DEVELOPMENT OF INNOVATIVE NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS FOR CANCER THERAPY

Carla Moutinho
Professora Auxiliar
Faculdade de Ciências da Saúde - UFP
carlamo@ufp.pt

Carla Matos
Professora Auxiliar
Faculdade de Ciências da Saúde - UFP
cmatos@ufp.pt

Victor Balcão
Professor Associado
Faculdade de Ciência e Tecnologia - UFP
vbalcao@ufp.pt

Abstract
Controlled drug delivery systems are not a new subject in the biomedical field. The continuously increasing need for the improvement of health care services has been the driving force for both search and development of such systems. Among these, micro- and nano-sized vehicles (e.g. nanocapsules, liposomes and mixed micelles) have received special attention over the last decade; they have been used for the delivery and vectorization of many pharmacologically active molecules, as is the case of anti-neoplastic drugs.

Key-words
Anti-neoplastic drugs; controlled delivery; liposomes; nanocapsules

Resumo
Os sistemas de libertação controlada de fármacos não são uma temática recente na área das ciências biomédicas: a crescente exigência no sector dos cuidados de saúde despoletou o desenvolvimento desta área científica. De entre os vários tipos de sistemas propostos, as micro- e nano-estruturas (i.e., nanocápsulas, lipossomas e micelas mistas) têm recebido especial atenção por parte dos investigadores ao longo da última década, tendo sido desenvolvidos sistemas de libertação e vectorização de muitas moléculas farmacologicamente activas, nomeadamente de fármacos anti-neoplásicos.

Palavras-chave
Fármacos anti-neoplásicos; libertação controlada; lipossomas; nanocápsulas

1. INTRODUCTION

The development of a new biologically active drug involves more than synthesis of a substance that has a particular effect on the human body. The researcher must also consider how the drug should be transported to the appropriate part of the body and, once there, make it available for use. However, this is not a trivial issue; in some cases, development of the appropriate system can be as complex as the development of the drug itself.

Cancer therapy is a paradigmatic example of this statement: chemotherapy remains the main weapon against cancer, but presents major disadvantages, such as poor accessibility of anti-carcinogenic drugs to the tumor, therefore requiring administration of higher doses, and intolerable systemic adverse effects, forcing discontinuation of therapy in many patients (Vasir and Labhasetwar, 2005). Other problems include multi-drug resistance, short *in vivo* half-lives and the need of repeated administration (McLeod and Evans, 1999).

Controlled drug delivery technology represents one of the frontier areas in biomedical science, involving a multidisciplinary scientific approach, contributing to human health care. Investment in this area has increased exponentially worldwide, over the last few years. A drug delivery system can be defined as a system that is capable of releasing a carried bioactive agent in a specific location within the body at a specific rate. Such delivery systems offer numerous advantages when compared to conventional dosage forms, including improved efficacy, reduced toxicity, and improved patient compliance and convenience. The idea is to convey a sufficient (optimal) dose of a drug to a lesion using a suitable carrier. Among the most promising approaches under development are nano- and microparticles (Liu *et al*., 2005; Orive *et al*., 2005).

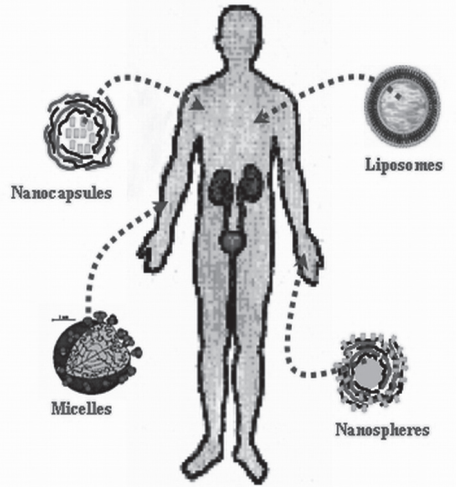

Figure 1 - Drug delivery systems that can be used in anti-cancer treatment.

