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PLGA-derived anticancer Nano therapeutics: Promises and challenges for the future

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ABSTRACT

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The World Health Organization (WHO) estimates that 84 million people will die of cancer between 2005 and 2015. The main weakness of most chemotherapeutic approaches to cancer treatment is that most of them are nonspecific. The polymeric nanoparticles can enhance the permeability and retention of anticancer drug and diminish the drug exposure to healthy tissues by limiting drug distribution to the target cancer. The properties of nanoparticles as precursor of a good nanomedicine are nanoparticle size, size distribution, surface morphology, surface chemistry, surface charge, surface adhesion, surface erosion, inner porosity, drug diffusivity, encapsulation efficiency, drug stability, drug release kinetics, hemodynamic and a high loading capacity to decrease the number of the carrier required for administration. Poly (lactic-co-glycolic acid) (PLGA) is one of the most effectively used biodegradable polymers for the development of nanomedicines due to its controlled and sustained release properties, low toxicity, and biocompatibility with tissue and cells. PLGA is approved by the US FDA and European Medicine Agency (EMA) in different drug delivery systems in humans. PLGA nanoparticles are commonly used for the encapsulation of various cancer related drugs and their successful delivery in vivo. The various anticancer drugs that have been investigated in PLGA nanoparticle preparations are discussed in this chapter. The application of PLGA nanoparticles has provided a promising future for the anticancer nanomedicine with high efficacy and few side effects.

Key words: PLGA, Anticancer drugs, Drug targeting, Nanoparticles, Nanomedicines.

INTRODUCTION

According to World Health Organization (WHO) cancer exists as one of the major public health problems worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012. The number of new cases is expected to rise by about 70% over the next 2 decades. Among men, the 5 most common sites of cancer diagnosed in 2012 were lung, prostate, colorectal, stomach, and liver cancer. Among women the 5 most common sites diagnosed were breast, colorectal, lung, cervix, and stomach cancer. More than 60% of world's total new annual cases occur in Africa, Asia and Central and South America. These regions account for 70% of the world's cancer deaths. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next 2 decades. The American Cancer Society estimated more than 1.5 million new cancer cases and approximately 500,000 cancer-related deaths in 2013 in the USA only [1-2].

One of the main causes of problem for cancer therapy is the lack of selectivity of anti-cancer compounds to neoplastic cells. Anti-cancer compounds undoubtly kills cancerous cells but it affects healthy cells also. As well as

high systemic exposure to anti-neoplastic agents often results in dose-limiting toxicity. Therefore, to overcome these limitations in cancer therapy, targeted drug delivery is out most important now a days [3]. As a result globally an effort is being done to develop new technologies that could overcome the inherent obstacles of chemotherapy and effectively kill the cancerous cells without hampering healthy cells while avoiding the appearance of resistant relapses. In this regard, over the past decade nanotechnology has drawn a remarkable attention as one of the most promising tools in cancer management [4].The European Science Foundation defines nanomedicines as "nanometre size scale complex systems, consisting of at least two components, one of which being the active ingredient". Although mainstream nanotechnology explores particles between 1 and 200 nm in diameter, the size of individual particles tested for drug delivery of therapeutic and imaging agents may range from 2 to 1000 nm. Most of the nanomedicines used now a days are nanoparticles, polymeric micelles, liposomes and self-assembling prodrugs [5]. Asthe name polymeric nanoparticle itself suggests, these nanoparticles can be thought of as solid particles composed of intertwining polymer strands. Polymeric nanoparticles have been made from many types of polymers ranging from basic polymers such as poly(lactide-*co*-glycolide) (PLGA) (*Figure 1*), poly(lactic acid) (PLA), chitosan, poly(alkyl cyanoacrylate) (PACA) such as polybutylcyanoacrylate, poly(ε- caprolactone) (PCL), etc., to more complex diblock and triblock polymers such as poly(ethylene glycol)-poly(lactide acid) (PEG-PLA)[6]. Among these the reasons for the widespread use of PLGA are its biodegradability, its biocompatibility, and the fact that drug products containing PLGA have been approved for parenteral use by regulatory authorities around the world like USFDA [7-8].

