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Nanotechnology: An introduction to future drug delivery system

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Abstract

Nanoparticles are capable of self-assembly and maintaining stability and specificity, which are crucial to drug encapsulation and biocompatibility. Recent progress in cancer nanotechnology raises exciting opportunities in which diagnosis and treatment are based on the molecular profiles of individual patients. The present review article elaborates various nanocarriers like nanotubes, quantum dots, nanoshells, liposomes, dendrimers, etc. and special emphasis on the recent advances of nanotechnology.

Keywords: Nanotechnology, cancer treatment, nanocarriers, nanomedicines.

Introduction

The prefix ‘nano’ comes from the Greek word ‘nanos’ meaning ‘a dwarf’. Hence, ‘nanotechnology’ might well simply mean a technology to do with ‘small’ things. Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger scale. Nanotechnologies are the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale. The size range of interest between a few nanometers and 100 nm is one where many interesting things happen[1]. All sorts of physical properties change and many biological systems function in this length scale. This technology has enabled the development of nanoscale devices that can be conjugated with several functional molecules simultaneously, including tumor-specific ligands, antibodies, anticancer drugs, and imaging probes. Since these nanodevices are 100 to 1,000-fold smaller than cancer cells, they

can be easily transferred through leaky blood vessels and interact with targeted tumor-specific proteins both on the surface of and inside cancer cells. Therefore, their applications as cancer cell-specific delivery vehicles are significant addition to the currently available armory for cancer therapeutics and imaging. Cancer is one of the major causes of mortality in the world and the worldwide incidence of cancer continues to increase. The most common cancer treatments are limited to chemotherapy, radiation, and surgery. Frequent challenges encountered by current cancer therapies include nonspecific systemic distribution of anti tumor agents, inadequate drug concentrations reaching the tumor, and the limited ability to monitor therapeutic responses. Poor drug delivery to the target site leads to significant complications, such as multidrug resistance. Greater targeting selectivity and better delivery efficiency are the 2 major goals in the development of therapeutic agents or imaging contrast formulations. A rational approach to achieve these goals is to conjugate therapeutic drugs with monoclonal antibodies (mAbs) or other ligands that selectively bind to antigens or receptors that are usually abundantly or uniquely expressed on the tumor cell surface. The development of tumor-targeted contrast agents based on a nanoparticle formulation may offer enhanced sensitivity and specificity for in vivo tumor imaging using currently available clinical imaging modalities. By applying a vast and diverse array of nanoparticles, whose design derives from the engineering, chemistry, and medicine fields, to molecular imaging and targeted therapy, cancer nanotechnology promises solutions to several of the current obstacles facing cancer therapies[2-9].

Nanotechnology and Nanomedicine has exploited the possibility of designing tumor-targeted nanocarriers able to deliver radionuclide payloads in a selective manner to improve the efficacy and safety of cancer imaging and therapy. The major nanocarriers include nanopores, carbon nanotube, nanoparticles, dendrimers, quantum dots, cantilevers**,** liposomes and s. In addition, the combining of tumor specific multifunctional and multimodality nanocarriers will hopefully achieve earlier tumor detection and better tumor treatment.

Nanotubes: Nanotubes are smaller than Nanopores. Nanotubes & carbon rods, about half the diameter of a molecule of DNA, also help to identify DNA changes associated with cancer cells. It helps to exactly pin point location of the changes. Mutated regions associated with cancer are first tagged with bulky molecules. Using a nano tube tip, resembling the needle on a record player, the physical shape of the DNA can be traced. A computer translates this information into topographical map. The bulky molecules identify the regions on the map where mutations are present. Since the location of mutations can influence the effects they have on a cell, these techniques are important in predicting disease.

Quantum Dotes (QD): These are tiny crystals that glow when these are stimulated by ultraviolet light. The latex beads filled with these crystals when stimulated by light, emit the color that lights up the sequence of interest. By combining different sized quantum dotes within a single bead, probes can be created that release a distinct spectrum of various colors and intensities of lights, serving as sort of spectral bar code.

