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Review

Drug delivery vehicles on a nano-engineering perspective

Betiana Felice [a,b,c], Molamma P. Prabhakaran [c,*], Andrea P. Rodríguez [a,b], Seeram Ramakrishna [c,d]

[a] Laboratorio de Medios e Interfases, Departamento de Bioingeniería, Universidad Nacional de Tucumán, Av. Kirchner 1800, Tucumán, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Buenos Aires, Argentina.
[c] START – Thrust 3, Create Research Wing, #03-08, 1 Create Way, National University of Singapore, Singapore 138602
[d] Department of Mechanical Engineering, National University of Singapore, Singapore

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ABSTRACT

Nanoengineered drug delivery systems (nDDS) have been successfully used as clinical tools for not only modulation of pharmacological drug release profile but also specific targeting of diseased tissues. Until now, encapsulation of anti-cancer molecules such as paclitaxel, vincristin and doxorubicin has been the main target of nDDS, whereby liposomes and polymer-drug conjugates remained as the most popular group of nDDS used for this purpose. The success reached by these nanocarriers can be imitated by careful selection and optimization of the different factors that affect drug release profile (i.e. type of biomaterial, size, system architecture, and biodegradability mechanisms) along with the selection of an appropriate manufacture technique that does not compromise the desired release profile, while it also offers possibilities to scale up for future industrialization. This review focuses from an engineering perspective on the different parameters that should be considered before and during the design of new nDDS, and the different manufacturing techniques available, in such a way to ensure success in clinical application.

Contents

* Corresponding author.
E-mail address: nnimpp@nus.edu.sg (M.P. Prabhakaran).

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1. Introduction

Drug Delivery Systems (DDS) are the systems used for administration of a pharmaceutical compound in a controlled manner to achieve a therapeutic effect in humans or animals. DDS appeared during the middle 1960s as macrosystems while a group of researchers were circulating rabbit blood inside a silicon rubber arterio-venous shunt and discovered that if the tube was exposed to anesthetic gases on the outside, the rabbits fell asleep [1]. Thus the possibility of a "constant rate drug delivery device" was proposed by this group. Further with the development of poly(hydroxy acids), macroscopic systems evolved to microscopic systems [2] where the commercialization of Lupron Depot®, namely microparticles loaded with leutinizing hormone for contraception therapies evolved [3]. But, with the introduction of liposomes in the 1960s [4] along with polymer-drug conjugates in the mid to late 1970s, the concept of nanoengineered Drug Delivery Systems (nDDS) was derived. These systems, against microDDS, offer possibility of specific targeting due to their smaller size, which consequently leads to decreased side effects and longer release period, among other advantages. With the FDA approval of AmBisome® in the 1990s as the first commercialized nDDS, the challenge towards better nDDS never stopped among the pharmaceutical companies. Nowadays there are multiple nDDS being studied (i.e. liposomes, nanoparticles, dendrimers, micelles and nanorods) and more than twenty nDDS are available commercially to treat different diseases including cancer, infections and hormonal disorders. The market value of drug delivery systems was estimated at around $53 billion during the year 2009, and is forecast to reach more than $136 billion in 2021, among which the nDDS might represent 40%[5].

Besides the biological advantages of nDDS over microDDS, there are other reasons that compel nDDS evolution towards better systems. The main reason is that the pharmaceutical companies are being forced to develop new formulations over those ones with going off-patents or with near expiring dates, thus maintaining their commercial monopoly over a specific drug, avoiding generic competition. Hence there is a need to either design a new drug or encapsulate the old drug within a new drug delivery system such as the nDDS. Design of a new drug involves a revenue as high as $500 million with longer time periods of development (10 years), and a high risk of failure during the last stages of the clinical trials [6–8]. Whereas with the development of new nDDS, the cost and time involved can be reduced to $20–$50 million and 3–4 years respectively[9], and it also involves reduced risk of clinical failure. The present review is focused on all the key factors that should be taken into account during the design of a successful nDDS, without losing the overall goal of FDA commercialization approval and clinical application.

1.1. Nanoengineered drug delivery systems approved by FDA

Almost 30 years after the discovery of liposomes, FDA approved the commercialization of AmBisome®, the first nDDS available in the market which encapsulated amphotericin B, a powerful antifungal drug [10]. During the following years multiple nDDS were approved by the FDA. Table 1 gives details of the most representative FDA approved nDDS. It is obvious from the table that cancer and infections were categorized as the main diseases targeted by nDDS [11] and in most cases, the approved nDDS were liposomes, followed by polymeric conjugates based on poly(ethylene glycol) (PEG). It is important to highlight that besides 30 years of research performed antitumoral-polymeric conjugates for anticancer drugs, they have not yet reached the market [12]. Most of the commercialized ones are focused on other disease applications, as can be seen in Table 1. Similar is the situation for biodegradable synthetic polymer-based systems. Besides that there are multiple pre-clinical studies conducted for nDDS made of just biodegradable synthetic polymers such as the PLGA, none of them have been approved for commercialization till date. The reason for this relies on the biocompatibility issues related to the acidic pH produced by the degradation residues of PLGA, which alter drug bioactivity [13] or induce irritation and inflammation to the patient [14]. Similar is the case of active-targeted and stimuli-responsive nDDS, where a few representative nDDS have been approved by FDA (though plenty of pre-clinical and clinical studies were carried out). The reasons are their high development costs, and other biological obstacles that have not been solved yet. For instance, it has been demonstrated that stimuli-responsive DDS either release a significant amount of drug before receiving the specific stimulus or are highly stable that they need to be stimulated by an intensive stimulus that might alter the normal physiological processes. Therefore, until now, the antibody–drug conjugates like Mylotarg®, Ontak®, Zevalin® and Bexxar® were approved for clinical application. Among the stimuli-responsive nDDS, it is only the ThermoDox®, a temperature sensitive liposomal nDDS that has entered phase III trials [15]. Most of the non conventional nDDS such as the dendrimers, lipoplexes or lipid-core micelles are still under pre-clinical or under early clinical trials and only a few of them entered the advanced clinical analysis. For instance, in year 2013, VivaGel® entered phase III human clinical trials, and it was also the first dendrimer-based product that received Fast Track Status from the FDA under an investigation for prevention of genital herpes [16].

2. Release profile

Drug delivery systems arose as a way to maintain blood drug concentration levels between therapeutic limits for prolonged times, thus reducing the number of drug dosages, initial drug concentration as well as their side effects caused by unspecific systemic distribution through simple diffusion [2]. Their release profile is a consequence of one or more release mechanisms: (i) desorption of drug bound to the surface (ii) diffusion through (iii) diffusion through the capsule shell (iv) matrix erosion and (v) a combined erosion–diffusion process [42]. Due to the decrease of diffusional distance in nDDS compared to microparticulate systems, surface desorption and erosion processes of drugs are the predominant release mechanisms followed by nDDS [42,43]. A typical example of naked drug release profile can be viewed from Fig. 1a, where the only advantage of conventional formulations is their low development cost [44]. On the other hand, a simple nDDS is characterized by release profiles shown in Fig. 1b, which tend to be of zero-order, if not for frequent initial burst effect, which we explained in Section 2.1 of this manuscript. As visible from Table 2, half life time of these types of systems can range from few hours to months.

Table 1
Representative nanoengineered drug delivery systems approved by FDA and in advanced clinical trial phase.

	Product name	Carrier type	Size	Drug	Diseases	Type of drug	Carrier material	Approval year/phase	Ref.
Liposomes	Doxil	PEGylated liposome	100 nm	Doxorubicin	Various types of cancer	Anthracycline	PEG/Phospholipids	1995	
	DaunoXome	Liposome	<100 nm	Daunorubicin	HIV	Anthracycline	Phospholipids	1996	[17]
	Ambisome	Liposome	<100 nm	Amphotericin B	Fungal infections	Polyene	Phospholipids	1997	[10]
	Depocyt	Liposome	<100 nm	Cytarabin	Lymphomatous meningitis	Nucleoside	Phospholipids	1999	[18]
	Visudyne	Liposome	<100 nm	Verteporfin	Macular degeneration	Benzoporphyrin derivative	Phospholipids	2000	[19]
	DepoDur	Liposome	<100 nm	Morphine	Severe pain	Opiate	Phospholipids	2004	[20]
	Octocog alfa	Liposome	<100 nm	Factor VIII	Hemophilia	Proteica	Phospholipids	2009	[21]
	Marqibo	Liposome	<100 nm	Vincristine sulfate	Hodgkin lymphoma	Alkaloid	Phospholipids	2012	[22]
	Myocet	Liposome	<100 nm	Doxorubicin	Metastatic breast cancer	Anthracycline	Phospholipids	Phase III	[23]
	OncoTCS	Liposome	<100 nm	Vincristine	Hodgkin lymphoma	Alkaloid	Phospholipids	Phase III	[24]
	Thermodox	Low temperature sensitive liposome	<100 nm	Doxorubicin	Metastatic malignant melanoma, liver cancer	Anthracycline	Low phase transition temperature phospholipids	Phase III	[25]
Conjugates	Adagen	Conjugate	1–50 nm	Adenosine deaminase	Severe combined immunodeficiency	Peptidic	PEG	1990	[26]
	Oncaspar	Conjugate	50–200 nm	Asparaginase	Leukemia	Peptidic	PEG	1994	[27]
	PEG-intron	Conjugate	1–50 nm	Interferon alfa 2b	Chronic hepatitis C	Proteic	PEG	2001	[28]
	Cimzia	Conjugate	1–50 nm	Interferon alfa 2b	Chron's disease	Proteic	PEG	2008	[29]
	Omontys	Conjugate	1–50 nm	Peginesatide acetate	Anemia	Peptidic	PEG	2012	[30]
	Xyotax	Conjugate	100–150 nm	Paclitaxel	Lung cancer, ovarian cancer	Anthracycline	Polyglumex	Phase III	[31]
	Puricase	Conjugate	50 nm	Uricase	Hyperuricemia	Proteic	PEG	Phase III	[32]
	Mylotarg	Monoclonal antibody	20–40 nm	Ozogamicin	Leukemia	calicheamicins	Anti-CD33 monoclonal antibody	2000	[33]
	Zevalin	Monoclonal antibody	20–40 nm	Yttrium-90	Non-Hodgkin's lymphoma	Radioactive material	Anti-CD20 monoclonal antibody	2002	[34]
	Bexxar	Monoclonal antibody	20–40 nm	Iodine-131	Non-Hodgkin's lymphoma	Radioactive material	Anti-CD20 monoclonal antibody	2003	[35]
	Kadcyla	Monoclonal antibody	20–40 nm	Emtansine	Breast cancer	maytansinoid	Anti-CD37 monoclonal antibody	2013	[36]
Polymeric particles	Livatag	Nanoparticle	<100 nm	Doxorubicin	Hepatocellular carcinoma	Anthracycline	poly(iso-hexyl-cyanoacrylate)	Phase II	[37]
	Lupron Depot	Microparticle	50 um	Leuprolid	Prostate and breast cancer	Peptidic	PLA	1989	[3]
	Estrasorb	Micelle	100 nm	Estradiol	Hot flushes during menopause	Esteroide	Lecithin	2003	[38]
	Risperdal Consta	Microparticle	1–50 um	Risperidone	Schizophrenia, bipolar disorder	Dopamine antagonist	PLGA	2003	[39]
	Abraxane	Nanoparticle	10 nm	Paclitaxel	Breast cancer	Anthracycline	Albumin	2005	[40]
	Genexol-PM	Micelle	10–100 nm	Paclitaxel	Breast cancer	Anthracycline	PEG–PLA	Phase II	[41]

Source: US Food and Drug Adminsitration website (http://www.accessdata.fda.gov) - US Clinical Trials website (http://clinicaltrials.gov/).

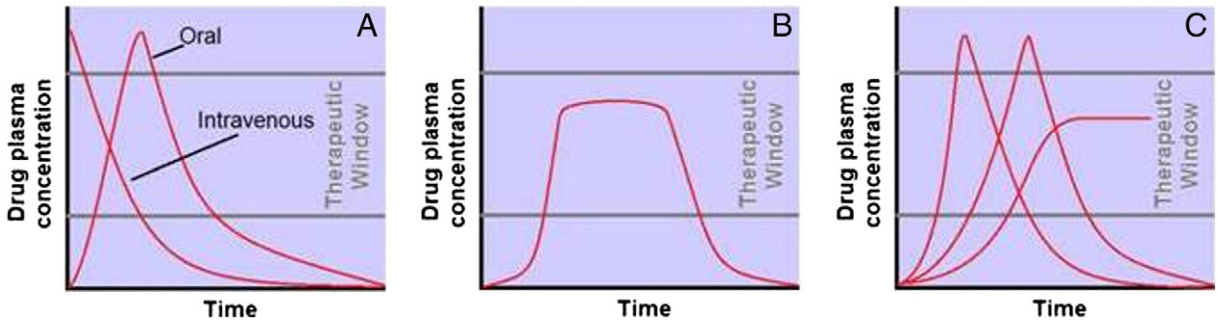

Fig. 1. Types of drug release profiles. (A) Naked drug where the release profile is based on simple diffusion and partition. (B) Zero-order release profile, (C) Programmed release profile. *Figure reproduced with copyright permission from Kim et al. [44] obtained from Elsevier.*

In addition to prolonged release times, an ideal response for nDDS also involves programmability of drug release profile according to the treatment conditions and patient needs (Fig. 1c). Material degradation rates of nDDS regulate the programmability and this is commonly achieved either using different molecular weights of the same polymer or using different formulations [45]. Another way to obtain programmability is through encapsulation of multiple drugs inside the same matrix but with different interaction mechanisms [46]. Programmability is also obtained using stimuli-repsonsive nDDS (see Section 2.2) and profiles similar to that shown in Fig. 1c can be achieved.

2.1. Burst release effect

Most of the simplest nDDS do not show an ideal zero-order release profile given that there is a burst effect immediately after systemic administration, followed by a zero-order response (Fig. 2). This phenomenon is related to the diffusion and desorption of drug located close to nDDS surface [42,57], which is considered undesirable because the released drug is not available for long term treatment and can cause damaging side effects. Too many factors affect the magnitude of burst release. It has been demonstrated in polymeric nDDS that burst effect is high for nDDS made of low molecular weight polymers, with high drug loading systems or with inhomogeneous drug distribution inside [58,59].

Different methods were proposed in different works for reducing the burst release. For instance, Hasan *et al.* encapsulated polymeric nanoparticles into polymeric microparticles by using W/O/W emulsion, thus reducing the diffusion of drugs through the double walled polymeric system [60]. PCL was used as the internal phase, while ethylcellulose and Eudragit® RS served as the external phase and prevented the dissolution of the internal phase. In another study, PEG–PLA microspheres were coated with gelatin film to reduce the initial burst release [61]. The hydrogen bonding between PLA–PEG copolymer and gelatin amine group made the encapsulation of the drug more stable within the microspheres, thus reducing the burst release effect.

2.2. Stimuli-responsive DDS

A method for drug programmability is also achieved through stimuli-responsive DDS, also known as the "smart DDS". These systems are able to release their load in response to one or more stimuli, due to changes of conformation, solubility, shape, size or charge [62]. This approach allows synchronization between biological rhythms and medical treatment [63]. Stimuli can be classified according to their origin (internal or external) or type of signal (chemical or physical). Only a few temperature-sensitive liposomal systems have entered the phase II or III of clinical trials such as ThermoDox®, which is under phase III [25] (doxorubicin, for breast and liver cancer), Allovectin® under phase II [64,65] (gene therapy, for metastatic melanoma) and Lipoplatin® under phase II [66] (cisplatin, for lung and pancreatic cancer). Most of these systems use lipids with a phase transition temperature of around 40 °C, which can be reached by radiofrequency or using ultrasound of high intensity [67]. However, there are rare cases where such systems showed promising results. Table 3 gives a summary of the representative "smart nDDS", which are mostly based on liposomes, polymers or their combinations. It is important to highlight that polymeric smart nDDS are still under initial or pre clinical trial phases given that the intelligent polymers such as the poly(methylvinyl ether) and poly(N-alkylacrylamides) are still being subjected to preclinical assessment of their biocompatibility or bioinertness [68,69]. Smart nDDS has been covered with more detail in excellent reviews elsewhere [70–75].

