Another approach to injectable DDS is via supramolecular assembly of shear-thinning and self-healing hydrogels. Here, non-covalent interactions between appropriately paired molecules are employed to form a hydrogel. On account of the reversible nature of the bonds, the gel will flow upon the application of force (shear-thinning for injection) and rapidly re-form once the force is relaxed (self-healing to comprise a depot following injection). Many strategies have been employed to form supramolecular hydrogels, including complementary pairs of adamantane-functionalized hyaluronan (Ad-HA) and *β*-cyclodextrin-functionalized hyaluronan (CD-HA) that self-assemble into a shear-thinning and self-healing gel upon mixing. (c) Intratumoral administration of a chemotherapeutic-loaded injectable DDS, for example, can enable (d) site-specific tumor cell death, limiting off-target effects and damage to the surrounding tissue.

responsive peptide. When this system encountered the low pH tumor microenvironment, the peptide conformation changed such that the therapeutic nucleic acid was directly inserted into the cytoplasm of the cell.

mRNAs are especially attractive therapeutic molecules, since they can act as gene therapies that replace deficient or dysfunctional protein.[106] However, the delivery of lncRNAs, mRNAs, and other large RNAs is made especially challenging by natural RNA biochemistry.[89] Unmodified RNAs are easily degraded and can be immunogenic.[107] Specific nucleotides on siRNAs, miRNAs, and other small RNAs can be chemically altered to improve stability, alter the duration of the therapeutic effect, and reduce immunostimulation.[89] The same is not currently true for large RNAs, and as a result, using biochemical modifications to reduce immunostimulation and increase large RNA stability remains an active area of investigation.[108,109] Despite these additional hurdles to successful delivery, nanoparticles have delivered mRNA and effectively increased gene expression in subcutaneous tumors and hepatocytes.[110,111]

4. DRUG DELIVERY SYSTEMS FOR LOCALIZED THERAPY

One potential limitation to systemic administration is insufficient therapeutic concentrations at the desired site of action. This is even true for DDS that target specific cellular markers; different cell types can express the same ligands, or

express them at densities that are insufficient for binding. One way to overcome these challenges is to implant drug delivery depots locally at the target site. For example, surgical implantation of chemotherapeutic (carmustine, or BCNU)-loaded polyanhydride wafers (Gliadel) at the site of tumor resection in the brain has been used to target therapeutics to the tumor margin in glioblastoma multiforme.[112,113] This strategy significantly improved patient survival and reduced systemic complications of the chemotherapeutic. Similarly, compressed wafers were fabricated from 1 kDa PEG and paclitaxel-containing polyphosphoester microspheres (Pa-climer) and implanted in the brain to treat malignant gliomas.[114] After implantation, the PEG dissolved, exposing microspheres that locally released paclitaxel for up to 90 days. However, these examples, while promising, are limited to situations where a surgeon can access the target site. When the target site is not accessible surgically, injectable drug delivery depots administered through a needle or catheter can be used to localize therapeutics. The depots can be tailored to release drugs over the course of hours to months, and as such, are particularly attractive for the management of chronic disease.

Locally administered DDS can improve drug efficacy by overcoming biological obstacles that vary from disease to disease. For example, chemotherapeutics are often constrained by dose-limiting toxicity. To avoid off-target effects and maximize potency, clinicians have used intratumoral implanta-

tion to directly apply chemotherapeutics. These systems stabilized the chemotherapeutics, enabled loading and release of insoluble drugs, lowered the overall required dose, directed the biological effect to target cells, and reduced off-target toxicity.[115] A second clinical problem that limits cancer therapeutics is the inherent biological complexity of the disease; it is difficult to predict which therapies will cause the most potent anti-tumor response. To simultaneously study anti-tumor response mediated by many drugs concurrently, scientists developed DDS that housed several chemotherapeutics on a single device.[116−118] After implanting the device in tumors, the response to all the potential therapies was analyzed at the same time. Notably, chemotherapeutics that worked best during the "in tumor screens" also worked best when delivered systemically.

