CHAPTER 2

REVIEW OF LITERATURE

NANOSIZED DRUG DELIVERY SYSTEMS FOR CANCER THERAPEUTICS
Chapter 2: Contents

2.1 Barriers in cancer drug delivery ................................................................. 41
2.2 Current cancer chemotherapy and challenges ........................................... 42
2.3 Tumor microenvironment ........................................................................... 44
  2.3.1 Angiogenesis in cancer ...................................................................... 44
  2.3.2 Enhanced Permeability and Retention (EPR) effect ........................... 45
  2.3.3 pH .................................................................................................... 46
2.4 Tumor drug targeting ................................................................................ 47
  2.4.1 Passive targeting .............................................................................. 48
  2.4.2 Active targeting ................................................................................ 49
2.5 Nanotechnology in medicine ................................................................... 56
2.6 Nanoparticles in medicine ....................................................................... 57
  2.6.1 Therapeutics ...................................................................................... 57
  2.6.2 Diagnostics ....................................................................................... 59
  2.6.3 Imaging ............................................................................................ 59
2.7 Advantages of nanoparticles as drug delivery systems ........................... 61
2.8 Nanoparticles as drug carriers ................................................................. 62
2.9 Types of nanoparticles for drug delivery .................................................. 63
  2.9.1 Polymeric nanoparticles ..................................................................... 65
  2.9.2 Liposomes ......................................................................................... 66
  2.9.3 Polymerosomes ................................................................................ 68
  2.9.4 Polymeric micelles (PMs) .................................................................. 68
  2.9.5 Solid lipid nanoparticles (SLNs) ......................................................... 70
  2.9.6 Dendrimers ...................................................................................... 71
  2.9.7 Carbon based polymers .................................................................... 75
  2.9.8 Viral nanoparticles ............................................................................ 77
  2.9.9 Inorganic nanoparticles .................................................................... 77
2.10 Clinical applications of nanomaterials .................................................... 81
2.11 Conclusion ............................................................................................. 82
2.12 References .............................................................................................. 84
2.1 Barriers in cancer drug delivery

Effective drug delivery and improvement of therapeutic index is a key factor towards better treatment of diseases like cancer. Inefficient drug delivery leads to poor tumor response, causes severe side effects, and gives rise to the drug resistance to cancer. Since anticancer drugs are typically toxic toward healthy proliferating cells as well, drug dosage must be restricted to avoid potentially lethal side effects (Brannon-Peppas & Blanchette 2012). Therapeutic efficacy of such restricted drug dosage can be further diminished by factors such as limited systemic circulation lifetime, undesirable biodistribution, non-specific cellular uptake, and poor tumor vascularity. As a result, each course of chemotherapy typically induces partial treatment, which subjects the surviving cancer cells to a selective pressure that favours mutations leading to drug resistance. Drugs that show favourable initial response are often rendered ineffective following repeated administrations, and the relapsed tumor would become much more difficult to treat (Cho et al. 2008, Sharma et al. 2010). This serious clinical challenge can be addressed by developing potent drug delivery vehicle that can deliver drugs to the cancerous part. A number of drug delivery systems are under investigation to circumvent these limitations and improve the efficiency of a specific drug. Mostly, they are nanoparticulate drug delivery systems or drug-polymer conjugates (Danhier et al. 2010, Petros & DeSimone 2010). In nanoparticulate drug delivery systems, the drugs are physically incorporated into it while in drug-polymer conjugates the drug is covalently linked to polymers like proteins, polysaccharides or synthetic polymers.
2.2 Current cancer chemotherapy and challenges

For the effective cancer treatment and minimize the chance to develop drug resistance, high doses of potent therapeutics need to be safely delivered specifically to tumoral site. These anticancer drugs face numerous barriers as they travel from the point of intravenous administration to their target site. These barriers can be classified into three separate levels which include physiological barrier, the cellular barrier, and the molecular barrier (Marcus et al. 2014, Alix-Panabieres & Pantel 2014).

At the physiological level, small-molecular drugs are rapidly cleared upon systemic injection by plasma degradation and reticuloendothelial system uptake. Majority of the drug molecules cannot stay in the circulation, long enough to reach the tumor cells because of their short circulation and poor pharmacokinetics. Therefore a major requirement for drug delivery system is to prolong the in vivo residence time of therapeutic compounds (Brezden-Masley & Polenz 2014, Clevers 2011).

On the cellular level, the membranes of the cancer cells are the major barrier to drug entry. Anticancer drugs rely on passive diffusion and membrane translocators to cross the cellular membrane, but the entry mechanisms preclude bulky and polar drugs from penetration. Presence of membrane bound drug-efflux pumps, which are overexpressed in drug resistant cancer cells, actively vacuums drug molecules from the intracellular to the extracellular space (Nieuwenhuis et al. 2010). Effective drug delivery needs to shuttle the drugs to the cellular cytoplasm.
and overcome these membrane barriers (Liechty & Peppas 2012).

Another major barrier exists on the molecular level in cancers, which can often survive drug-inflicted damage to a particular pathway is by activating and strengthening the alternative pathways. Such complexity of cancer biology can be likened to “webs of interconnected routes with multiple redundancies” (Stapleton-Gray & Woodcock 2011), in which single-drug therapies and their one-dimensional action mechanisms are usually inadequate. Frequent mutations prompt the emergence of chemo-resistance. Important mutations identified are compromised apoptotic signaling, enhanced damage-repair mechanisms, increased drug metabolism, altered drug targets and up-regulation of drug-efflux pumps (Gillet & Gottesman 2012). Therefore it is important to attack cancer cells through multiple pathways to minimize the possibility of cells acquiring favorable mutations that help to survive the treatment. Therapeutic efficacy can be improved by adopting treatment with multiple modes of mechanisms that can increase the evolutionary hurdle for the cancer cells to acquire drug resistance phenotypes (Cairns et al. 2011, Tennant et al. 2010).

A promising way to overcome all the aforementioned barriers is to associate anticancer drugs with nanoparticles (NPs). In the last a few decades, the advancement of nanotechnology has made possible to synthesize biocompatible and biodegradable drug delivery vehicles of size in the nanoscale. Many types of nanocarriers like liposomes, solid lipid and polymeric NPs were developed to deliver drugs (Horcajada et al. 2010, Liu et al. 2008). These nanocarriers have demonstrated desirable drug delivery characteristics such as prolonged systemic
circulation lifetime, reduced non-specific cellular uptake, targeting abilities, controlled drug release, and multidrug encapsulation for combinatorial treatment. Recently, NPs with a size range of 50~150 nm are emerging as a promising drug delivery platform for cancer treatment as a number of NP based cancer drugs are showing up on the market and in clinical trials (Allen & Cullis 2013, Wang et al. 2012). Numerous chemotherapeutic drugs, including many that are otherwise insoluble in the blood, have been successfully encapsulated in NPs. The other features such as functionalization with tumor targeting ligands and co-encapsulation of multiple drugs have also been implemented on nanobased polymer therapeutics.

2.3 Tumor microenvironment

In cancer therapy, a clear understanding of the tumor microenvironment can help to design new therapeutic strategies based on numerous differences compared with the normal tissue. These include vascular abnormalities, oxygenation, perfusion, pH and metabolic state. The differences in terms of morphology of tumor vasculature and the pH are the more relevant characteristics for the design of nanocarriers as tumor targeted drug delivery systems.

