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Targeted Nanotherapies for the Treatment of Surgical Diseases

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Abstract

Objective—To describe the components of targeted nanotherapeutics and to review their applications in the treatment of surgical diseases.

Background—Targeted nanotherapeutics is a novel strategy for treating a variety of diseases and is an emerging technology that offers advantages over current treatment strategies. The nanoscale size, combined with the ability to surface functionalize the delivery vehicle to enable targeting and incorporate a therapeutic payload, provide a new and innovative therapeutic platform to treat surgical diseases that has yet to be fully realized in the surgical arena.

Methods—A comprehensive literature review of nanotherapeutics, targeting strategies, and their utility in treating surgical diseases is performed.

Results—Targeted nanotherapeutics have demonstrated safety and biocompatibility in treating surgical diseases. The ability to surface functionalize the nanoparticles affords a unique tailorability that enables targeting specificity and therapeutic payload delivery to treat a variety of surgical diseases. Moreover, the small size and targeting capabilities allow access to biological compartments, such as the blood-brain-barrier, that have previously been difficult to treat.

Conclusions—Targeted nanotherapeutics represents a novel therapeutic platform and has great potential to impact the treatment of surgical diseases.

Mini-Abstract

Targeted nanotherapies represent a growing field of therapeutics with the potential to impact the care of surgical diseases. This review describes the components of targeted nanotherapies, including the delivery vehicle platform, targeting moiety, and method of delivering a therapeutic agent by providing examples relevant to the treatment of surgical diseases.

INTRODUCTION

Nanotechnology is the term used to describe the research, engineering, and technology of materials on the nanoscale, or approximately 1–100 nm in size.¹ The application of nanotechnology to the field of medicine and surgery is termed nanobiomedicine.² The first liposome-based nanotherapeutic to gain the approval of the US Food and Drug Administration (FDA) was Doxil (OrthoBiotech), in 1995.³ Doxil, or liposome-encapsulated

doxorubicin, was initially approved to treat HIV-related Kaposi's sarcoma and was subsequently approved to treat ovarian cancer and multiple myeloma. By encapsulating the cytotoxic anti-cancer drug doxorubicin and coating it with surface polymers, this nanobiotherapeutic demonstrated a prolonged circulatory half-life and increased passive deposition into tumors, with decreased cardiotoxicity in comparison to free doxorubicin.⁴⁻⁶ However, this is an example of a non-targeted nanotherapeutic.⁷ In this review, we will discuss the components of a targeted nanoscale delivery vehicle including the nanotechnologic platform, the targeting methodology, and the delivery of a therapeutic in relation to surgical diseases.

DELIVERY VEHICLE

The first component of a nanotherapy is the delivery vehicle itself. Examples of nanotechnologic platforms include liposomes (spherical lipid bilayers), dendrimers (macromolecules with tendrils extending from a central core), polymeric micelles (core-shell structures of spontaneously self-assembled amphiphilic co-polymers), biodegradable polymeric nanoparticles, fullerenes, metal nanoparticles (gold, copper, silver), and quantum dots (colloidal semiconductor nanocrystals composed of atoms from groups II-VI or III-V of the periodic table) (Table I).^{2, 8-10} The shapes of these engineered nanoparticles, including nanospheres, ellipsoids, nanotubes, nanorods, and nanofibers, are equally as varied.

The size of nanoscale technology is comparable to endogenous biological entities such as red blood cells (3–8 μ m), white blood cells (8–10 μ m), and platelets (2 μ m) (Figure 1).^{11, 12} Thus, delivery vehicles sized on this scale have the advantage of being able to passively traverse cellular environments in a size-dependent fashion, and are able to affect tissues previously difficult to target at non-toxic doses.¹³ As an example of the size advantage of nanoscale technology, gold nanoparticles have demonstrated the ability to passively cross the blood-brain barrier as well as the blood-tumor barrier.^{14,15} This discovery of small molecule penetration through the blood-brain barrier has resulted in an explosion in neurosurgical basic science literature in the targeted treatment of glioblastoma multiforme and other brain tumors.

