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A holistic approach to targeting disease with polymer nanoparticles

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PREFACE

The primary goal of nanomedicine is to improve clinical outcomes. Toward this end, targeted nanoparticles are engineered to reduce non-productive distribution while improving diagnostic and therapeutic efficacy. Paradoxically, as this field has matured, the notion of 'targeting' has been minimized to the concept of increasing affinity of a nanoparticle for its target. This review outlines a holistic view of nanoparticle targeting, in which nanoparticle route of administration, molecular characteristics, and temporal control are potential design variables that must be considered simultaneously. This comprehensive vision for nanoparticle targeting will hasten the integration of nanomedicines into clinical practice.

INTRODUCTION

The central promise of targeted drug delivery technologies is improved efficacy by increasing drug concentration at a desired (or target) site, while simultaneously minimizing toxicity by reducing off target accumulation. The last several years have seen the development of an enormous array of systems engineered to fulfill this drug targeting promise. Such systems range from the conjugation of a hydrophilic polymer to a hydrophobic drug[1], up to more complex nanocarrier systems that can dynamically respond to local environmental cues[2]. Ultimately, the utility of any drug delivery system—regardless of the materials used or mechanism of action—should be judged with respect to the definition of targeting; i.e. does the targeting system significantly improve efficacy and reduce toxicity by providing control over the drug biodistribution and pharmacokinetics. Many current approaches for targeted drug delivery systems assume that the best mechanism for controlling the fate of a therapeutic agent is via hijacking cellular receptor-ligand interactions. Often less consideration is given to other factors that can dramatically affect the ability to control the delivery of a drug to a specific site, properties such as: route of administration, the surface adsorption of serum proteins, drug release kinetics, and biological timing. In order to realize the full potential of targeted drug delivery, we believe

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that targeting should be more comprehensively defined to also include these non-canonical aspects. A holistic view of targeting encompasses all aspects of delivery from the macro-scale, e.g. where and how the therapeutic is introduced into the body, to the micro-scale, e.g. the molecular interactions that govern how a delivery system interacts with cells and the extracellular milieu (Figure 1).

More than any other class of drug delivery vehicle, polymer-based nanoparticles have the capacity to fully realize on this holistic view of targeting. Polymer nanoparticles (with a sub-300 nm diameter) are structurally defined as solid nanoparticles, micelles, polyplexes, or dendrimers (Box 1). These colloidal polymer systems have gained considerable commercial and translational attention in large part due to their improved stability, biocompatibility, and potential for extended drug release kinetics compared to non-polymeric nanosystems[3]. Additionally, polymeric nanoparticles provide versatility via the use of polymers of different chemical composition, hydrophilic-lipophilic balance, charge, physical structure, etc. As a result of this adaptability, nanoparticles can be formulated to deliver a range of drugs and should be adaptable to many clinical settings. Moreover, the ability to control the degradation or disassembly of polymeric nanoparticles imparts the ability to control temporal aspects of drug delivery over a wider range than permitted by other forms of nanoparticles. This diversity of potential applications makes polymeric nanoparticles attractive as therapeutic delivery vehicles. However, for each new particle formulation, this diversity must be matched with a comprehensive understanding of how both the biology of the target disease and the properties of a nanoparticle therapeutic influence delivery.

Box 1

Classes of polymer nanoparticles

Solid nanoparticles are composed of a dense polymer matrix typically stabilized by hydrophobic interactions of the constituent polymer(s). A key advantage of these systems is the ability for controlled release of various cargo ranging from hydrophobic small molecules to large proteins. BIND-014 is a solid PLA nanoparticle formulation (synthesized using an emulsion-solvent evaporation process) coated with PEG and prostate-specific membrane antigen-targeting ligands and loaded with doxorubicin; currently BIND-014 is in clinical trials for the treatment of prostate and lung cancer[8].

Micelles are composed of amphiphilic components that are organized by the hydrophobic effect to have a distinct lipophilic core and hydrophilic outer layer. In these systems, the drug cargo is typically limited to hydrophobic molecules entrapped in the core. Genexol-PM is composed of a PEG-PLA block co-polymer loaded with paclitaxel, and has been investigated in clinical trials for breast, pancreatic, lung, and ovarian cancer[106].

Dendrimers are branched tree-like structures which allow excellent control over size, dispersity, and functionalization during synthesis. Various types of drug cargo can be made to associate with the branched polymer matrix or attach to the dendrimer surface. SPL7013 (Vivagel™) is a lysine-based dendrimer with antimicrobial properties used for the prevention of HIV, genital herpes, and HPV[107].

Polyplexes are self-assembled nanoparticles that are stabilized by hydrophobic or electrostatic interactions with the constituent polymer(s) and drug cargo. CALAA-01 is a polyplex of PEGylated cyclodextrin loaded with siRNA (to knockdown the M2 subunit of ribonucleotide reductase) and coated with transferrin; CALAA-01 has been in clinical trials for the treatment of solid tumors[9]. Similarly, CRLX101 is a polyplex of PEGylated cyclodextrin conjugated to camptothecin that has been involved in clinical trials for renal, ovarian, and rectal cancer[108].