3. Factors that affect the drug release

There are several factors that affect the drug release profile and these factors should be taken into account during the design stages, aiming for an optimal therapeutic effect. Mainly, they are grouped based on drug-material interaction, morphologies and architectures of the

Table 2
Comparison of Half life time of various nDDS.

Product name/System name	Half life time	Carrier type	Drug	Reference
Doxil	40–70 h	Liposome	Doxorubicin	[47]
Lipoplatin	10–36 h	Liposome	Cisplatin	[48]
Marqibo	7.66 h	Liposome	Vincristine	[49]
Myocet	2–3 h	Liposome	Doxorubicin	[47]
Oncaspar	4–14 days	Polymeric conjugate	Asparaginase	[50]
PEG/PLGA Conjugates	1 month	Polymeric conjugate	Doxorubicin	[51]
PEG/PLGA Conjugates	1 month	Polymeric conjugate	Doxorubicin	[52]
Bexxar	30–90 h	Monoclonal antibody conjugate	Iodine 131	[53]
Zevalin	16–44 h	Monoclonal antibody conjugate	Yttrium-90	[54]
Abraxane	15–18 h	Albumin nanoparticle	Paclitaxel	[55]
–	5 h	Naked drug	Paclitaxel	[56]
–	0.2 h	Naked drug	Doxorubicin	[47]

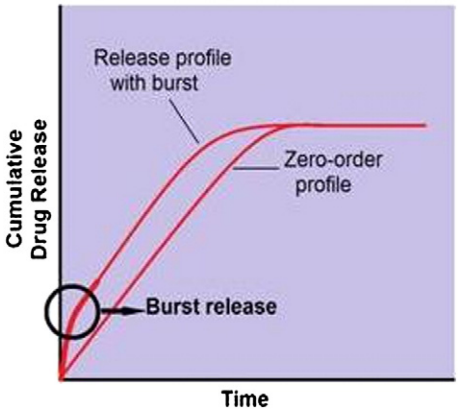

Fig. 2. Schematic illustration of the burst effect in zero-order drug delivery system. *Figure reproduced with copyright permission from Huang et al. [59] obtained from Elsevier.*

encapsulated forms, biodegradability mechanisms and clearance time regulation.

3.1. Drug-Material interaction

The interaction of drug with the carrier material is a primordial factor of concern even towards the beginning of drug release design, together with basic properties like biocompatibility of the material and degradation residues or intended therapeutic drug concentration. The fundamental features that directly determine the drug-material interaction are: hydrophobicity/hydrophilicity, chemical structure, molecular weight and net charge. However, these factors are also closely related to drug loading capacity and encapsulation efficiency and here we discuss this in detail.

The importance of hydrophobicity match (or mismatch) between drug and material can be understood via liposomal nDDS. It is known that the inner zone of liposomes is hydrophilic and their lamellaes are hydrophobic, and this allows for the loading of hydrophilic drugs in the inner zone and hydrophobic ones in the lamellae [96,97]. However, this is not easy to achieve mainly because liposomes present reduced values of drug loading for hydrophobic drugs, given that they just can be transported inside the liposomal membrane [96,98]. Also, they are characterized by low retention of highly lipophilic drugs (eg; Paclitaxel), due to high membrane permeability to them [99]. In addition, the loading of hydrophilic drugs into liposomes is difficult due to lipid-hydrophilic drug repulsion [100] and to solve these effects, multilamellar liposomes were developed [101]. Another option is to alter the liposomal membrane composition or to use active trapping (Section 3.2). Mohammed *et al.* combined all the aforementioned solutions to design an efficient liposomal system for ibuprofen delivery. Through the use of multilamellar liposomes made of long alkyl chain lipids and increased levels of cholesterol, liposomes with increased drug loading capacity, increased encapsulation efficiency and reduced burst effect were produced. The drug loading capacity was also regulated by utilization of lipid dicetylphosphate, an anionic lipid which by charge repulsion could decrease the drug concentration within the membrane [102]. Chemical structure is another factor that points out the possibility of chemical bonding between the drug and the carrier material, or capable of covalent or hydrogen bond formation such that it increases the drug retention time inside the nDDS and keep it inactive until it is released. For instance, Yokoyama *et al.* encapsulated covalent bonded Adriamycin, an anticancer drug, into the polymeric micelles made of poly(ethylene glycol)-poly (aspartic acid) block copolymers. Their results show better stability, increased water solubility and increased retention times of Adriamycin [103]. Analyzing the molecular weight in two different perspectives, it was obvious that the molecular weight of the drug mainly affects its diffusion into the biological medium after release as well as the intensity of burst release [58]. Secondly, with respect to the material holding the drug, the molecular weight affects the degradation rate of the carrier. Net charge is mainly related to the amount of drug retained inside the nDDS. The use of opposite charges between drug and material is a common technique applied to increase the drug retention time without chemical alterations (physical entrapment) [44]. It is through this attribute that nucleic acids (anionic molecules) are encapsulated in lipoplexes(cationic liposomes)[104, 105]. For instance, Morille *et al.* encapsulated plasmid DNA by conjugating it with DOTAP:DOPE (cationic lipids). Similarly, they produced PEGylated lipoplexes to acquire increased half- life times of circulation in blood [106].

3.1.1. Carrier materials used for DDS

Different kinds of materials are being used for nDDS development including lipids, biodegradable polymers, non biodegradable polymers, antibodies, metals [107,108], magnetic substances [109,110], carbon [111,112], ceramics [113,114], and viral capsids [115], among others. But most of them are still under pre-clinical trial. As in this review we prioritize to focus on FDA approved nDDS as a model for targeted design, here after we discuss only on those carrier materials associated with commercially successful nDDS or those that have entered advanced clinical trials.

Table 3
Representative smart nDDS and their applications.

Stimulus	Mechanism	Carrier type	Ref.
Temperature	Polymers: Competition between hydrophobic and hydrophilic interactions between polymer–polymer and polymer–water. Changes in viscosity, solubility, swelling / Liposomes: Phase transitions[67,68,76]	Thermosensitive polymeric micelle	[77]
		Traditional thermosensitive liposomes	[78,79]
Ultrasound	Disruption of nanocarrier structure by cavitation or indirect temperature increase; enhanced drug diffusion through biological fluids [80]	Modified liposomes with thermosensitive polymer	[81]
		Thermosensitive liposomes	[82]
Magnetic field	Disruption of the structure of the nanocarriers by temperature increase; particle oscillation control by changing wave frequency [72,83]	Magnetic nanoparticle-organic hybrid sensitive to AC magnetic field	[84]
Light	Light-induced shape changes (i.e. hydrophobicity–hydrophilicity transition); nanocarrier disruption by temperature increase [85–87]	Two-photon sensitive micelle	[88]
pH	Protonation or deprotonation of ionizable groups; changes in viscosity,solubility, swelling [76,89]	Polymeric nanoparticle with pH-dependent solubility	[90]
		Targeted magnetic mesoporous silica/polymer nanoparticles	[91]
		Polymer–liposome complex	[92]
Ionic strength	Changes in size and solubility [63]	Cu(II)-polymer–drug conjugate	[93]
Enzymes	Enzymatic action produces disruption or swelling of nanocarrier [94]	Polymeric nanoparticles	[94]
Biomolecules	Glucose: Indirect pH changes, swelling due to charge changes	Mesoporous silica nanoparticle with sensitive shell	[95]
	Antigen: swelling of nanocarrier structure due to dissociation of polymer coupled antibody because of free antigen and polymer-bound antigen competition [63,76]		

3.1.1.1. Lipids. This group contains phospholipids and cholesterol, the main liposomal components. Phospholipids are the major component of liposomes as well as the constituent of all cell membranes. Basically, they are composed of glycerol or sphingosin covalently linked to one or two fatty acids with a phosphate head group. They are amphiphilic molecules, a feature that allow them to form the bilayered membranes present in liposomes. Whereas ordinary amphiphiles have critical micelle concentrations (CMCs) of 10^{-2}–10^{-4} M, the CMC of phospholipids is four to five orders of magnitude smaller, meaning that the water solubility of these materials are extremely low and that its stability after administration is high, compared with ordinary micelles [116]. Phospholipids are mostly neutral or anionic molecules due to the presence of phosphate group. The most studied group includes the phosphatidylcholine family, a natural and neutral lipid present in plants and animals. These contain the primary alcohol choline which has a quaternary ammonium group [117]. Most commonly used natural anionic phospholipid are phosphatidic acid, phosphatidylglycerol, phosphatidylserine, and phosphatidylethanolamine [97]. Cationic phospholipids are less used, such as the stearylamine for nucleic acid encapsulation [104,105]. Against synthetic phospholipids (i.e. dimyristoylphosphatidylcholin, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine), natural phospholipids are preferred because of their greater chemical biostability (i.e. against phospholipases and/or esterases, bile salts, and serum proteins) and thermodynamic stability of the vesicle (e.g. to oxidative stress, high temperature and/or alkaline pH) [118]. However, the regulation of membrane permeability is demonstrated easier in liposomes made of synthetic phospholipids. Demel *et al.* showed that the permeability of glucose was significantly reduced by cholesterol in liposomes made of synthetic phospholipids. [119]. Phospholipids are degraded by lipolysis in the physiological environment and are characterized by its low toxicity. An important property of phospholipids is their viscosity, which significantly affects the fluidity of the liposomal membrane. This can be controlled by utilization of phospholipids with higher phase transition temperatures [96,97,118]; or by inclusion of cholesterol between phospholipids chains.

Cholesterol is the second most used lipid in nDDS. It is a sterol naturally found in cell membranes. As was mentioned before, it is used for fluidity regulation of phospholipidic membranes, decreasing the permeability of hydrophilic molecules, and increasing the liposomal membrane stability [97,120,121]. Membrane stability regulation through cholesterol content manipulation has been proved in multiple works. For instance, Senior and Gregoriadis showed cholesterol effect on different liposomes *in vivo*. These researchers used carboxyfluorescein as a marker and changed the saturation degree of phospholipid chains as well as their length. In all cases, higher cholesterol content implied higher stability [122]. On the other hand, Kirby *et al.* probed cholesterol effect on liposomes made of natural phospholipids [123], where they found that the cholesterol-rich liposomes remained stable in blood for at least 400 min. The cholesterol-poor liposomes lost their stability within 2 minutes of intravenous injection.

3.1.1.2. Polymers. Many types of polymers are used in nDDS, both biodegradable and non-biodegradable: chitosan [124,125], poly(lactic acid) (PLA), poly(lactic co-glycolic acid) (PLGA), PEG, poly (ε-caprolactone) (PCL) [126], and poly(cyano acrylates) [127] among others. Among these polymers, the ones which are being used in nDDS are either approved or entered the clinical trial phases and these are based on PLA, PLGA and PEG.

PLA is a biodegradable thermoplastic linear aliphatic polyester that has been used for the manufacture of implants, stents, and medical devices [2]. Typically, the polymer is characterized by its inherent viscosity and demonstrates a sustained release depending on the chain length of the PLA molecule. PLA is degraded by hydrolysis to units of lactic acid, a natural intermediate of anaerobic respiration [128]. PLA is commercially available just in microparticles like Lupron Depot® [129] and Risperdal Consta® [39]. Among the current PLA matrices, the PLA-PEG micelles are widely used. For instance Genexol PL®, is a PLA-PEG micelle which encapsulates paclitaxel. Its clinical application has been approved in South Korea and Europe [41] but is still under phase II clnical trial in the United States. Also Xu *et al.* encapsulated paclitaxel in micelles, either in the conjugated or non conjugated form. These micelles demonstrated a reduced burst effect and increased stability [130]. Amphotericin B [112] was also encapsulated within PLA micelles, using polymeric chains with different lengths and a sustained release of the drug was evidenced. About 80% of the Amphotericin B was released in 80 h and it was demonstrated that the release rate was reduced when PLA chain was longer. PLA-based nanoparticles were also subjected to pre-clinical studies by other groups. For example, Jain *et al.* encapsulated temozolomide for brain drug delivery, a powerful drug against C6 glioma [131]. These nanoparticles effectively maintained a higher and constant concentration of the drug over the tumor, during *in vitro* studies. PLA nanoparticles are also being used for nucleic acid encapsulation [132].

PLGA is a copolymer of lactic acid and glycolic acid. Similar to PLA, the polyester is sensitive to hydrolysis and produces biodegradable metabolite monomers. These monomers participate in a number of physiological and biochemical pathways and demonstrate minimal systemic toxicity [128]. Degradation rate depends on its molecular weight and monomers ratio [43]. So far, there aren't any approved PLGA-based nDDS, but the ongoing pre-clinical and clinical trials show some promising results. PLGA nanoparticles have been used mainly for encapsulation of multiple anti-cancer drugs such as paclitaxel, cisplatin, vincristine sulfate, doxorubicin and curcumin [133]. Kim *et al.* encapsulated dexamethasone, a known anti-inflammatory drug, in PLGA nanoparticles embedded in alginate hydrogel matrices [134]. It was aimed for the reduction of scar formation around the electrodes during neuronal stimulation at the peripheral and central nervous system. *In vitro* test showed reduced gliotic scar around hydrogel/nanoparticles-coated electrodes, without significant change in electrode impedance which meant an improved electrical pathway for long term stimulations.

PEG is a non biodegradable hydrophilic polymer, mainly used in polymer–drug conjugates, micelles [51,130,135] and for nDDS stabilizations and stealth. More features of PEG and its applications are described in Section 3.5.2.

3.1.1.3. Human serum albumin. Albumin represents the major fraction of the human serum proteins with a blood concentration between 35 and 50 g/L and a molecular weight of 65 kDa. It has a half-life time in blood of 19 days and is stable in the pH range between 4 and 9. It provides two binding sites for small molecules, two binding sites for long-chain fatty acids, and two binding sites for metal atoms. Due to its human native origin, albumin demonstrates low toxicity and immunogenicity. Albumin has been used in different forms. For instance, Dosio *et al.* encapsulated paclitaxel in PEG–Albumin conjugates. Their results showed that albumin significantly increased stability and solubility of PEG conjugates without bioactivity losses [136]. Paclitaxel was also encapsulated in albumin nanoparticles, which indeed served as the first natural polymeric nDDS approved by FDA for intravenous injection (Abraxane®) [137]. In this case, paclitaxel and albumin are not covalently linked but rather associated through hydrophobic interactions during an emulsion-evaporation based process, called Nanoparticle Albumin-Bound (NAB) Technology [138]. The clinical data have shown that Abraxane® offers several improvements over the conventional Cremophor EL®, including lower toxicities, shorter administrating time, higher efficacy and no premedication [139]. Other types of anti-cancer drugs were also encapsulated inside albumin nanoparticles. For example, Wartlick *et al.* manufactured albumin nanoparticles loaded with antisense oligonucleotides, which are currently being evaluated as anticancer agents in clinical trials and for antiviral therapies [140].

3.1.1.4. Monoclonal antibodies. Monoclonal antibodies are laboratory versions of natural antibodies, also known as immunoglobulines.

