Navigare necessere est

Improved navigation would help to solve two crucial problems in modern drug therapy: toxicity and precise delivery

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The availability of the human genome sequence together with major advances in biological technologies have an enormous potential to usher in a new era of medicine. We can imagine a situation in which a physician uses advanced molecular and imaging techniques—individually or collectively—to diagnose an illness and determine the exact molecular ‘fingerprint’ of the disease, be it cancer, cardiovascular disease or an infection. The physician will not only know what has gone awry in the patient’s body but also have access to a wide range of new, highly effective therapeutics that directly tackle molecular defects or invading pathogens without causing side effects. Such drugs could be, for example, peptides that specifically instruct cancer cells to initiate apoptosis, or molecules that repair a defect in the DNA of the cell. Many biomedical researchers are optimistic that such a scenario will be realized through the current advances in genomics, proteomics, systems biology and advanced diagnostic and imaging techniques, in conjunction with new methods of drug development.

This scenario challenges our current concept of treatment, whereby physicians identify a disease and prescribe a drug to treat it. Quite probably, the concept of a drug targeted against a single illness will no longer be accurate. Many diseases induce changes to cells and tissue, such as those that occur in tumour development, which involve numerous genes and proteins. Even a simple infection triggers a complex cascade of gene and protein actions both in the infected cells and in the cells of the immune system. Instead of using a

therapeutic that tackles rapidly dividing cells to kill tumour cells—which in the course of treatment affects normal body cells—or an antibiotic that kills beneficial bacteria as well, it would be much safer and more efficient to use therapeutics that target only the molecules that are involved in pathogenesis. It is logical to assume that, as soon as we know the precise sequence and pattern of molecular events for a specific disease, we can devise such drugs to interfere with pathological processes and correct what has gone wrong.

Some of these new, so-called ‘smart’ drugs are already available or are under development. They include small peptides that target specific surface receptors on cancer cells and also gene therapeutics—gene constructs, antisense oligonucleotides and small interfering RNAs (siRNAs)—that induce changes specifically in metabolism or gene expression. Another example is the protease inhibitor that blocks HIV protease and thus prevents the virus from maturing. On the diagnostic side, there are further advances: new diagnostic tools based on gene or gene expression analyses allow physicians to select the most effective drugs to treat various cancers or to circumvent drug resistance in anti-HIV therapy. These are the first harbingers of this new concept of medicine, and the next few years will probably see the arrival of many more peptides, antibodies and other molecules.

However, no matter how good and ‘smart’ these therapeutics are, the problem remains of how to target these drugs to the appropriate cells or tissues. Furthermore, many of these drugs

require carrier systems as they are either hydrophobic or need to be protected from enzyme destruction while they are on their way to the target cells. Even existing therapeutics of organic and inorganic origin could be vastly improved in terms of safety and efficacy using methods that deliver them safely to their designated area. Regardless of whether a ‘smart’ drug is new or old, one of the main challenges for biomedical research is the refinement of drug delivery. Precise navigation together with better drug protection would thus solve two crucial problems in drug development: toxicity, as a significantly smaller dose of the drug would be administered, and efficacy, because the drug would concentrate only in affected tissues and cells.

...one of the main challenges for biomedical research is the refinement of drug delivery

It is therefore not surprising that targeted drug delivery has become a leading area in medical research. However, many technologies that are being exploited have various limitations, and it seems that appropriate solutions could be found in nanotechnology. So, what is it that makes nanotechnology appealing for drug delivery? First, it makes it possible to produce devices and constructs smaller than 1 μm , which enhances function and creates completely new material properties. Second, it fits neatly in the biological setting, as biological molecules and cellular structures are about 100 nm or smaller—the size scale of this technology. Nanotechnology also creates

Fig 1 | Polymeric spherical micelles increase the solubility and bioavailability of 'smart' drugs, as well as increasing their stability in biological surroundings, improving their pharmacokinetics and biodistribution and decreasing the toxicity of the drug. Moreover, they are stable both *in vitro* and *in vivo* and efficiently deliver drugs or genes into pathological areas with compromised vasculature, such as tumours.

new possibilities in engineering devices with enormous precision through self-assembly and directed assembly (Hilt, 2004). In terms of drug delivery, it offers various advantages over traditional systems. Nanoscale drug-delivery devices—nanocarriers—are able to overcome biological barriers and may provide targeted delivery of drugs because of their small size. They may also be able to solubilize drugs for intravascular delivery, improve the stability of therapeutic agents and control drug release in target tissues for optimal therapeutic efficacy.