Particle size affects not only passive movement into various biological compartments, but it also affects the mode of cellular internalization within that microenvironment (i.e. phagocytosis, macropinocytosis, caveolar versus clathrin-mediated endocytosis) thus enabling engineered nanotechnology to be tailored for targeted internalization into specific microenvironments.³ As an example, cellular internalization of gold nanoparticles surface functionalized to carry Herceptin (the monoclonal antibody targeting the HER2/neu receptor and used for the treatment of breast cancer) has been demonstrated to vary depending on nanoparticle size, with the most efficient uptake occurring in a size range of 25–50 nm likely due to the balance between multivalent crosslinking of membrane receptors and receptor-mediated endocytosis.^{16, 17} In addition to size, nanoparticle geometry also influences the rate of cellular internalization. Evaluation of how particle size and shape of non-spherical nanoparticles effected the rate of cellular internalization in HeLa cells demonstrated a clear correlation between both size and shape on the rate of cellular internalization.¹⁸

Delivery vehicles sized on the nanoscale have characteristic biodistribution and clearance mechanisms that make them particularly advantageous for engineered manipulations and intravenous administration. Measurement of sieving coefficients of the kidney glomerular capillary wall have demonstrated that the threshold for first-pass renal elimination is ~10 nm in diameter. As such, nanoparticle delivery vehicles less than 5 to 10 nm in diameter are rapidly cleared through the kidneys.^{3, 19} As the size of the nanoscale delivery vehicle increases, particle filtration and sequestration occurs in the cells of the mononuclear phagocytic system in the spleen and liver.¹³ In the spleen, sinusoid fenestrations are approximately 200–500 nm in width. Mechanical filtration by these sinusoids allows for trapping of larger particles, greater than 500 nm in diameter, but escape of smaller particles and thus allows these latter particles to remain in the circulation for a longer period of time.²⁰ Moreover, due to the large blood flow to the liver and its fenestrated endothelia, Kupffer cells of the liver play a key role in nanoparticle removal from the blood stream.²¹

Intravascular administration of nanotherapeutics must overcome the body's innate immune system and the mononuclear phagocytic system in order to achieve prolonged circulation of the delivery vehicle, and hence, carrying capacity of the therapeutic payload. Macrophages are able to recognize and remove nanoparticles within seconds of intravascular administration and larger nanoparticles are more easily recognized and cleared by the liver and the mononuclear phagocytic system. Recognition is not of the nanoparticle delivery vehicle itself, but rather of opsonin proteins that adsorb to the surface of the nanoparticle upon entry into the bloodstream.²¹ Opsonization is the process by which non-self particles become covered with opsonin proteins, typically immunoglobulins and components of the complement system (C3, C4, C5), making these foreign particles recognizable to the phagocytic cells for eventual removal from the bloodstream. Opsonization and the body's rapid clearance of non-self particles render nanotherapeutic delivery vehicles ineffective. To counteract this mechanism, nanotherapeutic engineering advancements have enabled the development of "stealth" delivery vehicles that are able to evade opsonization and delay clearance from the bloodstream, thus allowing for circulation longevity and prolonged therapeutics delivery. The key to the development of these stealth nanotherapeutics involves surface modifications to inhibit opsonin adsorption to the delivery vehicle and complement activation.²² One breakthrough was the discovery that opsonization of hydrophobic particles occurs more quickly than opsonization of hydrophilic particles due to the enhanced adsorbability of opsonins to hydrophobic surfaces.^{21, 23, 24} Additionally, charged particles have been shown, *in vitro*, to be opsonized at a faster rate than neutrally charged particles.²⁵ Therefore, to alleviate the issue of rapid opsonization and clearance by the mononuclear phagocytic system, neutrally charged and hydrophilic polyethylene glycol (PEG) polymers were developed for adsorption to the surface of nanotherapeutic delivery vehicles.²¹ These neutrally charged and highly hydrophilic polymers are covalently grafted to or adsorbed to the surface of the nanoparticle, creating a physical barrier, or steric brush, to block opsonin adsorption to the surface of the delivery vehicle, to neutralize charged particles from blood proteins, and ultimately to sterically shield these nanotherapeutic delivery vehicles from phagocytic cells, resulting in increased intravascular stability and increased circulation time.^{3, 4, 21} Gref et al analyzed this theory by covalently attaching a PEG coating to a nanosphere core and assessed its ability to evade opsonization and the mononuclear

