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Nanobiotechnology in Drug Delivery

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Contents

Abstract

Nanotechnology is a novel branch of science that deals with the characterization, creation, and utilization of materials, devices, and systems at the nanometer scale. Advances in nanotechnology are spurring a revolution in science, engineering and therapeutics, particularly in drug delivery. Targeted delivery of therapeutic molecules is the most desirable feature of an effective drug therapy. Conventional chemotherapy faces major drawbacks such as poor specificity of the drug, increased adverse effects, and reduced therapeutic efficacy. Application of nanotechnology in drug delivery systems has provided new avenues for engineering materials with molecular precision. This aids in fabricating nanoscale delivery devices that combine diagnostic and therapeutic actions for immediate administration of therapy. Nanotechnology can generate a library of sophisticated drug delivery systems that integrate molecular recognition and site-specific delivery of the therapeutic agents. It formulates therapeutic agents in biocompatible nanomaterials such as nanoparticles, nanocapsules, liposomes, and micelles. This review focuses on some of the nano-sized systems used in drug delivery and discusses the potential applications of nanotechnology in the delivery of macromolecular therapeutic agents.

Nanotechnology is a multidisciplinary branch in science that has the ability to characterize, manipulate, and organize matter systematically at the nanometer scale. The term 'nanotechnology' is used to describe various research areas where the characteristic dimensions are in the nanometer range, typically between 0.1 and 100nm.[1] Advances in science have now made it possible to manipulate atoms and molecules in order to construct materials with nanometer-scale accuracy. Although in its infancy, nanotechnology is revolutionizing science, engineering, and therapeutics by novel technological breakthroughs. Apart from areas such as electronics and robotics, this technology is expected to make important developments in biomedical sciences, including gene therapy, tissue engineering, drug delivery, and development (figure 1).

The potential linkage between nanotechnology and biological sciences is enormous. Physiological functions depend greatly on nano-sized units such as viruses, nucleic acids, ribosomes, and components of the extracellular matrix.[2] Nanotechnology facilitates the utilization of small-sized materials in the body that makes them suitable for these diverse biological functions. Properties such as subcellular size, controlled-release capability, and susceptibility to external activation make nanodevices more suitable for new applications in medical science, especially in pharmaceutical industries.

The major challenge faced by pharmaceutical industries in the discovery and development of novel drugs is the delivery of drugs at the right time to the specific target site in a safe and reproducible manner.[3] In the last few decades, there has been considerable

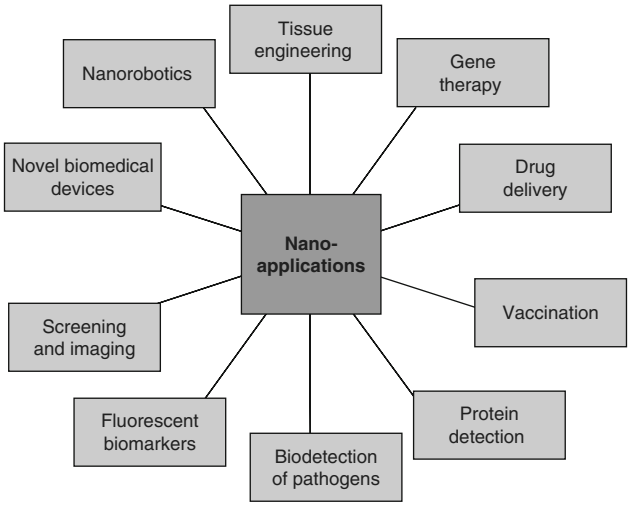

Fig. 1. Various applications of nanotechnology in science.

progress in drug delivery technology; however, the major requirements such as continuous release of therapeutic agents over extended period of time, targeted delivery of agents, and improved ease of administration remain unmet. Several potential therapeutic agents have been limited by their inability to reach target tissues or cells. For instance, cytostatic chemotherapeutic agents damage both malignant and normal cells due to nonspecificity of action.[4] Nano-sized systems can be manipulated at the molecular level for site-selective targeting of encapsulated therapeutic agents.