Nanoshells (NS): These are another recent invention. NS are miniscule beads coated with gold. By manipulating the thickness of the layers making up the NS, the beads can be designed that absorb specific wavelength of light. The most useful nanoshells are those that absorb near infrared light that can easily penetrate several centimeters in human tissues. Absorption of light

by nanoshells creates an intense heat that is lethal to cells. Nanoshells can be linked to antibodies that recognize cancer cells. In laboratory cultures, the heat generated by the light-absorbing nanoshells has successfully killed tumor cells while leaving neighboring cells intact.

Liposomes: Liposomes are self-assembling, spherical, closed colloidal structures composed of lipid bilayers that surround a central aqueous space. Liposomal formulations have shown an ability to improve the pharmacokinetics and pharmacodynamics of associated drugs. Liposome based formulations of several anticancer agents have been approved for the treatment of metastatic breast cancer and Kaposi's sarcoma.[10-18]

Cantilevers: Tiny bars anchored at one end can be engineered to bind to molecules associated with cancer. These molecules may bind to altered DNA proteins that are present in certain types of cancer monitoring the bending of cantilevers; it would be possible to tell whether the cancer molecules are present and hence detect early molecular events in the development of cancer cells[19].

Dendrimer: A number of nanoparticles that will facilitate drug delivery are being developed. One such molecule that has potential to link treatment with detection and diagnostic is known as dendrimer. These have branching shape which gives them vast amounts of surface area to which therapeutic agents or other biologically active molecules can be attached. A single dendrimer can carry a molecule that recognizes cancer cells, a therapeutic agent to kill those cells and a molecule that recognizes the signals of cell death. It is hoped that dendrimers can be manipulated to release their contents only in the presence of certain trigger molecules associated with cancer. Following drug releases, the dendrimers may also report back whether they are successfully killing their targets[20].

Nanopores: Nanopores (holes) allow DNA to pass through one strand at a time and hence DNA sequencing can be made more efficient. Thus the shape and electrical properties of each base on the strand can be monitored. As these properties are unique for each of the four bases that make up the genetic code, the passage of DNA through a nano pore can be used to decipher the encoded information, including errors in the code known to be associated with cancer[21].

Recent advances in nanotechnology in cancer Treatment

Fluorescent Nanoparticles: The diagnosis and treatment of cancer have been greatly improved with the recent developments in nanotechnology. One of the promising nanoscale tools for cancer diagnosis is fluorescent nanoparticles (NPs), such as organic dye-doped NPs, quantum dots and upconversion NPs that enable highly sensitive optical imaging of cancer at cellular and animal level. Furthermore, the emerging development of novel multi-functional NPs, which can be conjugated with several functional molecules simultaneously including targeting moieties, therapeutic agents and imaging probes, provides new potentials for clinical therapies and diagnostics and undoubtedly will play a critical role in cancer therapy. In this article, we review the types and characteristics of fluorescent NPs, in vitro and in vivo imaging of cancer using fluorescent NPs and multi-functional NPs for imaging-guided cancer therapy[22].

Monoclonal antibodies and Nanobodies: In the past decades, the mainstay of systemic therapy for solid and haematological malignancies was chemotherapy; nevertheless this modality has the drawbacks such as drug resistance and eliciting sever cytotoxicity in the normal tissue. To resolve such downsides, the cancer therapy modalities need to be advanced with more effective and tolerable treatments to specifically target the malignant cell with minimal adverse consequences. In fact, characteristically, the malignant diseases are self sufficiency in growth signals along with insensitivity to growth inhibition. They can also evade from apoptosis, have limitless replicative potential, induce angiogenesis and possess metastasis potential. Given that the most of these characteristics are often due to genetic defects, thus key to the development of targeted therapies is the ability to use such processes to phenotypically distinguish the tumor from its normal counterpart by its specific/selective markers. The therapeutic monoclonal antibodies (mAbs) are deemed to be a class of novel agents that can specifically target and disrupt molecular pathways underlying tumorigenesis. The mAbs are produced by a single clone of B-cells, and are monospecific and homogeneous. Since Kohler and Milstein heralded a new era in antibody research and clinical development by the discovery of hybridoma technology in 1975, more than 20 mAbs have been approved by the US Food and Drug Administration (FDA) for treatment of obdurate diseases, including different types of cancers. Mouse hybridomas were the first reliable source of monoclonal antibodies which were developed for several in vivo therapeutic applications. Accordingly, the recombinant antibodies have been reduced in size, rebuilt into multivalent molecules and fused with different moieties such as radionuclides, toxins and enzymes. The emergence of recombinant technologies, transgenic animals and phage display technology has revolutionized the selection, humanization and production of antibodies. This review focuses on implementation of the mAbs and nanobodies fragments for cancer therapy[23.]

