REVIEW

PLGA-Based Nanoparticles as Cancer Drug Delivery Systems

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Abstract

Poly (lactic-co-glycolic acid) (PLGA) is one of the most effective biodegradable polymeric nanoparticles (NPs). It has been approved by the US FDA to use in drug delivery systems due to controlled and sustained-release properties, low toxicity, and biocompatibility with tissue and cells. In the present review, the structure and properties of PLGA copolymers synthesized by ring-opening polymerization of DL-lactide and glicolide were characterized using 1H nuclear magnetic resonance spectroscopy, gel permeation chromatography, Fourier transform infrared spectroscopy and differential scanning calorimetry. Methods of preparation and characterization, various surface modifications, encapsulation of diverse anticancer drugs, active or passive tumor targeting and different release mechanisms of PLGA nanoparticles are discussed. Increasing experience in the application of PLGA nanoparticles has provided a promising future for use of these nanoparticles in cancer treatment, with high efficacy and few side effects.

Keywords: Nanotechnology - poly (lactic-co-glycolic acid) (PLGA) - drug delivery - anticancer drugs

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Introduction

Nanoparticles have become extremely attractive for their applications in the fields of biology and medicine in recent years (Xu et al., 2007; Mahapatro and Singh, 2011). Nanoparticles are solid and spherical structures ranging around 100 nm in size and prepared from natural or synthetic polymers. A wide variety of drugs can be delivered using nanoparticles like hydrophilic small drugs, hydrophobic small drugs, vaccines and biological macromolecules. Nanoparticles also allow a targeted direction to particular organs or cells or controlled drug delivery (Hans and Lowman, 2002; Hillaireau and Couvreur, 2009; Danhier et al., 2012).

The main goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to attain the site-specific action of the drug at the therapeutically optimal rate and dose regimen (Vila et al., 2002; Mu and Feng, 2003; Mohanraj and Chen, 2007). Nanoparticles used for drug delivery must gather some requirements, such as biocompatibility, drug compatibility, proper biodegradation kinetics and mechanical properties(Sahoo and Labhasetwar, 2003; Wickline et al., 2006; Lü et al., 2009), in addition to ease of processing the nanoparticle delivery systems are attractive because they target tumors and increase the tumor accumulation of anticancer agents in tumor cells more than in healthy tissues(Liu et al., 2007; Lü et al., 2009). From a broader perspective in medicine, nanoparticle have been used in specific applications such as tissue engineered scaffolds and devices, site specific drug delivery systems, cancer therapy and clinical bioanalytical diagnostics and therapeutics(van Vlerken and Amiji, 2006; Vasir and Labhasetwar, 2007; Liu et al., 2007; Mahapatro and Singh, 2011).

For targeted delivery, persistence of nanoparticles is needed in systemic circulation of the body, but the body identifies hydrophobic particles as alien (Kumari et al., 2010; Brigger et al., 2012). The reticulo-endothelial system (RES) removes these from the blood stream and takes them up in the liver or the spleen. This process is one of the most main biological barriers to nanoparticles-based controlled drug delivery (Kumari et al., 2010; Danhier et al., 2012) The binding of opsonin proteins present in the blood serum to injected nanoparticles causes attachment of opsonized particles to macrophages and consequently to their internalization by phagocytosis (Owens and Peppas, 2006; Danhier et al., 2012). The

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surface modification protected nanoparticles from being phagocytosed and eliminated from the blood vascular system after intravenous injections. (Park et al., 2009b; Mahapatro and Singh, 2011)

Polymer-based nanoparticles are submicron-sized polymeric colloidal particles in which a therapeutic agent of interest can be fixed or encapsulated inside their polymeric matrix or adsorbed or conjugated onto the surface (Labhasetwar et al., 1997; Mahapatro and Singh, 2011). Polymers may be linear, branched or globular, and their size can be firmly controlled. Polyamides, poly (amino acids), poly (alkyl-α-cyano acrylates), polyesters, poly orthoesters, polyurethanes and polyacrylamides have been used to set up different drug-loaded devices (Jain, 2000; Lü et al., 2009; Panyam and Labhasetwar, 2012) between them, the thermoplastic aliphatic polyesters, like polylactic acid, poly glycolic acid and specially the copolymer poly (lactic co-glycolic acid) (PLGA), have a long history of use as biomaterials because of their exceptional biocompatibility and biodegradability. (Studer et al., 2005; Wickline et al., 2007; Lü et al., 2009). Langer and Folkman were the first to demonstrate the controlled release of macromolecules via polymers, which facilitated the development of antiangiogenic drug delivery systems for cancer therapy and opened up novel areas for the delivery of macromolecules (Langer and Folkman, 1976; Dinarvand et al., 2011). Polymer-based nanoparticles act as an excellent vehicle for delivery of several biomolecules, drugs, genes and vaccines to the site of interest in-vivo(Hans and Lowman, 2002; Mahapatro and Singh, 2011).

By encapsulating these molecules inside a nanocarrier, the solubility and stability of drugs can be enhanced, providing a chance to re-evaluate the therapeutic potential of drugs because of poor pharmacokinetics (Langer, 1998; Dinarvand et al., 2011). The variety of drug delivery systems allows nanoparticles to be developed with a diverse array of shapes, sizes, and components, enabling them to be tailored for particular applications. However, the primary consideration as designing any drug delivery system is to control the drug concentration in the therapeutic window, while plummeting side effects and improving patient compliance. This allows useful treatment cycles to be maintained, and at the same time decreases damage to healthy cells and diminishes the recovery period (Peer et al., 2007; Davis, 2008; Lammers et al., 2008; Dinarvand et al., 2011). Poly (lactic-co-glycolic acid) (PLGA) is one of the most successfully used biodegradable polymers because its hydrolysis leads to metabolite monomers, lactic acid and glycolic acid. As these two monomers are endogenous and simply metabolized by the body via the Krebs cycle, a negligible systemic toxicity is associated with the use of PLGA for drug delivery or biomaterial applications (Kumari et al., 2010; Danhier et al., 2012). Additionally PLGA-based nanoparticles are currently under examinations for applications in cancer imaging and cancer therapy (Matsumura and Maeda, 1986; Danhier et al., 2012). So PLGA is discussed comprehensively here.

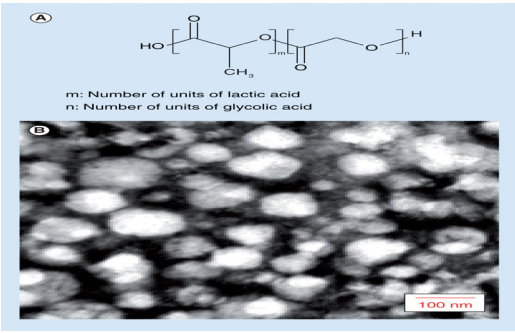

Figure 1. Chemical Structure of Poly (Lactic-Coglycolic Acid) (PLGA), (b) PLGA Nanoparticles (NPs)

Properties of PLGA

Poly (lactic-co-glycolic acid) is a copolymer synthesized via random ring opening copolymerization of two different monomers, the cyclic dimers (1, 4-dioxane-2, 5-diones) of glycolic acid and lactic acid. General catalysts used in the preparation of this copolymer includes tin (II) 2-ethylhexanoate, tin (II) alkoxides or aluminum isopropoxide. During polymerization, consecutive monomeric units (glycolic or lactic acid) are linked together in PLGA by ester linkages, so yielding a linear, amorphous aliphatic polyester products (Astete and Sabliov, 2006; Lü et al., 2009). The forms of PLGA are usually recognized by the monomers ratio used. For instance, PLGA 50:50 identifies a copolymer whose composition is 50% lactic acid and 50% glycolic acid (Vasir and Labhasetwar, 2007; Danhier et al., 2012). PLGA is a widely used polymer due to biocompatibility, long-standing track record in biomedical functions and well-documented utility for continued drug release compared to the conventional devices up to days, weeks or months, and ease of parenteral administration via injection (Mundargi et al., 2008; Acharya and Sahoo, 2011).

PLGA is one of the most effectively used biodegradable polymers for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid.(Acharya and Sahoo, 2011) These monomers are simply metabolized in the body via the Krebs cycle and removed as carbon dioxide and water (Jain, 2000; Panyam et al., 2002; Dinarvand et al., 2011). So result in minimal systemic toxicity. (Acharya and Sahoo, 2011) PLGA is approved by the US FDA and European Medicine Agency (EMA) in different drug delivery systems in humans. The polymers are commercially accessible with diverse molecular weights and copolymer compositions. Depending on the molecular weight copolymer ratio, the degradation time can vary since several months to and several years. (Vert et al., 1994; Prokop and Davidson, 2008; Danhier et al., 2012). Lactic acid is more hydrophobic than glycolic acid and, thus, lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and consequently, degrade more gradually (Park, 1994; Schliecker et al., 2003; Dinarvand et al., 2011).

