Pharm Res (2011) 28:1241–1258
DOI 10.1007/s11095-011-0392-y

EXPERT REVIEW

Controlled Delivery Systems: From Pharmaceuticals to Cells and Genes

Elizabeth Rosado Balmayor • Helena Sepulveda Azevedo • Rui L. Reis

Received: 30 September 2010 / Accepted: 3 February 2011 / Published online: 19 March 2011

ABSTRACT During the last few decades, a fair amount of scientific investigation has focused on developing novel and efficient drug delivery systems. According to different clinical needs, specific biopharmaceutical carriers have been proposed. Micro- and nanoparticulated systems, membranes and films, gels and even microelectronic chips have been successfully applied in order to deliver biopharmaceuticals via different anatomical routes. The ultimate goal is to deliver the potential drugs to target tissues, where regeneration or therapies (chemotherapy, antibiotics, and analgesics) are needed. Thereby, the bioactive molecule should be protected against environmental degradation. Delivery should be achieved in a dose- and time-correct manner. Drug delivery systems (DDS) have been conceived to provide improvements in drug administration such as ability to enhance the stability, absorption and therapeutic concentration of the molecules in combination with a long-term and controlled release of the drug. Moreover, the adverse effects related with some drugs can be reduced, and patient compliance could be improved. Recent advances in biotechnology, pharmaceutical sciences, molecular biology, polymer chemistry and nanotechnology are now opening up exciting possibilities in the field of DDS. However, it is also recognized that there are several key obstacles to overcome in bringing such approaches into routine clinical use. This review describes the present state-of-the-art DDS, with examples of current clinical applications, and the promises and challenges for the future in this innovative field.

KEY WORDS cell encapsulation · DDS routes of administration · gene therapy · growth factors · nanotechnology · regenerative medicine

E. R. Balmayor (✉) • H. S. Azevedo (✉)
3B's Research Group—Biomaterials, Biodegradables and Biomimetics
Headquarters of the European Institute of Excellence on Tissue Engineering & Regenerative Medicine
University of Minho
AvePark,
4806-909 Taipas Guimarães, Portugal
e-mail: erosado@dep.uminho.pt

H. S. Azevedo
e-mail: hazevedo@dep.uminho.pt

E. R. Balmayor • H. S. Azevedo • R. L. Reis
Institute for Biotechnology and Bioengineering
PT Government Associated Laboratory
Guimarães, Portugal

ABBREVIATIONS

BMP-2	bone morphogenetic protein 2
BSA	bovine serum albumin
cDNA	complementary DNA
DDS	drug delivery systems
ECM	extracellular matrix
HEMA-MMA	Hydroxyethylmethacrylate- Methyl methacrylate
IGF-I	insulin-like growth factor I
PC12	cell line derived from a pheochromocytoma of the rat adrenal medulla
PDGF	platelet-derived growth factor
PLA-PEG	Polylactic acid-polyethylene glycol
PLGA-m-PEG	Poly(lactic-co-glycolic acid)-methoxy-polyethylene glycol
VEGF	vascular endothelial growth factor

INTRODUCTION

When thinking about methods to administer medication, one may initially consider the traditional routes: oral,

subcutaneous, intramuscular, intravenous, and topical. These routes use traditional medication delivery systems such as needles, syringes, infusion pumps, and catheters (1). These medication delivery systems may not, however, allow the delivery of satisfactory concentrations of medication to the appropriate site, nor do they necessarily minimize local or systemic toxicity. Towards this goal, innovative drug delivery systems have been developed which are described in the following sections.

DRUG DELIVERY SYSTEMS (DDS)

When a drug is introduced in the human body using traditional administration methods, a cascade of biotransformations occurs as result of its interaction with the biological environment (drug metabolism). Drug metabolism is very complex and comprises oxidation, reduction, hydrolysis and conjugation reactions leading to final excretion from the body. Depending on the anatomical route, administered drugs are passing, upon absorption, through several tissues and organs (e.g. liver) before reaching the systemic circulation. In those organs, drugs may be subjected to chemical or enzymatic degradation. For example, in per os medication, a significant portion of the drug is destroyed (the drug may suffer degradation by digestive enzymes in the upper digestive tract before being exposed to the highly acidic gastric juice (2)), making a higher dose necessary to ensure relevant therapeutic levels.

Drug delivery systems (DDS) were first developed with the purpose of raising the level of bioactive drugs in the blood. Nowadays, the intent of DDS is to alter the pharmacokinetics of drugs so that sustained therapeutic concentrations can be maintained at specific locations in the body with minimal side effects. It is also imperative that DDS provide efficient and precise delivery in a way that the patient finds acceptable and tolerable.

Pioneering investigations proposed the creation of a matrix or carrier that could increase drug bioavailability while minimizing drug waste and local and systemic side effects. The matrix is supposed to act as a rate-controlling device to deliver the bioactive drug in a pre-determined place and controlled fashion for a certain time period (3) while protecting the therapeutic agent from the body's clearing mechanisms. Several polymers have been selected as suitable carrier materials, taking into account their recognized biocompatibility and non-toxicity, hydrophilicity and biodegradability (3).

Hydrophilicity and biodegradability are two important properties controlling the release mechanism of entrapped drugs (Fig. 1). Hydrophilic matrices release the contained drug by diffusion phenomena due to the swelling of the polymer upon contact with fluids (Fig. 1A). Similarly, for biodegradable matrices, the rate of degradation in the physiological environment controls the release of the drug (Fig. 1B). Natural and biodegradable polymers have been widely used as carriers in DDS (4–6). They present important advantages, such as non-toxicity and rapid clearance in the body with degradation products easily metabolized. Furthermore, being biodegradable, no additional surgeries are required for their removal from the body once they have performed their therapeutic function.

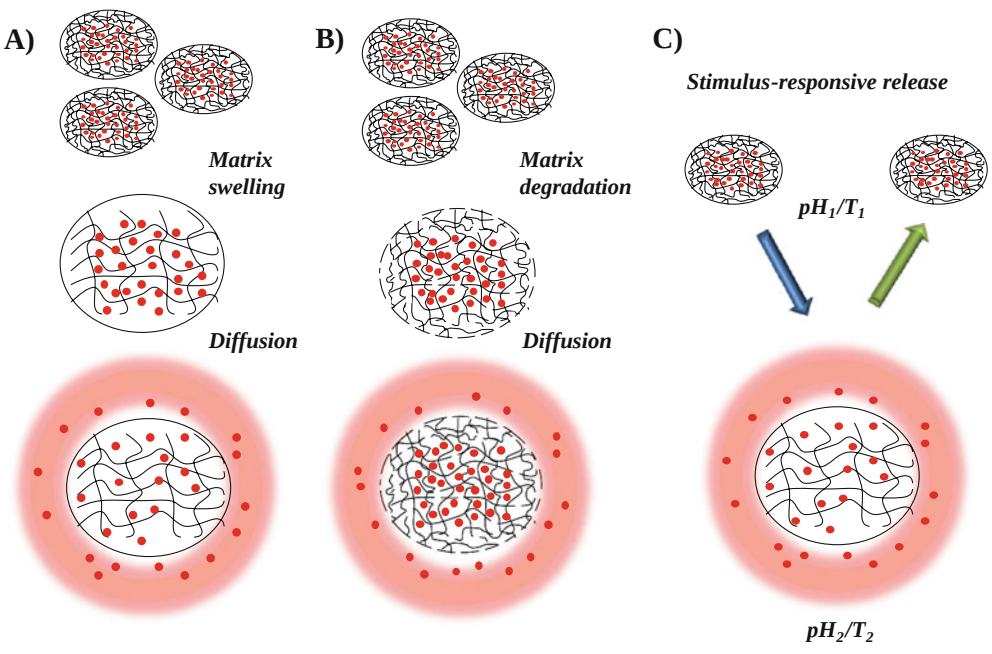

Fig. 1 Illustration of different drug release mechanisms. A polymeric nanoparticle is loaded with a specific drug and its release is controlled by (**A**) swelling of the matrix with subsequent drug diffusion, (**B**) matrix degradation with subsequent drug diffusion, (**C**) external stimuli (pH or temperature), which causes changes in the matrix properties (e.g. swelling) with subsequent drug release.

The first generation of devices developed as DDS were mainly based on polymeric implants (3,7,8). Two main approaches were used to obtain a drug-implant complex. The drugs were either coated on the implant surface or directly incorporated into the polymeric implant (9). These implants were extensively used in the field of orthopedics (9,10). However, the complications generated by the presence of these devices (toxicity, inflammation, infection, pharmacological side effects), together with the need for additional surgical intervention for implant removal, have been pointed out as important disadvantages of these systems. Moreover, the use of polymer implants as DDS has shown some difficulties over controlling the drug release rate (9).

Nowadays, novel molecules with high therapeutic value are being discovered for which the traditional administration routes may not provide adequate delivery or ensure maximum efficacy. These molecules will require refined strategies and sophisticated delivery systems capable of a coordinated control over their release. Many of these therapeutic molecules are proteins that have limited half-lives *in vivo* and are therefore particularly difficult to administer to certain sites at therapeutic concentrations and for prolonged periods of time. Local administration is likely necessary to achieve the desired result but presents delivery problems. Localized delivery of these agents without involvement of non-target organs has also proven to be problematic. These limitations may be overcome by using a materials technology to provide sustained local release of therapeutic molecules to cells and tissues with minimal collateral exposure of non-target tissues.

Progress in the development of novel drug delivery systems is joining researchers from different areas (materials science, pharmacology) and clinicians to ensure maximum efficacy, minimal toxicity and patient convenience. The goals behind the rationale for designing drug delivery systems are represented in Fig. 2. The advantages offered by DDS, compared to other methods of medication administration, are summarized in Table I.

Thus, novel devices have been proposed, such as those in which pharmaceutical agents are encapsulated within smart polymers or attached to them (Fig. 1C) (2,3,14). The challenge is, however, to move from research to product development, clinical implementation and commercial exploitation. The lack of technological feasibility, reproducibility and marketability that characterizes most of the proposed DDS at the research level has been the main problem impeding these systems from moving forward to clinical application. The challenge consists, therefore, in developing an effective formulation that combines the drug of interest with a suitable delivery system. In other words, effective DDS that provide reliable and consistent performance. This also includes that a different part of the body can been considered and investigated as delivery route. Transdermal patches (15–18), oral capsules and pills (19–21), injectable gels (22), drug carrier suspensions (23–25) and novel inhalation systems (25,26) are examples of DDS available to date with satisfactory results in medical use. However, there are a number of key challenges that need to be addressed when developing new DDS before they can enter clinical development. This article reviews the recent advances in drug delivery systems, their current clinical applications, advantages and limitations. We also describe very exciting opportunities that are emerging, which include gene therapy and nanotechnology, but are still waiting clinical implementation. The paper concludes with current challenges faced in this highly innovative research field.