Coronary stenting and other catheter-based interventions have revolutionized the treatment of coronary artery disease; however, they can still be complicated by restenosis after implantation.[119] In-stent restenosis has been attenuated by engineering drug-eluting stents that target smooth muscle cell proliferation locally.[120−123] Since the stents act within the complex flow environment of the vasculature, the local effect of the drug-eluting stent can be tuned by engineering the stent design and release rate to match the local tissue and physicochemical properties of the drug.[124] Finally, many vaccines are limited by an insufficient immune response, owing to limited interactions between the antigen and the cells of the adaptive immune system.[125] To overcome these limitations, synthetic biomaterials-based vaccines have been developed. In one example, synthetic vaccines housed in microparticles were locally injected into the lymph nodes; these biodegradable PLGA microparticles controlled the release of both the antigen and adjuvant, and sustained interaction with naïve T and B lymphocytes.[126] A single dose achieved 8-fold improvement in immune system activation compared to a standard intramuscular injection. These examples illustrate how specific design criteria for DDS can vary with disease physiology. However, all local DDS must regulate release rate, house sufficient quantities of drug, and confine drug to the site of administration.

In Situ **Forming Injectables.** To localize therapeutics in a minimally invasive manner via direct injection and provide a controlled release depot at the site of application, *in situ* forming materials have been designed that transition from a liquid precursor solution to a solid in the body (Figure 6). These materials can adapt to the geometry of the site and form a strong interface with tissue, without destroying natural tissue structure. *In situ* forming materials have been synthesized using a range of chemical strategies. Polymer precipitation is common; in this case, water insoluble polymers are prepared in a water miscible and physiologically compatible solvent.[127] Following injection, the organic solvent diffuses away, and the water insoluble polymer precipitates into a drug-releasing matrix. However, because the kinetics of precipitation are slow, they often suffer from a rapid burst release.[128] They can also be limited by toxicity, since organic solvents often cause adverse effects *in vivo*.

Injectable materials can also be engineered to spontaneously form three-dimensional structures in physiological conditions. For example, mesoporous silica rods that form macroporous three-dimensional structures *in vivo* have been designed.[129] These macroporous structures recruited naïve dendritic cells to the site of injection by releasing granulocyte-macrophage

colony-stimulating factor. The material also exposed dendritic cells to tumor antigens; these antigens programmed the dendritic cells, which primed the immune system to attack tumors. This material driven "recruit and train" strategy can be extended beyond cancer by programming the immune system to fight other diseases.

Temperature changes can also be used to induce liquid-gel transitions in polymers, thereby forming injectable drug depots. Polymers can be designed with a lower critical solution temperature (LCST) close to 37 °C. The LCST is the temperature at which a polymer will precipitate out of aqueous solution; polymer precipitation forms structures that store and release drugs. Notably, polymer structure influences both the absolute LCST as well as the "sharpness" of the LCST curve.[130,131] For example, poly(*N*-isopropylacrylamide) (NI-PAAM) is one of the most commonly used thermosensitive polymers.[46] It possesses a sharp sol−gel transition near physiological temperatures, which makes it attractive for biomedical applications. Similarly, block copolymers consisting of PEG and poly(propylene oxide) are commonly used and FDA approved.[132,133] However, because the LCST for these materials is often above 37 °C, a sol−gel transition is only observed at high polymer fractions (>10 wt%). High polymer fractions, in turn, increase the viscosity of the liquid and increases off-target effects, which can limit the application of these materials. As our understanding of how polymer structure affects LCST has improved, additional gelling systems with sharp LCSTs near 37 °C have been rationally designed using synthetic polymers including polylactide (PLA) and PEG as well as natural polymers including chitosan, hyaluronic acid, and peptides.[134−138] Once a thermal gelling polymer with an effective LCST is designed, it can tailored to protect and release drugs with poor aqueous solubility; for example, thermal-gelling PLA−PEG−PLA copolymers have been used for the controlled release of paclitaxel following intratumoral injection.[139]

Supramolecular Biomaterials as Injectable DDS. Materials that self-assemble in the body can also be designed using supramolecular chemistry. Unlike the materials described above, which are formed by stochastic intra- and intermolecular forces, supramolecular chemistry relies on selective and directional interactions.[140−142] For example, self-assembling peptides that form gels upon injection in the body have been designed by exploiting amino acid charge and protein secondary structure.[143−146] Peptide-based supramolecular materials have released a number of therapeutic molecules locally, including VEGF-mimics that increase blood perfusion and dexamethasone to suppress local inflammatory response.[147,148] DNA-based supramolecular materials, which are formed by exploiting hydrogen bonds and base pairing, have also been designed.[149−152] Notably, these materials can be formed into rationally designed two- and three-dimensional shapes that may influence biological activity.