As a general rule, the degradation time will be shorter for low molecular weight, more hydrophilic, and more amorphous polymers, and for copolymers with a higher glycolide content (Dinarvand et al., 2011). The

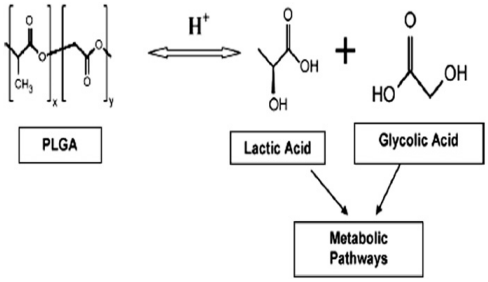

Figure 2. Hydrolysis of PLGA

polymer degradation process both in vitro and in vivo is affected by a number of factors, including the method of preparation, the presence of low molecular weight compounds (monomers, oligomers, catalysts), size, shape and morphology, the inherent properties of the polymer (molecular weight, chemical structure, hydrophobicity, crystallinity, and glass transition temperature) (Jain, 2000; Dinarvand et al., 2011), physicochemical parameters (pH, temperature, and ionic strength of the environment), site of implantation, and mechanism of hydrolysis (Dinarvand et al., 2011). PLGA nanoparticles are internalized in cells partly through liquid phase pinocytosis and in addition through clathrin-mediated endocytosis. PLGA-nanoparticles quickly escape the endo-lysosomes and enter the cytoplasm in 10 min of incubation. This facilitates interactions of nanoparticles with the vesicular membranes leading to transient and localized deterioration of the membrane resulting in the escape of nanoparticles into the cytosol (Vasir and Labhasetwar, 2007; Danhier et al., 2012).

Surface charges of nanoparticles also have a significant influence on their interaction with cells and on their uptake (Foged et al., 2005; Vasir and Labhasetwar, 2008; Danhier et al., 2012). Cationic surface charge is desirable as it promotes interaction of the nanoparticles with the cells and thus augments the rate and extent of internalization (Shenoy et al., 2005; Kumari et al., 2010). PLGA nanoparticles have negative charges which can be changed to neutral or positive charges by surface modification, for instance by PEGylation of the PLGA polymer or chitosan coating respectively (Tahara et al., 2009; Danhier et al., 2010; Danhier et al., 2012).

Methods for Preparation of PLGA Nanoparticles

There are several methods to organize nanoparticles. Depending on the process of preparation, the structural organization may be different. The drug is either entrapped inside the core of a "nanocapsule" as well as entrapped in or adsorbed on the surface of a matrix nanosphere (Danhier et al., 2012). Dispersion of preformed polymers is the most generally used technique to prepare biodegradable nanoparticles from poly-lactic acid (PLA); poly -D-L-glycolide (PLG); poly-D- L-lactide-co-glycolide (PLGA) and poly cyanoacrylate (PCA). These methods commonly include two important steps. The first step is to prepare an emulsified system, and this is common to all the techniques used. The nanoparticles are formed through the second step, which varies according to the method used. Usually, the principle of this second step gives its name to the method (Vauthier and Bouchemal, 2009; Dinarvand et al., 2011).

Single- or double-emulsion-solvent evaporation method

The most generally used method for PLGA NP formation is the single or double-emulsion-solvent evaporation. Single-emulsion process involves oil-in-water (o/w) emulsification, while the double-emulsion process is a water-in-oil-in-water (w/o/w) technique. The w/o/w method is best suited to encapsulate water-soluble drugs, like peptides, proteins and vaccines, whereas the o/w method is perfect for water-insoluble drugs, such as steroids (Jain, 2000; Lü et al., 2009). In some cases, solid/oil/water (s/o/w) techniques have been used with PLGA-based microspheres, specially for a higher drug loading of large water-soluble peptides, such as insulin (Zambaux et al., 1998; Lü et al., 2009). In o/w method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. The drug is dissolved or dispersed into the preformed polymer solution, and this mixture is emulsified into an aqueous solution to make an oil (O) in water (W) i.e., O/W emulsion via using a surfactant/emulsifying agent like gelatin, poly(vinyl alcohol), polysorbate-80, poloxamer-188, etc. Following the formation of a stable emulsion, the organic solvent is evaporated either by mounting the temperature/under pressure or by nonstop stirring. Both the above ways use a high-speed homogenization or sonication. However, these procedures are excellent for a laboratory-scale procedure, but for a large-scale pilot production, alternative methods using low-energy emulsification are necessitated (Scholes et al., 1993; Soppimath et al., 2001).

The size can be controlled by regulating the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature (Tice and Gilley, 1985; Pinto Reis et al., 2006). Although different types of emulsions may be used, oil/water emulsions are interest because they use water as the nonsolvent; this simplifies and thus improves process economics, because it eliminates the require for recycling, facilitating the washing step and minimizing agglomeration (Pinto Reis et al., 2006). However, this technique can only be applied to liposoluble drugs, and limitations are imposed by the scale-up of the high energy requirements in homogenization (Soppimath et al., 2001).

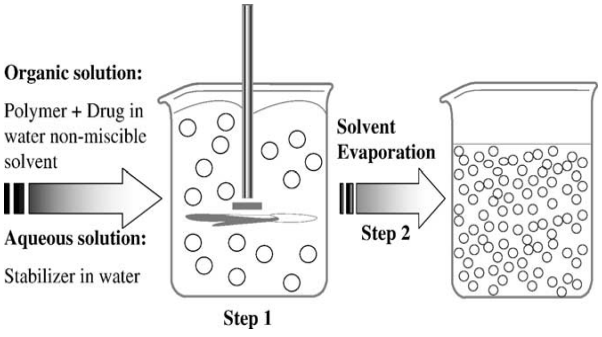

Figure 3. Schematic Representation of the Emulsification- Evaporation Technique

Emulsification solvent diffusion(ESD) method

In the method developed by Quintanar-Guerrero et al. (D'Mello et al., 2006; Yezhelyev et al., 2006), the solvent and water are mutually saturated at room temperature before use to ensure the initial thermodynamic equilibrium of both liquids. Later, the organic solvent containing the dissolved polymer and the drug is emulsified in an aqueous surfactant solution (typically with PVA as a stabilizing agent) by using a high-speed homogenizer. Water is subsequently added under regular stirring to the o/w emulsion system, therefore causing phase transformation and outward diffusion of the solvent from the internal phase, leading to the nano precipitation of the polymer and the formation of colloidal nanoparticles. At last, the solvent can be removed by vacuum steam distillation or evaporation (D'Mello et al., 2006). This method presents several advantages, for example high encapsulation efficiencies (generally 70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be removed from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase through emulsification, reducing encapsulation efficiency (Quintanar-Guerrero et al., 1998; Pinto Reis et al., 2006).

Emulsification reverse salting-out method

The emulsification reverse salting-out method involves the addition of polymer and drug solution to a water-miscible solvent, like acetone, and to an aqueous solution containing the salting-out agent, like magnesium chloride, calcium chloride, and a colloidal stabilizer, like polyvinyl pyrrolidone, under forceful mechanical stirring. As this oil-in-water emulsion is diluted with a plenty amount of water, it induces the creation of nanoparticles

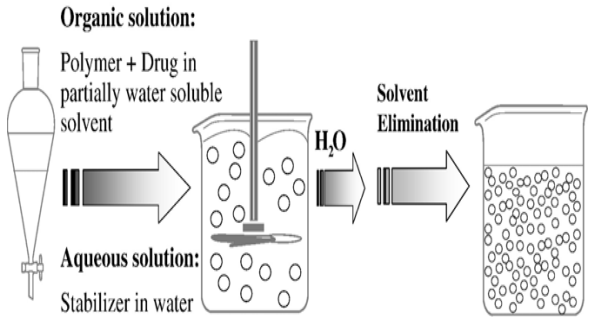

Figure 4. Schematic Illustration of the ESD Technique

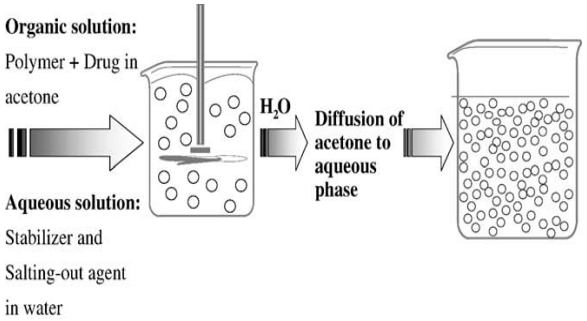

Figure 5. Schematic of the Salting-out Technique

by increasing the diffusion of acetone into the aqueous phase. The dilution produces an abrupt decrease in the salt concentration in the continuous phase of the emulsion, inducing the polymer solvent to migrate out of the emulsion droplets. The residual solvent and salting-out agent are removed by cross-flow filtration (Ibrahim et al., 1992; Allemann et al., 1993; Konan et al., 2002; Dinarvand et al., 2011). Although the emulsification-diffusion technique is a modification of the salting-out process, it has the advantage of avoiding the use of salts and thus eliminates the require for severe purification steps (Bala et al., 2004; Dinarvand et al., 2011). The most important advantage of salting out is that it minimizes tension to protein encapsulants (Jung et al., 2000). Salting out does not need a raise of temperature and, thus, may be useful when heat sensitive substances have to be processed (Lambert et al., 2001). The greatest disadvantages are exclusive function to lipophilic drugs and the extensive nanoparticle washing steps (Couvreur et al., 1995; Pinto Reis et al., 2006).