phagocytic system, as well as the effect this PEG coating had on circulation times and organ distribution.⁷ After injection of these PEG-coated nanospheres into mice, blood circulation times increased as the molecular weight of the PEG coating increased. Moreover, there was decreased particle uptake by the liver with the addition of the PEG-coating. At 5 minutes post-injection, 66% of the non-PEG-coated particles were removed by the liver, while at 5 hours post injection, less than 30% of the 20 kD PEG-coated nanospheres were removed by the liver. Particle distribution was confirmed by gamma scintigraphy, which demonstrated particle distribution localized to the liver and spleen in the non-PEG-coated nanosphere group at 15 minutes post-injection in comparison to persistence of PEG-coated particle in the bloodstream at the same time-point.

TARGETING

The ultimate goal of nanobiomedicine for the treatment of surgical diseases is the targeted application of a therapeutic agent to an intravascular target of interest. Engineering nanoscale delivery vehicles to evade rapid clearance from the blood stream, i.e. by addition of PEG, results in a prolonged circulating reservoir of a therapeutic agent. The next step in disease management is the targeting of that therapeutic agent to the site of disease. Targeting of a nanoscale drug delivery vehicle to a specific site can be a passive or an active process. Passive targeting of nanotherapeutics relies on endogenous biological conditions that enable the passive diffusion of nanoparticles through pathologically fenestrated vascular endothelium. Specifically, nanotherapeutic delivery vehicles take advantage of the leaky vasculature of inflamed and diseased endothelium in a process known as the enhanced permeability and retention (EPR) effect.²⁶ Under normal, nonpathologic conditions, nanoscale delivery vehicles are not able to passively diffuse through continuous endothelium and free drug diffuses slowly and nonspecifically.^{27–29} However, during inflammation and infection, endothelial cells become activated by chemokines and cytokines and the endothelium becomes discontinuous and leaky. This allows for extravasation of leukocytes to the site of inflammation or infection, but also allows for penetration of nanoscale therapeutics.²⁹ Similar to inflammation and infection, tumor vasculature is likewise discontinuous and permeable secondary to rapid and defective angiogenesis with hasty proliferation of endothelial cells and decreased number of pericytes.⁹ Nanoparticles of >8 nm diameter and up to 150 nm diameter are able to penetrate the inter-endothelial junctions (40 nm to 1 μ m) and accumulate within the tumor microenvironment.^{5, 13, 14, 27} Moreover, the tumors additionally lack effective lymphatic drainage due to compression and collapse of lymphatic vessels by proliferating tumor cells, further enhancing the EPR effect.^{9, 30} Animal studies have demonstrated up to a 50-fold increase in nanoparticle accumulation within the tumor microenvironment versus healthy tissue.³⁰ Combining engineered stealth nanobiotherapeutic technology and prolonged delivery vehicle circulation times with the EPR effect takes tremendous advantage of passively targeted nanotherapeutics (Figure 2).

There are limitations to passive targeting of nanobiotherapeutics that limit its efficacy and durability. Not all tumors, including gastric and pancreatic cancer, exhibit the EPR effect and as such passively targeted nanotherapeutics are of limited efficacy.³¹ Some tumors are less well perfused than others or vessel permeability may be variable throughout the same tumor. Additionally, some delivery vehicles with their surface functionalized therapeutic payload

are simply unable to penetrate the endothelial fenestrations. As such, active targeting of nanoscale delivery vehicles plays an important role in nanobiomedicine. Active targeting involves equipping the nanoparticle with a targeting ligand that recognizes a molecule expressed at the site of interest (Figure 3). Targeting ligands vary widely from naturally occurring antibodies, antibody fragments, and small molecules to engineered targeting ligands such as nucleic acid aptamers and peptides.