2.3.1 Angiogenesis in cancer

Angiogenesis is defined as the formation of new blood vessels from existing ones. For solid tumors (1-2 mm³), oxygen and nutrients can reach the center of the tumor by simple diffusion. Because of their non-functional or non-existent vasculature, non-angiogenic tumors are highly dependent on their
microenvironment for oxygen and the supply of nutrients. When tumors reach a size of 2 mm³, a state of cellular hypoxia begins, initiating angiogenesis. Angiogenesis is regulated by the fine balance of activators and inhibitors (Prager & Zielinski 2013, Holopainen et al. 2011)

2.3.2 Enhanced Permeability and Retention (EPR) effect

Structural changes in vascular pathophysiology could provide opportunities for the use of long-circulating particulate carrier systems. The ability of vascular endothelium to present open fenestrations was described for the sinus endothelium of the liver (Abel et al. 2013), when the endothelium is perturbed by inflammatory process, hypoxic areas of infarcted myocardium or in tumors (Starzl et al. 1989). More particularly, tumor blood vessels are generally characterized by abnormalities such as high proportion of proliferating endothelial cells, pericyte deficiency and aberrant 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 10-1000nm (Hobbs et al. 1998). Moreover, lymphatic vessels are absent or non-functional in tumor that contributes to inefficient drainage from the tumor tissue. Nanoparticles entered into the tumor are not removed efficiently and are thus retained in the tumor. This passive phenomenon is called the “Enhanced Permeability and Retention (EPR) effect,” discovered by Matsumura and Maeda (Maeda et al. 2000, Iyer et al. 2006). The abnormal vascular architecture plays a major role for the EPR effect in tumor for selective macromolecular drug targeting at tissue level. A comparison of normal and tumor tissue is shown in the Figure 2.1.
Figure 2.1 Differences between normal and tumor tissues: A. Normal tissues contain linear blood vessels maintained by pericytes, collagen fibers, fibroblasts and macrophages are in the extracellular matrix. Lymph vessels are present. B. Tumor tissues contain defective blood vessels with many sac-like formations and fenestrations. The extracellular matrix contains more collagen fibers, fibroblasts and macrophages than in normal tissue. Lymph vessels are lacking. Adapted from (Heldin et al. 2004)

2.3.3 pH

Intracellular pH within healthy tissues and blood are around 7.4, while that around the tumor cell is 6.0-7.0. The tumor pH may vary according to tumor area. Low pH and low pO₂ are intimately linked and a variety of insights now support their roles in the progression of tumor from in situ to invasive cancer. The resulting pH gradients between intra and extracellular tumor cells and the resulting tumor mass to normal tissue are therefore used as potential target for differential drug partitioning and distribution. In low pH extracellular environment, uncharged fraction of a weak acid increases and such a drug can thus more easily diffuse through the cell membrane. Basic intracellular compartment favour the ionization of the molecule and promote the cytosolic accumulation of the drug. Alteration in this process can contribute to multidrug resistance (MDR). Continuous exposure to chemotherapeutic agents may favour the selection tumor cell clones with very
acidic organelles which will trap drugs and thereby reduce their activity; if this organelle is part of the secretory pathway then the drug will be transported out of the cell by exocytosis (Lee et al. 2008, Bae et al. 2005).

2.4 Tumor drug targeting

Targeted delivery of anticancer drugs, which provides therapeutic concentration of anticancer agents at the desired sites of actions and spare normal tissues, promises reduced systemic toxicity and enhanced therapeutic efficacy. There are a wide range of strategies available for drug delivery in cancer therapy, among which systemic delivery using nanoscale drug carriers (e.g., Liposomes, polymeric nanoparticles or polymer-drug conjugate) has been demonstrated to be efficacious. Having a passive and/or active targeting mechanism, these nanoscale drug carriers are able to selectively target cancer sites where they locally deliver the incorporated drugs (Craig et al. 2014, Lammers et al. 2012, Vasir et al. 2005, Torchilin 2011). There are two main mechanisms through which nanoscale drug carriers achieve tumor targeting, i.e. passive targeting and active targeting. Passive targeting is based on the prolonged circulation time due to the hydrophilic outer shell thus avoiding phagocytic and renal clearance and selective tumor accumulation via enhanced permeability and retention (EPR) effect (Iyer et al. 2006). Active targeting builds on passive targeting and adds specific interaction of the targeting ligands on delivery systems with the receptors of cancer cells/tissues (Figure 2.2).
2.4.1 Passive targeting

In passive drug targeting nanocarriers is transported through leaky tumor capillary fenestrations into the tumor interstitium and to cells by convection or passive diffusion (Figure 2.2 A). The convection refers the movement of molecules within the fluids. Convection is the predominating transport mode for majority of large molecules across large pores when net filtration rate is zero.

![Figure 2.2](attachment:image.png)

**Figure 2.2** Active and passive drug targeting of nanomedicines: A. Passive targeting: Nanocarrier reaches selectively through the leaky vasculature of the tumors. B. Active targeting: Ligands grafted at the surface of the nanocarriers bind to over expressed receptors by (1) cancer cells or (2) angiogenic endothelial cells. Adapted from (Danhier et al. 2010)

The low molecular weight compounds like oxygen are mainly transported by diffusion, which is the process of transport of molecules across the cell membrane, according to a gradient of concentration and without contribution of cellular energy. Convection through the tumor interstitium is poor due to interstitial hypertension,
leaving diffusion as the major mode of drug transport (Haley & Frenkel 2008). EPR effect is responsible for the selective accumulation of the nanocarriers and the drugs and therefore it is now considered as the gold standard for novel drug carrier design to target solid tumor and drug designing (Maeda et al. 2009). The EPR effect will be optimal if nanocarriers can evade immune surveillance and circulate for a long period. This will help them to accumulate at the tumor site that results a local concentration of about 10-50 folds higher compared to that in normal tissue within 1-2 days (Iyer et al. 2006). In passive drug targeting, high interstitial fluid pressure of tumors and the poor lymphatic drainage explain the size relationship with the EPR effect. Larger and long circulating nanocarriers (100 nm) are more retained in the tumor compared to smaller molecules that can easily diffuse (Bouzin & Feron 2007). The passive targeting is also depends on degree of tumor vascularisation and angiogenesis

2.4.2 Active targeting

In active targeting, targeting ligands are incorporated to the nanocarrier surface to help its binding with appropriate overexpressed receptors at the target site (Figure 2.2 B). Targeting ligands are either monoclonal antibodies (mAbs) and antibody fragments or non-antibody ligands (peptidic or not). The binding affinity of the ligands influences the tumor penetration because of the ‘binding site barrier’. For targets in which cells are readily accessible, typically the tumor vasculature, because of the dynamic flow environment of the blood stream, high affinity binding appears to be preferable (Adams et al. 2003, van Bracht et al. 2014)
The anticancer therapeutics is grouped under the name “ligand targeted therapeutics”, divided into different classes based on the approach of the drug delivery but all aims the delivery of drugs specifically to the cancer cells. Antibodies (monoclonal antibody or fragments) target specific receptor, can interfere with signal-transduction pathways and regulate proto-oncogenes involved in cancer cells proliferation such as trastuzumab (anti-ERBB2, Herceptin), bevacizumab (anti-VEGF, Avastin) or etaracizumab, a humanized anti-αvβ3 antibody (Abe grin). Here the active molecule plays the role of both targeting ligand and drug while the antibodies (fragments) play only the role of targeting ligand.

**Figure 2.3 Main classes of ligand-targeted therapeutics** (A) Targeting antibodies are generally monoclonal immunoglobulin g (IgG) (a) or Fab’ fragments (b) or F(ab’)2 fragments (c). (B) Immunoconstructions are formed by the linking of antibodies or fragments to therapeutic molecules. (C) Targeted nanocarriers with targeting ligands at its surface. The ligands are either monoclonal antibodies and antibody fragments (immune-nanocarriers) or non antibody ligands. Targeted nanocarriers contain therapeutic drugs. Adapted from (Danhier et al. 2010)

The first radio immunotherapeutic received for clinical approval is ⁹⁰Yttrium-ibritumomab tiuxetan (Zevalin) that target anti-CD-20 (Wiseman et al. 2001). The
first immunotoxin approved in clinical was denileukin diftitox (ontak), an interleukin (IL)-2 diphtheria toxin fusion protein (Duvic et al. 2002). The only immunoconjugate to receive clinical approval is gemtuzumab ozogamicin (Mylotarg) (van der Velden et al. 2001) (Figure 2.3 A). Immuno-nanocarriers (Figure 2.3 B) used a different approach; cytotoxic drug is encapsulated into a nanocarriers and antibodies (or fragments), the targeting ligands, are coupled to the particle surface. Finally, or targeted nanocarriers (Figure 2.3 C) antibodies are replaced by molecule (peptidic or not) binding to specific receptors. In the active targeting strategy, two cellular targets can be distinguished. i.e. The targeting of cancer cell and the targeting tumoral endothelium.