As the field of nanoparticle delivery matures (Table 1), so must the principles guiding further innovation. Adopting a holistic view—which includes all aspects of nanocarrier design and deployment as inter-related tools to mediate targeting—will facilitate clinical translation of technologies and treatment paradigms. This integrated view may also shift the focus away from development of complex nanoparticle formulations—that are likely to be very difficult to scale for commercial availability—and towards simple, judiciously constructed solutions that are more likely to find clinical success. In this review we present a new outlook on targeting polymer nanoparticles for drug delivery, which we divide into anatomical, molecular, and temporal aspects (Figure 1). While our primary focus is on polymer nanoparticles, other drug delivery platforms, such as liposomes, are often employed under similar circumstances and therefore face many of the same challenges. As such, our conclusions present a broad perspective on targeting that will impact the design of all types of nanotherapeutics.

CURRENT STATE OF THE ART IN NANOPARTICLE TARGETING

The majority of current targeted nanoparticles are engineered to treat cancer by intravenous administration (Table 1); the details have been covered in several recent reviews[4–6]. Previous generations of tumor-targeted nanoparticles were designed to maximize passive targeting, in which systemically circulating nanoparticles penetrate the leaky vasculature often associated with tumors and accumulate due to slow clearance from poor lymphatic drainage (i.e. the enhanced permeability and retention, or EPR, effect)[7]. In the passive targeting approach, increasing accumulation at the tumor correlates with longer circulation times and so the surface of these particles are coated with inert materials (such as polyethylene glycol or PEG) to reduce elimination of the particles via the host immune system and thereby maximize the circulation time. Seeking to further improve upon passive targeting, many current-generation nanoparticles rely on the promise of molecular (or active) targeting enhancements. In active targeting the surfaces of nanoparticles are endowed with molecules (e.g. native ligands or antibodies) that can, in theory, increase affinity for specific cells or tissues. Often active targeting is used in combination with nanoparticle formulations designed to have enhanced passive targeting. Two such polymer nanoparticle systems in clinical trials, BIND-014 and CALAA-01, are surface-modified with both PEG and targeting molecules that bind receptors enriched on some cancer cells[8, 9]. While it is not the primary focus of this review, it is worth noting that there have also been some promising advances in the targeting of free drugs, such as the chemical stabilization of oligonucleotides[10] and ligand-oligonucleotide conjugates[11].

Despite an abundance of attention on molecular approaches to targeting, the results have been mixed. While there are some intriguing successes, current technologies for ligand-receptor targeting of nanoparticles do not produce predictable outcomes[12, 13]. There is growing evidence that the presence of targeting ligands can have a negative impact on passive targeting by reducing circulation times through enhanced immune elimination[6, 13]. Additionally, the assumption that the specificity of a targeting ligand will be retained after conjugation to a nanoparticle surface is flawed, particularly when particles are delivered *in vivo*[12]. Furthermore, even if a highly specific targeted nanoparticle can be achieved, there is no guarantee that a unique receptor will be significantly expressed solely on the cell population of interest. These challenges have led to questions about whether the added complexity that comes with the introduction of active targeting ligands is valuable, particularly since complex nano-delivery systems are both difficult and expensive to develop to the point of clinical availability[14]. Thus, in spite of the promise arising from recent innovations in nanoparticle synthesis, it is unclear how much closer these innovations have gotten us to fulfilling the promise of drug targeting and where exactly the field should focus its attention moving forward.

We believe a more nuanced view of targeting will speed design of clinically useful systems to better deliver on the potential of nanoparticle drug delivery. A typical approach for design of targeted therapies focuses first on the development of a drug delivery platform, which is then screened against a variety of diseases to find the setting in which the greatest efficacy can be achieved. We prefer a “top-down” strategy in which a given disease indication fuels the design of nanomedicines. Recently, others have expressed a similar approach that starts by selecting a particular disease and then sampling relevant drug delivery platforms to identify the best method for treating that pathology[15]. Such an approach focuses attention on the biological characteristics that define a given disease, and how nanoparticles are likely to interact with cells and tissues in that context, and thus emphasizes design of a targeting system that is specifically tailored to these characteristics.

In addition to considering the disease state first, we argue that it is also essential to broaden the focus of nanoparticle delivery beyond the current emphasis on active targeting by intravenous delivery. For many diseases, alternative routes of administration may be more effective than systemic delivery. Further, regardless of whether targeting molecules are employed or not, it is critical to consider how the physicochemical properties of the nanoparticle and the surrounding biological milieu affect the ability to target at the molecular level. Finally, there are also important temporal aspects to targeting including the relationship between pharmacokinetics, drug release kinetics, and the therapeutic window for effective disease treatment. In the following sections, we expand upon this more comprehensive notion of targeting by providing examples of how a holistic approach can better fulfill the promise of targeted nanomedicines.