Targeting ligands not only localize to the site of disease, but can play an important role in the uptake mechanism of the delivery vehicle to enhance drug delivery. Liposomal-based nanoparticles (Figure 4) are ideal delivery vehicles for incorporating targeting ligands on their surface, as they can also encapsulate a desired drug. For example, immunoliposomes, which contain antibodies coupled to a liposomal drug delivery system, were one of the first examples of active targeting. Schnyder et al sought to improve the delivery of cytotoxic drugs to the brain. Transferrin is enriched at the blood brain barrier, thus representing an optimal target. They utilized OX26 mAb, a monoclonal antibody to the transferrin receptor, as the targeting moiety, and coupled it to a liposome encapsulating danomycin. This not only localizes to the brain, but the interaction between OX26 mAb and the transferrin receptor results in receptor mediated transcytosis, improving delivery to brain tissues. They were able to demonstrate prolonged cytotoxic effects in cell culture, and higher levels of brain accumulation in a rat model with the targeted nanotherapy as compared to free drug.³² In another example, Clark et al designed gold nanoparticles with transferrin coupled by acid-cleavable links. This allowed for targeting to the blood brain barrier followed by nanoparticle release into the brain by cleavage from the transferrin receptor during the low pH process of transcytosis. They were able to demonstrate an increased ability of these nanoparticles to cross the blood brain barrier and found that these nanoparticles were present in greater quantities compared to non-cleavable nanoparticles in the brain parenchyma of a mouse model.³³ The folate receptor is another attractive target as it also results in cellular uptake. Folic acid is a small molecule that is easily conjugated to a delivery vehicle and upon ligand binding, the folate receptor, along with the delivery vehicle, is internalized into the cell.³⁴ Additionally, the folate receptor is overexpressed in multiple types of cancers making it an ideal target for many types of cancer nanotherapeutics. For example, Pan et al coupled folate to liposomal doxorubicin to treat a murine xenograft model of human oral squamous cell carcinoma. They demonstrated greater tumor growth inhibition as well as a 31% increase in lifespan compared to animals receiving non-targeted liposomal doxorubicin.³⁵ In addition, folate has been used as a targeting ligand on multiple delivery vehicles including dendrimers,³⁶ liposomes,³⁵ polymeric micelles,³⁷ and superparamagnetic iron oxide nanoparticles³⁸ to treat animal models of cancer including epithelial,³⁶ oral³⁵ and hepatocellular carcinoma.³⁹

Often, a naturally occurring targeting ligand does not exist for a disease of interest. As such, targeting ligands which are small enough to be coupled to a nanoparticle are engineered. For example, tissue factor, which is an important trigger of the coagulation cascade, is only exposed to the blood stream when the endothelium is disrupted. Thus, this represents an important target for bleeding and thrombosis.⁴⁰ Factor VII is the natural ligand for tissue factor, however is too large itself to serve as a targeting ligand. Shi et al utilized the EGF-1 domain of the light chain of factor VII, which is known to be important in binding to tissue

factor, to create a targeting protein. They coupled this targeting protein to a polymeric nanoparticle and demonstrated both *in vitro* and *in vivo* targeting specificity of this nanoparticle using a rat model of cerebral hemorrhage.⁴¹ Although early in development, this therapy could have applications in the neurosurgical field to stop bleeding.

Another example of an engineered targeting molecule is nucleic acid aptamers. Aptamers are RNA or DNA oligonucleotides selected for their high affinity to their target ligands. Due to this specificity, small size, and lack of immunogenicity, they are ideal targeting ligands for nanotechnology.⁴² Farokhzad et al demonstrated use of an aptamer as a targeting ligand by targeting the prostate specific membrane antigen, which is highly expressed on prostate cancer cells, with an RNA aptamer coupled to a polymeric nanoparticle to deliver docetaxel to an animal model of prostate cancer.⁴³