2.4.2.1 Cancer cell targeting

Active targeting aims the overexpressed cancer cell surface receptors to improve the cellular uptake of nanocarriers. It aims the intracellular delivery of macromolecular drugs, such as DNA, siRNA and proteins. Nanocarrier design is aimed to enhance cellular internalization rather than its accumulation in tumor cells (Kirpotin et al. 2006). Important internalization-prone receptors are

i. The transferrin receptor.

Transferrin, a serum glycoprotein, transports iron through the blood and into cells by binding to the transferrin receptor and subsequently being internalized via receptor mediated endocytosis. This receptor is involved in iron homeostasis and the regulation of cell growth. In cancer cells transferrin receptor is overexpressed (up to 100 fold excess) compared to that in normal cells. Its extracellular accessibility, ability to internalize and role in the cellular pathology of human
cancer, make it an attractive target for cancer therapy (Daniels et al. 2006, Davis 2008)

ii. The folate receptor

Tumor marker that binds to the vitamin folic acid and folate-drug conjugates or folate grafted nanocarriers with high affinity can carry the bound molecules into the cells via receptor mediated endocytosis. Folic acid is required in single carbon metabolic reactions are essential for the synthesis of nucleotide bases. The alpha isoform, folate receptor-α is overexpressed in about 40% of human tumors while the folate receptor-β is expressed in activated macrophages and in the surfaces of malignant cells of hematopoietic origin (Minko et al. 2004).

iii. Glycoproteins expressed on cell surfaces

Lectins are proteins of non-immunological origin are able to recognize and bind to carbohydrate molecules attached to glycoproteins expressed on cell surface. Cancer cell often express different glycoproteins compared to normal cells. Lectins interaction with certain carbohydrate is very specific. Lectins can be incorporated into nanoparticles as targeting moieties that are directed to cell-surface carbohydrates (direct lectin targeting) and carbohydrate moieties can be coupled to nanoparticles to target lectins (reverse lectin targeting). The use of lectins and neoglycoconjugates for direct or reverse targeting strategies is a tradition approach of colon drug targeting (Smetana Jr et al. 2014, Zhai et al. 2011).

iv. The Epidermal growth factor receptor (EGFR)

EGFR is a member of the ErbB family, a family of tyrosine kinase receptors. Its activation stimulates key processes involved in tumor growth and progression,
including proliferation, angiogenesis, invasion and metastasis. EGFR is overexpressed in a majority of tumors, especially in breast cancer played a significant role in the progression of several human malignancies. Human epidermal receptor-2 (HER-2) was expressed in 14-91% breast cancer patients (Meng et al. 2010, Levy et al. 2012). EGFR was overexpressed in a variety of solid tumors, including colorectal cancer, non-small cell lung cancer, squamous cell carcinoma of head and neck, ovarian, kidney, pancreatic and prostate cancer (Lurje & Lenz 2010)

2.4.2.2 Targeting of tumor endothelium

Destruction of the endothelium in solid tumors can lead to the killing of tumor cells induced by the lack of oxygen and nutrients. In 1971, Folkman suggested that tumor growth can be inhibited by preventing tumors from recruiting new blood vessels (Folkman 1971) and formed the base for the design of nanomedicines actively targeted to tumor endothelial cells (Lammers et al. 2008). By attacking the growth of the blood supply, the size and metastatic capabilities of tumors can be regulated. Here the ligand-targeted nanocarriers bind and kills angiogenic blood vessels in tumor core. The advantages of tumor endothelium targeting are; There is no need of extravasation of nanocarriers to reach targeted site and after the intravenous injection it is possible to bind to their receptors directly. The potential risk of emerging resistance is lower than the genetical stability of endothelial cells compared to tumor cells and most of endothelial cell markers are expressed in all tumor type an eventual broad application spectrum. Main targets of tumoral endothelium include;
i. The vascular endothelial growth factors (VEGF) and receptors

VEGFR-1 and VEGFR-2, mediate vital functions in tumor angiogenesis and neovascularisation. Two major approaches to target angiogenesis via the VEGF are targeting VEGFR-2 to decrease VEGF binding and induce an endocytotic pathway and targeting VEGF to inhibit ligand binding to VEGFR-2 (Byrne et al. 2008, Gomez-Manzano et al. 2008)

ii. The $\alpha_v\beta_3$ integrin

It is an endothelial cell receptor for extracellular matrix proteins which includes fibrinogen (fibrin), vibronectin, thrombospondin, osteopondin and fibronectin (Winter et al. 2003). The $\alpha_v\beta_3$ integrin is highly expressed on neovascular endothelial cells but poorly expressed in resting endothelial cells and most normal organs, and is important in the calcium dependent signaling pathway leading to endothelial cell migration (Graf et al. 2012, Murphy et al. 2008).

iii. Vascular Cell Adhesion Molecule-1 (VCAM-1)

It 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 process. Overexpression of VCAM-1 is found in various cancers, including leukemia, lung and breast cancer, melanoma, renal cell carcinoma, gastric cancer and nephroblastoma (Hernot et al. 2012)

iv. The Matrix metalloproteinases (MMPs)

These are family of zinc dependent endopeptidases that degrade the extracellular matrix, play an important role in angiogenesis and metastasis, particularly in endothelial cell invasion and migration, in the formation of capillary
tunes and in the recruitment 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 (Deryugina & Quigley 2006, Overall & Kleifeld 2006). An overview of clinically most relevant drug targeting strategies used nowadays is shown in the Figure 2.4.

Figure 2.4 Overview of clinically relevant drug targeting strategies
A. Upon the intravenous injection, the low molecular weight chemotherapeutic agent will rapidly cleared from blood and only low levels of the drug accumulate in tumors and in tumor cells while their localization in certain tissues was relatively high. B. Passive targeting of the drug delivery system by EPR effect enhances the drug accumulation in tumor cells. C. Active drug targeting of internalization-prone overexpressed cancer cell surface receptors improve the cellular uptake of the nanomedicine which is useful for the intracellular delivery of macromolecular drugs such as DNA, siRNA and proteins. D. Active drug targeting to overexpressed receptors by angiogenic endothelial cells reduces blood supply to tumors and thus depriving tumor cells from oxygen and nutrients. E. Stimuli sensitive nanomedicine like Thermodox was activated by externally applied physical triggers such as hyperthermia, ultrasound, magnetic fields and light. F. Tumors that are easily accessible during surgery, a sustained release delivery device can be implanted or injected directly into the tumors. Adapted from (Lammers et al. 2008)
2.5 Nanotechnology in medicine

Nanotechnology is a rapidly advancing, innovative field in science which involves interdisciplinary research that is aimed towards the production, characterization, development and application of “molecular” materials with sizes ranging between $10^{-9}$ m (nanometre) and $10^{-6}$ m (micrometre) (Calderon et al. 2010, Saad et al. 2008). The prefix “nano” derives from the Greek word for “dwarf”. One nanometer (nm) is equal to one-billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules. A human hair is approximately 80,000 nm wide, and a red blood cell is approximately 7000 nm wide. Atoms are smaller than 1 nm, whereas many molecules including some proteins range between 1 nm and larger (Figure 2.5).

![Figure 2.5 Relative size of nanoparticles compared with familiar items. Adapted from (McNeil 2005)](image)

The conceptual underpinnings of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman in his lecture, “There’s plenty of room at the bottom”. Feynman explored the possibility of manipulating material at the scale of individual
atoms and molecules, imaging the whole of the *Encyclopedia Britannica* written on the head of a pin and foreseeing the increasing ability to examine and control matter at the nanoscale. The term ‘nanotechnology” was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to engineer materials precisely at the nanometer level. In the past few years nanotechnology has grown by leaps and bounds, and this multidisciplinary scientific field is undergoing explosive development. It can prove to be a boon for human healthcare, because nanoscience and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. Human healthcare nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be trace to the promise of revolutionary advances across medicine, communications, genomics, and robotics. A complete list of the potential applications of nanotechnology is too vast and diverse to review, but without doubt, one of the greatest values of nanotechnology will be in the development of new and effective medical treatments (Arora *et al.* 2014, Gadde *et al.* 2014)

### 2.6 Nanoparticles in medicine

#### 2.6.1 Therapeutics

Therapeutic applications of nanoparticles are diverse, ranging from cancer therapeutics, antimicrobial actions, vaccine delivery, gene delivery and site-specific targeting to avoid the undesirable side effects. Loading them onto the nanoparticles can reduce the side effects and increase therapeutic index of chemotherapeutic
agents like carboplatin, paclitaxel, doxorubicin and etoposide etc. Surface functionalized multifunctional nanoparticles incorporated with biomolecules are also tested as potential therapeutic agents. Functionalized nanoparticles targeted to gene silencing offers exciting prospects. Nanoparticles can also used as antimicrobial agents. Table 2.1 highlights some of the nanoparticles that can be effectively used for therapeutics.