1.1. Physicochemical properties of PLGA

In order to design a better controlled and targeted drug delivery device, it is essential to understand the physical, chemical and biological properties of PLGA [8].PLGA has generated tremendous interest due to its excellent biocompatibility, biodegradability, and mechanical strength. PLGA can be synthesized by a polycondensation reaction or via ring-opening polymerization of cyclic diesters. Ring-opening polymerization is currently the preferred method for the synthesis for PLGA and polylactic acid due to shorter reaction times and higher monomer conversionrates [9]. 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 include 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 linear, amorphous aliphatic polyester products[10]. In order to improve the formulation of controlled drug delivery devices, an understanding of the physical, chemical, and biological properties of polymers is helpful. The polylactic acid polymer can exist in an optically active stereo regular form (L-polylactic acid) and in an optically inactive racemic form (D, L-polylactic acid). L-polylactic acid is semi crystalline in nature due to the high regularity of its polymer chain structure, while D, L-polylactic acid is an amorphous polymer because of irregularities in its polymer chain structure. Polyglycolide is highly crystalline because it lacks the methyl side groups of polylactic acid. PLGA copolymers prepared from L-polylactic acid and polyglycolide are crystalline, while those from D, L-polylactic acid and polyglycolide are amorphous in nature. It has been found that PLGAs containing less than 70% glycolide are amorphous. The degree of crystallinity and the melting point of the polymers are directly related to their molecular weight. The mechanical strength, swelling behaviour, capacity to undergo hydrolysis, and, subsequently, the biodegradation rate, are directly influenced by the crystallinity of the PLGA polymer, which depends on the type and molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain [11].The Tg (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 37 °C and hence they are glassy in nature, thus shows rigid chain structure. It is also reported that Tg of PLGAs decrease with a decrease of lactide content as well as molecular weight [8]. Some of the commercially available PLGA grades are listed below.

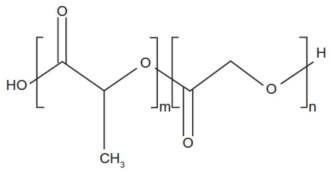

Figure 1: Chemical formula of PLGA (m: number of units of lactide, n: number of units of glycolic acid)

1.2. Biodegradation of PLGA

A fundamental understanding of the *in vivo* phenomenon of PLGA biodegradation is important because this determines the rate and mechanism of the release of therapeutic agents. PLGA copolymers are degraded in the body

by hydrolytic cleavage of the ester linkage to lactic and glycolic acid (*Figure 2*). These monomers are easily metabolized in the body via the Krebs cycle and eliminated as carbon dioxide and water[15-16].The polymer degradation process both in vitro and *in vivo* is affected by several factors, including the method of preparation, the presence of low molecular weight compounds (monomers, oligomers, catalysts), size, shape and morphology, the intrinsic properties of the polymer (molecular weight, chemical structure, hydrophobicity, crystallinity, and glass transition temperature), physicochemical parameters (pH, temperature, and ionic strength of the environment), site of implantation, and mechanism of hydrolysis. In general, the degradation time will be shorter for low molecular weight, more hydrophilic, and more amorphous polymers, and for copolymers with higherglycolide content [8, 17].

Table 1: Commercially available PLGA grades

Company (Commercial name)	Grade	Lactide/Glycolide ratio	Molecular weight	Tg (°C)	Inherent viscosity (dl/g)	Reference (s)
Sigma Aldrich (Resomer®)	P2191	50:50	30,000 -60,000 g/mol	-	0.55 - 0.75	[12]
	RG 502	50:50 ester terminated	7,000- 17,000 g/mol	42-46	0.16-0.24	
	RG 502 H	50:50 acid terminated	7,000- 17,000 g/mol	42-46	0.16-0.24	
	RG 503	50:50 ester terminated	24,000-38,000 g/mol	44-48	0.32-0.44	
	RG 503 H	50:50 acid terminated	24,000-38,000 g/mol	44-48	0.32-0.44	
	RG 504	50:50 ester terminated	38,000-54,000 g/mol	46-50	0.45-0.60	
	RG 504 H	50:50 acid terminated	38,000-54,000 g/mol	46-50	0.45-0.60	
	RG 505	50:50 ester terminated	54,000-69,000 g/mol	48-52	0.61-0.74	
	P2066	65:35	40,000-75,000 g/mol	-	0.55 - 0.75	
	RG 653 H	65:35 acid terminated	24,000-38,000 g/mol	46-50	0.32-0.44	
	P1941	75:25	66,000-107,000g/mol	-	0.55 - 0.75	
	RG 752 H	75:25 acid terminated	4,000-15,000 g/mol	42-46	0.14-0.22	
	RG 756 S	75:25 ester terminated	76,000-115,000g/mol	49-55	0.71-1.0	
	430471	85:15	50,000-75,000 g/mol	45-50	0.55 - 0.75	
	RG 858 S	85:15 ester terminated	190,000-240,000g/mol	-	1.3-1.7	
	790214	5:95	-	-	1.1	
PCAS (Expansorb ®)	10P022	50:50 ester terminated	70-100 kDa	-	0.50-0.65	[13]
	10P024	50:50 ester terminated	15-30 kDa	-	0.15-0.25	
	10P028	50:50 acid terminated	40-67 kDa	-	0.40-0.55	
	10P012	50:50 acid terminated	80-120 kDa	-	0.65-0.90	
	10P016	50:50 ester terminated	70-100 kDa	-	0.60-0.70	
	10P017	50:50 acid terminated	15-30 kDa	-	0.25-0.40	
	10P001	50:50 acid terminated	40-67 kDa	-	0.40-0.55	
	10P019	50:50 acid terminated	5-20 kDa	-	0.15-0.25	
	10P010	65:35 ester terminated	45-85 kDa	-	0.50-0.65	
	10P013	65:35 acid terminated	45-85 kDa	-	0.50-0.65	
	10P027	75:25 ester terminated	105-145 kDa	-	0.8-1.10	
	10P011	75:25 ester terminated	105-145 kDa	-	0.66-0.80	
	10P015	75:25 acid terminated	5-10 kDa	-	0.08-0.2	
	10P002	75:25 acid terminated	37-84 kDa	-	0.38-0.64	
	10P003	75:25 acid terminated	76-130 kDa	-	0.70-0.90	
	10P004	75:25 acid terminated	110-166 kDa	-	0.8-1.10	
	10P021	75:25 acid terminated	74-120 kDa	-	0.60-0.70	
	10P008	85:15 acid terminated	74-120 kDa	-	0.55-0.75	
	10P009	85:15 ester terminated	80-115 kDa	-	0.55-0.75	
	10P020	85:15 acid terminated	10-15 kDa	-	0.15-0.25	
	10P007	90:10 acid terminated	40-65 kDa	-	0.35-0.55	
	10P029	90:10 acid terminated	50-100 kDa	-	0.60-0.70	
	10P023	95:05 acid terminated	5-20 kDa	-	0.15-0.25	
	10P025	95:05 acid terminated	10-15 kDa	-	0.25-0.40	
Corbion Purac Biomaterials (Purasorb®)	PDLG 5002	50:50	-	-	0.2	[14]
	PDLG 5002A	50:50 acid terminated	-	-	0.2	
	PDLG 5004	50:50	-	-	0.4	
	PDLG 5004A	50:50 acid terminated	-	-	0.4	
	PDLG 5010	50:50	-	-	1	
	PDLG 7502	75:25	-	-	0.2	
	PDLG 7502A	75:25 acid terminated	-	-	0.2	
	PDLG 7507	75:25	-	-	0.7	