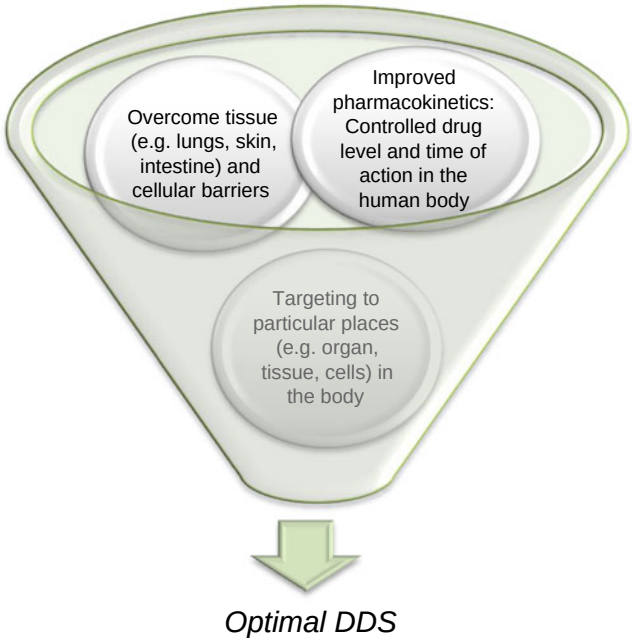

Fig. 2 Goals in the development of drug delivery systems (adapted from ref (11)).

PRINCIPAL ADMINISTRATION ROUTES IN DDS

Transdermal DDS

Delivering drugs through the skin is regarded as an alternative to oral delivery or hypodermic injections (15,16,18). The main advantages and drawbacks of transdermal drug delivery systems are listed in Table II. The fact that by simply removing an external patch applied to the patient's skin immediately stops the administration of the drug highlights the security of this DDS. This advantage,

Table I Advantages of DDSs Compared with Traditional Medication

i) Protein- and peptide-based drugs have short *in vivo* half-lives because they are commonly destroyed after oral intake due to the action of hydrolytic enzymes or environmental conditions (e.g. acidic gastric juice). DDSs can provide a protective effect against degradation and enhanced stability and bioactivity of the drugs.
ii) Absorption and therapeutic concentrations of the medications within the target tissue or organ are improved. Hence, DDSs allow for maintaining bioactive drugs at therapeutically desired doses.
iii) DDSs allow for reproducible, controlled, and long-term administration of therapeutic drugs.
iv) DDSs allows for the possibility of delivering drugs at desired place in the body, i.e., site-specific treatments and local administration.
v) The frequency of drug administration is reduced.
vi) Harmful/adverse side effects related to systemic administration and over-dosages are eliminated or may be reduced by delivering continuously small amounts of drugs instead of large doses.
vii) Drug administration may be improved and facilitated in deprived areas where medical supervision may be needed and is not available.
viii) Patient compliance and comfort are improved. Patients' negligence in the treatments is avoided since they do not need to remember taking daily doses of long-term medication routines. Uncomfortable injections and pills can be avoided.
ix) From an economic perspective, DDSs may result in less expensive products, great variability on the market, and less drug waste. Well-established and classical drugs with expiring patents, can be reformulated with novel administration mechanisms improving their therapeutic action.

Compiled from ref. (2, 3, 12, 13)

together with the simplicity in use, makes such patches the most available and used DDS nowadays (Table III).

Currently available transdermal patches can be classified into two main categories: reservoir-and-matrix or drug-in-adhesive type (18). A reservoir system holds the drug in a solution or gel, from which the delivery can be controlled by a rate-controlling film located between the drug reservoir and the skin. In contrast, the drug-in-adhesive type combines the drug, adhesive and mechanical backbone of the patch into a simple design. The latter does not involve rate-controlling films or membranes. The skin permeability controls the rate of the drug delivery, but it normally functions as a barrier to the exterior. This is mainly achieved by the *stratum corneum*, the outermost layer of the epidermis (15,17,18). Nevertheless, possibilities exist to overcome this barrier if low molecular weight drugs (100–500 Daltons) are used (15,18). This is, however, a limitation of this route of administration, as it excludes the application of molecules with higher molecular weight with relevance in clinical applications.

To circumvent the skin barrier and be able to administer higher molecular weight drugs, several strategies have been investigated, including the use of molecular absorption enhancers. These are substances that promote the passage of drugs through the different skin layers (17,18,27,28). For example, terpene-derived compounds and phenol derivatives seem to improve transdermal absorption (18). Clinical trials have been performed using this type of substance, e.g. linalool, alpha terpineneol and carvacrol. They have proved

Table II Advantages and Limitations of Transdermal DDS

Advantages	Limitations
Low dose and low frequency lead to improved patient safety	Low thermal stability of the drug
Simple and fast-interrupt routines make the system easily stopped by removing topic patch	Chemical reactions of the drug with polymers and excipients
Easy accessibility aids high patient compliance	Difficult to pass the skin natural barrier (stratum corneum)
Simple to administer, can be done by the patient	
Steady drug levels in the blood	
Gastrointestinal irritation avoided	
First-pass effect by liver detoxification step avoided	
Ability to achieve sustained release	

Compiled from ref. (15–18)

Table III Transdermal DDS: Current Applications and Commercially Available Products

Application (Active drug)	Commercial product
Menopause symptoms (estradiol)	Esclim®, Vivelle®, Vivelle-Dot®, Climara®
Smoker's addiction (Nicotine)	Nicoderm CQ®, Nicotrol®
Chest pain due to heart disease (Nitro-glycerine)	Nitro-Dur®, Nitrodisc®
Low levels of male sex hormone (Testosterone)	Androderm®
Continuous analgesia (Fentanyl)	Duragesic®, Transdermal System®
Motion sickness (scopolamine)	Transderm-Scop®
Contraceptive (ethinyl estradiol-norelgestromin)	Ortho-Evra®

Compiled from ref. (15–18) and www.drugs.com

to enhance the absorption of haloperidol. The most satisfactory result was obtained when linalool was used, allowing therapeutic levels of haloperidol (18,29).

Another possibility of increasing the transport of drugs through the skin is by applying different forms of energy (16–18), e.g. an electric field, ultrasound. These "active" methods of skin permeation include iontophoresis, which induces a potential difference across the skin (16–18). Hence, it promotes the transfer of charged ionic drugs or high molecular weight compounds. Current clinical applications include the administration of lidocaine and iontocaine (Phoresor®), local anesthetics (2,18) and dexamethasone, as well as local anti-inflammatory agents (18). Besides applying an electric gradient, sonophoresis and electroporation are alternative methods. However, these have been less studied. The use of low frequency ultrasound to enhance the absorption of mannitol has been reported (30).

Microfabrication for Transdermal Drug Delivery: Microneedles

In addition to the above-mentioned chemical and physicochemical procedures, physically disrupting the stratum corneum has been used for improving transdermal drug delivery (3,15–17,31). This technique uses microneedles to create "micro-holes" that allow for transdermal passage of drug molecules (3,15,31,32). Needles have been fabricated out of silicon, metals and polymeric materials. Moreover, these micro-devices have been coated with different compounds, like proteins, DNA or virus particles (3,16). Needle sizes vary from sub-micron to millimeter scale (31,32). Recently, micron-sized needles have been used for transdermal drug delivery. These microneedles support delivery of drugs in combination with passive patches through the skin layers (Table IV).

In vitro experiments have shown a remarkable increase in skin permeability. Rates of transdermal transport were determined by piercing human cadaver epidermis with microneedles. In these studies, skin permeability for calcein, insulin, and BSA was increased by orders of magnitude (32,33). Animal experiments have shown a major increase in the transdermal delivery capacity of vaccines, oligonucleotides, insulin, desmopressin and human growth hormone (32,34–36).

Subsequent human trials have proven that microneedles are painless, effective and reliable in delivering drugs (37,38). Naltrexone, for instance, was transdermally administered to healthy volunteers by using those microneedles where therapeutic serum concentrations were achieved (39). Additional transdermal drug delivery systems based on microneedle technology in clinical development include systems for the influenza vaccine (40) and osteoporosis treatment (15,16). The influenza vaccine has successfully completed a phase III clinical trial and was recently submitted for registration in Europe by a collaborative effort of Becton Dickinson and Sanofi-Pasteur (40). A clinical phase II for the delivery of parathyroid hormone to treat osteoporosis is currently being conducted by the company Zosano Pharmaceuticals (15,16). During these clinical trials, no severe adverse effects have been reported. Furthermore, no infections or bleedings have been diagnosed. Only mild and temporary skin irritations could be observed.

Table IV Microneedles as Transdermal DDS

Fabrication of small needles in the micron scale offers persuasive possibilities to improve transdermal administration of drugs:

i) The outmost layer of the skin can be selectively "pierced" with small needles. The size should be large enough to allow the drug molecules to enter, but small enough to avoid reaching nerve terminations and causing pain or significant damage.

ii) The permeability of the skin is increased (micron-scale pathways can be created into the skin).

iii) Targeted effect reaches the stratum corneum layer of the skin.

iv) Drugs can be delivered into the skin in a minimally invasive and controlled manner.

v) Batch-processing techniques allow for greater device reproducibility and uniformity.

vi) Offers possibilities of incorporating different components for pulsatile release of drugs in response to physiological requirements.

vii) Appears to be safe and well tolerated by patients and allows rapid skin recovery post-administration.

Compiled from ref. (2, 3, 14–17)

Oral DDS

In addition to the parenteral, transdermal delivery route, the oral route is an easy mode for drug delivery (3,41–43). It is non-invasive and constitutes a convenient administration procedure with good patient compliance. However, there are some limitations related to oral intake of drug molecules. As mentioned previously, the destruction or inactivation of drugs, especially proteins and peptides, due to enzymatic degradation and acidity of the gastrointestinal tract, is the major limitation of this administration route. Furthermore, the intestinal epithelium may form a barrier, inhibiting the uptake of large molecules (3). An opportunity to overcome this problem lies in the reformulation of these oral drugs as DDS. Thus, this may offer protection from degradation and inactivation of the drug molecules to be delivered.

Various approaches based on the use of protective coatings, targeted delivery, permeation enhancers, protease inhibitors and bioadhesive agents have been extensively investigated (3,44–46). These methods have shown to increase the bioavailability of these drug molecules upon oral administration. Nano- and microparticulate DDS have been developed for oral administration of bioactive agents. Microfabricated devices have been designed with different shapes, sizes and surface morphologies by using a variety of materials (3,47), maximizing the contact area with the intestinal epithelium.