**Table 2.1 Nanoparticles as therapeutic agents**

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Encapsulant</th>
<th>Indicator</th>
<th>Therapeutic uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-isohexylcyanoacylate NPs</td>
<td>Doxorubicin</td>
<td>Hepatocellular carcinoma</td>
<td>Higher antitumor efficiency than native doxorubicin and overcome MDR</td>
<td>(Felice et al. 2014)</td>
</tr>
<tr>
<td>PLGA NPs</td>
<td>Paclitaxel</td>
<td>Various cancers</td>
<td>Effective in chemotherapeutic and photothermal destruction of cancer cells</td>
<td>(Wang et al. 2010)</td>
</tr>
<tr>
<td>Gold NPs (AuNPs)</td>
<td></td>
<td>Various cancers</td>
<td>Radiation sensitizers for cancer therapy</td>
<td>(Jain et al. 2014)</td>
</tr>
<tr>
<td>Chitosan NPs</td>
<td>SiRNA</td>
<td>Lung cancer</td>
<td>Increased selective intratumoral delivery and significant inhibition of tumor growth</td>
<td>(Babu et al. 2013)</td>
</tr>
<tr>
<td>Cetylalcohol/Polysorbate NPs</td>
<td>Paclitaxel</td>
<td>Brain tumor</td>
<td>Higher brain and tumor cell uptake, thus leading to greater cytotoxicity and effective towards p-glycoprotein expressing tumor cells</td>
<td>(Koziaraet al. 2004)</td>
</tr>
<tr>
<td>Lipid nanocapsules</td>
<td>Etoposide</td>
<td>Glioma</td>
<td>Can overcome p-glycoprotein dependent MDR</td>
<td>(Maupas et al. 2011)</td>
</tr>
<tr>
<td>P(4-vinylpyridine) particles</td>
<td>Antimicrobial agent</td>
<td></td>
<td>Can inhibit bacterial growth for various bacteria as biocolloids</td>
<td>(Ozay et al. 2010)</td>
</tr>
<tr>
<td>Chitosan-alginate NPs</td>
<td>Carboplatin</td>
<td>Retinoblastoma</td>
<td>Enhanced antiproliferative activity and cytotoxicity of NPs in comparison with native carboplatin</td>
<td>(Parveen et al 2012)</td>
</tr>
<tr>
<td>Poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) NPs</td>
<td>Doxorubicin</td>
<td>Various cancers</td>
<td>Selective delivery of anticancer agent to folate receptor overexpressed cancer cells</td>
<td>(Zhang et al. 2010)</td>
</tr>
</tbody>
</table>
2.6.2 Diagnostics

The researchers have a high demand for simple, rapid, efficient and user-friendly alternative methods for the detection of cells in general and in particular for the detection of cancer cells. Cancer is a debilitating disease and its early and accurate detection is often a bottleneck that is responsible for its delayed treatment. To address these limitations, various types of nanoparticle based diagnostics are developed (Table 2.2).

Table 2.2 Nanoparticles as diagnostic agents

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Diagnostic strategy</th>
<th>Advantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs</td>
<td>The selectivity and specific affinity of aptamers is combined with spectroscopic advantages of AuNPs to detect diseased cells</td>
<td>For sensitive detection of cancer cells; diagnosis of breast cancer</td>
<td>(Hainfeld et al. 2014)</td>
</tr>
<tr>
<td>Magnetofluorescent particle systems</td>
<td>Bimodel contrast agents allows detection of cancer cells</td>
<td>Non-invasive diagnosis of breast cancer</td>
<td>(Huang et al. 2011b)</td>
</tr>
<tr>
<td>Semiconductor Fluorescent QDs</td>
<td>Fluorescent biomarkers analysed by their resulting fluorescence</td>
<td>Fast and precise cancer diagnosis</td>
<td>(Algar et al. 2011)</td>
</tr>
<tr>
<td>Aptamer conjugated NPs</td>
<td>Selective targeting cell extraction and aptamer conjugated fluorescent NPs can be used for sensitive cancer detection</td>
<td>Collection and detection of multiple cancer cells</td>
<td>(Medley et al. 2011)</td>
</tr>
<tr>
<td>Fluorescent europium (III)-chelate doped NPs</td>
<td>Labels high affinity monoclonal antibodies and microtitration wells provides a sensitive adenovirus immunoassay</td>
<td>Sensitive screening of viral analytes</td>
<td>(Peng et al. 2010)</td>
</tr>
</tbody>
</table>

2.6.3. Imaging

The development of the effective carrier system does not only mean the execution of delivery, but also the positive confirmation of the site-specific delivery of the drug. Consequently, the ability to track and image the fate of any nanomedicine from the systemic to the subcellular level becomes essential.
Nanoparticles are successfully exploited to improve the utility of fluorescent markers for medical imaging and diagnostic purposes. Although various fluorescent markers are widely used in research and clinical diagnostic applications, current techniques have several disadvantages, such as the requirement of colour matched lasers, fluorescence bleaching and lack of discriminatory capacity of multiple dyes, etc. Fluorescent nanoparticles can overcome these problems will serve as the best solution for in vivo imaging of tumors and other diseases.

**Table 2.3 Nanoparticles as imaging agents**

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Diagnostic strategy</th>
<th>Advantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(alkyl cyanoacrylate) NPs</td>
<td>Rhodamine B tagged poly(alkyl cyanoacrylate) NPs for specific human brain endothelial cell imaging</td>
<td>Used for human brain endothelial cell imaging</td>
<td>(Brambilla et al. 2010)</td>
</tr>
<tr>
<td>AuNPs</td>
<td>Au NPs bioconjugate with poly (2-hydroxypropyl methacrylamide) coating</td>
<td>Used for human brain endothelial cell imaging</td>
<td>(Freese et al. 2013)</td>
</tr>
<tr>
<td>AuNPs</td>
<td>AuNPs surface functionalized with prostate specific antigen (PSMA) RNA aptamer that binds to PSMA enables specific imaging of cancer cells expresses PSMA protein</td>
<td>Combined prostate cancer imaging by computed tomography (CT) and anticancer therapy</td>
<td>(Kim et al. 2010)</td>
</tr>
<tr>
<td>Streptavidin NPs</td>
<td>Biotinylated anti-Her2 Herceptin antibody to provide tumor targeting</td>
<td>Multimodality imaging of tumor in mice by fluorescence and nuclear detection</td>
<td>(Quarta et al. 2014)</td>
</tr>
<tr>
<td>Multifunctional SPIONs</td>
<td>Folate provides specific targeting and Doxorubicin conjugated SPIONs serves as therapeutic agent as well as MRI contrast agent.</td>
<td>Promising candidate for treating liver cancer and monitoring the cancer using MRI</td>
<td>(Kievit et al. 2012)</td>
</tr>
<tr>
<td>CNPs</td>
<td>Tumor targeted CNPs conjugated with dual imaging agents and can be designed as dual modality imaging agents</td>
<td>Optical/ MR (magnetic resonance) dual imaging agent</td>
<td>(Della Rocca et al. 2011)</td>
</tr>
<tr>
<td>QD loaded micelles</td>
<td>Lipid conjugated QDs and herceptin enhances tumor cell uptake</td>
<td>imaging and treatment of cancer</td>
<td>(Nurunnabi et al. 2010)</td>
</tr>
</tbody>
</table>
Recently, new classes of engineered optical probes, fluorescent silica nanoparticles (FSNPs), which are consisting of silica nanoparticles loaded with fluorescent dye were developed for cancer imaging. The use of water-soluble, functionalized quantum dots that are highly stable against oxidation for biological and biomedical applications is currently one of the fastest growing fields of nanotechnology. Quantum dots manifest stable fluorescent properties also offer new prospects for live cells, in vivo imaging and diagnostics. Magnetic iron oxide nanoparticles with the capability of deep-tissue imaging, non-invasiveness and low toxicity were developed as novel contrasting agents for biomedical imaging. Dynamic magneto motion of magnetic nanoparticles (MNPs) detected with magnetomotive optical coherence tomography (MM-OCT) also represents a new methodology for contrast enhancement and therapeutic interventions in molecular imaging. Gold nanoparticles are widely in use for cellular imaging. Table 2.3 gives an overview of functionalized nanoparticles used for imaging of diseased cells.

