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Nanomedicine for cancer targeted drug delivery, early detection mechanism and therapeutics

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Abstract

Nanotechnology leads to the platform of development of novel products with excellent qualities and potential applications in medicine and materials. The materials with one of the dimensions fall within the range from 1nm to 100nm are called as nanomaterial. The study of these materials has given more focus on to the neglected dimension in materials research. The potentiality of nanostructured materials in biomedical sciences has gradually been recognized and significant advances have been achieved. Suitable control over the properties of nanoscale structures can lead to new science, new products for various applications, devices and technologies. Nanomedicine is a broad area that diagnosing the biomedical system, treating the disease and make prevention activities to improve the human health. In this chapter we intends to describe the different kinds of nano structured materials and their applications in medicine especially in cancer biology.

Abbreviations: NCI: National Cancer Institute; RNA: Ribonucleic Acid; DNA: Deoxyribonucleic acid; QDs: Quantum Dots; AuNps: Gold Nanoparticles; HIV: Human Immunodeficiency Virus; DOX: Doxorubicin; PLM: Photo Localization Microscopy; PET: Positron Emission Tomography; CT: Computed Tomography; MNPs: Magnetic Nanoparticles; SWCNT: Single-Walled Carbon Nanotubes; MWCNT: Multiwall Carbon Nanotubes; MTT: (3-[4, 5-dimethylthiazol-2-yl] -2, 5-diphenyltetrazolium bromide reduction assay); NRC: Neutral Red Cellular uptake assay; LDH: Lactate Dehydrogenase assay; EPR: Enhanced Permeability and Retention; CTC: Circulating Tumor Cells; mRNA: Messenger RNA; EpCAM: Epithelial Cell Adhesion Molecule; TME: Total Mesorectal Excision; PDAC: Pancreatic Ductal Adenocarcinoma; CAFs: Cancer-Associated Fibroblasts; EGFR: Epidermal Growth Factor Receptor; PSMA: Prostate-Specific Membrane Antigen; DR5: Death Receptor Complexes; PEG: Polyethylene Glycol; MRI: Magnetic Resonance Imaging; WBC: White Blood Cells; MIT: Massachusetts Institute of Technology; PDMS: Polydimethylsiloxane; FDA: Food and Drug Administration; TNFα: Tumor Necrosis Factor Alpha; PDT: Photodynamic Therapy

Introduction

Nanotechnology is defined as the understanding, manipulating and controlling things at nanoscale, where the unique properties of material emerge that enable novel applications. It leads to the pathway of development of novel products with excellent qualities and potential applications in medicine, materials, scientific and technological field [1]. The materials with one of the dimensions fall within the range from 1nm to 100nm are called as nanomaterial. It attracts an extensive attention and relation with various fields of physics, chemistry, material science, medicine, engineering and photonics. Nanoparticles usually refers as a kind of material with a spherical-like appearance with a large surface area-to-volume ratio and fascinating properties and applications are derived from its quantum size effect. Nowadays, the potentiality of nanostructured materials in biomedical sciences has gradually been recognized and significant advances have been achieved. The general vision of nanoscience depends strongly on the ability of creating and manipulating matter at the nanoscale. Suitable control over the properties of nanoscale structures can lead to new science, new products for various applications, devices and technologies. Figure 1.1 illustrating the size of a tennis ball compared to a Carbon 60 (C_{60}) molecule. It was recognized that the properties of materials change drastically as their sizes decrease from the bulk material to nonoscale.

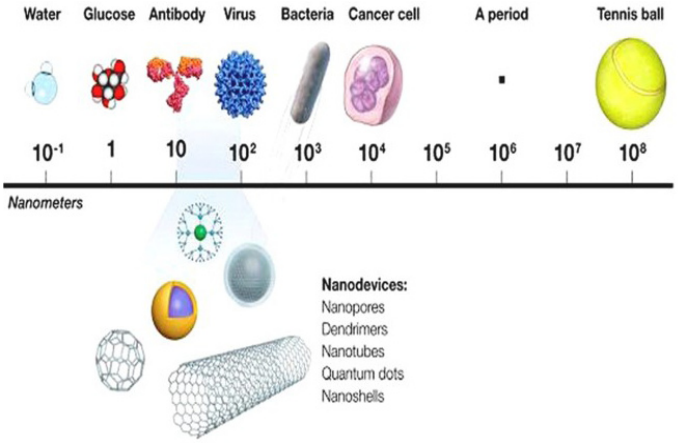


Figure 1.1: Nanoscale devices are one hundred to ten thousand times smaller than human cells (Courtesy: National Cancer Institute, USA)

The study of these materials has given more focus on to the neglected dimension in materials research. Materials at the nanoscale obeys neither classical physics nor quantum chemistry and also, the properties at this stage differs markedly from the bulk materials. This has led to new ways of creating materials and products having a wider range of options through which the process can be influenced. A great lecture given by the Nobel Physicist Richard P. Feynman in the year1959 at the session at American Physical Society laid down the concept of creating machines at nanoscale by manipulating atoms. In his visionary talk, there is plenty of room at the bottom. He proposed to make a machine which is small, then small so on all the way down to the atomic level [7]. In Tokyo, Norio Taniguchi coined the word nanometer in 1974 during the international conference in which he described the super thin films processing of materials. In 1986, K. Eric Drexler proposed the possibility of creating nanomachines in his famous book, Engine of Creation: The coming Era of Nanotechnology, [2] which was published in 1986. Feynman's early vision come to true in the writings of Drexler [3,4] in the years 1980s and 1990s and also, in the technical writings of Freitar [5,6]. The miniaturization of medical tools will be more accurate, reliable and more versatile that enhancing the quality of human life. The early genesis of the concept of Nanotechnology spracing from the visionary idea that tiny nanorobots and related machines could be designed, manufactured and introduced into the human body to perform cellular repairs at the molecular level [8].

Figure 1.2: Lycurgus Cup that was manufactured during 5th to 4th century B.C. When light is transmitted from the inside of the vessel it looks red but green in reflected light due to the presence of gold colloids (Courtesy: British Museum)

The British Museum showcases Lycurgus Cup, Figure 1.2 a glass bowl made up of nano gold and nano silver in an ancient Rome during 4th Century has an unusual properties for color during transmission and reflection. Change of physical properties of nanomaterial leads to an impact on chemical reactivity, boiling point and melting point compare to their basic bulk counterparts. Nanomedicine is a broad area that diagnosing the biomedical system, treating the disease and make prevention activities to improve the human health. In a simple way, we can define nanomedicine is the application of nanotechnology in medicine.

In this chapter we intends to describe the different kinds of nanoscale structured materials and their applications in medicine especially in cancer biology. Today nanomedicine branched out in hundreds of domains that able to structure the materials for medicinal applications and to develop molecular devices for research and practice of medicine.

Nanomaterial of biomedical interests

Nanopores

The surface of the nanoparticles are perforated with holes are called nanopores. This is one of the earliest material for biomedical applications jointly developed by Desai and Ferrari in 1997 [9]. The size of the pores are large enough to allow small molecules such as insulin, glucose, oxygen but impede the passage of immune system molecules such as graft-borne virus particles and immunoglobulins. These nanopores, Figure 1.3(a) are used for treating diabetes patients [10], harmone deficiency diseases [11] and Alzheimer's or Parkinson's disease. The flow of materials through nanopores can also be externally regulated with voltage-gated molecular nanosieve which has the diameter of 1.6 nanometers [12]. In 1995, Martin and colleagues [13] fabricated a nanosieve which consists of an array of gold nanotubes which excludes the positive ions when it is positively charged

and allows the negative ions to move through it and vice-versa. Daniel Branton's team at Harvard University has demonstrated to drive a variety of RNA and DNA polymers through the central nanopores of an alpha-hemolysin protein channel mounted in a lipid bilayer similar to the outer membrane of a living cell [14]. Nanopore based DNA sequencing devices could allow per-pore read rates more than 1000 base per second [15]. Nowadays low cost high throughput method for very rapid genome sequencing has launched by the nanotechnologist.