Nanoprecipitation method

The nanoprecipitation technique is a one-step process, also known as the solvent displacement method (Fessi et al., 1989; Dinarvand et al., 2011). Nanoprecipitation is performed using systems containing three basic components, the polymer, the polymer solvent, and the nonsolvent of the polymer (Thioune et al., 1997; Dinarvand et al., 2011). Usually, this method is used for hydrophobic drug entrapment, but it has been suited for hydrophilic drugs additionally. Polymers and drugs are dissolved in a polar, water-miscible solvent like acetone, acetonitrile, ethanol, or methanol. The solution is poured in a controlled manner (drop-by-drop addition) into an aqueous solution with surfactant. Nanoparticles are formed immediately by rapid solvent diffusion. Lastly, the solvent is removed under reduced pressure (Govender et al., 1999; Mahapatro and Singh, 2011).

Nanoparticle Characterization Techniques

Characterization of nanoparticles is necessary for a thorough understanding of their properties previous to developing them further for pharmaceutical function. Nanoparticle size is significant, not only in determining the release profile and degradation manners, but also in determining the efficacy of the therapeutic agent in terms of tissue penetration and cellular uptake (Gaumet et al., 2008; Dinarvand et al., 2011). Particle size, size distribution and morphology determined by Dynamic light scattering or photon correlation spectroscopy (Govender et al., 1999; Fonseca et al., 2002; Cheng et al., 2008), Scanning electron microscopy (Mu and Feng, 2003; Ricci-Júnior and Marchetti, 2006; Esmaeili et al., 2008b), transmission electron microscopy (Panyam et al., 2003; Mo and Lim, 2005; Yang et al., 2007) and Atomic force microscopy (Ravi Kumar et al., 2004; Dong and Feng, 2005; Song et al., 2006).

The molecular weight of the polymer influences the nanoparticles size, encapsulation efficiency, and degradation rate of the polymer (Dunne et al., 2000;

Dinarvand et al., 2011). Molecular weight is indicative of polymer chain length, and the higher the molecular weight, the longer the chain length. In addition, chain length reflects the hydrophilicity or lipophilicity of the polymer. An increase in chain length raises the lipophilicity and reduces the degradation rate of the polymer. Consequently, by varying the molecular weight, the degradation rate of the polymer and release kinetics of the drug can be managed (Jain, 2000; Mittal et al., 2007; Dinarvand et al., 2011). The molecular weight determined by Size exclusion chromatography (Chacon et al., 1999; Garinot et al., 2007; Danhier et al., 2009).

The physical state of both the drug and the polymer need to be determined because this will have an influence on the in vitro and in vivo drug release characteristics. The zeta potential can influence nanoparticle constancy and mucoadhesion, as well as intracellular trafficking of particles as a function of pH. Hydrophobicity determines the distribution of nanoparticles in the body after administration. Hydrophilic particles lean to remain in the blood for a longer time (Soppimath et al., 2001; Bala et al., 2004; Astete and Sabliov, 2006; Dinarvand et al., 2011). Zeta potential determined by Zetasizer (Ravi Kumar et al., 2004; Esmaeili et al., 2007b; Esmaeili et al., 2008a). The zeta potential values may be positive or negative depending on the nature of the polymer or the material used for surface modification. This is a widely used method to recognize the surface charges of NPs (Ratner et al., 1987; Soppimath et al., 2001). Hydrophobicity and hydrophilicity determined by Water contact angle measurements and hydrophobic interaction chromatography respectively (Storm et al., 1995; Manuela Gaspar et al., 1998; Mosqueira et al., 2001; Prior et al., 2002; Avgoustakis et al., 2002; Zhang et al., 2006). There are many sensitive methods for characterizing nanoparticles, depending upon the factor being investigated (Dinarvand et al., 2011).

PLGA Nanoparticles for Drug Delivery to Tumors

Cancer is a universal public health problem, and tens of millions of people suffer from this lethal disease.(Utreja et al., 2010; Dinarvand et al., 2011) Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Mathers et al., 2008; Jemal et al., 2011). The World Health Organization estimates that 84 million people will die of cancer between 2005 and 2015 (Byrne et al., 2008; Danhier et al., 2010). Cancer research is a rigorous scientific attempt in order to recognize the causes and develop exact strategies for prevention, diagnosis and treatments. Many forms of cancer are treatable via current therapies, such as surgical procedure, chemotherapy, radiation therapy and immunotherapy (Liu et al., 2007; Lü et al., 2009). The main weakness of most chemotherapeutic approaches to cancer treatment is that most of them are nonspecific. Therapeutic (generally cytotoxic) drugs are administered via intravenous, leading to systemic distribution. The nonspecific nature of this method results in the well known side effects of chemotherapy because the cytotoxic drug attacks normal healthy cells besides its primary target and tumor cells (Tannock and Rotin, 1989; Teicher, 2000; Akbarzadeh et al., 2012a). The rationale of using nanoparticles for tumor targeting is based on: 1) NP's capability to deliver the requisite dose load of drug in the area of the tumor because of the enhanced permeability and retention effect or active targeting by ligands on the surface of NPs and 2) NP's ability to diminish the drug exposure to healthy tissues by limiting drug distribution to the target organ (Nobs et al., 2006; Mahapatro and Singh, 2011). The properties of nanoparticles as precursor of good nanomedicine are nanoparticle size, size distribution, surface morphology, surface chemistry, surface charge, surface adhesion, surface erosion, inner porosity, drug diffusivity and encapsulation efficiency, drug stability, drug release kinetics and hemodynamic (Feng, 2004; Kumari et al., 2010). A successful NP system may be the one, which has a high loading capacity to decrease the number of the carrier required for administration. Drug loading in to the NPs is achieved by two methods: first, by incorporating the drug at the time of NP production or secondly, by adsorbing the drug after the formation of NPs by incubating them in the drug solution. It is so obvious that a large amount of drug can be entrapped by the incorporation method when compared to the adsorption (Alonso et al., 1991; Ueda et al., 1998; Soppimath et al., 2001).

PLGA nanoparticles are commonly used for the encapsulation of various cancer related drugs and their successful delivery in vivo (Kumari et al., 2010). Among the diverse forms of PLGA-based drug delivery systems microspheres or microparticles are the most common (Fredenberg et al., 2011). Other types consist of nanoparticles(Sharma et al., 2007), films(Klose et al., 2008), cylinders(Desai et al., 2010), in situ forming implants or microparticles(Dong et al., 2006), scaffolds(Xiong et al., 2009) and foams(Fredenberg et al., 2011; Ong et al., 2009). Protocols have been optimized for PLGA nanoparticles synthesis and many cancer related drugs have been incorporated in PLGA (Hans and Lowman, 2002; Kumari et al., 2010). These loaded nanoparticles protect poorly soluble and unstable payloads from the biological milieu and are tiny enough for capillary penetrations, internalization and endosomal escape (Soppimath et al., 2001; Panyam et al., 2002; Mahapatro and Singh, 2011). In addition, their surface is modified for targeted delivery of molecules to tumor or other tissues (Nobs et al., 2004; Mahapatro and Singh, 2011). They may have controlled-release properties owing to their biodegradability, pH, ions, and/or temperature sensitivity (Dinarvand et al., 2011). Mainly anticancer drugs that have been investigated in PLGA nanoparticle preparations are discussed below.

