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REVIEWS

Advances in Cancer Nanomedicine

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Abstract | Scientific advances have significantly improved the basic understanding of biology of cancer. Now it is clear that a series of genetic alterations leading to abnormal cell division, loss of growth control and the capability to develop their own blood supply resulted in this complex scenario of cancer. Due to the lack of drug availability, adverse side effects and drug resistance, the conventional therapy failed to achieve proper treatment. During the past few years, nanomedicine has showed considerable progress in improving the cancer treatment and this review highlighted some of the recent advancement in this field of research. Development of first generation nanomedicine such as cytotoxic drug loaded polymeric nanoparticles, micelles, liposomes, dendrimers, carbon nanostructures, inorganic nanoparticles, etc were discussed in detail. A detailed understanding of the mechanism of cancer leading to the evolution of second generation of nanomedicine aids more efficient targeted cancer therapy. Nanoparticle mediated magnetic hyperthermia, photothermal therapy and radiofrequency hyperthermia is also reviewed as new generation cancer nanotherapeutics.

1 Introduction

Cancer is a major human health problem and leading cause of death worldwide. It was estimated that every year about 7.6 million people are dying due to cancer and about 70% death in developing counties are because of cancer. Cancer develops due to deregulated cell division of an abnormal cell or from the changes that cause normal cells to acquire abnormal functions. Major factors for cellular abnormality include inherited mutations or mutation induced by environmental factors such as viruses, X-rays, UV light, chemicals, tobacco etc. Cancer comprises almost 100 different diseases and it was found that most of them are not due to a single factor. All evidence suggests that cancer results from a series of molecular events that fundamentally alter the normal function and properties of cells.

2 Characteristics of Cancer

Six characteristics alterations (figure 1) in cell physiology are associated with malignant growth includes self sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (Apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[1] Each of these physiological changes acquired during tumor development leads to the successful violation of an anti-cancer defense mechanism by the cells and tissues.

2.1 *Self-sufficiency in growth signals*

Appropriate behavior of different cell types in a tissue at varying time points is regulated by a homeostatic mechanism. Entry of a normal cell

Physiology: The term physiology indicates normal function living system which includes how cells, organs, organ system and organism do the chemical and physical functions of living system.

Homeostatic: The regulation and maintenance of stable, constant internal environment is called homeostasis.

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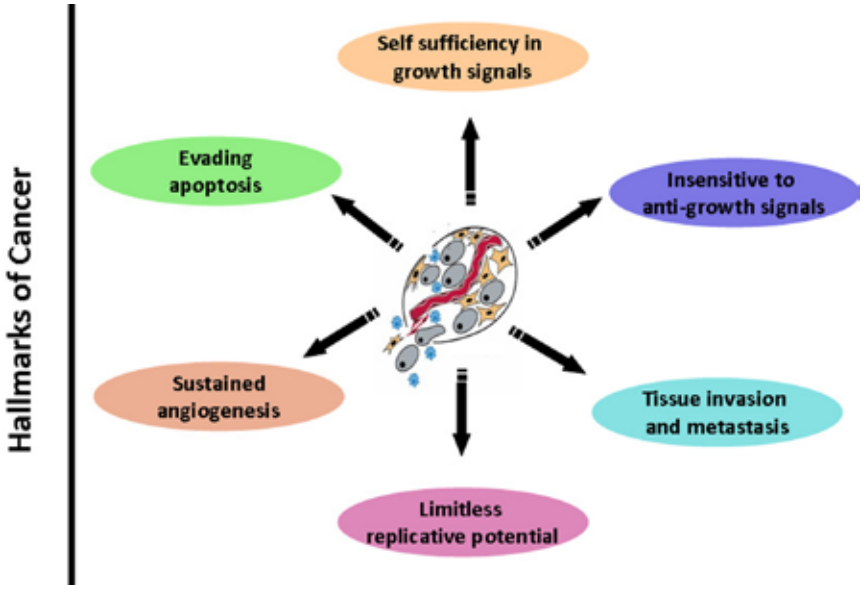

Figure 1: Six acquired capabilities of cancer. Most of the cancers have acquired these functional capabilities during tumor development.

into proliferative phase depended on the mitogenic growth signal, which transmitted in to the cells through trans-membrane receptor that bind to specific signaling molecules. Signaling molecules includes diffusible growth factors, extracellular matrix components, and cell-to-cell adhesion/ interaction molecules etc. Tumor cells show a reduced dependence on such extracellular growth signals and they have the capability to synthesize their own growth factors responsible for cellular division and proliferation.[2,3] Alternatively, cancer cells may send signals to stimulate normal cells within the supporting tumor-associated stroma, to supply various growth factors.[4,5] Example for such self sufficiency by cancer cells includes the expression of Vascular endothelial growth factors (VEGF), Epidermal growth factors (EGF), transferrin, PDGF (platelet-derived growth factor) by brain tumor and TGFa (tumor growth factor a) by sarcomas.[6] Expression of certain receptors such as epidermal growth factor receptor up regulation in the case of lung, stomach, brain and breast cancers is another means for achieving self sufficiency. In addition integrin switching is also reported to transmit growth signals by tumor cells.[7,8] Mutations, that activate downstream protein kinase pathways such as phosphoinositide 3- Kinase (PI3 kinase) pathway along with hyper activated protein kinase B (or Akt) transducer is detected in several tumors.[9] Disruption of negative feedback mechanism for proliferative signals is also found in many cancers. MTOR kinase, a coordinator of cell growth and metabolism is disrupted in various cancer cells resulting in the loss of negative feedback mechanism leading to the hyper activation of PI3kinase pathway.[10]

2.2 *Insensitivity to anti-growth signals*

Anti growth signals can inhibit cells to pass through cell division cycle (Cell cycle). Cell cycle involves an interphase (consist of G1, S and G2 phases) and a mitotic phase (M phase, where the cell physically divides into two) (Figure 2). Anti growth signal causes cells to either stay in the quiescent G_0 phase of cell cycle until appropriate growth signals received by the cell or it allows the cells to enter into post mitotic phase as in the case of specific differentiated cells. The retinoblastoma protein (pRB), a tumor suppressor protein, has a key role in the regulation of anti growth signal through the expression of several other proteins necessary for a cell to enter into S phase. In tumor cells the pRB proteins are inactivated and hence the cells become insensitive to anti-growth signals. pRB signaling pathway is primarily governed by a growth factor, TGF-β. Disruption of pRB pathway can also occur due to the down regulation or mutation of TGF-β receptor. In some cancers, such as cervical cancer, pRB function is eliminated because of the intercalation of viral oncoproteins into the human gene. Moreover cancer cells can also down regulate the expression of integrins responsible for transmitting the anti-growth signals. Hence in conclusion the pathways that ultimately leads to

Mitogenic: The capacity of a single cell to devide into two identical cells, each contain same number of chromosome and genetic content as that of the original cell.

Receptors: Receptors are molecules present on the surface of the cell which receive external chemical stimuli and transfer it into the cell.

Growth factors: Growth factors are proteins capable of stimulating cellular growth, proliferation and differentiation.

Cell cycle: Cell cycle is otherwise called cell division cycle. It consist of a series of events that leads to the division of a single cell into two.

Stroma: Functionally supportive frame work of a cell, tissue or organ, basically consist of connective tissue.

Sarcoma: Cancer originating from cells of mesenchymal origin are called sarcoma and epithelial origin is called carcinoma.

Integrin: Receptors seen on the plasma membrane of the cells for cell-cell adhesion and cell-extra cellular matrix adhesion.

Kinases: Kinases are regulatory enzymes that transfer phosphate groups from high energy molecules such as ATP to a substrate molecule inorder to activate/ deactivate the substrate.

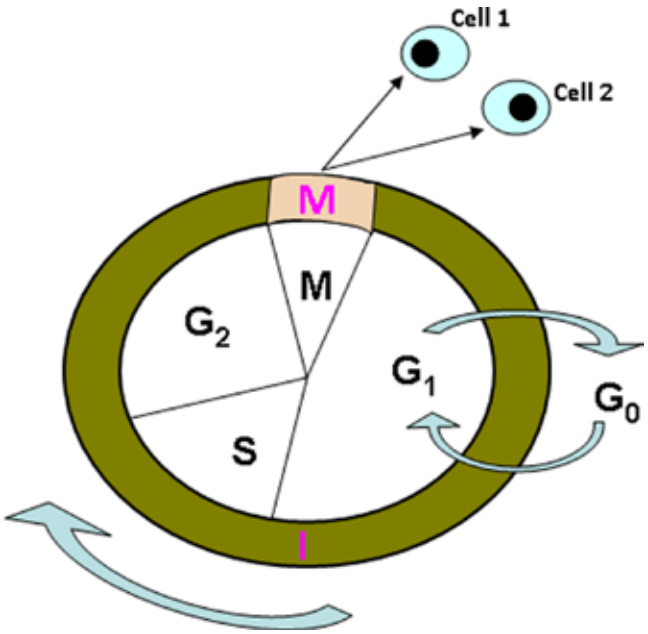

Figure 2: Schematic representation of cell cycle. Cell cycle have two distinct phases, Mitotic phase (M) and interphase (I). Interphase is divided into Gap phase 1 (G_1), synthetic phase (S) and Gap phase 2 (G_2). G_0 is the resting phase where cells has left the cycle and stopped dividing.

the activation of pRB is disrupted in cancer cells in one way or other for developing insensitivity to antigrowth signals.

2.3 *Evading apoptosis*

Acquired resistance to the programmed cell death mechanism, viz. apoptosis, is an important hallmark of cancer.[11] Apoptosis involves a series of physiological steps which is characterized by the disruption of cell membrane, loss of cytoplasmic and nuclear skeletal arrangement, increased volume of cytoplasm, fragmented nucleus and DNA damage. Apoptotic machinery involves sensors which monitor the intracellular and extracellular abnormalities and effectors which act on cells to enter into programmed cell death machinery.[12] Mitochondria are the cellular organelle primarily involved in programmed cell death by the release of a protein, cytochrome c, which triggers a cascade of caspase activity leading cell death. In normal physiology, p53 gene which is a tumor suppressor gene, elicit apoptosis by expression of pro-apoptotic proteins such as BAX, BID, and BAD in response to DNA damage, reactive oxygen stress, etc. Inactivation of p53 gene by mutation will upset the normal apoptotic process in our body leading to tumrogenesis.[13] In addition, proto-oncogenes such as bcl-2, RAS, MYC, WNT, ERK etc and oncogenes such as BCR-ABL can also activate anti-apoptosis signaling and enhance the uncontrolled proliferation of cancer cells.

2.4 *Limitless replicative potential*

Acquired capabilities such as self sufficiency in growth signals, insensitivity to anti-growth signals and evading apoptosis are not sufficient for the fast growth and enlarged volume of tumor tissue observed in most cancer patients. Apart from cell-cell signaling and programmed cell death, normal cells have another cell autonomous program that limits their multiplication.[14] i.e., After a certain number of cell division one cell must enter into a process called senescence. This process 'senescence' must be disrupted in order to develop a macroscopic life threatening tumor. Disabling pRB and p53 tumor suppressor genes leads to the replicative capacity of tumor cells. This was proved *in vivo* by Hayflic *et al*, 1997 and suggested that similar effects must be happening *in vivo* during the tumor development.[14]

2.5 *Sustained angiogenesis*

Oxygen and nutrients supplied by blood vessels (capillaries) are important for normal growth and function of a cell. Hence once a tissue is formed, new blood vessels were also starts developing (angiogenesis) and their growth is carefully regulated during organogenesis. Normal regulation of

Apoptosis: Apoptosis (in contrast to necrosis, which is a form of traumatic cell death) is programmed cell death occurs in multicellular organism, characterized by cell shrinkage, blebbing, nuclear fragmentation etc. Autophagy, another form of cell death, is degradion of cells own component with help of lysosomal machinery.

Mitochondria: Mitochondria are membrane bound organelle of cell. They are called power house of the cell since they supply high energy molecule ATP for various functions. They involved in cell signaling, cell differentiation, cell cycle and cell death.

Caspases: Caspases are cystein dependent proteases, involved in apoptosis, necrosis and inflammation.

Senescence: Senescence is a phenomenon in which a normal cell loses its capacity of cell division after several division like 50 division in vitro. Senescence causes a cell to enter into cell death.

Tumorogenesis: Tumorogenesis is the formation of new tumor or tumors.

Proto-oncogene: A normal gene that can mutated or over expressed to form oncogene.

Oncogene: A gene that has the potential to cause cancer.

Angiogenesis: Angiogenesis is the development of new blood vessels from existing blood vessels.

angiogenesis is carried out by signals transmitted by angiogenic inducers (soluble growth factors) such as VEGF and FGF as well as angiogenic inhibitors such as throbospondin-1. Integrin mediated signaling is also involved in the regulatory balance. Angiogenesis is a crucial pre-requisite for the development of macroscopic tumors. Sustained angiogenesis by tumor tissue is acquired during the tumor progression through angiogenic switch from vascular quiescence.[15] In most cancers, the angiogenesis is associated with altered expression of angiogenic inducers and countervailing inhibitors. In some cases the angiogenic inducers such as VEGF or FGF is upregulated. However in some other cancers the downregulation of endogenous inhibitors such as thombospondin 1/interferone β were associated with angiogenesis in tumor. Alteration in both inducers as well as inhibitors was observed in some tumors as well.[16]

Upregulation and downregulation: An increase in cellular component in response to an external signal is called upregulation. A decrease in cellular component in response to an external signal is called downregulation.

