

## A REVIEW ON NANOPARTICLES: TINY SOLDIERS FOR GIANT TUMORS

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### ABSTRACT

Nanoparticles (NPs) as drug delivery systems (DDS) enable unique approaches for cancer treatment. Cancer is a leading cause of deaths. Millions of people are diagnosed with cancer every year. Owing to the development of nanotechnology and biotechnology, NPs of biodegradable polymers as effective DDSs have received significant attention. They have the ability to carry various therapeutic agents including anticancer drugs, DNA, peptides, and proteins. Over the past decade, there has been an increasing interest in using nanotechnology for cancer therapy. The development of smart targeted NPs that can deliver drugs at a sustained rate directly to cancer cells may provide better efficacy and lower toxicity for treating primary and advanced metastatic tumors. Thus, metastatic cancer can also treat by NPs. Recently, albumin-based NPs have received much attention by the researchers owing to its biodegradability, biocompatibility, and the ability to deliver a wide range of drugs. This review explores recent work directed toward more targeted treatment of cancer. Treatment opportunities using NPs-targeted therapies are summarized. In this review, we will review the available NP technology platforms and their impact for cancer therapy along with nanoeconomics, future products, and patents on NPs.

**Keywords:** Cancer, Nanoparticles, Drug delivery, Targeted metastatic cancer.

### INTRODUCTION

#### Cancer [1,2]

According to the National Cancer Institute, cancer is a term used for disease, in which abnormal cells divide without control and are able to invade other tissues. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells, which can result in death (Fig. 1). Cancer develops when cells in a part of the entire body start to grow out of control. Normal body cells grow, divide, and die in an orderly fashion.

Throughout the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. Because cancer cells keep growing and divide, they are different from normal cells. Instead of dying, they outlive normal cells and continue to form new abnormal cells. Cancer cells can spread to other parts of body through the blood and lymph systems. Cancer is not just one disease but also many diseases. There are more than 100 different types of cancer.

In the developed countries, cancer is the second leading cause of death accounting for 21% (2.5 million) of all mortality. In the developing countries, cancer ranks third as a cause of death and accounts for 9.5% (3.8 million) of all deaths. Cancer has become one of the 10 leading causes of death in India [6]. It is estimated that there are nearly 2.5 million cancer cases any given point of time. Over 8 lakh new cases of cancer and 4 lakh deaths occur annually due to cancer. Nearly, 15 lakh patients require facilities for diagnosis, treatment, and follow-up at a given time [7].

Types: Cancer types can be grouped into broader categories. The main categories of cancer include:

- Carcinoma-cancer that begins in the skin or in tissues that line or cover internal organs.
- Sarcoma-cancer that begins in bone, cartilage, fat, muscles, blood vessels, or other connective or supportive tissue.
- Leukemia-cancer that starts in blood-forming tissue such as the bone marrow and causes a large number of abnormal blood cells to produce and enter the blood.

- Lymphoma- and myeloma-cancer that begins in the cell of immune system. Central nervous system cancer-cancer that begins in the tissue of the brain and spinal cord.

#### Tumor

The abnormal cancer cell gains the ability to keep dividing without control or order, and this may form a mass of tissue called tumor. Not all tumors are cancerous.

#### Benign

Benign tumors are not cancerous. They can often remove, and in most cases, they do not come back. Cells in benign tumors do not spray to the other parts of the body.

#### Malignant

Malignant tumors are cancerous; cells in these tumors can invade nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastasis [1].

#### Background

The word "cancer" is actually derived from the Latin word for crab. Cancer is a popular and generic word, as the actual medical term for cancer is "neoplasia," which is derived from a Greek word, which means "new formation [3]." The oldest known description and surgical treatment of cancer were discovered in Egypt and a date back to approximately 1600 B.C. Cancer is a poison that slowly spreads and concludes as contagious.

#### Causes

Physical carcinogens - such as ultraviolet and ionizing radiation; Chemical carcinogens - such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant), and arsenic (a drinking water contaminant); Biological carcinogens - such as infections from certain viruses, bacteria, or parasites [10].