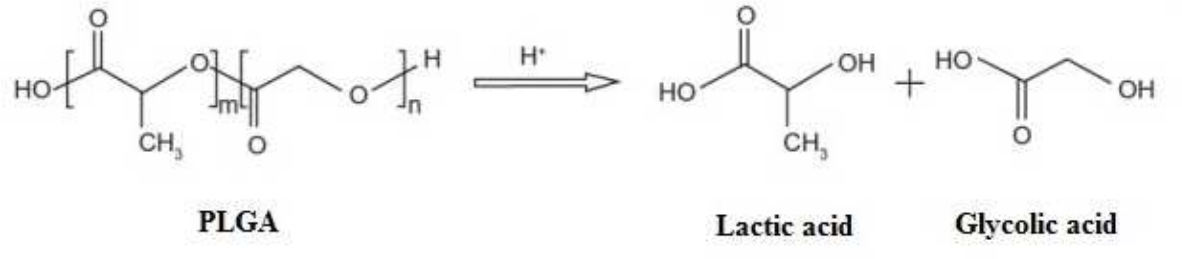

Figure 2: Degradation of polylactide-co-glycolide to lactic and glycolic acid

1.3. Preparation of PLGA nanoparticles

Number of methods for polymeric nanoparticle production has been developed by researchers. These are done by mainly two approaches. The first approach generally includes two main steps. The first step is to prepare an emulsified system, and this is common to all the methods used. The nanoparticles are formed during the second step, which varies according to the method used. In general, the principle of this second step gives its name to the method. The second approach consists in conduction of one-step procedures where emulsification is not required prior to formation of nanoparticles, and are based on spontaneous precipitation of a polymer or through self-assembly of macromolecules [18]. The commonly used methods for preparation of PLGA nanoparticles are briefly described.

1.3.1.Two-Step Procedures Based on Emulsification

1.3.1.1. 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 [17, 19]. In some cases, solid/ oil/water (s/o/w) techniques have been used with PLGA-based microspheres, especially for a higher drug loading of large water-soluble peptides, such as insulin [19-20]. 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 using a surfactant/emulsifying agent like gelatine, Poly(vinyl alcohol), Polylobate80, Poloxamer188, etc. Following the formation of a stable emulsion, the organic solvent is evaporated 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 is necessitated [21].

The size can be controlled by regulating the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature. Although different types of emulsions may be used, oil/water emulsions are of prime interest because they use water as the nonsolvent. Thus this simplifies and improves process economics, because it eliminates the requirement for recycling, facilitating the washing step and minimizing agglomeration [22]. 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 [21].