Interferons: Interferons are proteins secreted by a cell in response to the presence of pathogens such as virus, bacteria, tumor cells etc.

2.6 *Tissue invasion and metastasis*

Malignant cells from primary tumor mass spreading to adjacent tissue is called invasion, while traveling of the cells to distant sites (either through blood or lymphatics) where they develop new colonies are called metastases. Metastasis is the major reason for 90% cancer deaths. Successful invasion and metastasis depends on all other 5 acquired capabilities of tumor. Metastasis is a complex process which involves changes in the activation of extracellular proteases (proteolytic enzymes). Protease genes up regulation or protease inhibitor down regulation or activation of zymogens into active form are associated with protease mediated metastasis. Proteins involved in metastasis include cellular adhesion molecules (CAM) and integrins. CAM protein such as E cadherin serves as a suppressor of invasion and metastasis and the loss of function E-cadherin is associated with metastatic cancers such as metastatic epithelial cancer.[17] A switch in highly adhesive form to poor adhesive isoform of N-CAM is associated with invasive tumors in neuroblastoma, small cell lung cancer, pancreatic cancer and colorectal cancer.[18] The multistep process of invasion and metastasis involves a sequence of discrete steps, termed the invasion-metastasis cascade.[19]

Proteases: Proteases are enzymes that cleaves other proteins (proteolysis).

3 Conventional Treatment Methods

3.1 *Surgery*

Surgery, often the first line of treatment for cancer, is used to remove solid tumors (benign tumor as well as in early stage of cancer). Surgical resection is often not advised in cases of large tumor size, location close to other vital structures and presence of distant metastases (high grade tumors) requiring assistance treatment. Surgery has no great effect if the tumor is already spread to other organs.

3.2 *Radiation therapy*

High energy radiation which is targeted primarily to the tumor tissue, kills cancer cells by either directly damaging DNA or by generating reactive oxygen species (ROS) preventing cellular division. It is of two forms: a) Brachytherapy—where the radioactive source (in pellets) is placed close to the tumor, eg: uterine cancer and b) Teletherapy—where the patient is irradiated from a source place some distance away from the body, eg, skeletal tumors. However this method also kills the normal cells through which the radiation has to pass to reach the tumor. Yet another technique for radiation therapy is with the use of unsealed radio active sources, which emit a high energy beta of alpha radiation. Eg: ^{131}Iodine used for thyroid cancers. For better result a combination of surgery and radiation therapy is advisable. Major drawback of radiation therapy is that, certain tumors develop radiation resistance due to the expression of specific ROS scavengers. In addition not all tumors are eligible for radiation therapy due to the adjacency of radiosensitive organs.

3.3 *Chemotherapy*

Chemotherapy is the most widely used therapy method where chemotherapeutic agents (chemicals) are injected or orally delivered to kill rapidly growing cells. Most these chemicals interfere with normal DNA replication primarily blocking the cells to complete the S phase of cell cycle. In addition there are chemotherapeutic agents cause extensive DNA damage as well as spindle fibre inhibitors.

Although chemotherapeutic agents are relatively specific, they also kill normal cells which are dividing very rapidly such as gastrointestinal tract, bone marrow cells, and hair follicles. This causes some of the side effects of chemotherapy, including gastrointestinal distress, low white blood cell count, and hair loss. Moreover many of the clinically used chemotherapeutics require high tissue concentrations which are associated with systemic toxicity. Hydrophobic drugs which are administered intravenously with solubilizing adjuvant like ethanol or Cremophor EL often cause toxic side effects. Drug resistance has emerged as a major obstacle limiting the therapeutic efficacy of chemotherapeutic agents. These mechanisms allow tumors to evade chemotherapy. For example, multidrug resistance (MDR), a term to describe the broad spectrum resistance to chemotherapy in

human cancer, is one of the most important problems in chemotherapy. MDR is the phenomenon in which exposure of tumor cells to a single cytotoxic agent accounts for cross-resistance to other structurally unrelated classes of cytotoxic agents.

Therefore improving the therapeutic index by increasing the therapeutic effects to tumor cells with decreasing toxicity to normal cells is a central issue in improving cancer therapy. Delivering therapeutically active molecule to a target site is a challenging task. Poor aqueous solubility and non specific targeting are limitations of current treatment method. Nanostructures have the potential to overcome these limitations by acting as carriers for therapeutic agents such as drugs, genes etc targeted to specific cancer cells and as imaging agents.[20] Recent development of nanomedicine for cancer treatment surmounts various challenges in this field.

4 Nanomedicine for Cancer

After the commencement of nanotechnology in 1959, the field of nanomedicine has developed quickly and we are now successfully approaching towards solutions to various challenges. The term liposome was described and the drug encapsulated liposome developed before 70's itself. From there, the advancement of nanomedicine passed through various achievements starting from gold nanoparticle, polymeric nanoparticles, quantum dots, fullerenes etc to the clinically approved nanomedicines for chemotherapy. Figure 3 briefly describes 50 years of nanomedicine developments for cancer treatment.

Rationale for developing nanomaterial for cancer treatment includes; (i) multifunctionality (ii) increased potency and multivalencey (iii) increased selectivity for targets (iv) theranostic potential (v) altered pharmacokinetics (vi) controlled synthesis (vii) controlled agent release and kinetics

Theranostic: A single technique which functions both as a therapeutic as well as a diagnostic method for a particular disease condition.

Pharmaco kinetics: It is the analysis of absorption, distribution and bioavailablity etc of a drug, hormone and toxin administered externally to the living organism.

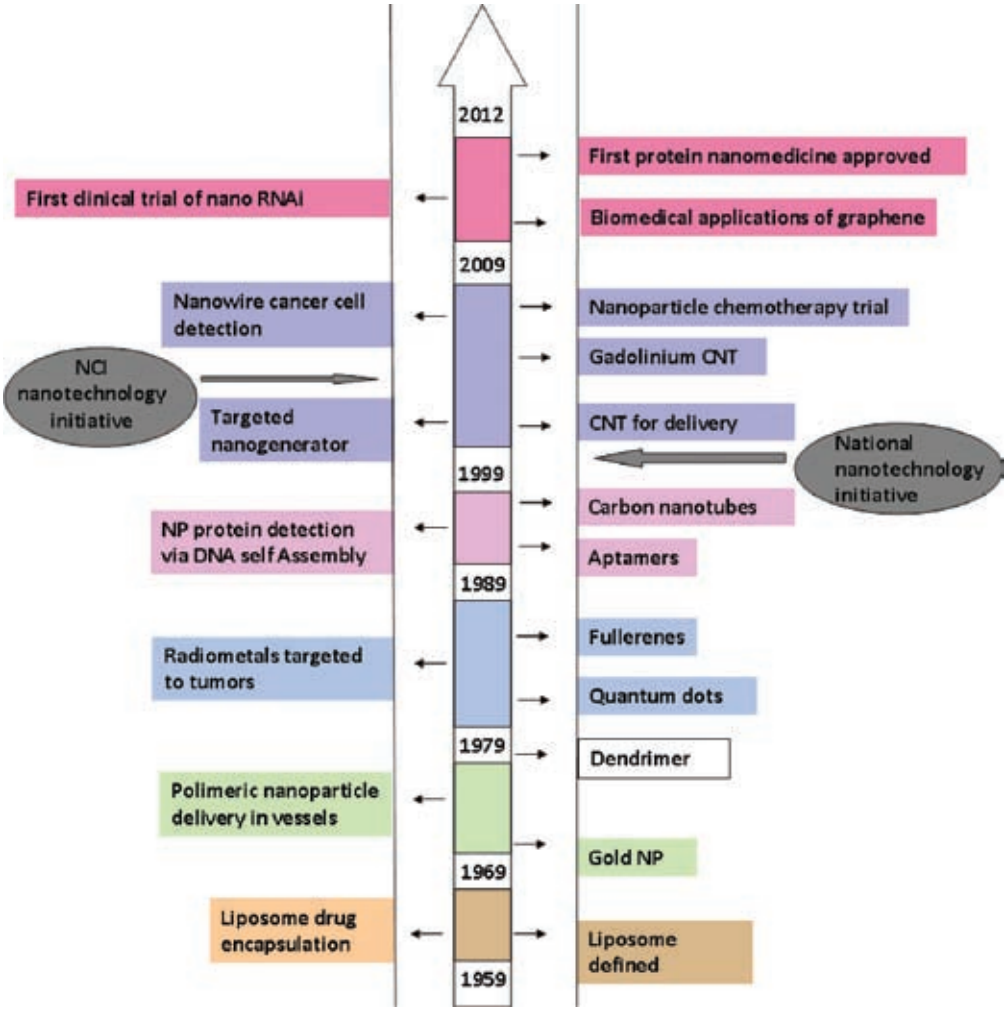

Figure 3: 50 years of nanomedicine development for cancer. Abbrevations: NP—Nanoparticles, NCI—National Cancer Institute, CNT—Carbon nanotube.

(viii) novel properties and interactions (ix) lack of immunogenicity (x) enhanced physical stability.[21]

Nanoparticle mediated delivery of cancer therapeutic agents can be done either by passive targeting or by active targeting. Active targeting utilizes specific biomarkers such as cell surface receptors for specifically targeting to tumor tissue. This is described elaborately in subsequent sections. In the case of passive targeting, the drug loaded nanoparticles entered into the tumor tissue owing to the enhanced permeability and retention effect (EPR) provided by the anatomical and pathophysilogical abnormality of tumor vasculature (figure 4). EPR effect is considered as a gold standard in the design and development of anticancer nanomedicines. Important hallmarks of EPR effect includes, extensive angiogenesis, imperfect vascular architecture and impairs lymphatic drainage system of the tumor. It was reported that this enhanced permeability of tumor tissue is due to the elevated levels of bradykinin, nitric oxide, peroxynitrite, vascular endothelial growth factors etc. Hence the drug loaded nanomaterials can extravasate through the leaky architecture of tumor vasculature and moreover the absence of lymphatic drainage from the tumors causes retention of the nanodrugs at the site of the leision.

5 Factors Influencing the Targeting of Nanomaterials

Importance of nanomaterials as carriers for cancer medicine is the ability to control their size, shape, surface charge and surface chemistry, etc to improve the pharmacokinetics of the chemo drugs and enhance their specificity by way of conjugating with cancer specific ligands. Suitably designed nanomedicines can also cargo multiple pay loads for combinatorial drug delivery or image guided therapy (theragnostics).

5.1 *Size*

Biological behavior of the nanoparticle is partly dependent upon the size of the nanoparticles. Significant difference in cellular uptake, function and toxicity can occur for a same material with different size scale.[22,23] As small as few nanometers itself can alter function of nanomaterials. For example nanoparticle with size scale 40–60 nm can more readily bind to cells and elicit endocytosis than particles less than 40 nm in diameter.[23] Hence by fine tuning of particle size, it is possible to direct them for accurately inhibiting different pathways and specific targeting to cancer cells without adversely affecting the normal cells.[23,24] In addition, nonspecific uptake by reticulo endothelial system, renal clearance and the pharmacokinetics of the nanomedicine etc found to be dependent on particle size.[25,26]

5.2 *Shape*

Molecular architecture of the nanomaterial has important role in pharmacology and function.[27] Several studies based on the shape effect on endocytosis revealed that endocytosis of non-spherical

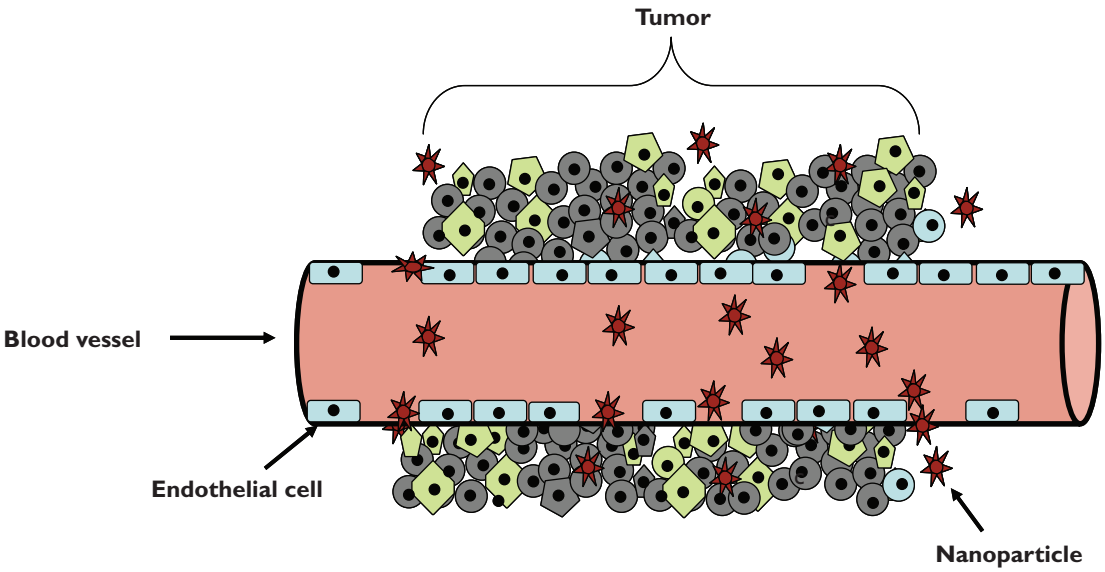

Figure 4: Passive targeting of nanoparticles by EPR effect. Tumor blood vessels possess an anatomical and pathophysiological abnormality in vasculature. Nanoparticles extravasate through the leaky vasculature into the tumor tissue and due to the absence of lymphatic drainage; the nanocarriers accumulate in the tumor.