Nanomedicine: Nanotechnology has been extensively merging into biomedical research to develop a new research field. Nanobiomedicine. It provides a unique approach and comprehensive technology against cancer by early diagnosis, prediction, prevention, personalized therapy and medicine[24]. Matters et al[25] studied the effects of gastrin messenger RNA (mRNA) down-regulation on growth of human pancreatic cancer. Gastrin expression was examined in human pancreatic cancer cell lines by reverse transcriptase-polymerase chain reaction, and peptide expression was assessed by immunocytochemistry. Gastrin was down-regulated using either stable transfection of an antisense gastrin cDNA or 1 of 3 shRNA (short hairpin RNA) constructs. Stable transfection of gastrin antisense or shRNAs into BxPC-3 cells resulted in clones with more than 90% reduction in gastrin mRNA. Immunofluorescence analysis confirmed that gastrin peptide levels were decreased in antisense and shRNA tumors.

Nanomicelles: Emerging nanotechnology has already developed various innovative nanomedicines. Nanomicelles, self-assemblies of block copolymers, are promising nanomedicines for targeted drug delivery and imaging. Stimulus-responsive targeted nanomicelles are designed to release drugs based on stimuli such as pH, temperature, redox potential, magnetism and ultrasound. This article will focus on recent advancements in the design of stimulus-responsive targeted nanomicelles loaded with anticancer drugs to fulfill the challenges associated with cancer cells (e.g., multidrug resistance) for the effective treatment of cancer[26].

Thermoresponsive polymer-coated magnetic nanoparticles: Thermoresponsive polymer-coated magnetic nanoparticles loaded with anti-cancer drugs are of considerable interest for novel multi-modal cancer therapies. Such nanoparticles can be used for magnetic drug targeting followed by simultaneous hyperthermia and drug release. Purushotham S et al synthesized Gamma-Fe(2)O(3) iron oxide magnetic nanoparticles (MNP) with average sizes of 14, 19 and 43 nm. Composite magnetic nanoparticles (CNP) of 43 nm MNP coated with the thermoresponsive polymer poly-n-isopropylacrylamide (PNIPAM) were prepared by dispersion polymerization of n-isopropylacrylamide monomer in the presence of the MNP. In vitro drug release of doxorubicin-(dox) loaded dehydrated CNP at temperatures below and above the lower critical solution temperature of PNIPAM (34 degrees C) revealed a weak dependence of drug release on swelling behavior. The particles displayed Fickian diffusion release kinetics; the maximum dox release at 42 degrees C after 101 h was 41%. In vitro simultaneous hyperthermia and drug release of therapeutically relevant quantities of dox was achieved[27].

Carbon Nanotubes: A vast majority of applications are based on CNTs, ranging from miniaturized biosensors to organ regeneration. Nevertheless, the complexity of biological systems poses a significant challenge in developing CNT-based tissue engineering applications. This review focuses on the recent developments of CNT-based tissue engineering, where the interaction between living cells/tissues and the nanotubes have been transformed into a variety of novel techniques. This integration has already resulted in a revaluation of tissue engineering and organ regeneration techniques. Some of the new treatments that were not possible previously become reachable now. Because of the advent of surface chemistry, the CNT's biocompatibility has been significantly improved, making it possible to serve as tissue scaffolding materials to enhance the organ regeneration. The superior mechanic strength and chemical inert also makes it ideal for blood compatible applications, especially for cardiopulmonary bypass surgery. The applications of CNTs in these cardiovascular surgeries led to a remarkable improvement in mechanical strength of implanted catheters and reduced thrombogenicity after surgery. Moreover, the functionalized CNTs have been extensively explored for in vivo targeted drug or gene delivery, which could potentially improve the efficiency of many cancer treatments[28].