To minimize the effect of local physiology on material properties and drug release, shear-thinning and self-healing hydrogels have been developed. These materials are designed with strong, reversible, non-covalent bonds. As a result, they form gels outside the body, become liquid when a shearing force is applied during injection, and quickly re-form into solid hydrogels in the body.[153] For example, self-healing colloidal gels have been made from drug-loaded, charged PLGA microspheres.[154] The microsphere charge attracted the particles together until the shearing force was applied. Controlled release of dexamethasone from these PLGA colloidal gels also
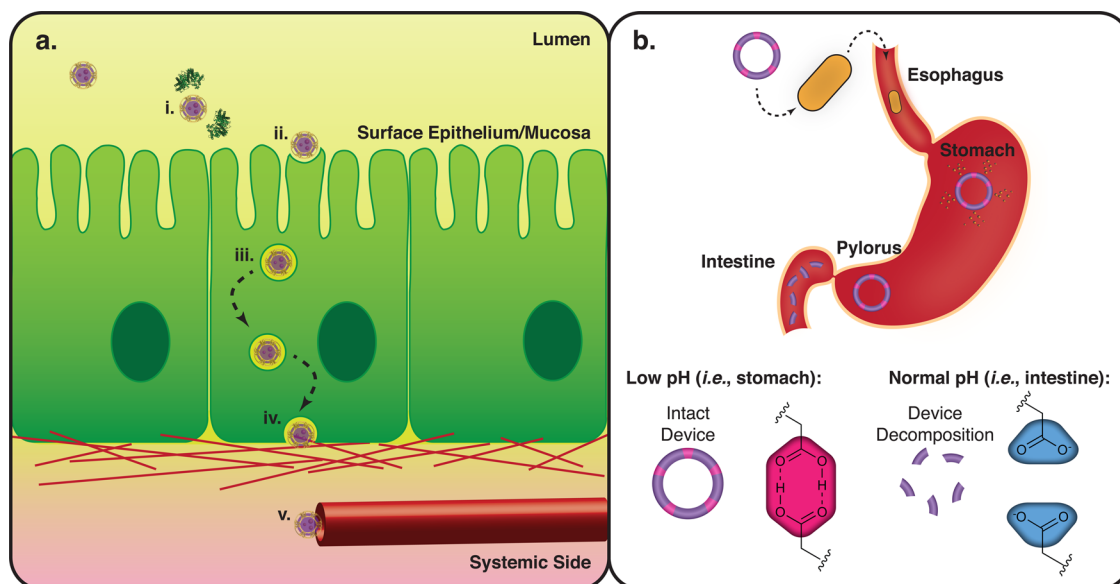
Figure 7. Oral DDS must overcome unique physiological hurdles. (a) Oral delivery of biologics requires that the drug (i) avoid protease degradation in the lumen, (ii) migrate toward and enter epithelial cells, (iii) transit across the epithelium, (iv) exit the cell at the basolateral side, and (v) enter systemic circulation. These significant barriers limit bioavailability of oral biotherapeutics. (b) Extended release is constrained by the average transit time through the human GI tract, which is generally less than 30 h. Gastroretentive devices reside within the stomach for days to weeks and can release drugs in a controlled manner, improving medical adherence. Here, a composite DDS held together by an enteric elastomer (pink material) can be delivered orally and retained within the stomach for 2−5 days.[204] Upon exit through the pylorus, the enteric elastomer dissolves and the device falls apart, preventing intestinal blockage. The enteric elastomer is bound together through hydrogen bonds that remain bound in the acidic pH (∼1.5) of the stomach, which protonates the free acid groups and facilitates hydrogen bonding. However, in the normal pH (∼7) of the intestine the acid groups deprotonate, causing the hydrogen bonds to disassemble and the enteric elastomer to dissolve.