particles are dependent on the local shapes that makes at the interface of the cells with nanomaterials.[28] They have also studied about the alignment of particles (parallel or perpendicular) to the cellular membrane for cellular uptake. Gratton *et al* revealed that particles with higher aspect ratios and size greater than 100 nm showed higher phagocytic uptake by the cells. Particle shape has also shown to affect biodistribution and renal clearance.[30]

5.3 *Surface charge*

Properties of nanomaterials can be further enhanced by conjugating with different moieties on their surface.[31] The biomoieties includes ligands (folic acid, thiamine), antibodies specific to cancer cells, proteins (BSA, transferring, lectins, and cytokines), polymers (Poly ethylene glycol), polysaccharides (dextran, oligosaccharides, heparin), fatty acids (palmitic acid, phospholipids), aptamers (synthetic molecules of DNA or RNA), siRNA, plasmid etc. According to Kobayashi *et al*, surface charge of ligands such as antibody conjugated on nanomaterial surface for targeting showed important role in its bio-distribution profiles.[32,36] Pegylation (conjugation with polyethylene glycol) is an efficient way to improve the stability as well as bio availability of nanoparticles *in vivo*.[35] For example conjugation of polyethylene glycol on the surface of particle improved biodistribution as well as reduced the RES uptake.[33] Increase in charge of the nanomaterial has important role in tubular re-absorption by kidney. Gotthardt *et al* showed that positively charged nanoparticles are retained in renal cortex.[34]

6 First Generation Cancer Nanomedicine

Development of first generation nanomedicines centered on the concept of simply improving the bio-availability and reduce the toxicity of some of the clinically used, highly hydrophobic drugs such as paclitaxel, doxorubicin etc. The best examples are Abraxane™, which is albumin bound paclitaxel and Doxil, which is a liposomal formulation of Doxorubicin. Both these nanomedicines are approved for clinical use and are found to be highly successful in improving the specificity with minimal toxic side effects. Abraxane uses albumin to deliver chemotherapeutic agent without chemical solvents such as Cremophor™ as well as premedication with steroids and thereby reduced risk of hypersensitivity reaction. Side effects of this formulation were less and the overall response rate was superior to Taxol (Paclitaxel). DOXIL is a pegylated liposome- encapsulated form of doxorubicin mainly for treating Kaposi's sarcoma, ovarian cancer and multiple myeloma. Other formulations that are in clinical trials includes DaunoXome, Myoset, and Oncasper etc. Table 1 shows the list of approved anticancer nanomedicines. Nanoparticles used for anticancer drug delivery can be made from a variety of materials such as polymers, dendrimers, liposomes, viruses, carbon nanotubes and inorganic materials.

6.1 *Polymers*

Polymers are very convenient material for nanoparticle synthesis with excellent drug and protein loading/release properties and enhanced shelf life.[37] The essential characteristics of polymeric nanoparticles are biocompatibility, biodegradability, ease of surface modification through chemical transformations, high entrapment efficiency, excellent pharmacokinetic control and controlled drug release.[38] Polymeric nanoparticles are generally grouped into two, natural or synthetic polymers. Commonly used polymeric materials include poly-lactic acid, polyglycolic acid, polylactic glycolic acid, poly caprolactone, polyglutamic

Table 1: FDA approved nanomedicines for anti cancer therapy.

Trade name	Compound	Nanocarrier
Abraxane	Paclitaxel	Albumin bound paclitaxel
DaunoXome	daunorubicin	Pegylated Liposome
Doxil	doxorubicin	Pegylated Liposome
Bexxar	anti-CD20 conjugated to idodine131	Radioimmunoconjugate
Zevalin	anti CD 20 conjugated to yittrium-90	Radioimmunoconjugate
Zeladex	goserelin acetate	Polymer rods
Myoset	doxorubicin	Non-pegylated liposome
Oncasper	PEG-L-asparaginase	Polymer-protein conjugate
Ontak	IL-2 fused to diphtheria toxin	Immuno toxin fusion protein
SMANCS	Zinostatin	polymer protein conjugate

acid, polymalic acid and their copolymers.[39] Polyethylene glycol is another extensively studied polymer used for drug delivery application. It is hydrophilic in nature and hence used to coat the surface of other nanoparticles. PEG coating considerably improves the stability and bioavailability of nanoparticles.[40] Polymeric nanoconjugates are another effective carrier system for nanomedicine. They have several functional groups on the surface and are useful in conjugating with biochemically active groups to direct them to tumor tissue.[41] Since a single nanoconjugate can accommodate several targeting ligands, they can be efficiently targeted to kill cancer cells by inhibiting several signaling pathways (figure 5). Polymeric nanocarriers currently in the clinical trials include N-(2-hydroxypropyl) methacrylamide copolymer camptothecin [MAG-CPT][42] Paclitaxel poliglumex [Xyotax],[43] HPMA-DOX [PKI].[44] HPMA Dox revealed a 5 fold reduction in anthracycline related toxicity and enhanced antitumor activity. Xyotax was reported to be effective for patients with non-small-cell lung cancer in phase III clinical trials.

6.2 *Liposomes*

Liposomes are self-assembled, colloidal nanostructures of globular shape, composed of lipid bilayers, with the outer lipid bilayer surrounding a central aqueous space (figure 6). So far in literature liposomal formulations of therapeutic agents showed enhanced pharmacokinetic and pharmacodyanamics. Lipopsomal based formulations of anticancer agents such a *stealth* liposomal doxorubicin (Doxil), liposomal daunorubicin (DaunoXome) and liposomal doxorubicin (Myocet) have been approved for the treatment of metastatic breast cancer. Furthermore liposome conjugated with antibody specific to CD44 has showed improved therapeutic efficacy.[45] Cisplatin, an anti cancer agent with poor efficacy, demonstrated enhanced results when loaded in antibody conjugated liposomes.[46] Several studies also reported the influence of pegylated liposomes in treatment of metastatic stomach cancer. Small interfering RNA (siRNA) mediated therapy is potential for the treatment of pediatric bone and soft tissue sarcomas such as Ewing sarcoma, rhabdomyosarcoma and synovial sarcoma.[47] Moreover exciting applications to pediatric cancer therapeutics using liposome mediated siRNA involve neuroblastoma, chronic myeloid leukemia and hepato blastoma. In addition, liposome mediated siRNA delivery is successful for several adult malignancies such as melanoma, lung cancer, breast cancer and ovarian cancer etc.[48] Liposomal formulations that are available and currently in pediatric clinic trials includes Depocyte "Cytorabine", 1-Annamycin "Annamycin", Marquibo "Vincristine sulfate", ThermoDox "Doxorubicin", CPX-351 "Daunorubicin + Cytarabine".[47]

6.3 *Dendrimers*

Dendrimers are extensively studied nanocarriers, they are uniformly distributed complex molecules with branched architecture.[49] The structure consists of a core, branches and end groups. Dendrimers are able to carry hydrophobic as well as

Pharmacodyanamics: Study of biochemical and physiological changes occurs in a living organism due to the administration of drugs, hormone, toxins etc.

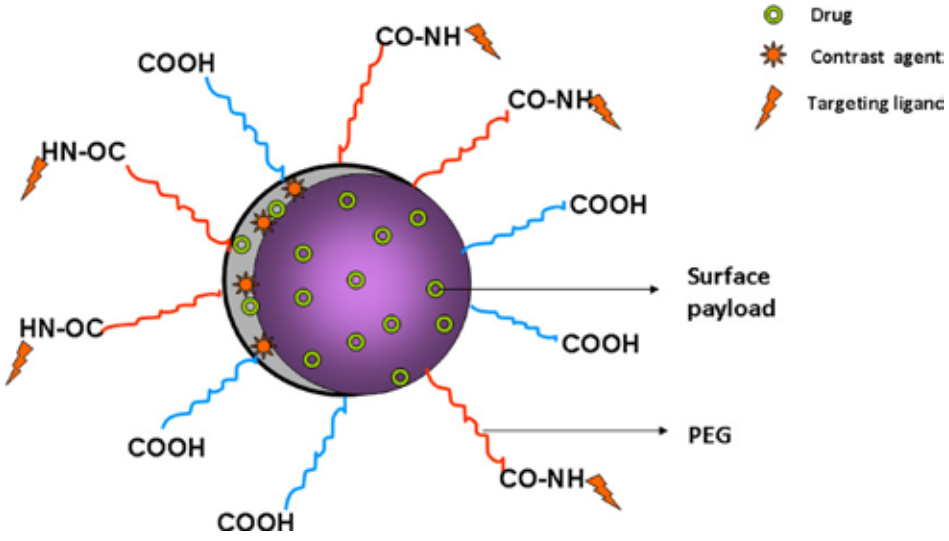

Figure 5: Schematic representation of a polymeric nanoparticle with multiple functional groups to carry drug, contrast agent and targeting ligand.

hydrophilic drugs due to presence of hydrophobic core and hydrophilic surface in them. Size of these molecules can vary from 1–10 nm depending on the addition of new generation of branches. The size, shape and pharmacokinetics of dendrimers depend on the generation number, chemical composition of core and branches as well as surface function group. Chemical modification also can significantly alter the pharmacokinetics and bio-distribution of dendrimers. Dendrimers have been used for various applications such as solubility enhancement, photodynamic therapy, drug deliver, bio imaging and cancer treatment.[50] Gillies and Frechet reported the use of dendirmers as delivery systems for cisplatin and doxorubicin.[51] Various researchers have reported their use in cancer therapy and as contrast agent for magnetic resonance imaging.[52] Gold nanoparticle conjugated dendrimers are also reported for targeting cancer cells with specific targeting molecules such as folic acid.[53]

6.4 *Micelle*

Micelles are small spherical, colloidal particles with size 1–100 nm, formed by self assembling amphiphilic diblock or triblock—copolymer. Size of micelle depends on the chemical nature of the drug and the micelle core. Micelles have core shell architecture which consists of two distinct regions: a hydrophillic head and a hydrophobic tail in aqueous media (figure 6). At low concentration in an aqueous medium, the components exist as separate monomers. However as concentration increases they agglomerate to form typical micelle. The concentration of monomers at which agglomeration takes place is called critical micelle concentration (CMC). The CMC of a micelle depends on the composition and type of block copolymer,[54] which are usually in the order of 10^{-6} to 10^{-7} M for polymers which is very low compared to low molecular weight surfactants. Moreover polymeric micelles remain stable at very low polymer concentration resulting in enhanced circulation time compared to surfactants.[55] Commonly used polymers for hydrophobic core formation are polyesters, polyethers and polyamino acids. Generally used molecules includes poly (propylene oxide) (PPO), poly (D, L-lactic acid) (PDLLA), poly (ε-caprolactone) (PCL), poly (L-aspartate) and poloxamers.