ANATOMICAL TARGETING

Targeting implies a direct focus on one object of interest, while simultaneously ignoring everything else. For nanoparticles that are intended to target a single cell population, this concept is difficult to translate into practice—particularly when the particles are

administered systemically. While delivery to the blood circulation has the potential benefit of providing access to any vascularized tissue in the body, this pervasive access also increases the likelihood that unintended tissues will be targeted. It is typically observed that systemic delivery of nanoparticles results in significant accumulation in both liver and spleen as a result of immune clearance by the mononuclear phagocyte system (MPS; also known as reticuloendothelial system or RES)[16]. Unless these phagocytic cells of the liver (or spleen) are the intended target, this off-target accumulation provides a major impediment to achieving specificity with systemic delivery. Alternatively, directed local delivery of drugs is a simpler and (for some diseases) potentially more effective form of targeting, allowing a physical means for enrichment of nanoparticle concentration at a specific site and reduced accumulation in non-target tissues[17–20]. Local delivery has been shown useful in treatment of brain tumors; implantation of a macroscopic polymer wafer provides sustained effective concentrations of a chemotherapy drug in the brain, while sparing other tissues to drug exposure[21]. Local administration can also be achieved with nanoparticles through a range of physical and minimally invasive targeting methods that are appropriate for specific diseases (Table 2). The following examples provide a sampling of how local modes of nanoparticle administration can be utilized as a means to enhance disease targeting.

Brain infusion

The most basic method for targeting by local administration of nanoparticles is direct injection into the tissue of interest. Such injections are invasive, but in some cases physiological barriers significantly impair the effectiveness of any other delivery route. The blood-brain barrier is one such physiological impediment where, despite decades of effort, no systemically targeted nanoparticle systems provide adequate transport across the BBB to allow effective treatment of serious brain diseases, such as glioblastoma multiforme[17, 22–25]. Alternately, nanoparticles can be directly injected into the brain (circumventing the BBB) using catheters stereotactically targeted to precise anatomic locations; approaches such as convection-enhanced delivery (CED) can then be used to target larger volumes of the brain to ensure that the injected particles fully cover the targeted area[22, 26, 27]. Nanoparticles are potentially useful here because they allow for sustained intracellular delivery of the encapsulated agent. Sustained drug release is essential in this setting, because repeat dosing is not practical. In addition, effective delivery relies on the ability of nanoparticles to penetrate away from the site of injection and into proximal surrounding diseased tissue. For CED, nanoparticle size appears to be the main property that influences susceptibility to convective transport within the brain. For example, atypically small PLGA nanoparticles can be transported large distances from the site of infusion via convection[23]. The invasiveness of this delivery method will limit its broad application, but it is potentially useful for the delivery of agents that reverse Parkinson's disease[28] or Huntington's disease[29]. In addition, this approach can be extended to other parts of the nervous system, as shown in the injection of protein growth factor-loaded nanoparticles for treatment of spinal cord injury[30] or the injection of various nanoparticles into the eye for treatment of retinal diseases[31, 32].

Dermal administration

Direct local administration to target nanoparticles need not be as invasive as delivery to the brain. For example, topical application to the skin is non-invasive and yet still provides

physical targeting. As evidence of the safety of this approach, chitosan, PLGA, poly(DL-lactide) (PLA), polyalkylcyanoacrylate, and polycaprolactone (PCL) polymers are routinely used in dermal delivery for both therapeutic and cosmetic purposes[18]. Generally, topically applied nanoparticles are unable to deeply penetrate the stratum corneum (i.e. the outermost layer of the epidermis) and instead localize to proximal glands and hair follicles[33]. As such, topically applied nanoparticles typically cannot reach subepithelial capillaries to access the circulatory system. Nevertheless, particles can be formulated to penetrate deeply enough to have significant therapeutic benefits. For example, PLGA nanoparticles coated with a cationic lipid that induced swelling and opening of the stratum corneum were used to co-deliver siRNA against TNF and capsacin (an anti-inflammatory drug) to treat the chronic inflammation of psoriasis[18]; importantly, this strategy has the potential to avoid the widespread immunosuppressive side-effects associated with systemic delivery of TNF antagonists[34]. The limited spread to surrounding tissues observed in dermal delivery provides a substantial safety benefit. In addition to this spatial advantage, the local retention of topically applied therapeutics imparts a temporal advantage by increasing treatment duration.

Both brain infusion and topical skin administration demonstrate the potential benefit of physically administering therapeutics directly at the intended site of action. However, local administration of nanoparticles via alternate routes does not always restrict targeting to the initial site of delivery. When subcutaneously injected, nanoparticles smaller than 100 nm are prone to clearance by lymphatic vessels, while larger nanoparticles are generally retained in the interstitial space near the site of injection[35, 36]. As such, subcutaneous injection of sub-100 nm particles can be a method for targeted delivery to surrounding lymph nodes[37–40], provided the particles are not so small as to leak into blood capillaries[35]. While targeting lymphatics by subcutaneous administration of sub-100 nm nanoparticles can be inefficient[41], this approach nevertheless has potential for the development of DNA-based vaccines[42] and in the treatment of certain lymphomas[43].