Some studies have indicated that liposome-incorporated anti-tumoral agents displayed prolonged drug retention, reduction in tumor growth and prolonged survival of tumor-bearing animals. Several liposomal formulations, which target drugs to tumors, are currently

available commercially, viz. liposome-encapsulated daunorubicin (Daunoxome™, from NeXtar Inc.) and doxorubicin (Doxil™, from Sequus Pharmaceuticals), both designed to provide prolonged blood circulation, tumor accumulation and extended drug release at the cancer site. These characteristics are intended to increase the effectiveness of, and reduce the side effects of, the micro-encapsulated drug. More recently, the Inex Pharmaceuticals Corporation has required licence for commercialization of its proprietary Marqibo™, a vincristine sulphate liposome formulation, as well as of its DepoCyt™, a sustained release formulation of the chemotherapeutic agent cytarabine, used for the treatment of patients with lymphomatous meningitis, A disease that can be controlled with cytarabine, but due to the drug's short half-life, a spinal injection is required twice per week, whereas DepoCyt™ is administered once every two weeks. DepoCyt™ gradually releases cytarabine into the cerebral spinal fluid, resulting in a significantly extended half-life, prolonged exposure to the therapy, and a more uniform distribution.

Nanocapsule-bound anti-cancer drugs have also been made available for commercialization: Abraxene™ (from Abraxis Oncology, Inc.) for injectable suspension (nanoparticle, albumin-bound paclitaxel) (Gradishar *et al.*, 2005) received approval in June 2006 from the Canadian Therapeutic Products Directorate of Health for the treatment of breast cancer.

CytImmune Sciences, Inc. is developing colloidal gold nanoparticles as a platform for tumor targeted drug delivery; Aurimune-T™, which avoids systemic toxicity of TNF-α (alpha tumor necrosis factor) *vs* native TNF-α, is manufactured by covalently linking molecules of TNF onto the surface of 25 nm colloidal gold particles. Intravenously administered Aurimune-T™ rapidly accumulates within solid tumors implanted in mice, and shows little to no-accumulation in the reticuloendothelial system or in other healthy organs. The second nanoparticle-based drug, AuriTax™, consists of a chemotherapeutic agent (paclitaxel) bound to the same nanoparticle. Like Aurimune-T™, AuriTax™ delivers 10 times more TNF and paclitaxel to the solid tumor when compared to each drug alone (Tamarkin *et al.*, 2006).

2. **BIOMEDICAL APPLICATIONS OF MICRO AND NANOTECHNOLOGIES**

Anti-cancer drugs can be associated with colloidal drug carrier systems such as polymeric micelles, nanocapsules, and liposomes, which can then be actively targeted to specific tumor cells by means of ligands or antibodies against tumor associated cell surface receptors. For the purposes of this manuscript, the term "nanosystems" includes all the nanosized (< 1000 nm) drug carrier systems, such as polymeric nanocapsules, liposomes, micelles and polymer-drug conjugates. Some of the newer nanosystems currently under development include nanocages, nanogels, nanofibers, nanoshells, nanorods and nanocontainers. All these nanosystems differ not only in their structure but also in their biopharmaceutical characteristics and therapeutic uses. The production protocol of each particle differs also considerably, and scaling-up could well be a challenge for some of these devices (Orive *et al*., 2005; Panyam and Labhasetwar, 2003).

Nanocapsules have received considerably more attention than liposomes, mainly due to their therapeutic potential and greater stability in biologic fluids as well as during storage. Their smaller particle size makes colloidal preparations well suited for parenteral administration and also potentially useful as sustained-release injections for delivery to a specific organ or

target site. Furthermore, nanosized particles are small enough to prevent occlusions of the vascular system, especially the capillaries (Jurgons *et al*., 2006).