#### Clinical features-symptoms

Local symptoms: Unusual lumps or swelling (tumor), hemorrhage, pain, and/or ulceration.

Symptoms of metastasis (spreading): Enlarged lymph nodes, cough and hemoptysis, hepatomegaly, bone pain, fracture of affected bones, and neurological symptoms. Advanced cancer may cause pain [12].

#### Treatments of cancer

1. Radiation therapy (radiotherapy): Controlled doses of radiation are targeted at the tumor to destroy the cancer cells. Usually, radiotherapy is used after surgery. Side effects of radiation therapy may include fatigue, lymphedema, darkening of the breast skin, and irritation of the breast skin.
2. Surgery: Lumpectomy - surgically removing the tumor. Mastectomy - surgically removing the breast.
3. Hormone therapy: Used for breast cancers those are sensitive to hormones. The aim is to prevent cancer recurrence. Hormone blocking therapy is usually used after surgery but may sometimes be used beforehand to shrink the tumor. Hormone therapy usually lasts up to 5 years after surgery.
4. Chemotherapy: Medications are used to kill the cancer cells - these are called cytotoxic drugs. The oncologist may recommend chemotherapy if there is a high risk of cancer recurrence, or cancer spreading elsewhere in the body. Chemotherapy may help stop estrogen production. Estrogen can encourage the growth of some breast cancer. Side effects of chemotherapy may include nausea, vomiting, loss of appetite, fatigue, sore mouth, hair loss, and a slightly higher susceptibility to infections. Many of these side effects can be controlled with medications the doctor can prescribe. Women over 40 may enter early menopause.
5. Biological therapy (targeted drug therapy): Trastuzumab (herceptin), lapatinib (tykerb), bevacizumab (avastin), low dose aspirin, other antibiotics such as anthracyclines-doxorubicin, epirubicin.
6. Drug targeting: The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, whereas the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system (DDS) that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task [17]. Chemotherapy has become an integral component of cancer treatment for most cancers. Conventional chemotherapeutic agents still exhibit poor specificity in reaching tumor tissue and are often

restricted by dose-limiting toxicity. The combination of developing controlled release technology and targeted drug delivery may provide a more efficient and less harmful solution to overcome the limitations found in conventional chemotherapy [18]. The efficacy of cancerous chemotherapy is often limited by serious side effects because of the toxicity of anticancer drugs to both tumor and normal cells [19]. The need for intravenous (IV) formulations and the advantage of enlarging surface contact with an external medium to control release kinetics have encouraged the development of nanoparticles (NPs) [20]. Despite several advancements, the drug transport at high concentrations to solid tumors seems still to be a challenge. NPs have been widely attempted for delivering cancer agents to tumors [21].

#### NPS FOR CANCER TARGETING

##### What are NPs?

NPs are subnanosized colloidal structures composed of synthetic or semi-synthetic polymers. Its size ranges from 1 to 1000 nm.

They consist of macromolecular materials, in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, and/or to which the active principle is adsorbed or attached [23]. NPs have been around since Michael Faraday's time of 1857 when he first developed the gold colloidal particles [24]. Recently, polymer NPs have been widely investigated as a carrier for drug targeting [25].

NPs are just as small, or even smaller than many blood proteins. They can, therefore, pass through the walls of healthy and sick cells, which make them interesting carriers of drugs against cancer and other disease.

One can distinguish two types [6] of NP shown in Fig. 2.

##### Polymers used for preparing NPs

NPs can be made from a broad number of materials such as poly (alkyl cyanoacrylates), poly acetates, polysaccharides, and copolymers (Tables 1 and 2). Recently, the idea of using NPs made from natural, biodegradable polymers to deliver drugs has provoked great interests [26].