2.7 Advantages of nanoparticles as drug delivery systems

Many types of nanoparticles formulated from diverse materials with unique architectures loaded with drugs are used as drug delivery systems to treat a particular disease. Drugs loading were done by encapsulation, surface attachment or entrapment. These delivery systems because of their small size and small surface to volume ratio can easily penetrate across the barriers through small capillaries into individual cells, thus allowing efficient drug accumulation at the target site. This will reduce unwanted side effects and the toxicity of the therapeutic agent and enhance
the therapeutic efficacy. Nano drug delivery systems can (1) overcome lack of target specificity of anticancer drugs (2) overcome aqueous solubility and avoid drug degradation (3) produce prolonged release of drugs (4) overcome multidrug resistance and rapid formulation development. However colloidal stability of the nanoparticles is the major concern. Their stability can be improved by absorbing or grafting polymeric surfactants or other modifiers to their surface forming a layer that can generate an effective repulsive force between nanoparticles and prevents flocculation. Nanoparticles with improved therapeutic index of drugs are the solution for the delivery problems associated with the upcoming biotechnological products such as recombinant proteins and oligonucleotides.

2.8 Nanoparticles as drug carriers

Nanosized particles as drug delivery vehicle hold great promise as pharmaceutical carriers and can be prepared from a wide range of materials such as polymers, lipids, viruses, and organometallic compounds. These nanoparticles-drug complexes have the ability to mitigate toxicity and side effects associated with raw pharmaceuticals such as chemotherapy drugs (Dobrovolskaia & McNeil 2012, Naahidi et al. 2013), by allowing the drug release at the target. The solubility of the drug in the complex can be improved by encapsulation, micellization, and protein cage architecture (Jolck et al. 2011).

Adverse toxicological responses like lung inflammation, platelet aggregation in blood, and impaired mitochondrial function in cells will vary with nanoparticles composition. The material composition may include metals such as gold, silver, and
metal oxides (Fadeel & Garcia-Bennett 2012) or like polymer based materials such as PLGA, and lipid based particles such as nanoliposomes, solid lipid nanoparticles, and nanoemulsions. Each substance exhibits its own inherent physicochemical properties such as surface charge, hydrophobicity, solubility, size, shape and aggregation tendencies which can be engineered to trigger different biological responses. Influence of such parameters on biocompatibility is well known (Figure 2.6). Manipulation of these properties for the purpose of function and biocompatibility represents a prominent area of the study of nanomedicine (Dobrovolskaia & McNeil 2007).

**Figure 2.6 Properties of nanoparticles with respect to their size and charge**
Adapted from (Dobrovolskaia & McNeil 2007)

### 2.9 Types of nanoparticles for drug delivery

Fine-tuning of the compositions and structures of the nanocarrier determine their specific applications and targets. Polymeric NPs provide more flexibility in terms of chemistry and structure for fabricating nanoparticles in contrast to
inorganic materials. Inorganic based nanomaterials, polymeric nanoparticles, micelles, and liposomes primarily consist of a biocompatible amphiphilic copolymers, cross linked nanogels possess a network with highly porous structure and the dendrimers possess tree like branched structures. Inorganic nanocarriers such as mesoporous silica, magnetic nanoparticles, gold nanoparticles, and quantum dots have unique properties and provide capabilities of tracking, while their rigid surfaces are amenable to functionalization.

![Diagram of nanocarriers targeting cancer](image)

**Figure 2.7 Nanocarriers targeting cancer** (a) A typical nanocarrier include a targeting moiety and a cargo (chemotherapeutic drugs) (b) Schematic diagram of the drug incorporation either by conjugation or by entrapment processes. Adapted from (Peer et al, 2007)
Appropriate nanoparticles need to be designed rationally according to specific situation and needs. The different types of multifunctional nanoparticles with diverse structure and fabrication have been developed for drug delivery, cellular targeting and biomedical imaging (Figure 2.7).

2.9.1 Polymeric nanoparticles

Polymeric nanoparticles include synthetic polymers, natural polymers (e.g. proteins), and pseudosynthetic polymers (synthetic polypeptides) are broadly used for drug delivery. Polymer architecture composition, backbone stability, as well as water solubility are important factors that specify the effectiveness of drug delivery carriers. Depending on the process used for their synthesis they can be nanoparticles (NPs), nanospheres or nanocapsules. Nanospheres have a matrix like structure, where active compounds can be firmly adsorbed at their surface, entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In that case, an active substance is usually dissolved in the core but can also be adsorbed at their surface. Biodegradable polymeric nanoparticles formulated from poly D, L-lactide co-glycolide (PLGA) and polylactide (PLA) have been investigated for sustained drug delivery (Anderson & Shive 2012). Analysis and understanding of various parameters that are critical to efficient intracellular trafficking and cellular uptake and retention is very important. These particles can escape rapidly from the endo-lysosomal compartment to the cytoplasmic compartment. Paclitaxel-loaded PLGA nanoparticles have greater and sustained antiprolifereative activity in HeLa cells (Yang et al 2009). NPs formulated from PLGA
can enhance the tissue uptake, permeation and targeting of zinc(II)phthalocyanine (ZnPc) for Photodynamic therapy.

Another characteristic function of polymeric NPs is their ability to deliver drugs to the target sites across biological barriers such as blood brain barrier (BBB). The brain delivery of a wide variety of drugs, such as anticancer and anti-HIV drugs is markedly hindered because they have great difficulty to cross the BBB. Nanotechnological approaches can help to overcome this barrier and to improve the pharmacokinetics of drugs used for the treatment of central nervous system (CNS) diseases. Poly-(butylcyanoacrylate) nanoparticles coated with Polysorbate-80 are effective in carrying different drugs to brain (Costantino & Boraschi 2012).

2.9.2 Liposomes

Liposomes are closed bi-layer vesicles made up of phospholipids containing an aqueous core which can carry hydrophilic or hydrophobic payloads embedded either in the interior core or in the lipid bi-layer. These biocompatible drug delivery agents are successfully used for the delivery of a variety of drugs including anticancer agents. Liposomes accumulate at tumors through passive targeting via EPR effect (Qin et al. 2014). But they are vulnerable to rapid clearance through phagocytosis by macrophages derived from reticuloendothelial system (RES). PEGylation or lipid cross-linking increases their half-life in the blood circulation. pH sensitive liposomes release their cargo either by a pH-provoked change in the lipid structural order or by the pH responsive lipid hydrolysis. Proteins and fusogenic peptides trigger the drug release after fusion with the endosomal membrane. The incorporation of pH sensitive polymers into the lipid bi-layer of the liposomal
structure also helps the drug release. The mechanism of drug efflux from the liposome to the target cell depends upon the type of polymer used; it may be a simple destabilization of the lipid bi-layer, or fusion between the liposome and the endosomal membrane (Felber et al. 2012b, Liu et al. 2013).