1.3.1.2. Emulsification solvent diffusion(ESD) method (Figure 3)

In the method developed by Quintanar-Guerrero *et al*[23-24] 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 magnetic stirrer/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 [23]. 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 [22, 25].

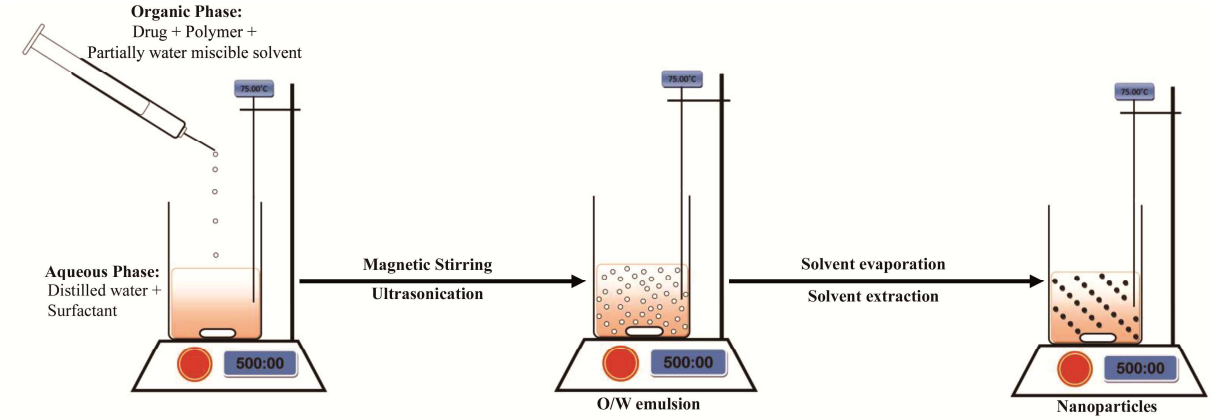

Figure 3: Emulsification solvent diffusion (ESD) method

1.3.1.3. 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 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 [26-29]. 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 requirement for severe purification steps [29-30]. The most important advantage of salting out is that it minimizes tension to protein encapsulates[31]. Salting out does not need a raise of temperature and, thus, may be useful when heat sensitive substances have to be processed [32]. The greatest disadvantages are exclusive function to lipophilic drugs and the extensive nanoparticle washing steps [22, 33].

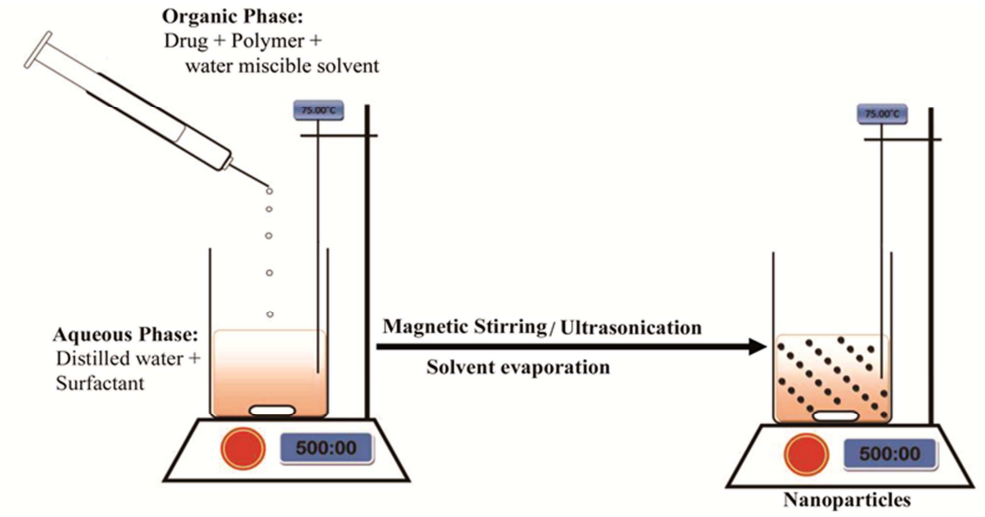

Figure 4: Nanoprecipitation method

1.3.2.One-Step Procedures

1.3.2.1. Nanoprecipitation method (Figure 4)

The nanoprecipitation technique is a one-step process, also known as the solvent displacement method [29, 34]. Nanoprecipitation is performed using systems containing three basic components, the polymer, the polymer solvent, and the nonsolvent of the polymer [29, 35]. 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 [36-37].

1.4. PLGA nanoparticles 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 [29, 38]. Particle size, size distribution and morphology determined by Dynamic light scattering or photon correlation spectroscopy [36, 39-40], Scanning electron microscopy [41-43], transmission electron microscopy [44-46] and Atomic force microscopy [47-49].