##### Types of NPs

According to material used for synthesis of NP, five types of particle are:

1. Polymeric NP,
2. Solid lipid NP,
3. Peglyted NP,
4. Magnetic NP,
5. Metallic NP.

##### Polymeric NP

Polymeric NPs are NPs, which are prepared from polymers shown in Fig. 3. The drug is dissolved, entrapped, encapsulated, or attached

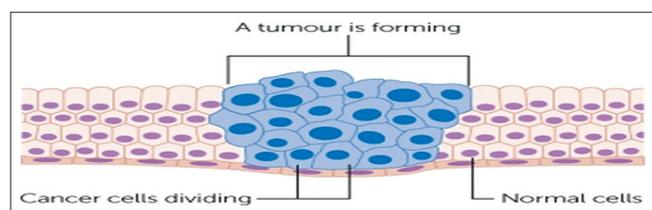


Fig. 1: Spread of abnormal cells, i.e., tumor

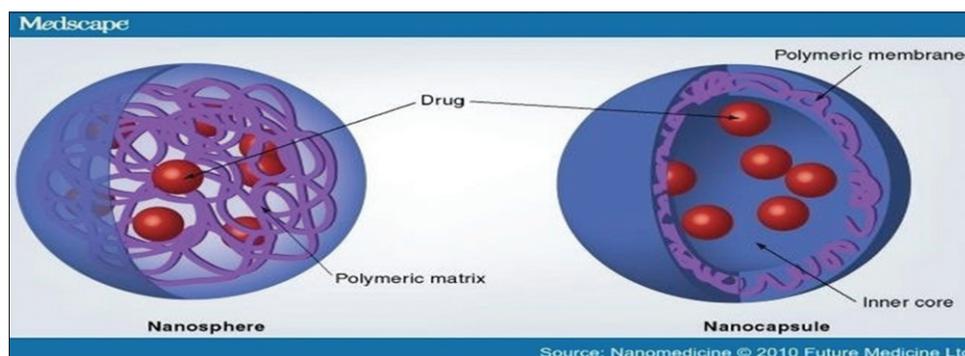


Fig. 2: Nanosphere and nanocapsules. Nanospheres - Matrix systems. Nanocapsules - Which are reservoir systems composed of a polymer membrane surrounding an oily or aqueous core

to an NP and depending on the method of preparation, NPs, and nanospheres can be obtained. Some of the polymeric materials are used for syntheses such as cellulosic, poly(vinyl alcohol), poly(acrylic acid), and polyacrylamide.

#### Advantages of polymeric NPs [6]

1. They offer a significant improvement over traditional oral and IV methods of administration in terms of efficiency and effectiveness.
2. Delivers a higher concentration of pharmaceutical agent to desired location.
3. The choice of polymer and the ability to modify drug release from polymeric NPs have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives, and delivery of targeted antibiotics.
4. Polymeric NPs can be easily incorporated into other activities related to drug delivery such as tissue.
5. Engineering.
6. To overcome drug resistance and to improve the effectiveness and safety of cancer chemotherapy, new DDSs such as microspheres, NPs have been studied. In comparison to the conventionally used drug solutions, these systems generally exhibit lower toxicity, and thus, allow higher doses of drugs to be safely administered.
7. NP-based DDSs have been shown to overcome drug resistance in cancer cells mainly by avoiding the activation of efflux pumps in tumor cells. NPs carrying anticancer drug can effectively overcome microsomal glutathione transferase 1-mediated drug resistance in breast cancer cells.

#### Advantages over microparticles

They have higher intracellular uptake compared to microparticles.

They are better suited for IV delivery since the smallest blood capillaries. In the body is about 5-6  $\mu\text{m}$ .

#### Advantages over liposomes

They have better stability in biological fluids and during storage. Their preparation is more amenable to scale up. They have the unique ability to create a controlled release product.

#### METHOD FOR THE PREPARATION OF NPS [33-41]

Conventionally, NPs have been prepared mainly by two methods:

- i. Dispersion of the preformed and,
- ii. Polymerization of monomers.

#### Dispersion of preformed polymers

Several methods have been suggested to prepare biodegradable NPs from poly(lactic acid) (PLA), poly(lactide-co-glycolide) (PLG), polylactic-co-glycolic acid (PLGA), and poly( $\epsilon$ -caprolactone) by dispersing the preformed polymers.