Encapsulation of Various Anticancer Drugs on PLGA Nanoparticles

Paclitaxel

Paclitaxel (commercially available as taxol) interferes with the usual function of microtubule breakdown via binding with β- subunit of tubulin. It promotes

the polymerization of tubulin causing cell death by disordering the dynamics necessary for cell division. Paclitaxel has neoplastic activity against primary ovarian carcinoma breast and colon tumors. It is one of the powerful anticancer agent but less useful for clinical administration owing to its poor solubility. PLGA intermingled with vitamin E, and tocopheryl polyethylene glycol succinate (TPGS) has been used to encapsulate by solvent evaporation/extraction techniques and in vitro controlled release of this drug (Wang et al., 1996; Kumari et al., 2010). Mu and Feng used α-tocopheryl polyethylene glycol l000 succinate as well as a matrix material with other biodegradable polymers for the fabrication of a nanoparticle formulation of paclitaxel. They concluded that vitamin E TPGS was valuable either as an emulsifier or as matrix material mixed with PLGA for the production of nanoparticles enabling controlled release of paclitaxel (Mu and Feng, 2003; Dinarvand et al., 2011).

Vincristine sulphate

Vincristine sulphate (VCR) is an helpful chemotherapeutic agent, which has been used widely for the treatment of various cancers. Unfortunately, many tumour cells are not susceptible to VCR due to efflux from the tumour cells mediated by P-glycoprotein and associated proteins (Ambudkar et al., 2003; Borst et al., 2006; Acharya and Sahoo, 2011). The reason behind the association of drugs with colloidal carriers against drug resistance comes from the reality that P-glycoprotein probably identifies the drug to be effluxed out of the tumoural cell just when this drug is present in the plasma membrane. As a drug-loaded NP is typically present in the endolysosomal complex after internalisation by cells, it possibly escapes the P-glycoprotein pump. Based on the optimal parameters, it was found that vincristine-loaded PLGA NPs could be formulated with expectable properties by combining the o/w emulsion-solvent evaporation technique and the salting-out technique. This study also showed that two hydrophilic low-molecular-weight drugs, VCR and verapamil (VRP), a chemosensitiser, could be simultaneously entrapped into PLGA NPs, with a relatively high entrapment efficiency of 55.35±4.22% for VCR and 69.47±5.34% for VRP in small-sized particles of 100 nm. Furthermore, their studies showed that PLGA NPs simultaneously loaded with an anticancer drug and a chemosensitiser might be the formulation with the most probable in the treatment of drug-resistant cancers in vivo (Song et al., 2008; Song et al., 2009; Acharya and Sahoo, 2011).

Cisplatin

Cisplatin is known to crosslink DNA molecule in several ways to interfere cell division via mitosis. The damaged DNA elicits DNA repair mechanism. Cisplatin is a very strong anticancer drug but the full therapeutic exploitation of cisplatin is limited owing to its toxicity in healthy tissues (Rosenberg, 2006; Kumari et al., 2010). The selective delivery of cisplatin to tumor cells would considerably reduce drug toxicity, and improve its therapeutic index. This drug has been encapsulated on PLGA–mPEG nanoparticles prepared by double emulsion methods. (Avgoustakis et al., 2002; Kumari et al., 2010). Cisplatin-loaded PLGA-methoxy(polyethylene glycol) (mPEG) nanaoparticles also resulted in prolonged cisplatin residence time in the systemic circulation when used in mice with prostate tumor (Gryparis et al., 2007; Dinarvand et al., 2011).

Etoposide

Etoposide is an anticancer agent used in the treatment of a diversity of malignancies, including malignant lymphomas. It acts by inhibition of topoisomerase-II and activation of oxidation-reduction reactions to create derivatives that bind directly to DNA and cause DNA damage. The successful chemotherapy of tumors depends on incessant exposure to anticancer agents for prolonged periods. Etoposide has a short biological half-life (3.6 hour), and although intra-peritoneal injection would cause initial high local tumour concentrations, prolonged exposure of tumour cells may not be probable. It is envisaged that intra-peritoneal delivery of etoposide through NPs would be a better approach for effectual treatment of peritoneal tumours. In this perspective, etoposide- loaded NPs were prepared applying nanoprecipitation and emulsion-solvent evaporation methods using PLGA in the presence of Pluronic F68 by Reddy et al. The methods produced NPs with high entrapment efficiency of around 80% with continued release of the drug up to 48 hour (Acharya and Sahoo, 2011; Reddy et al., 2004).

9-Nitrocamptothecin

9-Nitrocamptothecin (9-NC) (derivative of camptothecin) and related analogues are a promising family of anticancer agents with an exclusive mechanism of action, targeting the enzyme topoisomerase-I. All camptothecin derivatives undergo a pH dependent quick and reversible hydrolysis from closed lactone ring to the inactive hydroxyl carboxylated form with loss of anticancer activity. The delivery of lipophilic derivatives of 9-NC is fairly challenging due to instability at biological pH and its low water solubility. PLGA has been used to encapsulate 9-NC effectively by nanoprecipitation techniques having more than 30% encapsulation efficiency with its complete biological activity and without disturbing lactone ring (Derakhshandeh et al., 2007; Kumari et al., 2010).

Doxorubicin

Doxorubicin, an anthracycline antibiotic and one of the most extensively used anticancer agents, shows high antitumor activity. However, its therapeutic properties are limited owing to its dependent cardio toxicity and myelosuppression (Misra and Sahoo, 2010; Acharya and Sahoo, 2011). PLGA NPs promise to be a successful system for the targeted and controlled release of doxorubicin with decreased systemic toxicity, increased therapeutic efficiency and patient compliance. Furthermore, multifunctional PLGA NPs for combined doxorubicin and photo thermal treatments were studied by Park et al. to deliver both drug and heat simultaneously to a selected tumorigenic section (Park et al., 2009a; Acharya and Sahoo, 2011).

Curcumin

Curcumin has been used in traditional medicine for several centuries in India and China (Shishodia et al., 2006; Dinarvand et al., 2011). The factor that limits the use of free curcumin for tumor therapy is its low solubility in water, which in turn limits its bioavailability when administered orally. Nanotechnology-based carriers (PLGA NPs) emerged as a novel hope in curcumin delivery to tumour sites. Curcumin-loaded PLGA NPs were prepared by the emulsion diffusion evaporation technique using different stabilizers such as cetyl trimethylammonium bromide (CTAB) or PVA or PEG-5000. The present comprehensible data definitely testifies a well-established product profile, opening up new ways for the miracle molecule curcumin on PLGA owing to higher cellular uptake and increased in vitro bioactivity and better in vivo bioavailability in comparison to native curcumin (Shaikh et al., 2009; Acharya and Sahoo, 2011). Mukerjee and Vishwanatha formulated curcumin-loaded PLGA particles, and suggested that a nanoparticle-based formulation of curcumin has high probable as adjuvant therapy in prostate tumor (Mukerjee and Vishwanatha, 2009; Dinarvand et al., 2011).

Xanthones

Xanthones are natural, semi synthetic and heterocyclic compounds. Xanthonemolecules having a range of substituents on the different carbons constitute a group of compounds with a wide spectrum of biological activities (Pinto and Sousa, 2003; Kumari et al., 2010). These molecules inhibited the nitric oxide production from the macrophages and thus have strong inhibitory action on human cancer cell line expansion. Xanthone loaded PLGA nanospheres have been prepared by solvent displacement techniques (Teixeira et al., 2005; Kumari et al., 2010).

Triptorelin

Triptorelin is a decapeptide analog of lutenizing releasing hormone (LRH) used for the treatment of sex hormone dependent tumors. Triptorelin diminishes the production of luteinizing hormone to considerably reduce the levels of testosterone production and accumulation. This may cause shrinkage or slowing down the growth of the tumor. In order to optimize the treatments via triptorelin, maintenance of steady plasma levels of drug for prolonged time periods is needed. Triptorelin loaded PLGA nanospheres have been prepared via double emulsion solvent evaporation technique with encapsulation efficiency varying from 4% to 83% (Nicoli et al., 2001; Kumari et al., 2010).

Dexamethasone

Dexamethasone is frequently administered previous to antibiotics in cases of bacterial meningitis. It acts to decrease the inflammatory response of the body to killed bacterial population by the antibiotics, therefore improving prognosis and outcome. Dexamethasone causes inhibitory consequence on leukocytes infiltration at the inflammatory site. It is a weakly soluble and crystalline corticoid that has been used for the treatment of diabetic macular edema administered as an implant. This drug has been incorporated into PLGA nanoparticles via solvent evaporation technique (Gómez-Gaete et al., 2007; Kumari et al., 2010). The highest drug loading was obtained using 100mg PLGA (75:25) in a mixture of acetone-dichloromethane 1:1 (v/v) and 10mg of dexamethasone (Kumari et al., 2010).