Preliminary studies have indicated increased efficiency of drug delivery, improved release profiles and drug targeting. In a controlled release device, the rate at which the drug is made available to the body once it has been delivered, is maintained constant. With each dose of a noncontrolled-release drug, the concentration of drug available to the body immediately peaks and then declines rapidly. It is desirable to release drugs at a constant rate, thereby maintaining drug concentration within the therapeutic range and therefore eliminating the need for frequent administrations.

A second goal in the use of delivery systems is drug targeting. Targeting the drug to the desired site of action would not only improve the therapeutic efficiency but also permit a reduction in the amount of drug that must be administered to achieve a therapeutic response, thus minimizing unwanted (side) toxic effects. Drug targeting can be achieved via a number of techniques, by taking advantage of the distinctive pathophysiological features of a tumor tissue. The vasculature in many solid tumors differs from normal blood vessels, containing gaps that increase permeability. The enhanced endocytic activity and leaky vasculature in a tumor could result in accumulation of intravenously administered nano- and microparticles, a phenomenon usually addressed as EPR (enhanced permeability and retention) effect (Maeda *et al*., 2000; Andresen *et al*., 2005). Due to their size, these structures are able to permeate cells for cellular internalisation and connective tissue permeation, and therefore to deliver the drug efficiently to the targeted tissue without clogging capillaries.

Drug targeting can also be actively achieved by conjugating the delivery systems with antibodies or ligands such as transferrin, lectin, or avidin (Reis *et al*., 2006) that can bind specifically to molecules or structures present in the tumor cells. Cancer cells express some new proteins and/or overexpress normal proteins due to their transformed nature, many of which can be used as biomarkers for diagnosis, progression of disease or effectiveness of drug therapy in patients (Vasir and Labhasetwar, 2005). These specific biomarkers have been named as "tumor associated antigens" (TAA), and antibodies or ligands specific to these TAA can be incorporated in the nanostructure, liposome or micelle in order to target drugs to tumor cells. In this context, some of the strategies used for targeting include (but are not limited to) (Brannon-Peppas and Blanchette, 2004; Andresen *et al*., 2005; Vasir and Labhasetwar, 2005): i) Antibody targeting: immunoliposomes are antibody-coated liposomes, able to bind to specific tumor antigens. The ideal antigen should be expressed on all tumor cells but not on the host cells; ii) Targeting through angiogenesis: numerous regulators, mediators and stimulatory molecules regulate the proliferative and invasive activity of the endothelial cells of tumor blood vessels. The delivery systems can be targeted to the neovasculature by coupling with ligands to these substances; iii) Folate receptor targeting: the cell surface receptor for folic acid (folate receptor) is unaccessible from the circulation to healthy cells, but frequently overexpressed on tumor cells as a consequence of increased folate requirements.

The recent scientific literature is fertile with examples of such applications of nanosystems delivery. Nanoparticles can be easily conjugated with a ligand to favour a targeted therapeutic approach, and as it has been reported, some nanoparticles can cross the blood-brain barrier (BBB). For example, doxorubicin bound to polysorbate-coated nanoparticles can cross the intact BBB, reaching therapeutic concentrations within the brain (Steiniger *et al*., 2004).

Surface modifications of the nanoparticles can be achieved which would allow specific biochemical interactions with the proteins/receptors expressed on tumor cells. Sahoo and co-workers (2004) have demonstrated increased efficacy of paclitaxel-loaded nanoparticles upon conjugation with transferrin, in a murine model of prostate cancer. Transferrin receptors are overexpressed by 2-10 folds in tumor cells than in normal cells, and thus transferrin and/or transferrin antibodies have been used for targeting drugs to tumor cells. Because of its broad spectrum activity, there has been significant interest in developing different sustained paclitaxel-based microspheres and nanospheres formulations. Lu *et al*. (2004) studied the development of paclitaxel-loaded gelatin nanoparticles for use in intravesical therapy of superficial bladder cancer.