Pharmaceutical industries can take advantage of the unique properties of these systems as drugs or as constituents of drugs, as well as design new strategies at the nanoscale level for controlled release and drug targeting. This technology can be used for designing delivery devices that interact specifically at the subcellular level and then translate this effect into cellular- and tissue-specific clinical applications for maximum therapeutic outcomes and limited adverse effects.[5] Moreover, nanodelivery systems can penetrate or overcome anatomical barriers such as the complex branching of the pulmonary system, the blood brain barrier, and the tight epithelial junctions of the skin that hinder the delivery of a drug to the desired physiological site.[6] These systems are generally believed to be a prerequisite for efficient drug targeting because carrier systems with a diameter larger than that of a capillary tend to physically clog in the blood vessels. Even though the diameter of capillary blood vessels is approximately 5μm, generally carrier systems between 10 and 200nm or smaller in size are most feasible for drug targeting, since carrier systems larger than 400nm in diameter are easily scavenged by the reticuloendothelial system (RES).[7] However, in some cases carrier systems smaller than the lower limits (approximately 5nm in diameter) are also used so as to enable the complete excretion of carrier systems, such as nonbiodegradable polymeric carriers through the renal route.[4]

Even though there are several ways of achieving nanoscale delivery systems, it is believed that the most stable and versatile systems are nano-sized versions of synthetic materials that have already been used in drug delivery applications, i.e. miniaturized versions of degradable polymers such as poly(lactide-co-glycolide) [PLGA].[2] Nanodelivery systems can be given by a variety of routes and can be easily internalized by different types of human cells by various mechanisms including clathrin- and caveoli-mediated endocytosis, pinocytosis, and phagocytosis.[6] Nanodelivery focuses on formulating drugs in biocompatible nano-sized carriers such as nanoparticles, nanocapsules, liposomes, micelles, dendrimers, emulsions, hydrogels, nanospheres, niosomes, and nanotubes. This review focuses on some of these nanodelivery systems used in drug delivery and their relevance in the emerging field of nucleic acid- and peptide-based delivery as well as vaccine delivery.

1. Nanotechnology in Drug Delivery

Novel drug delivery technologies are important strategic tools used by the pharmaceutical industry for expanding drug markets. Nanotechnology can be successfully applied in basic biology as well as in the development of new biological technologies such as biocompatible drug delivery systems, imaging probes, or nanodevices. In the past, most drugs have been formulated for delivery via the oral or parenteral routes, which may not always be the most efficient routes for a particular treatment.[6] For instance, most of the drugs administered through the oral or parenteral route follow first-order kinetics; however, the ideal release profile for most drugs involves a steady release rate (zero-order kinetics) so that the drug level in the body remains constant while the drug is being administered.

It is widely believed that precise control of the drug carrier can help in the modulation of drug release in order to achieve a desired kinetic profile. Therefore, effective delivery of the drug to various parts of the body is directly affected by the particle size of the drug or drug carrier encapsulating the drug. Additional problems include the instability of the drug in the biological environment and drug loss due to rapid clearance and metabolism.[1] Moreover, newer classes of macromolecular drugs, such as proteins and nucleic acids, require novel delivery technologies that will maximize therapeutic outcomes and minimize adverse effects.[8]

Several approaches have been developed to circumvent the problems associated with nonspecific drug delivery. Since most drugs act as protagonists or antagonists to different molecules and chemicals within the body, a delivery system that can respond to the concentrations of these molecules in the body becomes very important.[9] Therefore, designing a newer generation of nano-sized systems for target-specific drug therapy and early diagnosis of pathologies becomes the priority research area.

In recent years, significant research has been undertaken to develop nanotechnology for drug delivery since it is an appropriate system for delivering biotechnology-based drugs such as proteins, peptides or genes by either local or site-specific delivery to the target tissue.[10] The additional advantages of nanostructure-mediated drug delivery include its ability to enhance drug bioavailability, improve the timed release of drug molecules, and enable precision drug targeting. Nanodelivery systems can address issues associated with current pharmaceuticals such as the reformulation of old drugs to reduce their adverse effects and reformulation of drug candidates that did not pass through the trial phases of new drug development.[6] Additionally, innovative drug delivery systems may make it possible to use certain chemical entities or biologics that were good therapeutically but were difficult to administer or had serious adverse effects. For example, drug targeting enables the delivery of chemotherapeutic agents directly to tumors, reducing systemic adverse effects. Moreover, this technology may aid in increasing product life, performance, and acceptability, either by enhancing efficacy or improving safety and patient compliance.[1]

Other advantages of nanodelivery systems include the potential to deliver drug molecules intracellularly to the target site.[11,12] For example, a nanoscale delivery system encapsulating DNA or RNA can be transported to the target site inside the cell to repair genetic mutations or alter gene expression profiles.[6] However, nanodelivery systems may not be suitable for drugs that are less potent because higher doses of such drugs would require larger delivery systems, which would be difficult to administer.[1] On account of their small size, safe doses of nanoparticles have to be optimized that will provide a manageable mass or volume for administration. Nevertheless, several nanoscale systems, as mentioned earlier, can be successfully employed for the targeted delivery of macromolecular drugs including DNA, proteins, and peptides.