Rapamycin

Currently, rapamycin and its analogues supply as promising novel drugs that use alternative mechanisms to restrain the growth of breast cancer cells efficiently (Noh et al., 2004). Clinically, rapamycin analogues with advanced stability and pharmacological properties have been well tolerated by patients in Phase I trials, and these agents have shown a hopeful antitumor consequence in breast cancer (Hidalgo and Rowinsky, 2000; Garber, 2001; Chan et al., 2005; Acharya and Sahoo, 2011). However, regardless of the potency of rapamycin in preclinical studies, the clinical development of this drug

floundered because of its poor solubility in water (2.6 μgml-) (Simamora et al., 2001), no tumor tissue specificity, low bioavailability and dose limiting toxicity. Currently, nanoparticulate drug-delivery systems are being developed to deliver smaller doses of rapamycin in an effective form with a controlled drug distribution within the body to treat diverse diseases. Recently, the therapeutic efficacy of rapamycin has been optimised by developing efficient delivery system for the drug, that is, nano scale delivery vehicles (such as PLGA NPs), which are capable of controlled release of the drug, thus enhancing its regressive activity on dendritic cells through altering their maturation profile (Haddadi et al., 2008 ; Acharya and Sahoo, 2011).

Hypericin

A natural photo sensitizer extracted from Hypericum perforatum, is a tool for the treatment and detection of ovarian cancer and other cancers. Because of its hydrophobicity, administration of hypericin is problematic. Hypericin-loaded PLGA NPs suppress ovarian tumor growth efficiently (Zeisser-Labouèbe et al., 2006; Lü et al., 2009).

Multidrug Resistance

The development of multidrug resistance (MDR) is a main barrier to effective cancer chemotherapy. After a long period of chemotherapy, numerous patients suffer from MDR, which can decrease therapy efficiency and cause to treatment failure (Szakács et al., 2006; Li et al., 2012). The high level of resistance is typically caused by complex MDR mechanisms. Between them, over expression of the adenosine triphosphate (ATP)-binding cassette transporters (ABC), such as P-glycoprotein (P-gp), is one of the most common mechanisms (Fojo et al., 1987; Kohno et al., 1989; Li et al., 2012). Tumor cells have highly ordered internal resistance. This P-gp encoded via the MDR-1 gene acts as a drug efflux pump that exports a wide range of chemotherapeutic drugs and will decrease the accumulation of functional drugs in MDR cancer cells, resulting in low tumor chemotherapeutic efficiency

Table 1. PLGA Biodegradable Polymeric Nanoparticles for Drug Delivery

Drug loaded in PLGA nanoparticles	Main targets	In vitro application	In vivo application	EE	References
Paclitaxel	Microtubules	Efficacy of paclitaxel mediated NP delivery was tested on human small cell lung cancer (NCI-H69 SCLC), human adenocarcinoma (HT-29), human laryngeal cancer (Hep-2), breast carcinoma (MCF-7) and carcinoma cervicis (HeLa) cell lines.	In vivo efficacy of paclitaxel-loaded nanoparticles was accessed on transplantable liver tumor in male NMRI mice and in model glioblastoma tumors	>90%	(Fonseca, Simoes et al. 2002; Si-Shen, Li et al. 2004; Patil, Papadmitrakopoulos et al. 2007; Danhier, Lecouturier et al. 2009; Jin, Bai et al. 2009; Kumari, Yadav et al. 2010; Ranganath, Fu et al. 2010; Acharya and Sahoo 2011)
Cisplatin	DNA adducts	Cisplatin in PLGA nanoparticles exhibited higher therapeutic efficacy on human prostate cancer LNCaP cells	Pharmacodynamics of cisplatin-loaded PLGA or PLGA-mPEG nanoparticles upon administration to tumor-bearing mice/Balb C mice was investigated by different groups	90%	(Agrahari, Kabra et al. ; Mattheolabakis, Taoufik et al. 2009; Moreno, Zalba et al. 2010; Acharya and Sahoo 2011; Cheng, Jin et al. 2011)
Doxorubicin	Topo II	Multifunctional PLGA nanoparticles were used	In vivo pharmacokinetics of DOX loaded NPs was evaluated in	80%	(Yoo, Lee et al. 2000; Avgoustakis, Beletsi et al. 2002; Betancourt, Brown et al. 2007; Kalaria, Sharma et al. 2009; Park, Yang et al. 2009; Gelperina, Maksimenko et al. 2010; Acharya and Sahoo 2011; Dinarvand, Sepehri et al. 2011)
Curcumin	Cytoplasmic proteins	Nanoparticle encapsulation improves oral bioavailability of curcumin and was effective against metastatic ovarian , breast cancer and prostate cancer cells	In vivo bioavailability of curcumin-loaded PLGA nanoparticleswas performed in Balb/c mice. Another independent study proved the marked anticancer efficacy of curcumin microparticles in nude mice bearing MDA-MB-231 xenografts	>72%	(Bisht, Feldmann et al. 2007; Mukerjee and Vishwanatha 2009; Shaikh, Ankola et al. 2009; Anand, Nair et al. 2010; Shahani, Swaminathan et al. 2010; Yallapu, Gupta et al. 2010; Acharya and Sahoo 2011; Dinarvand, Sepehri et al. 2011)
Dexamethasone	Cytoplasmic receptors	Efficient suppression proliferation of vascular smooth muscle cells by drug loaded NP	Enhanced in vivo efficacy of drug loaded nanocarriers for the local treatment of arthritis and angiogenesis was studiedusing these NPs	6%	(Gómez-Gaete, Tsapis et al. 2007; Butoescu, Seemayer et al. 2009; Kumari, Yadav et al. 2010; Acharya and Sahoo 2011; Panyam and Labhasetwar 2012)

(Ambudkar et al., 2003).

With the development of nanotechnology, nano formulations have been extensively used to avoid MDR (Sharma et al., 2008; Ren et al., 2011; Li et al., 2012). Earlier studies have shown that these nano-sized particles, like lipids, micelles, and inorganic hybrid particles, can bypass the P-gp efflux pumps and modify the intracellular accumulation of chemotherapeutic drugs (Lee et al., 2005; Patel et al., 2011; Li et al., 2012). Multidrug resistance may be treated using a mixture of entrapped cytotoxic drugs and chemo sensitizers. Co encapsulation of an anticancer drug and chemo sensitizer may cause lower drug toxicity and fewer drug-drug interactions. Consequently, PLGA nanoparticles concurrently loaded with an anticancer drug and a chemo sensitizer may potentially be a very capable formulation for treatment of drug-resistant cancers in vivo (Song et al., 2009; Dinarvand et al., 2011).

Surface Modification of PLGA Nanoparticles

Most cancer drugs do not differentiate between normal and cancer cells. These drugs are administered in high doses to attain the tumor site. Hence, an optimum drug concentration in the tumor is achieved at the expense of exposing other organs to high drug concentrations, which results in severe side effects. Nanoparticles suggest a targeted approach, which can be used for improving cancer therapy. However, depending on their surface characteristics, nanoparticles will be taken up by the liver, spleen, and other parts of the reticuloendothelial system (RES) (Nie et al., 2007; Mozafari et al., 2009). Surface modification of nanoparticles is significant for escaping the body's natural defense systems when transporting drugs to the bloodstream (Storm et al., 1995; Dinarvand et al., 2011). A long circulation time increases the chance that the nanoparticles will reach their target. Nanostructures with a hydrophilic surface which is smaller than 100 nm have the greatest ability to escape from the molecular phagocytic system (Feng, 2004; Dinarvand et al., 2011). Hydrophobic nanoparticles will be preferentially taken up by RES organs. It has been shown that hydrophilic particles can remain in the circulation for a longer time and

are taken up by liver to a minor extent (Araujo et al., 1999; Moghimi et al., 2005; Mozafari et al., 2009). Different strategies have been used to make a hydrophilic cloud around the nanoparticles and reduce their uptake by RES organs. These strategies comprise coating of nanoparticles with Tween 80 (Gelperina et al., 2002), PEG (polyethylene glycol) (Tang et al., 2007), PEO (polyethylene oxide) (Soppimath et al., 2001), poloxamers and poloxamines (Moghimi and Hunter, 2000; Mozafari et al., 2009), polysorbate 80, TPGS and polysaccharides like dextran (Stolnik et al., 1995; Torchilin and Trubetskoy, 1995; Mahapatro and Singh, 2011). Hydrophilic polymers can be useful at the surface of NPs by adsorption of surfactants or by utilize of block copolymers or branched copolymers (Stolnik et al., 1995; Storm et al., 1995; Torchilin and Trubetskoy, 1995; Mahapatro and Singh, 2011). Surface chemistry Analysis is determined by X-ray photoelectron spectroscopy (Mu and Feng, 2003; Si-Shen et al., 2004; Kim et al., 2005), Fourier transform infrared spectroscopy (Li et al., 2001; Choi and Kim, 2007; Yang et al., 2007) and Nuclear magnetic resonance spectroscopy (Li et al., 2001; Avgoustakis et al., 2002; Cheng et al., 2007; Dinarvand et al., 2011).