Mucosal delivery

Mucosal administration offers the advantages of local tissue targeting[44–46], while also providing potential routes for sustained systemic administration that are less invasive than intravenous injection[47–49]. Mucus provides a protective barrier on the epithelia of numerous tracts and structures in the body that are potential administration routes for nanotherapeutics. For example, topical vaginal administration of polymer nanoparticles allows for targeted delivery to the vaginal epithelium, and can be used to deliver siRNA[50, 51] and drugs[52, 53] in order to prevent and treat infectious diseases[51, 54] or cancer[55, 56]. Molecular engineering of these systems circumvents obstacles to local drug action; in particular, design of the nanoparticle surface allows particles to penetrate cervical mucus and to reach the underlying epithelium[50, 57, 58]. For example, addition of PEG to the nanoparticle surface enhances mucus penetration[58, 59], which can also be improved by modulating NP size and attraction to mucin fibres[60, 6162]. Other surface elements, such as chitosan, can disrupt tight junctions and increase epithelial penetration[63], providing a tool for controlling nanoparticle targeting after topical delivery to vagina, bladder, and other mucosal epithelial sites[64, 65]. Alternately, another approach for mucosal delivery is to improve mucus adhesion[66] or binding[67], which

then limits penetration to the epithelium, allows topical release of drug payloads, and links nanoparticle clearance to the natural clearance of mucus.

Regardless of the specific route of delivery, a consistent theme in the preceding examples is the need to carefully consider how the molecular properties of the delivered nanoparticles—such as shape, size, charge, chemical character—will guide interactions with the local environment after delivery. Different aspects of targeting, even those that seemingly operate on very different scales (i.e. macroscopic versus microscopic), are nonetheless highly inter-dependent. Consequently, overcoming the obstacles associated with targeting requires moving beyond the optimization of individual elements of targeting, to instead focus on how all aspects work in concert. In the following sections, we introduce molecular aspects of targeted delivery and discuss specific issues that need to be addressed to ensure that molecular targeting strategies can be reliably incorporated within this holistic approach.

Molecular targeting

Given the potential for high affinity and specificity in conjugating a native ligand or relevant antibody to nanoparticles, it is understandable that so much attention in the field has been focused on these molecular targeting approaches. However, it is becoming clear that the highly specific targeting capabilities associated with molecules such as antibodies cannot simply be grafted on to the therapeutic functionality of a drug-loaded nanoparticle. The conjugation of targeting molecules to the surface of nanoparticles can not only impair the bioactivity of the targeting molecule[12], but also negatively impact the therapeutic efficacy of a nanoparticle by reducing passive targeting via enhanced immune elimination[13]. Moreover, it has been demonstrated that the addition of targeting ligands does not significantly alter the biodistribution of systemically administered nanoparticles[5, 68, 69]. In spite of these challenges, active targeting can still play a role as part of the broader toolkit of targeted drug delivery, particularly when the route of administration ensures that the conjugated ligand will find its intended receptor. However, issues that can impact both specificity and therapeutic efficacy must be addressed to achieve success with molecular targeting strategies (Table 3). The following sections highlight some of the most significant challenges.

Targeting specificity challenges

Most polymer nanoparticle formulations are taken up—at least to some extent—by many different types of cells, even in the absence of modifications intended to enhance nanoparticle internalization[70, 71]. While an inherent propensity for cellular uptake can be therapeutically beneficial, it nevertheless provides an impediment to specific targeting by reducing the ability to regulate unintended particle uptake. Cellular uptake can be sensitive to physical characteristics of nanoparticles—properties such as particle shape, size, charge, and hydrophobicity[71–75]—thereby providing a potential means for controlling the level of uptake in the absence of a specific targeting ligand. However, without a singular mechanism linking particle properties to cellular uptake, it is difficult to predict what combination of properties will be most relevant for a given nanoparticle formulation and cellular target. Moreover, uptake observed for one cell in one environment may not occur for all cells in all environments, making it risky to extrapolate from *in vitro* mono-cell culture models to *in vivo* conditions. These caveats aside, it has been shown both *in vivo* and *in vitro* that coating

the surface of polymeric nanoparticles with inert molecules such as PEG[76] or hyper-branched polyglycerol[77] can significantly reduce unwanted cellular interactions.

Many studies have focused on understanding the basic mechanisms underlying this intrinsic capacity of nanoparticles to be taken up by cells[71]. In some cases this non-specific particle uptake may be attributable to electrostatic properties of the particles; for example, cationic particles associate on the basis of ionic interactions with anionic cell membranes and cell surface components leading to enhanced particle uptake[78]. But perhaps more importantly (especially *in vivo*), there is now substantial evidence that surface adsorbed serum or plasma proteins are critical determinants of cell-nanoparticle interactions[12, 79, 80]. These adsorbed proteins, often referred to as a protein corona, can rapidly (within seconds) associate with nanoparticles upon exposure in serum containing medium or blood. Using a mass spectrometry based approach, polystyrene nanoparticles were shown to associate with hundreds of different serum proteins; the specific proteins adsorbed depended on particle charge, size, and chemical composition[79]. Because of this, the ability of surface-conjugated molecules (such as transferrin) to provide specific nanoparticle targeting can be stifled by the presence of serum proteins[12]. Moreover, many of the serum proteins that adsorb to nanoparticle surfaces may themselves activate receptor-mediated endocytosis[16]. Consequently, what is observed as a non-specific effect may actually result from an unintended, specific interaction.