These are a type of glycoprotein naturally present in the human body and synthesized by B cells. They are used by immunologic system for detection and marking of foreign elements. More than 20 monoclonal antibodies have been approved by FDA till date [141], among which the most well known are Zevalin® [54,142] and Bexxar® [35,142]. Similar to other FDA approved antibody conjugates, both Zevalin® and Bexxar® are used for targeted radioimmunotherapy. This type of therapy is appropriate for treatment of multiple tumor sites that cannot be readily excised surgically or irradiated using external beam radiation or brachytherapy. Their success was mainly due to prolonged half-life times (30–90 h for Bexxar® and 16–44 h for Zevalin®), their nanoscale size which enables better bio-distribution of drugs mainly through passive targeting, and their active interaction with cell membrane receptors due to natural antibody-antigen coupling that increase their internalization efficiency. Monoclonal antibodies by themselves are being used as carrier materials after conjugation with drugs, but they are also being used as ligands in active-targeted nDDS where they are attached directly on the nDDS surface. For instance, some authors proposed the use of targeted-stealth immunoliposomes (SIL), which utilize IgG1 f(ab)2, which positively reacts to greater than 90% of cancerous stomach tissues but negatively with normal tissues [143]. Wang *et al.* attached CD44 antibody to liposomes loaded either with doxorubicin or a plasmid for hepatocarcinoma treatment and imaging, and performed pre-clinical studies [144]. The plasmid was the result of a triple fusion between genes containing the herpes simplex virus truncated thymidine kinase (a non viral suicide gene for anticancer therapy), renilla luciferasa (Rluc) and red fluorescent protein (RFP), which are used for tracking of the specific targeting. Furthermore, the growth status of the tumor was monitored by optical bioluminescence imaging of green fluorescent protein, offering in this way a dual imaging system. Their results showed a higher action of the gene-loaded liposomes against doxorubicin-loaded ones and demonstrated the effectiveness of dual imaging for evaluation of molecular targets.

From the aforementioned material descriptions, it is obvious that for successful design of nDDS, natural biomaterials, namely phospholipids or proteins, are the best choice till today. This is mainly due to the low immunogenicity associated with both these materials and their degradation residues and higher internalization efficiency because of the presence of natural cell uptake mechanisms as well as their mild interaction with encapsulated drugs which diminishes bioactivity losses. On the other hand, it is remarkable to note the incongruence that exists between the big amount of clinical and pre-clinical studies over nDDS based on synthetic polymers, and the small amount of approved FDA products based on these materials. According to these assumptions, with exception of PEG conjugates and micelles, it was therefore obvious that the synthetic polymers have not been classified as good biomaterials for nDDS manufacture.

3.2. Drug entrapping techniques

During nDDS design, carrier manufacturing should be differentiated from drug entrapping technique. The second one is commonly achieved in parallel with the former one, but sometimes implies a completely different stage during global manufacturing process in order to reach an encapsulation efficiency of 50% or nearly 100%. The other factor of concern is the drug loading capacity and this depends on the type of the drug (therapeutic efficacy versus toxicity) and the nDDS manufacturing process (i.e. drug–carrier interaction, solvents used or mechanical loadings applied etc.) [145].

There are two groups of drug entrapping techniques:

(1) Passive trapping: These are techniques where drug and carrier are co-dispersed in the same medium, in such a way nDDS formation and drug loading happen at the same time. These processes require organic solvents, sonication or high temperatures, which could result in decreased encapsulation efficiency or bioactivity losses [146].

(2) Active trapping (or remote loading): In this case, drug is loaded after nDDS formation. Active trapping is significant when repulsion between drug and carrier exists. This is mainly used for the loading of hydrophilic drugs inside the liposomes and it is done via different techniques, either pH or potential gradients technique or diffusion filling method. When pH or potential gradients techniques are used, the interaction between the inner and external medium leads to concentration equalization of different species next to the membrane. Consequently the drug, commonly an acid or weak base, is dragged towards the inner region of the nDDS [145,147,148], thus increasing its load value. For instance, doxorubicin has been loaded in liposomes through pH gradients [149,150] and ammonium ions gradients [151], while camptothecin has been loaded in polymeric trilayered nanoparticles using pH gradient technique [89]. Retention time was increased in all cases. On the other hand, for diffusion filling method, the nDDS is exposed to a drug solution, and by effect of drug concentration difference it gets into the nDDS [152].

3.3. Encapsulating architectures

Selection of an encapsulating material is always done concurrently with the encapsulating architecture selection in order to obtain optimal release profile. Stability of the chosen system, possibility of regulation of drug loading capacity, and nDDS size are factors that affect the release profile, which we explain here in more detail. Low nDDS stability induces burst release of drugs, and the effect is more visible in polymeric micelles. Polymeric micelles are colloidal particles composed of amphiphilic block polymers, which are formed through 'self-assembly' that occurs as the concentration of amphiphilic polymer increases. This phenomena occurs only when the concentration is above a thermodynamical critical value known as the Critical Micelle Concentration (CMC) [153,154] The lower the CMC, the more stable the micelles are [154]. During the administration of the loaded micelles, the concentration of the copolymer drops and particle dissociation occurs with fast drug release. Therefore, to increase the global micelle stability, it is necessary to increase the hydrophobic core stability either by increasing the hydrophobicity, crosslinking or via electrostatic interactions [155].

Drug loading level must be controlled to ensure prolonged release times. However, if the loading amount is low, the release profile is shorter and the need for multiple doses becomes necessary. From the structural point of view, release mechanism depends on selected architecture. For instance, hydrophobic drug loading capacity in liposomes can be increased by utilization of multi-lamellar liposomes [156]. Meanwhile, when dendrimers are used, drug loading capacity can be altered by changing the number of brunch layers (generation number) composed of a dendrimer [157]. For particles, this parameter can be increased through size augmentation.

Finally, during the selection of encapsulating architecture, size is the key point (Fig. 3). This parameter mainly determines clearance time (Section 3.5). Though a wide variety of nDDS have been developed (i.e. micelles, lipoplexes, nanorods, dendrimers, nanocapsules, biological carriers and so on), the FDA approved systems namely liposomes and polymer–drug conjugates are the predominant products in current nDDS market and here we evaluate these two systems with examples.

3.3.1. Liposomes

Liposomes are the commonest lipid-based formulation for drug delivery and the most successful nDDS, known till date. There are more than 20 commercialized liposomal formulations and many more are under clinical and pre-clinical trials [158,159]. Their success can be attributed to the remarkable flexibility of lipid-based delivery systems, ability to efficiently encapsulate both small molecules and macromolecules,

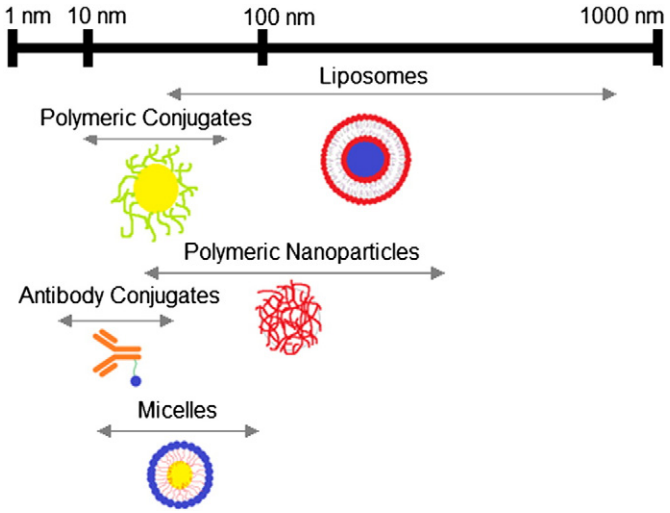

Fig. 3. Representative nanoengineered drug delivery systems and their sizes.

biodegradability and biocompatibility; possibility to be manufactured in sizes down to 20 μm in diameter; and their interaction with membrane components in a predictable manner [96].

Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids and cholesterol. Hydrophilic drug molecules can be incorporated in the internal aqueous phase while hydrophobic molecules are integrated into the bilayer membrane. Liposomes are spontaneously formed when phospholipids are dispersed in aqueous media due to the hydrophilic interaction of lipid head groups with water, resulting in the formation of vesicles [121]. Liposomes can be classified according to their lamellarity and size, or according to their phylogenetic scheme [47]. When lamellarity is considered, they are either unilamellar or multi-lamellar liposomes. The former ones comprise just one lipid bilayer with diameters of 50–250 nm. They contain a large aqueous core and are used for the encapsulation of water-soluble drugs. Multi-lamellar vesicles are composed of several concentric lipid bilayers in an onion-skin arrangement with diameters of 1–5 mm. The high lipid content allows these multilamellar vesicles to passively entrap lipid-soluble drugs. On the other hand, when phylogenetic scheme is considered, liposomes are divided into two subgroups: stealth or non-stealth liposomes. The high hydrophobicity of liposomes induces a small clearance time (Section 3.5). Because of this, some liposomes are conjugated with PEG in a manner to increase their blood circulation time and are known as Stealth® liposomes.

Most of the commercialized liposomes encapsulate anticancer drugs and among these, Myocet® and Doxil®, which encapsulate doxorubicin, are the most famous formulations. Doxorubicin is an anthracyclin produced by *Streptomyces peucetius* and used against a broad spectrum of neoplasms, including breast, ovarian, stomach, bladder and bronchogenic carcinomas [160]. It exerts its effect basically through inhibiton of DNA and RNA synthesis [161,162]. Severe myelosupression and cardiotoxicity among other consequences are its main side effects, which were reduced when doxorubicin was encapsulated in liposomes [101]. Initially, liposomal doxorubicin was encapsulated in multilamellar liposomes, in which the drug was passively entrapped. However, this formulation failed in following clinical trials mainly due to the rapid drug release and clearance by reticuloendothelial system *in vivo* [163]. Active loading was used to improve the drug loading efficiency and formulation stability, bringing about Myocet® and Doxil® in which doxorubicin was loaded by a pH and potential gradient, respectively. Myocet® is comprised of egg phosphatidylcholine and cholesterol whereas Doxil® uses hydrogenated soya phosphatidylcholine and cholesterol. However, the major advancement of Doxil® over Myocet® is its coating with PEG, which significantly improves its pharmacokinetic profile (Fig. 4). In a pharmacokinetic study of doxorubicin loaded liposomes, free doxorubicin had an elimination half life of 0.2 h and an area under the plasma concentration-time curve (AUC) of 3.81 mg h/ml, compared with 2–3 h and 46 mg h/ml for Myocet® and with a further increase to 41–70 h and 902 mg h/ml for Doxil®. Both Myocet® and Doxil® significantly reduced the toxic effects of doxorubicin [47]. Furthermore, Myocet® formulation releases more than half of its associated doxorubicin within 1 hour of intravenous administration and more than 90% of its entrapped contents within 24 h, whereas Doxil® releases less than 10% of the encapsulated doxorubicin within 24 h of administration [164]. This difference is also reflected in the toxicity studies performed by Waterhouse *et al.* using animal models, where they found Myocet® as more acutely toxic than Doxil® [160]. However, Doxil® produces specific toxicity called the plantar eryhtrodysaesthesia (PPE), which is a painful desquamating dermatitis which primarily affects hands and feet, and consequently implies that the maximal tolerated acute dose of Doxil® is lower than that of free doxorubicin for patients treated at short dose intervals of 3 weeks [165]. It must be highlighted that the equivalent survival rates between liposomes and free drugs were found in different studies, suggesting that the advantage of Myocet® and Doxil® lays only on the reduction of toxicities [101]. Besides that, the encapsulated anti-cancer drugs represent the majority of liposomal products, while there are other products like Ambisome®, which encapsulates antifungal drug, Depodur® which encapsulates morphine, (analgesic) and Visudyne®, which encapsulates verteporfin, a drug against macular degeneration.

There are much more liposomal systems under clinical phase II and III than other types of nDDS. Among them, ThermoDox® is the first heat-activated formulation of temperature-sensitive liposome that encapsulates doxorubicin. ThermoDox® is comprised of three synthetic phospholipids: DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine), MSPC (1-Stearoyl-2-hydroxy-sn-glyceSro-3-phosphocholine), and DSPE-MPEG-2000(1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-methoxypolyethyleneglycol-2000) [15,25]. ThermoDox® selectively releases its doxorubicin content when exposed to temperatures above 39.5 °C, due to its relatively low phase transition temperature (T_m). T_m is the temperature required to induce a change in lipidic chains from ordered gel phase, where the hydrocarbon chains are fully extended and closely packed, to the disordered liquid crystalline phase, where the hydrocarbon chains are randomly oriented and fluid. This temperature is reached commonly by heating the tumor with radiofrequency electromagnetic waves.

3.3.2. Polymer–drug conjugates

Polymer–drug conjugates occur as a result of covalent bonding between a polymer and a drug, where PEG is the most commonly used

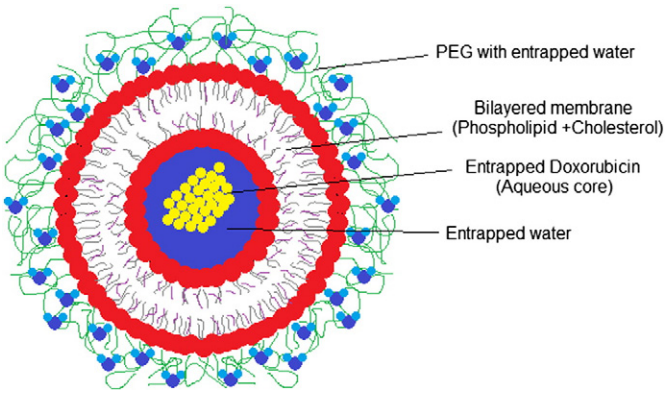

Fig. 4. Doxil®, in which $(NH_4)_2SO_4$ gradient is used for loading and protonation of encapsulated Doxorubicin in such a way that the protonated drug inside the liposome has difficulty in crossing the lipid bilayer which results in trapping efficiencies of >99%. Entrapped water creates a hydrated barrier and protects the liposome from fast elimination.

polymer until now [76]. Conjugation increases drug stability and solubility while it reduces drug immunogenicity, specially of proteins or peptide drugs which are liable to be quickly metabolized inside the body [166]. Also, it increases clearance time through hydrodynamic radio augmentation and particles concealing, which avoids renal filtration. If hydrophilic polymers are used, the clearance time might get increased. Nevertheless, conjugation can induce drug bioactivity losses due to obstruction of active site by covalent binding with conjugation polymer [44]. An example of commercialized conjugate is Oncaspar®, (encapsulates asparaginase, a drug used for leukemia treatment), which was approved by FDA in 1994. Asparaginase is an enzyme that catalyzes the decomposition of asparagine (a natural amino acid) to aspartic acid and ammonia. Asparagine is considered a non-essential amino acid but certain leukemias and other malignancies are unable to synthesize aspargine due to the lack of asparaginase synthetase activity. Therefore, asparagine becomes an essential amino acid for these cells which are dependent on extracellular sources of asparagine to be able to complete protein synthesis. When asparagine is depleted by the activity of asparaginase in plasma, these cells cannot derive asparagine from extracellular sources to maintain protein synthesis, providing the basis for selectivity of asparaginase against malignant cell. However, the use of naked asparaginases has been limited by a high rate of hypersensitivity reactions and development of anti-asparaginase antibodies, which neutralize its activity. Clinical trials (I and II) demonstrated that the mean half life time of PEGylated asparaginase was 357 h compared to 20 h for naked drug, which consequently implies that a single dose of PEG-asparaginase can replace 6–9 doses of native E. coli asparaginase injections. PEGylated asparaginase also reduced the toxicity in adults, but in kids despite the PEGylation, hypersensitivity reactions remain the main concern of toxicity [27,167,168]. Besides this consequence, it is still used as a primary drug for clinical trials in children because of its longer half life and reduced number of doses.