improved bone healing in a cranial defect model.[155] Again utilizing electrostatic forces, oppositely charged dextran nanoparticles formed a shear-thinning and self-healing nanoscale network that released insulin in response to glucose.[156] Supramolecular chemistries that generate strong, non-covalent interactions between polymeric constituents have also been used to form shear-thinning and self-healing hydrogels. Hydrogels have been formed via paired interactions between (strep)avidin and biotin,[157,158] self-assembling proteins,[159−162] and macrocyclic host chemistries.[163−165] By varying the on−off kinetics and mesh size within supramolecular biomaterials, drug release from these hydrogels can vary from days to months.[164] Moreover, rational design of interactions between drug-loaded nanoparticles and polymers has been exploited to release small molecule drugs and biologics simultaneously *in vivo*.[166]

5. ORAL DRUG DELIVERY SYSTEMS

Oral ingestion remains the preferred route for the application of pharmaceuticals, since it does not require a skilled health care professional and allows patients to self-administer drugs conveniently.[167] However, oral delivery of many therapeutics is challenging. The pH and the local biological environment (including the microbiota) of the GI tract vary spatially from the stomach to the intestine. There are also anatomical hurdles in the GI tract that prevent delivery. The drug must survive in the lumen, which has many proteolytic enzymes, traverse the mucosa and epithelial cells, and access the bloodstream on the systemic side (Figure 7). Natural eating cycles can also impede drug delivery, by introducing spikes in the concentration of lipids, carbohydrates, and digestive enzymes interacting with the drug.[168] Finally, typical oral administration requires the drug be released within ∼30 h, the normal time something takes to traverse from mouth to anus.[169] As a result, the

systemic bioavailability of drugs administered orally can be significantly lower than when administered intravenously.[170] There are significant opportunities in developing materials that improve oral administration of biologics and extend release from the GI tract.

DDS for Oral Administration of Biologics. Oral delivery is particularly challenging for biotherapeutics, since these drugs are readily degraded by proteases, nucleases, and other enzymes in the gut, and are much larger than traditional small molecules.[170] Certain biologics that function at the level of the epithelium have demonstrated clinical effect when delivered *in vivo*. Specifically, linaclotide, an FDA-approved peptide agonist of guanylate cyclase C, is commonly prescribed for the treatment of irritable bowel syndrome and chronic idiopathic constipation, while an antisense oligonucleotide targeting SMAD7 demonstrated clinical improvement in Crohn's disease by modulating TGF-β1 signaling in the GI epithelium.[171−174] However, even when the drug retains activity at the level of the epithelium, the systemic bioavailability remains poor.[174] Many biologics are large and hydrophilic, limiting passive diffusion across the cell membrane, and transport through the gaps in the paracellular space (1−5 nm).[175,176] While biologics can bind to cell surface receptors that promote transcytosis across the epithelium, a very small fraction is released into the bloodstream in a bioactive form.[177−179]

Early work to increase the oral bioavailability of biotherapeutics relied on adding protease inhibitors to minimize enzymatic degradation and permeation enhancers to increase transepithelial transport.[180] For example, the systemic bioavailability of the synthetic oligopeptide octreotide improved when different permeability enhancers were added to the formulation.[181] This led to promising Phase III results in the management of chronic acromegaly.[182] Similarly, Novo

Nordisk is currently developing an orally available long-acting GLP-1 analogue (semaglutide) for the treatment of obesity.[183,184] However, protease inhibitors and permeation enhancers do not always improve bioavailability, and can present additional safety concerns, especially with chronic use.[179]