Thus—whether by inhibiting the biological activity of a targeting ligand or by providing alternative, unintended routes of cellular uptake—proteins adsorbed from the environment can interfere with active targeting. This role of serum proteins on drug targeting extends beyond the field of nanoparticles: binding to albumin can prevent renal filtration of macromolecular drugs[81] and certain lipoproteins have been observed to direct the tissue accumulation and cellular uptake of various nucleic acid therapeutics[82]. These other fields have adapted to exploit drug interactions with serum components. Similarly, better means for either controlling the composition of the nanoparticle protein corona or minimizing its effects must be developed[80, 83]. Such strategies should focus not only on reducing uptake by phagocytic cells of the immune system (as is often the motivation with surface PEGylation), but also on preserving targeting specificity. Finally, it will be important to better understand how the protein corona varies with the physicochemical properties of a given particle, and with different anatomical routes of administration[80].

The ability to generate specific antibodies against any cell surface receptor would appear to promise a wide palette of potential targets from which to choose. However, the vast majority of these molecules are not unique to a single cell population, which severely restricts the number of surface receptors useful for targeting purposes. As a result, the majority of studies have focused on just a few specific targets typically overexpressed on cancer cells (e.g. transferrin receptor and HER2)[5]. Expanding the list of potential targets for a given pathology necessarily requires understanding the fundamental biology underlying the disease. It also requires understanding from a biochemical perspective the surface expression required on the targeted cell relative to non-targeted cells and nanoparticle avidity in order to ensure strong nanoparticle association. In instances where a suitable cell surface target does exist, it is also important to realize that the binding of the targeting

moiety to the targeted receptor may itself lead to a biologic response that can either enhance or inhibit the desired therapeutic effect[84].

Therapeutic efficacy challenges

Even if a nanoparticle can successfully navigate these pitfalls, and be specifically taken up in a targeted cell, this does not guarantee therapeutic efficacy. The intracellular fate of nanoparticles, and the timing of agent release, determines whether the active agent will find its intended site of action. A canonical example of this challenge is in the delivery of siRNA to the cytosol[85]. The therapeutic potential of siRNA is far-reaching, with the possibility to impact diverse disease states (such as chronic inflammatory disorders, neurodegenerative disorders, viral infections, organ transplant rejection, and cancer[86]) with high target specificity. In order to be effective, siRNA molecules need to reach the endogenous processing machinery in the cytosol, a difficult challenge given the instability and short half-life of extracellular siRNA. Various nanoparticle formulations have been engineered to successfully protect siRNA from degradation, but many of these formulations become trapped in endosomal compartments and/or trafficked to lysosomes where the particle and siRNA are ultimately degraded without ever reaching their target[87]. To combat this, a great deal of effort has been focused on facilitating endosomal escape by adding drugs[88], cationic polymers[89], lipids[90], or fusogenic peptides[91] to the nanoparticle formulation.

Enhancing siRNA delivery via endosome escape is just one example of controlling nanoparticle fate in cells; there is a diverse array of molecular modifications that can aid in regulating intracellular delivery and localization[92]. For example, cell-penetrating peptides (CPPs) are a widely used nanoparticle modification to improve cell uptake and modulate intracellular fate; however, the functional mechanisms of many CPPs (let alone those attached to nano-scale structures) are still unclear[93]. Gaps in the understanding between activity and mechanism (as well as potential for toxicity) suggest caution in the use of molecular modifications, providing another illustration of how each aspect of molecular composition must be considered as an element in a holistic design.

Achieving cellular specificity through active targeting remains the (non-trivial) goal of many nanomedicines. Active targeting is confounded by various factors including nanoparticle physicochemical properties, interactions specific to route of delivery, and challenges unique to the agent being delivered, e.g. cytosolic delivery of siRNA. To further complicate this issue, some efficacious actively targeted nanoparticles possess only a narrow window over which the targeting capacity of a conjugated ligand will be retained[94]. Consequently, it cannot be assumed that any given disease or nanoparticle platform will be compatible with active targeting approaches. This fact reinforces the argument that active molecular targeting should not be viewed as the only approach for targeting disease. Rather, ligand conjugation is appropriate only when: 1) the route of delivery can get the nanoparticle to the cell displaying the targeted receptor of interest without compromising the therapeutic efficacy of the delivered drug; and 2) the intracellular fate of the nanoparticle after uptake can support effective therapeutic delivery. These requirements will not be present in all clinical settings, or for all agents. By biasing the use of molecular targeting strategies strictly to diseases and

treatment modalities that meet these criteria, we will significantly improve the odds of successfully incorporating molecular targeting in therapeutic design.

TEMPORAL

In addition to selection of administration route and controlling molecular interactions, nanoparticle targeting can be further improved with a temporal perspective. Temporal targeting exploits therapeutic windows to maximize drug delivery. For example, lungs have a larger window of therapeutic opportunity for systemically administered agents than spleen; the respective times required for lungs and spleen to receive a volume equivalent to the total blood volume in humans are ~1 and 64 minutes[95]. In the context of nanoparticle therapeutics, temporal targeting can be achieved through an understanding of disease progression and pharmacokinetics, as well as through the engineering of nanoparticles that impart control over when a drug is delivered.