Unlike liposomes, which mostly encapsulate antitumoral molecules, conjugates cover a wider spectrum of therapeutic agents. For instance, Omontys®, a formulation that conjugates PEG with recombinant human erythropoietin (rHuEPO), is used for the treatment of anemia in patients with chronic kidney disease [8]. In the past two decades, pharmacological research has made a great effort in finding new agents with longer half-life and reduced receptor affinity compared to recombinant human erythropoietin (rHuEPO). This has been done by modifying the EPO molecule through changes in the aminoacid sequence and increase in the glycosylation pattern by addition of a PEGylated moiety (high-molecular weight erythropoietins) or more recently, by creating simpler molecules other than those with an EPO structure. rHuEPO is another option developed for achieving long half life time, consists of Omontys®, a small dimeric peptide conjugated to PEGylated moiety with a sequence completely unrelated to EPO and with a much lower molecular weight [30]. Omontys® has a half-life period between 21.5 and 59.7 h [169]. Clinical trials did not register any side effects, but recently (February 2013) there was a product recall of Omontys® due to the hypersensitivity reactions among patients, including life threatening and fatal events [30] and the reasons are still unknown.

3.4. Biodegradability

Using biodegradable materials is not an essential requirement for nDDS preparation. However, because of the toxicity related problems associated with liver and spleen, as well as the discomfort related to non-biodegradable materials, most of the currently used materials are biodegradables. As was exposed previously, biodegradation of nDDS is the dominant drug release mechanism over diffusion, and hence its control is key for temporal release profile. Biodegradation mechanisms mainly depend on the carrier material. For instance, liposomal and polymeric degradation rely on hydrolysis and peroxidation initiated by substances secreted by macrophages and other elements that belong to complementary system [121,170–172]. Enzymatic degradation of liposomes during blood circulation is not a significant process of biodegradation [173]. In general, oxidation is a slow process given that oxidative agents are produced by biological agents in small quantities and it is difficult to control. On the other hand, hydrolysis is a fast mechanism that can be controlled. In liposomes, hydrolysis is diminished via PEGylation [173], whereas for polymers the hydrolysis of polymers is highly dependent on the molecular structure and crystallinity. The more crystalline the polymer is, the lower the hydrolytic degradation rate of the polymer [76].

Biodegradation can be distinguished by the manner of erosion, like bulk and surface erosion. In surface erosion, biodegradation proceeds only at the surface such that the molar mass of the residual remains constant, while a fast mass loss is observed. In bulk erosion the mass loss is retarded but the molecular weight drops very fast due to degradation throughout the whole material [76]. The speed of degradation is also dependent on many factors such as the copolymer composition, autocatalysis by acidic degradation products inside a matrix and the presence of proteolytic drugs or other excipients. However, the impact of these parameters that increase or decrease the degradation velocity are not exactly clear [174].

PLGA-based nDDS offer various advantages as biodegradable systems, such as the possibility to control the resulting drug release accurately over prolonged periods of time, ease of administration, good biocompatibility and complete erosion [57]. However, its main mechanism of degradation is bulk erosion, which implies more intensive contact with the encapsulated drug. The degradation of PLGA is accelerated by an autocatalysis phenomena induced by the acidic pH produced by acidic residues of PLGA [57]. This acidic microclimate during degradation causes stability problems especially with acid-labile drugs (e.g. proteins) [175,176], and inflammatory processes. To solve this problem, alkaline salts or buffers were integrated to the polymer matrix [76], copolymerization of PLGA (or PLA) with hydrophilic domains such as PEG [177,178] forming di- or triblockcopolymers, or PLGA was replaced by poly(anhydrides), which undergo into surface erosion such that avoiding excessive interaction with the drug [179].

3.5. nDDS clearance time

Immediately after administration, nDDS can follow different routes (Fig. 5). The first and foremost thing expected of the nDDS is to circulate and extravagate until they reach the target tissue. However, unwanted interactions might also occur during this process that could lead to nDDS clearance from the body. For instance, interaction with endothelial walls occurs if the nDDS is cationic, and it inhibits systemic distribution and might leads to occlusion. Disintegration and decomposition of the nDDS are also possible, with consequent elimination from blood torrent through mononuclear phagocyte system (MPS), renal and lung filtration. Finally, nDDS might interact with plasma proteins and blood cells, leading to platelet aggregation, complementary system activation, or opsonization followed by phagocytosis or sequestration. Among these, opsonization is the most important phenomena and it is this process by which a foreign body is covered by opsonin proteins in such a way that they become more visible to phagocytic cells. The above mentioned processes are the route for clearance of foreign elements with sizes over critical value for renal filtration [180,181].

3.5.1. Factors affecting opsonization

Charge, molecular size and hydrophobicity are the factors that directly affect the opsonization process. It has been demonstrated that non-ionic particles are less opsonized than charged particles, and among the positively and negatively charged particles, the former ones interact more intensely with plasma proteins, cell membranes and connective tissues [73,182,183]. It is important to highlight that the charged particles show better internalization efficiency against neutral ones [73]. Hydrophobicity influences both the amount of opsonization and the identities of bounded plasmatic proteins on

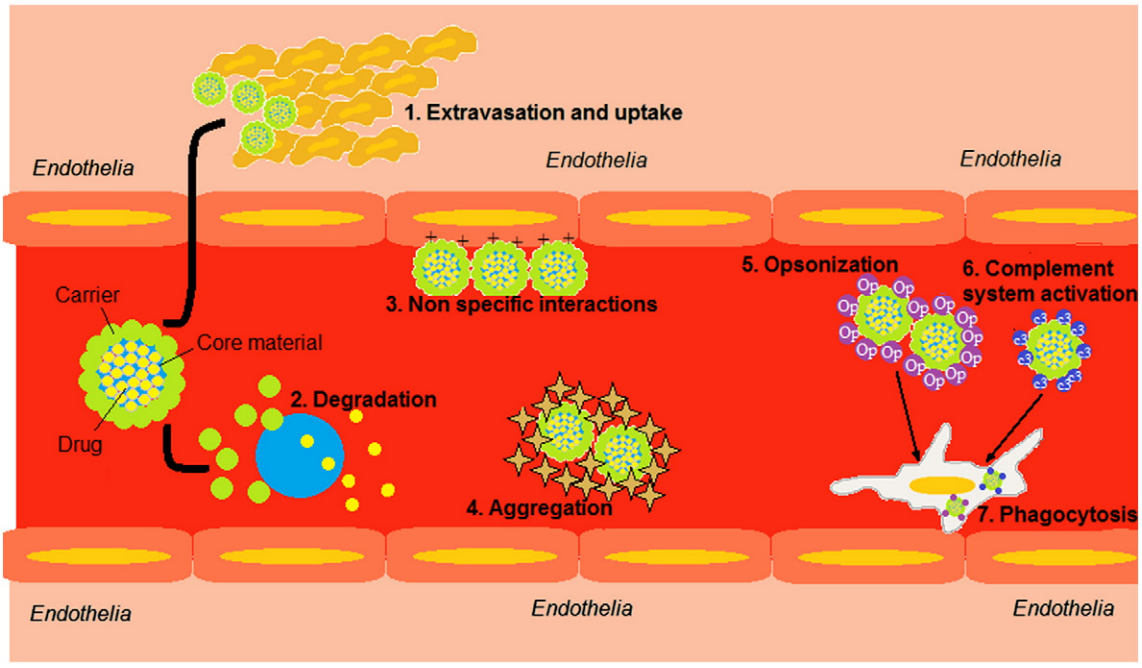

Fig. 5. Routes followed by nDDS after blood administration. (1) Extravasation through endothelia followed by nDDS uptake (2) nDDS can also be decomposed during circulation and the encapsulated drug gets degraded before internalization (3) Non specific adhesion of cationic carriers to negative endothelia of blood vessels leading to occlusion of the vessel (4) Platelet aggregation, an undesired event, which increase the risk of embolism (5) Opsonization with opsonin protein (6) activation of complement system leading to degradation and (7) phagocytosis.

particle surface, which affects the type of receptors with which the particles can interact [180,184,185]. The higher the hydrophobicity is, the faster the opsonization and clearance. It is the molecular size that determines whether the particles get filtered renally or not. DDS with a hydrodynamic radio of less than 150 nm [73,186,187] are rapidly eliminated through kidneys with almost no extravasation towards tissues [188]. Over this value, the MPS can act on them. Some researchers have also demonstrated that the smaller particle size show slower eliminations by the MPS [73,180,185], thus increasing their accumulation in targeted tissues.

3.5.2. Stealth nDDS

nDDS against microDDS have a short blood circulation time after systemic administration, which leads to minimized therapeutic effects. However, this problem was solved by surface modification through conjugation of nDDS or naked drug with PEG chains, the standard conjugation polymer. This surface treatment seeks to "hide" nDDS from MPS so that the opsonization degree is reduced [189–191].

PEG is a hydrophilic and bioinert polymer that functions as "proteic repellent" due to its flexible chains, high number of possible conformations and hydration degree. In this way, PEG sterically prevents non-specific interactions with biological medium and at the same time increases solubility, biocompatibility and circulation time as well as decreases platelet aggregation and immunogenicity. Furthermore, PEG is useful to get conjugated with small particles (i.e. enzymes, cytokines and antibodies) and thus increase their molecular sizes over renal filtration threshold. However, the disadvantage of PEGylation is that it decreases the internalization efficiency [189,192]. Until now, PEG is considered as the "gold standard" polymer for production of stealth particles. Currently, there are many commercialized nDDS that use PEG as stealth polymer (i.e. Doxil®, Xyotax®, Lipoplatin®). Nevertheless, multiple efforts have been done towards the introduction of choices with better compatibility and circumvention PEG patents. For instance, poly(hydroxypropyl methacrylate) is another polymer used for stealth and is currently under clinical trials (conjugated with doxorubicin, camptothecin and paclitaxel) [193]. Also, the use of bolaamphiphilic molecules for preparation of liposomes is a promising option to replace PEG. They are a unique class of lipids that bear two hydrophilic head groups situated at both ends of the hydrophobic domain, leading to the formation of a monolayer lipid-based membrane, increase the water solubility and produce higher clearance times. These structures are potentially more stable than classical bilayer liposomes and are less likely to fuse with each other or with cell membranes due to their reduced lipid exchange [16].

4. Specific targeting and control

The main advantage of nDDS over macrosystems is their ability to not only control the temporal release profile of drugs but also their spatial distribution in a precise manner. This property is known as specific targeting and can be achieved by two mechanisms, namely passive and active targeting.

4.1. Passive targeting

Passive targeting is the employment of some pathophysiological features of targeted tissue that allow accumulation of nDDS and drug release inside it, without using any ligands or external stimuli. For instance, the negative charge of mucosal membrane of some tissues (e.g. ocular, respiratory and gastrointestinal) [194–196] as well as the increased vascular permeability of infected and inflammed tissues [197] are the common pathophysiological features for passive targeting. The most applied pathophysiological phenomenon is called the Enhanced Permeability and Retention Effect (EPR) [198], which happens in tumors. It involves extensive angiogenesis, high vascular density, defective vascular architecture, impaired lymphatic clearance from the interstitial space of tumor tissue and increased permeability induced by various vascular mediators. The pore size of tumor vasculature range between 100–1000 nm, whereas the healthy vasculature pore size is around 10 nm [199]. The aforementioned points imply that nDDS will highly accumulate inside the tumors than in other tissues, and this serves as the basic functioning mechanism of almost all commercial nDDS for chemotherapy (i.e., Doxil®, Abraxane® and Myocet®), though it is not enough to ensure an efficient cell uptake of the drug.

4.2. Active targeting

In active targeting, the targeting ligands are grafted to the nDDS surface. The ligands are recognized by the target cells and interact with cognate receptors, thus mediating cell specificity and sometimes even enhancing the nanoparticle uptake. Different types of ligands such as the antibodies [200–203], growth factors [204,205], vitamins [206], carbohydrates [207,208] and aptamers [209,210] have been successfully tested *in vitro*.

Active targeting is not a targeting method (*per se*) and hence it cannot happen without the occurrence of passive targeting first. Therefore, all the nDDS reach target tissue through the passive targeting. Ligand addition do not increase the quantity of nanoparticles that accumulate within the target tissue but only increase the nanoparticle uptake efficiency that previously reached the target cell through passive process. This would also mean that the active targeted nDDS makes sense for tissues that can be previously accessed by passive way. For this reason, in many active targeted tissues there were either no or small improvement in comparison with passive systems, even if there was premature content release, low penetrability, no internalization or low receptor density at targeted cells. An additional drawback is that ligand utilization increases immunogenicity and proteic adsorption. Due to these reasons, there are only a few of active targeted nDDS that are under advanced clinical trials [15,211,212]. In tumors, for an effective active targeting, successful extravasation followed by an effective penetration is necessary until it reach the targeted cell. The first phenomenon can be hindered by different heterogeneities of each patient (i.e. partial increase of tumor vasculature permeability), whereas the second one can be stopped because of high interstitial pressures, high number of pericytes (or smooth muscle cells) surrounding vessels or high cell density [15]. All the above mentioned facts hamper the clinical approval of active targeted nDDS.

5. Manufacturing techniques

Selection of the manufacture technique is a key point for nDDS design because it can affect the response and release of drugs. For instance, system deformation might happen during mechanical loading involved in the process, or bioactivity changes might occur by interaction of materials with organic solvents. Also, scaling up of the technique must be taken into account to ensure successful industrialization. Table 4 presents a summary of the main DDS manufacturing techniques.

5.1. Conventional nDDS fabrication techniques

5.1.1. Nanoprecipitation

Nanoprecipitation, also called as the solvent displacement method, is one of the most commonly used methods for nDDS fabrication. Typically, this method is used for hydrophobic drug entrapment, but it has been adapted for hydrophilic drugs as well. It is performed by adding an organic solution which contains the carrier material and lipophilic drugs into an aqueous solution in a drop-wise manner under constant stirring. Nanoparticles containing drugs form instantaneously as the organic solvent diffuses to the aqueous phase. Finally, the solvent is removed under reduced pressure and particles are obtained (Fig. 6) [128]. The miscibility of the solvent with water is the most critical parameter governing the outcome of this process. The rate of solution addition and stirring speed also influence the size and drug loading level. Particle size formed by this method is around 200 nm, which is typically smaller than those produced by other processes. This method can be applied to a wide range of polymers, peptides and amphiphilic cyclodextrins. However, scaling up of the process might turn inefficient due to the pattern of drop-wise addition required during this process. High loading efficiencies are generally reported for lipophilic drugs using this method, but its usefulness is limited to water-miscible solvents [155,213].

5.1.2. Methods based on Emulsification

This group of methods is also known as two-step nDDS formation. In the first step, the organic phase containing the carrier material and drugs are vigorously agitated or sonicated in the aqueous phase to form emulsified droplets. Depending on the emulsified system (from nanoemulsion to macroemulsions) used, the eventual particle size and drug loading varies. Double emulsions are also used for the preparation of core-shell vesicular structures. In the second step, the solvent is eliminated by evaporation, diffusion or salting out (Fig. 6), and finally the particles are precipitated out. The application of emulsification techniques are greatly limited due to its disadvantages like working with toxic solvents (i.e. dichloromethane, chloroform) and the requirement of high energy apparatus (i.e. ultrasound probe or homogenizer). In contrast, nanoprecipitation involves spontaneous formation of particles and it does not require a source of external energy and uses less toxic organic solvents [214].