Quantum dots

The major domain of nanoparticles extensively explored for the applications in biomedicine and industry are Quantum Dots (QDs), Figure 1.3(b). It is diminutive and discrete unit of any physical system. The nanocrystals that have a peculiar optical properties are termed as 'Dots' which was coined by Quantum mechanics. QDs consisting of semiconductor core and over coated shell. Again its surfaces are coated with a capping agent enabling improved solubility in aqueous buffers [20]. During QDs synthesis, the organic surfactants are grown and ultimately forms ligands on the surface of core. The optical properties of QD's are greatly improved by passivating another inorganic shell before capping it with ligands. The enhancement of optical property is due to the greater band gap of shell over core so that the electrons and holes cannot get into shell. Also, shells plays a major role that it will not allow the core to interact with the surrounding medium. It eliminates trap sites by binding all the surface dangling bonds. Also, it helps to avoid loss of quantum yield, blinking of QDs and photo-bleaching. Primary amines, thiols, alcohols, carboxylic acids and long chain organophosphates are common ligands employed in QDs synthesis.

QDs are microscopically small spheres due to their distinct lack of length, width and depth. They are typically made out of Cadmium, Selenide, Zinc Selenide or Indium Phosphate. When these semiconducting materials are small enough, then quantum confinement occurs and electron behaves strangely. The peculiar effect is that when QDs absorbs light, it re-emits light of a specific wavelength depending on the QDs size. We can fine turn the color of emitted light by size controlled synthesis of QDs. In logically speaking, we can harness this behavior and use it to generate colors of our choice, effectively like manmade atoms whose electronic structure that we can construct. The emission characteristics of QDs have enabled us to construct the laser of wavelength that were previously impossible [21]. In biomedical science, it is a good imaging tool with broad band absorption and it has long life time that make them superior to conventional fluorescent dye markets [22]. The broad absorption and sharp emission characteristics of QDs make them ideal for use as sub pixels. They effectively purify the light, converting them into red, green and blue peaks rather than filtering them out.

Gold nanoparticles (AuNps)

Gold was one of the first metals discovered by humans and the history of its study spans over several thousands of years. First data on colloidal gold can be found in the treatises by Chinese, Arabs and Indians who prepared and used it for medical purpose as early as 4th century B.C [23]. The specific properties of AuNps are high electron density, the ability to scatter electrons and emit secondary electrons, the characteristic absorption and scattering in the visible region of the electromagnetic spectrum. The intense red color of gold bio markers enables easy detection of gold nanoparticles by different physicochemical methods. The possibility of preparing gold nanoparticle with different sizes and a narrow particle size distribution provides the application of gold nanoparticles in different fields. The present stage of development of physics of metal clusters dates back to 1993.

In recent years, gold is used in medical devices including pacemakers and gold plated stents [24,25] and gold alloys in dental restoration [26]. During the past few decades several organ gold complexes have emerged with promising antitumor, antimicrobial, and anti-HIV activities [27-29]. Some of the gold compounds like organo gold are now widely used to relieve arthritis symptoms such as joint pain, stiffness, swelling, bone damage, and also reduce the chance of joint deformity and disability [30]. The unique physical and chemical properties of nanoparticles provide excellent prospects for interfacing biological recognition event with electronic signal transduction and for designing a new generation of bioelectronics devices with novel functions. Especially, optical and catalytic properties which have made them a very attractive material for biosensors, Figure 1.3(c) cancer diagnosis, therapy and electro catalyst applications [31-33].

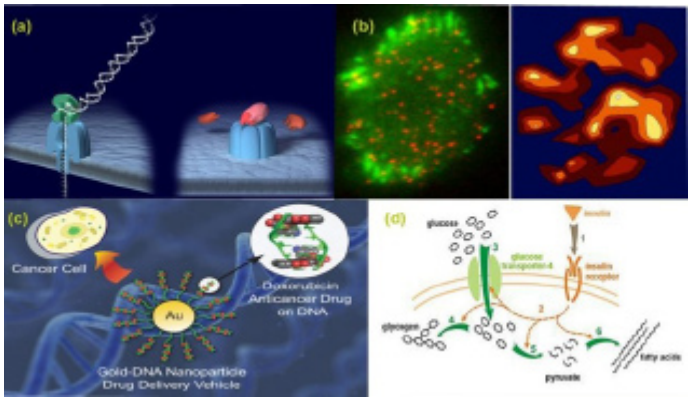


Figure 1.3: (a) Direct electronic analysis of single DNA and other molecules using nanopores (credit: Oxford Nanopore Technologies).
(b) Single molecule imaging of cell signaling using Quantum dots (Credit: Prof. Smith Lab, Illinois).
(c) Gold nanoparticles as a drug carrier using DNA to attach chemotherapy agent doxorubicin (DOX) to accumulate at the tumor site (Credit: Syracuse University office of technology transfer and Industrial development).
(d) Insulin binds to artificial binding sites which, in turn, starts many protein activation cascades (Credit: Revolvy).

Artificial binding sites and molecular imprinting

Artificial binding sites are prepared on the basis of how the biological molecular receptor works, Figure 1.3(d). The functionalized monomers interacts with a target molecule by noncovalently is termed as molecular imprinting [16,17]. The produced complexes are cross-linked and polymerized in a casting procedure, leaving behind a polymer having recognition sites for the adhesion of target molecules in both shape and functionality. Each such site have an induced molecular memory capable of selectively binding the target species. In an amino acid derivative target, an enzymatic transition state activity, chiral separations, and high receptor affinities have been demonstrated. Medically useful biopolymers are used in controlled drug release, quick biochemical separations and assays [18], drug monitoring devices, biological and receptor mimics, recognition elements in chemo sensors and biosensors [19].

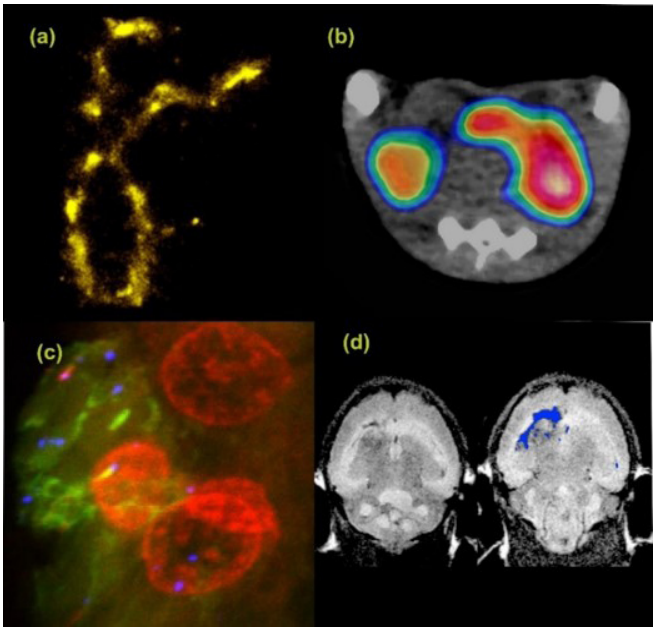


Figure 1.4: (a) Imaging a single human chromosome at the 20 nanometer scale Using super-resolution microscopy technique called DNA-PLM (single-molecule photo localization microscopy).
(b) PET/CT image of the targeted gold nanocluster (64Cu-AuNCs-DAPTA) was acquired 24 hours after intravenous injection in a mouse model of triple-negative breast cancer.
(c) A rabbit carrying a liver tumor with high demand for glucose was treated with titanium dioxide shell nanoparticles covered with glucose.
(d) Nanochains decorated with tumor-targeting molecules penetration into glioma tumors (Credit: National Cancer Institute, USA).

Magnetic nanoparticles

The application of Magnetic Nanoparticles (MNPs), Figure 1.5(a) for drug delivery was proposed in the year 1970 by the Widder, Senyl and Colleagues [34]. The therapeutic agents are attached to the nanoparticles or encapsulated within the magnetic nanoparticles. These magnetic core particles are coated with polymer or metals. Also some of the nanoparticles are prepared by filling the magnetic nanoparticles within the pores of porous polymer matrix. Magnetic nanoparticles functionalized with cytotoxic drugs are used for targeted chemotherapy to correct a genetic defect. The functionalized magnetic nanoparticles are injected into the blood stream to reach the target. A moderate external magnetic field is created near the target so that the nanoparticles are captured and extravasated at the target. This will be an effective mechanism once the target is very close to the skin. It is more difficult to reach the target when it is away from the surface. Concepts created to avoid these difficulties by implanting magnets near to the target. Magnetic nanoparticles techniques are used for in vitro gene transfection, particles are labeled with DNA and magnetic field increases sedimentation rates, particle internalization and gene expression. The process of endocytosis dependent on particle coating and cell type [35]. A wide range of research towards advances in drug targeting delivery and clinical trials are performed in recent years [36]. Magnetic nanoparticles with higher magnetic moments nonfouling surfaces, and increased functionalities are now being developed for applications in the detection, diagnosis and treatment of malignant tumors [37], cardiovascular diseases [38] and neurological disease [39,40].