Bioavailability of majority of anticancer drugs after oral administration is low due to poor absorption and most of these drugs require specific formulation inorder to administer intravenously to the patients. The unique architecture of micelle is very useful for the delivery of hydrophobic poorly soluble drugs[57] as well as preventing the interaction of drug with serum proteins thereby making it appropriate for intravenous administration.[57] Compared to other drug delivery systems polymeric micelles have several advantages. Because of their smaller size micelles are used for the delivery of drugs via cutaneous lymphatics as well as by simple extravasations from blood vessels into the tumor tissue.[58] Five different polymeric micelles currently in clinical trials include NK012, NK105, SP1049C, NC-6004 and Genexol PM. NK012 is a block copolymer of PEG and polyglutamate conjugated with 7-ethyl-10-hydroxy-compothecin. *In vivo* studies carried out with NK012 in mice showed potent antitumor activity.[59] Moreover the clearance was significantly lower. A combination of NK012 with 5-fluoruracil showed a significant antitumor effect in colon cancer model.[60] NK105 micelle formulation consists of PEG and polyaspartate hydrophobic block conjugated with paclitaxel. Paclitaxel is physically incorporated in the hydrophobic block by means of hydrophobic interaction. Preclinical *in vivo* studies revealed an AUC (Area under curve) over 50 times higher and maximum plasma concentration in tumors was three times higher compared to paclitaxel alone. Moreover side effects associated with systemic paclitaxel administration due to the solubilising agents such as cremophore and ethanol is significantly reduced with NK105.[61] The adverse effect of cisplatin such as neurotoxicity and drug resistance is reduced with micellar formulation NC-6004 (Nanoplatin™). NC-6004 is composed of PEG and a poly (γ- benzyl L-glutamate)/CDDP complex. NC-6004 was used for colorectal carcinoma, upper gastrointestinal cancer, non-small-cell lung carcinoma and melanoma. In addition in combination with gemcitabine, it is administered for patients with advanced pancreatic cancer and metastatic pancreatic cancer. (DLT phase I/II study). Genexol PM is made up of PEG and poly (D, L-Lactic acid) conjugated with paclitaxel. This micellar formulation was found to be effective for patients suffering from metastatic breast cancer and advanced pancreatic cancer.[62] In combination with cisplatin Genexol PM also has significant antitumor activity. On going studies are showing favorable results for its application in recurrent breast cancer.[63]

Ligands specific to receptors on cell membrane of tumor cells can be conjugated to the micelle for enhanced internalisation.[64,65] Kabonove *et al* reported actively targeted micelle conjugated with ligand α_2 glycoprotein specific to brain.[66] Monoclonal antibody based targeting to receptors such as EGFR was reported by several studies.[67] Folate

is another important molecule used as a ligand for targeting caner cells.[68] Folate receptor is over expressed in cancers like ovarian, brain, breast, and lung.[69] Nasongkla *et al* studied active targeting of $\alpha_v\beta_3$ using cyclic pentapeptide ARG-Gly-Asp-d-Phen-Lys.[70]

6.5 *Virus*

Viruses are considered as natural nanoparticles and they have been extensively studied for their application in cancer treatment. Major advantages of viral nanoparticles over synthetic nanoparticles include its precise dimensions, possible evasion of immune system, biocompatibility and biodegradability.[71] The applicability of viral nanoparticles such as cowpea mosaic virus (CPMV) and bacteriophages such as Qβ, MS_2 etc for targeted delivery, imaging and their modes of cellular uptake have been studied by various groups.

6.6 *Carbon nanostructures*

Carbon nanotubes (CNTs) are ordered carbon graphite nanomaterials. Carbon nanotubes are classified as single walled (SWCNT) or multiwalled (MWCNT). Liu *et al* showed that appropriately functionalized CNTs administered intravenously, excreted via biliary pathway without causing any side effects.[72] Therapeutic agents can be conjugated with CNTs by covalent and non-covalent interactions. Xu *et al* has demonstrated the covalent attachment of paclitaxel to SWCNTs through PEG molecules caused higher *in vivo* efficiency in drug delivery for cancer treatment.[73] 10-hydroxy camptothecin is another anti tumor agent covalently linked to MWCNTs.[74] CNTs can also be used for the delivery f platinum (IV) to cancer cells. Conjugation on Doxorubicin on SWCNTs is an example for CNT functionalization by non-covalent interaction. CNTs are also used for killing cancer cells by thermal ablation in conjunction with radio frequency and laser therapy.

6.7 *Inorganic materials*

6.7.1 Gold nanopoarticles: Gold nanoparticles are the most efficient inorganic platform for drug delivery application. Synthesis techniques for gold nanoparticles having size scale of 1–150 nm with limited dispersity have been well established.[75] They are non-toxic biocompatible and inert. Furthermore using ligand place-exchange reactions multifunctional monolayers can be fabricated thereby leading to the development of multiple targeting agents.[76] Several studies have reported the use of gold nanoparticle for drug delivery application. Visaria *et al* described the delivery of TNF-α using PEG coated gold nanoparticle and thereby maximize the tumor damage with reduced systemic toxicity.[77] In addition a combinational

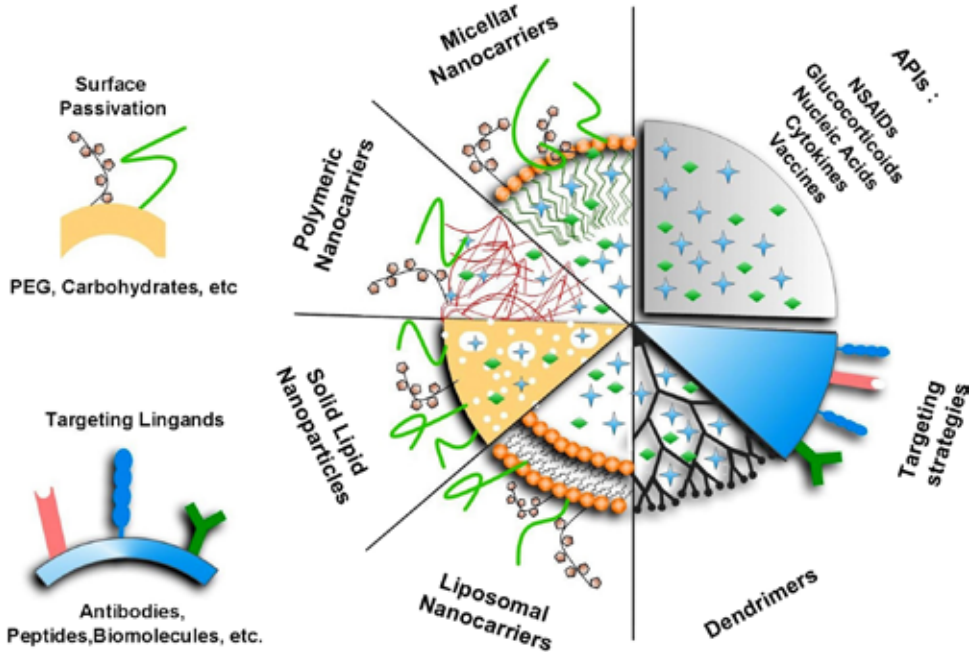

Figure 6: Different nanocarriers in nutshell. Various nanosystems (polymeric, micelles, liposomes, dendrimers, and solid lipid nanoparticles) that can form the nanoconstruct are depicted, along with the choice of active pharmaceutical ingredients, surface modifiers and targeting ligands.

therapy which uses heating as well as delivery of TNF-α showed enhanced therapeutic effect that gold nanoparticle alone. Phase 1 clinical trial of gold nanoconjugate called CYT-6091 is going on to study the pharmacokinetics and pharmacodyanamics of this formulation.[77] Another nanomedicine MTX-Gold nanoparticle conjugate, where gold particles are conjugated with methotrexate, an inhibitor of dihydrofolate reductase, is reported to have enhanced anti tumor effect *in vitro* and *in vivo*.[78] Controlled release of doxorubicin was achieved by a tumor mRNA dependent nanogold drug carrier in breast cancer cells.[79] Development of photothermally modulated drug delivery system using nanoshell is also established. Wherein, irradiation of nanoshells with 1024 nm electromagnetic waves leads to enhanced drug delivery.[80] Physical dimension of gold nanoparticle has crucial role in intracellular uptake.[81] Targeted drug delivery using gold nanoparticles *in vivo* has not yet been established because of its large size after conjugating with various ligands for targeting.[82] Recently, Khan *et al* made an attempt to target pancreatic cancer cells *in vivo* and *in vivo* by conjugating with EGFR antibody specific to EGFR receptors expressed on tumor cells.[83] Gene silencing of HDFG by siRNA in ovarian cancer cells by modulation of protein corona around surface functionalized gold nanoparticles have been reported by Arvizo *et al*.[84] PEG modified lipid gold porphyrin is another approach leads to the efficient cellular uptake and tumor destruction.[85]

6.7.2 Calcium phosphate: The potential of calcium phosphate nanoparticles (nCP) is being widely investigated for the delivery of nucleic acids and drugs. One of the main advantages of using calcium phosphate based delivery system is its high biocompatibility, which is due to the fact that it forms the mineral component of human bone and teeth and also due to the presence of calcium (Ca^{2+}) and phosphate ions (PO_4^{3-}) in millimolar concentration within the blood stream. Calcium phosphate is an excellent delivery vehicle for nucleic acids due to the high interaction between positively charged $Ca^{2+\text{ in}}$ nCP and the negatively charged phosphate group in the nucleotides. But the poor stability of the calcium phosphate suspension and the large size of the particles were obstacles for successful intracellular delivery applications. Capping of nCP with various polymers and adjustment of synthesis parameters such as pH of the reaction medium has helped to improve colloidal stability and reduce the particle size. Recently a number of studies have reported the development of nCP based nucleic acid delivery systems for DNA[86] as well as siRNA molecules.[87] The development of multi-shell nanoparticles consisting of alternate layers of calcium phosphate and nucleic acid helped to improve the stability of the nucleotides as well as the transfection efficiency (schematic).[88] In addition to nucleic acid delivery calcium phosphate nanoparticles were also tested for the *in vivo* delivery of drugs such as ceramide,[89] cisplatin,[90] docetaxel,[91] and methotrexate.[92] The major advantage of using calcium phosphate based drug delivery agent is the specific solubility of the material in acidic pH at ~4 which enable the release of the incorporated drug within lysosomes of cells or within tumor tissue which has a relatively more acidic microenvironment. The possibility of combined imaging and therapy of cancer cells using nCP was also demonstrated by Morgan *et al*.[89] The cytotoxic drug, ceramide, was incorporated into calcium phosphate nanoparticles along with fluorescent dyes such as fluorescein. The study proved that the Ceramide-fluorescein incorporated nCP was almost 25 fold more effective towards human vascular smooth muscle cells than the free ceramide drug dissolved in DMSO.[89] Preclinical evaluation of nCP based drug delivery systems need to be validated *in vivo*.

6.7.3 Silica nanoparticles: Another inorganic material, which is being extensively studied for drug delivery application, is silica nanoparticles. Recent advancements in better control of particle size, morphology and porosity of silica particles have made the material more attractive for drug delivery applications. The large surface area and porous interior structure of mesoporous silica nanoparticles (MSN) helps in the effective encapsulation of drugs and prevent degradation before reaching the target site. The size and shape of MSN can also be tuned to increase the particle uptake by cells. Hydrophobicity of certain chemodrugs limits their clinical applications which may be overcome by the effective incorporation of the drugs within MSN which was demonstrated by Lu *et al* using the chemodrug camptothecin.[93] There have also been reports on the effective encapsulation and controlled delivery of drugs such as ibuprofen,[94] erythromycin,[95] and alendronate[96] from MSN. The developed drug loaded MSN showed stability for over 2 months when stored at 4°C without loss of its cytotoxic property.[97] The safe delivery of MSN up to a concentration of 40 mg/kg has been demonstrated *in vivo*, but the material is lethal at a concentration of 1.2 g/kg.[98] More studies are required to study the treatment efficiency of MSN based drug delivery systems under *in vivo* conditions.

6.7.4 ZnO nanoparticle: Zinc oxide (ZnO) is an important II–VI group semiconductor material with direct band-gap of 3.37 eV and large exciton binding energy of ~60 meV, making it a promising candidate for many technology applications. Since a decade, ZnO have been extensively studied as a potential antimicrobial agent.[99,100] Owing to their fascinating physicochemical properties, recently, ZnO NPs have attracted the scientific community for their applications in cancer nanomedicine. Recently, Shen *et al*[101] have reported the low level cancer biomarker detection capability of polymethyl methacrylate capped ZnO nanoparticles using mass spectrometry. In another study by Dorfman *et al*[102] the high-throughput detection of ultra-low levels of telomerase activity using zinc oxide nanorods for cancer diagnosis and screening was explored. More recently tetrapod-like ZnO nanostructures have been synthesized for gene delivery applications.[103] This type of structures surface modified with silica and amine groups, are able to bind plasmid DNA and has been shown to enhance the transfection efficiency in A375 cells.[103] In addition, polycation capped luminescent ZnO quantum dots showed excellent DNA transfer capability and have been further explored for real time imaging of gene transfer.[104]

Recently, a number of reports have cited regarding the interesting anti-cancer property of ZnO NCs. Several studies have demonstrated the preferential *in vivo* toxicity exerted by ZnO nanoparticles, towards different origins of cancer cells such as glioma, breast, bone, colon, leukemias and lymphomas.[105] These studies showed that cancer cells were 30–40 times more susceptible to ZnO NPs induced toxicity compared with their corresponding normal healthy counter parts. ZnO NPs conjugated to a porphyrin photo drug, tested in *in vivo* ovarian cancer models, showed higher activity of the system under UV irradiation where as negligible cell death was observed in dark conditions.[104] Guo *et al*[106] have proposed to augment cancer therapy by synergistic administration of ZnO NPs with chemodrug, daunorubicin, which also showed enhanced cancer cell killing under UV irradiation. Yuan *et al*[107] have attempted to develop a new generation of cancer therapeutics using chitosan to encapsulate blue emitting luminescent ZnO QDs loaded with anticancer drugs for tumor targeted drug delivery. Lately, Abhilash *et al*[108] have done an extensive study to elucidate the preferential toxicity mechanism of ZnO NCs towards cancer cells. Authors have reported that irrespective of the size-scale and surface chemistry differences, ZnO NCs exhibited multiple stress mechanisms against cancer cells. The mechanism behind the preferential toxicity was found to be the acidic cancer microenvironment mediated rapid dissolution of ZnO NCs and associated oxidative stress mediated toxicity. These studies showed the promising potential of ZnO NPs in targeted gene delivery gene silencing and selective destruction of cancer cells for future cancer nanomedicine applications.