The molecular weight of the polymer influences the nanoparticles size, encapsulation efficiency, and degradation rate of the polymer [29, 50]. 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 [17, 29, 51]. The molecular weight determined by Size exclusion chromatography [52-54].

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 [21, 29-30, 55].Zeta potential determined by Zetasizer[47, 56-57]. 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 [21, 58]. Hydrophobicity and hydrophilicity determined by Water contact angle measurements and hydrophobic interaction chromatography respectively [59-64]. There are many sensitive methods for characterizing nanoparticles, depending upon the factor being investigated [29].

1.5. PLGA nanoparticles 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 [65]. Nanoparticle systems offer major improvements in therapeutics via site specificity, a capability to evade multidrug resistance, and proper delivery of anticancer agents [29, 66].

Targeted delivery can be passively (by taking advantage of the distinct pathophysiological features of tumour tissue) or actively (by targeting the drug carrier using target-specific ligands) accomplished [29, 67].

1.5.1.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 [68-69], when the endothelium is perturbed by inflammatory process, hypoxic areas of infracted myocardium [70] or in tumours[71]. More particularly, tumour 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 extravagate and accumulate inside the interstitial space. Endothelial pores have sizes varying from 10 to 1000 nm [69, 72]. Furthermore, lymphatic vessels are absent or non-functional in tumour which contributes to incompetent drainage from the tumour tissue. Nanocarriers entered into the tumour are not removed efficiently and are thus preserved in the tumour. This passive phenomenon has been called the “Enhanced Permeability and Retention (EPR) effect,” discovered by Matsumura and Maeda [69, 73-75]. 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 [65, 73, 76-78], resulting in the gathering of nanoparticles at the tumour 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 [65, 79-81]. 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 [65].Researcher 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 [29, 54].

1.5.2.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 tumour cells or tumour 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 tumour penetration owing to the "binding-site barrier." For targets in which cells are readily reachable, usually the tumour vasculature, because of the dynamic flow environment of the bloodstream, high affinity binding appears to be preferable [69, 82-83].Various anti-cancer therapeutics, grouped under the name "ligand targeted therapeutics,"is classified into different classes based on the approach of drug delivery [69, 84]. In the active targeting strategy, two cellular targets can be differentiated: *i*) the targeting of cancer cell and *ii*) the targeting of tumoralendothelium [69].

1.5.3.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 tumour 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 [69, 85]. The aptitude of the nanocarrier to be internalized after binding to target cell is so a significant criterion in the selection of proper targeting ligands [69, 86]. In this strategy, ligand targeted nanocarriers will result in direct cell kill, including cytotoxicity against cells that are at the tumour periphery and are independent on the tumour vasculature [69, 87]. 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 [69, 86, 88].

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 [69, 89].

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 [69, 90].

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 [69, 91-92]. 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 [69, 93].

1.5.4.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 [69]. This observation is the base of the design of nanomedicines actively targeted to tumor endothelial cells [69, 94]. 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 antigenic 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 nanocarriersto 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 [69, 83]. 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 [69, 95].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 endocytic pathway and 2) targeting VEGF to restrain ligand binding to VEGFR-2 [96-97].*ii*) The αvβ3 integrin is an endothelial cell receptor for extracellular matrix proteins which includes fibrinogen (fibrin), fibronectin, thrombospondin, osteopontin and fibronectin [98]. 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 signalling pathway leading to endothelial cell migration [97]. Cyclic or linear derivatives of RGD (Arg-Gly-Asp) oligopeptides are the most studied peptides which bind to endothelial αvβ3 integrin. The αvβ3 integrin is upregulated in both tumor cells and angiogenic endothelial cells [69, 97].*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 leukaemia, lung and breast cancer, melanoma, renal cell carcinoma, gastric cancer and nephroblastoma[69].*iv*) The matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptidases. 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 [99]. 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 [100]. NGR (Asn-Gly-Arg) peptide is reported to bind to the aminopeptidase [101].

RESULTS AND DISCUSSION

1.6. PLGA nanoparticles for cancer therapy

Cancer is a worldwide public health problem, and tens of millions of people presently suffer from this deadly disease[102]. Cancer research involves intensive scientific efforts to identify the causes of cancer and to develop specific strategies for its prevention, diagnosis, treatment, and cure. Despite considerable progress in its early diagnosis, but progress concerning its treatment has been less so. In current anticancer therapy, drugs are administered via the intravenous and/or oral route using conventional formulations, including injections, tablets, and capsules. Controlled and targeted delivery of an anticancer agent at the site of action is necessary to maximize the killing effect during the tumor growth phase and to avoid drug exposure to healthy adjacent cells, thereby reducing drug toxicity. It is also desirable to maintain a steady rate of infusion of the drug into the tumor to maximize exposure to dividing cells, resulting in tumor regression[103]. Nanoparticle-based drug delivery systems have many advantages for anticancer drug delivery, including an ability to pass through the smallest capillary vessels, because of their very small volume, and being able to avoid rapid clearance by phagocytes, so that their presence in the blood stream is greatly prolonged[31]. Nanoparticles can also penetrate cells and gaps in tissue to arrive at target organs,

including the liver, spleen, lung, spinal cord, and lymph. They may have controlled-release properties due to their biodegradability, pH, ions, and/or temperature sensitivity. All these properties can improve the utility of anticancer drugs and reduce their toxic side effects.