These DDS present side effects, associated with the release kinetics (peaks) of the drugs. Therefore, strategies to improve drug delivery profiles have been investigated (12). Controlled release systems based on osmotic delivery, or push-pull systems, were successfully used to reformulate nifedipine-based products (48,49). Extended bioavailability, leading to an optimized blood pressure control, with concomitant reduced side effects, was observed. Similarly, oxybutynin chloride and methylphenidate have been successfully converted into osmotic drug delivery system (50). Moreover, this technology allows for the delivery of hydrophobic substances and drugs with low permeability.

In addition to osmotic technology, multilayer matrices incorporating the drug in the matrix core (51,52) have been developed with different rates of swelling and biodegradation. This ensures controlling the rate of drug release through dissolution, diffusion and degradation of the matrix. The additional layers are regulating the diffusion of the drug(s) out of the device. This multilayer technology has been used to reformulate several immediate release formulations, such as diltiazem, paroxetine and diclofenac sodium (12).

Inhalation DDS

Inhalative drug delivery utilizes the huge surface area of the lungs to improve absorption. Inhalation represents an easy, needle-free and comfortable mode of drug administration through the respiratory tract. Inhalative products can be administered via the nasal or oral route. One of the oldest examples for this mode of drug administration is inhalative anesthesia, in which both nose and mouth are used when an anesthetic mask is applied to the face of the patient.

Nowadays, devices with dose counter and functional status indicator are already available in the market, especially for the treatment of asthma (13). The efficacy of the currently available nasal inhalers can be improved by using microspheres. Nano- and microspheres, developed for nasal administration for systemic delivery of drugs, generally use degradable starch, dextran, chitosan, microcrystalline cellulose, cellulose derivatives, and gelatin as a polymeric matrix (53). The mucoadhesive properties of these polymers are an important factor for their retention and, therefore, action in the nasal mucosa. Chitosan, which is a positively charged polymer with a strong mucoadhesive property, is frequently used in nasal application of drugs (53). In fact, chitosan microspheres have been used as nasal DDS for salbutamol administration. The effect showed a prolonged controlled release of the drug (54). A more recent example on the use of this specific material for nasal DDS is the delivery of ondansetron hydrochloride (a drug used to treat renal dysfunction). *In vivo* studies in rats indicate that ondansetron hydrochloride-loaded chitosan microspheres were able to achieve a sustained drug level in the plasma. In addition, a significant increased drug absorption was observed using this system in comparison with the use of drug aqueous solutions (55).

The nasal mucosa presents, however, a physical and metabolic barrier for drug permeation. Polar drug molecules and additive compounds have a poor absorption when using the nasal route (3). To overcome this limitation, beside the use of mucoadhesive polymers, cyclodextrins have been used as molecular carriers with promising results. As cyclic oligosaccharides, cyclodextrins have the possibility of forming highly stable molecular inclusion complexes with a wide range of drug molecules both in solution or solid state (3,56,57). The molecule of interest occupies the cyclodextrin hydrophobic cavity. The hydrophilic exterior allows dissolution in water. Thus, the drug molecules are being protected from the environment, and the hydrophilicity of the entire system is enhanced (Table V). Further absorption enhancers, such as poly-L-arginine and lipids, are also under investigation (2,57). Besides salbutamol administration, clinical trials have been conducted for testosterone, insulin, morphine and interferon, among others (58–61).

Injectable DDS

Several efforts are being made to develop new needle-free DDS, manly due to the pain and fear felt by patients when subjected to frequent injections. On the other hand, drug

Table V Cyclodextrins: Molecular Carrier for Traditional Formulations and DDS

The utilization of cyclodextrins in drug formulation design is based on: i) enhanced solubility in water of poorly soluble drugs ii) stabilization of labile agents against biodegradation iii) taste modification by covering with flavors, masking unpleasant odors
In DDS, cyclodextrins can control the release of encapsulated drugs. For example, in inhalation drug delivery formulations, cyclodextrins are able to reduce or minimize the enzymatic activity of nasal mucosa. They can also largely improve the permeation of various lipophilic drugs, and the fraction that is not absorbed is easily removed by the nasal mucociliary clearance system.

Compiled from ref. (2,3,56,57,62)

toxicity, related to transiently high plasma concentrations caused by intravenous administration, needs to be avoided, especially during chronic treatments. For example, some chemotherapeutics, steroids or antibiotics are potent drugs that may evoke severe adverse effects. The toxicity of these drugs limits their dosing and hence their efficacy. However, despite these drawbacks, injections are an extraordinarily efficient way of delivering systemic drugs to the body. Therefore, it is desirable to develop systems that have a high efficiency and an accelerated onset in action while minimizing the need for repeated injections

One possibility is the use of injectable biodegradable materials that can act as drug delivery vehicles. They can maintain a sustained and controlled release of the associated drug, avoiding the need for repetitive injections. Examples of these materials are biodegradable hydrogels (62,63). Their benefits and limitations are summarized in Table VI.

Another possibility to overcome the use of needle-based administration in injectable systems is the use of novel auto-injectors (65,66). They offer the same benefits of needle-based delivery but with fewer disadvantages. They minimize the pain and discomfort associated with injections and can be used by a wide range of patients, as they are easy to use and require minimal training. In the past five years, companies like Oval Medical Technologies Limited (UK) have been investing towards auto-injector development (66). This company is currently involved in improving the existing auto-injector products. Other companies have also developed and brought to the pharmaceutical market several products using this technology. One well-known auto-injector product is, for instance, the EpiPen (0.3 mg epinephrine) injector, successfully use for the treatment of acute allergic reactions (67). Others are the Rebiject and Rebiject II (interferon beta-1a) injectors used to treat multiple sclerosis (68) or SureClick^{TM} auto-injector (available for both drugs Aranesp for anemia and Enbrel for rheumatoid arthritis) (69).

NANOTECHNOLOGY IN DRUG DELIVERY

Nanotechnology has provided new ways for developing innovative and highly efficient DDS with great potential in medicine. Nano-delivery systems offer improved bioavailability, controlled and sustained release of drugs and lower systemic toxicity. In this section, we review nano DDS: drug

Table VI Hydrogels: Benefits and Limitations for Their Application in Drug Delivery

Hydrogels: highly hydrated, three-dimensional, cross-linked networks of polymers that are often processed under relatively mild condition and allow the encapsulation of labile drugs

Benefits	Limitations
Optimal pharmacokinetics: drugs can elute slowly, maintaining high local concentration in the surrounding tissues for an extended period	Low tensile strength
Highly biocompatible, promoted by the high water content which resembles the hydrated environment of native extracellular matrix	The quantity and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs
Biodegradability and/or matrix dissolution via enzymatic, hydrolytic and environmental means	Rapid drug release due to high water content and porosity
Smart matrices responding to physiological stimuli	Some hydrogels are not injectable and may need surgical implantation
Relatively deformable, allowing easy adjustments to the shape of the surface where they are applied	
Can be designed with muco/bioadhesive properties	

Compiled from ref. (62–64)

nanosuspensions and drug encapsulated into polymeric nanoparticles.

Particle Engineering: Design of Pharmaceutical Solids with Desired Chemical and Physical Properties

The term nanosuspension is related to colloidal suspension of pure, solid-state drug particles, frequently stabilized by surfactants (70). The sizes of these particle suspensions are below the micron range. They are based on the formulation of some drug candidates (mainly water insoluble) into a crystalline nanosized particle suspension. In such formulations, drugs or active principles are maintained in a preferred crystalline (solid) state. Thus, the suspensions are formed by building particles from the molecular state (i.e. precipitation) or by breaking larger particles of compounds down to the nanosize (70).

At this point, it is important to highlight the difference between nanosuspensions and nanoparticles. While nanoparticles are polymeric colloidal carriers of drugs, nanosuspensions are nanosized particle of drugs (without carrier polymeric material) stabilized by surfactants. A nanosuspension is intended to overcome the solubility problem of a large number of water-insoluble drug candidates. In such cases, the need to dissolve these drugs is overcome by maintaining the drug in a solid, crystalline state at very small size.

Advantages of these formulations include increased bioavailability, due to their small size and increased surface area, the possibility of targeting (e.g. cells) because of their particulate shape, and higher mass of drug per volume loading (70). The latter is clearly advantageous, especially when high doses are needed. In addition, a related benefit for high loading is the use of a reduced administration volume, particularly useful in intramuscular or ophthalmic applications (70).

Currently, nanosuspensions are being used for the sustained delivery of a variety of active principles via diverse administration routes. When used as components of oral formulations, nanosuspensions can overcome the main limitation of bioavailability described for the oral administration route. Decreased size and increased surface area enhance the concentration of the released drug. Furthermore, mucosa-adhesion is also improved, which can accelerate the transition to the systemic circulation over the gastrointestinal wall (70–72). As a result, bioavailability of the medication improves. Animal studies indicated that an improved pharmacokinetic profile (i.e. optimal drug concentration in plasma for a prolonged time period) and bioavailability have been obtained by the use of a nanosuspension formulation of danazol (72). Additionally, this system can reduce the gastric irritation associated with oral intake. Experiments in rats showed that reducing naproxen particle size, from micro- to nanometers scale, resulted in faster absorption, decreased gastric residency time and produced locally high and prolonged concentrations of the drug (73).

Injectable nanosuspensions provide the possibility of administering hydrophobic drugs without using solvents or additives. Generally, the use of nanosuspensions as injectable solutions can reduce the toxicity and adverse effects usually related to the high concentration of drugs administered by this route (70).

As the main component of inhalation systems, nanoparticulated drugs not only improve bioavailability and drug absorption, but are also beneficial with respect to homogeneity. Moreover, a significantly higher fraction can be administrated in each dose, lowering the need for systemic uptake. Clinical trials with budesonide in nanoparticle suspension have showed a two-fold increased drug concentration in plasma for longer periods of time and increased drug absorption, compared to currently commercialized inhalation products (74).

As mentioned before, nanosuspensions were initially developed to solve limitations related to poor drug solubility. To date, they have been proven to be very efficient to optimize pharmacokinetics, drug bioavailability, safety and efficacy in all types of DDS and for different administration routes (70,72). However, some limitations are also associated with these nanosuspension systems. The protection of sensitive drug molecules, like proteins, growth factors and peptides, cannot be achieved due to the lack of carrier material to entrap or encapsulate the active compound. In this case, the combination of this technology and encapsulating materials can result in nanosized devices for drug delivery (polymeric nanoparticles) (2,3,14).

Polymeric Nanoparticles as Carriers for Drug Molecules

Polymeric nanoparticles have been extensively investigated as drug carriers. They have been designed to augment drug concentrations in blood or tissues and aim to reduce a drug's toxicity and to improve its therapeutic effects. These nanoparticles are characterized by sizes ranging from 1 to 100 nm and exhibit unique physical and chemical properties (75). They consist of a polymeric matrix, usually formed by a biodegradable and biocompatible polymer, and a bioactive molecule. This bioactive molecule can be either entrapped within or immobilized onto the polymeric matrix (Fig. 3). Therefore, the drug loading can be achieved by entrapment of the drug molecules using the polymer to form nanostructures like nanoparticles or nanocapsules, or by chemically linking the drug molecule to the surface of the polymeric nanostructure that has been previously functionalized (Fig. 3A) (75).