6.7.5 TiO_2 nanoparticles: TiO_2 nanoparticles are also emerged as a drug carrier system for cancer treatment apart from its efficient photocatalytic activity. Their application in cancer therapy through drug delivery is studied by various researchers. Li *et al* incorporated anti cancer drug daunorubicin on titanium dioxide nano whiskers for drug delivery application. In this study they found that titanium dioxide particle efficiently enhance the concentration of daunorubicin drug in human hepato carcinoma cells indicating its efficiency as anti cancer nanocarrier system. Further more, daunorubicin incorporated TiO_2 nanoparticle have great potential in inhibition of multi drug resistance in leukemia cells.[109] Here Song *et al* observed that nano-TiO_2 enhanced the drug accumulation in target cancer cells and cause considerable membrane damage to the leukemia cells.[109] Recently, Chen *et al* demonstrated that the side effect of anticancer drug doxorubicin can significantly reduced by incorporating on nano TiO_2. Furthermore the anti cancer efficacy of doxorubicin is enhanced due to down regulation of Bcl-2 as well as up regulation of Bax and caspase 3.[110]

7 Second Generation Nanomedicine

Second generation nanomedicine primarily focused on the development of new nanocarriers, which specifically targeted to cancer cells, without affecting the function normal cells. Properly designed nanoparticles can specifically target to tumor tissue and can accumulate in tumor. Targeting can be either passive or active without being eliminated by the body. Targeting the major mechanism of drug resistance is found to be an effective way in cancer therapy. In drug resistant cancer cells, there is an activation in anti apoptotic cellular defense such as over-expression of BCL2, an anti apoptotic regulator and nuclear factor kappa B (NF-kB), a master transcription factor etc which controls the expression of various genes involved in suppression of apoptotic response. In combination with a chemical sensor the drug efflux can be prevented and thereby increase the in intracellular drug concentration, which further leads to enhanced cytotoxic effect of the therapeutic agent. Shapira *et al* described

Transcription factors: Transcription factors are protein that binds and control the transcription of genetic information from DNA into mRNA.

about a new strategy named quadrugnostic nanomedicine, which would synergize the benefits of current nanomedicines.[111]

New generation nanomedicine comprises of nanocarrier loaded with one or more cancer therapeutics, chemosensitizer, imaging components and an active targeting element such as folate receptor.[112] Pinhassi *et al* demonstrated the selective targeting and cytotoxicity of a FA (Folic acid) and methotrexate conjugate arabinogalactan to FR (Folate Receptor) over expressing cells.[113] Albumin-based nanoparticle carriers have been extensively studied by various group.[114–116] Pegylated liposome encapsulated Doxorubicin, carbon nanotubes, micellar NPs etc are also extensively studied nanoformulations for targeted delivery. A combination of cancer diagnostic aid and therapeutic agent loaded onto same NP system called theragnostic NPs are also studied.[117] Different strategies for targeted cancer therapy are discussed in detail in the following section.

7.1 *Targeting aberrant cancer kinome*

Kinome, the full complement of protein kinases in a genome,[118] drives signal transduction in eukaryotic cells to orchestrate virtually all cellular processes, including metabolism, transcription, cell cycle progression, cytoskeletal rearrangement, cell movement, apoptosis, and differentiation. However, deregulation of kinase activity has emerged as a major mechanism by which cancer cells evade normal physiological constraints on growth and survival.[119] This scenario have propelled kinases to the upfront as a new class of drug targets and led to the development of small molecule kinase inhibitors (SMI) for the treatment of cancer. SMI modulates kinase activity by competing with the ATP binding site of the catalytic domain of oncogenic tyrosine kinases.[120] The approval of the first kinase inhibitor, imatinib, for the treatment of chronic myelogenous leukemia (CML) and the success of antibody-based drugs that targeted the epidermal growth factor receptor (erbB2) in breast cancer (trastuzumab) and colon cancer (cetuximab) heralded the current period of intensive kinase drug development efforts. They have come up as well tolerated anti-cancer agents with high molecular specificity and a favorable safety profile. Conventional cytotoxic chemotherapy although directed toward certain macromolecules or enzymes, typically does not discriminate effectively between rapidly dividing normal cells and tumor cells leading to severe toxic side effects and partial, brief, or unpredictable tumor response. In contrast, targeted therapies interfere with molecular targets that have a role in tumor growth or progression and thus have a high specificity toward tumor cells, providing a broader therapeutic window with less toxicity.[119,120] Moreover they can work in combination with cytotoxic chemotherapy or radiation to produce additive or synergistic anticancer activity because their toxicity profiles often do not overlap with traditional cytotoxic chemotherapy. Thus, targeted therapies represent a new and promising approach to cancer therapy, one that is already leading to beneficial clinical effects. The small molecule kinase inhibitors which have been approved for the treatment of cancer includes imatinib, dasatinib, nilotinib, gefitinib, lapatinib, sunitinib, sorafenib and everolimus to name a few which have been developed to specifically target the kinases, c-Abl (and Bcr-Abl), PDGFR, cKit, EGFR, VEGFR2 and mTOR.[121]

Tyrosine kinase inhibitors for the treatment of cancer and its associated manifestations appears really promising, however the bioavailability of most of the small molecule inhibitors in the physiological environment is extremely poor owing to their hydrophobic nature.[122] This limits its dissolution potential in biological fluids and hence the active molecules fail to reach the target organs in the required concentration. Inorder to evoke a desired response in the target cells, high concentration of drug may be required which increases the risk of side effects. Furthermore, to improve aqueous solubility of drugs, currently approved formulations use toxic excipients such as dehydrated ethanol, DMSO, Tween 80, and castor oil (Cremophor EL) which cause additive toxicity.[123] Moreover, the genetic complexity of most human malignancies reveals derailing of multiple pathways, requiring their simultaneous inhibition to produce sustained effect. Accordingly, therapeutic interference of multiple inhibitors needs to be called into synergism to tackle this situation.[124] With the emergence of nanotechnology, improvised drug delivery platforms are developed which can carry multiple drugs of various chemical nature and actively target discrete cells, pass through biological barriers and deliver the payloads at the target site without potent drug-drug interferences, which might affect the pharmacology of drugs or in worst cases bring in adverse health effects. Nanoformulations of these hydrophobic kinase inhibitors can greatly improve the patient compliance by increasing the aqueous dissolution and hence the bioavailability without the use of any toxic excipients. Nanoparticles fabricated from the biodegradable and biocompatible polymers, polysaccharides, proteins etc are being intensively investigated for drug delivery applications. Natalie *et al*[125] have discussed in her review a

comprehensive list of recent US and World patents on developing and modifying nanoparticles for the detection, analysis, and treatment of cancer.

Preclinical *in vivo* studies of nab-rapamycin™ (ABI-009) is a credible example demonstrating enhanced antitumor activity compared to free rapamycin, suggesting potential clinical utility. Moreover nab-rapamycin™ was well tolerated (no observed hypercholesterolemia and hypertriglyceridemia) overcoming the limitations posed by the poor aqueous solubility of rapamycin. Specifically the binding of hydrophobic drugs to albumin nanoparticle permits albumin-mediated transcytosis of the drug by microvessel endothelial cells, and the SPARC-albumin interaction may further increase accumulation of albumin-bound drug in the tumor.[126] Therefore, suitably designed nanoconstructs loaded with tyrosine kinase inhibitors can deliver the drug in a targeted fashion to the cells thereby enhancing the therapeutic outcome.

7.2 *Nanoparticle mediated gene silencing*

RNA interference (RNAi) is a potent gene-silencing phenomenon induced by double-stranded RNAs.[127] The phenomenon was first described by Andrew Fire and Craig Melo in 1998 during their studies in *C.elegans*.[128] Later Tuschl and his coworkers demonstrated that short stretches of double stranded RNA ~22 nucleotides in length can achieve sequence specific gene suppression in mammalian cell lines.[129] The high efficiency as well as its remarkable specificity combined with the ease of application makes RNAi an attractive novel therapeutic approach as well as a major reverse genetic tool. Although other gene silencing approaches using ribozymes, DNAzymes and antisense oligonucleotides have evolved over the past few years, RNAi being endogenous machinery conserved in almost all eukaryotes generated considerable interest among the researchers[130] (Rutz *et al*, 2004). The endogenous machinery of RNAi is induced by micro RNAs (miRNAs) which is a mechanism that organisms use to silence genes when their protein products are no longer needed.[131] The cells utilize RNAi as a primitive antiviral response and as a mechanism to limit potentially deleterious retro-transposon movements. miRNAs have significant roles in the cell growth and division, development of tissue and organs and regulation of gene-expression[132] (Plasterk *et al*, 2001).

RNAi is triggered by the presence of long double stranded RNA stretches, which is cleaved in to short stretches of 21–23nt fragments known as short interfering RNA (siRNA) by the enzymatic activity of Dicer. It is an endogenous mechanism of gene regulation carried out by a multi-protein complex residing in the cytoplasm, known as RNA induced silencing complex (RISC). Short stretches of double stranded RNAs are then incorporated in to RISC. Argonaute 2 (Ago-2), a multifunctional protein in the RISC unwinds the double-stranded siRNA after which the sense strand/passenger strand is cleaved. The antisense/guide strand is directed to form an RNA duplex with its complementary mRNA transcript, which is further degraded by the RNase activity of activated RISC. The cleavage of the strand happens at a position between 10 and 11 on the anti-sense strand at 5' end. The potency of this silencing effect stays for 3–7 days in rapidly dividing cells and for several weeks in non-dividing cells[133] (Zamore *et al*, 2000, Myers *et al*, 2003, Martinez *et al*, 2002). The advantages of siRNAs over small molecule drugs are that, sequences can be rapidly designed for highly specific inhibition of the target of interest, even picomolar concentrations of siRNA can induce silencing and synthesis does not require a cellular expression system, complex protein purification, or refolding schemes.[134] Hence RNAi holds promise as a therapeutic tool to reduce the expression of disease causing genes through the sequence specific degradation of mRNA at the post-transcriptional level before translation in to oncoproteins. Yet, the success of RNAi depends mainly on the successful delivery of siRNAs in to cells overcoming all the barriers offered by the cells. The major limitation is the highly anionic charge offered by the phosphate backbone of siRNA and consequent electrostatic repulsion from the anionic cell membrane surface, which prevents its passive diffusion in to cytoplasm and also the rapid enzymatic degradation by serum nucleases.[135] Moreover, the presence of double stranded RNAs may switch on the immune response machinery. Hence, siRNAs can be delivered to the cells only by carrier mediated delivery. The carrier mediated delivery can be either viral mediated or non-viral delivery of siRNAs using polymers, lipids, proteins, peptides or electroporation. Because of the invasiveness of the electricity of electroporation and cytotoxicity of some lipid reagents, non-viral transfection methods have become less efficient for *in vivo* delivery. Moreover conventional non-viral siRNA delivery systems are limited by packaging efficiency and colloidal stability and non-specificity.[136] In the case of viral delivery systems there is possibility of immune and toxic reactions in addition to the potential for viral recombination[137] (Li *et al*, 2006). In this scenario, nanoparticles

hold great promise for the intracellular delivery of siRNAs.[136,137] Nanoparticle based delivery systems have already shown great promise in *in vivo* as well as *in vivo* systems.[138,139] Recently, phase I clinical trial had been conducted using siRNAs targeted against RRM2 gene overexpressed in melanoma. The cyclodextrin nanoparticles were modified using transferrin targeting ligand, to engage the transferrin receptors over-expressed in melanoma cells. The study holds great promise in terms of targeted delivery of siRNAs using nanoparticles.[140]

7.3 *Targeting tumor angiogenesis*

Angiogenesis is the formation of new blood capillaries from existing blood vessel, which is important for normal embryonic vascular development as well as various physiological processes in adults. Tumor cannot grow more than 2 mm diameter without proper angiogenesis. Unlike normal angiogenesis tumor angiogenesis resulted in leaky and convoluted blood vessels which are often inflammated. Such a deregulated angiogenesis in tumor is mainly associated with over expression of some growth factors and cytokines such as vascular endothelial cell growth factor (VEGF), angiopoetin etc as well as $\alpha_v\beta_3$ integrin. Anti-angiogenic therapy is basically blocking or disrupting newly formed blood vessels with the help of therapeutic agents. Examples for such molecules include TNP 470, endostatin, angiostatin, combretastatin etc. First anti angiogenic therapy uses avastin monoclonal antibody that specifically target VEGF over-expressed colorectal cancer cells.[141] Though these anti-angiogenic therapies are superior to conventional chemotherapy, limitations pertaining to conventional therapy are still associated with this.