PLGA nanoparticles linked to targeting ligands are used to target malignant tumors with high affinity. PLGA nanoparticles also have large surface areas and functional groups for conjugating to multiple diagnostic (eg, optical, radioisotopic, or magnetic) agents [19]. Nanoparticle carriers have high stability in biological fluids, and are more able to avoid enzymatic metabolism than other colloidal carriers, such as liposomes or lipid vesicles[104].

Paclitaxel

Many anticancer drugs can be used clinically to treat various cancers, but have limited efficacy due to poor cell penetration. For example, paclitaxel, a mitotic inhibitor, has had limited clinical application because of its low therapeutic index, and its low solubility in water and many other pharmaceutical solvents acceptable for intravascular administration. Incorporation of paclitaxel into PLGA nanoparticles strongly enhances its antitumor efficacy compared with the free drug, with this effect being more relevant after more prolonged incubation with cells. Based on these results, it can be concluded that the formulations developed so far may be considered promising systems for in vivo paclitaxel delivery [105]. Researcher encapsulated paclitaxel and the apoptotic signalling molecule, C6-ceramide, into a PLGA/poly (β-amino ester)-blended polymer. When this nanoparticle formulation was administered intravenously to MCF7 and MCF7TR-tumor-bearing mice, higher concentrations of paclitaxel were found in the blood due to a longer retention time and enhanced tumoral accumulation compared with the free drug. In addition, the PLGA/poly(β-amino ester)-blended nanoparticles were effective in enhancing the residence time of both drugs at the tumor site by reducing systemic clearance [106].In another study, Feng et al developed paclitaxel-loaded nanoparticles to achieve better therapeutic effects with minimum side effects. In this investigation, phospholipids, cholesterol, and vitamins were used to replace traditional chemical emulsifiers to achieve high encapsulation efficacy and the desired drug release rate [107].The methodology and experimental parameters used for nanoparticle preparation can impact the physicochemical properties of the resulting formulations. Danhier et al have reported significantly higher encapsulation efficacies for paclitaxel loaded into PLGA nanoparticles using the nanoprecipitation method (70%) compared with the emulsion/ solvent evaporation technique (40%) [54] Elsewhere, it was shown that an increase in the oil-to-water phase ratio [105] and the polymer concentration of the organic phase [108] could enhance the entrapment efficacy of paclitaxel within polyester-based nanoparticles produced by the nanoprecipitation technique.Mu and Feng used α-tocopheryl polyethylene glycol l000 succinate (vitamin E TPGS) 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 advantageous either as an emulsifier or as matrix material blended with PLGA for the manufacture of nanoparticles enabling controlled release of paclitaxel [41].Surfactants and stabilizers are used to increase the physical stability of nanoparticles. Reports of the positive surface charge of a quaternary ammonium salt, didodecyl dimethyl ammonium bromide (DMAB) provided the incentive to aid the delivery of paclitaxel, because it was expected to ensure better interaction with the negatively charged cell membrane. This could result in increased retention time at the cell surface, thus increasing particle uptake [109]. In another study, the safety and utility of DMAB for stabilizing PLGA nanoparticles was studied. The preliminary data from this study provide proof-of-concept of improved efficacy and safety of oral paclitaxel chemotherapy [110].

Docetaxel

PLGA nanoparticles containing docetaxel with the desired size and drug-loading characteristics suitable for intravenous administration can be prepared without using Tween 80. Esmaeili et al showed that the cellular cytotoxicity of the nanoparticles was higher than for the free drug.Docetaxel-loaded nanoparticles reached good plasma levels *in vivo* in comparison with a conventional formulation of docetaxel [111]. The nanoprecipitation process has been applied for the formation of docetaxel-loaded nanoparticles [112, 113]. Cheng et al showed that limiting drug loading to 1% (w/w) minimized particle aggregation and yielded docetaxel-loaded PLGA nanoparticles with narrower size distributions[112].

Cisplatin

Mattheolabakis*et al* prepared cisplatin nanoparticles with an average size of 150–160 nm and an approximately 2% w/w cisplatin content using a modified emulsification and solvent evaporation method. The cisplatin-loaded PLGAmonomethoxy (m) PEG nanoparticles appeared to be effective in delaying tumor growth in HT29 tumor-bearing mice with severe combined immune deficiency. The group of mice treated with cisplatin-loaded

nanoparticles had a higher survival rate compared with the free cisplatin group [114] Cisplatin-loaded PLGA-mPEG nanoparticles also resulted in prolonged cisplatin residence time in the systemic circulation when used in mice with prostate cancer [115].