A final example of an engineered targeting ligand is a peptide binding sequence (i.e., a short sequence of amino acids). A short peptide sequence can be coupled to the surface of the nanoparticle, thereby allowing for binding to the site where the target protein is expressed. While some targets have known binding sequences, often a sequence is not known for a particular target. A method of screening for a binding sequence called phage display can be useful to identify an optimal binding sequence. Phage display utilizes bacteriophages displaying peptides from a peptide library to a target or surface of interest, and then selecting for the phage displaying the optimal binding affinity to the target of interest.⁴⁴ An example of a targeting peptide developed through phage display is the fibrin binding peptide including the amino acids cysteine-arginine-glutamate-lysine-alanine (CREKA). CREKA was developed as a tumor targeting peptide by using a transgenic model of breast cancer as the phage display target. However, through animal studies, it was noted to localize specifically to the tumor vasculature. To further delineate the specific target, CREKA was tested in fibrinogen knockout mice, and it did not accumulate in tumors, making it appear to be a fibrin binding peptide rather than a specific tumor binding peptide.⁴⁵ The fibrin binding peptide has utility in oncology where it was shown to target animal models of breast cancer,⁴⁵ prostate cancer,⁴⁶ and brain tumors⁴⁷ because of its ability to localize to clotting which occurs in the tumor vasculature. It is also utilized in the field of vascular biology to target atherosclerotic plaques in a mouse model of atherosclerosis to deliver the anticoagulant hirulog, which decreases the thrombogenicity of atherosclerotic plaques.⁴⁸ Beyond its diverse clinical applications, CREKA represents an optimal binding peptide because as a short 5 amino acid sequence it is linear and less bulky; in addition, the single cysteine residue has a sulfhydryl group not required for binding, which is useful in coupling it to other moieties such as a nanoparticle or a fluorescent tag.

In addition to specific targeting moieties, several factors contribute to the ability of a nanoparticle to target to the site of interest. As we previously discussed, in the design of nanoparticles, the targeting ligand is often coupled to a PEG coating to make the ligand available to the target. These PEG arms can be designed at different lengths, which can vary the size of the nanoparticle itself, but may also have implications for binding affinity. This is demonstrated in the example of a nanotherapeutic for hemorrhage, the arginine-glycine-aspartate (RGD) peptide, which binds to activated platelets. The RGD peptide was attached to PEG arms of a poly lactic-*co*-glycolic acid core (PLGA), with the goal of acting as a

synthetic platelet. By varying the PEG arm length from 4600 Da to 1500 Da, it was found that the 4600 Da arm length had improved clotting both *in vitro* and *in vivo*.⁴⁹ Another important factor is ligand density. Ligand density is the percentage of PEG arms incorporated during synthesis of the nanoparticle, or the percentage of the surface area of the nanoparticle that contains the targeting ligand. Optimal ligand density can vary widely depending on the nanoparticle and the targeting ligand. Reasons for variations in optimal density are related to steric hindrance of the targeting ligand, and may also be due to solubility of the molecule itself. For example, the therapy for prostate cancer previously mentioned using an RNA aptamer to prostate specific membrane antigen was found to have an optimal ligand density of 5%,⁵⁰ however a nanoparticle with a binding peptide targeted to intercellular cell adhesion molecule-1 was found to have an optimal targeting ligand density of 50%.⁵¹ Duncan et al used monte carlo simulations to show that increasing ligand density paired with lower affinity but highly specific targets can optimize targeting.⁵² Alternatively, cancer cells that display a high density of the receptor of interest may bind more nanoparticles that contain the targeting ligand compared to normal, non-cancerous cells with fewer receptors (Figure 5). Finally, shape of the nanoparticle itself can play an important role in targeting. Peptide amphiphile monomers can self-assemble into either fibers or spheres depending on the molecular composition. Using a collagen targeted peptide to target the injured vasculature following balloon injury in a rat carotid artery model, it was shown that the targeted nanofiber was able to localize to the injured vasculature, however the targeted nanosphere was not, demonstrating the importance of shape in targeting efficacy.⁵³

THERAPEUTICS

While the diversity of delivery vehicles and wide range of biologic targets allows for the broad application of nanotherapeutics in treating surgical diseases such as cancer, hemorrhage, atherosclerosis, etc., the most important aspect of this technology is delivery of a therapeutic. The variety of therapeutics that can be delivered allows targeted nanotherapies to have application in many different surgical diseases. Therapies such chemotherapeutics for use in cancer, nucleic acids for gene therapy, immunotherapeutics for vaccine delivery, antithrombotic agents for hemorrhage, and protein therapeutics such as growth factors can treat a wide range of surgical diseases. In addition, nanoparticles can deliver contrast agents and fluorophores which aid in imaging and diagnostics, adding even more clinical applicability.¹⁴ Finally some nanoparticles serve as the therapeutic themselves, often by enhancing localized thrombosis.