Not surprisingly, drug-delivery systems based on nanotechnology have attracted much commercial interest in the pharmaceutical industry. According to a recent report, the global market for drug-delivery products and services is projected to exceed US\$67 billion in 2009 (NanoMarkets, 2005). The market value for delivery technologies in cancer treatment alone was US\$2.6 billion in 2002, and is expected to grow to US\$15.4 billion by 2007. As a part of this, nanotechnology-based drug-delivery systems are expected to create a market of about US\$1.7 billion by 2009 and more than US\$4.8 billion by 2012.

Some of the systems already used in drug delivery are nanospheres and nanoparticles—both composed of oligomeric or polymeric units—and nanocapsules consisting of a hydrophobic core surrounded by a polymer wall.

These drug carriers can entrap, dissolve or encapsulate therapeutic molecules, chemically attach them or adsorb them at the surface (Ravi Kumar *et al*, 2004a). Many polymers, such as polyvinyl alcohol, polyethylene glycol (PEG), poly-*N*-vinyl pyrrolidone, polyethylenimine (PEI), polyethylene oxide (PEO), phosphatidylethanolamine (PE), polylactides (PLA) and polylactide-co-glycolides (PLGA) are already in use. For example, two newly synthesized nanoparticles, methoxypolyethylene glycol(MPEG)–PLA copolymer and pluronic PEO–polypropylene oxide–PEO triblock copolymer, are now being investigated for the delivery and controlled release of paclitaxel, which could reduce unwanted side effects and improve therapy (Dong & Feng, 2004; Oh *et al*, 2005).

However, a major drawback of polymer carrier systems is their low drug-carrying capacity—each molecule of PEG, for example, can carry only two drug molecules. Thus, polymeric micelles—spherical, colloidal particles spontaneously formed by amphiphilic molecules in water—are progressively being exploited, as their inner cores have a higher drug-loading capacity (Fig 1; Otsuka *et al*, 2003). Micelles also increase the solubility and stability of hydrophobic agents, improve their pharmacokinetics and decrease the toxicity of the drug. The first generation of drug-delivery devices for anticancer therapeutics were liposomes—colloidal, vesicular structures based on

phospholipid bilayers of different sizes, some as small as 20 nm. But, as liposomes are made of phospholipids, they are taken up by macrophages. Additionally, they move from blood vessels into other tissues, such as the liver and spleen. Several researchers have therefore tried to increase the circulation time of liposomes in the bloodstream by adding different molecules that reduce their uptake by macrophages. However, lipid carriers, even if easy to produce, often display toxicity that limits their use. Le Garrec *et al* (2004) therefore investigated poly(*N*-vinylpyrrolidone)-block-poly(*D,L*-lactide), as an alternative drug carrier system. In water, it self-assembles into micelles that efficiently solubilize hydrophobic anticancer drugs, such as paclitaxel and etoposide. *In vitro* and *in vivo* studies showed that the micelles released paclitaxel in a controlled manner and were stable in aqueous media for several months.

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Platins are widely used cancer drugs that could benefit from improved drug navigation to reduce their severe side effects. Several carrier systems, such as micelles and liposomes, have been used to deliver platin drugs, but these formulations have shown a higher selectivity for the liver and spleen than for tumour tissue. A recent *in vivo* study used PEG–poly(glutamic acid) block copolymer micelles as carriers, which accumulated significantly and selectively in solid tumours. Administration of these cisplatin-incorporated micelles caused total eradication of primary tumours (Nishiyama *et al*, 2003). Li *et al* (2004) went a step further and prepared a new class of multi-compartment micelles in aqueous solution by combining a water-soluble PEO, a saturated hydrocarbon polyethylene and poly(perfluoropropylene) oxide. These multi-compartment cores could, for example, be used to deliver several incompatible drugs together, which opens up a new possibility for cancer therapy and other treatments.

