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Rational design and engineering of delivery systems for therapeutics: biomedical exercises in colloid and surface science

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Abstract

Engineering delivery systems of therapeutic agents has grown into an independent field, transcending the scope of traditional disciplines and capturing the interest of both academic and industrial research. At the same time, the acceleration in the discovery of new therapeutic moieties (chemical, biological, genetic and radiological) has led to an increasing demand for delivery systems capable of protecting, transporting, and selectively depositing those therapeutic agents to desired sites. The vast majority of delivery systems physically reside in the colloidal domain, while their surface properties and interfacial interactions with the biological milieu critically determine the pharmacological profiles of the delivered therapeutic agents. Interestingly though, the colloidal and surface properties of delivery systems are commonly overlooked in view of the predominant attention placed on the therapeutic effectiveness achieved. Moreover, the development and evaluation of novel delivery systems towards clinical use is often progressed by serendipity rather than a systematic design process, often leading to failure. The present article will attempt to illustrate the colloid and interfacial perspective of a delivery event, as well as exemplify the vast opportunities offered by treating, analysing and manipulating delivery systems as colloidal systems. Exploring and defining the colloid and surface nature of the interactions taking place between the biological moieties in the body and an administered delivery vehicle will allow for the rational engineering of effective delivery systems. A design scheme is also proposed on the way in which the engineering of advanced delivery systems should be practiced towards their transformation from laboratory inventions to clinically viable therapeutics. Lastly, three case

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studies are presented, demonstrating how rational manipulation of the colloidal and surface properties of delivery systems can lead to newly engineered systems relevant to chemotherapy, gene therapy and radiotherapy.
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1. Introduction

The development of delivery systems able to alter the biological profiles (biodistribution, tissue uptake, pharmacokinetics) of therapeutic agents is considered of utmost importance to biomedical research and the pharmaceutical industry, rendering an otherwise ineffective agent, a viable pharmaceutical once properly delivered [1–3]. Construction of novel delivery vehicles toward optimisation of a therapeutic agent's transport and release at the desired site is a challenging technology, requiring multidisciplinary knowledge. Application of fundamental colloid chemistry and particle engineering principles to modify and design delivery systems constitutes a direction, which can lead to novel biomaterials useful as delivery devices for various therapeutic applications [4,5].

The delivery of therapeutics is herein addressed as a complex engineering problem that requires definition of the stages involved in a delivery event and determination of all critical parameters. From such an 'engineering' treatment of the delivery of therapeutics, the critically important role of colloid and interface science tools and principles is exemplified. In the present review, apart from presenting an engineering framework for the design and development of delivery systems, examples of the most widely employed systems for the delivery of therapeutics will be presented in an attempt to illustrate how colloidal and interfacial forces and interactions have been employed in this field. Furthermore, three case studies will be described from our experiences on how colloid and surface science principles can be exercised for the development of delivery systems in three different therapeutic fields: chemotherapy, gene therapy and radiotherapy.

The present review will attempt to demonstrate that:

a. colloid and interface science is intricately implicated in the quest for any rationally designed pharmaceutical;
b. application of colloid and interface science principles can offer a powerful tool-kit for the development of effective delivery systems towards overcoming pharmacological obstacles associated with site-specific or controlled release of therapeutic agents;
c. treatment of certain complex, biological entities (such as viruses, bacteria) as 'biologically active colloidal particles' (or, in contemporary terms, 'nanoparticles'), can offer tremendous opportunities in terms of engineering novel delivery systems of therapeutic agents.

Throughout the present article one fundamental issue is asserted: the existing opportunity to rationally design optimum delivery systems for therapeutic applica-

Table 1
Definition of commonly used terms in pharmacology and drug delivery

Bioavailability The percent administered dose of a therapeutic agent that becomes available in the systemic circulation in its original molecular form
Biodistribution The percentage of an administered dose that gets deposited into specific organs throughout the body at specific time points. Biodistribution of an agent is usually time-dependent.
Pharmacokinetics The study of the time course in the distribution of an administered drug concentration in the different organs throughout the body.
Pharmacodynamics The quantitative evaluation of any clinical parameter, such as body temperature, decrease in viral load, therapeutic index, temporally fluctuating relative to the administered agent.
Circulation half-life The time point at which half of the administered dose of an agent is still in the blood compartment.
Reticuloendothelial system (*RES*) The cellular system in the body responsible for protection and clearance of 'foreign' material. The RES primarily consists of phagocytic cells (e.g. Kuppfer cells–liver macrophages) resident in the blood, liver, spleen and lymph nodes.

tions by exercising colloid and surface engineering principles and techniques to manipulate existing or newly developed agents (biological, radiological and chemical) towards their transformation to effective therapeutics.

2. The need for delivery of therapeutic agents

A common prevailing misconception about our failure to eliminate diseases such as cancer is the notion that we lack effective therapeutic molecules. In reality, there are ample numbers of particularly efficient therapeutic agents readily available and moreover, growing at an unprecedented rate. Mapping of the genetic code and the gradual identification of gene properties and their interrelationships, along with advances in proteomics will undoubtedly increase the availability of agents with a therapeutic potential. Despite that, most therapeutic agents are of limited use because they are: (a) only active against specific biological targets; (b) severely cytotoxic and unsafe for any administration or interaction with healthy tissues; (c) effective only if able to interact with specific cellular compartments (e.g. nucleus); and (d) of very limited solubility in aqueous phases, like blood or most of the bodily fluids. Therefore, the need to be able to control the bioavailability, biodistribution, pharmacokinetics [6,7] and, ultimately, the therapeutic effect of an administered therapeutic agent becomes apparent (Table 1). Apart from the pharmacological performance of the engineered delivery systems, evaluation of their toxicological

profiles should always be contemplated, along with other pharmaceutical technology issues such as capability for lyophilisation, storage and long-term stability.