The magnetic nanoparticles are investigated as the next generation magnetic resonance imaging contrast agents and targeted drug delivery agent [41,42]. Composition, size, morphology and surface chemistry can now be tailored by various processes to not only improve magnetic properties but also affect the behavior of nanoparticle in a biological system [43,44]. Magnetic nanoparticles have the potential to overcome the limitations associated with systematic distribution of conventional chemotherapies. In early clinical trials, the colloidal iron oxide particles demonstrated some degree of success with the technique and shown satisfactory toleration by patients [45,46]. To increase the effectiveness of MNPs several technique, including reducing size and grafting non fouling polymers have been employed to improve their stealthiness and increase their blood circulation time to maximize the likelihood of reaching targeted tissue [47,48].

Polymer micelles

Polymeric micelles based drug delivery is an effective way of delivering drugs at the target. The core of micelles are hydrophobic environment, insoluble drugs can be easily solubilized and loaded for drug delivery at the desired site. Targeted delivery system produce minimum drug degradation or loss, increase drug bioavailability, prevent harmful side effects, and increase the change of getting more drugs at the required site. A variety of drug carrier like polymers, lipoproteins, liposomes and amphiphillic polymers are extensively used [49-51]. Liposomes are preferred as a potential drug delivery moiety due to their ability of protecting drugs from less targeting the drug to the site of action and thus reducing the side effects [52]. But it shows no markable advancement due to inherent problems such as low encapsulation efficiency, rapid leakage of water soluble drugs in the presence of blood components and poor storage stability.

The importance of the hydrophilic part of the polymeric micelles (Figure 1.5(c)) is that its brush like structure allows the hydrophobic part from the biological invasion. The threshold of renal clearance of nanoparticles is approximately 5.5 nm [53] and the allowed size of the polymeric micelles for filtration by kidneys, so the polymeric micelles have maximum drug loading capacity and ability to carry more drugs with prolonged circulation time [54-56]. These release nanocarriers in blood and accumulates in sites with leaky vasculature because of the enhanced permeability and retention effect. Further, there is an improvement in drug loading efficiency, making transport facile through the cell membrane and stability in blood after injection [57,58]. Polyionic compounds can be incorporated through the formation of polyion complex micelles [59]. The chemical stability of the Dox incorporated into polymeric micelles can be explained on the basis of protection from aqueous environment [61]. There is an increased resistance of plasmid DNA in polyion complex micelles against enzymatic degradation [60].

Nanowires

Different types of nanowires are used for biomarker applications such as silicon nanowires [62-65], In_2O_3 nanowires [66], conducting polymer nanowires [67] and gold nanowires [68,69]. Silicon nanowires has exceptional physical, electronic and optical properties as well as excellent biocompatibility [70-73]. Silicon nanowire modified with peptide nucleic acid have used to detect miRNA extracted from Hela cells. Silicon nanowire functionalized QDs on a patterned gold substrate have been studied as an optical sensor for cancer biomarker applications. The fluorescent intensity was ten-fold than a silica substrate and the signal was labeled as IL-10 [74]. Functionalized gold nanowires has high surface area to volume ratio with enhanced sensitiv-

ity and selectivity for cancer biomarker detection. Gold nanowires functionalized with DNA-nanowire is a potential cancer diagnostic system [75]. A simple polypyrrole nanowire was integrated into FET system, just like carbon nanotubes and the measurements of changes in conductivity due to biomolecule recognition events were measured [76]. Micro cantilever based biosensors are nowadays used for the detection of cancer. Surface stress increased due to the increase of antigen concentration in the micro cantilever which gives mechanical deflection for cancer detection.

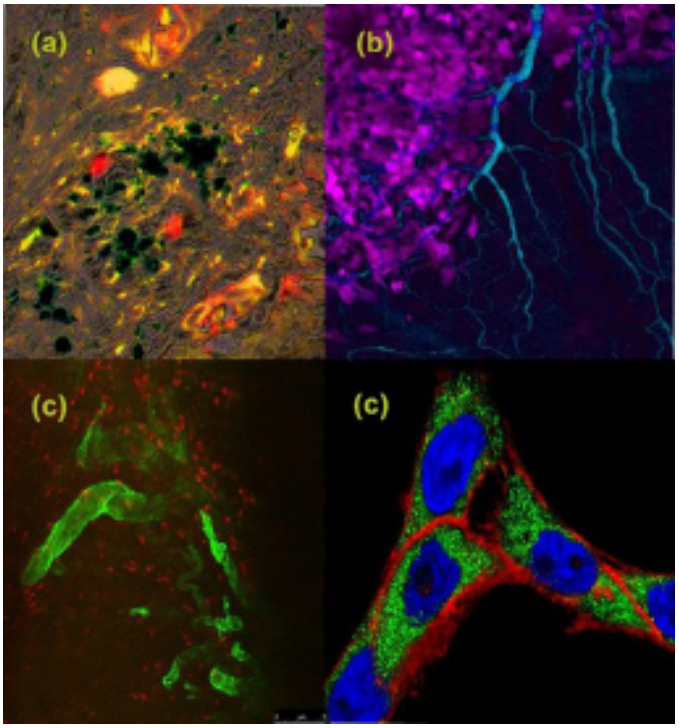


Figure 1.5: (a) Magnetic iron nanoparticles that target cells with IGF-1R receptors, and are conjugated to a chemotherapy drug of pancreatic cancer (dark blue), tumor-associated macrophages expressing IGF-1R (red) and CD68 (green).
(b) High resolution image of blood vessels (light blue) infiltrating brain cancer (purple) in a live mouse.
(c) Micelle-based nanoparticles (red) that have moved beyond the blood vessels (green) of a tumor in a mouse model of ovarian cancer suggesting excellent tumor-penetration capability.
(d) Cancer cells (cell nuclei in blue) were treated with antibody-conjugated nanoparticles, the antibodies (red) and the nanoparticle cores (green) separated into different cellular compartments (Credit: National Cancer Institute).

Carbon nanotubes (CNTs)

Carbon nanotubes are cylinders of one or more number stacked one over another. If it is a single cylinder, then it is Single-Walled Carbon Nanotubes (SWCNT) and more than one which are rolled over one another, then it is Multiwall Carbon Nanotubes (MWCNT), Figure 1.6(b). The length of the nanotubes may extend to several millimeter and their diameter is in the range of nanometer. Functionalization of SWCNTs with polyethylene oxide chains enables the binding of specific proteins of medical interest. CNTs are used in the therapeutic field as vectors for drug delivery. It was demonstrated that the functionalized CNTs are able to cross the membrane of fibroblasts in vitro and accumulate in the cytoplasm without any associated toxicity [77]. In a recent research, CNTs were used to destroy cancer cells selectively upon irradiation with NIR light without harming the receptor-free normal cells functionalization of SWCNT with folate moiety [78].

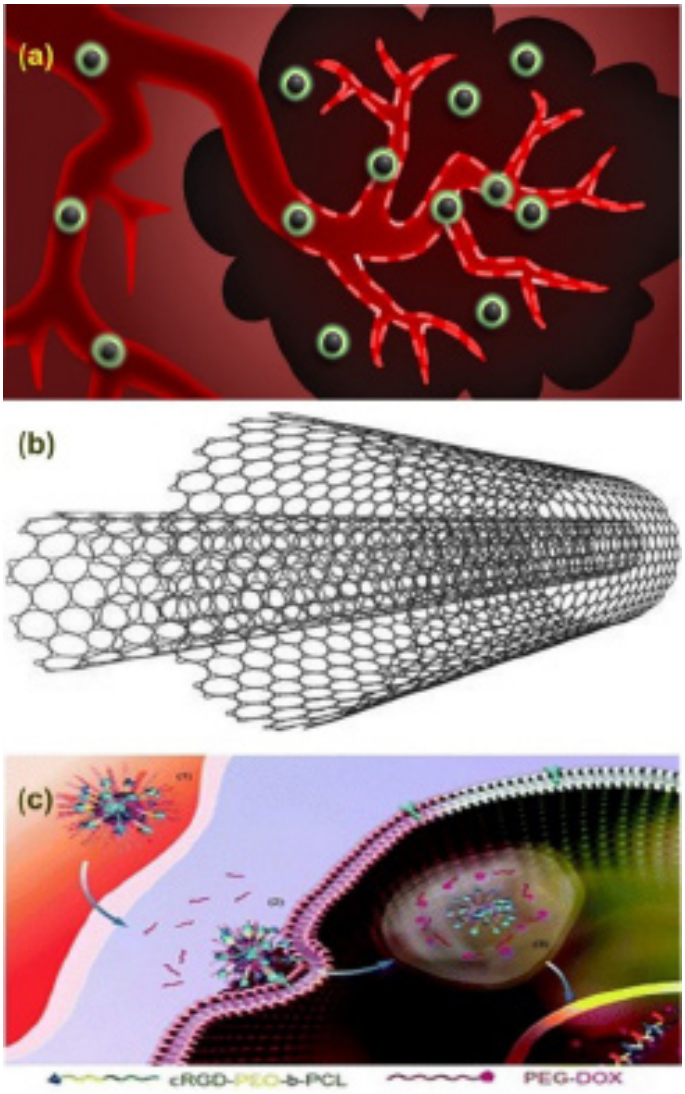


Figure 1.6: (a) After a quick intravenous injection of magnetic nanoparticles and just three minutes inside magnetic field, 80% of their test animals are completely cured of cancer (Credit: Nanoprobe, INC.).
(b) Carbon Nanotubes: Double-walled carbon nanotubes. Reprinted from Pichler [82].
(c) Polymeric micelles with combinatorial targeting ability to cancer cells was achieved by co-assembly of cRGDPEO-b-PCL and tumor pH sensitive PEG-DOX [83].