**Table 1: Various proteins and polysaccharides used for the preparation of nanoparticles**

Proteins	Polysaccharides
Gelatin	Alginate
Albumin	Dextran
Lectins	Chitosan
Legumin	Agarose
Vicilin	Pullulan

**Table 2: Various synthetic polymers used for the preparation of nanoparticles**

Pre-polymerized	Polymerized in process
Poly( $\epsilon$ -caprolactone)	Poly (n-butylcyanoacrylate)
Poly (lactic acid)	Poly (isobutylcyanoacrylate)
Poly (lactide-co-glycolide)	Poly (hexylcyanoacrylate)
Polystyrene	Poly (methylmethacrylate)

#### Emulsification-solvent evaporation [40-52]

The emulsification-solvent evaporation method was the first method used to prepare biodegradable and injectable lattices. Briefly, both the drug and polymer are dissolved in a volatile, water-immiscible organic solvent such as dichloro-methane, chloroform, or ethyl acetate. The organic phase is then emulsified as nanodroplets in an aqueous surfactant (such as polyvinyl alcohol and pluronic) solution using high energy homogenizer or sonicator. The polymer precipitates as nanospheres and subsequently the organic phase is evaporated using a rotary evaporator or by continuous stirring as represented in Fig. 4.

Various lipophilic and hydrophilic drugs such as indomethacin, cyclosporine A, loperamide, praziquantel, tetanus toxoid, and testosterone have been encapsulated in polymeric NPs using this method.

#### Solvent displacement and interfacial deposition method

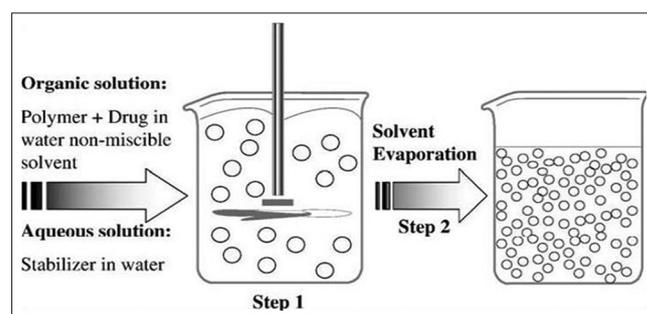
One of the easiest and reproducible techniques for preparing nanospheres was the solvent displacement (also called nanoprecipitation) method and has been widely used to prepare NPs. The method is based on the precipitation of preformed polymer following the displacement of a semi-polar solvent miscible with water in the presence or absence of surfactant. The basic principle of this technique is similar to spontaneous emulsification of the organic phase containing drug and polymer into the external aqueous phase. Three basic ingredients are needed for this method: Polymer, polymer solvent, and non-solvent for the polymer. In brief, both the polymer and drug are dissolved in a water-miscible organic solvent (polymer-solvent phase) of intermediate polarity (e.g., acetone and ethanol). The resulting organic phase is injected into a stirred aqueous phase (non-solvent phase) containing a surfactant as a stabilizer. The NPs are formed instantaneously during the rapid diffusion of the organic phase into the aqueous phase as shown in Fig. 5.

#### Emulsification-solvent diffusion

The emulsification-solvent diffusion or emulsification-solvent displacement method is the widely used method for preparing NPs due to several advantages. These include high drug entrapment efficiency for poorly water-soluble drugs, narrow particle size distribution,



**Fig. 3: Polymeric nanoparticle**



**Fig. 4: Schematic representation of emulsification-solvent evaporation method**

high batch-to-batch reproducibility, no homogenization required, simplicity, ease of scale up, and rapid organic solvent extraction. The drug and polymer usually PLA, PLGA, PCL, or Eudragit are dissolved in a partially water-soluble solvent. Commonly used solvents are propylene carbonate, benzyl alcohol, ethyl acetate, isopropyl acetate, methyl acetate, methyl ethyl ketone, butyl lactate, or isovaleric acid. The organic phase is saturated with water to ensure the initial thermodynamic equilibrium. It is then diluted with an extensive amount of pure water to facilitate diffusion of the organic solvent from the organic phase droplets leading to the precipitation of the polymer as presented in Fig. 6.