The need for engineered delivery systems was realised as the importance of quantitative pharmacological parameters became evident in determining the overall therapeutic index. Also, delivery systems have been clinically used as tools for rationalizing and executing different treatment modalities (dose escalations, administration sites, etc.). Even today, most drug approvals are still based on the traditional method of empirical determination of drug effectiveness with increased dosing of the therapeutic agent carried out in clinical trials [8]. However, determination of the occurring complex biological processes following the administration of a therapeutic agent and the quantitative assessment of the critical parameters involved, is gradually becoming an intricate part of the pharmaceutical development process. This has rendered the construction of delivery systems an increasingly valuable tool in pharmaceutical development, allowing rational manipulation of the pharmacological profiles of drugs and their concomitant therapeutic indices. Delivery systems are now used to modify potentially therapeutic agents towards: (a) creation of new pharmaceutical moieties (e.g. liposomal anthracyclines) [9]; (b) improvement in the effectiveness or reduction of the side-effects of an existing therapeutic [10]; and (c) extension of the patent lifetime for an already marketed drug. This has led to investors and analysts projecting the size of the 'Drug Delivery' industry to be between $12 and 20 billion annually [11].

3. The aims of delivering therapeutic agents

The design of delivery systems is largely dependent on a variety of factors that are intricately related to each specific therapeutic or diagnostic application. For example, engineering a system for delivery of a cytotoxic drug molecule to the brain will be inherently different from a system required for the delivery of nucleic acids to the lung. As mentioned above, the purpose for any delivery system is to control the pharmacological parameters (bioavailability, pharmacokinetics, biodistribution and pharmacodynamics) characteristic of the administered moieties. This can be achieved by designing delivery systems with the aim to either: (a) enhance the deposition or accumulation of the therapeutic in a particular tissue; (b) associate the therapeutic with a particular cell population; (c) associate the therapeutic with a specific intracellular component; (d) prolong the association of the therapeutic within a specific organ (e.g. brain, blood, etc.); or any combination of these. '*Targeting*' is a term commonly used to describe the above mentioned aims when delivering therapeutics [12], whereby selective delivery and prolonged retention is desired. The preferential accumulation of the delivered agents to specific sites in the body is dependent upon a fine interplay between specific biological interactions, physiological defense mechanisms, physicochemical and biomechanical factors (electrostatic interactions, flow dynamics and hydrostatic pressures) [13–15]. In the event that sufficient targeting of a therapeutic agent is achieved, all pharmacological parameters will be altered compared to those associated with administration of the free agent.

Table 2
Biological applications achieved by engineering the colloid and interface characteristics of delivery systems

Delivery aim	Colloid and interface characteristic of delivery vehicle engineered	Biological application	Refs.
(a) Target specific tissue	Surface charge	Pulmonary gene therapy	[29–31]
	Steric stabilisation	Solid tumor chemotherapy	[32,33]
(b) Target specific cell population	Surface charge	Hepatocytes	[34,35]
(c) Interact with specific intracellular components	Phase behaviour	Endosomal escape	[36,37]
(d) Prolonged tissue retention	Mean particle size	Blood circulation	[38–40]
	Hydrophilic surface coating		

There are different types of targeted delivery that can be employed. *Active targeting* refers to delivery systems designed with the ability to associate or interact with specific biological moieties, most commonly by attachment on their outer surface of ligands (peptides, antibodies, antibody fragments and proteins) with an enhanced binding affinity for complement cellular receptors. Therefore, active targeting is primarily used to denote active binding between biological moieties [16]. *Passive targeting* refers to all strategies attempting to achieve the defined delivery aim(s), without utilizing specific biological (ligand–receptor) interactions, by correlating the physicochemical and surface characteristics of the delivery systems with the pathophysiology and anatomy of the target sites [17,18]. Illustrative examples of passive targeting include the extravasation of sterically stabilised nanoparticles from leaky tumor capillaries into the interstitium [17], the extended blood circulation half-lives of polymer-coated moieties (proteins, drugs and liposomes) [19], and the enhanced intracellular delivery capabilities of molecules self-assembling in the H_{II} hexagonal phase (e.g. DOPE containing liposomes) [20]. A third type of targeting, gradually becoming more commonly studied, can be termed *Externally stimulated targeting*, whereby localisation of the therapeutic agent at a specific site is achieved by an externally applied stimulus such as temperature [21–23], light [24,25], ultrasound [26] and magnetic force [27,28]. Table 2 shows examples of specific biological applications whereby each of the delivery aims has been achieved by manipulation of a colloidal or interfacial property of the delivery system.

4. Principles for the design of delivery systems

Before attempting to design or engineer a novel delivery system for a specific therapeutic aim, the critical parameters behind the ensuing delivery event will have

to be identified clearly. These parameters lie at different spatial and temporal levels, and at different dimension scales within each level.