Nanomedicines are emerging as new paradigm to overcome the limitations of anti-angiogenesis research. The leaky vasculature as well as increased permeability of blood vessels associated with tumor tissue allowing nanoparticles with less than 200 nm to extravasate tumor tissue due to enhanced permeability and retention effect (EPR). Various studies reported the accumulation nanoparticle conjugated chemotherapeutic agents and anti-angiogenic molecules due to EPR effect.[142] However it is important to understand that EPR alone is not sufficient for targeting tumor tissue. Hence a new approach in which active targeting of tumor site using ligands that bind to over expressed receptors on tumor cells along with anti-angiogenic agents is established for effective killing of tumor. As already mentioned the key tags include growth factors, cytokines as well as integrin molecules. Nano carriers reported so far includes polymeric conjugates and nanoparticles, lipids such as liposomes and micelle, synthetic organic nanoparticles such as dendrimers, carbon nanotubes fullerenes, inorganic nanoparticles etc (Table 2).

Table 2: Various nanovectors and nanoconjugate with anti-angiogenic factors.

No	Material	Therapeutic agent/target	
1	Polymers	N-(2-hydroxypropyl) methacrylate (HPMA)	TNP 470 Caplostatin[148] Fosomax + Paclitaxel + TNP 470[149] Radiolabelled peptide to $\alpha_v\beta_3$[150]
		PLGA	PD98059 (MAPK inhibitor)[112] PI3K inhibitor[151] Doxorubicin + combretastatin[152]
		Chitosan	Chitosan nanoparticle[153]
		Dendrimers	$VEGF_{121}$[154]
2	Lipids		
	Micelle	Monomethoxy-polyethyleneglycol-polylactic acid copolymer Poly (ε-caprolactone) poly (ethylene glycol) (PCL-PEG)	TNP 470[155] Doxorubicin[70] $\alpha_v\beta_3$
	Liposome		APRPG peptide targeted to tum or vasculature[156] Membrane type Matrix Metalloproteinases[157] $EGFR_2$[158]
3	Carbon	MWCNT Fullerenes Graphites	VEGF and bFGF targeted[159]
4	Inorganic	Gold Silver Dextran coted Iron oxide	VEGF[160]

siRNA, small interfering RNA mediated silencing of angiogenic genes are new generation research in the field of nanomedicine. The specificity of siRNA as well as short hairpin RNA (sh RNA) in binding target proteins allows generation of new therapeutic modality. Nanoparticle conjugated with RGD sequences have been used to deliver siRNA specifically targeted VEGF receptor and inhibit tumor angiogenesis.[143] In addition lipid based[144] as well as chitosan nanoparticle loaded siRNA[145] have been studied extensively expression of VEGF in endothelial cells.

Aptamers are new nanotherapeutics where a 3D synthetic RNA or DNA oligonucleotide or peptides were used to target tumor tissue. Aptamers are ideally suited for diagnostics, imaging and targeting of angiogenic based diseases by conjugating with contrasting agent as well as therapeutic agents. VEGF targeting RNA aptamer in conjunction with polymeric as well as liposomal nanocarriers have been reported as potent inhibitor of angiogenesis *in vivo* and *in vivo*.[146]

Embryonic stem cells in combination with nanoparticles [iron oxide super paramagnetic nanoparticle have been used for tumor imaging particularly in the case of glioma. This finding got enormous implication because of the inability of other agents to pass through the blood brain barrier. Yang *et al* reported regarding pro-angiogenic stem cell therapeutics in which cells were transfected with VEGF DNA using poly (β-amino esters) nanoparticles.[147]

7.4 *Targeting metastatic cancer*

Metastasis, the spread of cancer cells from a primary tumor site to a distantly placed secondary site (figure 7). Metastasis is one of the greatest challenges in cancer treatment. Surgical intervention can cure few patients with metastatic cancer compared to other treatment modalities. Approximately 25% of cancer metastasis can be predicted by understanding the normal blood flow pattern. Table 3 represents the relative incidence of metastatic spread to different organs for specified cancer type. In the case of colorectal cancer since the blood flow is from gastrointestinal tract to liver through hepatic portal vein, most of the colorectal cancer and pancreatic tumor metastates to liver.

Conventional treatment using chemotherapy is inadequate to treat metastatic cancer, primarily because of the inefficiency of these drugs to reach various metastatic sites. Furthermore, the treatment modality for large well vascularised tumor is not appropriate for circulated malignant cells. Advancement in nanotechnology especially in targeting, detection and particle trafficking facilitate novel approaches for such cancer treatments. It is appropriate to select the size, surface charge and surface chemistry of the nanoparticle for the treatment of metastatic cancer. In the case of liver endothelium or in the tumor microenvironment only small sized particle will be able to exit. Hence the surface characteristic will ultimately define the fate of the nanoparticle mediated targeting as well as therapy.

Nanoparticle mediated targeting of metastasis can be either primary targeting or secondary targeting. Primary targeting refers to targeting diagnostic and therapeutic agents to the organ in which metastases located. It was reported that brain, lungs, liver, lymph and bone are most challenging organs to target nanomedicine.[161]

7.5 *Brain targeting*

In the case of brain, the blood brain barrier allows only certain materials to pass through the circulation in to CSF. Even though the integrity of blood brain barrier disrupts during tumor development,

Table 3: Metastatic spread to different organs for a specific cancer.

	Metastasis (%)							
Primary tumor	Lungs	Liver	Bone	Brain	Lymph nodes	Abdominal cavity	Adrenal gland	Pleura
Breast cancer	12	14	22	-	30	-	-	-
Lung cancer	12	12	19	12	29	-	-	-
Liver cancer	25	34	9	-	12	6	-	-
Pancreatic cancer	8	58	-	-	4	10	-	-
Kidnery crancer	33	9	17	-	7	-	6	-
Ovarian cancer	-	9	-	-	6	63	-	10
Colon cancer	10	39	-	-	26	14	-	-
Prostrate cancer	4	3	65	-	14		7	-

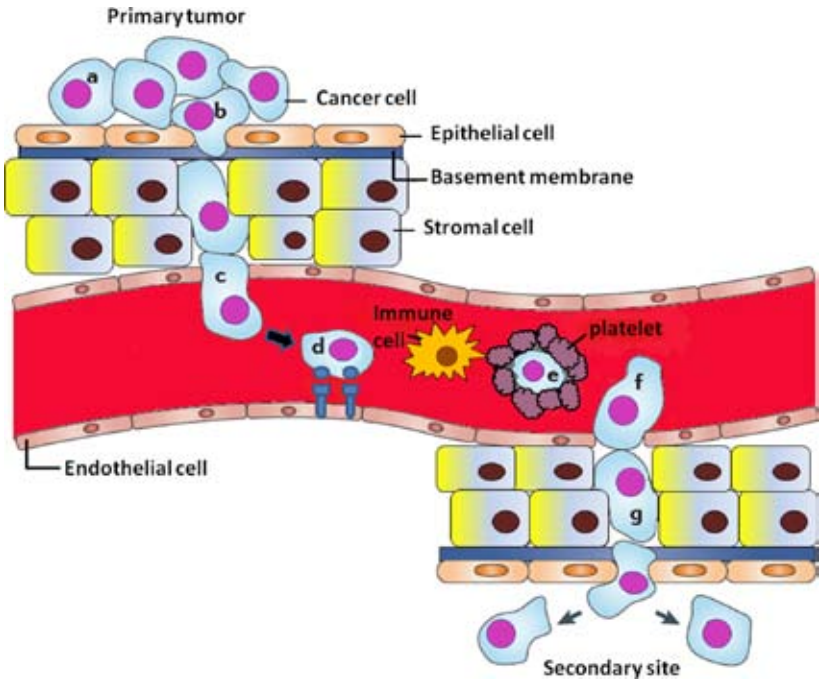

Figure 7: Steps of metastasis. The metastatic cells (a) reduce adhesion to neighboring cells and (b) migrate into the vasculature. In the vasculature, cells can move freely through the blood. If the vasculature is continuous the cells (c) intravasate by endothelial cell retraction or endothelial cell death. Cancer cells can trapped in the narrow capillaries (d) can express receptors that bind to metastasis supporting sites or to platelets (e) platelets protect the cancer cells from immune system. At the secondary site, cell can cause endothelial retraction or death. And at the secondary site the cells can proliferate with the help of growth factors released by the neighboring cells.

it has been reported that nanoparticle with size less than 15 nm can enter into the brain. The uptake efficiency of nanoparticle found to be decreases exponentially with size.[162] Recent finding suggest that conjugating nanoparticles with lipophilic moieties such as Apolipoprotein E can successfully mediate blood brain barrier passage as well as liver hepatocytes trafficking.

7.6 *Lymphatic system*

Lymphatic vessels are another means for metastatic spread and in such cases the patient survival rate is poor. Hence several researchers focused on targeting disseminated cancer cells in the lymphatic system. Recent studies reported the accumulation of dextran coated iron oxide nanoparticle in the lymph nodes.[163,164] The importance of particle size in lymph nodes targeting were also studied by several researchers. They found that particles with less than 80 nm administered subcutaneously results in trafficking to lymph nodes.

7.7 *Liver*

Liver is another common site of metastases due to its well vascularised nature. Specific particle engineering is necessary to target liver tissue. In many cases, administered particles accumulate in liver endothelium fenestrae as well as kupffer cells and will not reach hepatocytes.[165] Conjugating particles with apolipoprotein E as well as N-acetylgalactosamine were reported for efficient targeting of therapeutic drugs to liver.[166] Lipid nanoparticles as siRNA carriers for liver metastases is currently in clinical trial.[166]

Targeting specific cell type in a target organ is another challenge in treatment of metastatic cancer. Secondary targeting mainly focus on this aspect. Conventional approaches include use of magnetic field to accumulate iron oxide nanoparticle in cancer cells or use acoustic waves to trigger micro bubble localization.[167] Metastatic cancer cells always express certain cell surface markers as well as secreted factors which usually over expressed during embryonic development. In addition metastatic cells also express surface markers from its site of origin. This understanding favors active targeting of metastatic cells using nanotechnology. Conjugating antibodies specific to these surface markers as well as peptide for integrin binding domain were reported for enhanced targeting of specific cell types within a tissue.[168,169] Nanoparticles coated with folic acid demonstrated its affinity to folate receptor over-expressed in oral carcinoma, metastatic breast, colorectal cancer as

well as other cancers.[170] Although nanomedicine achieved greater advancement in cancer treatment, extensive investigations are required to develop better methods for targeting nanoparticle mediated therapeutic agents to metastatic site, particularly towards tumor environment, bone and brain.

8 Nanoparticles for Hyperthermia Therapy

While nanoparticles for better chemotherapeutic application progress over the years; another application of nanoparticles in cancer therapy has also simultaneously grown. This is the use of nanoparticles in hyperthermia therapy of tumors. Hyperthermia, in the clinical setting today refers to localized heating of tumours to a temperature of 41–43°C or whole-body heating to 40–42°C.[171] The term is often used synonymously with thermo ablation; but technically the latter refers to application of temperatures in excess of 50°C. Many techniques have been developed to deliver thermal energy to tumors; radiofrequency, microwaves, electrocautery, magneto/photo thermal and focussed ultrasound. A validated account of tumor regression following whole-body hyperthermia can be seen way back in 1866 when W. Busch reported the regression of a neck sarcoma after an erysipelas-induced fever.[172] The local control of tumor has an important impact on the quality of life and survival of patients with cancer, and hyperthermia is now recognised as a technique to achieve this.

Erysipelas: A streptococcal (bacterial) skin infection characterized by very high degrees of fever. Not common now due to availability of better antibiotics.

The exact target for heat-induced cell death is debateable. Unlike radiotherapy where the cells die during their attempt to divide after irradiation, cell death in hyperthermia occurs faster and at all stages of the cell cycle. The potential mechanisms proposed are denaturation of membrane proteins, repair enzymes and even chromosomal proteins—apoptotic death.[171] At temperatures between 50–60°C the tissues show **coagulative necrosis**.[173] Histologically the tissue after application of hyperthermia will show oedema and cellular infiltration, similar to a regular thermal burn.

Oedema: Swelling of a region due to increase in the extracellular fluid volume caused usually by an inflammatory process.

The rationale for using heat in treating cancer is based primarily on the difference in the blood supply to tumors when compared to normal tissues. Factors justifying use of hyperthermia in tumors are:

a) Deficiencies in tumor vasculature resulting in:
   i) Poorer cooling capacity and hence become hotter than the surrounding normal tissue
   ii) Hypoxia, doesn't affect response to hyperthermia (in contrast to conventional radiotherapy)
   iii) Acidic environment and/or deficient nutrition increases susceptibility to heat
b) Cells in the DNA synthesis phase which are particularly resistant to radiation are sensitive to heat.
c) Combining heat with chemotherapeutic agents has shown an increase in therapeutic effect.
d) Recent work has suggested that neoplastic cells may be intrinsically more sensitive to heat than normal cells.

Hypoxia: Decreased oxygenation of a regiondue to negative mismatch between the tissue oxygen demand and supply.

The hyperthermia and thermoablation methods currently practised are either invasive or else have to sufficiently penetrate normal tissue to reach the tumor. As these techniques cannot discriminate tumors from the surrounding normal tissue the heating and destruction of intervening normal tissue between the source and the tumor is a common side-effect. Moreover these heating methods are difficult to monitor and control. It was while addressing this dilemma, that researchers realised the potential of the unique properties of nanoparticles as a promising solution.