Using the entrapment approach, the drug is delivered mainly by either diffusion or carrier degradation. When the drug release is primarily controlled by diffusion, as soon as the polymeric nanoparticles come into contact

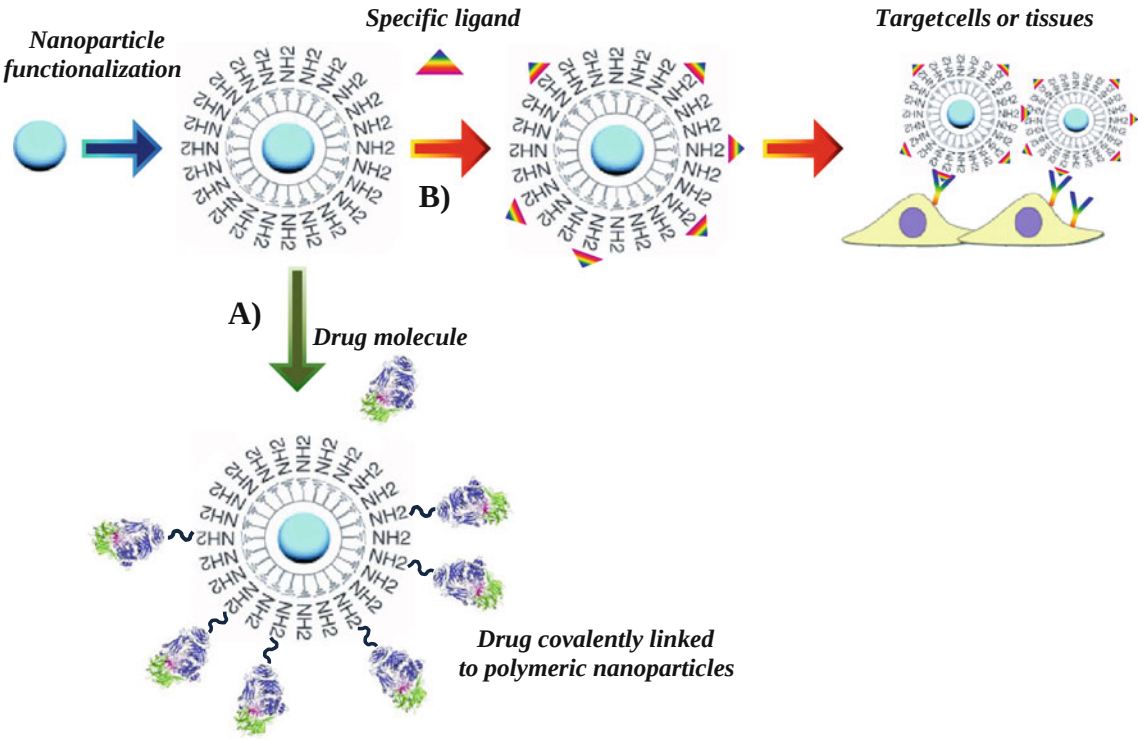

Fig. 3 Nanoparticle functionalization with chemical groups for binding to drug molecules (**A**) or to ligands that specifically interact with target cells or tissues (**B**).

with the external aqueous environment, the nanoparticles swell, allowing the diffusion of the bioactive drug into the external environment. On the other hand, when degradation of the carrier is the controlling factor, the polymeric nanoparticle will start to degrade as a result of its contact with the external environment, allowing a gradual and controlled release of the entrapped drug. In fact, the degradation process will affect and ultimately control the rate of drug release from the biodegradable nanocarrier. A schematic representation of the release of entrapped drugs as a result of diffusion or degradation mechanism is illustrated in Fig. 1A and B.

Examples of clinical use of nanoparticles are mainly related to applications for cancer treatment. Numerous nanoparticulate systems have been evaluated preclinically and clinically for the treatment of diverse malignancies (76,77). The most intensively studied formulations are related to paclitaxel delivery using nano DDS. This drug has been encapsulated in PLGA nanoparticles, resulting in higher and prolonged drug levels above the effective concentration *in vivo* (78). Cisplatin, another anticancer agent, has been satisfactorily loaded into poly(lactic-co-glycolic acid)-methoxy-polyethylene glycol (PLGA-m-PEG) co-polymer. *In vitro* studies confirm that cisplatin-loaded nanoparticles effectively target prostate cancer cells (79). Another interesting variation of this entrapment approach is the use of stimulus-responsive materials as a component of the nanoparticles. Stimuli-responsive polymers show a sudden change in properties upon a change in environmental condition, e.g. temperature, light, salt concentration or pH. Based on this behaviour, "smart" drug delivery systems can be developed, in which the drug can be released (on demand) in response to local environmental signals or externally applied cues (Fig. 1C). pH and temperature-responsive materials are currently under investigation for the development of novel DDS (80–84).

Moreover, nanoparticles can be previously functionalized with chemical groups on their surfaces to allow the binding of drug molecules to the polymer (Fig. 3). These chemical bonds can be subsequently cleaved *in vivo* (e.g. hydrolysis, enzyme cleavage, pH change). Surface modification approaches have been also used to prolong the presence of nanoparticles in the circulation by inhibiting recognition and phagocytosis by the mononuclear system (85).

The bioavailability of drugs in certain tissues is limited by a barrier to entry in those tissues (e.g. cartilage is an avascular tissue with dense ECM) as well as their rapid clearance. To overcome the limitations of bioavailability of therapeutic molecules in these situations, drug delivery faces two critical challenges: the size of the delivery system and its retention in the target tissue. It has been recently reported that only 1 of 100,000 molecules of intravenous therapeutic drugs reaches its desired site of action (1). As a result, drug dosages must be increased, leading to adverse

side effects. The availability of a drug delivery system which can be made sufficiently small to enter very dense tissues (e.g. tumors) is therefore broadly useful. Ultrasmall polymeric particles can be created using different self-assembling and nanofabrication approaches (86,87). These nanoparticles can be further modified with targeting ligands to bind cells or tissues (Fig. 3B), thus allowing their retention and avoiding clearance from the tissue site. Ligands can be incorporated into the nanostructures either by direct covalent binding to the polymeric surface or through the use of inert spacer groups (75). This possibility for specifically targeting cells and tissues with nanoparticles has been extensively investigated in cancer research. For instance, RNA A10 molecules specifically bind prostate membrane antigens. They have been successfully conjugated with polylactic acid-polyethylene glycol (PLA-PEG) co-polymers. As a result, increased drug delivery to prostatic tumor cells was observed compared to non-targeting nanoparticles (88).

Although polymeric nanoparticles have demonstrated great potential for future medicine development, they still face important limitations. Problems like aggregation during processing and storage, as well as poor formulation stability, are major challenges of this field today. In addition, in the dry form, e.g. tablets or lyophilized powder, nanoparticles often encounter size changes and/or stabilizer desorption. Typically, once the formulations are processed to these dry forms, their resuspension may lead to the loss of some of their important properties (89). Nevertheless, the constant advances in nanoparticle technology will allow improvement of the current systems and will facilitate their rapid application as clinical formulations.

DRUG DELIVERY TECHNOLOGY FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

When injured or inflicted by a chronic disease, the human body does not always show capacity to heal. Diseases like diabetes, cancer, Parkinson's, Alzheimer's, heart failure or lung failure show increasing incidence, and treatment options are limited. In addition, trauma-related injuries, like bone loss or cartilage damage, have traditionally been a complex surgical problem with unsatisfactory outcome for the patients.

Regenerative medicine offers unique opportunities for developing new therapeutic approaches to treat these chronic diseases as well as injuries. Based on the use of cells and/or growth factors, regenerative medicine has been defined as a new branch of medicine that attempts to change the course of diseases by the regeneration of damaged organs or tissues (90). Regenerative medicine strategies include the direct application of stem or progenitor cells (cell therapy) or the use of growth factors to instruct cells to regrow tissues. For both of these approaches, it is often beneficial to combine cells or growth factors with three-dimensional materials. The encapsulation of therapeutic cells within a biomaterial capsule prior to their application, so-called cell therapy, has several advantages. On the other hand, when tissue regeneration is achieved by the administration of growth factors to the cells, it is critical to control the amount of growth factor provided to the cells together with the maintenance of their biological activity. Therefore, it is often required to use drug delivery matrices like scaffolds, particles or fibres in which the growth factor can be encapsulated.

Growth Factor Delivery

Growth factors are naturally occurring proteins capable of stimulating cellular proliferation, migration and/or differentiation into a specialized phenotype (Fig. 4). Thus, being involved in the regulation of several cellular functions, they can enhance the healing and regeneration processes of diverse tissues (91,92). Nowadays, numerous growth factors are being identified, some of them produced by recombinant technology (93). For instance, in the field of bone regeneration, more than 40 bone morphogenetic proteins (BMPs) have been identified to date. However, only BMP-2

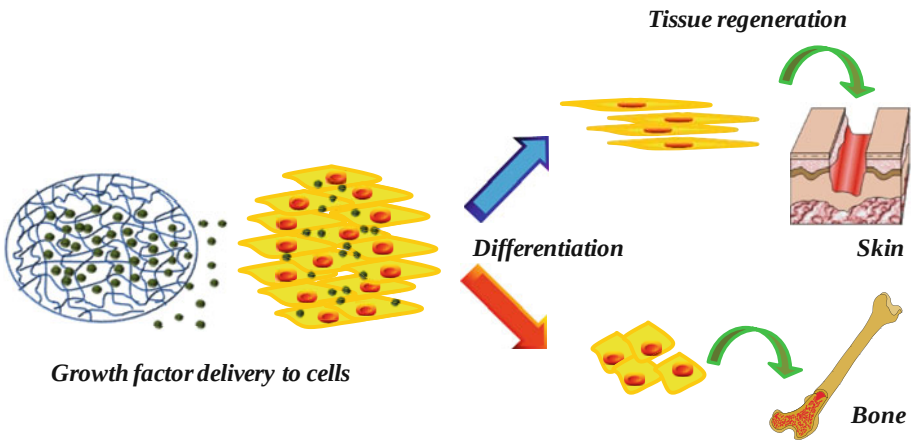

Fig. 4 Growth factor release based on a biomaterial approach to deliver signals to cells towards their differentiation for applications in tissue regeneration.

and 7 have been developed further for clinical trials. The reason why only few growth factors have been approved for clinical use in humans is concerned with the limitations of the existing recombinant technologies to obtain large amounts of purified growth factors together with the higher costs associated with their production (94). This is a major disadvantage considering that large quantities (about 400-1000X the physiological concentration) of those proteins are usually needed to obtain a significant effect. Another important concern is related with safety issues, namely in using molecules in human beings which are produced in bacteria and can carry potential toxins (94,95). Moreover, the effectiveness of some growth factors in the healing of chronic wounds has not been fully demonstrated (94). Due to the limited half-lives of many of these proteins *in vivo*, they are difficult to administer to sites of damaged tissue at therapeutic concentrations and for sustained periods of time. We believe that the way these molecules should be delivered to the injury site plays a crucial role for their success as therapeutic agents.