As an alternative to formulation enhancers, DDS have been engineered to deliver sensitive biologics orally. Nano and microparticles synthesized from polymers and lipids have emerged as a major driver in the clinical application of biologics.[185] For example, insulin loaded poly(anhydride) microspheres were targeted to the intestinal wall and released their payload over 6 h as the polymeric vehicle degraded.[186] Similarly, pH-responsive poly(methacrylic acid)-*graft*-poly-(ethylene glycol) hydrogels were rationally designed.[187] These hydrogels were synthesized to entrap and protect insulin in the low pH stomach. However, once the gels entered the intestine, an increase in pH caused them to swell and release insulin, resulting in a dose-dependent reduction of glucose in healthy and diabetic rats. Using a separate strategy to improve oral delivery across the epithelial barrier, nanoparticles were conjugated to the neonatal Fc receptor (FcRn) and loaded with insulin.[188] The FcRn helps transport immunoglobulin G antibodies across epithelial barriers; the authors utilized this native transcytosis pathway to guide the particles into systemic circulation.

**Extended Release DDS for Oral Administration.** Traditional oral administration requires frequent dosing as the normal residence time in the GI tract is less than 30 h.[169] Chronic medical therapy is associated with poor adherence to medications, which manifests in significant morbidity and mortality.[189] In fact, adherence to long-term therapies is only 50%, in developed nations, and even lower in the developing world.[190] Simply put, a well-designed medicine cannot work if it is not taken.[189] Strategies to mitigate non-adherence have focused on extended release oral delivery technologies. One approach utilizes DDS that adhere to the gut epithelial wall. These "mucoadhesive" patches and particles have been designed to target different regions in the gut.[191] For example, a three-layered patch consisting of a mucoadhesive layer, a drug containing middle layer, and an outer layer that inhibits enzymatic activity was designed to release granulocyte colony-stimulating factor.[192,193] Once formed, the device was loaded into an enteric capsule that degraded in the intestine, releasing the mucoadhesives. Similar results were achieved with a patch consisting of drug-loaded microspheres in the drug carrying layer, which was used to deliver insulin and restore glycemic homeostasis in diabetic rats.[194,195]

Gastroretentive devices that reside in the stomach for days to weeks have also been engineered.[196] Often, these rely on changes in material shape; a material is designed to be small and easily swallowed, and to expand or unfold in the stomach.[197−199] More specifically, devices expand so they are at least 2 cm in diameter, in order to prevent transit through the ~1.3 cm diameter pylorus.[200] However, when these devices are fabricated from non-degradable elastic polymers, they can create significant health complications. If a non-degradable polymer escapes the stomach and enters the intestine, it can require surgical removal.[201−203] To overcome this substantial hurdle, pH-responsive enteric elastomers were developed for the formation or gastroretentive CRS that disassemble upon accidental passage into the intestine.[204] The device was composed of polycaprolactone sections that were held together by flexible junctions composed of enteric elastomers. These were packed into tablets. Upon dissolution of the tablet, the device expanded and remained in the gastric environment for 2−5 days and disassembled rapidly when exposed to the increased pH of the intestine.

6. BIOLOGIC DRUG DELIVERY SYSTEMS

Biologic DDS, in which the delivery vehicle is composed of living systems, provide another approach to deliver therapeutics and treat disease (Figure 8).[205] Biological systems often
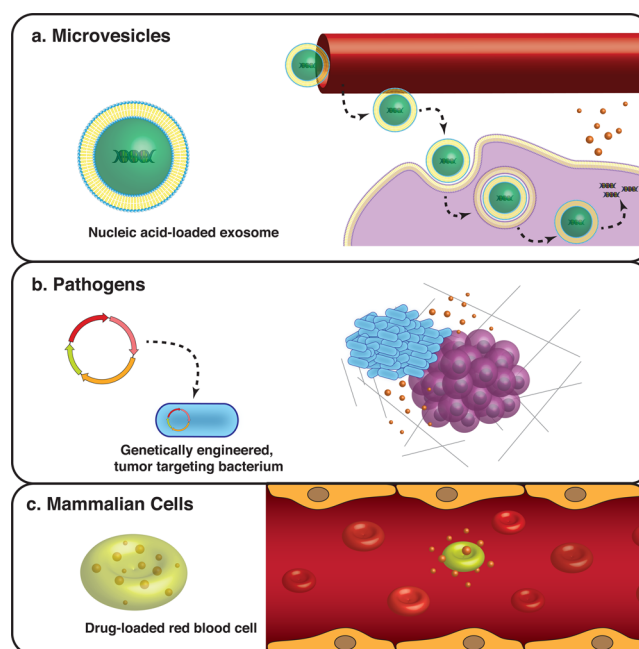
Figure 8. Biologic DDS hijack natural mechanisms to deliver therapeutics. As a complement to synthetic CRS, living systems that have evolved to produce, release, and target biomolecules are being re-engineered as DDS. For instance: (a) Exosomes can be loaded with nucleic acids to transfect cells *in vivo*. (b) Bacteria can be genetically engineered to communicate and collectively detect or treat tumors in the body. (c) Red blood cells can be loaded with therapeutics and circulate for up to 120 days.