Nanoparticles, liposomes, micelles, and nanocapsules are considered some of the most promising tools for drug delivery on account of their versatility for formulations, biocompatibility, and sustained-release properties. Several of these nanodelivery systems, such as dendrimers,[13,14] nanotubes,[12,15] micelles,[16,17] and emulsions,[18] have been reviewed recently. This review outlines some promising nanodelivery systems including nanoparticles, liposomes, and nanocapsules and their potential applications in gene, protein, and vaccine delivery.

2. Nanodelivery Systems

2.1 Nanoparticles

Nanoparticles are small polymeric colloidal particles varying in size from 10–100nm with a therapeutic agent adsorbed, attached, dissolved, dispersed, or encapsulated in its polymer matrix (figure 2).[19] Nanoparticles as delivery systems provide two basic advantages:

1. they can easily penetrate through small capillaries and are taken up by cells allowing efficient accumulation of the drug at the target sites;[20,21] and
2. biodegradable nanoparticles facilitate sustained release of the drug within the target site over an extended period of time.

In nanoparticulate delivery systems, a broad range of both natural and synthetic polymers have been employed to encapsulate drugs in the polymer matrix. Polymers used for the delivery systems exhibit several desirable properties as drug carrier including technical flexibility, biodegradability, and biocompatibility.[22] Polymers undergo hydrolysis forming biocompatible moieties, such as lactic acid and glycolic acid, that are easily removed from the body by the citric acid cycle.[23] Additionally, structural manipulation of polymer materials allows easy incorporation of drug molecules enabling greater control of the pharmacokinetic behavior of the active drug constituent. Some of the most commonly used polymers are synthetic polymers such as PLGA, polylactide (PLA), polylactide-co-poly(ethylene glycol) and polyacrylates, and natural polymers such as gelatin, albumin, collagen, and poliglusam.[10,24-26]

PLA and PLGA have been the most extensively employed and investigated polymers for drug delivery.[27,28] As compared with the natural polymers, synthetic polymers such as PLGA have the added advantage of sustained release of the encapsulated therapeutic agent over an extended period of time. The release rate of the drug can be extended from days to months via slowing the degradation process of the polymer by changing its composition and molecular weight.[29] PLGA microparticles have been formulated and are being successfully used in the depot formulation of

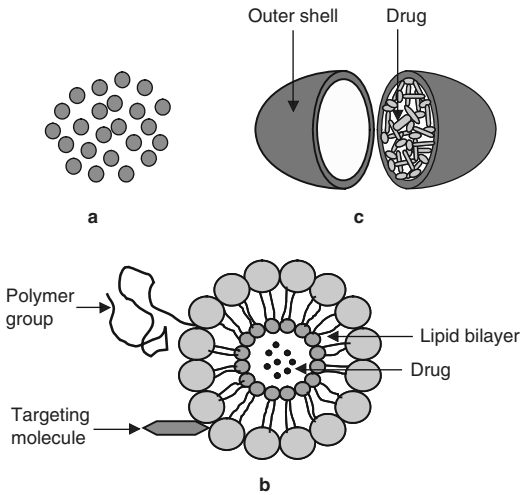

Fig. 2. Schematic representation of various nanotechnology-based delivery systems: (**a**) nanoparticles, (**b**) liposomes, and (**c**) nanocapsules.

leuprorelin acetate (Lupron Depot®)[1], a luteinizing hormone-releasing hormone for the treatment of patients with hormone-dependent cancers and precocious puberty.[30] PLGA nanoparticles can be formulated in a similar manner.