Doxorubicin

Betancourt *et al* formulated nanoparticles by nanoprecipitation of acid-ended PLGA to control the release of doxorubicin in a pH-dependent manner and deliver high loads of active drug to an MDA-MB-231 breast cancer cell line. The pH-dependent release behaviour could be a result of accelerated degradation of the polymer and decreasing ionic interaction between the drug and the polymer at an acidic pH [116]. Another approach to improve the efficacy and selectivity of cancer treatment is the application of hyperthermia in combination with traditional cancer therapeutics, such as radiation therapy and chemotherapy[117-118].Hyperthermia makes some cancer cells more sensitive to radiation and can also enhance the effect of certain anticancer drugs,thus allowing the use of decreased chemotherapy doses[118]. Indocyanine green is an optical tracer that can generate heat by absorbing near-infrared light. The significance of the Manchanda*et al* study is the synthesis of multifunctional PLGA nanoparticles and the incorporation of drugs with different physical properties (indocyanine green being amphiphilic and doxorubicin being hydrophobic). These indocyanine green-doxorubicin nanoparticles have potential applications as drug delivery systems for combined chemotherapy and localized hyperthermia[119].

Curcumin

Curcumin has been used in traditional medicine for many centuries in India and China [120]. It is chemically diferuloylmethane,a yellow polyphenol extracted from the rhizomes of turmeric (*Curcuma longa*). The only factor that limits the use of free curcumin for cancer therapy is its poor solubility in water, which in turn limits its systemic bioavailability when administered orally. Mukerjee and Vishwanatha formulated curcumin-loaded PLGA nanoparticles, and suggested that a nanoparticle-based formulation of curcumin has high potential as adjuvant therapy in prostate cancer [121]. Another study demonstrated that curcumin encapsulation in PLGA nanoparticles employing a nanoprecipitation approach in the presence of polyvinyl alcohol and poly L-lysine stabilizers not only produced a very stable nanoformulation but also enhanced cellular drug uptake and retention, as well as sustained release of curcumin. The optimized nanoparticle formulation has shown a greater inhibitory effect on the growth of metastatic cancer (A2780CP and MDA-MB-231) cells than free curcumin[122].

Vincristine sulphate

Vincristine sulphate (VCR) is a 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 [123-125]. 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 tumoral 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*[125-127].

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 [125, 128]

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 carboxylate 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 [129-130].Some of the PLGA anticancer nanoformulation developed are listed below (*Table 2*)

Table 2: PLGA anticancer nanoformulation

Polymer (s)	Drug (s)	Particle size (nm)	Zeta potential (mV)	Entrapment Efficiency (% w/w)	Drug loading (% w/w)	Cell line (s)	Reference (s)
PLGA	Paclitaxel	<200	(−23)–(−31)	70–90	1	NCI-H69 (SCLC)	[105]
PLGA	Paclitaxel	300	-20	75	4	C6 glioma	[131]
PLGA	Docetaxel	172	-12.2	68	0.34	T47D, MCF7, SKOV3, A549	[111]
PLGA-mPEG	Cisplatin	130-160	(-5.7)-(-9.3)		1.99-2.96	LNCaP	[115]
PLGA	Doxorubicin	230	-45	80	5	MDA-MB-231	[116]
PLGA	Curcumin	76	0.06	89.5		A2780CP, MDA-MB-231	[122]
PLGA	Docetaxel	217	-23.35	87.99	11.11	MCF-7 TAX30	[132]
PLGA	Vincristine Verapamil	98	-0.75	68 (VCR) 80 (VRP)		MCF-7/ADR	[127]
PLGA	Paclitaxel	182	−3.45			C6 rat glioma	[133]