naturally operate with the central goal of controlled drug delivery—to target molecules to specific cells at desired times. For example, exosomes transport proteins and RNAs between cells; bacteria and viruses efficiently deliver cargo to infect the body; and the immune system homes to disease and releases signaling molecules that restore homeostasis. The natural tropism these DDS exhibit, along with a growing capacity to engineer them, is increasing our ability to leverage living systems for drug delivery.

Microvesicles as Natural Drug Carriers. Cell-derived membrane vesicles or microvesicles (e.g., exosomes, shedding vesicles, apoptotic bodies) are secreted by most cells in the body and are found in most bodily fluids.[206] Initially termed "platelet dust" and observed to regulate coagulation of blood,[207] microvesicles are now known to facilitate cell−cell communication and paracrine transport of RNA and protein.[208,209] Microvesicles naturally transport biological molecules, and possess several potential advantages: they are stable in blood, can possess native targeting ligands, and can confer immune-tolerance.[206] An early demonstration of microvesicle-based drug delivery employed exosomes to deliver curcumin to

murine monocyte-derived myeloid cells.[210] Exosomes were isolated from cultured murine T lymphocytes (EL-4 cells) and complexed with curcumin. Upon injection, the exosomes delivered the curcumin to activated myeloid cells, inducing apoptosis and suppressing lipopolysaccharide-induced inflammation. Microvesicles have also been explored as delivery vehicles for biotherapeutics, including nucleic acids and proteins.[211] In one example, exosomes delivered siRNA to the brain in mice for selective suppression of BACE1 mRNA and protein expression.[212]

Pathogen-Based DDS. Infectious agents including viruses and bacteria can trigger disease by escaping the immune system and infecting target cells.[205] Researchers have studied whether these systems can be used for drug delivery. Viruses have evolved to transfer genetic material efficiently into host cells, with the aim of hijacking the cells' internal machinery for self-replication.[213] As such, viruses have been bioengineered to deliver genes; notably, specificity can be encoded into the virus by inserting a cell-type-specific promoter.[214] Retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAVs) have been used as vectors for gene delivery in the treatment of disease, including cancer, monogenic diseases, and vascular disease.[215] While some viral DDS are limited by off-target infection, immune activation, and random insertion into the genome, AAVs have been safely injected in many human patients, and have led to promising clinical results.[216] However, these viruses often lose efficacy upon re-administration, since the immune system can generate antibodies to the virus during the first administration.[205] To avoid some of these complications, bioengineered virus-like particles and virosomes have also been developed for gene delivery.[205]

Bacteria can also be engineered for drug delivery. Increasing evidence demonstrates that these microorganisms play an active role in human health and physiology; the gastrointestinal microbiome has been associated mental health, heart disease, and metabolic disorders, among others.[217] Strategies have focused on directly "drugging" the gastrointestinal microbiome through the use of prebiotics, probiotics, as well as fecal transplants, with the intention of manipulating or normalizing the microbiome. Fecal transplants have demonstrated clinical promise in the management of *Clostridium difficile*-induced colitis.[218,219] These studies suggest that GRAS ("generally recognized as safe") strains of bacteria may be useful clinically. Notably, bacteria can be engineered to express protein at a target site, and can even be engineered to do so in response to soluble factors.[220] Thus, bioengineered bacteria that already are naturally evolved to thrive in the GI tract may be useful for drug delivery. For example, *Lactococcus lactis* has been engineered to express human interleukin 10 (hIL-10) for the treatment of inflammatory bowel disease.[221] To limit potential runaway growth of genetically modified *L. lactis*, the strain was made dependent on thymidine or thymine such that their survival rate significantly decreased outside the body. This biotic approach is being commercialized by Intrexon. Non-pathogenic bacteria have also been used to colonize and deliver proteins to the oral, nasal, and vaginal mucosae.[205]