Once a delivery vehicle carrying a therapeutic payload is delivered to the site of interest, that therapeutic must be released in a controlled fashion. There are two main types of release profiles; those that release the therapeutic in reaction to exogenous stimuli, and those that release the therapeutic in reaction to stimuli endogenous to the target cell of interest. Examples of exogenous release include release secondary to temperature, magnetic, ultrasound, or light applied to the nanoparticle. Examples of endogenous release include release secondary to pH changes, the actions of endogenous enzymes, or redox reactions. Although exogenous release sounds promising in theory, there are several downsides to this technology. Namely, that most therapeutics require external application of the stimulant (i.e., heat, ultrasound, light, etc.), which limits the area of treatment to solid tumors that are

relatively close to the surface of the body. One example of a combination of thermoresponsive (i.e., endogenous release) and light sensitive (i.e., exogenous release) therapy utilizes thermosensitive gold nanorod liposomes to deliver doxorubicin to a mouse model of glioma (Figure 6). The liposome composition was engineered to be stable at body temperature (37°C), but breaks down and releases doxorubicin at 43°C. To attain this temperature *in vivo*, the liposomes were co-injected with gold nanoparticles which locally induce heat with near infrared light stimulation. This methodology demonstrated a reduction in tumor size and improvement in survival compared to control animals.⁵⁴

Endogenous triggers capitalize on differences within organs, tumors, or intracellular environments to trigger release of a therapeutic. For example, the liver has increased levels of glutathione, which reduces disulfide bonds compared with the rest of the body. By delivering N-acetyl-cysteine (NAC) coupled to a dendrimer by a disulfide bond, the NAC is released preferentially in the liver.⁵⁵ Tumor microenvironments are often at a more acidic pH than the rest of the body due to the higher rate of anaerobic glycolysis of cancer cells.⁵⁶ One method of triggering therapeutic release by pH change involves altering the ratio of poly(L-lactic acid) to PEG in nanoparticle monomers. This allows for release of the therapeutic in the pH range of 7.2–6.6 which is optimal for the tumor microenvironment.³⁷ An even starker pH contrast exists with intracellular organelles such as liposomes, which have a pH below 6. Linking a therapeutic for hepatocellular carcinoma such as triptolide (which has been shown to have systemic toxicity) to a nanoparticle coated with folate (which targets for cellular uptake) that contains a pH sensitive imidazole group allows for therapeutic release only below a pH of 6, which occurs intracellularly.³⁹

Finally, the therapeutic payload is frequently the nanoparticle itself. Several therapies which target specific intravascular molecules depend on the nanoparticle to create a localized clotting response either to reduce hemorrhage or thrombose the vasculature of a tumor. We previously mentioned the CREKA targeting peptide, used in multiple cancer applications including breast and prostate cancer. Both of these models were able to demonstrate that the CREKA targeted nanoparticles not only target to fibrin, but that the presence of the nanoparticles themselves accumulating in tumor vessels creates additional clotting, thus creating a self-amplifying clotting mechanism.^{45, 46} A similar idea is used in the setting of hemostasis where the previously mentioned RGD peptide is used to simulate platelets. The therapeutic mechanism of this peptide acts by targeting the particle to activated platelets. This mechanism not only induces clotting itself, but is chemotactic to platelets at the site of bleeding, thereby improving hemostasis even further. Thus, even without delivering a specific therapeutic these therapies can have huge applications in the field of surgical oncology to augment chemotherapy or serve as neoadjuvant therapy, and in the field of trauma surgery to improve hemostasis.