Functional analyses of water-dispersed carbon nanohorns with antitumor activity were performed to explore their potential as a drug carrier for local cancer chemotherapy. Water-dispersed carbon nanohorns were prepared by adsorption of polyethylene glycol-doxorubicin conjugate (PEG-DXR) onto oxidized single-wall carbon nanohorns (oxSWNHs). PEG-DXR-bound oxSWNHs were administered intratumorally to lung cancer-cell NCI-H460-bearing mice. When injected intratumorally, PEG-DXR-bound oxSWNHs caused significant retardation of tumor growth associated with prolonged DXR retention in the tumor. In accordance with this DXR retention, a large number of oxSWNH agglomerates was found in the periphery of the tumor. Histological analyses showed migration of oxSWNHs to the axillary lymph node, which is a major site of breast cancer metastasis near the tumor, possibly by means of interstitial lymphatic-fluid transport. These results suggest that water-dispersed oxSWNHs may thus be useful as a drug carrier for local chemotherapy[29]].Hampel,et al[30] prescribed CNTs as feasible carriers for carboplatin, a therapeutic agent for cancer treatment. The drug was introduced into CNTs to demonstrate that they are suited as nanocontainers and nanocarriers and can release the drug to initialize its medical virtue. The filling was accomplished by a wet-chemical approach after the CNTs were opened. The effect on cell proliferation and cytotoxicity of the carboplatin-filled

CNTS was investigated by using viability assays. Using different analysis methods such as electron energy loss spectroscopy and x-ray photoelectron spectroscopy the structure of carboplatin incorporated into the CNTs was found to be retained. In vitro studies showed that carboplatin-filled CNTs inhibited growth of bladder cancer cells whereas unfilled, opened CNTs barely affected cancer cell growth[31].

Gold Nanoparticles: Nanotechnology has been used to provide advanced biomedical research tools in diagnostic imaging and therapy, which requires targeting of nanoparticles (NPs) to individual cells and subcellular compartments. However, a complete understanding of the intracellular uptake, transport, and subcellular distribution of nanostructured materials remains limited. Hence, gold NPs were explored as a model system to study the intracellular behavior of NPs in real time. Our results show that the cellular uptake of gold NPs is dependent on their size and surface properties. The NPs were transported in vesicles of 300-500 nm diameter within the cytoplasm. The average velocity and diffusion coefficient of the vesicles containing NPs were 10.2 (+/-1.8) microm/hr and 3.33 (+/-0.52) microm 2/hr, respectively. Analysis of the time-dependent intracellular spatial distribution of the NPs demonstrated that they reside in lysosomes (final degrading organelles) within 40 minutes of incubation. These findings can be used to tailor nanoscale devices for effective cell targeting and delivery[32-33].

Radioprotection by nanoparticles: Radiolysis of water generates a series of its radical decomposition products that inactivate enzymes and damage cellular lipids, proteins and DNA. Postirradiation protection is another approach to reduce or reverse deleterious effects after exposure to ionizing radiation[34]. After World War II there was a great interest in developing chemicals, so-called radioprotectors, to protect humans from harmful effects of radiation. Already in 1949 it was shown that rats pretreated with the amino acid cysteine were protected against lethal doses of X-rays[35] Later it has been found that supplementation with antioxidants[36], selenium compounds [37] and a cytoprotective adjuvant amifostine [38] and[39] alters cell radiosensitivity.