4.1. Definition of spatial and temporal parameters in a delivery event

4.1.1. The spatial level—determination of barriers to delivery

In order to be effective, any administered delivery system will need to be absorbed, distributed, reach the desired site and interact with the target tissue and its cell population. From initial administration to 'target landing', the uptake and distribution of a delivery system primarily will depend upon its physicochemical and colloidal properties and the interaction with the various anatomical barriers, largely dependent on the chosen route of administration [1,41]. Moreover, as the delivery system travels closer to the target, it will encounter different anatomical barriers and physical conditions based on the environment of the bodily compartment it will transcend to. Illustrated in Fig. 1, is the delivery system as it travels within the body. The spatial scale at which interactions are taking place between the delivery system and the biological milieu significantly downgrades during transport, from the centimetre (in blood circulation) to nanometer range (intracellularly) as the delivery vehicle translocates through the physiological barriers. Another important factor that represents a considerable barrier to effective delivery is the increase in the total protein concentration towards the intracellular environment, and thus dramatic increases in viscosity as well as biological interactions, leading to increasingly restricted transport capabilities for the delivery system [42]. The quantitative pharmacological parameters that will be determined by the spatial distribution of the delivery systems include its bioavailability and biodistribution within each tissue. Spatial transport in vivo very much will be dependent on the colloidal and surface characteristics of the delivery systems and their dynamic interaction with the physicochemical factors prevalent in each scale and barrier.

4.1.2. The temporal level—temporal dissection of a delivery event

Living systems are characterised not only by their molecular composition but also by their dynamic behaviour. The role of kinetic processes during a delivery event is of great importance for evaluating effectiveness and designing better vehicles. The pharmacokinetics of a delivery system will greatly depend on its colloidal and surface properties (surface charge, size, interaction kinetics with other molecules), the route of administration (residence times at different barrier sites) and rate of clearance from the body. In Fig. 2, the temporal dissection of a delivery event can be described by approximation of each kinetic constant (k_{1-3}). Compartment modelling (single or multicompartmental) of the body is used to simulate and predict the temporal changes of the delivery system and therapeutic agent concentration [43–45]. Indicative examples of utilizing the colloid and surface characteristics of a delivery system to alter its pharmacokinetic profile are the cases of programmable desorption of polymer (PEG) chains from liposome surfaces while in circulation [46,47], and the biodegradation rate of microspheres either in circulation or deposited in tissues [48,49]. In the first case, the kinetic constant k_2

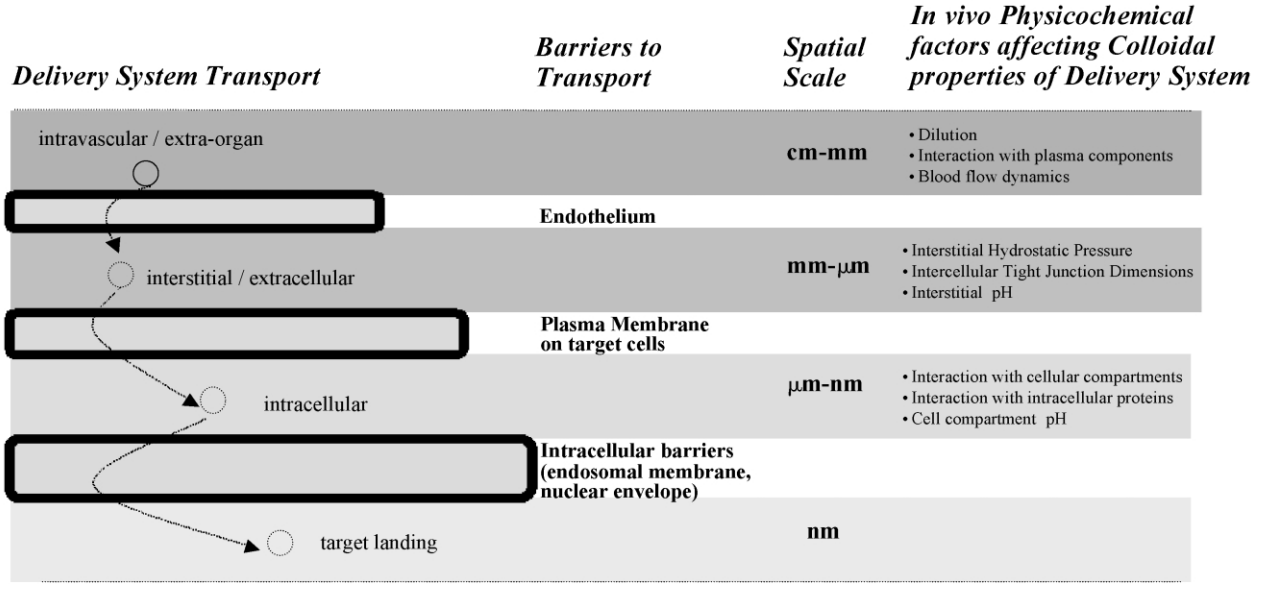


Fig. 1. The spatial level of a delivery event. Transport of the delivery vehicle is required through different barriers, a gradual reduction in the spatial scale in which a variety of physicochemical factors affect its colloid and surface characteristics.