Several administration methods have been used, some of them relying on the direct application of the growth factors (without the use of a DDS), but this method shows clear limitations. Infusion of growth factors into the systemic circulation or direct injection to the injured tissue has failed in numberous occasions. The lack of protection against biodegradation and the subsequent low local bioavailability are some of the major disadvantages related to the administration of growth factors by the traditional means. To overcome these limitations, several technologies have been explored to achieve a better control over the growth factor release. These technologies use biomaterials in the form of fibers, capsules and particles, three-dimensional porous scaffolds and injectable gels, which can be obtained by a variety of fabrication techniques.

Fibrin hydrogels releasing IGF-I, for example, have been investigated to repair articular cartilage defects in an animal model (96). Similarly, an alginate hydrogel carrying VEGF has been applied for treating ischemic disease in mice (97). Moreover, in the same model, a mixture of VEGF and PDGF loaded in a polymeric scaffold has been investigated (98). Nano- and microparticles made of poly (glycolic) and poly(lactic) acid (99), silk fibroin (6), and gelatin (100,101) have also been extensively investigated for the loading and release of growth factors.

Polymers as carriers for growth factor delivery allow a localized and controlled release to yield a desirable concentration over a certain period of time. The release profile can be optimized according to the regeneration process by tailoring the chemical properties of the polymeric matrix and/or the physical features of the carrier system (e.g. carrier size, porosity, pore size, pore distribution, surface area) (102). Moreover, the growth factor release can also be controlled by selecting adequate conjugation methods (encapsulation, adsorption, covalent binding).

There are, however, technical challenges for delivering growth factors using polymeric carriers. The use of aggressive processing techniques (e.g. organic solvents, high temperatures, freeze and towing cycles) during growth factor loading and short release periods (i.e. insufficient for injury healing) have been indicated as potential problems for the unsuccessful use of DDS for growth factor release in *in vivo* studies and clinical trials.

Based on those limitations, other technologies have been investigated for delivering growth factors in different parts of the human body. Cell and gene therapy are examples of alternative technologies. These investigations will lead to a future development where growth factors will be expressed/delivered at the site of interest and only at the levels and time at which they are required.

Cell Encapsulation for Drug Delivery

As previously described, there are still limitations with currently available DDS to deliver peptides and proteins. To circumvent some of the mentioned limitations, alternative approaches have been investigated for delivering these molecules to target sites. Cells may be considered as "biological factories." They can continuously produce and release therapeutic molecules. In cell-based therapies, there is the challenge of retaining the transplanted cells in the target tissue. Delivery could be performed by cell encapsulation or immobilization within a semipermeable membrane (103). This will ensure their protection against the immune system and will diminish mechanical stress. The membrane should allow diffusion of nutrients and oxygen towards the cells and waste products as well as the therapeutic agent in the opposite direction (Fig. 5).

In this manner, the proteins of interest are synthesized locally by cells and are presented to the microenvironment in a natural fashion. Furthermore, recombinant proteins produced by overexpression in bacteria may have altered activity, since post-translational modifications that normally take place during synthesis in mammalian cells are absent. Additional advantages of cell encapsulation technology as DDS include (103–106) no need for immunosuppression; biocompatibility of the materials that are used for the capsule formation that do not interfere with cellular function or exert toxicity, wherein the permeability of the capsule membrane can be tailored to obtain optimal conditions for cell encapsulation; therapeutic products can be continuously released in a sustained and controlled way for long time periods; bioactivity of the therapeutic agent can be guaranteed since they are produced by the cells; cells can easily be genetically modified to express the

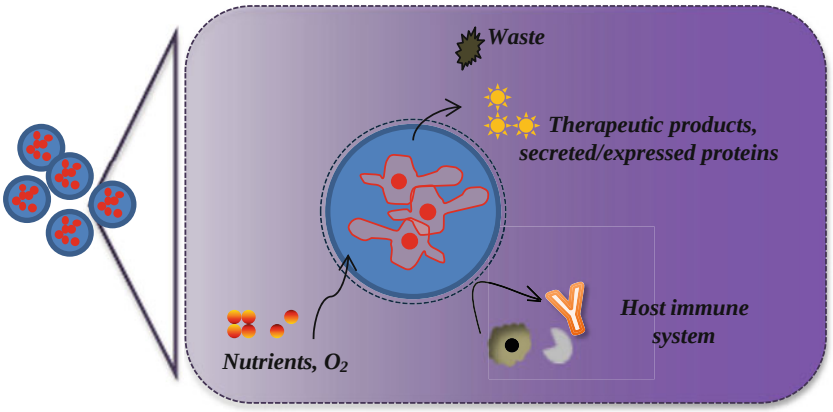

Fig. 5 Function of the semipermeable membrane in cell encapsulation technology (adapted from ref (103)).

desired protein for therapy; no safety concerns, as the capsule degrades the foreign cells will be eliminated immediately by the immune system; and typical toxicity due to high drug concentrations does not occur.

Capsules of small sizes can be developed and therefore easily administrated into the blood circulation. Furthermore, the higher surface-to-volume of capsules compared to other cell delivery systems with other shapes (e.g. fibres, scaffolds) allows for a good transfer of oxygen and nutrients into the capsules, ensuring high cell viability (107). The optimum capsule size is 100–500 μm (106), which can be easily obtained by established processing methods. The microencapsulation system based on alginates has been widely described. This is due to the unique property of alginates to form hydrogels when they react with multivalent cations. Divalent cations, such as calcium, barium, and strontium, cooperatively bind alginate chains, creating interchain bridges which cause gelling of the aqueous alginate solutions (106). Additionally, the so-called core-shell capsules can entrap cells in the free empty core surrounded by a semipermeable membrane. Usually, the diffusion is controlled by size-exclusion phenomena and diffusion rate. The permeability, composition and configuration of the membrane can be varied using different types of materials, which allows for extensive variations in the membrane properties.

Several materials can be used for cell encapsulation. In addition to alginates and combinations with chitosan (108) or agarose (109), biomaterials like collagen (110), hyaluronic acid (111) and dextran (112) have also been used. For these applications, the materials should have the ability to form a gel in physiological conditions (temperature, pH and ionic strength). These can be accomplished by ionic and/or polyelectrolyte complexation, self-assembling processes. In addition, they need to be biocompatible and should not interfere with cellular function. A key challenge in cell encapsulation is the availability of human cells (allogeneic cells). The use of xenogeneic cells from non-human sources has been proposed, because the polymeric membrane can exclude leukocytes and antibodies, resulting in protection from the immune response. In fact, a wide range of cells and cell sources have been described in the literature in cell encapsulation studies. For example, genetically modified cells expressing desired proteins, stem cells or specific cell types with therapeutic action for specific diseases have been encapsulated (Table VII). Pancreatic islet cells microencapsulated in alginate beads have been investigated in animal studies to treat diabetes (113,114) as well as in pilot clinical trials (115,116).

In another example, choroid plexus cells encapsulated in an alginate-based system were shown to release neurotrophic factors in the brain in a primate model of Huntington's disease to prevent degeneration of neurons (121). Similarly, entrapped bone marrow stromal cells encapsulated in poly-lactic glycolic acid capsules have shown enhanced bone regeneration in an animal model (122).

Although the use of encapsulated cells for drug delivery is relatively straightforward, and there are some routine clinical applications of such cells, research indicates that both xenografts and allografts might provoke an inflammatory cell response. An inflammatory response could be problematic in the long term, and in these cases a membrane with very limited permeability is required. These are major challenges that need to be resolved before moving to large-scale clinical trials. Key requirements include reliable and safe sources of cells, biocompatible and stable membranes with suitable molecular cut-off to prevent immune rejection, reproducibility of the product and long-term survival of encapsulated cells. In addition, the use of xenografts or genetically engineered cells raises additional ethical, political and regulatory questions that need to be resolved (123).

Gene Therapy

Techniques for transferring therapeutic vectors, encoding the necessary gene products to cells, for sustained local expression of therapeutic molecules are of great interest in regenerative medicine. In this case, a higher and localized

Table VII Cell Encapsulation Systems Recently Employed

Factors to consider in cell encapsulation: Allogenic *vs*. Xenogenic Source, Controlled Cell Proliferation Once Encapsulated, Possibility for Genetic Modification.

Developed systems		Application
Alginate	—Kidney cells	Hemophilia, neurotrophic factors
	—Parathyroid cells	Artificial organs
	—Chondrocytes	Bone-cartilage regeneration
	—Leydig cells	Hormone replacement
	—Adrenal chromatin cells	e.g. Parkinson's disease
	—Stem cells	e.g. bone regeneration
	—Myeloma cells	Hepatic growth factor release
Alginate/HEMA/MMA	—Fibroblast	Epilepsy, metabolic deficiencies
	—Myoblast	Cancer, metabolic deficiencies
	—Ovary cells	Fabry disease
	—Hepatocytes	Liver related diseases and transplantation
	—PC12 pheochromocytoma cells	Neurotrophic and neurotransmitters factors
Alginate/Chitosan	—Tumor cells	Cancer vaccine, interleukins
Chitosan	—Fibroblast —Chondrocytes	*In vitro* study: tissue engineering
Collagen	—Myoblast	*In vitro* study
Hyaluronic acid	—Chondrocytes	*In vitro* study: cartilage formation
Dextran/RGD	—Human embryonic stem cells	*In vitro* study: vascular differentiation
Agarose	—Murine embryonic stem cells and kidney cells	*In vitro* study: tissue engineering
Agarose/Gelatin	—Feline kidney cells	*In vitro* study: Increase in metabolic activity

Compiled from ref. (103–106,110–112,117–120)

expression of therapeutic molecules is the main objective. Gene therapy is clearly an example of a new emerging technology that facilitates the delivery of growth factors. High and sustained levels of growth factors at the site of injury cannot be achieved by any known means of protein administration. In the case of molecules that function completely intracellularly, they cannot be delivered in soluble form, and gene transfer might be the only way to harness these factors for repair (124). Delivery of growth factors by gene transduction would be a less invasive and more persistent means of supplying growth factors. Several studies have shown that exogenous cDNAs encoding growth factors can be delivered locally to sites of tissue damage, where they are expressed at therapeutically relevant levels. The use of gene-transfer techniques to facilitate musculoskeletal tissue repair offers perhaps an immediate opportunity for a clinical application of gene therapy, as it may only require transient, localized expression of a specific transgene product. Alternatively, delivery and expression of cDNAs encoding specific extracellular matrix (ECM) components may also be used to support production and maintenance of the proper tissue matrix when damaged.