The high aspect ratio makes CNTs an outstanding scholar allow loading large quantities of payloads along its longitude of tubes without affecting their cell penetration capability. It can carry drugs, genes and targeting molecules into cells to exert multivalence effects. In recent years, the devices made up of CNTs have been successfully utilized in stem cell based therapeutic application and tissue engineering. It has excellent optical properties like high absorption in the near-infra red range, photoluminescence and strong Raman shift [79]. The functionalized CNTs is preferable for the accumulation in tumor tissues, having ultrahigh surface area facilitate them to load the drugs and targeting the molecules to the tumor site. Since DNA and SiRNA are macromolecules, they cannot pass through cell membrane by themselves. Cationic reagents such as lipids and polymers are needed to take them inside of cell to take effects [80,81].

Cytotoxicity

Paracelsus is sometimes called the father of toxicology, Figure 1.7. Toxicity is the degree at which the substances may be toxic that can damage living or non-living organisms. The cen-

tral concept of toxicology is that toxic effects are dose dependent. Even water can lead to water intoxication when taken in large enough doses where as a very toxic substance such as snake venom, there is a dose below which there is no detectable toxicity. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. The chief criterion regarding the toxicity of a chemical is the dose or the amount of exposure to the substance. All substances are toxic under certain conditions. The term LD_{50} refers to the dose of a toxic substance that kills 50 percent of a test population (typically rats or other surrogates when the test concerns human toxicity).

The nanoparticles have been shown to adhere to cell membranes [84] and be ingested by cells [85]. The breaching of the cell membrane and the intracellular storage may have a negative effect on the cells regardless of the toxicity of the particles and their subsequent functionality. One of the research work shows that the gold nanoparticles stabilized with different capping agents and the size of the particles has different toxicity level in *in vitro* studies [86, 87].

Figure 1.7: Paracelsus (1493 – 1541), the father of toxicology

"All things are poison and nothing is without poison, only the dose permits something not to be poisonous"

The assays used are based on different modes of detection like (3-[4, 5-dimethylthiazol-2-yl] -2, 5-diphenyltetrazolium bromide reduction assay) (MTT), neutral red cellular uptake assay and Lactate Dehydrogenase (LDH) release assay. Further, it was found that the gold nanoparticles stabilized with citrate, starch and gum arabic are viable to different cells through different assays with different concentrations and time of exposure of gold nanoparticles.

Nanomaterial in cancer biology

The best chance of winning the war against cancer is to detect at the earliest stage prior to being increased. It is very difficult to detect from fewer number of cancer cells being available at the initial stage in the biological system. Also, due to the lower concentration of biomarker in the fluid and cancer site, it is very difficult to trace the cells in the whole body, Further, it was characterized that there are some differences in the properties of early stage cancer and late cancer. Increase of cancer cell counts leads to more changes in the genome, proteome, transcriptome and epigenome when characterized. These challenges can be addressed by nanotechnology based diagnostics as part of nanomedicine. Nanomedicine has a promises unprecedented innovations for early diagnosis, staging and therapy. Through molecular imaging, nanotechnology has a potential impact in vivo diagnostics for early cancer detection.

Nanotechnology offers great benefits for early to late cancer detection from single cell analysis to bulk system. Cancer is one of the leading causes of death with an estimated count of 7.6 million losses every year and it is expected to rise 13.1 million by 2030. Research identifies that 30% of cancer is due to life style changes like smoking and dietary practices [88-90]. Cancer is the second most common cause of death in the world, exceeded by heart disease. Genetic factors, physical activity, certain type of inflections, exposure to chemicals and radiations are the most leading factors of causes of cancer. The existing vaccine technologies should be integrated with nanotechnology to improve the vaccination efficiency [91-94]. Also, the researcher finding tools to distinguish malignant and non-malignant cells as well as delivery a lethal pay load at the site specific target using nanotechnology. The advancement of nanotechnology in a regular basis highlight its potentiality in biomedical sciences [95-104]. The technology enhances the bioavailability of nanoscale drugs, targeting and create multi-functional therapeutic agent with future vision of nano-machine and nano-robots [105,106]. According to National Cancer Institute about 227900 people died in 2007 due to lung cancer, breast cancer and prostate cancer. Cancer is also greatly feared due to recurrence although treatable, can reappear after a period of time, even after chemotherapy, surgery or radiotherapy. The traditional method of diagnosis and treating are not very powerful methods and also, very costly which is not affordable for middle income families.

The non-specific distribution of drugs limits the therapeutic dose within cancer cells, tissues and organs and thereby causing several adverse side effects including hair loss, weakness and organ function leading to very risky life for cancer patients [107-113]. Tumor cells obtain nutrients for growth by passive diffusion until it reaches size of 2-3 mm. to continue growth, the tumor synthesize new blood vessels by the process called angiogenesis. These blood vessels are proliferating endothelial cells in a large number with large gaps between adjacent endothelial cells ranging between 380 nm and 780 nm. The blood vessels have enhanced permeability for particle passage through the wall into the interstitial surrounding tumor cells [114-120]. Tumors lack in lymphatic networks and the drugs that gain interstial access may have extended retention times in the tumor. This feature is termed as Enhanced Permeability and Retention (EPR) effect and favors tumor interstitial drug accumulation [121,122]. This facilitate the nanotechnology to target and treat the tumor without affecting the healthy cells.

Tumor targeting

Tumor targeting is one of the potential specialty of nanotechnology in cancer research. The central concept of nanotechnology is to distinguish the malignant cells from non-malignant cells and also eradicate the malignant cells from the diseased persons. Passive and active targeting are the process involved to distinguish the malignant and non-malignant tumor. Passive targeting is a process to increase the tumor targeting and there by enhanced permeability and retention (EPR) effect [123,124]. Active targeting [125] involves selective molecular recognition of antigens such as proteins that are expressed on the surface of cancer cells to localize nanoparticles to malignant cells or to exploits biochemical properties associated with malignancy such as metalloprotein secretion [126]. Both active and passive

targeting may be taken individually or combined effect [127]. Drug carriers include microcapsules, synthetic polymers, dendrimers and liposomes has increased bioavailability and increased accumulation at the pathological site without affecting the healthy cells. Also, the nanocarriers are an ideal entities to deliver the payloads at the desired site which are poorly water-soluble agents [128].

Passive targeting

The tumor surface has leaky vasculature relative to the structural morphology because malignant cells are not responsive to cell signaling requires for orderly vasculogenesis [129,130]. Due to Enhanced Permeability and Retention effect (EPR) [131], the macromolecular enter the tumor through leaky vasculature and persists there because of reduced lymph clearance [132] in tumors. The preferable dimensions for the localization of proteins in tumor tissue is a minimum of 25 KDa with enhanced uptake for proteins whether it is larger or smaller. Passive targeting, Figure 1.8(a) were achieved by using variety of nanoparticles including single walled carbon nanotube [135,136], liposomes [137] and viral nanoparticles [138]. The gap size in the tumor interstitial space is present with a maximum diameter of 800 nm. Encapsulated drugs are loaded onto the tumor tissue by the nanoparticles. The phenomenon extravasation provides another passive method to concentrate the particles up to 300 nm diameters in inflamed tissues or tumors [139].