Polyethylene glycol (PEG)

The most preferred process of surface modification is the adsorption or grafting of poly-ethylene glycol (PEG) to the surface of nanoparticles. This is a hydrophilic, non-ionic polymer that has been shown to exhibit exceptional biocompatibility (Tobıo et al., 2000; Kumari et al., 2010). Insertion of PEG and PEG-containing copolymers to the surface of particles results in an augment in the blood circulation half-life of the nanoparticles. The precise mechanisms by which PEG prolonged circulation time of the surface modified NPs are still not well understood. It is usually thought that the increased residency of the nanoparticles in blood is mostly due to prevention of opsonization of nanoparticles by a certain serum or plasma proteins (opsonins). It is believed that PEG causes steric repulsion via creating hydrated barriers on nanoparticle surfaces that prevents coating of PEG modified NPs by serum opsonins. More surveys have shown that the degree to which proteins (opsonins) adsorb on to nanoparticles surface can be reduced by means of mounting the PEG density on the particle surface. Increasing the molecular weight of the PEG chains has also been shown to reduce opsonization of nanoparticles and improve retention in the circulation (Gref et al., 2000; Mahapatro and Singh, 2011). High surface density and long chain lengths of PEG are required for low protein adsorption. Though, surface density has a greater effect than the chain-length on steric repulsion and Vander Waals attraction (Soppimath et al., 2001). PEG is also believed to make easy mucoadhesion and consequent transport through the Peyer's patches of the GALT (gut associated lymphoid tissue) (Vila et al., 2002; Mahapatro and Singh, 2011). In addition, PEG may benefit nanoparticle's interaction with blood components. PEGylated particles showed moderately higher uptake of drug by the spleen and the brain than conventional non-PEGylated nanoparticles (Calvo et al., 2001; Kumari et al., 2010). Consequently, the presence of PEG on the nanoparticles imparts additional functionality during the use of polymeric NPs (Mahapatro and Singh, 2011).

Polysorbate

Polysorbate 20,40,60 and 80 have been used to coat the surface of PBC nanoparticles (Kreuter et al., 1997; Kumari et al., 2010). Polysorbate coating leads to the alteration of surface properties of the nanoparticles. This new surface seems to adsorb certain substances from the blood through endothelial cells (Alyautdin et al., 1998; Kumari et al., 2010). Polysorbate coated nanoparticles can cross the blood-brain barrier more efficiently. The mechanism of enhancement of drug transport from the coated NPs through BBB is due to the number of mechanisms: *i*) with binding the NPs to the inner endothelial lining of the brain capillaries and then, particles deliver drugs to the brain by providing a large concentration gradient, therefore enhancing the passive diffusion; *ii*) brain endothelial uptake by phagocytosis (Alyautdin et al., 1995; Soppimath et al., 2001; Kumari et al., 2010).

Vitamin E TPGS

TPGS is a form of vitamin E that has been used as an emulsifier, a solubilizer and a vehicle in drug delivery formulations. Vitamin E TPGS has been employed as an emulsifier for producing nanoparticles enclosing hydrophobic drugs and for advancing encapsulation, drug loading, and the release profile of particles (Esmaeili et al., 2007a; Dinarvand et al., 2011). TPGS augments the PLGA nanoparticles adhesion to the cells and hemodynamic properties of the nanoparticles (Zhang and Feng, 2006; Kumari et al., 2010). Random PLGA-TPGS copolymers could work as a novel and potential biocompatible polymeric matrix material proper to nanoparticle-based drug delivery systems for cancer chemotherapy (Ma et al., 2010; Dinarvand et al., 2011).

PLGA Nanoparticle Targeting Strategies

Most current anticancer agents do not significantly differentiate between normal and cancerous cells, leading to systemic toxicity and adverse effects. Therefore, systemic applications of these drugs often cause rigorous side effects in other tissues (such as bone marrow suppression, cardiomyopathy, and neurotoxicity), which greatly limits the maximal permissible dose of the drug. In addition, rapid removal and widespread distribution into nontargeted organs and tissues require the administration of a drug in large quantities, which is not cost-effective and often complicated owing to nonspecific toxicity. Nanotechnology suggests a more targeted approach and could provide important benefits to cancer patients. In fact, the exploit of nanoparticles for drug delivery and targeting is likely one of the most exciting and clinically significant applications of cancer nanotechnology (Nie et al., 2007). Nanoparticle systems offer major improvements in therapeutics via site specificity, a capability to evade multidrug resistance, and proper delivery of anticancer agents (Parveen and Sahoo, 2008; Dinarvand et al., 2011).

Targeted delivery can be passively (by taking advantage of the distinct pathophysiological features of

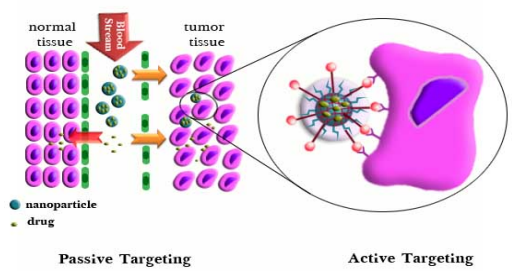

Figure 6. The Concept of Passive Targeting through the EPR Effect and Active Targeting through Ligand Display

tumor tissue) or actively (by targeting the drug carrier using target-specific ligands) accomplished (Lamprecht et al., 2001; Dinarvand et al., 2011).

Passive targeting

Structural changes in vascular pathophysiology could provide chances for the use of long-circulating particulate carrier systems. The aptitude of vascular endothelium to present open fenestrations was described for the sinus endothelium of the liver (Roerdink et al., 1984; Danhier et al., 2010) , when the endothelium is perturbed by inflammatory process, hypoxic areas of infracted myocardium (Palmer et al., 1984) or in tumors (Jain, 1989). More particularly, tumor blood vessels are usually characterized by abnormalities such as high proportion of proliferating endothelial cells, pericyte deficiency and abnormal basement membrane formation leading to an enhanced vascular permeability. Particles, such as nanocarriers (in the size range of 20-200 nm), can extravasate and accumulate inside the interstitial space. Endothelial pores have sizes varying from 10 to 1000 nm (Torchilin, 2000; Danhier et al., 2010). Furthermore, lymphatic vessels are absent or non-functional in tumor which contributes to incompetent drainage from the tumor tissue. Nanocarriers entered into the tumor are not removed efficiently and are thus preserved in the tumor. This passive phenomenon has been called the "Enhanced Permeability and Retention (EPR) effect," discovered by Matsumura and Maeda (Matsumura and Maeda, 1986; Maeda et al., 2001; Maeda et al., 2009; Danhier et al., 2010). Rapid vascularization in fast-growing cancerous tissues is known to result in leaky, defective architecture and impaired lymphatic drainage. This arrangement allows an EPR effect (Matsumura and Maeda, 1986; Jain, 1999; Jain, 2001; Duncan, 2003; Nie et al., 2007), resulting in the gathering of nanoparticles at the tumor site. To maximize circulation times and targeting capability, the optimal size should be less than 100 nm in diameter and the surface should be hydrophilic to circumvent clearance by macrophages (Ringsdorf, 1975; Moghimi and Hunter, 2000; Davis, 2002; Nie et al., 2007; Park et al., 2005). The covalent linkage of amphiphilic copolymers (polylactic acid, polycaprolactone, polycyanonacrylate chemically coupled to PEG) is usually preferred, as it avoids aggregation and ligand desorption when in contact with blood components (Nie et al., 2007). Danhier et al formulated Cremophor EL-free paclitaxelloaded PEGylated PLGA-based nanoparticles via a nanoprecipitation technique. In vivo tumor growth inhibition by the paclitaxel-loaded nanoparticles was then investigated in transplantable liver tumor-bearing mice. Paclitaxel was shown to reach the tumor site through the improved permeation and retention effect and maintain an efficient therapeutic concentration (Danhier et al., 2009; Dinarvand et al., 2011).