Several liposomal formulations have met with success over the years in a number of animal tumor models. Lipid prodrug-based liposomes have shown promise in drug and gene delivery (Roux et al. 2004). Currently several liposomal formulation are in the clinical practice containing different chemotherapeutics such as doxorubicin (Doxil/Caelyx1/Myocet1), daunorubicin (DaunoXome1), cytarabine (DepoCyte1) for treating ovarian cancer, AIDS related Kaposi’s sarcoma, multiple myeloma, lymphomas or leukemia with meningeal spread. Several other liposomal chemotherapeutic drugs containing doxorubicin, annamycin, mitoxantrone, cisplatin, oxaliplatin, camptothecine, 9-nitro-20 (S)-camptothecin, irinotecan, lurtotecan, topotecan, Paclitaxel, vincristine, vinorelbine and flouxuridine are at the various stages of clinical trials (Slingerland et al. 2012). Advances in cationic liposomes also helped successful delivery of small interfering RNA (SiRNA) (Yano et al. 2004). Targeted liposomal delivery has been explored through the use of low-density lipoprotein (LDL) and haloperidol associated ‘stealth’ liposomes for genetic therapy of breast cancer cells. Drug delivery and imaging has been combined in some studies of murine tumor model. Thermally sensitive liposomes containing doxorubicin and MnSO4 (Mn is paramagnetic similar to gadolinium) were used for the MRI based in vivo monitoring of the drug. Temperature responsive particles entered the tumor, shattered, and released the MnSO4 could be monitored through
the relaxivity of manganese nuclei under the applied magnetic field (Viglianti et al. 2004).

2.9.3 Polymerosomes

Polymerosomes consist of polymeric casing surrounding an aqueous internal core. The casing generally contains one hydrophobic layer with two hydrophilic polymer faces (Felber et al. 2012a). They are more stable than liposomes, and therefore the subject of much attention for drug delivery purposes. Polymerosomes that can assemble and disassemble with changes in pH are particularly attractive for the delivery of antitumor drugs. Polyanion based pH sensitive polymerosomes are less studied than those consisting of cationic non-pH sensitive polymers (Felber et al. 2012a). The spontaneous self-assembly of polyanionic block copolymers in water can be exploited to deliver sensitive drugs such as proteins and nucleic acids by simply adjusting the pH to an optimum value where vesicle formation ensures. Biodegradable polymerosomes prepared from poly(trimethylcarbonate)-b-poly(L-glutamic acid) were able to deliver doxorubicin with an increased release rate when the pH was lowered from 7.4 to 5.5 (Sanson et al. 2009). They have recently been tested for in vivo doxorubicin delivery to a murine tumor model and were found to be more effective than free doxorubicin (Gaoe et al. 2012). Polymerosomes usually have a smaller vesicle size and a thinner membrane.

2.9.4 Polymeric micelles (PMs)

PMs represent a class of micelles that are formed by the block copolymers consisting hydrophilic and hydrophobic monomer units. They composed of a hydrophobic blocks core stabilized by a corona of hydrophilic polymeric chains.
Although a variety of hydrophilic polymers can be used to synthesize PMs, PEG blocks with molecular weight ranging from 1 to 15 KDa are generally used to make corona forming blocks. The length of a hydrophobic core forming block is closer or somewhat lower than that of a hydrophilic block. Water solubility of a poorly soluble drug can be enhanced by using micelle forming surfactants. They also improve drugs’ bioavailability by enhancing their permeability across physiological barriers and results the substantial changes in drug biodistribution. They also help to minimize the toxicity and other adverse side effects associated with drugs. Moreover, the pharmaceutical polymeric micelles chosen for effective drug delivery also have a high drug loading capacity, a controlled release profile for the incorporated drug and good compatibility between the core-forming polymeric block and the incorporated drug (Torchilin 2007). Following intravenous administration they circulate in the blood for a longer period due to their smaller size and hydrophobic shell that minimize cell uptake by RES. In addition, micelles can be made target specific by incorporating a target specific moiety to its surface chemically. This can help efficiency of the local release of loaded drug at the target organ extensively. Micellar form en route to the target organ or tissues, the drug is well protected from possible inactivation by the biological surroundings. It also helps to do not provoke the undesirable side effects on non-targeted organs and tissues. The size of both core forming and corona-forming blocks of the micelle also influenced its efficacy of drug loading. Researchers showed that a narrow size range of micelles act as a crucial factor in determining their transport and retention in tissues showing EPR effect.
Polymeric micelles (PMs) can be formulated from a variety of biocompatible polymers such as chitosan, PEG, methacrylic acid (MAA), poly(amidoamine) (PAMAM), poly(L-aspartic acid) (PAsp), N-isopropylacrylamide (NIPAM), dimethylacrylamide (DMAA), 10-undeconic acid (UA), poly(10-undecenoic acid) (PUA), 2-hydroxyethyl methacrylate (HEMA), ethylacrylate (EA), ethyl methacrylate (EMA), butyl methacrylate (BMA), N-(2-hydroxypropyl) methacrylamide (HPMA), octadecyl acrylate (ODA), N-vinyl-2-pyrrolidone (VP), poly(N,N-dimethylaminoethyl methacrylate) (DMAEMA), polyglutamic acid (PGA), poly(D,L-lactide) (PLA), poly(ε-caprolactone) (PCL) and poly-D,L-lactide-co-glycolide (PLGA) (Felber et al. 2012).

Another subclass of nanoparticles, polyion complex micelles (PICMs), originates from oppositely charged polymers making up the micelle structure. The self-association of polymers in such micelles is due to electrostatic interaction between the oppositely charged polymeric chains (Felber et al. 2012a, Torchilin 2011, Torchilin 2007).

### 2.9.5 Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles (SLN) were developed as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles for controlled drug delivery (Couvreur 2013). They are made from solid lipids (solid at room/body temperature) and stabilized by surfactant(s). SLN can be formulated by using highly purified triglycerides, complex glyceride mixtures or even waxes. Compared with other particulate carriers, SLN has many advantages such as good tolerability, biodegradability, a high bioavailability by ocular administration and a targeting effect on the brain (Dilnawaz & Sahoo 2014, Martins et al. 2013). SLN synthesized
by high-pressure homogenization technique were used for parenteral, pulmonary and dermal applications. Because of their small size, SLN may be injected intravenously and used to target drugs to particular organs. The particles together with all intravenously injected and colloidal particulates are cleared from the circulation by the liver and spleen. In tumor tissues, delivery of the drugs by PEG-coated polymeric nanoparticles (stealth property) can help to escape from reticuloendothelial system (RES) (Pignatello et al. 2013). This may be achieved using block polyoxyethylene polypropylene copolymers like Pluronic F188 in which the hydrophobic portion of the molecule forms the NP matrix while the water-soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLN can help to increase the tumor accumulation, antibacterial activity of antiparasitic and antifungal drugs, and the brain delivery of anticancer drugs that cannot cross the BBB. Recently YU et al used SLNs for the targeted delivery of therapeutics to the alveolar macrophages. A mannan-based PEG grafted ligand was used for the surface modification of DNA-loaded cationic SLN to prepare Man-SLN-DNA produced the highest gene expressions, especially in vivo. Thus, these modified SLNs may have great potential for targeted gene delivery (Wang et al. 2014).

2.9.6 Dendrimers

Though physical aggregates such as liposomes and micelles are frequently used as drug delivery systems, they unstable under shear force and other environmental effects, such as high dilution (Haag & Kratz 2006), temperature, and pressure. In the early 1980’s, chemically designed macromolecules with precise structural
characteristics including a branch on branch, tree like architecture polymers was synthesized and named after the Greek word ‘dendron” for tree (Turk et al. 2004). Dendrimers consist of three critical architectural domains; (i) the multivalent surface with larger number of potential reactive sites (ii) the interior shells (branch cell layers defined by dendrons) surrounding the core, and (iii) a core were the dendrons are attached (Figure 2.8). These three domains can be tailored to serve various purposes, such as dendritic sensors, drug and gene carriers, or themselves as drugs.

Figure 2.8 Dendrimer generations and dendron

In dendrimers, the number of branch points present from the core to the periphery is defined as their generation number (G1, G2, G3 and G4). The generation number can be altered to enhance their drug-loading capacity. The high density of exo-presented surface functionalities make the dendritic surface nano-scaffold with close proximity of functional groups helps to target specific cell surface receptors for targeting purposes. The interior part is well suited for host-guest interaction and the encapsulation of guest molecules. The end groups present in the dendrimer surface can be modulated with hydrophilic moieties, which help to create a water-
soluble hydrophilic peripheral region and a hydrophobic interior region soluble in oil. A controlled degradation of the dendrimers can be achieved by the judicious choice of their chemistry, which also makes it biocompatible.