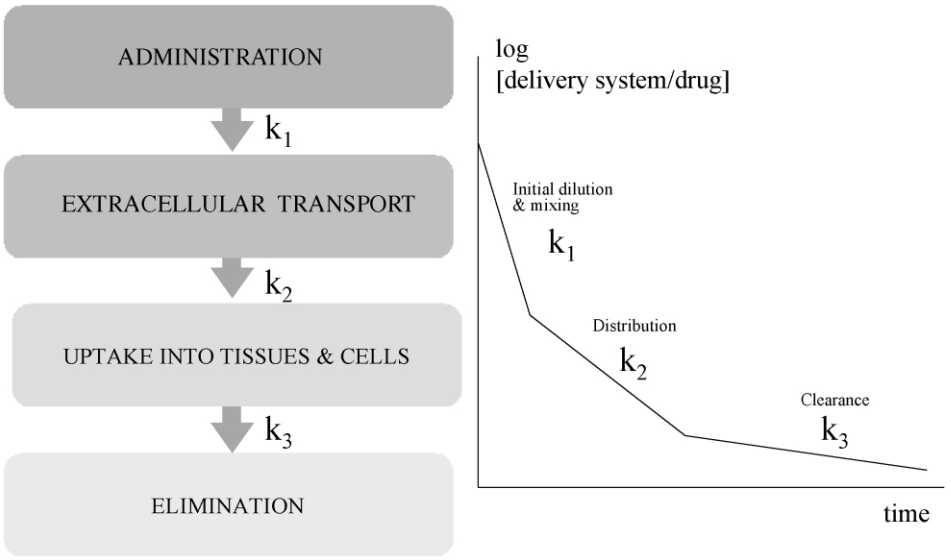


Fig. 2. The temporal level of a delivery event. From initial administration to uptake into cells and tissues and eventual elimination from the body, different constants are used to characterize the pharmacokinetics of each stage during the delivery event.

is altered as the polymer-coated delivery system gradually 'sheds' its polymer chains from its outer surface during blood circulation. This effect leads to a different pharmacokinetic profile for those systems that no longer are sterically stabilised, compared to the polymer-coated vehicles. In the case of biodegradable microspheres and their encapsulated material, alterations in the k_3 constant are obtained as the polymer particle degrades in vivo, leading to a sustained release profile [50,51].

5. Ways of delivering therapeutic agents: colloidal delivery systems

Therapeutic agents that are administered using a delivery system exhibit different biological profiles compared to those obtained when administered alone. The way to deliver therapeutic agents more effectively is either by incorporation, encapsulation, adsorption or binding with a delivery system. In some cases, as in non-viral gene therapy, hardly any therapeutic effect can be obtained at all in the absence of an effective delivery system. The construction of delivery systems most commonly involves application of colloid and surface engineering principles.

Pharmaceutically-relevant colloids have been used for decades primarily to assist in the modification of drug molecules exhibiting a low aqueous solubility [52]. These included emulsions, microemulsions and micelles [53]. Particularly in transdermal delivery applications, colloidal carriers constitute the formulation basis for most dermatological and cosmetic products on the market today [54]. Rapid development in the field of polymer synthesis and the growing knowledge of their

mesophase behaviour has led to a rich source of colloidal components for the engineering of pharmacologically-relevant delivery systems [55]. Furthermore, the introduction of biodegradable polymers and liposomes in the last three decades offers more colloidal systems with tremendous opportunities for effective delivery of therapeutics. Today colloidal systems (particles, gels, foams, etc.) are used to deliver almost any therapeutic or other agent under development towards human or other animal health application. In Scheme 1, the most commonly used colloid particle-based delivery systems are included with their typical average dimensions. In addition to more traditional particulate delivery systems, a variety of other nanomaterials more recently developed, such as quantum dots and nanocrystals, and their aqueous dispersions should also be considered as colloids due to their dimensions and surface properties. Treatment of those nanomaterials from a colloid and surface science perspective is thought to offer an efficient way towards optimisation of their production methodologies, as recently described in Ref. [56], as well as realisation of their potential as novel components of newly engineered delivery systems.

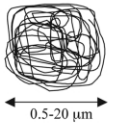
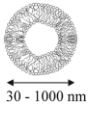
6. Practicing the delivery of therapeutics

Despite progress in the last decade towards development of more effective delivery systems, their engineering is still practiced on an empirical basis, with scarce success in the resolution of the biologically complex processes involved, or the establishment of clear and systematic structure–function relationships between the delivery system and the therapeutic effect [79]. Furthermore, the scarcity of experimental and computational models bridging the gap between the in vitro and in vivo assays of therapeutic responses renders rational design of delivery systems particularly intuitive.

The engineering design model proposed herein that aims to facilitate the development of clinically-effective delivery systems is represented in Fig. 3. It consists of three interconnected layers of processes: Layer I, leads to the construction and characterisation of the best candidate delivery systems following systematic determination of the aims, critical parameters, and their correlation with the available materials and knowledge. Layer II consists of the experimental and computational models used to pre-clinically evaluate the candidate delivery systems, optimise construction parameters, and formulate structure–function relationships. Feedback cycles between Layer I and II will produce the best candidate systems. Layer 3 involves the design and execution of studies for clinically evaluating the delivery systems best characterised and performed in Layers I and II. Clinical evaluation should be carefully designed for the specific therapeutic application for which the delivery system was initially designed (see first box in Layer I). Contrary to most currently practiced methods, the process of engineering an effective delivery system should include the pre-clinical evaluation as part of the developmental process and not merely as proof of therapeutic effectiveness.