5.1.2.1. Emulsification-solvent evaporation. Emulsification-solvent evaporation is the most common technique where solvent elimination is possible. In this method, the carrier material is dissolved in a volatile solvent and emulsified in an aqueous phase. Formation of nDDS is achieved by evaporation of the solvent under reduced pressure. Further, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants and finally, the product is lyophilized [213]. In the past, dichloromethane and chloroform preformed polymer were widely used, but nowadays they are replaced with ethyl acetate due to its better toxicological profile [213]. Nevertheless, this is a slow process compared to nanoprecipitation, which happens in milliseconds. The size of nanoparticles drops to minimal during the first 40 min, and increases in the second 40 min due to coalescence of emulsion droplets [215]. It is the coalescence that determines the final particle size which is largely dependent on the evaporation conditions. Adjusting solvent evaporation conditions such as the temperature and pressure would improve the quality of the nanoparticles. The use of a surfactant such as sodium dodecyl sulphate (SDS) or poly(vinyl alcohol) (PVA) might also minimize the coalescence effect and produce smaller nanoparticles [216].

Emulsion-solvent evaporation is widely used to encapsulate lipophilic drugs. However, the loading level for hydrophilic drugs, such as proteins and peptides is generally poor due to the diffusion of hydrophilic drug into the aqueous phase before the polymer can solidify to entrap the drug. To overcome this problem, water-in-oil-in-water (W/O/W) double emulsion is used to reduce the loss of drug and also to preserve the bioactivity of delicate drugs such as proteins in the aqueous phase [155].

Emulsification-solvent evaporation is the most common technique for PLGA-based nDDS manufacture [43]. Andreas *et al.* encapsulated insulin in PLGA particles through this technique. Insulin was intended to be used as growth factor for cartilage tissue. To reach that aim, they proposed the application of emulsification-solvent evaporation method. Three different approaches that differed in the sequence of solvents added were applied in this work: (i) solid-in-oil-in-water (s/o/w), (ii) water-in-oil-in-water (w/o/w), (iii) oil-in-oil-in-water (o/o/w). The W/O/W, as demonstrated as the most appropriate technique, produced high encapsulation efficiency and low initial burst release. Insulin released from these particles stimulated proteoglycans and collagen type II secretion that eventually supported cartilage formation [217].

5.1.2.2. Emulsification-solvent diffusion. In this method, the carrier is dissolved in a partially water-miscible solvent (eg., benzyl alcohol, propylene carbonate). This is mainly carried out to promote diffusion of the solvent of the dispersed phase due to its dilution with excess amount of water. A typical emulsification method is then used to produce oil-in-water (O/W) emulsion droplets from the water-polymer saturated solvent. The dispersed droplets are then diluted using copious amounts of water containing a stabilizer. The diffusion of organic solvent out

Table 4
Summary of main DDS manufacture techniques.

	Manufacture Techniques						
	Conventional				Non Conventional		
	Nanoprecipitation	Solvent Evaporation	Solvent Difussion	Salting Out	Microfluidics	Top-Down techniques	Electrospraying
Simplicity	Medium	High	Medium	High	Low	Low	High
Scalability	Low	Low	Low	Low	High	High	Medium
Safety of Compounds	Low	Low	Low	High	High	Medium	Medium

from the droplets leads to the condensation of materials within the droplet and NPs are formed. Finally, the solvent is eliminated by evaporation or filtration, depending on its boiling point. The solvent extraction process takes places within a few milliseconds, causing a drop in particle size. In general, the diameter of particles prepared by this method is around 150 nm. Due to the fast solvent extraction kinetics and well-defined solvent–water interaction, the physical properties of nDDS prepared by this method is highly reproducible while the polydispersity is significantly lower than nDDS prepared by other conventional methods [155,213].

Emulsification solvent-diffusion is suitable for hydrophobic drugs. For instance, Know *et al.* encapsulated estrogen in PLGA nanoparticles by using this method. These researchers used propylene carbonate as a partially water-soluble solvent, which was removed by dialysis after its diffusion towards aqueous phase and studied on the stabilizer effect towards particle sizes. Their results showed an encapsulation efficiency of 67% and particle sizes to range from 78 and 204 nm, depending on the type of stabilizer used [218]. High entrapment efficiency (generally >70%), high batch-to-batch reproducibility, and the use of low toxicity solvents are advantages of the method. However, the disadvantage of the method includes usage of copious amounts of water followed by its elimination from the suspension.

5.1.2.3. Emulsification-salting out. Emulsification-salting out is a derivative of the emulsification-solvent diffusion method. In this case, the organic solvent (e.g., acetone) used is totally miscible with water. The polymer-containing solvent is emulsified in an aqueous phase containing high concentration of a salt (magnesium chloride, calcium chloride) or sucrose. The saturated aqueous solution prevents acetone from mixing with water. Dilution of the emulsion droplets with large amounts of water results in an abrupt drop of salt concentration within the continuous phase, leading to the extraction of organic solvent and precipitation of NPs. This method works exclusively for lipophilic drugs. The choice of salting-out agent greatly influences the size of the particle and drug encapsulation efficiency, whereas mechanical mixing and stabilizer concentration had little effect. The main advantage of salting out is that it minimizes stress to protein encapsulants. Moreover, salting out does not require an increase of temperature hence beneficial for processing of heat sensitive substances. Emulsification-salting out also helps to avoid the use of organic chlorinated solvents and large amounts of stabilizer during formulation. High drug loadings can be achieved, depending on the solubility of the drug in acetone and on the nature of the salting-out agent. The greatest disadvantages include its exclusive application to lipophilic drugs and the extensive washing of nanoparticles [155,213].The use of acetone and large amounts of salts may raise some concern about recycling of the salts and compatibility with active compounds [214].

5.2. Non conventional techniques of fabrication

Conventional nDDS fabrication techniques have many disadvantages, such as particle-size polydispersity, low drug-loading efficiency, difficulties for incorporation of hydrophilic drugs and batch-to-batch variations [145,219]. For instance, the final size of the nDDS generated by emulsion-based technique is directly determined by the size of the emulsion droplets, which itself could be very heterogeneous during

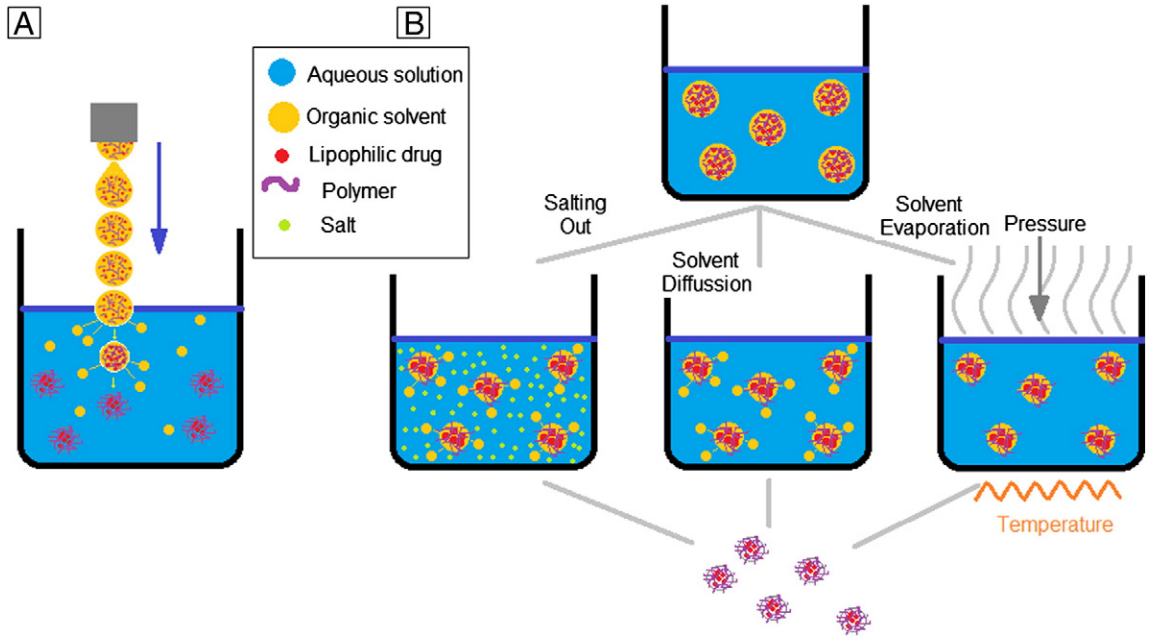

Fig. 6. Conventional DDS manufacturing techniques. (A) Nanoprecipitation (B) Emulsification-based methods. Emulsified oil-in-water droplets containing polymer and drugs are formed in the first step. In the second step, different methods are applied to remove the solvent and precipitate nanoparticles. (i) Salting out (ii) Solvent diffusion (iii) Solvent evaporation.

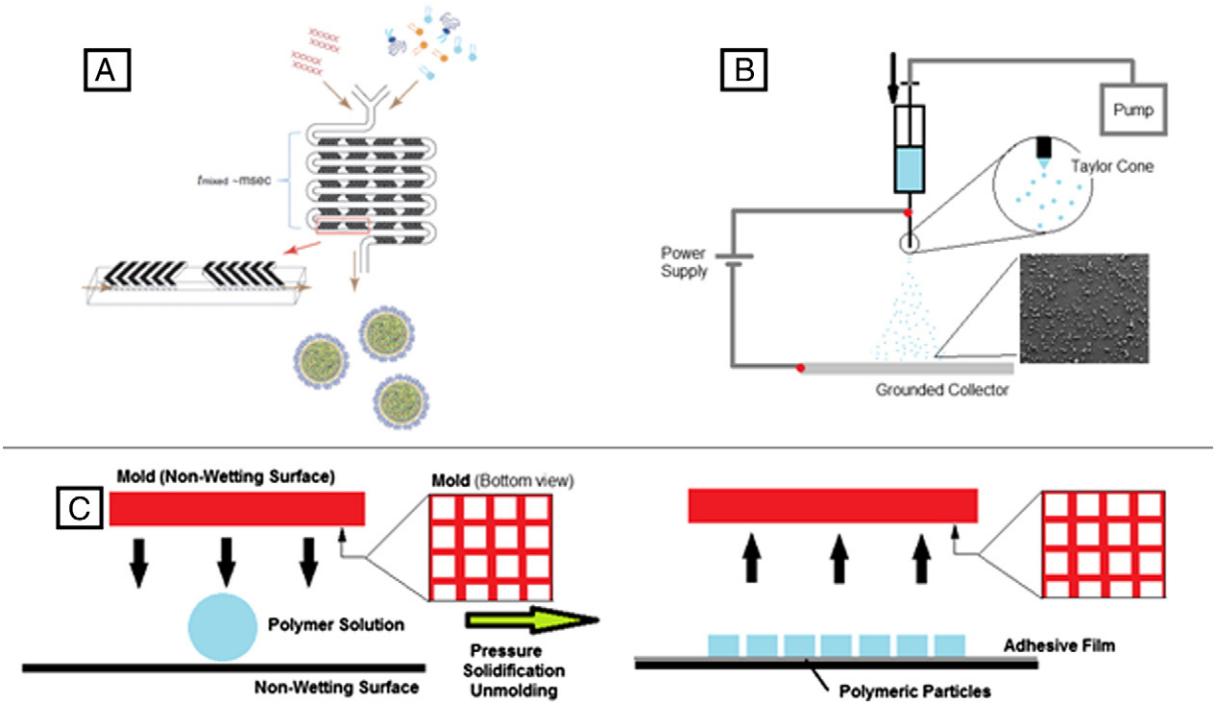

Fig. 7. Non conventional techniques (A) Schematics of microfluidic device for siRNA-loaded liposome manufacture. *Figure reproduced with copyright permission from Belliveau et al.* [220] *obtained from NPG* (B) Schematics of Electrospraying set up (C) Method of Particle replication in Non-wetting Template (PRINT®).

bulk mixing. Below are the described techniques that solve some of these problems. Fig. 7 shows a graphical summary of them.

5.2.1. Microfluidics

Microfluidics is the manipulation of fluid in nano/picoliter scale channels, and it presents exciting opportunities to improve the fabrication of nDDS. There are different approaches: flow-focusing configuration, in-flow focusing and concentric capillaries (Fig. 8). The most common is the flow-focusing approach, where basically two streams containing continuous and disperse phases are infused into two separate inlets, and the disperse phase is confined to isolated droplets or narrow stream at T-junction. This approach is mainly used for polymer based nDDS whereas liposomes are produced through electroformation-based microfluidic systems.

The second one involves spreading lipids dissolved in an organic solvent, such as chloroform, on the surface of a planar electrode and subsequent evaporation of the solvent by vacuum dessication to form a dry phospholipidic film which is immersed in an aqueous solution. Finally, an electrical field is applied across the lipid film and surrounding buffer. The lipids interact with the aqueous solution and electric field by "peeling off" the electrode surface in layers and self-assembling into giant but polydisperse, multilamellar vesicles (Fig. 7A). Emulsification-based, extrusion-based and pulsed-jeting microfluidic configurations are also used for liposome manufacturing [221]. Belliveau *et al.* [220] encapsulated small interfering RNA (siRNA) in liposomes through emulsification-based microfluidic device. This liposomal formulation can silence therapeutically relevant genes in a variety of animal models and are in clinical trials for treatment of cardiovascular disease, liver cancer and other disorders. Basically, lipids dissolved in ethanol and siRNA in aqueous solution were pumped into the two inlets of the microfluidic mixing device using a syringe pump. The shape of the channels (herringbone structure) induce chaotic advection of the laminar streams causing rapid mixing of the ethanol and aqueous phases with corresponding increase in the polarity of the lipid solution. At a critical polarity, liposomes get precipitated. Microfluidics devices are also used for the production of polymeric nanoparticles [222], polymersomes [223] and polymeric conjugates [224] among other architectures.

The general benefits of conducting reaction in microfluidics chips include rapid mixing of reagents, reaction flexibility for multi-step reaction design, enhanced processing accuracy and efficiency, better heat transfer due to high surface-to-volume ratio, miniaturization, cost savings from reduced consumption of reagents and better controllability over the physical properties of drug carriers. Also, it allows for on-line quality control and is amenable to scale-up [155,225].

5.2.2. Electrospraying

Electrospraying, also called EHD atomization (EHDA), is a promising technique for preparation of micro- and nanoparticles suitable as drug-delivery systems. The typical electrospraying setup consists of three main components: high-voltage power supply, spinneret, and conductive plate as collector, as can be seen in Fig. 7B. The principle of electrospraying is based on the theory of charged droplets; stating that an electric field applied to a liquid droplet exiting a capillary is

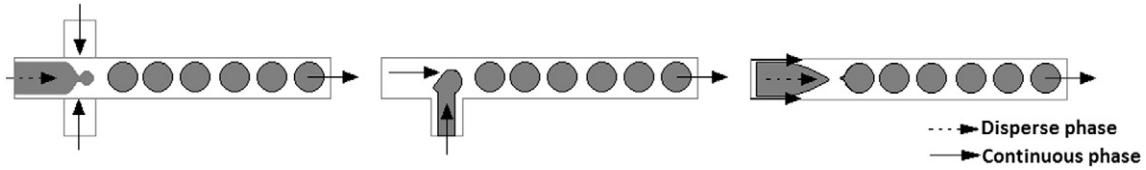

Fig. 8. Microfluidic approach (A) Flow focusing, (B) T-junction, and (C) Concentric capillaries. *Figure reproduced with copyright permission from Zhang et al.* [155] *obtained from Elsevier.*

able to deform the interface of the droplet. The electric charge generates an electrostatic force inside the droplet which competes with the surface tension of the droplet, forming the Taylor cone, characteristic of a charged droplet. Eventually, the electrostatic force, generated by the use of high voltage on the capillary, is able to overcome the surface tension of the droplet. The excess charge then needs to be dissipated and smaller charged droplets on the micro to nano-scale are ejected from the primary droplet, thus reducing its charge without significantly reducing its mass. Due to Coulomb repulsion of the charges, the droplets disperse well and do not coalesce during their flight toward the collector. Once the droplets are ejected from the Taylor cone according to the theory of charged droplets, solvent evaporation leads to the progressive contraction and solidification of droplets resulting in solid polymeric particles deposited on the collector [226–228]. In general, it is also the same setup that can be used for electrospinning of nanofibers, with modification of parameters such as solution properties, voltage applied or distance from tip to collector are modified. Many authors reported the use of electrospun mats as successful nanoengineered drug delivery systems [229,230]. Nanofibers possess high surface area with ability to incorporate a wide range of drugs and have the ease of fabrication, thus serving as a platform for controlled drug release. Nevertheless, they lack the features for passive targeting, which is the main property of the nDDS and hence they are not discussed further in this review. So far, with this technique only polymeric nDDS have been obtained. For example, Xie *et al.* encapsulated paclitaxel in PCL and PLGA electrosprayed nanoparticles. Depending on the flow rate, molecular weight of the polymer and solvent used, the encapsulation efficiencies varied between 78 to 82%, while loading capacities varied from 8 to 16% [231]. Albumin [232,233], PLA [234] and chitosan [235] are also electrosprayed for drug delivery applications. The main advantages of EHDA over other conventional encapsulating methods are higher loading efficiency, narrow particle-size distribution, and ease of particle synthesis due to single-step processing. Furthermore, electrospraying would eliminate the need for using a surfactant or additional template [227].