As drugs become larger and less water-soluble, new delivery systems such as nanoporous materials are needed (Shin *et al*, 2001). These have defined pore sizes of 1–100 nm or larger, and are being studied particularly for controlled drug release. By making the pores only slightly larger than the drug molecule, the rate of diffusion of the molecules can be controlled, regardless of the amount of the drug inside the capsule (Orosz *et al*, 2004). Among various approaches, dendrimers—polymers with a branching structure that are synthesized one step at a time to add a new monomer layer with each synthesis—have recently gained in popularity due to their low systemic toxicity and high drug-load ability. Dendrimers have a central core, an interior branched structure and external reactive groups, and move through vascular pores and into tissue more efficiently than larger carriers. Several researchers are therefore studying the possibility of using them as drug-delivery devices, including methotrexate (Quintana *et al*, 2002), indomethacin (Chauhan *et al*, 2004), paclitaxel (Ooya *et al*, 2004) and doxorubicin (Padilla De Jesus *et al*, 2002).

However, the synthesis of dendrimers is a long, difficult and expensive process, and the yield of functional particles declines successively with each synthesis step. A new, less time-consuming and more efficient method for dendrimer synthesis is consequently a promising development. Choi *et al* (2005) prepared two types of dendrimer that carry complementary single-stranded, non-coding DNA. The DNA molecules bound to each other in solution, thus forming two-dendrimer complexes. One dendrimer was prepared for imaging applications and the second was specifically designed to target cancer cells through the folate receptor. The self-assembled dendrimer clusters were shown to be well formed and functional.

Another important research field is nucleic-acid delivery for gene therapy. With the development of genomics and proteomics, exciting new possibilities for these therapeutic approaches have arisen (Kraljevic *et al*, 2004). But the clinical use of gene therapy will depend strongly on the development of safe and efficacious delivery systems to protect and deliver the drug to the target cell, as the carrier system has to overcome many natural

Table 1 | Weakness of some frequently used cationic polymers for gene delivery

Cationic polymer	Weaknesses
Polyethyleneimine polymers (PEI)	Rather high toxicity
Poly(L-lysine) polymers	Low transfection efficacy, modest to high toxicity
Polyamidoamine dendrimers (PAMAM)	Need for further <i>in vivo</i> studies
Imidazole-containing polymers	Need for charge density optimization step during preparation
Chitosans	Problems with endolysosomal escape

barriers. When arriving at the target cell, it must be taken up through endocytosis and escape the endosome by disrupting the membrane; only then can it deliver the nucleic acid to the nucleus.

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Two groups of vectors have been used for gene delivery: viruses and non-viral vectors. During recent years, it has become obvious that viruses harbour the risk of unwanted immune responses and have inadequate specificity for cell targeting. Non-viral systems could overcome some of these problems. Most importantly, they can be used with a higher degree of safety, as they do not integrate into the host chromosome and cause fewer problems with immune response. They can also carry more nucleic acids than viruses can, which allows the delivery of larger genes. Additionally, they are suitable for attaching various types of targeting molecule and provide better cell-type specificity. Finally, synthetic systems are much easier to characterize and control than viral vectors.

Some promising non-viral delivery systems include cationic lipids and polymers (Table 1), which bind negatively charged DNA to form stable complexes due to their charge (Ravi Kumar *et al*, 2004a). In addition to the examples listed in Table 1, other polymers are also being investigated. Ravi Kumar *et al* (2004a) prepared PLGA

nanospheres through an emulsion-diffusion-evaporation technique using ethylacetate as an organic solvent and polyvinyl acetate-chitosan as a stabilizer. Their work has shown that the resulting particles can be used for the binding and condensation of DNA. Csaba *et al* (2005) combined PLGA with polyoxyethylene derivatives (poloxamers and poloxamines) to create a carrier system that was able to encapsulate plasmid DNA and was well tolerated in cell culture. In addition, PEG-PEI nanoplexes were successfully tested as delivery vehicles for siRNA to tumour neovasculature. The siRNA nanocomplex self-assembled by exploiting the PEI's cationic domain, which binds to negatively charged nucleic acids. PEG was used for steric stabilization and further ligation of the targeting peptide sequence (Schiffelers *et al*, 2004). Similarly, PLGA nanospheres containing phosphothionated antisense DNA were shown in animal experiments to be an effective gene-delivery system for the treatment of restenosis—the narrowing of coronary arteries after angioplasty. Another group prepared a protein-based nanocarrier using albumin matrices to entrap different antisense oligonucleotides. The results were promising, as human albumin molecules showed good biocompatibility and transfection efficacy (Cohen-Sacks *et al*, 2002). However, a basic problem is the limited knowledge of the biological effects of polymeric complexes formed with DNA. For example, complexes formed by combining PEI and DNA have a higher transfection efficiency if the polymer is added to the DNA during preparation and not vice versa (Merdan *et al*, 2002).