PLGA nanoparticles are very useful in the delivery of therapeutic agents to the cells as these nanoparticles rapidly escape from the endolysosomal compartment into cytosol following their uptake.[31] It has been demonstrated that PLGA nanoparticles are taken up by human dendritic cells, thus implicating PLGA nanoparticles in the selective activation of T cell-mediated immune response.[31] Another report established that dexamethasone-loaded nanoparticles demonstrated greater and sustained antiproliferative activity in vascular smooth muscle cells as compared with the activity demonstrated with the drug alone.[32] This was due to the fact that dexamethasone-loaded nanoparticles were more effective in sustaining intracellular dexamethasone levels, which allowed for more efficient interaction with the cytoplasmic glucocorticoid receptors.[32]

Various therapeutic agents including low molecular weight lipophilic or hydrophilic drugs and high molecular weight DNA or antisense oligonucleotides can be encapsulated in PLGA nanoparticles.[23] Nanoparticles can also be used for targeting specific cell populations by conjugating them with specific ligands or for the delivery of antigens for vaccination as these systems are capable of enhancing mucosal immunity, which is extremely important in disease prevention. Some of the other polymers that have been used or are being investigated for nanoscale drug carriers include poly(3-hydroxybutanoic acid),[33] polyalkylcyanoacrylate,[34] poly(ethylene glycol) [PEG],[35] poly(ethylene oxide),[36] and copolymers such as PLA-PEG.[37,38]

Nanoparticles are very useful drug delivery systems; however, the process of assembling polymers together with different classes of drug molecules is usually difficult as most of the time a complex mixture of particles of different sizes and shapes is obtained. Finding methods of particle formation that are suitable and compatible with drugs has been one of the major challenges in this area.[2] Various methods including solvent emulsion evaporation or displacement, mold replication, colloidal lithography, interfacial polymerization, nanoprecipitation, and nanoimprinting have been developed to make nano-sized formulation of polymers.[39-41] The drug is coupled with the polymer by sequestering, conjugation, and micelle formation.[42] Unfortunately, most of these methods are limited by their incompatibility with most drugs. In the future, newer and better methods have to be developed to produce controlled particles that are compatible with drug incorporation.

2.2 Liposomes

Liposomes are spherical phospholipid vesicles typically ranging between 100 and 200nm in size with an inner compartment that can be used for the encapsulation of drugs[3] (figure 2). Liposomes are inert, biocompatible systems with little toxicity and antigenic reactions. Liposomal design can be easily manipulated to provide protection to the enclosed drug from enzymatic degradation and to increase its cellular uptake.[43] Anionic and neutral liposomes have received limited attention due to their poor encapsulation or association efficiencies of macromolecular drugs.[44]

Cationic liposomes are the most widely used systems for drug delivery owing to the electrostatic interaction between the negatively charged drug molecules and positively charged lipid carrier that results in a more stable complex. Typically these complexes have a net positive charge that interacts with the negative charge of the plasma membrane facilitating internalization of the complex into the cell by endocytosis[45] (figure 3). It has been suggested that membrane destabilization by cationic liposomes causes the anionic lipids from the cytoplasm-facing lipid layer to tumble to the luminal layer diffusing into the liposome. These anionic lipids then replace cationic lipids, releasing the drug moiety into the perinuclear area and finally into the cytoplasm and nucleus.[46] In order to further facilitate the release of nucleic acids from the endosomal or lysosomal compartment, helper lipids such as dioleylphosphatidylethanolamine are generally used. Although cationic liposomes are good in *in vitro* models, *in vivo* they demonstrate nonspecific binding to the cellular components, blood plasma components or the endothelial lining of the vessel, resulting in a short biological half-life.[45]

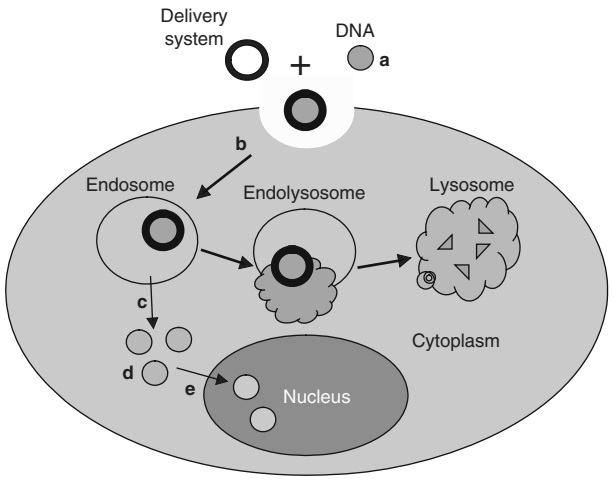

Fig. 3. Endocytosis-mediated uptake and various barriers hindering the delivery of DNA drugs into the nucleus: (**a**) extracellular nucleases; (**b**) endocytosis, (**c**) endosomal escape, (**d**) cytoplasmic stability, and (**e**) nuclear entry.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