Nanocapsules are successfully prepared by this method when a small amount of oil is incorporated into the organic phase. The disadvantages of this method include: Long time required to remove the high volume of water and leakage of water-soluble drugs during processing.

#### Salting out method

The salting out procedure can be considered as a modification of the emulsification/solvent diffusion method. The separation of a water-miscible solvent from aqueous solution is achieved via a salting out effect. Briefly, a water-miscible organic solvent, usually acetone, containing polymer, and drug are added dropwise to an aqueous phase saturated with an electrolyte or non-electrolyte (such as magnesium chloride, calcium chloride, or sucrose) with a colloidal stabilizer (such as polyvinyl pyrrolidone) under agitation to form an o/w emulsion. A sufficient volume of water is added to enhance the diffusion of acetone to the water phase, and nanospheres are thus obtained shown in Fig. 7.

The technique offers advantages such as the avoidance of chlorinated solvents and surfactants, minimization of stress for protein encapsulants, useful for heat-sensitive substances, high encapsulation efficiency, and easy scaling up. The method is not popular because of the extensive washing steps required to achieve purity of the NPs and the possibility of incompatibility between drugs and salts.

#### Polymerization method

In the polymerization method, monomers are polymerized to form NPs in aqueous solution. The polymerization method can be classified into the emulsion and interfacial polymerization. The emulsion polymerization method is the fastest and scalable method of producing NPs. It can be classified into two categories: Continuous organic phase or continuous aqueous phase methodology depending on the use of the continuous phase. In general, the monomer is dissolved in an organic or aqueous continuous phase. Additional monomer molecules are then emulsified into the emulsion droplets that are stabilized by a surfactant. The polymerization is started by chemical initiation, pH shift or by irradiation of gamma, ultraviolet, or visible rays. In the continuous phase, chain growth starts when the initiated monomer ion or monomer radical collide with each other and forms aggregates which are stabilized by polymeric emulsifier particles.

#### Production of NPs using supercritical fluid technology

Recently, supercritical or compressed fluids have been utilized as an alternative way to prepare biodegradable NPs. This new technique obviates the use of toxic organic solvents associated with conventional methods. Two techniques are most commonly used for preparing NPs – Supercritical Antisolvent (SAS) and Rapid Expansion of Critical Solution (RESS). In the SAS method, solutes are dissolved in methanol which is completely miscible with supercritical fluids. Dexamethasone phosphate NPs were prepared by this method. In the RESS method, solutes are dissolved in the supercritical fluid, and the solution is expanded through a small nozzle into a region of lower pressure. The solutes eventually precipitate as NPs. Insulin-loaded PEG/PLA NPs were prepared by this method.

#### APPLICATIONS OF NP

##### Delivery of specific agents by nanoparticulate systems

Cancer treatment using NPs: Paclitaxel: Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubulin causing cell death by disrupting the dynamics necessary for cell division. It

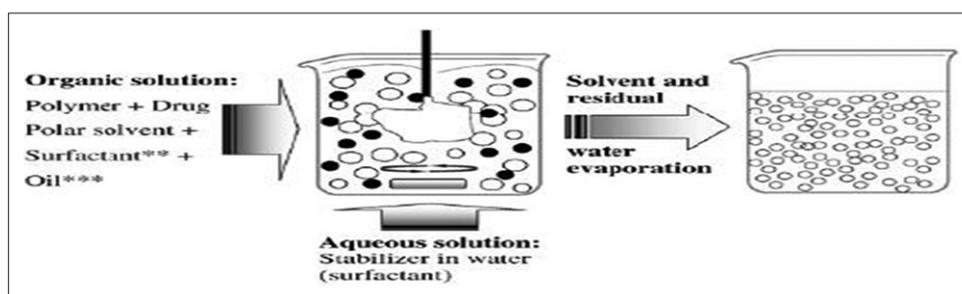


Fig. 5: Schematic representation of solvent displacement and interfacial deposition method