Given the fact that the degree of complexity in the therapeutic applications requiring advanced delivery systems is constantly increasing, more systematic

Table 3
Delivery system types, common delivery systems from each type and most widespread biomedical and pharmaceutical uses

Delivery system types and typical mean particle diameter	Representative systems of each type	Characteristic applications	Refs.
 Microspheres hydrogels	alginate, gelatin, chitosan, PLGA microspheres Synthetic, biodegradable, polymer hydrogels	• sustained release of therapeutics • scaffolds for cell delivery in tissue engineering	[48,57,58]
 microparticles	Polystyrene, microspheres	targeted delivery of therapeutics	[59–61]
 Emulsions, microemulsions	o/w, w/o/w, lipid, emulsions o/w microemulsions	controlled and targeted delivery of therapeutics	[62–64]
 liposomes	phospholid and polymer-based bilayer vesicles	targeted delivery of therapeutics	[65,66]
 micelles	natural and synthetic surfactant micelles	targeted delivery of therapeutics	[67–70]
 nanoparticles	lipid, polymer, inorganic nanoparticles	• targeted delivery of therapeutics • in vivo navigation devices	[27,71–74]
 nanocrystals	quantum dots	imaging agents	[75–78]

Scheme 1. Delivery system types, common delivery system from each type and most widespread biomedical and pharmaceutical uses [57–78].

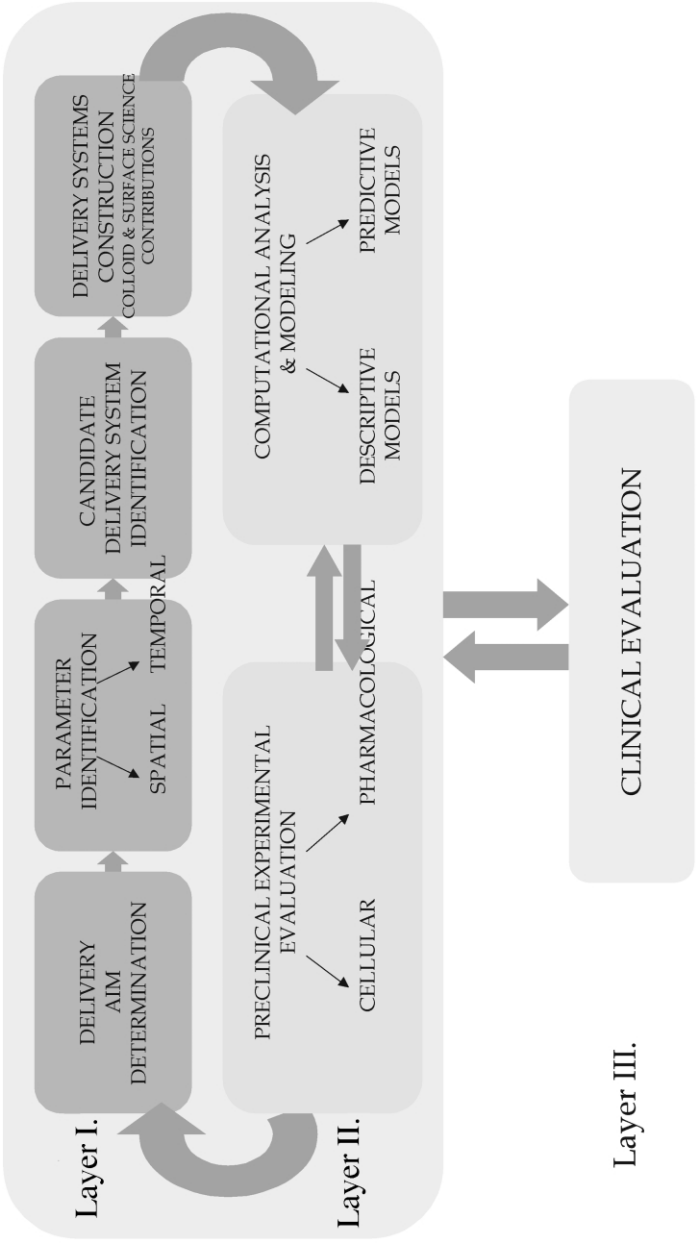


Fig. 3. The design model proposed for development of advanced delivery systems and evaluation of their clinical viability.

engineering designs and platforms are essential. Lack of such systematic approaches has been evidenced recently in the field of clinically applied genetic medicine, which has suffered severe drawbacks due to poor and non-systematic design and evaluation of effective delivery vehicles for genetic material [80]. Taking into account the complex and often unknown processes involved before therapeutic effects are obtained from novel treatment modalities such as genetic or regenerative medicines, systematic and integrative development approaches that will facilitate their optimum delivery to patients should be an unequivocal requirement.

7. Case studies in exercising colloid and surface science principles in the design of delivery systems for different therapeutic applications

Three case studies are described below, whereby application of colloid and surface science principles and techniques exemplify the valuable tools and diverse possibilities offered for optimisation of delivery systems toward different therapeutic applications. Each of the studies presented below was carried out towards development of delivery systems for chemotherapy, gene therapy and radiotherapy, respectively.

7.1. Polymer-coated liposomes in the delivery of chemotherapeutics: the effect of steric stabilisation

A wide variety of molecules have been delivered using liposomes or other lipid-based vesicular systems since the early 1970s when liposomes were first proposed as novel particulate systems for the effective delivery of drugs [81]. The realisation that the physicochemical characteristics of liposome carriers (mean particle size, surface charge and bilayer phase behaviour) determine the pharmacological profile and, consequently, the therapeutic index of the encapsulated drug molecules exemplified the critical importance of the *delivery system structure-drug function* paradigm [82–84]. The contribution of colloid and interface science in the development of liposomes from the laboratory to the clinic culminated during the last decade or so, by application of the principle of steric stabilisation [85,86]. Liposomes coated with hydrophilic polymer chains have been described as sterically stabilised colloidal systems by experimental and theoretical treatments [87–90]. The altered pharmacological profiles that sterically stabilised liposomes exhibit compared to standard, ‘naked’ liposomes has led to their successful application as passively targeted delivery systems to a variety of pathological conditions, such as malignacies, infectious sites, etc. [91–93].