Biological timing

Disease pathophysiology can significantly impair or improve the amenability to nanoparticle treatment. Developing targeted nanomedicines with a disease-first approach requires a detailed understanding of pathophysiology, since treatment susceptibility for diseases often lessens with time. Typically, treating disease at early stages will prevent further spread and pathogenesis, but early treatment may also be advantageous from a pharmacokinetic perspective. For example, in cancer, elevated tumor interstitial fluid pressure (IFP) results from a combination of poorly formed vasculature and lack of functional lymphatics, which in turn, reduces convective flow of nanoparticles into the tumor parenchyma[96]. As tumors grow, IFP typically intensifies and further impedes nanoparticle transport[97]. Thus, tumor delivery of nanoparticles can be improved through temporal targeting of early-stage tumors (Figure 2a). Similarly, cystic fibrosis involves thickening of mucus lining the airways and intestinal tract, which can exacerbate the mucosal barrier and impede delivery of nanotherapeutics[98]. Although nanoparticles with muco-adhesive and muco-penetrating properties have been engineered to exploit and overcome mucus retention[62, 67, 99, 100], early treatment of cystic fibrosis may circumvent this obstacle (Figure 2b). Note that in addition to the temporal aspects of disease pathology, other aspects of biological timing can have an impact on therapeutic windows. For example, the cycling of the female reproductive mucosa can impact the timing for administration of intravaginal or intrauterine therapies. Likewise, the cycle of expansion and contraction of the bladder influences tissue structure in the bladder wall and therefore likely influences tissue permeability to nanoparticles as well.

Designing nanomedicines that target specific therapeutic windows for a given disease may be limited in some cases by an incomplete understanding of disease pathogenesis. Pre-clinical models can be predictive, but not necessarily representative of disease progression in humans. For example, the EPR effect observed in many pre-clinical models may be less prevalent in human tumors[101]. A key benefit of nanoparticle platforms is their amenability to deliver a diverse array of cargos. Thus multifunctional nanoparticles can combine therapy with imaging modalities (i.e. theranostics[102]) in order to help identify times when a given disease is most susceptible to treatment. A common example is to fabricate nanoparticles loaded with a chemotherapeutic and functionalized with an imaging contrast agent[103].

However, similar to the concerns with molecular targeting, the added complexity of theranostic nanosystems suggests a careful approach.

Controlled release

In addition to the capacity for multifunctional properties, another property of polymer nanoparticles is the ability to protect drug cargo from the time of administration to delivery at the intended tissue, cell, or intracellular location, and to release it in a sustained fashion. Given the numerous physiological and cellular barriers facing the delivery of nanotherapeutics, control over when to release an encapsulated drug can significantly impact therapeutic efficacy. Unlike other classes of nanocarriers, most polymer nanoparticle systems can be tuned to yield desirable release kinetics. This process, known as controlled release, is typically achieved by regulating the rates of polymer biodegradation and drug diffusion outward through the polymer matrix. As such, polymer nanosystems have been developed with release durations ranging from minutes to weeks[104]; in particular, biodegradable poly(ester) polymers such as PLA and PLGA are well known for use in formulating controlled release nanoparticles. A primary goal in developing these systems is to align drug release profiles with nanoparticle pharmacokinetics. For example, the drug release profiles of various PLA nanoparticles (with similar composition to BIND-014) were tuned to release over several days in order to capitalize on the extended systemic circulation time of the PEGylated nanocarriers, which ultimately accumulated in tumors[8]. In another example, PLGA nanoparticles administered intranasally to a model of cystic fibrosis resided within diseased lungs and achieved pharmacological effects through sustained drug release for up to 11 days[19].

Another approach to controlling drug release is the development of modular nanoparticles in which delivery is triggered. For example, a polymer nanoparticle system was designed to sequentially deliver an anti-angiogenesis agent followed by a chemotherapy agent[105]. This approach triggered vascular shutdown and entrapped the nanoparticles within a tumor before releasing chemotherapy; thereby temporally targeting drug release to coincide with the time at which a disease was susceptible to treatment. With the ability to respond when needed, modular nanosystems may be paradoxically simpler than some complex molecularly targeted nanoparticles. Through the coordinated alignment of pharmacokinetics and drug delivery with therapeutic windows provided by disease pathophysiology, polymer nanoparticles present a unique technology for temporal targeting of disease, which has not yet been fully exploited.