Dendrimers are also appropriate vehicles for gene delivery (Cohen-Sacks *et al*, 2002; Nakanishi *et al*, 2003). Kim *et al* (2004) have synthesized a new triblock polyamidoamine (PAMAM) dendrimer copolymer with PEG, which formed highly water-soluble polyplexes with plasmid DNA. It revealed little cytotoxicity despite its poor degradability and achieved high transfection efficiency.

Cationic liposomes, such as Lipofectin®, are already being tested in clinical trials of non-viral gene therapy because of their low immunogenic response (Ravi Kumar *et al*, 2004b). These lipid-gene complexes have the potential to transfer large pieces of DNA—up to 1 million base pairs—into cells. However, their transfection efficiencies

are still low when compared with viral vectors, and problems with cytotoxicity remain. According to a recent study, it seems that, at present, the limiting factor for cationic lipid carriers seems to be the tight association of a fraction of the delivered exogenous DNA with cationic cellular molecules, which may prevent optimal transcriptional activity (Ewert *et al*, 2004).

A further challenge for improving drug delivery is creating carriers that specifically home in on target cells to deliver the drug while leaving normal cells untouched. There are two possible strategies to achieve this goal, namely passive and active targeting. Passive targeting requires an increase in the stability of the drug carrier in the body. As nanoparticles tend to accumulate in and around cancer tissues, the longer the circulation time, the more drug carriers accumulate at the tumour. Additionally, nanoparticles can be coated with PEG to reduce non-specific interactions and absorption by endothelial cells.

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Active targeting is achieved by incorporating homing moieties, which facilitate uptake by certain tissues or cell types into the drug-delivery vehicle. Such vehicles concentrate the drug at the target site, thus increasing efficacy and decreasing side effects. Much emphasis has been placed on finding adhesion molecules—antibodies and so-called small ‘homing peptides’—that are specific for different tissues, particularly for the vasculature. To find such markers, phage display libraries of small peptides were screened in live mice, and peptides that directed phages to a specific target in the body were selected (Ruoslahti, 2002). Interestingly, most organ- and tumour-specific receptors found so far have been peptidases. For example, dipeptidyl peptidase IV and membrane dipeptidase are selectively expressed in lung vessels, whereas aminopeptidase P was found to be expressed in breast gland vasculature (Ruoslahti, 2004). Essler & Ruoslahti (2002)

CGFECVRQCPERC	binds to membrane dipeptidase on the endothelial cells in lung blood vessels
KDEPQRRSARLSAKPAPPKPEPKPKKAPAKK	preferentially binds to blood vessels in various tumours
CGNKRTRGC	recognizes lymphatic vessels and tumour cells in certain tumours

Fig 2 | Tumour vessels express specific markers of angiogenesis. Some of the successfully tested tumour-homing peptides are presented (Akerman *et al*, 2002).

also identified a nonapeptide that targets aminopeptidase P in the breast vasculature in mice. Their results showed that this peptide was able to target not only normal breast tissue but also premalignant tissue and primary breast tumours.

Targeting drugs to blood vessels holds great promise. Because tumour vessels express specific markers of angiogenesis, cancer chemotherapy directed against angiogenic vessels induces tumour regression while avoiding drug resistance. Some tumour-homing peptides that have already been successfully tested are the RGD-motif tripeptide that binds to integrins $\alpha v \beta 3$ and $\alpha v \beta 5$, both overexpressed in tumour vasculature; the NGR-motif tripeptide that binds to aminopeptidase N, a membrane protein that is specific for angiogenesis if expressed in the vasculature; and peptides directed to nucleolin, a new marker for angiogenic vessels (Fig 2). Additionally, peptides that target the vasculature of various tumours have been recently discovered, such as markers for pancreatic islet cell tumours and skin tumours (Chen *et al*, 2001). Some authors have already successfully used NGR-motif peptides to target drug-delivery vehicles loaded with tumour necrosis factor and tachyplesin, which are both anticancer compounds (Curnis *et al*, 2000). Another homing device that is similarly interesting is a new pentapeptide; liposomes carrying this peptide and adriamycin effectively suppressed tumour growth and were able to target angiogenic vessels of glioblastomas (Oku *et al*, 2002). Also, Hong & Clayman (2000) described the isolation of a 12-amino-acid peptide that was able to direct drug-delivery systems to solid head and neck tumours and to translocate across the cell membrane. The peptide also binds preferentially to specific tumour cells and penetrates the cancerogenic tissue.