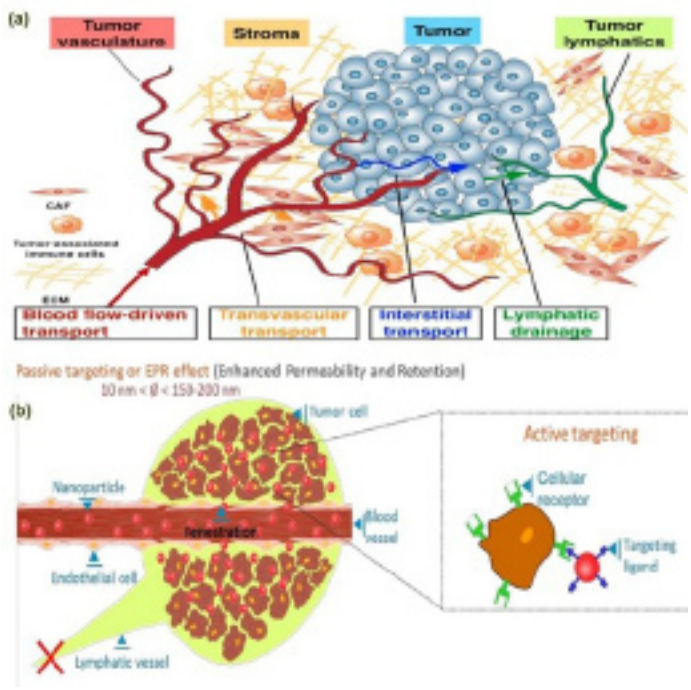


Figure 1.8: (a) Transport of drug molecules and nanoparticles in the TME of PDAC. It consists of dense stroma composed of Cancer-Associated Fibroblasts (CAFs), tumor associated immune cells, and dense ECMstructure [133].
(b) Fluorescent nanoprobes dedicated to in Vivo Imaging [134].

A network of blood vessels needs to expand very quickly to accommodate tumor cells in need of nutrient and oxygen. Oxygen while tumor mass grows rapidly. The leaky vessels allow relatively large nanoparticles to extravasate into tumor masses. In passive target, higher concentration of nanoparticles accumulates greatly in the tumor mass. The National Cancer Institute, USA has developed a nanoparticle which is capable of penetrating the entire mass of tumor within 48 hours of injection, suggesting excellent tumor-penetration capability. Polyethylene Glycol (PEG) often used for drug designing which has long circulating time in the blood pool. PEG coated liposomal Doxorubicin (DOXIL) is used clinically for breast cancer leveraging passive tumor accumulation. Passive accumulation through EPR effect is the most acceptable drug delivery system for solid tumor treatment. However, size or molecular weight of the nanoparticles are not the sole determinant of the EPR effect, other factors such as surface charge, biocompatibility and in vivo surveillance system for macromolecules should not be ignored in designing the nanomedicne for efficient passive tumor accumulation [140,141].

Active tumor targeting

It is capable to actively target and finding malignant cells or non-malignant cell site with any ligand is called as active targeting [142,143] the growth factor receptors such as transferrin [144-147], Epidermal Growth Factor Receptor (EGFR) [148], death receptor complexes, DR5 [149,150], folate ligand [151-154] as well as tumor-specific antigens, pSMA [155,156] have been utilized to localize nanoparticles to malignant cells through active targeting, Figure 1.8(b). In some cases, the active targeting is does not uniformly enhance tumor localization. The recent research evaluated that the surface modification of gold nanoparticles on the interaction with blood components and bio distribution of nanoparticles [157]. Also, low molecular weight chitosan has been developed as an alternative to PEGylation that may allow for retention of specific molecular interactions that are marked by PEG [158]. Inside the necrotic area of larger tumor cells shows poor vascularization that prevents the localization of the nanoparticles and makes local drug deposition impossible. A more sophisticated approach is to modify the surface of the nanoparticle by the addition of antibody or ligand with affinity for the desired target. In this case, the nanoparticles targets the folic acid receptor which is over-expressed in various human carcinomas [159]. It was demonstrated that the nanoparticles conjugated with folic acid as a receptor seeker and loaded with methotrexate as a chemotherapeutic agent has entered into cancer cell and inhibit its growth [160]. The active targeting is considered as an essential feature for next generation nanoparticle therapeutics.

Cancer detection

The imaging modalities such as plain radiography, Ultrasound, Compound Tomography (CT) and Magnetic Resonance Imaging (MRI) are rely on detecting cancer cells once it becomes visible entity at around 1 cm^3 which contains about approximately 1 billion cancer cells. Over the past decade, there has been a paradigm shift from anatomical imaging which detects macroscopic or gross pathology to molecular imaging which has the potential to detect cancer much earlier at the molecular level, long before phenotypic changes occur. Molecular imaging characterization helps us to identify the genetic changes involved in oncogenesis that can prove most beneficial molecular therapy for patient as well as it allows the repeated noninvasive monitoring of the disease for response, progression and transformation following therapy or recurrence [161,162]. Currently, several preclinical imaging tools are available with nanotechnology concept over the conventional methods. These includes magnetic nanoparticles or gadolinium chelate, functionalized nanoparticles-enabled for high resolution MRI [163-166], nanoparticle and intrinsic contrast based photo acoustic imaging [167], surface enhanced Raman Spectroscopy based endoscopy [168,169], smart optical nanoimaging agents [170-172], micro nuclear magnetic resonance imaging [173], PET-MRI [174], MRI-

photo acoustic and Raman [175].

Circulating tumor cells

In clinical care, the tissue based evaluation of biopsy samples remains the gold standard for diagnostics and prognosis. This is the process of obtaining tissue samples by surgical excision or radio-graphically directed needle extractions. This approach is complicated by several issues with the extractions are both invasive and costly overall, as well as the metastatic tissue there is a growing focus and concern for the impact of the tumor tissues temporospatial heterogeneity. Circulating Tumor Cells (CTCs) have been proposed as they provide a means to sampling tumors across all present disease sites including the primary tumor and metastases [179]. Over the course of therapy, CTCs creates new possibility for personalization cancer care by monitoring cancer progression, understanding pathogenic mechanisms and guiding the implementation of the most effective treatment interventions. Much progress has been made in CTC detection, isolation and characterization that has driven by the inter-disciplinary collaborative research spanning across material science, chemistry, oncology and bioengineering. Nanomaterial based CTCs gives in-depth characterization with reduced costs and ultimately brings the oncology to the goal of personalized care. CTCs detection assay include utilize capture agent labeled magnetic beads to either negatively deplete White Blood Cells (WBCs) using anti-CD45 or positively select CTCs using a cell surface marker. The cell search assay is a CTC diagnostic technology for metastatic breast colorectal and prostate cancer [180]. To improve the detection speed and efficiency a new system of CTCs are developed such as IsoFlux, Magsweeper, Magnetic Sifters, Cynvenio and AdnaGen.

Another CTCs detection assay is flow cytometry which uses florescent markers which is the most mature technologies for analyzing and sorting sub population of cells. Microscopy imaging of ICC-treated blood samples allows highly sensitive detection of CTCs accompanied with their morphometric characteristics and protein expression. Development of nanotechnology enabled CTC assays was developed by MIT General Hospital [181] which is a first generation device chemically etched microposts on a silicon substrate where on covalently functionalized anti-EPCAM antibodies are present. The embedded microposts increase the contact between the device surfaces and the flow through cells. A unique Ephesia which is an assay based on microposts of capture agent coated magnetic beads self-assembled in a microchip demonstrated combined advantages of both micro fluid and immunomagnetic cell sorting [182]. Micro scale herring bone patterns were engineered into the imprinted PDMS component to introduce micro vortices leading to enhanced contact between the CTCs and the antibody coated chip surface is termed as the MGHs second generation devices [183].