Polymer-coated, sterically stabilised liposomes loaded with the anthracycline molecules, doxorubicin and daunorubicin, are multimillion dollar anticancer drug products today, primarily due to the improved cardiotoxicity profiles and enhanced localisation to specific tumor sites (e.g. Kaposi sarcomas) compared to the free drugs [94]. The application of steric stabilisation theories in liposome research has led to numerous polymer-coated systems. A key factor that leads to the improved pharmacological and therapeutic profiles of encapsulated material is the prolonged

circulation half-lives obtained with sterically stabilised liposome systems. In Fig. 4, three different polymer-coated, sterically stabilised liposome systems and the respective blood circulation profiles of a model-encapsulated drug are schematically depicted. Circulation half-lives up to 2 days has been achieved with systems (b) and (d), however, system (d) exhibits increased leakage of encapsulated hydrophilic material [95]. Sterically stabilised system (c) quickly leads to only slightly improved blood half-life profile compared to system (a) due to polymer desorption following administration [96,97].

Numerous studies have investigated the reasons behind the observed prolonged blood residence times of sterically stabilised liposomes. The most prevalent mechanism is thought to be the liposome surface protection offered by the hydrophilic groups against protein adsorption and opsonisation [98,99]. The latter being the process whereby opsonic molecules, such as immunoglobulins, lipoproteins and others bind to the surface of delivery systems while in circulation, facilitating recognition and specific interaction with plasma membrane receptors on monocytes and macrophages, thus leading to clearance of the delivered therapeutics before reaching their target tissue [17]. Other mechanisms contributing to rapid clearance, leading to reduced circulation half-lives of delivery systems include: complement activation [100], filtration and blockade through anatomical barriers in specific tissues such as lung capillaries, spleen sinusoids, or entrapment in blood clotted regions in the circulation [17,99].

7.2. Virus particles in the delivery of gene therapeutics: the effect of hydrophobicity and particle surface charge

Gene therapy vectors are commonly distinguished in two generic categories: (i) viral and (ii) non-viral (or synthetic). *Viral vectors* include a wide array of genetically modified (usually non-wild type, replication defective) viruses of different families, of which the most widely used are retrovirus, adenovirus, adeno-associated virus and herpes simplex virus. *Non-viral vectors* are electrostatic complexes between various synthetic or natural cationic molecules with the gene-encoding plasmid DNA [101].

Two fundamental properties often disregarded in the gene therapy field, however, common to any type of viral particle, supramolecular complex or other delivery vehicle used for transferring genetic material to target cells and tissues, are that: (a) the complex, particulate gene delivery systems are all materials in the nanometer length scale (commonly of mean diameters below 150–200 nm), and thus can be treated as nanoparticles; (b) gene delivery vector systems obey the principles of molecular self-assembly, interparticle and intermolecular force interactions. Approaching gene delivery vectors from a colloid and surface science perspective is more common in the field of non-viral gene therapy vector development, since familiarity with the concepts of electrostatic interactions, flocculation and stabilisation of cationic–anionic molecular complexes are prerequisites for viable vector construction. However, in the field of viral gene therapy vector development,