Another promising approach is used to attack lymph-node tumour cells. These cells carry specific markers, which can be targeted specifically by a cyclic amino-acid

peptide (LyP-1) that does not bind to normal lymphatic tissues (Laakkonen *et al*, 2002). The authors reported that LyP-1 had a proapoptotic and cytotoxic effect on tumour cells, and that systemic administration of the LyP-1 peptide inhibited breast cancer xenograft growth in mice. It was later found that LyP-1 also binds to cells in other kinds of tumours, including prostate cancer in mice (Laakkonen *et al*, 2004). LyP-1 offers even more clinical value than simply being a homing device for lymph-node tumours: as cancer often spreads through lymphatic vessels, it could be used to target drugs to lymphatic vessels and to block metastases by destroying tumour cells close to the lymphatic system.

In addition to antibodies and small peptides, inorganic compounds are being investigated for their ability to specifically target tumours. In a recent experiment, Akerman *et al* (2002) showed that quantum dots (qdots) coated with PEG were able to avoid the reticuloendothelial system (RES), which reduced their accumulation in the liver and spleen but did not affect their accumulation in tumour tissue. The qdots were further coupled with homing peptides and showed excellent abilities for ‘homing’ to the vasculature of normal lungs and to tumours. Qdots have traditionally been used for molecular imaging, but their application could be expanded for drug-delivery purposes as well.

Colloidal silica nanoparticles with cationic surfaces are interesting vehicles for targeted gene delivery as they bind to plasmid DNA and transfect cells *in vitro*. The silica particles have been studied in the mouse, where they increased gene expression in the lung while causing either low or no cell toxicity (Ravi Kumar *et al*, 2004b). Layered double hydroxide (LDH) also performed well, both *in vitro* and *in vivo*. It was first described by Choy *et al* (2000) as a nanosized inorganic ‘clay’ with the ability to intercalate biomolecules such

as DNA, ATP and nucleosides. The inorganic lattice of LDH provides biochemical properties that could be exploited for gene delivery and for the prolonged release of drugs (Kwak *et al*, 2002). Kwak *et al* (2004) prepared uniformly sized LDHs in the range 100–200 nm and injected them into adult male rats to investigate their safety. No serious systemic effects were observed for LDHs at concentrations below 200 mg/kg, but if deposited extravascularly, LDHs were locally irritating. Recently, Tyner *et al* (2004) used LDHs as delivery vehicles for camptothecin. Their idea was to encapsulate camptothecin in an anionic micelle derived from a biocompatible surfactant with a negative charge, which allowed the intercalation of camptothecin-loaded micelles into LDH layers. These complexes inhibited the growth of 9L glioma cells *in vitro* similar to the 'naked' drug. However, the real advantage lies in the fact that these nanohybrids can be administered in a dose-controlled fashion to increase solubility. The authors successfully attached a few homing molecules to the outside surface of LDHs (Fig 3), which showed the good targeting abilities of the hybrids. In general, LDH-micelle constructs could become excellent drug-carrier systems as they are biocompatible, show good targeting properties and successfully avoid the RES. However, they are not biodegradable and further studies are needed to assess their safety.

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Nanotechnology supports and enhances advances in genomics and proteomics. Specifically, it offers many interesting possibilities for improving drug delivery. The rapid progress of nanotechnology-based applications in medicine is due to the huge interest from therapeutic and diagnostic companies, which have already introduced many nanotechnology-based devices to everyday practice, among them new drug-delivery systems. Future research should further improve their targeting properties, given that one of the most important problems in medicine is the controlled and exact

Fig 3 | LDH carrying therapeutic DNA and homing proteins as a potential targeted drug-delivery system

delivery of a drug to diseased tissue. However, the promising solutions offered by nanotechnology pose some inevitable questions. We still do not know whether organic and inorganic nanoparticles, once they have entered the body, can cause damage to other tissues. Additionally, we need new safety standards as the novel and unexplored characteristics of nanoscale materials demand appropriate quality-control measures. The further development of new strategies in drug delivery depends largely on the establishment of such standards and their subsequent implementation in research and manufacturing.

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