5.2.3. Top-down techniques

As discussed earlier, the capacity to achieve large size differences and shape variation is greatly limited by the nature of the self-assembly process. Consequently, the top-down methods are considered attractive because of its ability to produce particulate DDS with well-controlled size and shape.

5.2.3.1. Particle replication in non-wetting template (PRINT). PRINT® (Fig. 7C) first introduced in 2005, is a top-down technique to fabricate monodisperse particles with precise particle structures. A non-wetting perfluoropolyether (PFPE) elastomeric mold containing wells or cavities of predefined shape and size is used to fabricate particles. The polymer liquid solution containing the cargo is confined in the cavities by pressure applied between the mold and the PFPE surface, followed by crosslinking or solvent evaporation [236]. The low surface energy of PFPE prevents the overflow of polymer solution to non-cavity regions, leading to well-isolated NP formation. Using this method, particles from 80 nm to 20 μm are produced. Fabrication and applications of PRINT were described earlier by DeSimone and co-workers [236]. However, PRINT® technology has no significant influence on the pharmaceutical market, though it is an interesting approach that utilizes an available technology for the large-scale production of nanostructures.

From the aforementioned techniques (Table 4), and considering FDA approval as a target for design, electrospraying and microfluidics represent the best choice for nDDS manufacture. Indeed, the former one could be used at a laboratory stage for optimization of nDDS due to its low cost, time and easiness, whereas the second one could be applied at an industrial stage, based on the results obtained for electrosprayed nDDS.

6. Conclusion

Further to the approval and consequent commercialization of AmBisome® in the 1990s, competition between the pharmaceutical companies was triggered. Many new nDDS have been investigated (e.g. polymersomes, micelles, dendrimers) and approved (e.g. albumin nanoparticles, micelles, PEGylated liposomes) since then, but liposomes keep leading the nDDS market. Thanks to their biodegradability, biocompatibility, predictable interaction mechanism with membrane components, and ability to efficiently encapsulate a wide range of molecules. The design of successful nDDS implies not only in understanding the effect of multiple parameters affecting the release profile, but also other factors such as the possibility of scaling-up the manufacturing processes and product safety considerations. For instance, synthetic polymeric nanoparticles seem to be promising nDDS due to the ease of regulation of their release profiles and the possibility of using scalable manufacture techniques, but the inflammation produced by the acidic microenvironment after degradation and the intense interaction between drugs and carrier material during bulk erosion is delaying their clinical approval. The success of nDDS design must be centered on a clinical perspective aiming towards its commercialization. The transfer of knowledge from the academic world to the industrial one is expected to reach a climax with competition, and the real life nDDS that cause less pain will be embraced by the patients.

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References

[1] J. Folkman, How the field of controlled-release technology began, and its central role in the developmenot f angiogenesis research, Biomaterials 11 (1990) 615–618.
[2] A.S. Hoffman, The origins and evolution of “controlled” drug delivery systems, J. Control. Release 132 (3) (2008) 153–163.
[3] H. Okada, et al., Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers, Pharm. Res. 11 (1994) 1143–1147.
[4] A.D. Bangham, M.M. Standish, J.C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 13 (1965) 238–252.
[5] P. Boisseau, B. Loubaton, Nanomedicine, nanotechnology in medicine, C. R. Physique 12 (2011) 620–636.
[6] J.A. DiMasi, R.W. Hansen, H.G. Grabowski, The price of innovation: new estimates of drug development costs, J. Health Econ. 22 (2) (2003) 151–185.
[7] K.I. Kaitin, Deconstructing the drug development process: the new face of innovation, Clin. Pharmacol. Ther. 87 (3) (2010) 356–361.
[8] A.C. Eifler, C.S. Thaxton, Nanoparticle therapeutics: FDA approval, clinical trials, regulatory pathways, and case study, Methods Mol. Biol. 726 (2011) 325–338.
[9] R.K. Verma, S. Garg, Current status of drug delivery technologies and future directions, Pharm. Technol. On-line 25 (2001) 1–14.
[10] J. Adler-Moore, R.T. Proffitt, Am Bisome: liposomal formulation, structure, mechanism of action and pre-clinical experience, J. Antimicrob. Chemother. 49 (2002) 21–30.
[11] M.L. Etheridge, et al., The big picture on nanomedicine: the state of investigational and approved nanomedicine products, Nanomedicine 9 (1) (2013) 1–14.
[12] F. Canal, J. Sanchis, M.J. Vicent, Polymer–drug conjugates as nano-sized medicines, Curr. Opin. Biotechnol. 22 (6) (2011) 894–900.
[13] A. Aubert-Pouessel, et al., In vitro study of GDNF release from biodegradable PLGA microspheres, J. Control. Release 95 (3) (2004) 463–475.
[14] E. Igarashi, Factors affecting toxicity and efficacy of polymeric nanomedicines, Toxicol. Appl. Pharmacol. 229 (1) (2008) 121–134.
[15] T. Lammers, et al., Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress, J. Control. Release 161 (2) (2012) 175–187.
[16] A. Puri, L.K., B. Smith, J.H. Lee, A. Yavlovich, E. Heldman, R. Blumenthal, Lipid-Based Nanoparticles as Pharmaceutical Drug Carriers: From Concepts to Clinic, Crit. Rev. Ther. Drug Carrier Syst. 26 (6) (2009) 523–580.
[17] G. Pratt, et al., Liposomal Daunorubicin: In Vitro And In Vivo Efficacy In Multiple Myeloma, Hematol. Oncol. 16 (1998) 47–55.
[18] M. Benesch, C. Urban, Liposomal cytarabine for leukemic and lymphomatous meningitis: recent developments, Expert. Opin. Pharmacother. 9 (2008) 301–309.

[19] N.M. Bressler, S.B. Bressler, Photodynamic Therapy with Verteporfin (Visudyne): Impact on Ophthalmology and Visual Sciences, Invest. Ophthalmol. Vis. Sci. 41 (3) (2000) 624–628.
[20] M. Alam, C.T. Hartick, Extended-Release Epidural Morphine (DepoDur(tm)): An Old Drug with a New Profile, Pain Pract. 5 (2005) 349–359.
[21] J.E. Frampton, A.J. Wagstaff, Sucrose-Formulated Octocog Alfa A Review of its Use in Patients with Haemophilia A, Drugs 68 (2008) 839–853.
[22] L. Boehlke, J.N. Winter, Sphingomyelin/cholesterol liposomal vincristine: a new formulation for an old drug, Expert. Opin. Biol. Ther. 6 (2006) 409–415.
[23] C.E. Swenson, et al., Liposome technology and the development of Myocet TM (liposomal doxorubicin citrate), Breast 2 (2001) 1–7.
[24] K.A. Gelmon, et al., Phase I Study of Liposomal Vincristine, J. Clin. Oncol. 17 (1999) 697–705.
[25] R.T.P. Poon, N. Borys, Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer, Expert. Opin. Pharmacother. 10 (2009) 333–343.
[26] B.E. Bax, et al., In vitro and in vivo studies with human carrier erythrocytes loaded with polyethylene glycol-conjugated and native adenosine deaminase, Br. J. Haematol. 109 (2000) 549–554.
[27] P.A. Dinndorf, et al., FDA drug approval summary: pegaspargase (oncaspar) for the first-line treatment of children with acute lymphoblastic leukemia (ALL), Oncologist 12 (8) (2007) 991–998.
[28] Y.S. Wang, et al., Structural and biological characterization of pegylated recombinant interferon alpha-2b and its therapeutic implications, Adv. Drug Deliv. Rev. 54 (2002) 547–570.
[29] S. Schreiber, et al., Maintenance Therapy with Certolizumab Pegol for Crohn's Disease, N. Engl. J. Med. 357 (2007) 239–250.
[30] F. Locatelli, L. Del Vecchio, Peginesatide as a new approach for treating anemia of CKD patient: is it like a falling star? Expert. Opin. Pharmacother. 14 (2013) 1277–1280.
[31] J.W. Singer, Paclitaxel poliglumex (XYOTAX, CT-2103): a macromolecular taxane, J. Control. Release 109 (1–3) (2005) 120–126.
[32] M.R. Sherman, M.G. Saifer, F. Perez-Ruiz, PEG-uricase in the management of treatment-resistant gout and hyperuricemia, Adv. Drug Deliv. Rev. 60 (1) (2008) 59–68.
[33] P.R. Hamann, et al., Gemtuzumab Ozogamicin, A Potent and Selective Anti-CD33 Antibody-Calicheamicin Conjugate for Treatment of Acute Myeloid Leukemia, Bioconjug. Chem. 13 (2002) 47–58.
[34] G.A. Wiseman, et al., Radioimmunotherapy of Relapsed Non-Hodgkin's Lymphoma with Zevalin, a 9 yttrium Anti-CD20 Monoclonal Antibody, Clin. Cancer Res. 5 (1999) 3281–3286.
[35] J.M. Vose, Bexxar(r): Novel radioimmunotherapy for the treatment of low-grade and transformed low-grade non-Hodgkin's lymphoma, Oncology 9 (2004) 160–172.
[36] J. Deckert, et al., A novel anti-CD37 antibody–drug conjugate with multiple antitumor, Blood 322 (2013) 3500–3510.
[37] K. Alhareth, et al., HPLC quantification of doxorubicin in plasma and tissues of rats treated with doxorubicin loaded poly(alkylcyanoacrylate) nanoparticles, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 887–888 (2012) 128–132.
[38] H. Chen, et al., Nanonization strategies for poorly water-soluble drugs, Drug Discov. Today 16 (7–8) (2011) 354–360.
[39] T.S. Harrison, K. Goa, Low-acting Risperidone. A review of its use in schizophrenia, CNS Drugs 18 (2004) 113–132.
[40] M.R. Green, et al., Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer, Ann. Oncol. 17 (8) (2006) 1263–1268.
[41] T.Y. Kim, et al., Phase I and pharmacokinetic study of genexol-pm, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[42] A. Kumari, S.K. Yadav, S.C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, Colloids Surf. B Biointerfaces 75 (1) (2010) 1–18.
[43] F. Danhier, et al., PLGA-based nanoparticles: an overview of biomedical applications, J. Control. Release 161 (2) (2012) 505–522.
[44] S. Kim, et al., Engineered polymers for advanced drug delivery, Eur. J. Pharm. Biopharm. 71 (3) (2009) 420–430.
[45] A. Jaklenec, et al., Sequential release of bioactive IGF-I and TGF-beta 1 from PLGA microsphere-based scaffolds, Biomaterials 29 (10) (2008) 1518–1525.
[46] Z. Wang, P.C. Ho, A nanocapsular combinatorial sequential drug delivery system for antiangiogenesis and anticancer activities, Biomaterials 31 (27) (2010) 7115–7123.
[47] R.D. Hofheinz, et al., Liposomal encapsulated anti-cancer drugs, Anti-Cancer Drugs 16 (2) (2005) 691–707.
[48] M.E. Froudarakis, et al., Phase 1 Trial of Lipoplatin and Gemcitabine as a Second-line Chemotherapy in Patients With Nonsmall Cell Lung Carcinoma, Cancer 113 (10) (2008) 2752–2760.
[49] J.A. Silverman, S.R. Deitcher, MarqiboC (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine, Cancer Chemother. Pharmacol. 71 (2013) 555–564.
[50] H.J. Müller, et al., Pegylated asparaginase (OncasparTM) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols, Br. J. Haematol. 110 (2000) 379–384.
[51] H.S. Yoo, T.G. Park, Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer, J. Control. Release 70 (2001) 63–70.
[52] J. Park, et al., PEGylated PLGA nanoparticles for the improved delivery of doxorubicin, Nanomedicine 5 (4) (2009) 410–418.
[53] J.M. Korth-Bradley, et al., Impact of age and gender on the pharmacokinetics of gemtuzumab ozogamicin, Pharmacotherapy 21 (2001) 1175–1180.
[54] G.A. Wiseman, et al., Biodistribution and dosimetry results from a phase III prospectively randomized controlled trial of Zevalin(tm) radioimmunotherapy for low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma, Crit. Rev. Oncol. Hematol. 39 (2001) 181–194.
[55] D.W. Nyman, et al., Phase I and Pharmacokinetics Trial of ABI-007, a Novel Nanoparticle Formulation of Paclitaxel in Patients With Advanced Nonhematologic Malignancies, J. Clin. Oncol. 23 (31) (2005) 7775–7793.
[56] A.K. Singla, A. Garg, D. Aggarwal, Paclitaxel and its formulations, Int. J. Pharm. 235 (2002) 179–192.
[57] N. Faisant, J. Siepmann, J.P. Benoit, PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release, Eur. J. Pharm. Sci. 15 (2002) 355–366.
[58] Y. Yeo, K. Park, Control of encapsulation efficiency and initial burst in polymeric microparticle systems, Arch. Pharm. Res. 27 (2004) 1–12.
[59] X. Huang, C.S. Brazel, On the importance and mechanism of burst release in matrix-controlled drug delivery systems, J. Control. Release 73 (2001) 121–136.
[60] A.S. Hasan, et al., Effect of the microencapsulation of nanoparticles on the reduction of burst release, Int. J. Pharm. 344 (1–2) (2007) 53–61.
[61] Y.Y. Huang, T.W. Chung, T.W. Tzeng, A method using biodegradable polylactides: polyethylene glycol for drug release with reduced initial burst, Int. J. Pharm. 182 (1999) 93–100.
[62] R. Cheng, et al., Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery, Biomaterials 34 (14) (2013) 3647–3657.
[63] P. Bawa, et al., Stimuli-responsive polymers and their applications in drug delivery, Biomed. Mater. 4 (2) (2009) 022001.
[64] A.T. Stopeck, et al., Phase II study of direct intralesional gene transfer of allovectin-7 and HLA-B7/β2-microglobulin DNA–liposome complex, in patients with metastatic melanoma, Clin. Cancer Res. 7 (2001) 2285–2291.
[65] R. Chowdhery, R. Gonzalez, Immunologic therapy targeting metastatic melanoma: Allovectin-7(r), Immunotherapy 3 (2011) 17–21.
[66] N. Mylonakis, et al., Phase II study of liposomal cisplatin (Lipoplatin) plus gemcitabine versus cisplatin plus gemcitabine as first line treatment in inoperable (stage IIIB/IV) non-small cell lung cancer, Lung Cancer 68 (2) (2010) 240–247.
[67] T. Ta, T.M. Porter, Thermosensitive liposomes for localized delivery and triggered release of chemotherapy, J. Control. Release 169 (1–2) (2013) 112–125.
[68] D. Schmaljohann, Thermo- and pH-responsive polymers in drug delivery, Adv. Drug Deliv. Rev. 58 (15) (2006) 1655–1670.
[69] I. Tan, et al., PEGylated Chromatography: Efficient Bioseparation on Silica Monoliths Grafted with Smart Biocompatible Polymers, ACS Appl. Mater. Interfaces 1 (9) (2009) 1869–1872.
[70] J. Kost, R. Langer, Responsive polymeric delivery systems, Adv. Drug Deliv. Rev. 64 (2012) 327–341.
[71] A.S. Hoffman, Stimuli-responsive polymers: biomedical applications and challenges for clinical translation, Adv. Drug Deliv. Rev. 65 (1) (2013) 10–16.
[72] V. Torchilin, Multifunctional and stimuli-sensitive pharmaceutical nanocarriers, Eur. J. Pharm. Biopharm. 71 (3) (2009) 431–444.
[73] C. He, et al., Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles, Biomaterials 31 (13) (2010) 3657–3666.
[74] Y.S. Tarahovsky, "Smart" liposomal nanocontainers in biology and medicine, Biochem. Mosc. 75 (7) (2010) 811–824.
[75] S.R. MacEwan, D.J. Callahan, A. Chilkoti, Stimulus-responsive macromolecules and nanoparticles for cancer drug delivery, Nanomedicine (Lond) 5 (5) (2010) 793–806.
[76] S. Grund, M. Bauer, D. Fischer, Polymers in Drug Delivery—State of the Art and Future Trends, Adv. Eng. 13 (3) (2011) 61–87.
[77] M. Nakayama, et al., Molecular design of biodegradable polymeric micelles for temperature-responsive drug release, J. Control. Release 115 (2006) 46–56.
[78] T. Tagami, M.J. Ernsting, S.D. Li, Optimization of a novel and improved thermosensitive liposome formulated with DPPC and a Brij surfactant using a robust in vitro system, J. Control. Release 154 (2011) 290–297.
[79] H. Grüll, S. Langereis, Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound, J. Control. Release 161 (2012) 317–327.
[80] W.G. Pitt, G.A. Husseini, B.J. Staples, Ultrasonic drug delivery — a general review, Expert Opin. Drug Deliv. 1 (1) (2004) 37–56.
[81] K. Ninomiyaa, et al., Ultrasound-mediated drug delivery using liposomes modified with a thermosensitive polymer, Ultrason. Sonochem. 21 (2014) 310–316.
[82] A. Schroeder, et al., Controlling Liposomal Drug Release with Low Frequency Ultrasound: Mechanism and Feasibility, Langmuir 23 (2007) 4019–4025.
[83] G.B. Sukhorukov, et al., Nanoengineered Polymer Capsules: Tools for Detection, Controlled Delivery, and Site-Specific Manipulation, Small 1 (2) (2005) 194–200.
[84] K. Hayashi, et al., High-Frequency, Magnetic-Field-Responsive Drug Release from Magnetic Nanoparticle/Organic Hybrid Based on Hyperthermic Effect, ACS Appl. Mater. Interfaces 2 (7) (2010) 1903–1911.
[85] C. Alvarez-Lorenzo, L. Bromberg, A. Concheiro, Light-sensitive Intelligent Drug Delivery Systems, Photochem. Photobiol. 85 (2009) 848–860.
[86] G. Liu, W. Liu, C.M. Dong, UV- and NIR-responsive polymeric nanomedicines for on-demand drug delivery, Polym. Chem. 4 (2013) 3431–3443.
[87] N. Fomina, J. Sankaranarayanan, A. Almutairi, Photochemical mechanisms of light-triggered release from nanocarriers, Adv. Drug Deliv. Rev. 64 (2012) 1005–1020.
[88] J. Babin, et al., A New Two-Photon-Sensitive Block Copolymer Nanocarrier, Angew. Chem. Int. 48 (2009) 3329–3332.
[89] Y. Shen, et al., Multifunctioning pH-responsive nanoparticles from hierarchical self-assembly of polymer brush for cancer drug delivery, AIChE J. 54 (11) (2008) 2979–2989.