Successful clinical application of these cationic reagents depends on a number of factors, such as its chemical structure, target cell type, length, the method of complex formation, and the charge ratio.[47] The major problem with the use of liposomes is the toxicity associated with the high charge ratio of cationic lipid species and the drug. Therefore, cationic liposomes should be delivered to the target site so as to minimize adverse effects. Adding a targeting ligand to liposomes, such as an antibody, facilitates specific cell targeting (figure 2). Several studies have demonstrated that antibody-associated liposomes can augment cell-specific delivery and therapeutic activity of nucleic acids.[48,49]

Another concern is the size of the liposomes. Small liposomes have longer circulation half-life than large liposomes. Liposomes administered systemically are rapidly cleared *in vivo* from the blood circulation by the RES.[50-52] Liposomes were actually introduced as drug delivery systems in the 1960s; however, due to rapid clearance by the RES, liposomal delivery systems had limited success. In 1987, this drawback was overcome by the introduction of GM-1 glycolipid into liposomal formulations, which radically reduced the nonspecific scavenging of liposomes by the RES.[53] Later, PEG-bound lipid was used instead of GM-1 glycolipid as it was easier to supply and could be easily manipulated for specific applications.[4] This liposome has been shown to circulate in the bloodstream for a longer time and can be targeted in cancer therapy to the solid tumor sites by the enhanced permeability and retention (EPR) effect.[54] Encapsulating doxorubicin in PEG-coated liposomal systems demonstrated excellent EPR-based tumor therapy results and reduced toxicity of the original drug.[54] A doxorubicin-containing PEG-coated liposome (Doxil®) has been approved in the US and EU for chemotherapy against Kaposi's sarcoma and ovarian cancer.[55] Cationic liposomes have also been extensively employed for the delivery of DNA, small-interfering RNA, and antisense oligonucleotides.

Even though liposomes are not very good *in vivo* models, the fact that they can be administered *in vivo* via the vascular system and stored in water for an extended period of time makes them attractive candidates for drug delivery.[46,50] Liposomes have been used successfully as *in vitro* delivery systems; however, there is still a limited understanding of their behavior *in vivo* and particularly inside the cell. For successful clinical applications, a more lucid correlation between the drug-liposome complex and all the steps that lead to efficient translocation needs to be established.

2.3 Nanocapsules

Colloidal drug delivery systems such as nanoparticles and liposomes have been studied extensively for site-specific delivery of drugs.[56] More recently, nanocapsules have been proposed as a promising colloidal polymeric drug delivery system. Nanocapsules are vesicular carrier systems containing the drug in a cavity that is surrounded by a unique polymer membrane[57] (figure 2). Therefore, nanocapsules are essentially nanoparticles consisting of the drug in a hollow space that is enclosed by a shell. These systems can be designed such that the breakdown of the capsule and release of the drug are controlled.[5]

Polymeric nanocapsules can be manipulated at the molecular level and can be prepared in specific sizes, shapes, and in reasonable quantities. Depending on the method of preparation, nanocapsules with different properties and release characteristics can be obtained. For instance, some nanocapsules swell or shrink in response to changes in pH, facilitating controlled release of the enclosed drug. Furthermore, the encapsulation of the drug protects it from degradation both during storage and after administration, allowing site-specific drug delivery.[58] Since the drug is not in direct contact with tissue at the site of administration, irritation due to the drug is reduced. In addition, nanocapsules can reduce the harmful adverse effects of the drug by allowing multifold decreases in drug dosages. The main advantage of this system is the low polymer content and high loading capacity for both lipophilic as well as hydrophilic drugs.[57] Additional advantages include increased bioavailability of the drug, higher safety and efficacy, and improved patient compliance.

Nanocapsules can either be produced as monodisperse particles with exactly defined properties or tailored for the specific application. Nanocapsules can be synthesized directly by interfacial polymerization of monomers or by means of nanodeposition of preformed polymers.[35] Surface engineering by the interfacial deposition method can provide suitable size distribution and necessary surface characteristics to the nanocapsules.[59] The basic principle involved in all the methodologies proposed for preparing nanocapsules entails the preparation of either oil/water emulsions (oily core suspended in water) or water/oil emulsions (aqueous core suspended in oil). Oil-based nanocapsule delivery systems prepared by interfacial polymerization of alkylcyanoacrylate have been proposed for the delivery of several drugs by various routes of administration. Because of their oily vesicular nature and ultrafine particle size, poly(alkylcyanoacrylate) [PACA] nanocapsules can be utilized for sustained drug release.