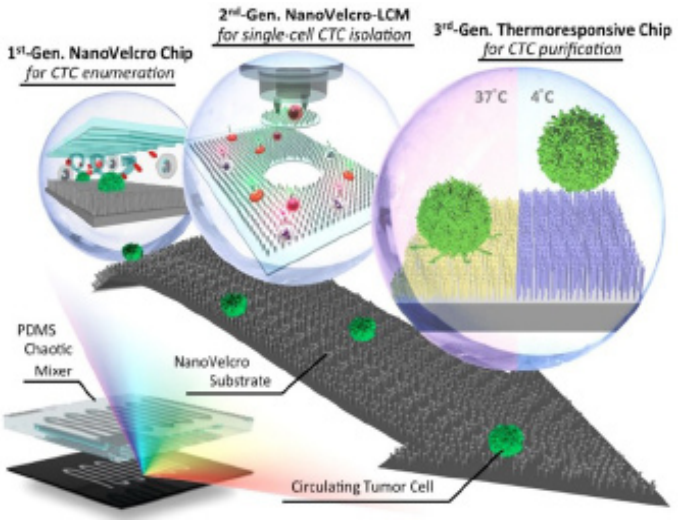


Figure 1.9: Conceptual illustration of the three generations of NanoVelcro CTC Assays developed by the UCLA team to achieve different clinical utilities. 1st-gen NanoVelcro Chip, composed of a Silicon Nanowire Substrate (SiNS) and an overlaid microfluidic chaotic mixer, was created for CTC enumeration. In conjunction with the use of the Laser Microdissection (LMD) technique, 2nd-gen NanoVelcro-LMD technology, was developed for single-CTC isolation. The individually isolated CTCs can be subjected to single-CTC genotyping. By grafting thermoresponsive polymer brushes onto SiNS, 3rd-gen Thermoresponsive NanoVelcro CTC Chips were developed for purification of CTCs via capture and release of CTCs at 37 and 4°C, respectively. The surface-grafted polymer brushes were responsible for altering the accessibility of the capture agent on NanoVelcro substrates, allowing for rapid CTC purification with desired viability and molecular integrity. (Reprinted with permission from Tseng et al, 2014) [184].

The third generation chip, Figure 1.9 combines negative immune magnetic depletion processes with an inertial focusing setting in an integrated microchip [185]. A microfluidic device with designated sections for selectively capturing CYCs according to the amount of magnetic beads grafted on their surfaces has been developed [186]. The presence of nanoscale materials in the tissue microenvironment like in extracellular matrix and cells surface structure provide structural and biochemical support to cellular behavior. CTC capture agent coated nanostructured substrates called as nanovelcro are utilized to immobilize CTCs with high efficiency [187]. When the two fabric strips of a velcro fastener are pressed together, tangling between the hairy surfaces on two strips leads to strong affinity between cell and nanosubstrates.

Table 1: Nanotechnology Based Oncology Products FDA and/or EMA (Credit: NCI, USA)

Product	Nanoplatform/ agent	Indication	Status	Company
Doxil	PEGylated liposome/ doxorubicin HCl	Ovarian cancer	Approved 11/17/1995 FDA50718	Ortho Biotech (acquired by JNJ)
Myocet	Non-PEGylated liposome/ doxorubicin HCl	Metastatic breast Cancer	Approved in Europe and anada, in combination with cyclophos-phamide	Teva Pharma B.V.
DaunoXome	Lipid encapsulation of dauno-rubicin citrate	First-line treatment for advanced HIVassociated Kaposi's Sarcoma	Approved in USA	Galen Ltd
ThermoDox	Heat activated liposomal encapsulation of doxorubicin	Breast cancer, primary liver cancer	In Phase III in USA	Celsion
Abraxane	Nanoparticulate albumin/ paclitaxel	Various cancers	Approved 1/7/2005 FDA21660	Celgene
Rexin-G	Targeting protein tagged phospholipid/microRNA122	Sarcoma, osteosarcoma, pancre-atic cancer, and other solid tumors	Fully approved in Philippines in 2007, Phase III Fast Track Desig-nation, Orphan Drug Status Acquired in USA	Epeius Biotechnologies Corp
Oncaspar	PEGylated asparaginase	Acute lymphoblastic Leukemia	Approved 6/24/2006	Sigma-Tau Pharmaceuticals
Resovist	Iron oxide nanoparticles coated with carboxydextran	Liver/spleen lesion Imaging	Approved 2001 for European market	Bayer Schering Pharma AG
Feridex	Iron oxide nanoparticles coated with dextran	Liver/spleen lesion Imaging	Approved in 1996 by FDA	Berlex Laboratories
Endorem	Iron Oxide nanoparticles coated with dextran	Liver/spleen lesion Imaging	Approved in Europe	Guerbet
DepoCyt	Liposome/ cytarabine	Lymphomatous Meningitis	Approved in USA	Sigma-Tau Pharmaceuticals

An extended research was devoted to exploiting different nanomaterials such as polymer dots, nanotubes, TiO_2 nanowires, Fe_3o_4 nanoparticles and graphene nanosheets to achieve high affinity capture of CTCs and other type of rare cells [188].

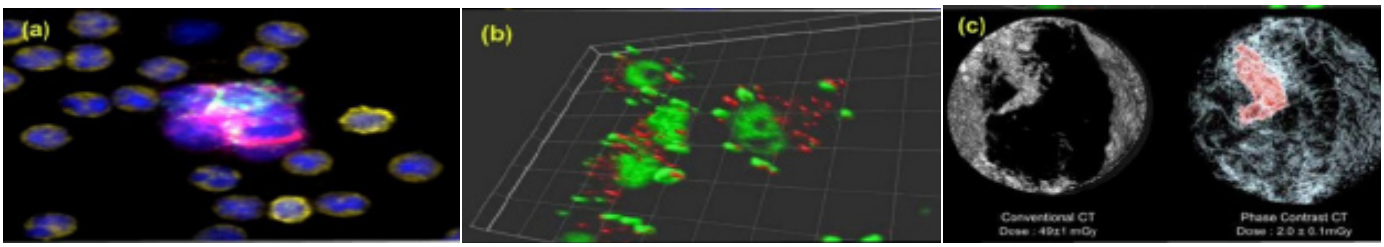


Figure 1.10: (a) Five-cell CTC cluster expressing drug target from a clinical research study participant. Cytokeratin (red) / EpCAM (magenta) / Drug target (green) / CD45 (yellow) / nuclei (blue) (Credit: Rarecyte, Inc, USA).
(b) Intracellular localization and interaction of mRNA binding proteins as detected by FRET (Credit: Wikimedia Organization).
(c) New X-ray breast cancer imaging possible with 25 times reduced radiation dose (Credits: European Synchrotron Radiation Facility).

Intracellular mRNA detection

Nucleic acids has emerged as a powerful tool in biomedicine with far reaching implications in cancer research [189]. Spherical Nucleic Acids (SNAs) consists of nanoparticle core such as gold [190], silver [191],iron oxide [192], silica [193] and infinite coordination polymers [194] heavily functionalized with oligonucleotide shells like mRNA, Figure 1.10(b) [195], single or double stranded DNA [196], PNA [197], SiRNA are used for detection. The unique architecture of the oligonucleotide shall have novel chemical and physical properties that make this material very useful in cancer research. SNAs are considered as a scavenger receptor that enters cells as a single entity without the aid of ancillary transfection agents [198-200] which exhibits high affinity for complementary DNA strands [201]. In cancer research and oncology, SNAs are designed to target any gene associated with a wide variety of cancer type in intracellular and extracellular biodetection and therapeutic schemes.

SNAs were first synthesized in Chad Mirbin laboratory at North Western University in 1996 and then formulated as nanoflare constructs in 2007 in the same lab. Nanoflare are gold-nanoparticle based SNAs that are hybridized with short, fluorophore labeled complementary DNA strands. The nanoflare encounter a longer complementary target like mRNA strand in a biological environment, it displaces one of the shorter flare strands and the fluorescence signal is observed. It is capable of engaging in gene regulation, SiRNA and micro RNA delivery vehicles. Also, it has theranostic potential to detect and treat cancer simultaneously [202]. It was demonstrated that nanoflares

could be used to detect oncogenes, more specifically surviving an anti-apoptotic gene that is up regulated in a variety of cancer types [203]. Increase of fluorescence occur when nanoflare targeting surviving were added to breast cancer cell line (SKBRJ). The potential physicochemical properties of nanoflares used to distinguish cancerous or non-cancerous cells based on the expression of an mRNA target of interest [204]. Even a single nanoflare can able to target multiple genes [205-207] in breast and cervical cancer cell lines. Nanoflares are used to capture live breast cancer circulating tumor cells, MDA-MB-231 from human whole blood samples and metastatic triple negative breast cancer from orthotropic marine model. These results and approach provide an opportunity to isolate cancer stem cells and may improve cancer diagnosis and prognosis.