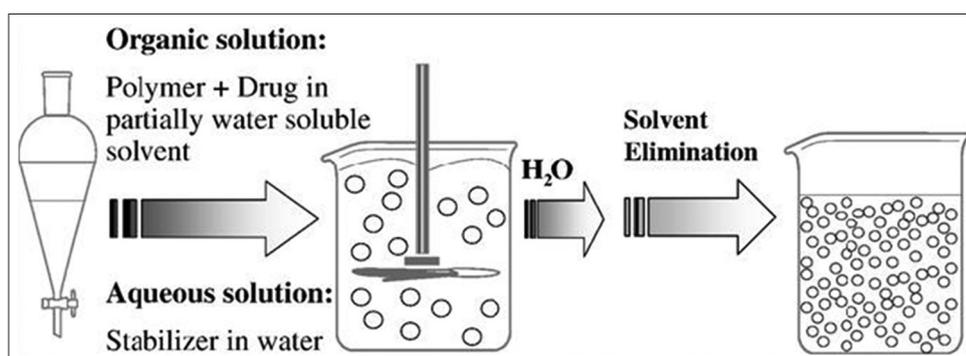


Fig. 6: Schematic representation of the emulsification-solvent diffusion method

has neoplastic activity, especially against primary epithelial ovarian carcinoma, breast, colon, and non-small cell lung cancers.

Paclitaxel is poorly soluble in aqueous solutions but soluble in many organic solvents such as alcohols. It, therefore, lends itself well to more advanced formulation strategies.

A paclitaxel-albumin NP also receives much attention due to its biodegradability as shown in Fig. 8.

**Present application of NPs (Table 3) [46]**

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**Future applications of NPs**

Researchers are developing a method to release insulin that uses a sponge-like matrix that contains insulin as well as nanocapsules containing an enzyme.

Researchers at MIT have developed an NP that can be taken orally and pass through the lining of the intestines into the blood stream. This should allow drugs that must now be delivered with a shot to be taken in pill form.

Researchers are also developing an NP to defeat viruses. The NP does not actually destroy virus molecules but delivers an enzyme that prevents the reproduction of viruses molecules in the patients bloodstream.

**Recent innovation in nanoparticulate system (Table 4)**

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**Recent patents related to NPs for treatment of cancer (Table 5)**

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**NANO ECONOMICS [56]**

Drug improvements and innovations in cancer medicines have traditionally been assessed and analyzed with respect to safety and efficacy. An often overlooked factor is cost, which is especially important in the face of ever-increasing health-care expenses. At present, there are few cost-benefit studies available for nanomedicine products.

As an example, the cost-effectiveness of Doxil® (Janssen Biotech Inc, PA, USA; PEGylated liposomal doxorubicin) and Abraxane® (Celgene Corporation, NJ, USA; nano-albumin bound paclitaxel) is evaluated

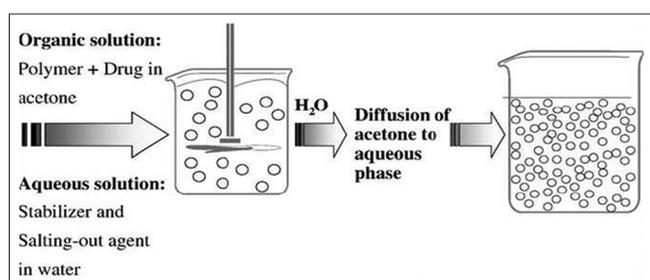
compared with their conventional standard-of-care generic alternatives, doxorubicin, and paclitaxel, respectively.