The ability of PACA nanocapsules to deliver drug to blood after oral administration led to widespread utilization of this system for oral delivery of unstable molecules such as peptides and compounds that caused local adverse effects on the mucosae, such as anti-inflammatory agents.[60,61] Oral administration of indometacin encapsulated in PACA nanocapsules has been shown to dramatically reduce the mucosal adverse effects of the drug with increased efficacy.[62] Similarly, oral delivery of insulin and ocular delivery of ganciclovir encapsulated in PACA nanocapsules has demonstrated increased efficacy and site-specific delivery of these drugs.[42,63] Furthermore, intramuscular administration of diclofenac nanocapsules showed decreased inflammation at the injection site with increased therapeutic efficacy.[64]

Nanocapsules are being investigated for the site-specific delivery of various drugs including oligonucleotides, proteins, peptides, and small molecular agents. Despite several successful applications, *in vivo* delivery of nanocapsules is limited by their distribution, particularly their recognition by the mononuclear phagocyte systems after intravenous administration.[58] Other drawbacks include improper encapsulation of the active molecule and modulation of its release rate that largely depends on the surface properties of the nanocapsules. Further development of nanocapsule delivery systems with precise manipulation of the surface properties and appropriate particle size can lead to successful application of these systems for the delivery of various drugs.

3. Pharmaceutical Applications of Nanotechnology

3.1 Gene Therapy

With the human genome sequenced, gene or DNA delivery is becoming the main focus of gene therapy. Delivery of exogenous DNA plasmid into the cells is a powerful tool in genetic diseases as it controls gene structure, regulation, and function. The nucleus is considered the ultimate delivery site for DNA; however, DNA delivery to mitochondria is also important.[65]

Entry into the nucleus is essential for drugs targeting DNA and the delivery of therapeutic genes in gene therapy.[66] Basically, in gene therapy, genetic material including DNA is introduced into cells either to block the expression of harmful proteins or to produce therapeutic proteins. Therefore, gene therapy is a practical approach for curing diseases rather than treating the symptoms alone. In order to access the nuclear transcription machinery, therapeutic DNA must cross the extracellular and intracellular barriers (figure 3). DNA is degraded by extracellular nucleases and, hence, to begin with, DNA must be condensed or protected with vectors that prevent it from nuclease degradation.[67] DNA then enters the cell by endocytosis and is generally degraded in this process due to low pH and enzymatic action. Nonetheless, the DNA molecules that survive finally escape from the endosome and enter the nucleus through nuclear pores or during cell division when the nuclear envelope ruptures.[68] As a result of all these barriers, the number of potential DNA molecules decreases at each step of their passage from the extracellular environment to the nucleus.

DNA delivery presents the challenge of not only delivering DNA to the right cell type but also to the correct cellular organelle, i.e. the nucleus. Appropriate delivery systems are required to protect DNA from nuclease degradation and to enhance its therapeutic efficacy. Among the DNA delivery systems developed so far, viral vectors are one of the most widely used strategies for clinical applications owing to their inherent ability to transport genetic material into the cell and nucleus, leading to enhanced gene expression. However, the engineered virus may revert back to its wild form causing harmful effects. Large-scale production of viral vectors is difficult and some viruses, such as adenoviruses, can also cause immunogenic and inflammatory reactions leading to *de novo* carcinogenesis.[69] Due to these safety issues, alternative nonviral gene delivery systems have been developed. These systems provide several advantages, including the possibility of transfecting cells with large DNA molecules, low cytotoxicity, less immunogenic reactions, reduced cost, and reproducibility.[70,71] Nonviral delivery systems also have their own drawbacks, such as being restricted to extrachromosomal plasmid expression; furthermore, the delivered plasmid may even escape from the nucleus during cell division resulting in plasmid dilution and integration into inactive chromatin.[72,73] However, these delivery systems can be manipulated for efficient delivery and enhanced site specificity.

Nonviral nanodelivery systems such as cationic lipids and polymers are one of the most extensively studied systems for the delivery of nucleic acids as their structure can be easily manipulated, enabling the investigation of structure-function relationships.[74-76] Condensing DNA with positively charged nanomolecules such as cationic lipids and cationic polymers, protects it from nuclease degradation and results in a more stable complex.[77] Both *in vivo* and *in vitro* delivery of DNA has been studied using various nanocarriers such as polyethylenimine, 2-diethylaminoether (DEAE)-dextran, artificial lipids, proteins, and peptides.[78,79]