The gene encoding the desired growth factor can be transferred into cells by the use of viral or non-viral vectors (Fig. 6). The transfected cells will subsequently either secrete the desired protein (Fig. 6A) or differentiate into a desired phenotype (Fig. 6B).

Local gene delivery can be achieved by either *in vivo* or *ex vivo* approaches (125–128). Basically, the *in vivo* approach (126) consists of administering directly the vector into the injured tissue. However, serious safety concerns exist for a viral approach due to lack of control of the virus. As many gene products can have detrimental side effects, if over-expressed in non-target organs such as the heart, lung or kidney, the characterization of the duration of expression *in vivo* and the biodistribution of vector and/or genetically modified cells following delivery is critical. On the other hand, the *ex vivo* strategy (125) provides more control over

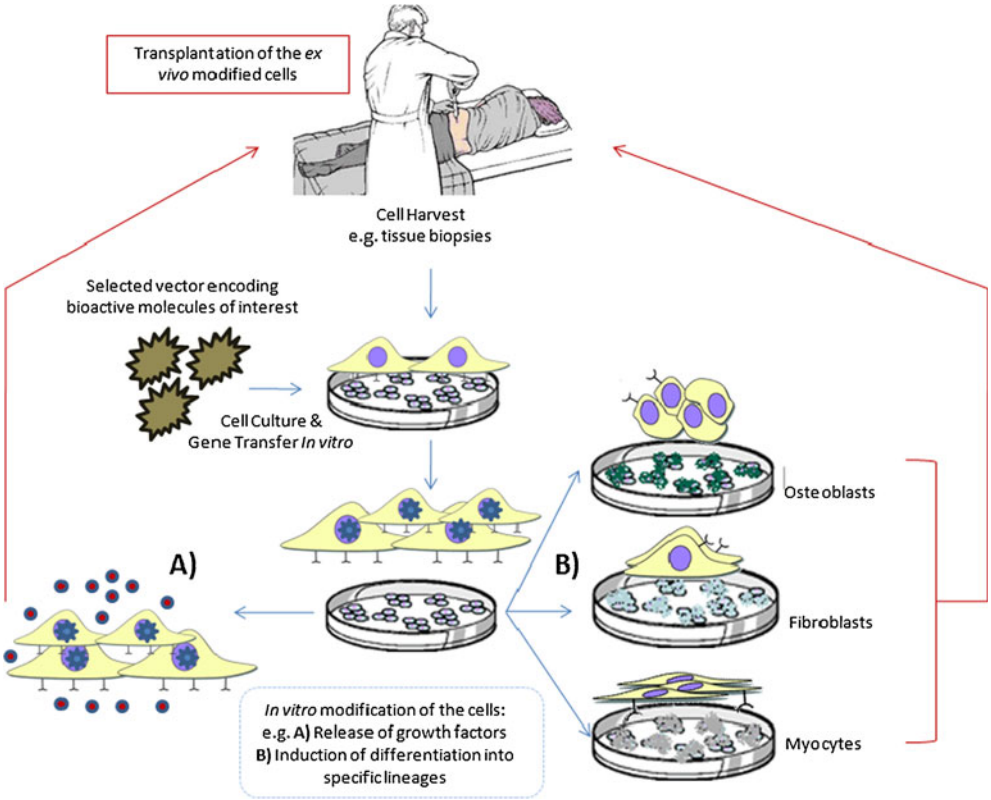

Fig. 6 Representation of an autologous *ex vivo* gene therapy. The cells are isolated, grown and transfected *in vitro* in a controllable fashion before re-administration into the injured site. Two examples are represented: the transfected cells can release therapeutic molecules of interest (**A**) or can be induced to differentiate into different phenotypes for tissue regeneration (**B**).

each step. This strategy is based on the *in vitro* genetic modification of cells (which can be autologous cells), where the growth and transfection of the cells can be carefully controlled. Finally, the modified cells can be re-injected or transplanted into site of injury. Numerous advantages are claimed (125–128) for the *ex vivo* strategy: the patient's cells can be cryopreserved at any stage of the process to be used in subsequent therapies, and the concentration of the expressed protein can be regulated by controlling the amount of vector during transfection, thereby diminishing systemic adverse effects or toxicity.

Several studies on the use of gene therapy have recently been reported, including studies on gene therapy to treat urological dysfunction (125), bone and cartilage injuries (126–130) and cardiovascular dysfunction (131). An adenovirus expressing BMP-2 has been used to induce bone formation (132). A polyhedron promoter of baculovirus encoding BMP-2 loaded onto collagen sponges has induced ectopic bone formation in rats after four weeks (133). Inducible nitric oxide synthase adenovirus transduced cells were more effective than plasmid or adenoviral solutions for the treatment of erectile dysfunction when injected to the corpus cavernosum of adult rats (134).

Ex vivo approaches are generally more invasive, expensive and technically tedious. However, they permit control of the transduced cells and safety testing prior to transplantation. *In vivo* approaches are simpler, cheaper and less invasive, but viruses are introduced directly into the body, which poses safety risks.

Despite the fact that a significant portion of gene therapy research is being conducted for bone (127,129) and cartilage regeneration (135–137), only a few have been tested in clinical trials for treating human joint diseases. Viruses such as retrovirus, adenovirus and lentivirus are still the preferred vectors due to their potential efficiency (126,128,138). However, they present safety concerns related to their intrinsic cytotoxicity, immunogenicity and possible mutagenesis that prevent them from being transferred to clinical applications. This is the main limitation of this field. Major concerns arise within the scientific community today on the biosafety of this technology. Therefore, numerous efforts are still required to increase the transfection efficiency of non-viral vectors. When non-viral vectors will be fully implemented in gene therapy, one may expect that gene therapy can be acceptable for human use. The potential of this technology for clinical use strongly depends on the use of safe and efficient vectors, transgenes and delivery systems.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This field of DDS has evolved from the simple delivery of pharmaceuticals to the local delivery cells, anticancer drugs,

growth factors and therapeutic genes. DDS has become, therefore, a powerful tool in healthcare.

Currently, transdermal DDS are the most employed drug delivery products, and skin seems to remain an excellent route for drug delivery. They have shown a good combination of safety and efficacy in *in vitro*, *in vivo* and human studies, but constant improvements of the current systems are being observed. DDS for oral, inhalative, and injectable applications will need additional efforts to increase reproducibility, control of drug concentrations and release profiles. The possibility of overcoming their limitations and successfully entering human clinical trials seems to be close to reality.

Major advances have been observed over recent years in the development of nano-delivery systems for applications in regenerative therapies. Progress in polymer chemistry has also made available new and modified materials that allow the bottom-up fabrication of nano-delivery systems with sophisticated properties. Furthermore, the functionalization of the nano-delivery systems, for selective and target delivery, is currently a very exciting promise to deliver anticancer therapies, and this technology is expected to have a major impact in cancer treatment.

New challenges for the future, besides the improvement of known DDS, will be the delivery of therapeutic entities using "biological devices." Cells are being recognized as a source for potential delivery of therapeutics. Cell and gene therapy concepts have already been introduced into the clinical arena. Although they are not yet established as approved therapeutic techniques, remarkable results have been obtained, mainly in the field of bone and cartilage regeneration. At the experimental level, many studies have been reported demonstrating the feasibility of these therapies for tissue healing. A fair amount of research will still be needed to successfully transfer cell- and gene-based technologies to the medical practice. The unavailability of safe vectors carrying therapeutic genes appears to be the main obstacle in these technologies. The optimization of the use of non-viral vectors will be of major importance in the future of cell- and gene-related therapies.

We believe that technology will improve the success of DDS to deliver new therapeutics and will accelerate the clinical realization of the many exciting potential applications.

ACKNOWLEDGMENTS

This work was supported through the European Union funded projects Marie Curie Host Fellowships for Early Stage Research Training (EST) "Alea Jacta EST" (MEST-CT-2004-008104), which provided E. R. Balmayor with a PhD fellowship, and the European Network of Excellence EXPERTISSUES (NMP3-CT-2004-500283).

REFERENCES

1. Berkowitz AC, Goddard DM. Novel drug delivery systems: future directions. J Neurosci Nurs. 2009;41:115–20.
2. Orive G, Hernández RM, Gascón AR, Domínguez-Gil A, Pedraz JL. Drug delivery in biotechnology: present and future. Curr Opin Biotechnol. 2003;14:659–64.
3. Paolino D, Fresta M, Sinha P, Ferrari M. Drug Delivery Systems. In: Webster JG, editor. Encyclopedia of medical devices and instrumentation. New York: Wiley; 2006. p. 437–95.
4. Balmayor ER, Tuzlakoglu K, Azevedo HS, Reis RL. Preparation and characterization of starch-poly-caprolactone microparticles incorporating bioactive agents for drug delivery and tissue engineering applications. Acta Biomater. 2009;5:1035–45.
5. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev. 2007;59:207–33.
6. Wenk E, Wandrey AJ, Merkle HP, Meinel L. Silk fibroin spheres as a platform for controlled drug delivery. J Control Release. 2008;132:26–34.
7. Ranade W. Drug delivery systems. Implants in drug delivery. J Clin Pharmacol. 1990;30:871–89.
8. Nitsch MJ, Banakar UV. Implantable drug delivery. J Biomater Appl. 1994;8:247–84.
9. Pioletti DP, Gauthier O, Stadelmann VA, Bujoli B, Guicheux J, Zambelli PY, *et al.* Orthopedic implant used as drug delivery system: clinical situation and state of the research. Curr Drug Deliv. 2008;5:59–63.
10. Parvizi J, Antoci V, Hickok NJ, Shapiro IM. Selfprotective smart orthopedic implants. Expert Rev Med Devices. 2007;4:55–64.
11. Langer R, Peppas NA. Advances in biomaterials, drug delivery, and bionanotechnology. AIChE J. 2003;49:2990–3006.
12. Rosen H, Abribat T. The rise and rise of drug delivery. Nat Rev Drug Discov. 2005;4:381–5.
13. Jain KK. Strategies and technologies for drug delivery systems. Trends Pharmacol Sci. 1998;19:155–7.
14. Orive G, Gascon AR, Hernandez RM, Dominguez-Gil A, Pedraz JL. Techniques: new approaches to the delivery of biopharmaceuticals. Trends Pharmacol Sci. 2004;25:382–7.
15. Arora A, Prausnitz MR, Mitragotri S. Micro-scale devices for transdermal drug delivery. Int J Pharm. 2008;364:227–36.
16. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26:1261–8.
17. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov. 2004;3:115–24.
18. Scheindlin S. Transdermal drug delivery: past, present, future. Mol Interv. 2004;4:308–12.
19. Vilivalam VD, Illum L, Iqbal K. Starch capsules: an alternative system for oral drug delivery. Pharm Sci Technolo Today. 2000;3:64–9.
20. Majuru S. Advances in the oral delivery of heparin from solid dosage forms using emisphere's eligen® oral drug delivery technology. Drug Deliv Technol. 2004;4:9–14.
21. Hosny EA, Al-Shora HI, Elmazar MM. Oral delivery of insulin from enteric-coated capsules containing sodium salicylate: effect on relative hypoglycemia of diabetic beagle dogs. Int J Pharm. 2002;237:71–6.
22. Rosenblatt JS, Berg RA. Collagen-based injectable drug delivery system and its use. 1998. US Patent Specification 5807581. United State
23. Bernstein G. Delivery of insulin to the buccal mucosa utilizing the RapidMist™ system. Expert Opin Drug Deliv. 2008;5:1047–55.