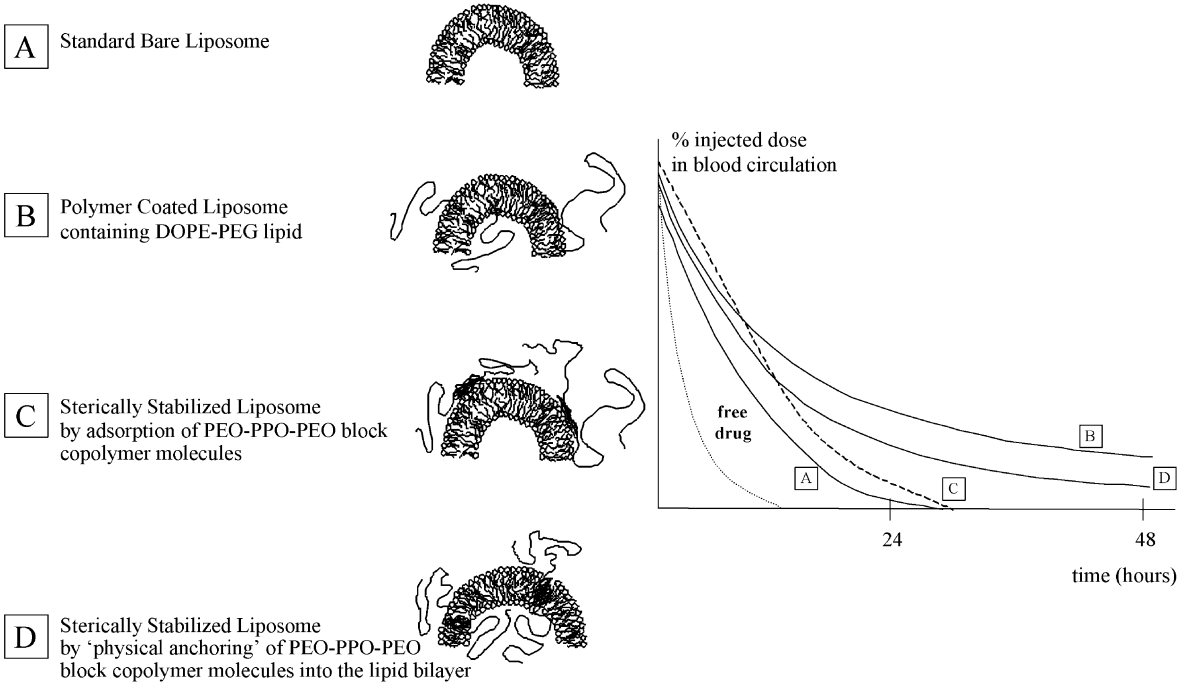


Fig. 4. Different types of polymer-coated, sterically stabilised liposomes and their respective blood residence half-lives. (a) is a 'bare', non-coated, liposome; (b) is polymer-coated by a lipopolymer molecule, while (c) and (d) are different types of polymer-coated liposomes using tri-block copolymer molecules.

colloidal approaches that would allow particle engineering and manipulation are very scarce.

The case of adenovirus development can serve as an illustration of the existing opportunities when adenoviruses are treated as colloidal particulate dispersions. With adenoviruses, cell entry is facilitated by attachment to the coxsackie and adenovirus receptor (CAR) and formation of a high-affinity complex with the viral fiber knob [102,103]. Moreover, adenovirus particles are monodispersed nanoparticles of 70–80 nm in diameter (Fig. 5a), anionic surface charge characteristics with a ζ-potential of approximately -40 mV at pH 7 (Fig. 5b), and a specific and well-characterised viral capsid structure [104]. A variety of cell lines present significantly low levels of the essential cellular receptors facilitating binding of the viral particles onto the plasma membranes, thus posing a serious impediment to effective adenoviral gene transfer of therapeutic genes [105,106]. In Fig. 5, manipulation of the adenovirus surface with cholesterol molecules led to viral particles of enhanced hydrophobic surface character, lower surface charge and often resulted in formation of multi-virion clustering [107]. Interestingly, these alterations in the surface characteristics of the adenovirus nanoparticles led to enhanced interaction with cells that lack the adenovirus CAR receptor (Fig. 5c). Moreover, this was followed by an upregulation in the gene expression for the cholesterol-coated viruses [108], and a dramatic alteration in the pharmacokinetics of in vivo gene expression [109] compared to adenoviruses with unmodified surface characteristics. Overall, those studies exemplified a novel strategy to manipulate adenovirus surfaces by taking advantage of molecular physicochemical interactions based on self-assembly theories.

7.3. Liposomes in the delivery of radiotherapeutics: the effect of particle size and surface charge

The use of liposomes as carriers of radionuclides has been primarily focused on imaging and diagnostic applications [110,111]. Conjugation of the isotope indium-111 (^{111}In) with liposomes has been also approved for human use, particularly towards solid tumor detection applications [112,113]. Moreover, numerous studies have recently explored the possibility to efficiently image infectious sites using polymer-coated radiolabeled liposomes [114]. Surprisingly enough, evaluation of liposomes as delivery vehicles of heavier particle-emitting radionuclides for therapeutic purposes has been almost completely ignored [115].

We decided to systematically explore the possibility of using liposomes as delivery vehicles of radionuclides used in internal radiotherapy clinical protocols [116,117]. Therefore, a variety of liposome systems were considered and an analytic radiodosimetric evaluation was carried out to assess which combinations of liposomes and radionuclides offered the best tumor-to-normal-tissue energy deposition and were therefore most promising for development [118].

Initial studies were based on previously published in vivo data and a well-established, first order approximation model was used to calculate radiation doses deposited to human tissue for a variety of liposome-radionuclide conjugates. In Fig.