Recent studies also show radioprotective effect of various nanoparticles. Amifostine is not effective when given orally. However, amifostine containing copolymer nanoparticles, prepared using spray drying technique, have been shown to protect mice against injury induced by whole body gamma irradiation after oral administration of the nanoparticles [40]. When encapsulated in transferrin-coupled liposomes neuroprotective agent citicoline exhibits radioprotective effect in human ovarian adenocarcinoma cells overexpressing transferrin receptor. Such effect is considerably smaller in endothelial cells. However, free citicoline was found to be less radioprotective in the ovarian adenocarcinoma cells than in the endothelial cells [41]. Carbon nanoparticles also exhibit radioprotective effect. Fullerenes (C60) diminished toxicity of radiation on zebra fish embryos by reducing generation of reactive oxygen species [42]. Fulleronols (C60[OH]X) protected unicellular eukaryotes organisms against gamma radiation [43]. Cerium oxide nanoparticles (nanoceria) increased longevity of cells by reducing hydrogen peroxide and ultraviolet radiation induced injury[44]. Autoregenerative reaction cycle Ce3+ ⇔ Ce4+ occurs on the surface of the ceria nanoparticles: changing oxidation state from Ce3+ to Ce4+ might scavenge free radicals produced by radiation [44].

Monitoring toxicity in real time using novel impedance technique

Interaction of mammalian cells with surfaces and focus on the kinetic aspects of this phenomenon is of great interest for science. Cell attachment is an important parameter to assess cancer cell potential for metastasis and tumour healing caused by their dynamic interaction with substrates and drugs. Most commonly cell behaviour is studied by imposing an effect to attached cells and quantifying cell density, morphology and number resisting to this treatment by microscopic or ultrastructural techniques. Non-destructive methods to monitor cell responses in real time are also available. Array of microelecrodes in chambers is used to record electrophysiological activity of cells[45]and[46]. Bioelectrical potential of primary isolated epithelial cells was studied to determine whether the cells can maintain epithelial structure and function when isolated from mesenchymal framework [47]. In another study, cell-to-cell communications were studied by injecting a dye Lucifer yellow to a single cell through a microelectrode: electrical characteristics of the cells were measured with the electrode and transfer of the dye from the single cell to its neighbours by cell-to-cell gap junctions was observed in cell monolayers [48]. The method that applies external electrical field to sense cell spreading upon artificial surfaces in real time is referred to as Electric Cell-substrate Impedance Sensing (ECIS) with frequencies 400 Hz, 4 kHz and 40 kHz being used in most experiments 38. Although the ECIS technique has been first described by Giaever and Keese [49], it can still be considered novel. When a cell attaches to a small gold electrode, it reduces the detecting area in contact with culture medium and measured electric impedance increases because the cell membranes gradually block the current flow. Amount and type of protein coating the gold surface of the electrode are one factor effecting cell attachment, and protein type is significant[50]. When cells cover a gold electrode, various cell lines will attach at different rates; this in turn will be affected by exposing the cells to a treatment. From the impedance changes, several important properties of the cell layer can be determined such as barrier function, membrane capacitance, cell proliferation, motility and motion [51]. Use of ECIS to study cytotoxicity to chemical compounds experiences growing interest [52] and [53]. Dynamics of cell invasiveness can also be studied looking at how cells with different metastatic potentials affect confluent monolayer of for example endothelial cells[54]. Measurements using ECIS of repopulation of mechanically disrupted cells in culture have been as well suggested as wound healing assay[55].

The ECIS technique is becoming now a well-established technique to study chemical and physical factors and other dynamic processes [56]. Recently the first paper on the use of this technique to assess toxicity effects of quantum dots was published[57]. Concentration to achieve 50% inhibition was determined in fibroblast V79 cells for free metals and quantum dots: around 6 μM for Cd, 98 μM for Te, 140 μM for Zn, 154 nm for red CdSe/ZnS and 240 nM for green CdSe/ZnS[58]. For the cadmium selenide and telluride quantum dots, toxicity could be assigned to release of free cadmium. The quantum dots synthesized with indium gallium phosphide and gold nanoparticles were not cytotoxic for concentrations studied up to 200 nM and 45 μM, respectively[58].

Conclusion

More effective delivery of nanoparticles has resulted in the development of novel methods to treat cancer. Nanoparticle systems are able to target various portions of the tumor using specific targeting moieties and evade the problems associatedwithmulti-drug resistance. Furthermore,

formany of the targetingmoieties, there is a large overlap in the types of targetsproviding synergistic antitumor effects. The search for more molecular targets will advance the ability to improve delivery at the tumor level while decreasing toxicity to normal tissue.

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