24. Rogueda P. Novel hydrofluoroalkane suspension formulations for respiratory drug delivery. Expert Opin Drug Deliv. 2005;2:625–38.
25. Yang JZ, Young AL, Chiang P-C, Thurston A, Pretzer DK. Fluticasone and budesonide nanosuspensions for pulmonary delivery: Preparation, characterization, and pharmacokinetic studies. J Pharm Sci. 2008;97:4869–78.
26. Engstrom JD, Tam JM, Miller MA, Williams RO, Johnston KP. Templated open flocs of nanorods for enhanced pulmonary delivery with pressurized metered dose inhalers. Pharm Res. 2008;26:101–17.
27. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev. 2004;56:603–18.
28. Smith EW, Maibach HI. In: Smith EW, Maibach HI, editors. Percutaneous penetration enhancers. Boca Raton: CRC Press. Taylor & Francis Group; 2006. p. 4–14.
29. Vaddi HK, Ho PC, Chan SY. Terpenes in propylene glycol as skin-penetration enhancers: Permeation and partition of haloperidol, fourier transform infrared spectroscopy, and differential scanning calorimetry. J Pharm Sci. 2002;91:1639–51.
30. Tang H, Blankschtein D, Langer R. Effects of low-frequency ultrasound on the transdermal permeation of mannitol: Comparative studies with *in vivo* and *in vitro* skin. J Pharm Sci. 2002;91:1776–94.
31. McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles for gene and drug delivery. Annu Rev Biomed Eng. 2000;2:289–313.
32. McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, Allen MG, *et al.* Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. Proc Natl Acad Sci USA. 2003;100:13755–60.
33. Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: a novel approach to transdermal drug delivery. J Pharm Sci. 1998;87:922–5.
34. Lin W, Cormier M, Samiee A, Griffin A, Johnson B, Teng CL, *et al.* Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux) technology. Pharm Res. 2001;18:1789–93.
35. Martanto W, Davis SP, Holiday NR, Wang J, Gill HS, Prausnitz MR. Transdermal delivery of insulin using microneedles *in vivo*. Pharm Res. 2004;21:947–52.
36. Cormier M, Daddona PE. Macroflux technology for transdermal delivery of therapeutic proteins and vaccines. In: Rathbone MJ, Hadgraft J, Roberts MS, editors. Modified-release drug delivery technology. New York: Marcel Dekker, Inc.; 2003. p. 589–98.
37. Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, Allen MG, *et al.* Lack of pain associated with microfabricated microneedles. Anesth Analg. 2001;92:502–4.
38. Laurent PE, Bonnet S, Alchas P, Regolini P, Mikszta JA, Pettis R, *et al.* Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. Vaccine. 2007;25:8833–42.
39. Wermeling DP, Banks SL, Hudson DA, Gill HS, Gupta J, Prausnitz MR, *et al.* Microneedles permit transdermal delivery of a skin-impermeant medication to humans. Proc Natl Acad Sci USA. 2008;105:2058–63.
40. Dean CH, Alarcon JB, Waterston AM, Draper K, Early R, Guirakhoo F, *et al.* Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax)-JE) in non-human primates. Hum Vaccin. 2005; 1:106–11.
41. Mutwiri G, Bowersock TL, Babiuk LA. Microparticles for oral delivery of vaccines. Expert Opin Drug Deliv. 2005;2:791–806.
42. Lavelle EC, O'Hagan DT. Delivery systems and adjuvants for oral vaccines. Expert Opin Drug Deliv. 2006;3:747–62.
43. Maroni A, Zema L, Cerea M, Sangalli ME. Oral pulsatile drug delivery systems. Expert Opin Drug Deliv. 2005;2:855–71.
44. Rhodes CT, Porter SC. Coatings for controlled release drug delivery systems. Drug Dev Ind Pharm. 1998;24:1139–54.
45. Lambkin I, Pinilla C. Targeting approaches to oral drug delivery. Expert Opin Biol Ther. 2002;2:67–73.
46. Peppas NA, Robinson JR. Bioadhesives for optimization of drug delivery. J Drug Target. 1995;3:183–4.
47. Simone EA, Dziubla TD, Muzykantov VR. Polymeric carriers: role of geometry in drug delivery. Expert Opin Drug Deliv. 2008;5:1283–300.
48. Anderson RU, Mobley D, Blank B, Saltzstein D, Susset J, Brown JS. Once daily controlled *versus* immediate release oxybutynin chloride for urge urinary incontinence. OROS Oxybutynin Study Group. J Urol. 1999;161:1809–12.
49. Versi E, Appell R, Mobley D, Patton W, Saltzstein D. Dry mouth with conventional and controlled-release oxybutynin in urinary incontinence. The Ditropan XL Study Group. Obstet Gynecol. 2000;95:718–21.
50. Swanson J, Gupta S, Lam A, Shoulson I, Lerner M, Modi N, *et al.* Development of a new once-a-day formulation of methylphenidate for the treatment of attention-deficit/hyperactivity disorder: proof-of-concept and proof-of-product studies. Arch Gen Psychiatry. 2003;60:204–11.
51. Conte U, Maggi L, Colombo P, Lamanna A. Multilayered hydrophilic matrices as constant release devices (Geomatrix(Tm) Systems). J Control Release. 1993;26:39–47.
52. Conte U, Maggi L. Modulation of the dissolution profiles from Geomatrix multi-layer matrix tablets containing drugs of different solubility. Biomaterials. 1996;17:889–96.
53. Ozsoy Y, Gungor S, Cevher E. Nasal delivery of high molecular weight drugs. Molecules. 2009;14:3754–79.
54. Jain SK, Chourasia MK, Jain AK, Jain RK, Shrivastava AK. Development and characterization of mucoadhesive microspheres bearing salbutamol for nasal delivery. Drug Deliv. 2004;11:113–22.
55. Gungor S, Okyar A, Erturk-Toker S, Baktir G, Ozsoy Y. Ondansetron-loaded chitosan microspheres for nasal antiemetic drug delivery: an alternative approach to oral and parenteral routes. Drug Dev Ind Pharm. 2010;36:806–13.
56. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci. 1996;85:1017–25.
57. Merkus FWHM, Verhoef JC, Marttin E, Romeijn SG, van der Kuy PHM, Hermens WAJJ, *et al.* Cyclodextrins in nasal drug delivery. Adv Drug Deliv Rev. 1999;36:41–57.
58. Hayes RP, Muchmore D, Schmitke J. Effect of inhaled insulin on patient-reported outcomes and treatment preference in patients with type 1 diabetes. Curr Med Res Opin. 2007;23:435–42.
59. Otulana B, Okikawa J, Linn L, Morishige R, Thipphawong J. Safety and pharmacokinetics of inhaled morphine delivered using the AERx system in patients with moderate-to-severe asthma. Int J Clin Pharmacol Ther Toxicol. 2004;42:456–62.
60. Davison S, Thipphawong J, Blanchard J, Liu K, Morishige R, Gonda I, *et al.* Pharmacokinetics and acute safety of inhaled testosterone in postmenopausal women. J Clin Pharmacol. 2005;45:177–84.
61. Jiang RG, Pan WS, Wang CL, Liu H. Use of recrystallized lactose as carrier for inhalation powder of interferon a2b. Pharmazie. 2005;60:632–3.
62. Hoare TR, Kohane DS. Hydrogels in drug delivery: progress and challenges. Polymers. 2008;49:1993–2007.
63. Malafaya PB, Silva GA, Baran ET, Reis RL. Drug delivery therapies II.: strategies for delivering bone regenerating factors. Curr Opin Solid State Mater Sci. 2002;6:297–312.
64. Lee KY, Mooney DJ. Hydrogels for tissue engineering. Chem Rev. 2001;101:1869–79.