Dendrimers can be synthesized either by divergent or by convergent approach (Boas & Heegaard 2004). In divergent approach, the synthesis is started from a multivalent core unit onto which the consecutive ‘layers’ of branching units are added. Layers can be extended by adding the building blocks in a stepwise manner still reach the exterior surface. In the convergent approach, on the other hand, the exterior part of the molecule is designed first with the successive synthesis of different size branches starting from building blocks of surface groups. This approach helps to minimize structural defects during synthesis and to incorporate versatile functionalities or morphologies in such assemblies (Figure 2.9)

Dendritic polymers can be classified as (a) perfect dendrimers (b) dendrons (c) dendronized polymers and (d) hyperbranched polymers. Perfect dendrons and dendrimers are unique nanosystems because of their monodispersity (PDI≈1.0), nanometre dimensions (1-10 nm), low viscosity, multiple functionality at the terminal groups, high solubility and biocompatibility. Hyperbranched and dendronized polymers have broadened dimensions up to micrometre scale (PDI ≥ 1.1), with the concomitant increase in the field of applications.
Figure 2.9 Schematic representation of dendrimer synthesis (A) Divergent synthesis (B) Convergent synthesis. Adapted from (Mintzer & Grinstaff 2011)

The synergy between their multi-functionality and size on the nanoscale enables a chemical “smartness” along their molecular scaffold that helps to achieve environmentally sensitive modalities. Therefore these functional materials may help to revolutionize the existing therapeutic practice. Dendritic molecules such as polyamidoamine (PAMAM) (Esfand & Tomalia 2001), poly(propylene imine) (Hummelen et al. 1997), Polyaryl ethers (Gilat et al. 1999), polylysine (Han et al. 2007), polyester (Ma et al. 2009), polyamide (Ishida et al. 2000), polyglycerol (PG) (Haag et al. 2000), and triazine dendrimers (Simanek et al. 2009) have been introduced for biomedical applications to amplify pharmacological effects.

Dendrimers can be used as potential gene delivery vehicle because of their ability to form compact polycations under physiological condition. Functionalized
PAMAM, poly (propylene imine) and partially hydrolyzed PAMAM dendrimers have been effectively used as DNA delivery vehicle. PAMAM dendrimers functionalized with α-cyclodextrin showed about 100 fold higher luciferase gene expression than the unfunctionalized PAMAM or noncovalent mixtures of PAMAM and α-cyclodextrin. Sophisticated drug delivery systems can also be prepared by using dendrimers. A “bow-tie” structured dendrimer was synthesized by the covalent conjugation of two polyester dendrons, in which one dendron provides multiple functional handles for drug incorporation and other for solubilizing poly(ethylene oxide) (PEO) chains. By varying the generations of dendrons and the mass of the PEO chains, molecular weight, architecture and drug loading capacity can be controlled. Though small interfering RNA (siRNA) holds great promise for cancer treatment, the field has been hindered by the availability of suitable delivery vehicles. Cationic dendrimers can improve the stability of siRNA, intracellular trafficking, silencing efficacy and their accumulation in tumor cells by the EPR effect (Liu et al. 2012). Polyplexes showed very low cell toxicity and resulted no significant weight loss after IV administration. In vitro, dendrosomes are highly effective in delivering siRNA targeting E6 and E7 proteins of cervical cancer cells (Braun et al. 2005, Luo et al. 2012). Dendrimers showed great promising as future effective drug and gene delivery vehicles.

2.9.7 Carbon based polymers

Carbon based polymers such as fullerenes, carbon dots, nanodiamonds, and nanofoams also represent a prominent area of nanoparticle research. Fullerenes consist of C-60, single-walled nanotubes and multi-walled nanotubes. Carbon
nanotubes forms simple layers of graphite rolled in a tubular shape capable of exhibiting a single or multi walled morphology are very promising and may be used from structural reinforcement of existing materials to drug carriers (Bianco et al. 2005, Moghimi & Hunter 2012, Pastorin et al. 2006). Their cell penetrating and conjugative properties make them a promising tool for the in vivo delivery of nucleic acids, peptides and antibodies (Goodwin et al. 2014). In addition, surface functionalization can render typically heterogeneous nanotubes water soluble (Salvador-Morales et al. 2006, Goodwin et al. 2014).

Recently carbon nanotubes have attracted attention due to their use in controlled drug release as well as delivery of nucleic acids, peptides and antibodies. Their inner core allows the insertion of specific payload into the small inner core. The outer surface can be modified to achieve the necessary biocompatibility within the body or to attaching targeting ligands or drug payloads. Single walled carbon nanotubes (SWNT) coated with doxorubicin can release the drug in a pH responsive manner (Dang et al. 2011, Gu et al. 2011). Carbon nanotubes till now reached only the phase I trials but to compete against micelles, liposomes, polymeric systems and dendrimers, a compelling argument based on their unique characteristic is required but it is not yet achieved.

Fullerenes are the spheroidal carbon nanostructures, with exceptional physical, photochemical and electrochemical properties. Their surface functionalized with receptor agonists and antagonists have been remodeled to carry gadolinium atoms for MRI of tumors. The water soluble gadolinium metallofullerenes showed prolonged blood circulation (48h) and delayed clearance by the
excretory system. Their in vivo safety aspects and all the findings need to be tested at clinically applicable MRI field strengths (Bolskar 2012, Montellano et al. 2011, Shi et al. 2014).

2.9.8 Viral nanoparticles

A variety of viruses including cowpea mosaic virus, cowpea chlorotic mottle virus, canine parvovirus, and bacteriophages have been developed for biomedical and nanotechnology applications like tissue targeting and drug delivery. A number of targeting molecules and peptides can be displayed in a biologically functional form on their capsid surface using chemical or genetic means. Several ligands or antibodies including transferrin, folic acid, and single chain antibodies have been conjugated to viruses for specific tumor targeting in vivo (Yildiz et al. 2011). A subset of viruses, such as canine parvovirus has natural affinity for receptors such as transferrin receptors, which are up-regulated in a variety of tumors. A dual function protein cage with specific targeting capability and doxorubicin encapsulation has been developed to target the heat shock protein. (Molino & Wang 2014, Koppers-Lalic et al. 2014).

2.9.9 Inorganic nanoparticles

The various forms of inorganic nanoparticles like quantum dots (QDs), super paramagnetic iron oxide nanoparticles (SPIONS), gold nanoparticles and other metallic and non metallic nanoparticles or nanoclusters are used for the diagnosis and treatment of various forms of cancers (Figure 2.10)
Figure 2.10 Inorganic nanoparticle used for the imaging and treatment of tumors (A) Superparamagnetic iron oxide nanoparticles (SPION) (B) Inorganic core-shell nanoparticles such as quantum dots (QDs) (C) Gold nanoparticles (AuNPs) (D) Inorganic nanoclusters. Adapted from (Nazir et al. 2013)

2.9.9.1 Quantum dots

Fluorescent QDs are inorganic semiconductor nanocrystals of 2-10 nm in size, exhibited a broad absorption band and a symmetric, narrow emission band, typically in the visible to near infrared (NIR) range. As size decreases, their excitation by single wavelength laser light increases absorption probability and is shifted towards the blue end of the spectrum. The quantum confinement effect comes into play when the size of the semiconductor crystal becomes less than the Bohr exciton radius, leading to unique electronic and optical features (Wang & Chen 2011, Singh 2011). The surface properties of the QD crystals can be tailored to achieve better solubility, biocompatibility and target selectivity. The surface functionalities help to incorporate targeting proteins, including transferrin or
antibodies which can help to bind to specific cell surface receptors. The surface tailoring and bio-conjugation with peptides, antibodies, proteins, and DNA has greatly enhanced the in vivo applications of QDs. Such bio-conjugation can take the form of either covalent bonding or electrostatic interaction. Proteins containing cysteine or histidine can be directly attached to the ZnS surface through in-situ disulfide linkages between the sulphur atoms of ZnS and the respective sulphur containing amino acid residues (Sun et al. 2013, Zhan et al. 2014, Blanco-Canosa et al. 2014).