1.7. Patented Anticancer PLGA nanoformulation(*Table 3*)

Bin B*et al* filed a patent on preparation of particles containing antitumor drug and its preparation method. The formulation with multidrug resistance between mesenchymal stem cells microparticles as carriers of anticancer drugs. The present invention is a good solution to the problem of mesenchymal stem cells leads to necrosis of anticancer drugs between accumulation, antineoplastic particle transport can be localized to cancer and slow release drugs toachieve targeted cancer therapy to improve efficacy, reduced toxicity side effects, and has broad application prospects [134]. Zhou D *et al* filed a patent on Nanoparticle formulation containing glycolipid antigens for immunotherapy. A composition for stimulating NKT cells to produce anticancer and antiviral cytokines without causing energy of NKT cells includes a glycolipid antigen and a nanoparticle conjugated with the glycolipid antigen. The glycolipid antigen and the nanoparticle are not antigenic in mouse and human being. The composition can further include covalent or noncovalent connection between the glycolipidantigen and the nanoparticle. Theglycolipid antigen is α-galactosylceramideor an analogue of that. The nanoparticle can be a polymer. A production method ofthe composition includes preparing a nanoparticle and a glycolipid antigen andloading the glycolipid antigen to the nanoparticle. The glycolipid antigen can becoated onto the surface of the nanoparticle or encapsulated within thenanoparticle. A method of stimulating NKT cells to produce anticancerand antiviral cytokines without causing energy of NKT cells is also provided [135]. Braden ARC *et al* filed a patent on formulation of active agent loaded activated PLGA nanoparticles for targeted cancer nanotherapeutics. The present invention includes compositions and methods of making an activated polymeric nanoparticle for targeted drug delivery that includes a biocompatible polymer and an amphiphilic stabilizing agent noncovalently associated with a spacer compound that includes at least one electrophile that selectively reacts with any nucleophilic on a targeting agent and places the targeting agent on the exterior surface of a biodegradable nanoshell, wherein an active agent is loaded with the nanoshell [136].

Table 3: Patented Anticancer PLGA nanoformulation

Patent Publication number	Title	Claim (s)
CN102225054 B	Preparation carried with particles of antitumordrug and preparation method thereof.	1. PLA or PLGA particles were prepared by emulsion solvent evaporation method with particle size 10nm~500 μ m, drug content O. 01 ~ 30% 2. PLA or PLGA polymer were used in a weight ratio of 40 /60~60 / 40. 3. Antineoplastic particle transport can be localized to cancer and slow release drugs to achieve targeted cancer therapy to improve efficacy, reduced toxicity side effects, and has broad application prospects.
US8546371 B2	Nanoparticle formulated glycolipid antigens for Immunotherapy.	1. A composition for stimulating NKT cells to produce anticancer and antiviral cytokines without causing energy of NKT cells includes a glycolipid antigen and a nanoparticle conjugated with the glycolipid antigen. 2. PLGA nanosphereswere prepared by nanoprecipitation method with particle size of 50 nm to 500 nm. 3. The nanoparticle comprises an acyl group exposed on the surface of the nanoparticle and wherein the nanoparticle is conjugated to the glycolipid by means of an amine group positioned on the glycolipid through an amide linkage.
US9023395 B2	Formulation of active agent loaded activated PLGA nanoparticle for targeted cancer nanotherapeutics.	1. An activated polymeric nanoparticle for targeted drug delivery comprising of curcumin embedded in PLGA nanoshell comprises a polylacticcoglycolic acid biocompatible polymer in contact with an amphiphilic polyol stabilizing agent 2. Nanoparticle formulation was carried out through a modified double emulsion technique. 3. Greater than 90% entrapment of curcumin was observed at concentrations of 1 mg/ml or less with particle size of 121nm to 409nm

CONCLUSION AND FUTURE PROSPECTIVE

Nanomedicine represents one of the fastest growing research areas and is regarded as one of the most promising tools for cancer treatment, but it is still in the early stage of development. They have the potential to overcome the limitations of conventional cancer chemotherapy by their ability to selectively target the cancer cells without doing too much damage to healthy tissue. Current polymeric nanocarrier technologies have demonstrated remarkable advantages for cancer therapy when compared with conventional drugs. Among the polymers utilized to date, PLGA is very promising for the preparation of novel anticancer drug delivery systems due to its desirable characteristics, including good biodegradability and biocompatibility. PLGA nanoparticles can achieve tumor targeted drug delivery via passive targeting based on the enhanced permeation and retention effect, or active targeting by an appropriate ligand, which improves antitumor efficacy and reduces toxicity on healthy tissues. 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. For further advancement, it will be necessary to focus more research attention on the pharmacokinetics, biodistribution, and safety of these novel drug delivery systems. Despite significant progress so far, a large gap between the cost of preparing PLGA nanoparticles and that of conventional delivery systems is seen as an impediment to their commercial application. Till now, most of the methods reported for preparation of PLGA nanoparticles have involved small batches. Scale-up to large production volumes will certainly introduce additional challenges. As a result, the preparation process for PLGA nanoparticles needs to be further developed to achieve the reproducibility and scalability necessary in the marketplace. Nevertheless, nanomaterial characterization, safety concerns, and regulatory as well as manufacturing issues hamper the widespread application of cancer nanomedicine therapeutics. Comprehensive and reproducible characterization of nanomedicine products to predict their efficacy and safety in humans is warranted. Timely contact with regulators is recommended to discuss new nanotechnology platforms at an early stage and to expedite evaluation and approval processes. In this promising area of cancer nanomedicine, the cooperation of all parties (i.e., research laboratories, regulatory agencies, and industry) involvement is necessary to afford patients rapid access to innovative, safe, and efficacious treatment options.

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