With the advancements in nanotechnology, new nanoparticle based probes are being developed for localized/targeted hyperthermia. Nanoparticles (NPs) exhibit unique energy absorption properties and their biodistribution can be controlled by simple manipulation of their structure, thus producing the concept of "silver-bullets" that specifically kill only diseased cells. NPs also exploit the tumor's rapidly formed vasculature, and enhanced permeability to aid in the targeting—EPR effect. As biomaterials, NPs are composed of elements generally understood to be biocompatible. To further improve biocompatibility, 'stealthing' polymers like polyethylene glycol (PEG) can be grafted on their surface.

Here we explore three techniques of hyperthermia which employ nanoparticles—magnetic hyperthermia with superparamagnetic iron oxide, radiofrequency induced hyperthermia with gold and carbon based nano structures and photothermal therapy using gold nanoshells. The basic principle, advantages, current progress and associated limitations in each method will be discussed.

8.1 *Magnetic hyperthermia*

Magnetic hyperthermia or magnetic fluid hyperthermia (MFH) refers to a hyperthermic treatment technique based on the observation that magnetic nanoparticles (MNPs) when subjected to an alternating (AC) magnetic field exhibit significant heating. Incorporation of the MNPs within a tumour and exposing the region/whole patient to an AC magnetic field of appropriate

frequency, heats the malignant cells which are either destroyed or made susceptible to other treatments like chemotherapy and radiation. Apart from achieving localization of heat, MNPs offer the possibility of self-limitation of temperature increase by using a magnetic material with suitable Curie temperature.[174]

The power dissipated by a magnetic material subjected to an alternating magnetic field is referred to as 'Specific Loss Power' (SLP). It is measured in watts per gram (W/g) and must be high enough to be useful therapeutically. The following groups of nanoparticles for magnetic hyperthermia have been described:

i. Superparamagnetic iron oxide nano particles (SPIONs); magnetite (Fe_3O_4) nanoparticles stabilized by a variety of ligands such as dextran, cationic liposomes, polyvinyl alcohol, hydrogel, lauric acid and maghemite (γ-Fe_2O_3) nanoparticles stabilized by ligands such as dextran.
ii. Ferrites such as cobalt ferrites ($CoFe_2O_4$), manganese ferrite ($MnFe_2O_4$), nickel ferrite ($NiFe_2O_4$), lithium ferrite ($Li_{0.5}Fe_{2.5}O_4$), mixed ferrites of nickel-zinc-copper ($Ni_{0.65}Zn_{0.35}Cu_{0.1}Fe_{1.9}O_4$) and cobalt-nickel ferrite ($Co_xNi_{(1-x)}Fe_2O_4$).
iii. Ferromagnetic nanoparticles such as Fe doped Au nanoparticles, Zn-Mn doped iron oxides ($Zn_xMn_{(1-x)}Fe_3O_4$) and Mn-Zn-Gd doped iron oxides ($Mn_xZn_xGd_xFe_{(2-x)}O_4$) composites. And
iv. Metallic nanoparticles of iron, cobalt or alloys of the two, have recently gained attention due to their high heating performance.

8.1a Magnetic loss processes: The heating in magnetic hyperthermia arise due primarily to three processes involving magnetisation reversal of the particle system: (i) hysteresis losses, (ii) relaxation losses, and (iii) frictional losses in viscous suspensions. Though magnetic losses by generation of eddy currents has been mentioned by some workers, their contribution has been considered negligibly small.[175]

Hysteresis losses—the energy dissipated as heat per cycle of magnetisation reversal can be determined by integrating the area of their hysteresis loops (figure 8). This is the dominant contributor to the thermal energy for >1 μm diameter (or

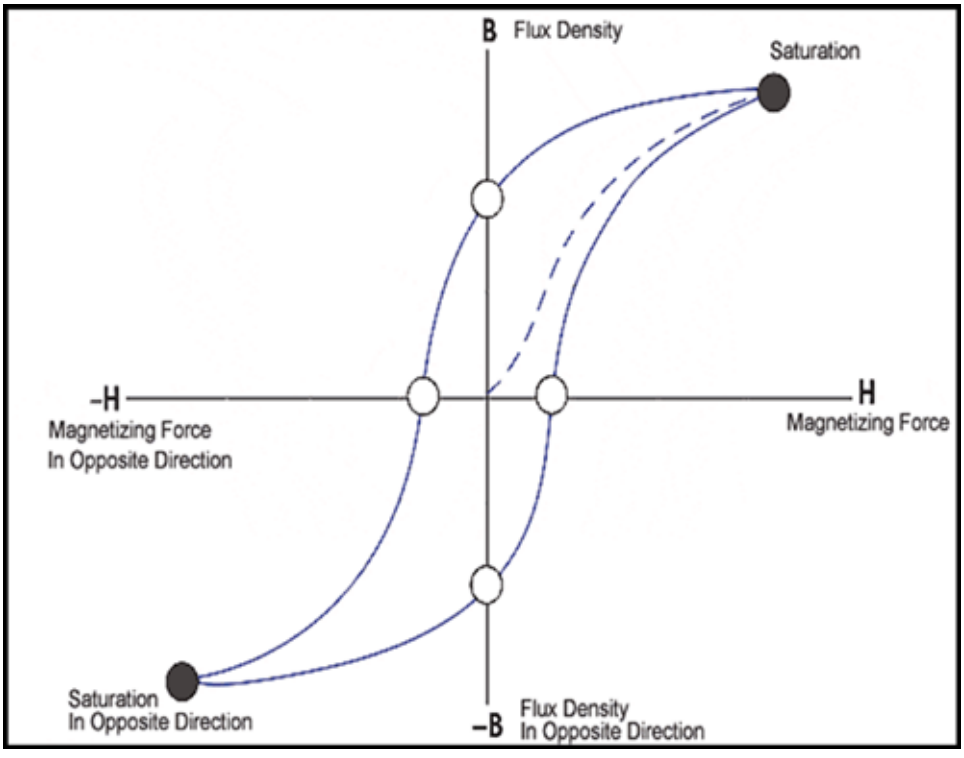

Figure 8: A typical Hysteresis loop—closed curve showing the variation of the magnetic flux density of a ferromagnetic material with the external magnetic field producing it, when this field is changed through a complete cycle.

multidomain) particles.[175] Nano scale magnetic particles behave as single domain particles and their magnetization reversal can be explained using the uniform mode described by Stoner and Wohlfarth.[176] The factors affecting hysteresis losses in magnetite particles for field amplitudes below 10kA/m are particle size, shape and microstructure. A maximum hysteresis loss in SPIONs is expected near a mean diameter of 30 nm.

Relaxation losses—as the particle size decrease, so do the energy barrier for magnetization reversal and consequently the hysteresis loops narrow.[175] Therefore the SLP calculated from the loop is less than the value measured directly by calorimetry. The relaxation loss in single domain MNPs is explained by two modes: rotational (Brownian) mode and Néel mode. [figure 9(a) and (b)] The Néel mechanism is analogous to the hysteresis loss in multi-domain magnetic particles whereby there is an 'internal friction' due to the movement of the magnetic moment in an external field that results in heat generation. In the Brownian mode, the whole particle oscillates towards the field with the moment locked along the crystal axis under the effect of a thermal force against a viscous drag in a suspending medium.[177]

Viscous losses—the Brownian mechanism (mentioned above) essentially represents the mechanical friction between the rotating particles and the surrounding medium. The energy loss per cycle of rotation where the viscous drag of the fluid is countered by a magnetic torque T equals the value $2\pi T$.[177]

8.1b Factors determining hyperthermia of MNPs: On evaluating the different principles explaining the loss mechanisms, it can be realised that, in principle, specific loss power is an increasing function of the frequency and amplitude of the magnetic field. The enhancement of SLP by increasing either of these factors is limited for medical reasons and the technical difficulty in realization of large field amplitudes at high frequency in a volume appropriate for hyperthermia. The most important factor however, is considered to be the particle size. A steep decline of SLP with decreasing particle size has been demonstrated.[175]

8.1c SPIONs: Superparamagnetic iron oxide nanoparticles sometimes referred to as 'ultrasmall superparamagnetic iron oxide particles' (USIOP) are considered as an important class of MNPs and have been tested by several authors (figure 10). Despite their many advantages, development of SPIONs is also fraught with challenges like; high frequency of oscillating magnetic fields required, the need to be injected directly into tumors as tumour targeting is still being studied, low SLP which directly correlates with the hyperthermic effect and those with higher SLP like cobalt and manganese ferrite nanoparticles have bio-incompatibility issues.[178] Recently, incorporation of optically active components onto MNPs has attracted a lot of attention. Dumbbell-like gold coated SPIONs[179,180] are very attractive composite systems due to their interesting optical and magnetic properties. However the decrease in the magnetic moment in such composite materials needs to be addressed.

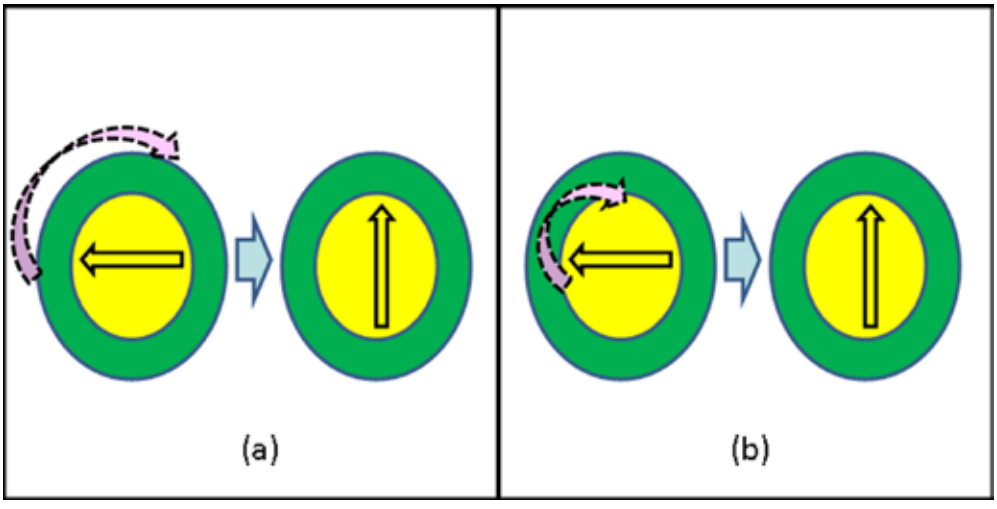

Figure 9: Relaxation mechanisms of MNP: (a) Brownian relaxation, entire particle rotates influid; (b) Néel relaxation, direction of magnetization rotates in core. The structure of MNP: core (yellow), shell (green). The arrow inside the core represents the direction of magnetization.

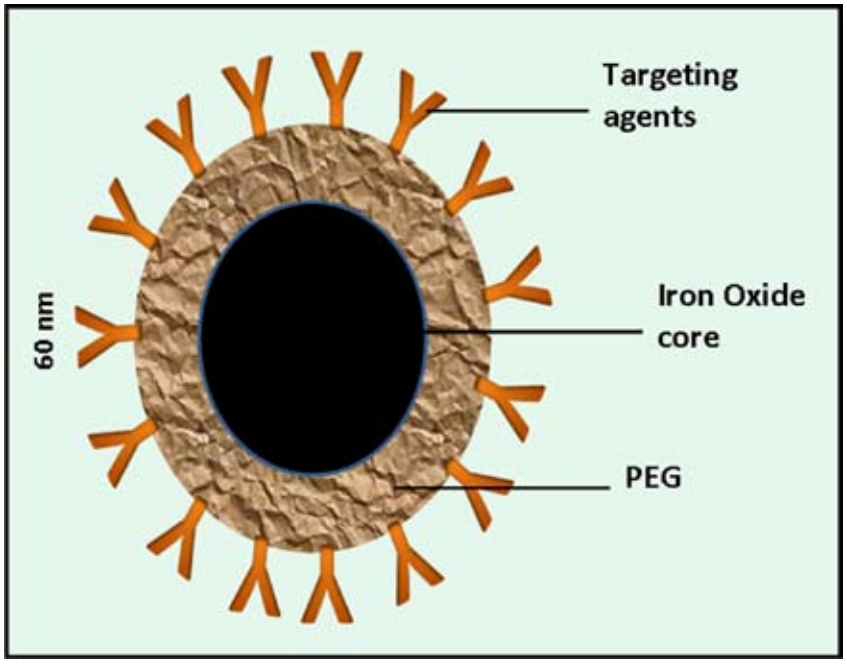

Figure 10: Diagrammatic representation of SPION showing the iron-oxide core which has been pegylated to allow better solubility *in-vivo*. Also an outer layer of targeting ligands attached for tissue specific localization.