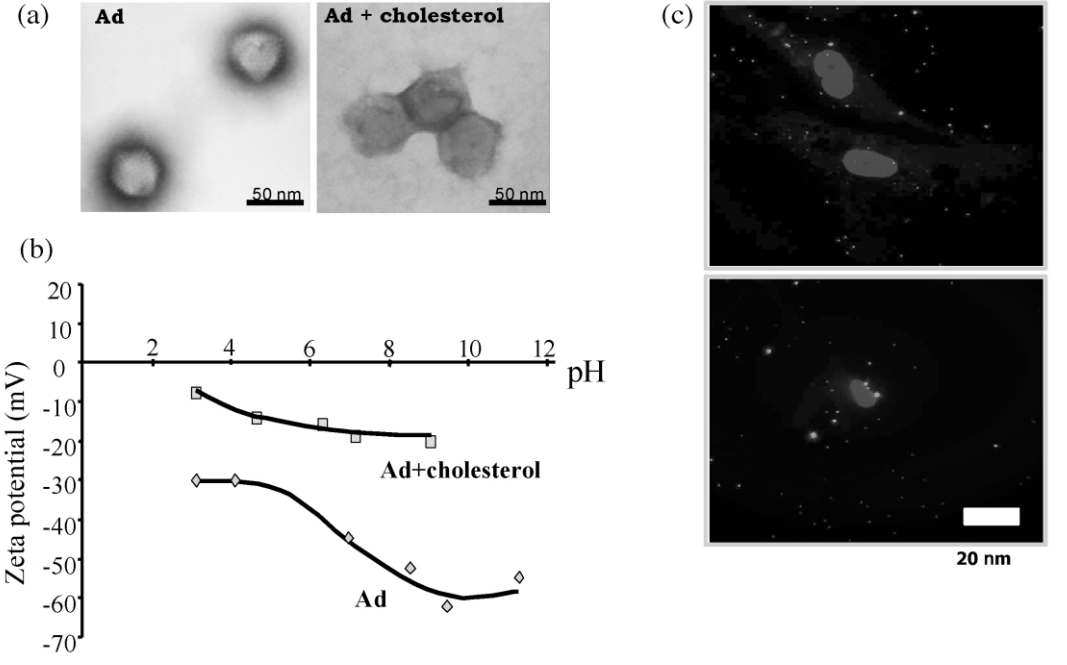
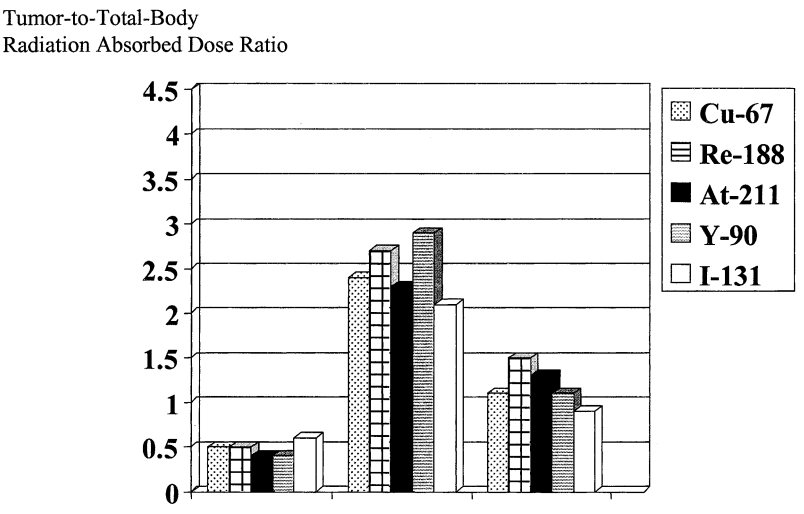


Fig. 5. Treatment of adenovirus particles as nanoparticles of 60–80 nm and their surface interaction with cholesterol molecules (a), leading to virion clustering, reduction in their zeta potential (b), and a more efficient interaction with human skin fibroblasts (cell nuclei shown stained with DAPY dye) that do not carry the natural receptor to facilitate viral binding and uptake (c).



	MLV	SUV	PEG
Mean diameter	800-1.5 μm	70 nm	110 nm
Surface charge (ζ-potential)	_	-30 mV	-4 mV

Fig. 6. Radiation absorbed doses for a variety of radionuclides in tumor-bearing mice as calculated from the tissue biodistribution data for three different liposome systems: multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and polyethylene glycol-coated vesicles (PEG) of different physicochemical characteristics.

6, values of tumor-to-total body (i.e. healthy tissue) radiation doses are depicted for multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and pegylated small unilamellar vesicles (PEG). Typical values of mean vesicle size and surface charge are shown, correspondingly, for the three types of liposome systems evaluated. A dramatic display of the effects that mean liposome size and surface charge have on the radiation absorbed dose ratios were obtained. The large mean particle size of MLV systems seem to be the worst in terms of the amount of radiation energy absorbed by the tumor tissue compared to the rest of healthy organs. More interestingly though, the sterically stabilised pegylated liposomes that have been clinically successful in carrying cytotoxic agents selectively to tumors, in the case of radionuclide delivery seem to perform worst than the more traditional SUV system (at least from the radiodosimetric point of view).

Once again, and in the case of a radiotherapeutic application, manipulation of the colloidal characteristics attained by the delivery systems leads to a dramatically different biological profile, thus therapeutic effect. Manipulation of those properties allows for rational engineering of vehicles for effective delivery of radiotherapeutic agents.

8. Delivery systems and the vanity of 'the magic bullet'

Ever since Paul Ehlrich's introduction over a century ago of the 'magic bullet' concept as a means to develop effective therapeutics for a variety of diseases, scientists from a broad range of disciplines have been fascinated with the challenge. Today the field of therapeutic agent delivery is a truly interdisciplinary area, innovated continuously by contributions from almost every scientific field. However, the realisation should prevail that the quest for 'magic bullets' has to be treated on the basis of the specific pathological condition to be treated, rather than a universal tool. In the present article, we attempted to illustrate two main points:

a. treatment of delivery systems from a colloidal and surface perspective offers tremendous opportunities in engineering novel tools for the effective targeting of almost every therapeutic agent (chemical, biological, genetic and radiological) to diseased sites;
b. discovery of novel therapeutic agents and engineering of advanced delivery systems will only realize their potential as effective medicines only by following a systematic development process. Towards that goal, a design scheme was proposed on how to practice the development of novel delivery of therapeutic agents in their transformation to clinically viable therapeutics.

The development of colloidal delivery systems and systematic approaches that will allow their transfer from the laboratory to the clinic is an engineering task of great complexity that requires colloid scientists with interdisciplinary knowledge and analytical skills. The quest for a single 'magic bullet' may seem futile, however, the construction of novel delivery systems offers the possibility for a myriad of clinically viable 'magic bullets', once treated in an integrative and systematic manner.

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