In addition, although not a therapeutic, targeted therapies are being used to deliver contrast agents to enhance imaging modalities such as computed tomography (CT), magnetic resonance (MR), and position emission tomography (PET). As an example, Seo et al designed a targeted contrast agent for PET/CT. They designed a dendrimer using the LyP-1 ligand which targets macrophages in atherosclerotic plaques, incorporating copper into the dendrimer, which was found to enhance PET/CT imaging of atherosclerotic plaques in the

aortas after injection into atherosclerotic mice.⁵⁷ By altering this targeting ligand, this modality could enhance imaging for a wide range of surgical diseases. A similar application is described by Li et al in regards to visualization of brain tumors. They utilized a quantum dot nanoparticle, and incorporated both gadolinium and the near infrared (NIR II) probe Ag2-S into a single nanoprobe. By injecting this into a mouse model of glioblastoma multiforme, the nanoprobes localize by the EPR effect, and help to precisely locate the edges of the tumor by imaging with MR preoperatively and NIR II fluorescence imaging intraoperatively. This technology has great potential to assist surgeons in achieving precise surgical margins during excision.⁵⁸ Overall, the application of targeted nanotherapies to imaging has the potential to improve our ability to detect disease and more precisely localize it, both preoperatively and intraoperatively.

MULTIMODAL THERAPIES

As technology advances and therapies are transformed through nanoscale design, a future direction of targeted nanotherapies is the ability to integrate functions into complex systems, such as the use of more than one therapeutic element with targeting information, diagnostic capabilities, or therapeutic benefit. The idea of treating and enhancing imaging or diagnosis in one modality is termed theranostic. For example, Yang et al used polymeric nanovesicles targeted to the folate receptor, with pH sensitive release of the chemotherapeutic doxorubicin, which also contained superparamagnetic iron oxide nanoparticles that function as a magnetic resonance imaging contrast agent. This allowed not only for targeted delivery of a chemotherapeutic, but also contrast enhanced imaging.³⁸ Another example of theranostics was demonstrated by Bagalkot et al who created lipid-latex nanoparticles that target M1 macrophages with a phagocytic signal, so that they are taken up at sites of inflammation. These nanoparticles encapsulate a lipid soluble drug, and are labeled with the MR contrast agent, gadolinium, allowing both drug delivery, and contrast enhanced imaging at sites of inflammation such as atherosclerosis, or excess adipose tissue.⁵⁹ As another example, high density lipoprotein (HDL) conjugated to gold nanoparticles (AuNPs) is a newly evolving and innovative nanostructure that combines both biocompatibility and functional cholesterol efflux. HDL is a serum nano-protein that protects against the development of atherosclerotic plaque build-up in arteries and ultimately protects against coronary and peripheral arterial disease by a process known as reverse cholesterol transport, whereby cholesterol is effluxed from foam cells and transported to the liver for eventual biliary excretion.⁶⁰ Thaxton et al developed an AuNP conjugated to HDL with a size, shape, and surface composition that mimics natural HDL and cholesterol binding capability similar to native HDL.⁶¹ Moreover, this nanostructure is not only biocompatible but has been shown to efflux cholesterol similar to native HDL, thus demonstrating functional bio-mimicry.⁶² This type of therapeutic could have broad applicability, especially when conjugated to a target for atherosclerotic plaques, potentially leading to a targeted injectable therapy for the treatment of atherosclerotic vascular disease.

CONCLUSION

Targeted nanotherapies represent a rapidly emerging scientific arena that has significant implications for the care of surgical diseases. We discussed examples that are applicable

across multiple fields of surgery as well as direct applicability to specific surgical diseases. Several therapies currently in development in animal models will influence neurosurgery, either by improving delivery of chemotherapeutics in a targeted fashion across the blood brain barrier,^{33, 54, 58} or acting as a targeted therapy for cerebral hemorrhage.^{41, 47} There are numerous therapeutics that will influence oncologic resection in the fields of surgical oncology,³⁶ breast cancer, urology,^{43, 46} and otolaryngology³⁵ with therapeutics in development to directly target those types of tumors, potentially reducing the need for large surgical resections. In addition the field of hepatology and transplant surgery may see improved treatment of liver toxicity with direct targeting of liver enzymes to deliver NAC,⁵⁵ and to deliver specific therapeutics in a targeted manner to treat hepatocellular carcinoma.³⁹ The fields of vascular and cardiac surgery will see the influence of targeted therapies to treat atherosclerotic lesions as well as prevent re-stenosis after interventions.^{48, 53, 57, 63} Finally, the field of trauma surgery and military medicine will benefit from therapeutics being developed to stop hemorrhage.^{49, 64} In summary, we have described how targeted therapeutics are designed in regards to the delivery vehicle platform, targeting moiety, and method of delivering a therapeutic agent, and discussed the many applications of this emerging field to the treatment of surgical diseases.