The use of liposomes and nanoparticles as anti-cancer drug delivery systems was originally hampered by the realisation that liposomes are rapidly cleared from blood circulation. One of the main reasons for such event is the macrophage uptake by both the liver and spleen (the reticuloendothelial system - RES) (Uchegbu, 1999). These systems will usually be taken up by the liver, spleen and other parts of the RES, depending on their surface characteristics: particles with more hydrophobic surfaces will preferentially be taken up by the liver, followed by the spleen and lungs, while more hydrophilic systems exhibit higher retention times (Brannon-Peppas and Blanchette, 2004). Other parameters, such as particle size, surface charge and composition have a strong influence on the clearance profile (Andresen *et al*., 2005).

Liposomes had a head start on studies aiming at a longer body circulation. In the late 1980s and early 1990s it was discovered that the presence of liposome surface ligands, such as polyethylene glycol (PEG), decreased liposome clearance by producing a hydrophilic surface. Such structures were named "Stealth liposomes". The reduced RES uptake of these structures is due to a reduced opsonisation by plasma proteins, thus enabling them to escape recognition by both the liver and spleen. It is likely that the reduced aggregation of Stealth liposomes in the blood is also responsible for their increased circulation time. Liposome size also affects biodistribution and a size between 70 and 200 nm is necessary to achieve prolonged circulation times with Stealth liposomes (Uchegbu, 1999).

In the case of nanoparticles, the observed behaviour is similar: particles with diameters under 100 nm and hydrophilic surfaces (coated with a polyoxyethylene polymer) undergo less opsonization and clearance by RES uptake. Such long-circulating nanoparticles have been designed as "biomimetic nanoparticles" or "sterically stabilized nanoparticles" (Vasir *et al*., 2005).

Oral delivery, in which the therapeutic agent is absorbed from within the gastrointestinal tract (GIT), is the most desirable approach, but success with both peptides and proteins is limited by barriers to peptide and protein absorption from the GIT. In this respect, nanoparticles can be used to protect a labile drug from degradation in the GIT, protect the GIT from drug toxicity, and deliver antigens to the Payer's patches for oral immunization. Briefly, nanoparticles have been used as oral drug carriers for several reasons (Reis *et al*., 2006): **i)** improvement of the bioavailability of drugs with poor absorption characteristics; **ii)** prolongation of the residence time of drugs within the intestine; **iii)** high dispersion at the molecular level and subsequent increase of absorption; **iv)** delivery of vaccine antigens to gut-associated lymphoid tissue; **v)** control of the drug release; **vi)** targeting of therapeutic

agents to a particular organ and thus reducing toxicity; **vii)** reduction of the GIT mucosal irritation caused by drugs; and **viii)** assurance of the stability of drugs in the GIT.
A better appreciation of biology, together with advances in polymer chemistry and their interaction with nanotechnology has brought a renowned interest in the field of drug delivery, whereby nanosized drug delivery systems are emerging as an answer for the global quest of drug targeting.

3. DEVELOPMENT OF NANO AND MICRO SYSTEMS

Nanoparticles are a collective name for both nanospheres and nanocapsules. Nanospheres have a matrix-type structure. Drugs or tracers may be absorbed at their surface, or entrapped or dissolved within the particle. Nanocapsules are vesicular systems in which the drug is confined to a cavity or inner liquid core surrounded by a polymeric membrane. In this case, the active substances are usually dissolved in the inner core but may also be adsorbed at their surface (Reis *et al*., 2006).