Cancer imaging

Cancer imaging is a very important visualization tools to enhance surgical vision, facilitate minimally invasive surgical procedures and alter surgical outcomes of oncological patients. Each year, nearly 13 million new cancer cases and 7.6 million cancer deaths occur worldwide [208] cancer diagnosis, treatment option, spreading of cancer to regional lymph nodes are challenging due to lock of clear surgical vision [209]. Technical advances that have enabled large scale imaging instruments such as PET-CT and MRI to impact pre-operative cancer diagnosis and staging. Nanomaterial based advanced cancer imaging leads to efficient approaches for early stage detection and treatment. The emergence of NIR fluorescence probes, Figure 1.11 and molecular agents that enhances soft tissue contrast, detection sensitivity and depth penetration. Nanoparticles based imaging system offers higher resolution images that enabled lesions to be detected down to sizes smaller than 10 µm which increased sensitivity and specificity of detection over human vision [210]. By administering non-specific optical agents [211,212] has included particle based probes like quantum dots [213], fluorescent dyes [214,215] to detect cancer bearing tissue very preciously.

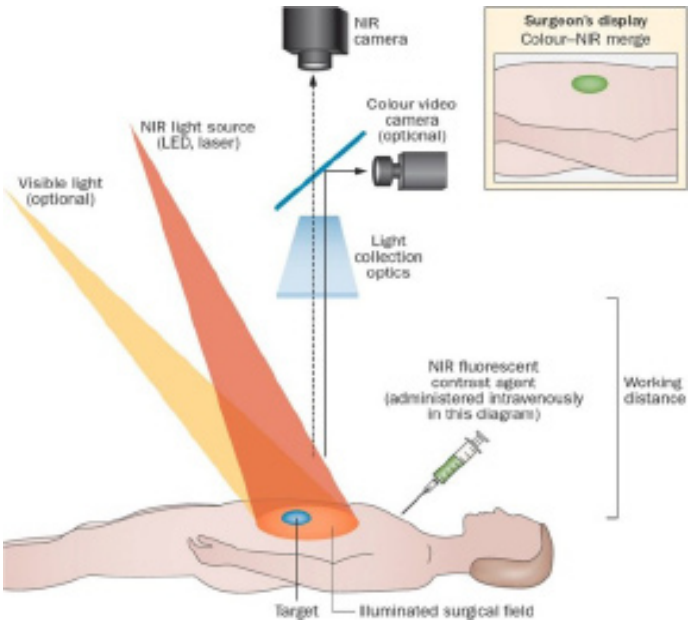


Figure 1.11: During surgery, an NIR optically-active agent is visualized using a fluorescence camera system. Current imaging systems operate at working distances that enable illumination of a sizable surgical field. LED, light-emitting diode (Reprinted with permission from Vahrmeijer et al, 2013) [218].

The emergence of NIR fluorescent nanoparticle designed to improve the sensitivity and reliability of lesion detection over the organic dyes has revealed exiting opportunity for probing and characterizing new molecular targets and novel biomarkers within the human subjects [216]. The nanoparticle couples advanced imaging devices have mainly focused on selective mapping of cancerous lymph nodes, precise identification of surgical borders, accurate detection and treatment of remnant disease and reliable assessment of tissue function. Particles in the triple modality imaging system, MR-photo acoustic –Raman imaging, has address surgical margins. Multispectral opto-acoustic tomography (MSOT) utilize multiple optical wavelengths and spectral demixing algorithms to permit imaging at depths which is greater than that of fluorescence imaging. This system was enhanced with the use of gold nanorods capable to detect a broad range of novel light absorption [217].

Therapy

Nanoparticles assisted chemotherapeutics

Chemotherapy is a clinical procedure to deliver cytotoxic drug that attack critical component of the cell in a non-specific manner. The agent of chemotherapy damages the DNA template, microtubules and inhibits DNA, RNA or protein. The lack of tumor specificity of chemotherapy seriously limits drug distribution and penetration of tumors and neutralizes the activity of drug agents. Doxorubicin was the first nanoparticle based chemotherapy, Figure 1.12(a) agent approved by the Food and Drug Administration (FDA). Nanoparticles have stable association with drug and carrier in circulation and release the active drug at the specified site at a satisfactory rate. Nanoparticle formulation enhances drug delivery to tumors with long circulation time to increase the number of potential passages through the tumor microvasculature. There is an urgency for the new treatment strategies for cancer using nanomaterials with chemotherapy.

Gold Nanoparticles (AuNps) are a versatile and nontoxic material which has attracted widespread interest for therapeutic applications in humans [219-221]. The unique quenching efficiencies of gold nanoparticles show that these particles are potential agent for treating malignant tumors [222-224]. Gold nanoparticles are biocompatible and accepts surface modifications [225-229]. The PEGylated colloid gold particles can effectively deliver the Tumor Necrosis Factor Alpha (TNFα) to tumor sites. Further, numerous gold nanoparticles based chemotherapy mediators such as Auroshell are striving for success in clinical trials [230-232]. Due to ease surface modification of nanoparticles as well as the physicochemical properties of nanoparticles leads to the development of drugs for efficient cancer treatments and targeting the tumor [233-236].

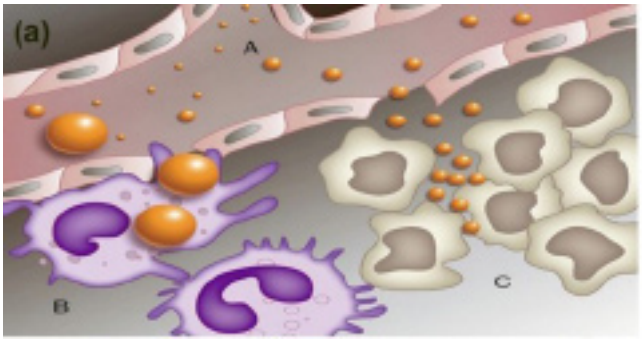
Recently hybrid AuNps have been synthesized using dicarboxylic terminated PEG by using a one-step technique [237,238]. Surface modified anticancer nano-carriers which cause no or minimal side effects and at the same time provide enhanced drug loading capacity and better blood circulation time are of great interest for cancer therapy. Chemotherapeutic drug efficacy at the tumor site is hindered either by poor bioavailability or dose limiting side effects. Various biocompatible polymers have been used for the surface decoration of nanoparticles to enhance their stability, cellular uptake and payload capacity. Gold nanoparticles assisted combination chemotherapy using anti-cancer drugs bleomycin and doxorubicin at optimized effective concentrations which act via different mechanisms thus

decreasing possibilities of development of the cancer drug resistance, reduction of systematic drug toxicity and the improvement of outcomes of chemotherapy [239].

Regard to drug loading, one of the imperative concerns for combination therapy based on chemotherapy, there are two main mechanisms to load drugs in nanoparticles non-covalent interaction and covalent interactions [240,241]. Even though, there is an advantages of unique functional drugs and nanocarriers which are having diverse chemical conjugations like ester bond, hydrazine cleaved at the tumor environment [242-244]. DOX is conjugated to methoxypoly (ethylene glycol)-β-poly (amidoamine) (MPEG-b-PAMAM) is easily cleave under acid environment of tumor sites. There have been several dual-drug-loaded nanomedicne under preclinical or clinical trials over the past few year such as irinotecan/floxuridine, paclitaxel, tanespimycin and cytarabine/daunorubicin [245,246].

Photodynamic therapy

Photodynamic therapies are new kind of cancer therapy attracted wide attention in nanomedicine. It was photosensitizing drug at the tumor site and activated by exposure of light at a specific wavelength. Upon light activation, the photosensitizer produce cytotoxic reactive oxygen and hydroxyl radicals leading to cancer cell death. PDT is a selective and minimally invasive and may be used repeatedly to the targeted site without causing incurred resistance. However, because the excitation source is near infrared light, their potential therapeutic outcomes are still heavily surface weighted. Recently, X-rays are used as an excitation source which permits deep tissue therapy [247]. X-ray assisted PDT can kill cells that are resistant to radiotherapy. It induces cell damage rather than radiation caused DNA damage. Further, it uses low dose of radiation with control over tumor within a few or even single treatment sessions. The radiation dose is very less which is less than 10 Gy and in the conventional method, the radiation dose is 60-80 Gy is often needed [248]. Irradiation time is 15-30 min for typical radiation therapy whereas in PDT it is about a minute which potentially reduces the toxicity with proper surface coating and by conjugating with a tumor targeting ligand, nanotranducers may accumulate in tumors with high efficiency. AuNps have been widely used to deliver photosensitizer agents for photodynamic therapy, Figure 1.12(b) of cancer. Significant advancement of nano assisted therapy is nowadays practically possible. More specifically, PEGylated gold nanoparticles provide aqueous compatibility and stealth properties for in-vivo use. Further, functionalization of AuNp surface with biological ligands to specifically target overexpressed receptors on the surface of cancer cells and the creation of nanorods and nanostones to enable combined PDT and photothermal therapies [249].