Mohammad *et al* have demonstrated a 4- to 5- fold increase in the amount of heat released with gold coated SPIONs in comparison with regular SPIONs, on application of low frequency oscillating magnetic fields (44–430 Hz).[181]

Two clinical studies have been published.[182,183] The first, a 67 year-old patient with prostate cancer received an intratumoral injection of aminosilan-coated SPIONs followed by exposure to an alternating magnetic field (100 kHz) once weekly for 6 weeks. The results showed that the particles were retained at the tumor for the entire period and adequate temperatures for ablation of the tumor cells were achieved. The second report is a feasibility and tolerability study on 14 patients with glioblastoma multiforme.[183] Again intratumoral administration of aminosilan-coated SPIONs was made followed by the magnetic field exposure. The overall findings were that the technique does not cause any adverse affects on the patients. Further studies are under way to determine the efficacy of this technique.

8.2 *Photothermal therapy*

Photothermal interactions result from light energy conversion to heat. Nanoparticle induced hyperthermia is explained by the resonant phenomenon where light induces collective oscillations conductive metal electrons at the surface of nanoparticles—*plasmonic absorption/resonance*. This basically determines the absorbing and scattering properties of the particle. Near infrared (NIR) laser light (wavelength approximately between 700 nm and 900 nm) is considered ideal for *in-vivo* hyperthermia applications because of its low absorption by tissue chromophores like haemoglobin and water. Simple gold nanospheres (2 nm to several 100 nm in diameter) have characteristic extinction spectra due to plasmonic absorptions which are influenced by their size and composition. The materials studied so far include gold nanorods, gold nanocages, gold nanoclusters and gold nanoshells.

Naomi Halas from the Rice University is credited with having introduced gold nanoshells in hyperthermia therapy. Her basic design of the metal nanoshells consists of a spherical dielectric core NP (silica) which is surrounded by a thin metal shell (figure 11). They possess a highly tuneable plasmon resonance which is easily controlled by adjusting the gold shell thickness to core diameter ratios. The group has also demonstrated the multifunctional theranostic properties of gold-gold sulphide NPs which were conjugated with antibodies against breast cancer.[183] They further successfully demonstrated *in vivo*; the use of a single gold nanoshells formulation to first increase the optical contrast in tumors for optical coherence tomography (OCT) imaging and second to subsequently for absorption of NIR light for photothermal ablation of the tumor. Though

Intratumoral: Within the tumor.

Dielectric: A dielectric material is one which is a poor conducti\or of electricity, but an efficient supporter of electrostatic fields.

Tomography: Word tomography is derived from the Greek tomos ("part") and graphein ("to write"). It refers to imaging by sectioning using any penetrating wave (X-rays) so that the relation of a lesion to the adjacent structures can be visualized.

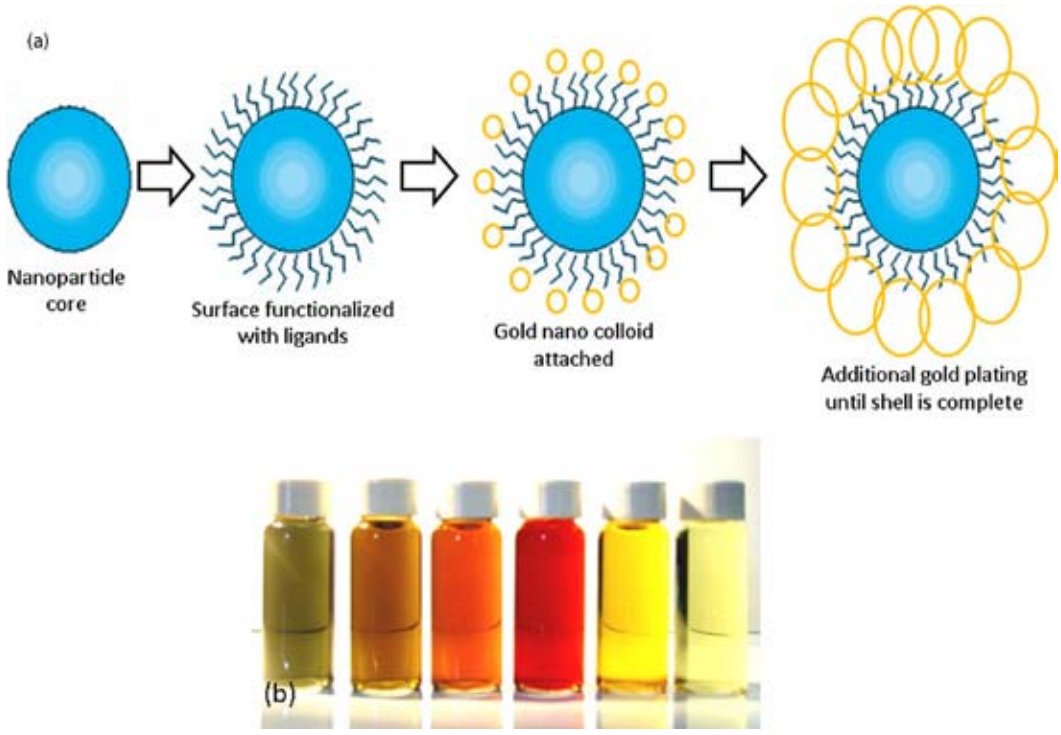

Figure 11: (a) Gold nanoshells consisting of a silica NP core and surrounded by gold nano colloid. (as demonstrated by Naomi Halas) (b) shows the GNPs developed at our centre for hyperthermia therapy.

gold nanorods too have been widely studied, their biological use has been restricted due to concerns about the toxicity of a surfactant used during the particle synthesis, hexadecyltrimethylammonium bromide (CTAB) which is known to degrade membranes and peptides.

8.2a Factors influencing NIR hyperthermia: The *in vivo* penetration depth of NIR light is dependent on a variety of factors including the degree of light scattering and absorption within tissue.[184] The heating of the tissue depends on the intensity of NIR light at the given point, the absorptive cross-section of the NP, the distribution and concentration of NPs within the tissue and the degree of NIR absorption by chromatophores in the surrounding tissue. Accurate modelling the heating profile if NP-laden tissue is important for optimising the ablation process. Regarding location of the NPs relative to the tumor cells, one group[185] studying folate-conjugated gold nanorods noticed the hyperthermia to be most effective when the particles are adsorbed on the cell surface rather than when internalized. Also, NIR therapy is enhanced by using a pulsed-mode laser instead of a continuous-wave one. The former permit more efficient photothermal conversions because of lapses between the pulses allowing additional time for electron-phonon relaxation.[186,187]

8.3 *Radiofrequency induced hyperthermia*

Conventional radiofrequency (RF) ablation is a routinely performed procedure for solid tumors (esp. liver) in clinical practice today. It is presently an invasive technique where an electrode is percutaneously/surgically introduced into the tumor using image guidance. An RF generator then delivers a high frequency (375–500 kHz) alternating current through the electrode and the circuit through the body of the patient is completed using a 'grounding pad' on the skin close to the tumor site. Heat is produced through friction caused by rapid oscillation of ions within the tissue and is highest close to the electrode. The FDA limit for RF induced energy deposition is 200 watts. The thermal conduction is inversely proportional to distance and hence decreases as we move further from the point of application. Different types of electrodes are now available, (monopolar, multipolar clusters, cool-tipped) to choose the one best suited depending upon the site and size of the tumor.

Despite its many applications, RF ablation is fraught with many shortcomings:

i) Though minimal, it is still an invasive procedure requiring specialized training
ii) The non-specific heating destroys normal tissue also
iii) In monopolar electrodes, an equal amount of heat is also produced at the site of contact of 'grounding pad' causing skin burns
iv) Heat produced is highest at the tip of electrode, thus heating is non-uniform
v) Difficult to control the heating and temperatures ≥110°C produces tissue charring resulting in rising circuit impedance and therefore decreases the RF output.
vi) Works best only for tumors <5 cm in diameter and significantly higher recurrence rates observed in those >6 cm because of incomplete destruction.[189]

To overcome the above limitations, nanotechnology was brought into the picture, allowing a more predictable and reproducible technique so that the energy is dissipated in a uniform volume which is matched to the tumor size. The heating rate of nanoparticles is defined by their Specific Absorption Rate (SAR), which is similar to the SLP seen in the previous section and is also measured in watts per gram (W/g). Two groups of NPs with sufficiently high SAR are currently being investigated:

i) Single-walled Carbon nanotubes (SWNTs)
ii) Gold nanoparticles (GNPs)

8.3a SWNTs: Single walled carbon nanotubes posses a one dimensional structure consisting of a honeycomb pattern of carbon that is rolled into a seamless cylinder and this provides a wide dynamic range of electromagnetic absorptions.[188] Though initially designed as hyperthermic enhancers when using NIR (Near Infrared) absorptions, their property of significant absorption and intense heat release under capacitively-coupled RF fields has also been studied.[189] The fact that a relatively small concentration of nanotubes (5–500 mg/l) significantly enhance RF-induced heating of the cells is explained based on the resistive conductivity of SWNTs and their high aspect ratios (length of the individual SWNT/greatest dimension of SWNT).[190] Individual SWNTs which typically measure 1 nm in greatest dimension, have an aspect ratio of approximately 300 to 1000.

SWNTs for RF heating was first reported from the M.D. Anderson Cancer Centre.[191] They showed promising results in 2 human hepatocellular cell lines (Hep3B and HepG2) and in 1 pancreatic cancer cell line (Panc-1). Their data indicated that RF-induced heating of intracellular SWNTs is dose dependent, and therefore methods to enhance cell-specific delivery and uptake of SWNTs will be crucial. A simple intratumoral injection of the particles results in incomplete thermal destruction of the malignant cells due to their heterogeneous concentration in the tumor environment thus once again underscoring the need for specific tumor targeting.

It was initially suggested that SWNTs would not be naturally compatible with biological systems. This is being overcome by chemically functionalizing them making them more soluble and biocompatible without critically compromising their inherent properties. Functional groups are often covalently attached to the benzene rings lining the SWNT's sidewall (sidewall functionalization), the fullerene caps, and even structural defects (defect functionalization). Though no studies report incidences of acute toxicities, long term studies to evaluate possible chronic toxicities needs to be performed in preclinical models.

8.3b GNPs: These are either simple gold nanospheres or composite gold nanoshells similar to those discussed in photothermal therapy. The limited penetration of NIR light in tissues (as compared to RF waves) confines their use to solid tumors that are either directly accessible, such as skin/oral cancers, or to those which can be indirectly accessed via endoscopy or intestinal fiber-optic placement. Thus RF energy appears to have a potentially broader clinical application. When comparing heating rates under RF field exposure, GNPs seem to provide a more efficient system over SWNTs.[192]

A recent work from the University of Pittsburgh.[193,194] has claimed that contrary to the previously made assumption, gold NPs do not make any measurable contribution to RF energy absorption. Their electrical conductivity measurements of solutions with and without GNPs revealed that the Joule heating via ionic conduction in the electrolyte solution is the dominant mechanism of RF-radiation-to-thermal conversion. This report however needs to be more critically evaluated.

Joule heating: Also known as ohmic heating and resistive heating, is the process by which the passage of an electric current through a conductor releases heat.

8.3c The Kanzius Machine: John S Kanzius, an American inventor who was undergoing chemotherapy for lymphoma envisioned this device as a potential method to treat not just his condition but almost all forms of cancer. Kanzius built a prototype *Kanzius RF device* in his home, and formed Therm Med LLC to test and market his invention.

It is composed of a variable power (0–2 KW) RF signal generator coupled to the High Q system consisting of a transmitting and receiving head. The High Q system is mounted on a bracket that allows the field to be oriented horizontally or vertically and the distance between the heads is adjustable. The system generates a field that is 30 cm in diameter with a peak intensity located within the center radius that is 7 cm. Preliminary research with the device using SWNTs and GNPs at The University of Texas M. D. Anderson Cancer Center and the University of Pittsburgh Medical Center have been promising. Clinical trials using this system are scheduled to begin later in 2012.

9 Conclusion

The paradox of standard cancer therapy practiced today is the deterioration of the quality of life of the patients due to the adverse side effects, because the adage at the heart of the physician's creed is "not to do harm". Development of superior diagnostic and therapeutic tools for cancer is crucial. Recent advances have helped to identify molecular mechanisms underlying the pathogenesis of cancers which can now be specifically targeted. The development of new approaches to improve treatment of cancer is an area of intensive research. However, these new treatments also contribute to the rising costs of health care. To justify this increased cost, state-of-the-art treatment should have enhanced efficacy, decreased invasiveness and fewer side effects than current therapies.

Nanotechnology has shown immense potential to achieve the above mentioned targets. Due to appropriate size and surface chemistry, allowing conjugation to biologically active molecule, several nanoparticles are being investigated for targeted delivery of chemotherapeutic agents. Protein and liposome based nanomedicine formulation are already in the clinical use and many new formulations are in the phase 2 and phase 3 stages of evaluation. In addition to nanodrug delivery, other nanoparticle enabled physical therapy methods such as hyperthermia is also an attractive concept to address the issue as they provide a more localized affect and enable a non-invasive approach. Extensive progress is being made in terms of the different techniques that can be applied and different agents in each technique. However, rigorous evaluation is still warranted regarding; the short and long term toxicity effects by nanoparticles, targeting efficacy of nanoparticles, the off-target effects of radiothermal and magneto-thermal therapies, clearance of nanoparticles from the body, etc.

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