[90] S. Hao, et al., Rapid preparation of pH-sensitive polymeric nanoparticle with high loading capacity using electrospray for oral drug delivery, Mater. Sci. Eng. C 33 (2013) 4562–4567.
[91] H. Wen, et al., pH-responsive composite microspheres based on magnetic mesoporous silica nanoparticle for drug delivery, Eur. J. Pharm. Biopharm. 84 (2013) 91–98.
[92] E.C. Cho, et al., Role of pH-sensitive polymer-liposome complex in enhancing cellular uptake of biologically active drugs, Mater. Sci. Eng. C 29 (2009) 774–778.
[93] A. Kumar, I.Y. Galaev, B. Mattiasson, Affinity Precipitation of -Amylase Inhibitor from Wheat Meal by Metal Chelate Affinity Binding Using Cu(II)-Loaded Copolymers of 1-Vinylimidazole with -Isopropylacrylamide, Biotechnol. Bioeng. 59 (1998) 695–704.
[94] R. de la Rica, D. Aili, M.M. Stevens, Enzyme-responsive nanoparticles for drug release and diagnostics, Adv. Drug Deliv. Rev. 64 (2012) 967–978.
[95] L. Sun, et al., Oral glucose- and pH-sensitive nanocarriers for simulating insulin release in vivo, Polym. Chem. (2013), http://dx.doi.org/10.1039/C3PY01416A.
[96] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, Adv. Drug Deliv. Rev. 65 (1) (2013) 36–48.
[97] A. Jesorka, O. Orwar, Liposomes: technologies and analytical applications, Annu Rev Anal Chem (Palo Alto, Calif) 1 (2008) 801–832.
[98] A. Sharma, U.S. Sharma, Liposomes in drug delivery: progress and limitations, Int. J. Pharm. 154 (1997) 123–140.
[99] P. Kan, et al., A liposomal formulation able to incorporate a high content of Paclitaxel and exert promising anticancer effect, J Drug Deliv. 2011 (2011) 629234.
[100] E.V. Tazina, K.V. Kostin, N.A. Oborotova, Drug synthesis methods and manufacturing technology. Specific features of drug encapsulation in liposomes (A review), Pharm. Chem. J. 45 (2011) 481–490.
[101] Y. Fan, Q. Zhang, Development of liposomal formulations: From concept to clinical investigations, Asian J. Pharm. Sci. 8 (2) (2013) 81–87.
[102] A.R. Mohammed, et al., Liposome formulation of poorly water soluble drugs: optimisation of drug loading and ESEM analysis of stability, Int. J. Pharm. 285 (1–2) (2004) 23–34.
[103] M. Yokoyama, et al., Adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer, J. Control. Release 11 (1990) 269–278.
[104] W. Li, F.C. Szoka Jr., Lipid-based nanoparticles for nucleic acid delivery, Pharm. Res. 24 (3) (2007) 438–449.
[105] C.R. Dass, Lipoplex-mediated delivery of nucleic acids: factors affecting in vivo transfection, J. Mol. Med. (Berl) 82 (9) (2004) 579–591.
[106] M. Morille, et al., Long-circulating DNA lipid nanocapsules as new vector for passive tumor targeting, Biomaterials 31 (2) (2010) 321–329.
[107] S.D. Brown, et al., Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin, J. Am. Chem. Soc. 132 (2010) 4678–4684.
[108] B. Duncan, C. Kim, V.M. Rotello, Gold nanoparticle platforms as drug and biomacromolecule delivery systems, J. Control. Release 148 (1) (2010) 122–127.
[109] J.H. Maeng, et al., Multifunctional doxorubicin loaded superparamagnetic iron oxide nanoparticles for chemotherapy and magnetic resonance imaging in liver cancer, Biomaterials 31 (18) (2010) 4995–5006.
[110] O. Veiseh, J.W. Gunn, M. Zhang, Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging, Adv. Drug Deliv. Rev. 62 (3) (2010) 284–304.
[111] S.Y. Madani, et al., A new era of cancer treatment: carbon nanotubes as drug delivery tools, Int. J. Nanomedicine 6 (2011) 2963–2979.
[112] Z. Liu, et al., Drug delivery with carbon nanotubes for in vivo cancer treatment, Cancer Res. 68 (16) (2008) 6652–6660.
[113] J. Forsgren, et al., A ceramic drug delivery vehicle for oral administration of highly potent opioids, J. Pharm. Sci. 99 (1) (2010) 219–226.
[114] J.M. Rosenholm, C. Sahlgren, M. Linden, Towards multifunctional, targeted drug delivery systems using mesoporous silica nanoparticles—opportunities & challenges, Nanoscale 2 (10) (2010) 1870–1883.
[115] J.W. Yoo, et al., Bio-inspired, bioengineered and biomimetic drug delivery carriers, Nat. Rev. Drug Discov. 10 (7) (2011) 521–535.
[116] M. Shimomura, T. Sawadaishi, Bottom-up strategy of materials fabrication: a new trend in nanotechnology of soft materials, Curr. Opin. Colloid Interface Sci. 6 (2001) 11–16.
[117] M. Wacker, Nanocarriers for intravenous injection—the long hard road to the market, Int. J. Pharm. 457 (1) (2013) 50–62.
[118] J.L. Arias, Liposomes in drug delivery: A patent review (2007–Present), Expert Opin. Ther. Pat. 23 (2013) 1–16.
[119] R.A. Demel, S.C. Kinsky, L.L. Van Deenen, Effects of temperatura and colesterol on the glucose premeability of liposomes prepared with natural and synthetic lecithins, Biochim. Biophys. Acta 150 (1968) 655–665.
[120] S.B. Kaye, V.J. Richardson, Potential of Liposomes as Drug-Carriers in Cancer Chemotherapy: A Review, Cancer Chemother. Pharmacol. 3 (1979) 81–85.
[121] S. Vemuri, C.T. Rhodes, Preparation and characterization of liposomes as therapeutics delivery systems: a review, Pharm. Acta Helv. 70 (1995) 95–111.
[122] J. Senior, G. Gregoriadis, Stability of small unilamellar liposomes in serum and clearance from the circulation: The effect of the phospholipid nd cholesterol components, Life Sci. 30 (1982) 2123–2136.
[123] C. Kirby, J. Clarke, G. Gregoriadis, Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in vitro, Biochem. J. 186 (1980) 591–598.
[124] K. Nagpal, S.K. Singh, D.N. Mishra, Chitosan nanoparticles: A promising system in novel drug delivery, Chem. Pharm. Bull. 58 (2010) 1423–1430.
[125] J.J. Wang, et al., Recent advances of chitosan nanoparticles as drug carriers, Int. J. Nanomedicine 6 (2011) 765–774.
[126] H. Xin, et al., Enhanced anti-glioblastoma efficacy by PTX-loaded PEGylated poly(varepsilon-caprolactone) nanoparticles: In vitro and in vivo evaluation, Int. J. Pharm. 402 (1–2) (2010) 238–247.
[127] M.T. Peracchia, et al., Stealth(r) PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting, J. Control. Release 60 (1999) 121–128.
[128] A. Mahapatro, D.K. Singh, Biodegradable nanoparticles are excellent vehicle for site directed in-vivo delivery of drugs and vaccines, J. Nanobiotechnol. 9 (2011) 55.
[129] H. Okada, et al., Pharmacokinetics of once-a-month injectable microspheres of leuprolide acetate, Pharm. Res. 8 (1991) 787–791.
[130] X. Xu, et al., Comparative study of paclitaxel physically encapsulated in and chemically conjugated with PEG-PLA, Polym. Adv. Technol. 20 (11) (2009) 843–848.
[131] D.S. Jain, et al., Poly lactic acid (PLA) nanoparticles sustain the cytotoxic action of temozolomide in C6 Glioma cells, Biomed. Aging Pathol. 3 (4) (2013) 201–208.
[132] S. Munier, et al., Cationic PLA nanoparticles for DNA delivery: comparison of three surface polycations for DNA binding, protection and transfection properties, Colloids Surf. B: Biointerfaces 43 (3–4) (2005) 163–173.
[133] S. Acharya, S.K. Sahoo, PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect, Adv. Drug Deliv. Rev. 63 (3) (2011) 170–183.
[134] D.H. Kim, D.C. Martin, Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery, Biomaterials 27 (15) (2006) 3031–3037.
[135] K. Osada, R.J. Christie, K. Kataoka, Polymeric micelles from poly(ethylene glycol)-poly(amino acid) block copolymer for drug and gene delivery, J. R. Soc. Interface 6 (Suppl. 3) (2009) S325–S339.
[136] F. Dosio, et al., Poly(ethylene glycol)-human serum albumin-paclitaxel conjugates: preparation, characterization and pharmacokinetics, J. Control. Release 6 (2001) 107–117.
[137] E. Miele, et al., Albumin-bound formulation of paclitaxel (Abraxane(r) ABI-007) in the treatment of breast cancer, Int. J. Nanomedicine 4 (2009) 99–105.
[138] I.N. Desai, Nab Technology: A Drug Delivery Platform Utilising Endothelial gp60 Receptor-based Transport and Tumour derived sparee for Targeting, in: Pharmadeals (Ed.), Drug Delivery Report Winter 2007/2008, 2007.
[139] Q. Fu, et al., Nanoparticle Albumin-Bound (NAB) Technology is a Promising Method for Anti-Cancer Drug Delivery, Recent Pat. Anticancer Drug Discov. 4 (2009) 262–272.
[140] H. Wartlick, et al., Tumour cell delivery of antisense oligonuclceotides by human serum albumin nanoparticles, J. Control. Release 96 (3) (2004) 483–495.
[141] S.C. Alley, N.M. Okeley, P.D. Senter, Antibody–drug conjugates: targeted drug delivery for cancer, Curr. Opin. Chem. Biol. 14 (4) (2010) 529–537.
[142] S.J. Goldsmith, Radioimmunotherapy of lymphoma: Bexxar and Zevalin, Semin. Nucl. Med. 40 (2) (2010) 122–135.
[143] D.C. Julien, et al., Utilization of monoclonal antibody-targeted nanomaterials in the treatment of cancer, MAbs 3 (5) (2011) 467–478.
[144] L. Wang, et al., CD44 antibody-targeted liposomal nanoparticles for molecular imaging and therapy of hepatocellular carcinoma, Biomaterials 33 (20) (2012) 5107–5114.
[145] P.R. Cullis, et al., Generating and loading of liposomal systems for drug-delivery applications, Adv. Drug Deliv. Rev. 3 (1989) 267–282.
[146] A.L. Weiner, Liposomes for protein delivery: Selecting manufacture and development processes, Immunomethods 4 (1994) 201–209.
[147] B. Ceht, D.D. Lasic, A Rigorous Theory of Remote Loading of Drugs into Liposomes, Langmuir 11 (1995) 3356–3368.
[148] A. Fritze, et al., Remote loading of doxorubicin into liposomes driven by a transmembrane phosphate gradient, Biochim. Biophys. Acta 1758 (10) (2006) 1633–1640.
[149] N. Dos Santos, et al., pH gradient loading of anthracyclines into cholesterol-free liposomes: enhancing drug loading rates through use of ethanol, Biochim. Biophys. Acta 1661 (1) (2004) 47–60.
[150] I.V. Zhigaltsev, et al., Liposome-encapsulated vincristine, vinblastine and vinorelbine: a comparative study of drug loading and retention, J. Control. Release 104 (1) (2005) 103–111.
[151] E.M. Bolotin, et al., Ammonium sulfate gradients for efficient and stable remote loading of amphipathic weak bases into liposomes and ligando liposomes, J. Liposome Res. 4 (1994) 455–479.
[152] S. Sajeesh, C.P. Sharma, Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery, Int. J. Pharm. 325 (1–2) (2006) 147–154.
[153] G.A. Husseini, W.G. Pitt, Micelles and nanoparticles for ultrasonic drug and gene delivery, Adv. Drug Deliv. Rev. 60 (10) (2008) 1137–1152.
[154] C. Oerlemans, et al., Polymeric micelles in anticancer therapy: targeting, imaging and triggered release, Pharm. Res. 27 (12) (2010) 2569–2589.
[155] Y. Zhang, H.F. Chan, K.W. Leong, Advanced materials and processing for drug delivery: the past and the future, Adv. Drug Deliv. Rev. 65 (1) (2013) 104–120.
[156] L. Tayebi, D. Vashaee, A.N. Parikh, Stability of uni- and multillamellar spherical vesicles, ChemPhysChem 13 (1) (2012) 314–322.
[157] E.R. Gillies, J.M.J. Fréchet, Dendrimers and dendritic polymers in drug delivery, Drug Discov. Today 10 (2005) 35–43.
[158] Food and Drug Administration, Available from: www.accessdata.fda.gov .
[159] J. Buse, A. El-Aneed, Properties, engineering and applications of lipid-based nanoparticle drug-delivery systems: current research and advances, Nanomedicine 5 (8) (2010) 1237–1260.
[160] D.N. Waterhouse, et al., A Comparison of Liposomal Formulations of Doxorubicin with Drug Administered in Free Form Changing Toxicity Profiles, Drug Saf. 24 (2001) 903–920.
[161] T.L. Jackson, Intracellular Accumulation and Mechanism of Action of Doxorubicin in a Spatio-temporal Tumor Model, J. Theor. Biol. 220 (2) (2003) 201–213.
[162] G. Minotti, et al., Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity, Pharmacol. Rev. 56 (2) (2004) 185–229.