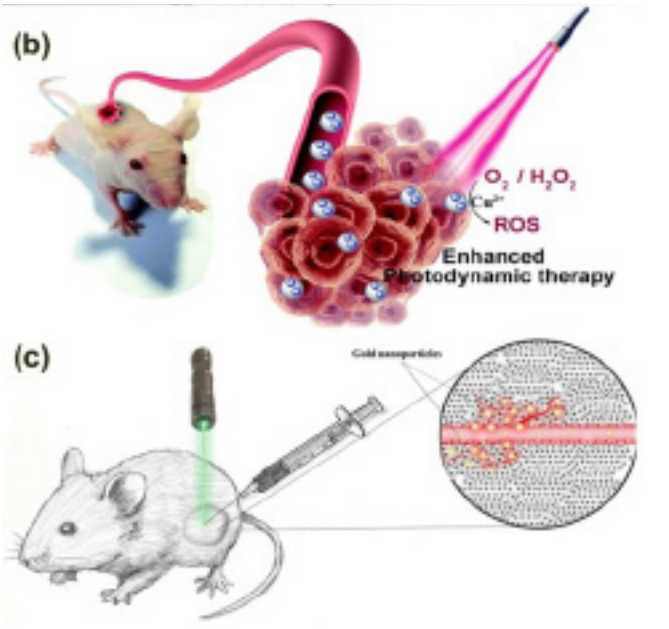


Figure 1.12: (a) Nanoparticle chemotherapeutic targeting is a size dependent process ranging from 10 to 100 nm in diameter [176].
(b) Nanoparticles penetrate leaky gap junctions between tumor endothelial cells and accumulate into tumor sites for photodynamic therapy [177].
(c) Nanoparticle therapeutics for non-small cell lung cancer for photothermal therapy [178].

Combination of positive surface charge with mitochondria targeting domain onto AuNps allowed their accumulation in the mitochondria of breast cancer cells to significantly elevate reactive oxygen species formation in 5-aminolevuclinic acid enabled photodynamic therapy and improve selective destruction of breast cancer cells. PDT is considered as a promising treatment modality which gives significant effectiveness and limited side effects, while numerous side effects are associated with chemotherapy [250]. Non-toxic photosensitizer is administered at the tumor site and it is illuminated with light which damages the tumor with the help of molecular oxygen [251]. Some of the photosensitizer agents used nowadays are methylene blue, hematoporphyrin, chlorins, photodithazine, curcumin, phthalocyanine and hypericin [252-254]. Type I or Type II reactions can result from an effective activation of Ps in the presence of molecular oxygen. Type-I reaction is related to a low oxygen level in treated tissues (neoplastic) and depends on the interaction between the triplet excited state of Ps (3Ps) and the treated tissues, which act as a substrate for 3 Ps. This reaction generates radicals, which are able to interact with oxygen to produce Reactive Oxygen Species (ROS). Generated ROS cause damage to the treated tissues and subsequently result in cell death [255,256]. Type II reaction is related to a high level of oxygen in treated tissues and depends on the direct interaction between triplets excited state of Ps (3Ps) and oxygen to generate single excited state of oxygen. The latter is a highly reactive and toxic molecule, able to readily damage treated tissues and cause subsequent cell death [257,258]. Nanocarriers used in drug-delivery systems are very capable of penetrating the cancer cells and eradicating drug-resistant cancer stem cells, yielding therapeutic efficiency of up to 100 fold against drug-resistant cancer in comparison with free drugs [259].

Gene therapy

Gene therapy, Figure 1.13 is a process of killing of tumor cells via transfection with Plasmid DNA (pDNA) that contains gene which produces a protein results in the apoptosis of cancerous cells. The introduction of pDNA alone at the tumor site is a very challenging task. The use of polymeric or inorganic nanoparticles which have physiochemical and biological properties allow them to carry pDNA into the tumor site. For target delivery, nanoparticles are functionalized with a specific chemical moieties which is capable of recognize the molecular marker on the surface of tumor cells. After binding, the tumor cells induces specific endocytosis by avoiding toxicity in healthy cells. Nanoparticles can carry nucleic acids as a therapeutic agents and preclinical efficiency in gene therapy is highly appreciated [260-263]. Liposomes have an excellent in vitro transfection capacity however, it shows less capable in vivo experiment due to agglomeration when blood protein interacts with liposomes.

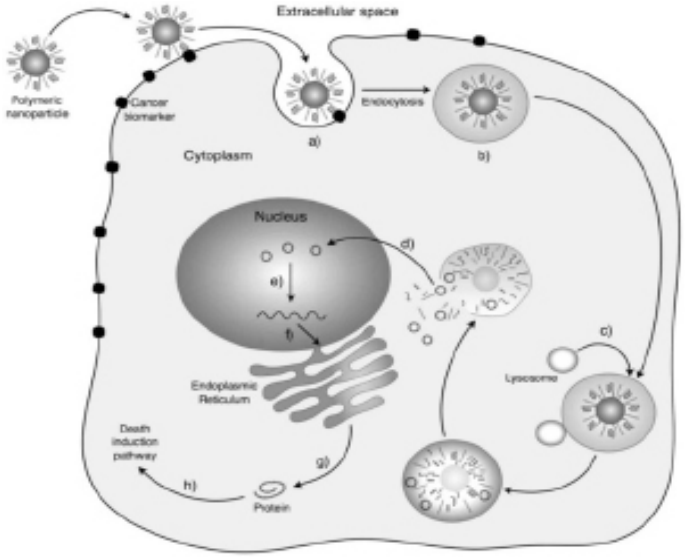


Figure 1.13: Mechanism of the death-induced gene therapy.
(a) The scFv on the surface of the nanosystem binds with the cancer biomarker onto the cell membrane.
(b) Ligand-mediated endocytosis takes place inside a vesicle.
(c) Biodegradation occurs, mediated by the endosomal cellular system, releasing all the nanosystem components in the cytoplasm.
(d) All the non-DNA elements of the nanosystem are eliminated from the cell or integrated into the natural cellular metabolism. Recombinant plasmid enters the nucleus by nuclear pores.
(e) Once the plasmid DNA is located inside the nucleus, DNA is transcribed into mRNA.
(f) This mRNA goes to the cytoplasm.
(g) The ribosomes traduced it into a protein.
(h) Protein induces cell death by different cellular pathways, Single-Stranded Variable Fragment (scFv) [264].

In contrast, polymeric and inorganic nanoparticles can be easily modified to be more stable, more reproducible and easily sterilize [265,266] and also they are considered as promising vectors for gene transfection [267,268]. Currently biodegradable cationic polymer nanoparticles are widely used for cancer treatment. The degradation of polymeric nanoparticles releases the plasmid into the cellular cytoplasm. Inorganic nanoparticles can be easily manipulated to obtain optimal conditions to improve transfection [269]. The inorganic nanoparticles are also used for gene therapy which includes carbon nanotubes [270], calcium phosphate [271], gold [272], silica [273] and magnetic nanoparticles [274]. In the use of magnetic nanoparticles, an external magnetic field should be applied to make a site specific deposition at tumor site. In gene therapy, the process of directing nucleic acids with the support of external magnetic field is known as magnetofection. To avoid the immediate recognition of nanomedicine by the immune system, the particles are must be deigned with some unique properties. If the size of the nanoparticles used for gene therapy is lower than 30 nm, then the nanoparticles are immediately discarded by the kidneys and liver from the circulatory system. The greatest challenge of cancer treatments is to selectively release the therapeutic agent in cancerous tissues. The nanosystem should be circulated through the circulatory blood systems avoiding natural elimination mechanisms of the host, such as opsonization and subsequent phagocytosis by macrophages. When functionalized nanoparticles binds with the tumor, protein receptors attached on the surface of cancer cells. This binding mechanism is called ligand.

The ligand activates different molecular pathways conducing to endocytosis. Ligand-mediated endocytosis increases the affinity and specificity of the nanosystem endocytosis [275]. The nanosystem is internalized in the cell into a vesicle generated by the cell membrane. The vesicle enters into the endosome cellular system where it is degraded. The nanosystem is released into the cytoplasm where it begins to degrade and it releases all its components into the medium. Nanosystem residues are removed from the cell by standard excretion mechanisms, or they are incorporated into cellular metabolic pathways depending on their nature [276]. The recombinant plasmid, which is the active substance, enters into the nucleus through the nuclear pores. Once inside, it is recognized by the transcription system, generating the mRNA of the death-induced gene. This mRNA goes from the nucleus to the cytoplasm and it is translated into protein. These proteins trigger different cellular pathways ending in cellular death [277].

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