**Table 3: ???**

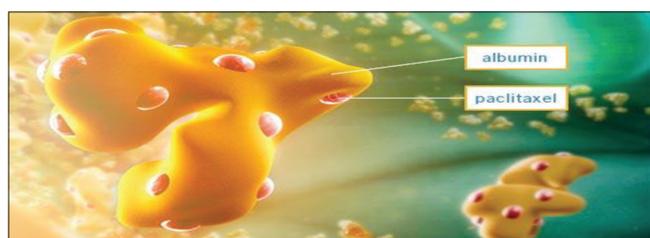
Application	Material	Purpose
Cancer therapy	Poly (alkylcyanoacrylate) nanoparticles with anticancer agents, oligonucleotides	Targeting, reduced toxicity, improved <i>in vitro</i> and <i>in vivo</i> stability
Intracellular Targeting	Poly (alkylcyanoacrylate)	Target reticuloendothelial system for intracellular infections
Prolonged systemic circulation	Popolyesteryethylene with adsorbed polyethylene glycols or pluronics derivatized polyester	Systemic drug effect, avoid uptake by reticuloendothelial system
Vaccine adjuvant	Poly (methylmethacrylate) nanoparticles with vaccines	Enhance immune response, alternate adjuvant
Peroral absorption	Poly (methylmethacrylate) nanoparticles with proteins therapeutic agents	Enhance bioavailability, protection from gastrointestinal enzymes
Ocular delivery	Poly (alkylcyanoacrylate) nanoparticles with steroids anti-inflammatory agents, antibacterial agents for glaucoma	Improved retention of drug or reduced wash out
DNA delivery	DNA-gelatin nanoparticles, DNA-chitosan nanoparticles, PDNA-poly (DL-lactide-co- levels glycolide) nanoparticles	Enhanced delivery higher and significantly higher expression
Oligonucleotide delivery	A Alginate nanoparticles, poly (D, L) lactic acid nanoparticles	Enhanced delivery of oligonucleotide

**Table 4: Products in pipeline**

Company	Technology	API	Route of administration
Pfizer	i.v. solution	Epirubicin	I.v.
Novavax, USA	Micellar nanoparticles	Testosterone	S.c.
BioAlliance, France	Polydsohexyl cyanoacrylate nanoparticles	Doxorubicin	I.v.
American Bioscience, USA	Albumin-Drug nanoparticles	Paclitaxel	I.v.
Wyeth Pharmaceutical, USA	Drug Nanoparticles	Rapamycin	Oral
Biosante, USA	Calcium phosphate nanoparticles	Insulin	Oral



**Fig. 7: Schematic presentation of salting out method of preparing nanospheres**



**Fig. 8: Albumin-bound paclitaxel nanoparticles**

Table 5: ???

Number	Patent application number	Title of patent
1	PCT/US2008/003096	Nanoparticle comprising rapamycin and albumin as anticancer agent [40]
2	CN20121018172	Compound epirubicin hydrochloride Poly(lactic-co-glycolic acid) (PLGA) nanoparticles and preparation method thereof [41]
3	EP20110305364	Nanoparticles loaded with chemotherapeutic antitumoral drug [42]
4	EP20110305364	Folacin receptor mediated targeted acetyl pullulan polysaccharide nano granule and preparation thereof [43]
5	US 12/059,496	Cancer cell targeting using NPs [54]

## CONCLUSION

Research activity aimed toward achieving specific and targeted delivery of anticancer agents has expanded tremendously in the last 5 years or so with new avenues of directing drugs to tumors as well as new types of drugs. Metastasis is still an extremely complex disease with multiple questions remaining. While 90% of human cancer deaths are due to cancer metastases, the hope for fighting cancer is sustained by the fact that there were more than 50 new agents approved in the past 10 years for cancer treatment and hundreds of new agents in clinical development. The development of NP DDSs is expected to have a big impact on the clinical approaches for cancer therapy.

By rationally designing NPs based on improved knowledge of cancer biology and the tumor microenvironment, improved efficacy can be achieved. In addition, multifunctional NPs able to carry imaging agents and deliver multiple drugs are now being developed for enhanced detection and treatment of breast cancer. The application of nanotechnology to cancer has already produced some exciting results and holds even greater promise for cancer patients in the future.

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