Nanoparticles are generally produced via a layer-by-layer (LBL) deposition method onto colloidal templates (with 0.05 - 4 μm diameter) and consist of nanospheres covered with bioactive films or nanocapsules that can act as reservoirs. Both kinds of (nano)vehicles have advantages: the nanospheres are easy to process but they are not as flexible as nanocapsules. As a matter of fact, such nanocapsules are of particular interest due to their potential for encapsulation of large quantities of guest molecules or large-sized guests within their empty core domain. These materials could be useful in applications in areas as diverse as biological chemistry, synthesis and enzymatic catalysis. In fact, for polymeric nanocapsules, a multitude of different applications have already been proposed, such as confined reaction vessels, drug carriers, protective shells for cells or enzymes, transfection vectors in gene therapy, carrier systems in heterogeneous catalysis, dye dispersants or as materials for removal of contaminated wastes (Vasir *et al*., 2005). Depending on the application, nanocapsules can be produced in any size and endowed with almost any pharmaceutical, biochemical, electrical, optical or magnetic property.

The capsules are manufactured by LBL deposition in several steps. First, the substance to be encapsulated is suspended in an aqueous solution. The addition of a positively or negatively charged polymer constitutes the first actual step in capsule production (Figure 2: 1). Polyelectrolyte molecules are then applied on a layer-by-layer fashion (self-assembling), thus creating an ultra-thin polymer film, where each new layer has the opposite charge of the previous layer. The polymer coating is propelled by electrostatic gravities, which create shells of well-ordered polyelectrolyte complex layers (Figure 2: 2). The resulting capsule walls generally encompasses 4 to 20 of these layers and have a thickness of 8 - 50 nm (Figure 2: 3). The complete capsules are endowed with precisely defined properties. Often, their surfaces take on additional functions, providing, for instance, connections for antibodies to dock (Figure 2: 4). When requested by product development schemes, the core of the capsule can be removed (Figure 2: 5) or the empty capsule shells can then, upon demand, be filled with various substances (Figure 2: 6).

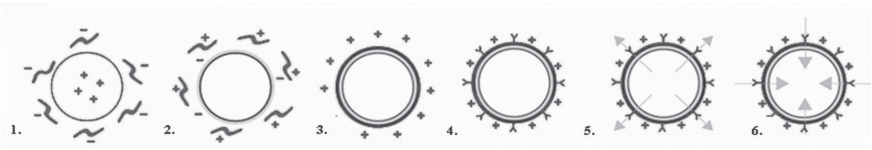

Figure 2 - Overview of the encapsulation process. Adapted from LBL Thechnology® [www.capsulation.com].

Liposomes have been extensively investigated as a potential drug delivery system due to the enormous diversity of structures and compositions that can be achieved (Garnett, 2001; Andresen *et al*., 2005).

Liposomes generally have a large carrying capacity, but usually not large enough to ferry large molecules such as proteins. For liposomes, hydrophilic drugs can be readily entrapped within the aqueous interior of the vesicles, while neutral and/or hydrophobic molecules may be carried within the hydrophobic bilayers of the vesicles (Dass and Choong, 2006). Liposomes are one of the most well-known drug delivery carriers employed in the treatment of cancer. Due to their advantages, liposomal formulations provide a substantial increase in anti-tumor efficacy when comparing with the free drug or standard chemotherapy regimens.

Liposomes are prepared by the thin film hydration method, in which a lipid solution in chloroform/methanol is evaporated to dryness under a stream of nitrogen and the resultant lipid film hydrated with the appropriate buffer. This mixture is vortexed to yield multilamellar vesicles (MLV) or mixed micelles. MLV are then extruded 10 times through polycarbonate filters with 100 nm pore size to form large unilamellar vesicles (LUV) (Matos *et al*., 2004a,b).

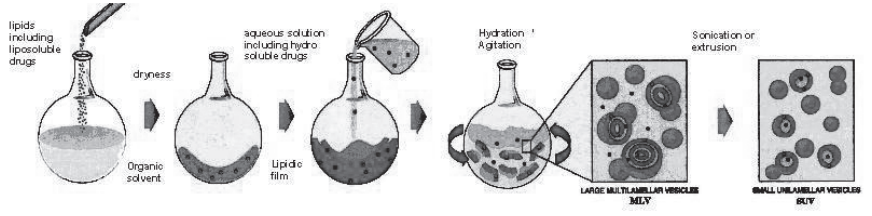

Figure 3 - Preparation of MLV and LUV by the thin film hydration method followed by extrusion (adapted from Lasic, 1996).