Bacterial species have evolved to survive in different physiological environments. For example, certain bacteria naturally survive in hypoxic environments; these bacteria have been used to treat tumors, which can also exhibit hypoxia.[222] Tumor-targeting bacteria have delivered cytosine deaminase, tumor necrosis factor, colicin E3, and other proteins in the tumor microenvironment.[223−229] They have also been en-gineered using quorum sensing; in this strategy, entire bacterial colonies produce genes based on cell density.[230,231] In this manner, tumor-targeting bacteria have been designed to function collectively at the tumor site for the detection and clinical management of cancer.[232−234]

Mammalian Cell-Based DDS. Certain mammalian cell types exhibit natural functions that can be exploited for drug delivery. Autologous or donor-matched red blood cells (RBCs) are particularly attractive, given that they are inherently biocompatible, circulate for up to 120 days, and are cleared naturally by the immune system.[235] RBCs can carry large amounts of drug, owing to their considerable volume (mean corpuscular volume of a human RBC is ∼90 μm^3).[236] Drugs can be loaded into RBCs using hypotonic dialysis; in this process, the RBC membrane is disrupted in a solution of drug. After the drug is loaded, the RBC membrane can be resealed.[237] The drug-loaded RBCs are then infused into the circulation, enabling the sustained release of small molecules and extended conversion of toxic metabolites.[235] This approach has been employed for the long-term delivery of antiretroviral drugs, enzymes, steroids, and cardiovascular small molecules.[238−241] In another approach, nanomaterials were adsorbed onto the surface of RBCs, in order to target the lung and avoid accumulation in the liver and spleen.[242,243] Nanoparticles can also be coated with RBC components to reduce immunostimulation and increase targeting to inflamed vasculature.[244] Notably, RBC-based delivery of L-asparginase is in clinical development for the treatment of cancer and RBC-based release of dexamethasone is in clinical development for the management of Louis−Bar syndrome.[245]

Immune cells, which can naturally home to inflamed tissues, have been bioengineered to release drugs. Macrophages, which naturally phagocytose drugs and other DDS, have received particular focus.[243,246] In this manner, "Trojan horse" macrophages can be generated with cargo that includes small molecules, enzymes, drug-loaded nanocarriers, and metal nanoparticles use for imaging.[247−249] The Trojan horse approach has carried lipid nanoparticles containing indinavir to HIV-infected sites, including to the brain.[248,250] The concept has also been applied to cancer therapy, since macrophages accumulate in the hypoxic areas of solid tumors,[251−253] which can be difficult to target using traditional nanoparticles.[205] Tumor infiltration by cargo-loaded macrophages has been used to deliver gold nanoparticles for photothermal ablation therapy and oncolytic viruses to treat pancreatic cancer, as well as liposomal doxorubicin for chemotherapy.[249,254,255] Bioengineered leukocytes have also targeted circulating tumor cells (CTCs).[256,257] Liposomes were first functionalized to present E-selectin and the cancer-specific TNF-related apoptosis inducing (TRAIL). These liposomes were conjugated to leukocytes, which bind E-selectin; once tethered to the leukocytes, the liposomes targeted CTCs in the blood, which express ligands that bind TRAIL.