Active targeting

In active targeting, targeting ligands are attached at the shell of the nanocarrier for binding to proper receptors expressed at the target site. The ligand is chosen to bind to a receptor over expressed by tumor cells or tumor vasculature and not expressed by normal cells. Furthermore, targeted receptors should be expressed homogeneously on all targeted cells. Targeting ligands are either monoclonal antibodies (mAbs) and antibody fragments or nonantibody ligands (peptidic or not). The binding affinity of the ligands influences the tumor penetration owing to the "binding-site barrier." For targets in which cells are readily reachable, usually the tumor vasculature, because of the dynamic flow environment of the bloodstream, high affinity binding appears to be preferable (Adams et al., 2001; Gosk et al., 2008; Danhier et al., 2010). Various anti-cancer therapeutics, grouped under the name "ligand targeted therapeutics," are classified into different classes based on the approach of drug delivery (Allen, 2002; Danhier et al., 2010). In the active targeting strategy, two cellular targets can be differentiated: *i*) the targeting of cancer cell and *ii*) the targeting of tumoral endothelium (Danhier et al., 2010).

The targeting of cancer cell

The aim of active targeting of internalization-prone cell-surface receptors, over expressed by cancer cells, is to improve the cellular uptake of the nanocarriers. Therefore, the active targeting is mainly attractive for the intracellular delivery of macromolecular drugs, such as DNA, siRNA and proteins. The improved cellular internalization rather than an increased tumor accumulation is responsible of the anti-tumoral efficacy of actively targeted nanocarriers. This is the foundation of the design of delivery systems targeted to endocytosis-prone surface receptors (Kirpotin et al., 2006; Danhier et al., 2010). The aptitude of the nanocarrier to be internalized after binding to target cell is so an significant criterion in the selection of proper targeting ligands (Cho et al., 2008; Danhier et al., 2010). In this strategy, ligand targeted nanocarriers will result in direct cell kill, including cytotoxicity against cells that are at the tumor periphery and are independent on the tumor vasculature (Pastorino et al., 2006; Danhier et al., 2010). The more considered internalization-prone receptors are:

i) The transferrin receptor. Transferrin, a serum glycoprotein, transports iron through the blood and into cells by binding to the transferring receptor and then being internalized via receptor-mediated endocytosis. The transferrin receptor is a crucial protein involved in iron homeostasis and the regulation of cell growth. The high levels of expression of transferring receptor in cancer cells, which may be up to 100-fold higher than the regular expression of normal cells, its extracellular

accessibility, its ability to internalize and its central role in the cellular pathology of human cancer, make this receptor an attractive target for cancer therapy (Cho et al., 2008; Danhier et al., 2010; Daniels et al., 2006). *ii*) The folate receptor is a famous tumor marker that binds to the vitamin folic acid and folate-drug conjugates or folate grafted nanocarriers with a high affinity and carries these bound molecules into the cells through receptor-mediated endocytosis. Folic acid is needed in one carbon metabolic reactions and as a result, is essential for the synthesis of nucleotide bases. The alpha isoform, folate receptor-α is over expressed on 40% of human cancers. On the contrary, folate receptor-β is expressed on activated macrophages and also on the surfaces of malignant cells of hematopoietic origin (Danhier et al., 2010; Low and Kularatne, 2009). *iii*) Glycoproteins expressed on cell surfaces. Lectins are proteins of non-immunological origin which are able to identify and bind to carbohydrate moieties attached to glycoprotein's expressed on cell surface. Cancer cells often express diverse glycoprotein's compared to normal cells. Lectins interaction with certain carbohydrate is extremely specific. Lectins can be incorporated into nanoparticles as targeting moieties that are directed to cell-surface carbohydrates (direct lectin targeting) and carbohydrates moieties can be coupled to nanoparticles to target lectins (reverse lectin targeting). The use of lectins and neoglyco conjugates for direct or reverse targeting strategies is a usual approach of colon drug targeting (Minko, 2004; Danhier et al., 2010). *iv*) The Epidermal growth factor receptor (EGFR). The EGFR is a component of the ErbB family, a family of tyrosine kinase receptors. Its activation stimulates key processes involved in tumor growth and progression. EGFR is commonly over expressed in a lot of cancer, particularly in breast cancer. Also it has been found to play an important role in the progression of several human malignancies. Human epidermal receptor-2 (HER-2) is reported to be expressed in 14-91% of patients with breast cancer (Scaltriti and Baselga, 2006; Acharya et al., 2009; Danhier et al., 2010). EGFR is expressed or over expressed in a diversity of solid tumors, including colorectal cancer, non-small cell lung cancer and squamous cell carcinoma of the head and neck, as well as ovarian, kidney, pancreatic, and prostate cancer (Lurje and Lenz, 2009; Danhier et al., 2010).

Targeting of tumoral endothelium

Demolition of the endothelium in solid tumors can result in the death of tumor cells induced by the lack of oxygen and nutrients. In 1971, Judah Folkman suggested that the tumor growth might be inhibited by preventing tumors from recruiting new blood vessels (Folkman, 1971; Danhier et al., 2010). This observation is the base of the design of nanomedicines actively targeted to tumor endothelial cells (Lammers et al., 2008, Danhier et al., 2010). By attacking the growth of the blood supply, the size and metastatic capabilities of tumors can be controlled. Consequently, in this strategy, ligand-targeted nanocarriers bind to and kill angiogenic blood vessels and indirectly, the tumor cells that these vessels support, mostly in the tumor core. The advantages of the tumoral endothelium targeting are; *i*) there is no need of extravasation of nanocarriers to achieve to their targeted site, *ii*) the binding to their receptors is directly possible after intravenous injection, *iii*) the possible risk of emerging resistance is reduced due to the genetically stability of endothelial cells as compared to tumor cells, and *iv*) the majority of endothelial cells markers are expressed whatever the tumor type, involving an ubiquitous approach and an ultimate broad application spectrum (Gosk et al., 2008; Danhier et al., 2010). The major targets of the tumoral endothelium include:

i) The vascular endothelial growth factors (VEGF) and their receptors, VEGFR-1 and VEGFR-2, mediate imperative functions in tumor angiogenesis and neovascularization (Shadidi and Sioud, 2003; Danhier et al., 2010). Tumor hypoxia and oncogenes upregulate VEGF levels in the tumor cells, resulting in an upregulation of VEGF receptors on tumor endothelial cells. Two major approaches to object angiogenesis via the VEGF way have been studied: 1) targeting VEGFR-2 to reduce VEGF binding and induce an endocytotic pathway and 2) targeting VEGF to restrain ligand binding to VEGFR-2 (Carmeliet, 2005; Byrne et al., 2008; Danhier et al., 2010). *ii*) The αvβ3 integrin is an endothelial cell receptor for extracellular matrix proteins which includes fibrinogen (fibrin), vibronectin, thrombospondin, osteopontin and fibronectin (Danhier et al., 2010; Desgrosellier and Cheresh, 2010). The αvβ3 integrin is extremely expressed on neovascular endothelial cells but poorly expressed in resting endothelial cells and most normal organs, and is significant in the calcium dependent signaling pathway leading to endothelial cell migration (Byrne et al., 2008; Danhier et al., 2010).

Cyclic or linear derivatives of RGD (Arg-Gly-Asp) oligopeptides are the most studied peptides which bind to endothelial αvβ3 integrins. The αvβ3 integrin is upregulated in both tumor cells and angiogenic endothelial cells (Danhier et al., 2010; Desgrosellier and Cheresh, 2010). *iii*) Vascular cell adhesion molecule-1 (VCAM-1) is an immunoglobulin- like transmembrane glycoprotein that is expressed on the surface of endothelial tumor cells. VCAM-1 induces the cell to cell adhesion, a key step in the angiogenesis procedure. Over expression of VCAM-1 is found in various cancers, such as leukemia, lung and breast cancer, melanoma, renal cell carcinoma, gastric cancer and nephroblastoma (Danhier et al., 2010). *iv*) The matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptideases. MMPs degrade the extracellular matrix, playing an important role in angiogenesis and metastasis more particularly in endothelial cell invasion and migration, in the formation of capillary tubes and in the employment of accessory cells. Membrane type 1 matrix metalloproteinase (MT1-MMP) is expressed on endothelial tumor cells, including malignancies of lung; gastric, colon and cervical carcinomas; gliomas and melanomas (Danhier et al., 2010; Genís et al., 2006). Aminopeptidase N/CD13, a metalloproteinase that eliminates amino-acids from unblocked N-terminal segments of peptides or proteins, is an endothelial cell-surface receptor involved in tumor-cell invasion, extracellular matrix degradation by tumor cells and tumor metastasis in vitro and in vivo (Saiki et al., 1993; Danhier et al., 2010). NGR (Asn-Gly-Arg) peptide

is reported to bind to the aminopeptidase (Pasqualini et al., 2000; Danhier et al., 2010).