CONCLUSION

Polymer nanoparticles can be synthesized with control over particle composition, incorporation of many kinds of drug agents with tunable release kinetics, and presentation of targeting ligands. But the effective use of these nanomaterials for targeted treatment of human disease is still limited. We believe that effective targeting requires thinking along several length scales – from macroscopic to microscopic. Here, we present a holistic approach to nanoparticle design, which benefits from the enormous progress that has been made over the past decade. This new approach—encompassing the route of administration,

molecular composition of the nanocarrier, and temporal coordination—requires that nanoparticle design be directed by pathophysiology and be integrated over all of these elements. This holistic perspective has already been applied in some settings; for example, the aforementioned local delivery of small nanoparticles for controlled drug release in the brain engages all of these targeting elements. Additionally, these principles are not mutually exclusive. For example, molecular modifications and drug release kinetics should be tuned for a given nanoparticle administration route. We suggest that deliberate application of this holistic approach will substantially reduce the hurdles in developing polymer nanoparticles for effective treatment of cancer and many other difficult to manage diseases. In fact, the holistic view we have outlined here is not fully inclusive; in the emerging era of personalized medicine, drugs themselves can impart another layer of targeting. Ultimately, the definition of different aspects of targeting does not matter as much as the approach taken to achieve targeting, which we believe should consider every possible tool in the arsenal in order to achieve the desired therapeutic end.

GLOSSARY

Active targeting	Targeting that is mediated by specific receptor-ligand interactions. For active targeting with nanoparticles, ligands are usually attached to the particle surface to enhance cell- or tissue-specific binding and nanoparticle uptake through receptor-mediated endocytosis
Enhanced permeability and retention (EPR) effect	Accumulation of particles in tumors due to extravasation from the blood through leaky vasculature (enhanced permeability) and lack of lymphatic drainage (retention). Nanoparticles of diameter near 100 nm appear to be optimal for the EPR effect in many tumors
Passive targeting	Targeting that occurs due to physical properties of a nanoparticle, such as surface charge or size, that decrease protein opsonization and phagocytic elimination, enhancing circulation time and subsequently retention in tumors
Targeting	The preferential accumulation of nanoparticles in a preferred (or target site) when compared to other (non-target) sites

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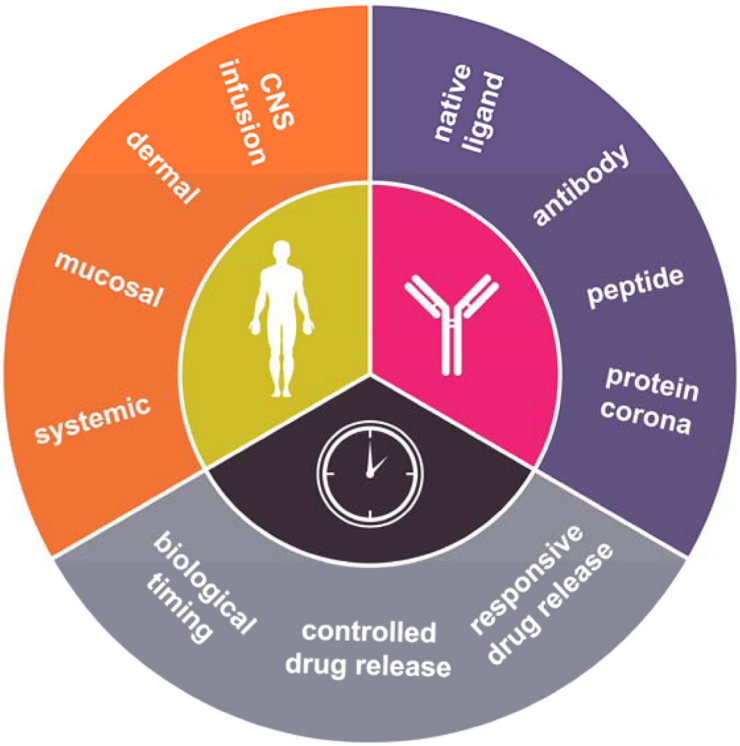

Figure 1.

Holistic perspective of targeting. Representation of key factors influencing nanoparticle targeting organized into anatomical route of delivery, molecular, and temporal aspects. The outer wheel highlights some ways in which nanoparticles can be targeted within the three subgroups. First, different anatomical routes of delivery will affect particle biodistribution providing an initial level of targeting. Second, molecular modifications, either in the form of chemically conjugated targeting ligands and/or proteins adsorbed from the local environment have the capacity to modulate the nature of cellular interactions. Third, the choice of when to treat a disease (biological timing) combined with engineering nanoparticles to have defined drug release profiles (nanoparticle timing) provide an additional mechanism for targeting. Design must be holistic, considering all aspects simultaneously, as the three areas are interconnected; any choice made in one aspect of delivery will have significant effects on the other areas.