[163] A. Gabizon, et al., Enhancement of adriamycin Delivery to Liver Metastatic Cells with Increased Tumoricidal Effect Using Liposomes as Drug Carriers, Cancer Res. 43 (1983) 4730–4735.
[164] A. Gabizon, et al., Prolonged Circulation Time and Enhanced Accumulation in Malignant Exudates of Doxorubicin Encapsulated in Polyethylene-glycol Coated Liposomes, Cancer Res. 54 (1994) 987–992.
[165] O. Lyass, et al., Correlation of Toxicity with Pharmacokinetics of Pegylated Liposomal Doxorubicin (Doxil) in Metastatic Breast Carcinoma, Cancer 89 (2000) 1037–1047.
[166] R. Duncan, Polymer conjugates as anticancer nanomedicines, Nat. Rev. Cancer 6 (9) (2006) 688–701.
[167] M.J. Keating, et al., L-Asparaginase and PEG Asparaginase — Past, Present, and Future, Leuk. Lymphoma 10 (1993) 153–157.
[168] C.H. Fu, K.M. Sakamoto, PEG-asparaginase, Expert. Opin. Pharmacother. 8 (12) (2007) 1977–1984.
[169] L. Pollaro, C. Heinis, Strategies to prolong the plasma residence time of peptide drugs, MedChemComm 1 (5) (2010) 319.
[170] H. Harashima, et al., Size Dependent Liposome Degradation in Blood: In vivo/In vitro Correlation by Kinetic Modeling, J. Drug Target. 3 (1995) 253–261.
[171] M.A. Schubert, et al., Role of oxygen in biodegradation of poly(etherurethane urea) elastomers, J. Biomed. Mater. Res. 34 (1997) 519–530.
[172] C.S. Ha, J.A. Gardella, Surface Chemistry of Biodegradable Polymers for Drug Delivery Systems, Chem. Rev. 105 (2005) 4205–4232.
[173] C. Vermehren, K. Jørgensen, S. Frokjaer, Influence of lipopolymer concentration on liposome degradation and blood clearance, Int. J. Pharm. 183 (1999) 13–16.
[174] F. Von Burkersroda, L. Schedlb, A. Göpferich, Why degradable polymers undergo surface erosion or bulk erosion, Biomaterials 23 (2002) 4221–4231.
[175] A. Göpferich, Mechanisms of polymer degradation and erosion, Biomaterials 17 (1996) 103–114.
[176] K.A. Athanasiou, G.G. Niederauer, C.M. Agrawal, Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers, Biomaterials 17 (1996) 93–102.
[177] E. Kiss, I. Bertoti, E.I. Vargha-Butler, XPS and wettability characterization of modified poly(lactic acid) and poly(lactic/glycolic acid) films, J. Colloid Interface Sci. 245 (1) (2002) 91–98.
[178] E.J. Cho, et al., Tools to Rapidly Produce and Screen Biodegradable Polymer and Sol–gel-Derived Xerogel Formulations, Appl. Spectrosc. 56 (11) (2002) 1385–1389.
[179] A. Göpferich, Polymeric bulk erosion, Macromolecules 30 (1997) 2598–2604.
[180] D.E. Owens III, N.A. Peppas, Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles, Int. J. Pharm. 307 (1) (2006) 93–102.
[181] S.D. Li, L. Huang, Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting, J. Control. Release 145 (3) (2010) 178–181.
[182] M. Rosera, D. Fischerb, T. Kisselb, Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats, Eur. J. Pharm. Biopharm. 46 (1998) 255–263.
[183] B. Rihova, Biocompatibility of biomaterials: hemocompatibility, immunocompatiblity and biocompatibility of solid polymeric materials and soluble targetable polymeric carriers, Adv. Drug Deliv. Rev. 21 (1996) 157–176.
[184] P. Aggarwal, et al., Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy, Adv. Drug Deliv. Rev. 61 (6) (2009) 428–437.
[185] F. Alexis, et al., Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles, Mol. Pharm. 5 (2008) 505–515.
[186] A.J. D'Souza, E.M. Topp, Release from polymeric prodrugs: linkages and their degradation, J. Pharm. Sci. 93 (8) (2004) 1962–1979.
[187] H.S. Choi, et al., Renal clearance of quantum dots, Nat. Biotechnol. 25 (10) (2007) 1165–1170.
[188] T. Yamaoka, Y. Tabata, Y. Ikada, Distribution and Tissue Uptake of Poly(ethylene glycol) with Different Molecular Weights after Intravenous Administration to Mice, J. Pharm. Sci. 83 (1994) 601–606.
[189] F.M. Veronese, G. Pasut, PEGylation, successful approach to drug delivery, Drug Discov. Today 10 (21) (2005) 1451–1458.
[190] M.J. Roberts, M.D. Bentley, J.M. Harris, Chemistry for peptide and protein PEGylation, Adv. Drug Deliv. Rev. 64 (2012) 116–127.
[191] J.M. Harris, R.B. Chess, Effect of PEGylation on pharmaceuticals, Nat. Rev. 2 (2003) 214–221.
[192] K. Knop, et al., Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives, Angew. Chem. Int. Ed. Engl. 49 (36) (2010) 6288–6308.
[193] E. Jäger, et al., Self-assembly of biodegradable copolyester and reactive HPMA-based polymers into nanoparticles as an alternative stealth drug delivery system, Soft Matter 8 (2012) 9563–9575.
[194] S.K. Lai, Y.Y. Wang, J. Hanes, Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues, Adv. Drug Deliv. Rev. 61 (2) (2009) 158–171.
[195] L.M. Ensign, R. Cone, J. Hanes, Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers, Adv. Drug Deliv. Rev. 64 (2012) 557–570.
[196] R.S. Bhatta, et al., Mucoadhesive nanoparticles for prolonged ocular delivery of natamycin: In vitro and pharmacokinetics studies, Int. J. Pharm. 432 (1–2) (2012) 105–112.
[197] H. Maeda, et al., Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review, J. Control. Release 65 (2000) 271–284.
[198] H. Maeda, The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting, Adv. Enzym. Regul. 41 (2001) 189–207.
[199] G.A. Hughes, Nanostructure-mediated drug delivery, Nanomedicine 1 (1) (2005) 22–30.
[200] P. Kocbek, et al., Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody, J. Control. Release 120 (1–2) (2007) 18–26.
[201] A. Cirstoiu-Hapca, et al., Differential tumor cell targeting of anti-HER2 (Herceptin) and anti-CD20 (Mabthera) coupled nanoparticles, Int. J. Pharm. 331 (2) (2007) 190–196.
[202] H. Kouchakzadeh, et al., Optimization of an anti-HER2 monoclonal antibody targeted delivery system using PEGylated human serum albumin nanoparticles, Int. J. Pharm. 447 (1–2) (2013) 62–69.
[203] Y.M. Lu, et al., Targeted therapy of brain ischaemia using Fas ligand antibody conjugated PEG-lipid nanoparticles, Biomaterials 35 (2014) 530–537.
[204] R. Heukers, et al., Targeting hepatocyte growth factor receptor (Met) positive tumor cells using internalizing nanobody-decorated albumin nanoparticles, Biomaterials 35 (1) (2014) 601–610.
[205] H. Lee, et al., Apoptotic Epidermal Growth Factor (EGF)-Conjugated Block Copolymer Micelles as a Nanotechnology Platform for Targeted Combination Therapy, Mol. Pharm. 4 (2007) 769–781.
[206] K.B. Chalasani, et al., Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles, J. Control. Release 122 (2) (2007) 141–150.
[207] T. Hiratsuka, et al., Copolymers for hepatocyte-specific targeting carrying galactose and hydrophobic alkyl groups, Macromol. Biosci. 8 (3) (2008) 231–238.
[208] S. Mahajan, et al., Receptor Specific Macrophage Targeting by Mannose-Conjugated Gelatin Nanoparticles-An In Vitro and In Vivo Study, Curr. Nanosci. 6 (2010) 413–421.
[209] S. Dhar, et al., Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles, Proc. Natl. Acad. Sci. U. S. A. 105 (45) (2008) 17356–17361.
[210] O.C. Farokzhad, et al., Nanoparticle-Aptamer Bioconjugates: A New Approach for Targeting Prostate Cancer Cells, Cancer Res. 64 (2004) 7668–7672.
[211] D.B. Kirpotin, et al., Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models, Cancer Res. 66 (13) (2006) 6732–6740.
[212] T.M. Allen, Ligand-targeted therapeutics in anticancer therapy, Nat. Rev. Cancer 2 (10) (2002) 750–763.
[213] B.V.N. Nagavarma, et al., Different Techniques For Preparation Of Polymeric Nanoparticles — A Review, Asian J. Pharm. Clin. Res. 5 (2012) 16–23.
[214] P.A. Grabnar, J. Kristl, The manufacturing techniques of drug-loaded polymeric nanoparticles from preformed polymers, J. Microencapsul. 28 (4) (2011) 323–335.
[215] S. Desgouilles, et al., The Design of Nanoparticles Obtained by Solvent Evaporation: A Comprehensive Study, Langmuir 19 (2003) 9504–9510.
[216] D. Lemoine, V. Preát, Polymeric nanoparticles as delivery system for influenza virus glycoproteins, J. Control. Release 54 (1998) 15–27.
[217] K. Andreas, et al., Biodegradable insulin-loaded PLGA microspheres fabricated by three different emulsification techniques: investigation for cartilage tissue engineering, Acta Biomater. 7 (4) (2011) 1485–1495.
[218] H.Y. Kwon, et al., Preparation of PLGA nanoparticles containing estrogen by emulsification–diffusion method, Colloids Surf. A 182 (2001) 123–130.
[219] C.W. Pouton, Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification, Int. J. Pharm. 27 (1985) 335–348.
[220] N.M. Belliveau, et al., Microfluidic Synthesis of Highly Potent Limit-size Lipid Nanoparticles for In Vivo Delivery of siRNA, Mol. Ther. Nucleic Acids 1 (2012) e37.
[221] D. van Swaay, A. deMello, Microfluidic methods for forming liposomes, Lab Chip 13 (5) (2013) 752–767.
[222] R. Karnik, et al., Microfluidic Platform for Controlled Synthesis of Polymeric Nanoparticles, Nano Lett. 8 (2008) 2906–2912.
[223] H.C. Shum, J.W. Kim, D.A. Weitz, Microfluidic Fabrication of Monodisperse Biocompatible and Biodegradable Polymersomes with Controlled Permeability, J. Am. Chem. Soc. 130 (2008) 9543–9549.
[224] N. Kolishetti, et al., Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy, PNAS 107 (2010) 17939–17944.
[225] P.M. Valencia, et al., Microfluidic technologies for accelerating the clinical translation of nanoparticles, Nat. Nanotechnol. 7 (10) (2012) 623–629.
[226] N. Bock, T.R. Dargaville, W.A. Woodruffa, Electrospraying of polymers with therapeutic molecules: State of the art, Prog. Polym. Sci. 37 (2012) 1510–1551.
[227] M. Zamani, M.P. Prabhakaran, S. Ramakrishna, Advances in drug delivery via electrospun and electrosprayed nanomaterials, Int. J. Nanomedicine 8 (2013) 2997–3017.
[228] A. Gomez, et al., Production Of Protein Nanoparticles By Electrospray Drying, J. Aerosol Sci. 29 (1998) 561–574.
[229] J. Venugopal, M.P. Prabhakaran, S. Low, A.T. Choon, Y.Z. Zhang, G. Deepika, S. Ramakrishna, Nanotechnology for Nanomedicine and Delivery of Drugs, Curr. Pharm. Des. 14 (2008) 2184–2200.
[230] J. Venugopal, M.P. Prabhakaran, S. Low, A.T. Choon, G. Deepika, V.R. Giri Dev, S. Ramakrishna, Continuous Nanostructures for the Controlled Release of Drugs, Curr. Pharm. Des. 15 (2009) 1799–1808.
[231] J. Xie, J.C. Marijnissen, C.H. Wang, Microparticles developed by electrohydrodynamic atomization for the local delivery of anticancer drug to treat C6 glioma in vitro, Biomaterials 27 (17) (2006) 3321–3332.
[232] R. Pareta, M.J. Edirisinghe, A novel method for the preparation of biodegradable microspheres for protein drug delivery, J. R. Soc. Interface 3 (9) (2006) 573–582.
[233] J. Xie, et al., Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray, J. Colloid Interface Sci. 317 (2) (2008) 469–476.
[234] H. Valo, et al., Electrospray encapsulation of hydrophilic and hydrophobic drugs in poly(L-lactic acid) nanoparticles, Small 5 (15) (2009) 1791–1798.
[235] K. Pancholi, et al., Novel electrohydrodynamic preparation of porous chitosan particles for drug delivery, J. Mater. Sci. Mater. Med. 20 (4) (2009) 917–923.
[236] J.M. DeSimone, et al., Methods for fabricating isolated micro- and nano-structures using soft of imprint lithography, 2004.

Betiana Felice is currently a Doctoral Research Fellow with the National Council of Scientific and Technical Researches (CONICET, Argentina). She obtained her degree as Biomedical Engineer from National University of Tucumán (Argentina). Her research interests include biomaterials, nanotechnology and tissue engineering.

Molamma P. Prabhakaran is a Post-Doctoral Research Fellow with the Nanoscience and Nanotechnology Initiative in the National University of Singapore (NUS, Singapore). She obtained her Ph.D. in Chemistry from the Faculty of Science in NUS and her research interests include electrospinning, scaffold modifications, nanoparticles, tissue engineering and drug delivery.

Andrea P. Rodríguez is an assistant researcher in the National Council of Scientific and Technical Researches (CONICET, Argentina) and associate professor at Bioengineering Department in National University of Tucumán (UNT, Argentina). She obtained her Ph.D. in the Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan. Her research interests include tissue engineering, cell culture and cell-biomaterial interactions.

Seeram Ramakrishna, *FREng* is a professor of materials engineering at the National University of Singapore Mechanical Engineering department. He is advancing science and engineering aspects of electrospun nanomaterials to realize their full potential in healthcare, food, energy, water and environment. He is a highly cited materials scientist. He authored over 600 journal papers and six books with 32,000 citations and 83 H-index. He is an international fellow of major professional societies in Singapore, Asia, India, UK and USA.