PLGA Release Mechanisms

PLGA is the most commonly used biodegradable polymer in the controlled release of encapsulated drugs. The interval of drug release can be varied from hours (Ratajczak-Enselme et al., 2009) to several months (D'Souza et al., 2004; Lagarce et al., 2005; Fredenberg et al., 2011). Moreover, pulsed drug release is also possible (Dorta et al., 2002; Fredenberg et al., 2011). The term "release mechanism" has been defined in slightly different ways. True release mechanisms is the processes defining the way in which the drug is released, and Rate-controlling release mechanisms is the processes that control the release rate (Fredenberg et al., 2011).There are four true release mechanisms:

i) Diffusion via water-filled pores: In many studies, this release mechanism has only been used to describe the first stage of the release period, previous to the onset of polymer erosion (Alexis et al., 2004; Fredenberg et al., 2011; Johnson et al., 1997; Lam et al., 2000). *ii*) Diffusion via the polymer: It is probable for small hydrophobic drugs (Wischke and Schwendeman, 2008). Unlike diffusion through water-filled pores, diffusion through the polymer is not particular dependent on the porous structure. However, the drug must be dissolved in water before being released, and this process could reduce the overall release time. High porosity augments the surface area for drug dissolution and could improve drug release (Fredenberg et al., 2011). *iii*) Osmotic pumping: It is an incident that occurs when osmotic pressure, caused by water absorption, drives the transport of the drug. This release mechanism is more frequent for drug delivery systems (DDSs) using materials other than PLGA. However, there have been some reports of osmotic pumping from PLGA-based DDSs (Fredenberg et al., 2011). *iv*) Erosion (no drug transport): as a true release mechanism, i.e. drug release devoid of drug transport, results in identical profiles of drug release and polymer erosion, assuming that the drug is homogeneously distributed throughout the DDS. Erosion could be the major release mechanism for low-Mw PLGA formulations, in which an important part of the polymer has a molecular weight just above the limit for water solubility (Fredenberg et al., 2011).

The two major release mechanisms associated with drug release from PLGA-based DDSs are diffusion and degradation/erosion. The release rate is often said to be diffusion-controlled initially and degradation/erosion controlled during the final stage of the release period (D'Souza et al., 2005; Fredenberg et al., 2011). The encapsulated drug may be released by more than one true release mechanism at once, and the dominating mechanism may vary with time (Wischke and Schwendeman, 2008; Fredenberg et al., 2011).

However, numerous processes or events influence the rate of drug diffusion and the degradation kinetics, for example dissolution of the drug (in combination with diffusion) (Wong et al., 2001), Diffusion through water-filled pores (Kim et al., 2006), Diffusion through the polymer matrix (Sun et al., 2008), Hydrolysis (Bishara and Domb, 2005), Erosion (Shah et al., 1992), Osmotic pumping (Jonnalagadda and Robinson, 2000), Water absorption/Swelling (Mochizuki et al., 2008), Polymer-drug interactions (Manuela Gaspar et al., 1998), Drug-drug interactions (Zhu and Schwendeman, 2000), Polymer relaxation (Gagliardi et al., 2010), Pore closure (Kang and Schwendeman, 2007), Heterogeneous degradation (Park, 1995), Formation of cracks or deformation (Matsumoto et al., 2006) and Collapse of the polymer structure (Friess and Schlapp, 2002; Fredenberg et al., 2011). Controlled drug release from PLGA-based DDSs is complex, and many processes that influence drug release affect each other in many ways. The effects of different factors on drug release may differ in time and position through a polymer matrix. The complexity of drug release from PLGA-based DDSs makes it complicated to generalize results obtained with specific DDSs (Fredenberg et al., 2011)

Pitfalls

One of the main pitfalls of PLGA-based nanoparticles relates to the poor loading. In reality, while PLGA-based nanoparticles often present high encapsulation efficiencies, the drug loading is usually poor. This low drug loading constitutes a major problem for some drugs in the design of PLGA-based nanoparticles. A second significant pitfall consists in the high burst release of drug from nanoparticles. This fact is described for the majority of PLGA-based nanoparticles. As a result, the drug might not be able to reach the target tissue or cells, leading to a loss of efficacy. Because of the application of nanoparticles in sustained release drug delivery, the drug release mechanisms are also imperative to understand. Drug release mechanisms depend on the polymer used and on the loading efficiency. Usually, the rapid initial, or burst release is attributed to adsorbed drug to the nanoparticles surface (Danhier et al., 2012; Kumari et al., 2010). The disadvantage associated with PLGA is the production of acids upon degradation, as is the case of many other biodegradable polymers. Several methods for the stabilization of acid-sensitive drugs have been investigated, and this continues to be an area of concentrated research (Zhu and Schwendeman, 2000; Bilati et al., 2005; Houchin and Topp, 2008; Fredenberg et al., 2011). A novel subdiscipline of nanotechnology called "nanotoxicology" has emerged. Indeed, the interactions of nanocarriers with biological systems are really complex. As expected, the size and surface properties of nanocarriers adjust the behavior of these components in the body. More data are required to understand their structure– property relationships. Some nanomedicines received regulatory approvals showing their biocompatibility as others were not tested. Toxicology studies and regulations are compulsory in order to fully define the biocompatibility of nanocarriers in humans. In most of case, in vitro studies supply encouraging results. Unfortunately, these results are often far away from reality in vivo. In the same idea, animal models routinely used in preclinical trials are far from being representative for the clinical condition. In conclusion, to commercialize a new drug

delivery system, the financial aspect has to be taken in account, not only for the pharmaceutical industry but also for patients. The production of GMP PLGA with well defined properties can be expensive. An extra limitation for the commercialization of nanoparticles is the scaling-up. Many steps in experimental production are unfeasible to reproduce industrially such as dialysis, ultra centrifugation, sonication, etc (Danhier et al., 2012).

Conclusions and Future Out Look

PLGA is a polymer approved by the US FDA for drug delivery because of its biodegradability, drug biocompatibility, suitable biodegradation kinetics, mechanical properties and ease of processing. PLGA-based nanoparticles present many advantages for drug delivery. They can protect drugs from degradation and increase their stability. Moreover, due to their size, nanoparticles can penetrate specific tissues via the fenestrations present in the endothelium of cancer and inflamed tissue or via receptors over expressed by target cells or in the blood brain barrier. This allows a specific delivery of drugs, proteins, peptides or nucleic acids to their target tissue. PLGA based nanoparticles can increase the efficacy of treatments because of the sustained release of the therapeutic agent from stable nanoparticles. Another major advantage of PLGA over other polymers is that PLGA is approved by the FDA and EMA in various drug delivery systems, which leading PLGA-based nanoparticles in a good position for clinical trials. Drug delivery using PLGA or PLGA-based polymers is an attractive area with innumerable opportunities for biomedical research with the primary goal of increasing therapeutic (antitumour) effect while minimising side effects. The therapeutic advantages of PLGA NPs are becoming apparent and will soon be associated with every route of drug administration, making them feasible candidates for drug-delivery systems. We have witnessed the use of drug-loaded PLGA nanoparticulate technology in developing a new generation of more effective cancer therapies capable of overcoming the many biological, biophysical and biomedical barriers that the body stages against conventional cancer therapies. Drug delivery using PLGA or PLGA-based polymers is an attractive area with innumerable opportunities for biomedical research. These polymers are increasingly becoming feasible candidates for drug delivery systems, anticancer agents and vaccine immunotherapy. Along with better understanding of diseases, new methods will be designed to improve the treatment and diagnosis. The PLGA NP materials need to be further developed and to be accepted by the market. However, in the next 5 years, more attention will be focused on the thorough in vivo evaluation for pharmacokinetics, biodistribution and toxicity before the use of PLGA NPs in more clinical trials. Further solid proof of efficacy is expected to be achieved from clinical trials, particularly from patients with CVD and cancer. The studies of PLGA NPs as vaccine candidates will focus on improving such features as providing delivery vehicles with the sufficient surface molecules for recognition via the immune system and for more-effective targeting. These systems present also some disadvantages such as the low drug loading described for many drugs, the high cost of production and the difficulty of the scale-up. Even though this issue is seldom addressed, the relatively low drug loading efficiency is probably the major hurdle limiting the use of drug-loaded PLGA-based nanoparticles in clinical trials. Nevertheless, the future remains exciting and wide open, and further advances are needed to turn the concept of drug-loaded PLGA NP technology into a realistic practical application as the next generation of drug-delivery systems (Akbarzadeh et al., 2012a; Akbarzadeh et al., 2012b; Akbarzadeh et al., 2012c; Akbarzadeh et al., 2012d; Akbarzadeh et al., 2013)

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