2.9.9.2 Superparamagnetic iron oxide nanoparticles (SPIONs)

SPIONs acquire a large magnetic moment in an externally applied magnetic field, thus attaining superparamagnetic properties. They are capable of producing high contrast per unit of particles, so that smaller quantities of SPIONs are sufficient for imaging therapy, thereby reducing the toxicity issues (El-Dakdouki et al. 2014). Their surface can be engineered with a variety of functionalities that helps to enhance biocompatibility and biodegradability. These properties make them attractive materials for advanced biomedical applications, which include medical imaging, contrast agents in MRI; killing malignant and infected cells through thermal therapy by acting as miniaturized heaters and can be employed for targeted drug delivery to disease sites including cancer cells. SPIONs degraded to iron ions, which are added to the body's native iron pool, incorporated into heamoglobin, and degraded through the normal iron recycling pathway (Kievit & Zhang 2013, Mahmoudi et al. 2011). SPIONS can be synthesized by the co-precipitation of Fe(OH)$_2$ suspensions and can be functionalized with biodegradable and
biocompatible polymers like PEG, polyethylene oxide (PEO), dextran and polysaccharides to achieve stability in blood plasma (Huang et al. 2011a).

### 2.9.9.3 Gold nanoparticles

Gold nanoparticles (AuNPs) are easy to synthesize in a range of sizes by changing simple parameters. Moreover, due to presence of negative charge, AuNPs can be easily functionalized with various biomolecules and they are biocompatible and nontoxic. AuNPs have unique physicochemical characters like ultra small size, large surface area to mass ratio, high surface reactivity and the presence of surface plasmon response (SPR) bands. SPR is responsible for their large absorption and scattering cross-sections in the 4 to 5 orders of magnitude larger than that of the conventional dyes. Though AuNPs used for a variety of drug delivery applications, they showed enhanced cytotoxicity in all the cell lines (Pissuwan et al. 2011). AuNPs can helps to improve the delivery of anticancer drug oxaliplatin, showed ability to penetrate into the nucleus of lung cancer cells (Brown et al. 2010). 3-mercapto propionic acid capped AuNPs can deliver drugs to drug resistant leukemia K562/ADM cells. This could be explored as a novel strategy to inhibit multidrug resistance in targeted tumor cells (Wang et al. 2011).

### 2.9.9.4 Other metal based nanoparticles

Currently many metal-based nanoparticles and hybrid surface functionalized NPs are used for drug delivery applications. New hybrid systems consisting of anticancer drugs, such as methotrexate (MTX) or 5-fluorouracil (5-FU) and carrier like layered double hydroxide (LDH) that contain inorganic vectors with biocompatible metal ions, were developed by Choi et al. LDH NPs showed sustained
drug release, prolonged drug half-life and high ability to accumulate the drugs in targeted tumor tissue. These hybrid systems can be promising anticancer chemotherapy agents for tumor targeting with biocompatibility (Furukawa et al. 2010)

2.10 Clinical applications of nanomaterials

A variety of nanomaterials are being investigated for the drug delivery, diagnosis, imaging, and therapy of cancers. Significant advances have been made in their synthesis with controlled geometry, physicochemical properties, surface charge and tailoring with bioactive polymers. These diverse properties improved the biocompatibility and active targeting of tumor tissues. This leads to the development of a diverse range of nanomaterials that can recognize a cancer cell and deliver anticancer drugs to the tumor. In addition to drug delivery applications, NPs can also be used in thermotherapy and Photo Dynamic Therapy (PDT). Within the last 10 years, the number of clinical trials has increased continuously and it is likely that this trend will continue in the future due to innovative research. A variety of organic and inorganic nanoparticles are available for preferential uptake and residence at tumor sites due to the EPR effect and RES mediated uptake into tumor cells. Self-assembled polymeric nanoparticles such as liposomes, micelles, polymerosomes, and nanospheres have the ability to release the drug payloads at targets sites triggered by the variations in pH or temperature at the site of its action. Some of these nanomedicines are in the later stages of approval for medical treatment and detection of cancers, which are listed in the table (Table 2.4)
### Table 2.4 Approved/Marketed nanomaterials in cancer therapy

<table>
<thead>
<tr>
<th>No</th>
<th>Product(s)/device</th>
<th>Nanoplatform/active agent</th>
<th>Status</th>
<th>Active Mechanism</th>
<th>Company/Ref</th>
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<tbody>
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<td>Doxorubicin HCl, Liposome(Pegylated)</td>
<td>Approved</td>
<td>Chemotherapy</td>
<td>OrthoBiotech (Wang et al. 2013)</td>
</tr>
<tr>
<td>2</td>
<td>Daunoxome</td>
<td>Liposomal daunorubicin</td>
<td>Approved</td>
<td>Chemotherapy</td>
<td>Galen Ltd (Fonseca et al. 2014)</td>
</tr>
<tr>
<td>3</td>
<td>Myocet</td>
<td>Liposomal doxorubicin(Non-PEGylated)</td>
<td>Approved</td>
<td>Chemotherapy</td>
<td>SopherionTherapeutics (Eitan et al. 2014)</td>
</tr>
<tr>
<td>4</td>
<td>Abraxane</td>
<td>Paclitaxel Albumin NPs</td>
<td>Approved</td>
<td>Chemotherapy</td>
<td>Calgene (Ciruelos &amp; Jackisch 2014)</td>
</tr>
<tr>
<td>5</td>
<td>Feridex</td>
<td>Dextran coated FeO NPs</td>
<td>Approved</td>
<td>MRI imaging</td>
<td>Berlex Laboratories (Jenkins et al. 2014)</td>
</tr>
<tr>
<td>6</td>
<td>Endorem</td>
<td>Carboxydextran coated FeO NPs</td>
<td>Approved</td>
<td>MRI imaging</td>
<td>Guerbet (Visscher et al. 2014)</td>
</tr>
<tr>
<td>7</td>
<td>Resovist/sup-ravist</td>
<td>Dextra coated Iron oxide NPs</td>
<td>Approved</td>
<td>MRI imaging</td>
<td>Baer Schering Pharma AG (Sato et al. 2014)</td>
</tr>
<tr>
<td>8</td>
<td>ThermoDox</td>
<td>Heat inactivated liposomal encapsulation</td>
<td>Approved Phase III</td>
<td>Chemotherapy</td>
<td>Celsion (Harris 2014)</td>
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<td>9</td>
<td>Lumirem, Sinerem.</td>
<td>Iron oxide NPs</td>
<td>Approved/Investigational</td>
<td>Enhanced MRI Contrast</td>
<td>Guerbet (Liao et al. 2014)</td>
</tr>
</tbody>
</table>

### 2.11 Conclusion

Nanomedicine is driven by the success in the creation of innovative and safe ‘nanosized materials’ through the controlled synthesis on the nanometer scale. The definable architectures, synthetic tenability, and chemical versatility of nanocarriers
have increased the perspectives in the field of nanotherapeutics and nanodiagnosticstics. The recent advances in the design and synthesis may lead to the development of intelligent nanomaterials that are stable in the body, with long circulation times and improved localization to diseased areas without compromise their therapeutic efficiency. These nanomaterials may result short-term therapeutics without any adverse effects of long-term exposure. But extensive research is required to ensure confidence in translating nanomaterials into clinical applications. Medicine needs new solutions to achieve highly effective side effect free, and cost efficient solutions for major diseases. The emerging field of intelligent nanomaterials for medical diagnosis, therapy and their combinations “theranostics” is based on a range of well studied carrier platforms, a number of targeting strategies each offering advantages and challenges and a “smart” payload. This combination has the goal of increasing specificity and efficacy in the diseased tissue, while abolishing toxicity largely. Although pioneering work has been done in oncology, both in preclinical development and clinical application, it is widely felt that the added capabilities of intelligent systems beyond “target cell killing” may render intelligent nanosystems a shaping force for a much broader range of diseases. To advance in such applications, a thorough knowledge about clinical challenges, material properties, physicochemical properties, nano-bio interactions, toxicity, regulatory pathways and clinical trial results are required.
2.12 References


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