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Figure 1.

Sizes of various nanoparticles in relation to known biological entities such as DNA, proteins, bacterial and red blood cells. Reprinted from *Advances in Carbohydrate Chemistry and Biochemistry, Glyconanoparticles: Vol 64*, Marradi M, Martín-Lomas M, Penadés S, Polyvalent Tools to Study Carbohydrate-Based Interactions, Page 213, Copyright (2010), with permission from Elsevier.¹²

Figure 2.

Scanning electron microscopy images of blood vessels. Images A-C depict capillaries in normal: A) pancreas tissue; B) colonic intestinal villi; and C) liver sinusoids. Images D-F depict metastatic liver tumors: D) malignant tumor nodule (T, circled area) within normal (N) liver tissue; E) higher magnification of tumor blood vessels with arrows showing extravasation of tumor through the blood vessel via the enhanced permeability and retention (EPR) effect; and F) destruction of a tumor following systemic injection of an anti-tumor drug and deposition of the therapeutic to the tumor site via the EPR effect. G) Cartoon

graphic depicting the EPR effect, showing nanoparticle delivery vehicles containing therapeutics that are able to extravasate through the leaky endothelium and target surface receptors on tumor cells. Figure 2A-F reprinted from *Advanced Drug Delivery Reviews*, Vol 63, Fang J, Nakamura H, Maeda H., The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Page 142, Copyright (2011), with permission from Elsevier.²⁶ Figure 2G reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews: Drug Discovery* (Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: An emerging treatment modality for cancer, Vol 7:p. 775), Copyright (2008).⁵

Figure 3. Nanoparticle delivery vehicle with its surface functionalized to contain targeting molecules, a therapeutic payload, and an imaging contrast agent.

Figure 4. Self-assembled liposomal nanoparticle that encapsulates a drug. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews: Drug Discovery (Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: An emerging treatment modality for cancer, Vol 7:p. 770), Copyright (2008).⁵

Figure 5.

Cartoon graphic showing that more targeted nanoparticles carrying a drug bind to cancer cells that have a higher receptor density compared to non-cancerous cells. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews: Drug Discovery (Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: An emerging treatment modality for cancer, Vol 7:p. 772), Copyright (2008).⁵

Figure 6.

Cartoon graphic depicting a gold nanorod delivery vehicle surface functionalized with PEG, a therapeutic payload, and a targeting moiety. Application of an external stimuli, such as near infrared light, results in a gold particle-mediated rise in temperature followed by release of the therapeutic agent to the site of interest.

Table 1

Category	Example	Size (nm)	Particle Structure	Therapeutic Use
Organic	Liposomes	20 – 250	Spherical vesicle with lipid bilayer membrane structure and aqueous core	– Delivery of vaccines, toxoids, genes, anti-cancer, and anti-HIV therapeutics
	Dendrimers	1 – 30	Highly branched, monodispersed macromolecule with tendrils extending from a central core	– Delivery of therapeutics – Conjugation of chemotherapeutics
	Polymeric Micelles/ Nanofibers	30	Core-shell structures of spontaneously self-assembled amphiphilic co-polymers	– Delivery of therapeutics, including pH sensitivity drug release – Imaging contrast agent – Solid tumor therapeutics
Carbon-based	Fullerenes	1	Carbon nanomaterial, in the form of a hollow sphere, tube, ellipsoid, cylinder, etc.	– Delivery of therapeutics – Conjugation to antibiotics as an antimicrobial agent – Imaging contrast agent – Tumor imaging and therapeutics – Gene delivery
Inorganic	Metal Nanoparticles	1 – 100	Gold, copper, silver	– Optical, photoelectric, fluorescent, and photothermal properties – Imaging contrast agent – <i>In vivo</i> tumor imaging – Gene delivery – Breast cancer diagnostics
	Quantum Dots	2 – 10	Colloid semiconductor nanocrystals composed of atoms from groups II-VI or III-V of the periodic table of elements	– Optical and fluorescent properties – Therapeutics delivery – Imaging contrast agent – Biomedical implants – Quantum optical semiconductor device