65. Thompson I. Market trends: disposable mono-dose auto-injectors and pen-injectors. ONdrugDelivery. 2008;15–17.
66. Young M. The next generation of auto-injectors. ONdrugDelivery. 2010:4–7.
67. Dey-Pharma. EpiPen Autoinjector. www.dey.com; www.epipen.com (accessed December 2010).
68. EMD Serono Inc. and Pfizer Inc. Rebif. www.rebif.com (accessed December 2010).
69. Amgen and Pfizer Inc. SureClick. www.enbrel.com (accessed December 2010).
70. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov. 2004;3:785–96.
71. Jia L, Wong H, Cerna C, Weitman SD. Effect of nanonization on absorption of 301029: *ex vivo* and *in vivo* pharmacokinetic correlations determined by liquid chromatography/mass spectrometry. Pharm Res. 2002;19:1091–6.
72. Liversidge GG, Cundy KC. Particle-size reduction for improvement of oral bioavailability of hydrophobic drugs.1. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm. 1995;125:91–7.
73. Liversidge GG, Conzentino P. Drug particle-size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int J Pharm. 1995;125:309–13.
74. Kraft WK, Steiger B, Beussink D, Quiring JN, Fitzgerald N, Greenberg HE, *et al*. The pharmacokinetics of nebulized nanocrystal budesonide suspension in healthy volunteers. J Clin Pharmacol. 2004;44:67–72.
75. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci. 2009;30:592–9.
76. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov. 2003;2:347–60.
77. Qiu LY, Bae YH. Polymer architecture and drug delivery. Pharm Res. 2006;23:1–30.
78. Win KY, Feng SS. *In vitro* and *in vivo* studies on vitamin E TPGS-emulsified poly(D, L-lactic-co-glycolic acid) nanoparticles for paclitaxel formulation. Biomaterials. 2006;27:2285–91.
79. Gryparis EC, Hatziapostolou M, Papadimitriou E, Avgoustakis K. Anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP prostate cancer cells. Eur J Pharm Biopharm. 2007;67:1–8.
80. Bajpai AK, Shukla SK, Bhanu S, Kankane S. Responsive polymers in controlled drug delivery. Prog Polym Sci. 2008;33:1088–118.
81. Frutos G, Prior-Cabanillas A, París R, Quijada-Garrido I. A novel controlled drug delivery system based on pH-responsive hydrogels included in soft gelatin capsules. Acta Biomater. 2010;6:4650–6.
82. Guo B-L, Gao Q-Y. Preparation and properties of a pH/temperature-responsive carboxymethyl chitosan/poly(N-isopropylacrylamide)semi-IPN hydrogel for oral delivery of drugs. Carbohydr Res. 2007;342:2416–22.
83. Suedee R, Jantarat C, Lindner W, Viernstein H, Songkro S, Srichana T. Development of a pH-responsive drug delivery system for enantioselective-controlled delivery of racemic drugs. J Control Release. 2010;142:122–31.
84. Zhang K, Wu XYXY. Temperature and pH-responsive polymeric composite membranes for controlled delivery of proteins and peptides. Biomaterials. 2004;25:5281–91.
85. Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, Kawano T, *et al*. PEG-modified gold nanorods with a stealth character for *in vivo* applications. J Control Release. 2006;114:343–7.
86. Li H, Carter JD, LaBean TH. Nanofabrication by DNA self-assembly. Mater Today. 2009;12:24–32.
87. Ozin GA, Hou K, Lotsch BV, Cademartiri L, Puzzo DP, Scotognella F, *et al*. Nanofabrication by self-assembly. Mater Today. 2009;12:12–23.
88. Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Deliv Rev. 2008;60:1615–26.
89. Shah P. Use of nanotechnologies for drug delivery. MRS Bull. 2006;31:894–9.
90. Kaiser LR. The future of multihospital systems. Top Health Care Financ. 1992;18:32–45.
91. Anitua E, Sanchez M, Orive G, Andia I. Delivering growth factors for therapeutics. Trends Pharmacol Sci. 2008;29:37–41.
92. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83:835–70.
93. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). J Tissue Eng Regen Med. 2008;2:1–13.
94. Wound Biotechnology, Department of Dermatology, School of Medicine, Boston University. Growth factors: their development and testing. http://www.bu.edu/woundbiotech/growthfactors/gfdevtest.html (accessed January 2011).
95. Alaoui-Ismaili MH, Falb D. Design of second generation therapeutic recombinant bone morphogenetic proteins. Cytokine Growth Factor Rev. 2009;20:501–7.
96. Fortier LA, Mohammed HO, Lust G, Nixon AJ. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. J Bone Joint Surg. 2002;84:276–88.
97. Silva EA, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J Thromb Haemost. 2007;5:590–8.
98. Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm Res. 2007;24:258–64.
99. Meinel L, Zoidis E, Zapf J, Hassa P, Hottiger MO, Auer JA, *et al*. Localized insulin-like growth factor I delivery to enhance new bone formation. Bone. 2003;33:660–72.
100. Patel ZS, Young S, Tabata Y, Jansen JA, Wong ME, Mikos AG. Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. Bone. 2008;43:931–40.
101. Holland TA, Tabata Y, Mikos AG. *In vitro* release of transforming growth factor-beta 1 from gelatin microparticles encapsulated in biodegradable, injectable oligo(poly(ethylene glycol) fumarate) hydrogels. J Control Release. 2003;91:299–313.
102. Langer R, Tirrell DA. Designing materials for biology and medicine. Nature. 2004;428:487–92.
103. Orive G, Gascon AR, Hernandez RM, Igartua M, Pedraz JL. Cell microencapsulation technology for biomedical purposes: novel insights and challenges. Trends Pharmacol Sci. 2003;24:207–10.
104. Orive G, Hernandez RM, Gascon AR, Igartua M, Pedraz JL. Encapsulated cell technology: from research to market. Trends Biotechnol. 2002;20:382–7.
105. Orive G, Hernandez RM, Gascon AR, Calafiore R, Chang TMS, de Vos P, *et al*. History, challenges and perspectives of cell microencapsulation. Trends Biotechnol. 2004;22:87–92.
106. Murua A, Portero A, Orive G, Hernandez RM, de Castro M, Pedraz JL. Cell microencapsulation technology: towards clinical application. J Control Release. 2008;132:76–83.
107. de Vos P, Andersson A, Tam SK, Faas MM, Hallé JP. Advances and barriers in mammalian cell encapsulation for treatment of diabetes. Immunol Endocr Metab Agents Med Chem. 2006;6:139–53.
108. Sakai S, Hashimoto I, Kawakami K. Development of alginate-agarose subsievesize capsules for subsequent modification with a polyelectrolyte complex membrane. Biochem Eng J. 2006;30:76–81.
109. Baruch L, Machluf M. Alginate-chitosan complex coacervation for cell encapsulation: effect on mechanical properties and on long-term viability. Biopolymers. 2006;82:570–9.

110. Wu TJ, Huang HH, Hsu YM, Lyu SR, Wang YJ. A novel method of encapsulating and cultivating adherent mammalian cells within collagen microcarriers. Biotechnol Bioeng. 2007;98:578–85.
111. Chung C, Mesa J, Miller GJ, Randolph MA, Gill TJ, Burdick JA. Effects of auricular chondrocyte expansion on neocartilage formation in photocrosslinked hyaluronic acid networks. Tissue Eng. 2006;12:2665–73.
112. Ferreira LS, Gerecht S, Fuller J, Shieh HF, Vunjak-Novakovic G, Langer R. Bioactive hydrogel scaffolds for controllable vascular differentiation of human embryonic stem cells. Biomaterials. 2007;28:2706–17.
113. Black SP, Constantinidis I, Cui H, Tucker-Burden C, Weber CJ, Safley SA. Immune responses to an encapsulated allogeneic islet beta-cell line in diabetic NOD mice. Biochem Biophys Res Commun. 2006;340:236–43.
114. Dufrane D, Goebbels RM, Saliez A, Guiot Y, Gianello P. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept. Transplantation. 2006;81:1345–53.
115. Calafiore R, Basta G, Luca G, Lemmi A, Racanicchi L, Mancuso F, *et al.* Standard technical procedures for microencapsulation of human islets for graft into nonimmunosuppressed patients with type 1 diabetes mellitus. Transplant Proc. 2006;38:1156–7.
116. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, *et al.* Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes - First two cases. Diab Care. 2006;29:137–8.
117. Hong Y, Song HQ, Gong YH, Mao ZW, Gao CY, Shen JC. Covalently crosslinked chitosan hydrogel: properties of *in vitro* degradation and chondrocyte encapsulation. Acta Biomater. 2007;3:23–31.
118. Inanc B, Elcin AE, Koc A, Balos K, Parlar A, Elcin YM. Encapsulation and osteoinduction of human periodontal ligament fibroblasts in chitosan-hydroxyapatite microspheres. J Biomed Mater Res A. 2007;82A:917–26.
119. Sakai S, Hashimoto I, Kawakami K. Production of cell-enclosing hollow-core agarose microcapsules via jetting in water-immiscible liquid paraffin and formation of embryoid body-like spherical tissues from mouse ES cells enclosed within these microcapsules. Biotechnol Bioeng. 2008;99:235–43.
120. Sakai S, Hashimoto I, Kawakami K. Agarose-gelatin conjugate for adherent cell-enclosing capsules. Biotechnol Lett. 2007;29:731–5.
121. Emerich DF, Thanos CG, Goddard M, Skinner SJM, Geany MS, Bell WJ, *et al.* Extensive neuroprotection by choroid plexus transplants in excitotoxin lesioned monkeys. Neurobiol Dis. 2006;23:471–80.
122. Kaigler D, Krebsbach PH, Wang Z, West ER, Horger K, Mooney DJ. Transplanted endothelial cells enhance orthotopic bone regeneration. J Dent Res. 2006;85:633–7.
123. Chang TMS. Therapeutic applications of polymeric artificial cells. Nat Rev Drug Discov. 2005;4:221–35.
124. Torchilin VP. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. Annu Rev Biomed Eng. 2006;8:343–75.
125. Chancellor MB, Yoshimura N, Pruchnic R, Huard J. Gene therapy strategies for urological dysfunction. Trends Mol Med. 2001;7:301–6.
126. Adachi N, Pelinkovic D, Lee CW, Fu FH, Huard J. Gene therapy and the future of cartilage repair. Oper Tech Orthop. 2001;11:138–44.
127. Wright VJ, Peng HR, Huard J. Muscle-based gene therapy and tissue engineering for the musculoskeletal system. Drug Discov Today. 2001;6:728–33.
128. Huard J, Li Y, Peng HR, Fu FH. Gene therapy and tissue engineering for sports medicine. J Gene Med. 2003;5:93–108.
129. Kimelman N, Pelled G, Helm GA, Huard J, Schwarz EM, Gazit D. Review: gene- and stem cell-based therapeutics for bone regeneration and repair. Tissue Eng. 2007;13:1135–50.
130. Usas A, Huard J. Muscle-derived stem cells for tissue engineering and regenerative therapy. Biomaterials. 2007;28:5401–6.
131. Sakai T, Ling Y, Payne TR, Huard J. The use of *ex vivo* gene transfer based on muscle-derived stem cells for cardiovascular medicine. Trends Cardiovasc Med. 2002;12:115–20.
132. Meinel L, Hofmann S, Betz O, Fajardo R, Merkle HP, Langer R, *et al.* Osteogenesis by human mesenchymal stem cells cultured on silk biomaterials: comparison of adenovirus mediated gene transfer and protein delivery of BMP-2. Biomaterials. 2006;27:4993–5002.
133. Hosseinkhani H, Yamamoto M, Inatsugu Y, Hiraoka Y, Inoue S, Shimokawa H, *et al.* Enhanced ectopic bone formation using a combination of plasmid DNA impregnation into 3-D scaffold and bioreactor perfusion culture. Biomaterials. 2006;27:1387–98.
134. Tirney S, Mattes CE, Yoshimura N, Yokayama T, Ozawa H, Tzeng E, *et al.* Nitric oxide synthase gene therapy for erectile dysfunction: comparison of plasmid, adenovirus, and adenovirus-transduced myoblast vectors. Mol Urol. 2001;5:37–43.
135. Madry H, Kaul G, Cucchiarini M, Stein U, Zurakowski D, Remberger K, *et al.* Enhanced repair of articular cartilage defects *in vivo* by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). Gene Ther. 2005;12:1171–9.
136. Nixon AJ, Haupt JL, Frisbie DD, Morisset SS, McIlwraith CW, Robbins PD, *et al.* Gene-mediated restoration of cartilage matrix by combination insulin-like growth factor-I/interleukin-1 receptor antagonist therapy. Gene Ther. 2005;12:177–86.
137. Steinert AF, Nöth U, Tuan RS. Concepts in gene therapy for cartilage repair. Inj Int J Care Injured. 2008;39:S97–S113.
138. Jo J, Tabata Y. Non-viral gene transfection technologies for genetic engineering of stem cells. Eur J Pharm Biopharm. 2008;68:90–104.