The transfection efficiency of DNA can be enhanced by coupling molecules such as transferrin, asialoorosomucoid, folate, antibodies (CD3, CD34), or calcium phosphate to the DNA polyplexes.[80-82] Furthermore, nuclear localization sequence (NLS) tags can be attached directly to the DNA molecule or to the delivery system in order to increase the efficiency of DNA uptake through the nuclear pore complex (NPC) into the nucleus.[67] Zanta and coworkers[83] reported successful nuclear targeting of a reporter gene plasmid by covalently linking it to a single Simian Virus 40-derived NLS-containing peptide. However, binding to a NLS may modify the chemical nature of DNA and interfere with the plasmid transcription. Future studies should focus on understanding the mechanism of the uptake of carrier-DNA complex which will help in the processing and targeting of these macromolecules in the intracellular compartment in a more efficient way.

3.2 Protein Delivery

Proteins are polymers of amino acids that perform a wide range of cellular functions. Proteins and peptides possess and control biological activities that mark them as important therapeutic agents. Some of the examples include antimicrobial peptides, anti-inflammatory enzymes, and antioxidants such as catalase and superoxide dismutase.[84] However, most proteins and peptides are

rapidly eliminated from the circulation by renal filtration, proteolytic degradation, receptor-mediated clearance, and accumulation in nonspecific organs and tissues.[85] High molecular weight proteins may generate neutralizing antibodies leading to an immune response. Additionally, most of them have limited biodistribution *in vivo* as they are unable to cross biological membranes in the absence of specific transport systems.

Clinical application of proteins and peptides is further limited by their short biological half-lives due to low solubility or poor stability.[86] For instance, a number of commercial peptides, including insulin for diabetes, Fuzeon® for HIV, and Forteo® for osteoporosis, require regular injections. Protein drugs normally exert their action either extracellularly by interacting with receptors on the cell surface or have targets inside the cell. In the case of intracellularly acting peptides and proteins, low permeability of cell membranes to these macromolecules presents an additional obstacle for the development of proteins and peptides as therapeutic agents. Endogenous proteins that are about 40kDa in size cannot easily diffuse into the nucleus via the NPC. Moreover, proteins and peptides administered by the oral route show poor bioavailability due to their rapid degradation by proteolytic enzymes in the gastrointestinal tract.[87] Parenteral delivery of proteins and peptides is the most popular route as it bypasses the biological barriers that deter the passage of proteins and also leads to pharmacologic levels of proteins in a relatively short time.[88]

Advances in the field of molecular biology have led to the use of protein drugs for the treatment of a number of diseases. However, these formulations require a delivery system that would protect protein and peptide drugs from enzymatic degradation and deliver them to the target site. To address these problems several approaches have been explored. Conjugating proteins and peptides with nanodelivery systems such as nanoparticles or liposomes prolongs their blood circulation by reducing glomerular filtration. In these systems, the protein drug is encapsulated or attached to the polymeric or liposomal matrix that releases the protein in a controlled manner by undergoing enzymatic digestion or hydrolysis. With the exceptions of BioPORTER® (Gene Therapy Systems, Inc., San Diego, CA, USA) which is a cationic lipid-based carrier[89] and TransIT® (Panvera, Madison, WI, USA) which is a histone-based polyamine,[90] most of these systems do not efficiently deliver proteins to their intracellular targets. This could be due to the fact that the protein encapsulated in nanocarrier systems may undergo denaturation due to exposure to organic solvents during the formulation procedure and/or the acidic environment generated during the degradation of the polymer matrix.[91,92]

PEGylation that involves linking specific PEG polymers to protein and peptide macromolecules is considered to be a useful method for protein delivery. Currently, PEG is the most widely used polymer for modifying therapeutically active proteins because it is less toxic, economical and many of its molecular weight variants are commercially available. Additional advantages include optimized pharmacokinetics, decreased dosage frequency, enhanced efficacy, bioavailability, solubility, and stability. Mathiowitz et al.[93] were able to deliver insulin in diabetic rats using polyanhydride nanospheres. Small molecular carriers are being used to deliver insulin and heparin in animals.[94,95] Endogenous proteins are generally imported into the nucleus via the NPC in an NLS-dependent and ATP-dependent fashion.[96] Numerous proteins and peptides including growth factors, hormones, monoclonal antibodies, and cytokines are undergoing clinical investigation for a wide range of clinical conditions.

A major hindrance in the development of protein/peptide-based drugs is the cost, as large quantities of proteins are required for formulation and thorough bioavailability studies.[84] Further development of suitable delivery systems is required for site-specific delivery of protein drugs for maximum efficacy and minimum adverse effects.