The chemical composition of liposomes can be widely varied. Most of them contain neutral phospholipids like phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), or sphingomyelin (SM), supplemented, if desired, with negatively charged phospholipids, like phosphatidyl serine (PS) and phosphatidyl glycerol (PG). In addition, single chain amphiphiles like cholesterol (C) and detergents can be incorporated into the bilayer membrane, modulating its fluidity and transition temperature (Sharma *et al*., 2006). Liposomes have been used as carriers for radioisotopes and contrast agents to be used in diagnostic imaging. Liposomes carrying contrast agents accumulate more in surrounding cells than in tumor cells, due to the low phagocytic activity of tumor cells. This can be used for tumor imaging, wherein tumor cells are observed as holes within the surrounding background (Koukourakis *et al*., 2000; Reis *et al*., 2006).

The role of liposomes in reducing toxicity of anti-cancer drugs has been described by many researchers. For the treatment of breast cancer, liposomal drug delivery has primarily involved the use of anthracyclines (Campos, 2003) such as daunorubicin and doxorubicin. Liposomal encapsulation of anthracyclines does not alter toxicities as such, but the severity is greatly reduced due to altered pharmacokinetics and tissue distribution of the drug. In addition to this, toxicity buffering is also achieved as a result of relatively slow release of drug from these formulations into systemic circulation.

Vinca alkaloids, like vincristine and vinblastine, and paclitaxel, methotrexate and cisplatin derivatives are also frequently studied anti-cancer drugs (Sharma *et al*., 2006), and display efficacy against the transformed or aggressive non-Hodgkin's lymphomas and present less neurotoxicity than the free-drug (Orive *et al*., 2005).

Sharma and co-workers (2006) described the use of anti-HER2 immunoliposomes. As a target antigen, HER-2 is a readily accessible cell surface receptor over expressed in cancerous cells. It provides selective immunotargeting of tumor cells. The second generation of liposomal formulations for anti-cancer therapy includes HER-2, as this receptor plays an important role in the development and progression of many breast cancers (Sharma *et al*., 2006).

Another interesting approach is the immobilization of therapeutic product-secreting cells within microcapsules. For instance, when using CYP2B1-transfected cells, which activate the prodrug ifosfamide at the site of tumor, the median survival of mice transplanted with a human pancreatic carcinoma was the double than that of the control group (Orive *et al*., 2004).

4. **CONCLUSIONS**

(Controlled) delivery systems can overcome many problems involving solubility, *in vivo* stability, pharmacokinetics, tumor uptake and toxicity of the drug. The increasing repertoire of sophisticated delivery systems may thus allow the development of new classes of potent anti-cancer agents such as nucleic acid-based agents (antisense oligonucleotides) to reach clinical applications (Menezes *et al*., 2000). As discussed above, currently approved liposomal drug delivery systems provide stable formulation, improved pharmacokinetics and passive or physiological targeting to tumor tissues.

It is clear that (bio)pharmaceutical formulations and delivery systems became a major limiting step in providing increasingly efficient treatments and introducing new molecular entities into clinical practice.

Long-circulating carriers such as liposomes and nanoparticles can exploit the EPR effect for preferential extravasation from tumor vessels. The structural versatility of nanosystems, their ability to incorporate a wide spectrum of anti-cancer drugs and their ability to carry tumor cell specific ligands, renders them clinically and therapeutically important drug delivery systems in the treatment of many different cancers.

In the future, some challenges need to be addressed including adaptation of each drug delivery system to the particular needs of each malignancy, improvement of interactions between the drug and some of the components of the carrier and development of multi-

functional systems able to deliver several drugs at the same (or different) time with different kinetic releases.

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