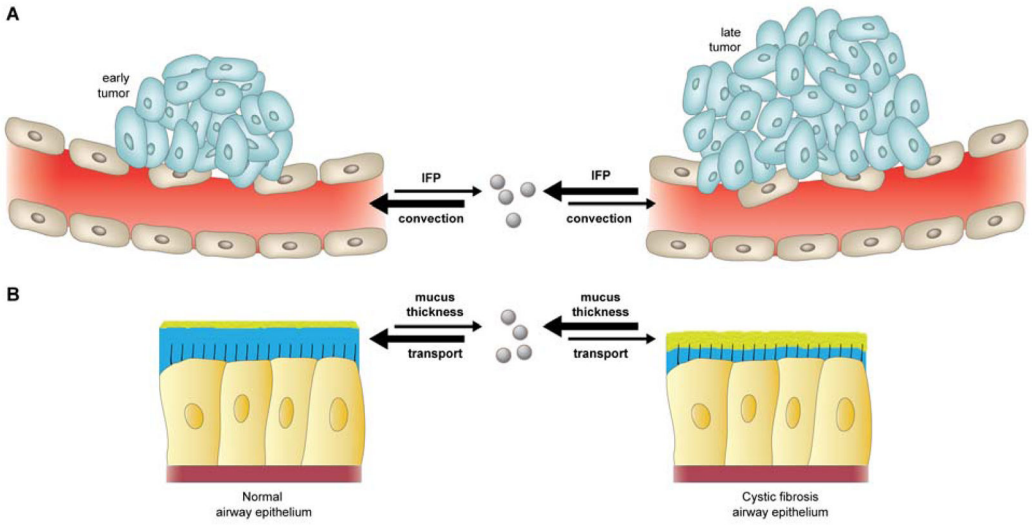

Figure 2.

Temporal targeting of therapeutic windows. **a.** As a result of heightened IFP as tumors grow, systemically circulating nanoparticles more readily distribute to early-stage tumors. **b.** Mucus thickening is a phenotype of cystic fibrosis that can impede nanoparticle transport.

Table 1

Selected clinical studies in the United States involving polymer nanoparticles

Name	Formulation	Targeting molecule	Disease indication	Clinical phase	References
BIND-014	Docetaxel | PLA-PEG	Prostate-specific membrane antigen ligand	Non-small cell lung cancer, prostate cancer	Phase II	8
SEL-068	Nicotine-*t*SVP™	Agonist/T cell helper peptide	Nicotine addiction	Phase I	109
CALAA-01	siRNA | cyclodextrin-PEG	Transferrin	Solid tumors	Phase I	110
CRLX101	Camptothecin | cyclodextrin-PEG	None	Pancreatic cancer, ovarian cancer, and small cell lung cancer	Phase II	111
Genexol-PM	Paclitaxel | PLA-PEG	None	Breast, lung, pancreatic cancer	Phase III–IV	112, 113

Table 2

Routes of administration for polymer nanoparticles in pre-clinical studies; * indicates incomplete understanding, with more research required.

Category	Route	Examples of potential disease targets	Typical Fate	References
Non-mucosal local administration	Brain infusion	Glioblastoma multiforme, Pediatric brain tumors, Parkinson's disease	Size-dependent penetration, Local retention, Clearance by perivascular spaces possible*	23, 24, 114, 115
	Spinal cord infusion	Traumatic spinal cord injury	Local retention*	116, 117
	Periocular injection	Glaucoma, retinoblastoma	Delivery to intraocular tissues	118
	Intravitreal injection	Macular degeneration	Delivery to vitreus chamber and retina	118–120
	Topical skin application	Insufficient wound healing	Local retention	18, 33, 121
	Subcutaneous injection	Vaccines for infectious diseases, lymphoma	Local retention, Clearance by lymphatic system	35, 36, 41, 122
	Intraperitoneal injection	Ovarian cancer	Retention in peritoneum, Systemic distribution	123–125
	Intramuscular injection	Ischemia, Vaccines for infectious diseases	Local retention, Clearance by lymphatic system	36, 122, 126, 127
	Intradermal injection	Vaccines for infectious diseases	Local retention, Clearance by lymphatic system	36, 126, 128
	Intra-articular injection	Osteoarthritis	Size-dependent penetration, Local retention, Clearance by unknown mechanisms*	129
Mucosal local administration	Topical vaginal delivery	Prevention of sexually transmitted infections	Mucosal retention and tissue penetration	55, 57, 58
	Inhalation delivery	Cystic fibrosis, Interstitial lung disease, Lung cancer	Delivery to respiratory tract	130, 131
	Intranasal delivery	Respiratory infectious diseases	Delivery to respiratory tract, CNS delivery possible*	132–134
	Oral ingestion	Insulin-dependent diabetes	Delivery to gastrointestinal tract	135–139
	Topical eye delivery	Glaucoma	Delivery to cornea, Lateral diffusion to ocular tissues	118, 120
Systemic	Intravenous	Diverse	Systemic distribution, MPS clearance	4, 5, 8, 95, 110

Table 3

Select challenges facing molecular targeting of polymeric nanoparticles (NP)

	Issue	Contributing factors	Potential Solution
Factors affecting targeting specificity	Unintended NP uptake	NP size/shape/charge Cell specific uptake properties	Inert particle coatings (e.g. PEG, HPG)
	Protein corona	Unintentional receptor mediated uptake Steric hindrance	Composition characterization and control via NP formulation
	Receptor identification	Lack of unique targets for cells of interest	Combine with local delivery
Factors affecting therapeutic efficacy	Reduced Passive targeting	Increased immunogenecity Shorter circulation time	Control ligand density to maximize avidity and minimize immunogenicity
	Intracellular fate	Endosomal entrapment Lysosomal degredation	Combine with endosomal escape ligands (e.g. CPP)