Many diseases, including diabetes mellitus, arise from cell dysfunction or death and could be reversed by autologous or allogeneic transplantation of appropriate cells.[258,259] This approach can also be viewed as drug delivery as the transplanted cells secrete factors in a controlled manner to restore function. Additionally, cell transplantation can assist in the management of many protein deficiency diseases, such as anemia, by providing living factories in the body to supplement protein production.[260] A clinical example of cell trans-plantation-based drug delivery is the Edmonton protocol in

the treatment of diabetes.[261] Here, insulin producing pancreatic islets are recovered from a cadaveric donor and transplanted into the recipients portal vein. The cells are able to produce insulin to aid in the management of diabetes; however, chronic immunosuppression is required to limit rejection of the allogeneic islets. Advances in stem cell biology, including induced pluripotent stem cells and controlled differentiation, and mammalian cell genetic engineering provide new cellular sources for cell-based therapies.[57,262−264] Clinical success of these approaches will depend on materials that promote cell survival and engraftment, protect cells from the immune system, and allow secreted factors to diffuse to the body.

7. CONCLUDING THOUGHTS

The clinical and commercial impact of drug delivery and controlled release systems over the recent decades have been directly enabled by advances in synthetic chemistry, polymer physics, materials science, and bioengineering. However, despite the successes, many challenges and unmet clinical needs remain. New classes of therapeutics (e.g., biologics) and administration demands (e.g., orally administered extended release devices and injectable materials for site-specific delivery) necessitate advanced DDS that protect sensitive molecules, specifically target diseased regions of the body, and release drugs over the course of months to treat chronic disease. Medicine is no longer limited to orally available formulations that require two or three times daily ingestion. We can now tackle pathology at the site of action, engaging biological mechanisms that underpin its origin to manage and reverse the progress of disease.

The emerging frontiers of drug delivery discussed in this paper have the potential for tremendous clinical impact in the coming decades. Systemic delivery of RNAs can treat disease at the genetic level, seeking out aberrant regions of the body and repairing their function at the most basic level. Injectable materials can localize therapeutics to the site of action in order to mitigate off-target toxicity and increase clinical effect. Oral delivery of biologics can increase the indication and impact of this growing field of therapeutics. Extended release devices delivered to the GI tract can aid in the management of chronic disease and avoid adherence issues. Finally, living systems can be re-engineered to work with the body, and not against, to treat disease using the outstanding delivery mechanisms of microvesicles, pathogens, and cells (e.g., selective targeting, prolonged circulation, and immune tolerance).

Indeed, as therapeutics continue to improve, there will be a growing need for improved DDS. Materials will be required to control the delivery of gene editing technologies including CRISPR-Cas9, zinc-finger nucleases, and transcription activator-like effector nucleases, to ensure permanent modifications to the genetic code are localized to diseased cells. Chemical strategies for the safe delivery of gene editing technologies will require improved nucleic acid delivery, since the targeting RNA needs to be delivered concurrently with mRNA encoding the nuclease, and since this protein/RNA complex needs to form in the cytoplasm and migrate to the nucleus. Nucleic acid delivery will likely improve as we understand how biological pathways affect nanoparticle targeting and endosomal escape.[61,91] Gene editing may also be accomplished by complexing protein and RNA together in a nanoparticle before delivering the complex into cells.[265] This protein-based approach is especially promising for gene editing, since a brief pulse of DNA-editing drug can induce permanent changes in the genome. In fact,

unlike traditional gene therapies, short acting gene editing drugs are likely to be more beneficial than long lasting drugs, since durable expression of nucleases may increase the number of off-target mutations. As CRS enable the extended release of therapeutics for the management of chronic disease, materials will need to be engineered to ensure dosing can be turned off if adverse effects are observed. In one iteration, this has been achieved using microelectronics for remote-controlled drug delivery.[53,54] Additional advances in "on−off" dosing may rely on materials that, through chemical interactions, respond directly to specific biological stimuli. For example, glucose-responsive DDS based on phenylboronic acid derivatives or glucose oxidase that induce material properties alterations with changes in glucose concentration could control the release of insulin directly as needed by the body. Further, despite the advances in biotargeting, materials that seek out target cells in the body are still difficult to design. It will be important to better understand precisely how materials interact with the body, and how differences in cell-specific gene expression and disease physiology can be exploited to improve targeting. Despite these challenges, several decades of scientific evidence has already demonstrated that the intersection of chemistry, nanotechnology, materials, and medicine is a fruitful one, and that further advances in DDS will have a significant effect on human health.

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Notes

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