3.3 Vaccine Delivery

Vaccines are one of the most valuable medical interventions as they have the capability of completely eradicating a disease. Vaccine development faces major challenges such as noncompliance for needle systems, the requirement of large number of doses, and insufficient availability of vaccines in certain parts of the world.[97] Therefore, new vaccine delivery strategies that require fewer doses and provide lifelong, complete immunity against diseases are being developed.

An ideal theoretical vaccine will never be convincing unless formulated and delivered suitably. Currently, various formulation techniques including adjuvants are being evaluated to deliver vaccines in a safe and effective manner. Adjuvants are substances that when co-administered with the desired antigen can modulate and produce an enhanced antigen-specific immune response.[98,99] Adjuvants can either be used as immunostimulatory agents that stimulate the innate immune system or as vaccine delivery systems that target antigen-presenting cells.[94] Adjuvants play a critical role in surmounting the poor immunogenicity of subunit vaccines in which the isolated component containing antigen fails to induce immunity on its own.

Since 1926, when it was first reported that aluminum compounds increased the immune response elicited by diphtheria toxoid vaccine,[100] several natural and synthetic compounds having adjuvant properties have been established. However, to date, the only adjuvants licensed for human use by the US FDA are the so-called 'alum' adjuvants composed of aluminum salts. However, alum adjuvants have been associated with allergic reactions in some populations and increased levels of IgE antibody response in others, making them generally unsuitable for mucosal delivery.[101-103] In addition, they produce T-helper type 2 biased immunogenicity with decreased levels of T-helper type 1 responses

restricted only to the stimulation of antibodies with little or no induction of cell-mediated immunity.[104,105]

Other potential adjuvants that have advanced to the clinical trial phase have been verified to be very toxic for regular clinical use. As a result, there is a need for the development of safer and effective adjuvants for delivery of vaccines that will provide a complete immune response including stimulation of antibodies and cell-mediated immunity. Additionally, these adjuvants should be biocompatible, biodegradable, stable, and easily manufactured.[24,98]

Although the mechanism of action of most adjuvants is only partially understood, adjuvants administered through mucosal or topical route are drawing interest as they are capable of stimulating the innate immune system providing the added advantage of easy administration and better patient compliance.[106] Modified cholera toxin and *E. coli* heat labile enterotoxin are the most commonly used adjuvants for mucosal delivery.[25,107] Nanodelivery systems such as nanoparticles have also been studied for oral immunization in order to induce systemic and mucosal immunity.[108] Nanoparticles containing entrapped or adsorbed antigens are being investigated as vaccine adjuvant alternatives as they can facilitate sustained release of the antigen, minimizing the frequency of immunization.[109]

In 1976, the adjuvant properties of poly(methyl methacrylate) nanoparticles were demonstrated for the first time.[110] Desai and coworkers[111] demonstrated adjuvant properties of PLGA nanoparticles containing encapsulated staphylococcal enterotoxin B toxoid. It was reported that animals injected with PLGA nanoparticles demonstrated systemic immune response that was comparable with that obtained following injection of alum. In another study it was demonstrated that injecting tetanus toxoid-loaded nanoparticles coupled with alum induced a synergistic immune response.[109] It was reported that the mean immune response obtained with this combination was comparable with the response obtained from two injections of alum alone. These studies suggest that nanoparticles could be used as vaccine adjuvants; however, extensive research is required to further validate these systems.

In the future, novel adjuvants with well defined mechanisms of action and site-specific delivery systems should be developed. Such improved adjuvants will be more potent, requiring fewer revaccinations and will help in future disease eradication programs.

4. Summary

An appropriate delivery system is the major determinant of the efficacy of any therapeutic agent. Despite the development of a large number of new therapeutic systems that improve drug efficacy and specificity, undesirable adverse effects still deter the potential therapeutic outcome of the drugs. With the plethora of knowledge amassed in the field of molecular biology, which has led to better understanding of disease conditions and their cellular mechanisms, delivery systems customized for site-specific delivery and increased therapeutic efficacy are required.

Nanoscale carriers are promising drug delivery systems, as they provide several advantages over conventional delivery systems including a subcellular size, sustained release properties, and biocompatibility. In the future, developing nanodelivery systems that are economical, reproducible and easily inclusive with current infrastructure will definitely improve current drug treatment. With positive development in the field of nanoscale science, the goal of site-specific drug delivery will soon be attained. Advances in nanotechnology coupled with improved therapeutic methods will revolutionize medical science and the way healthcare is administered.

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