Prof. S. Kannaiyan Memorial Oration

Nanovectors and Drug delivery Systems in Cancer Therapy

Delivered by

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Respected Chairperson of the Biodiversity Authority of India, MOEFC, Hon'ble Vice Chancellor Prof. Dr. A. Ganapathi, Most beloved President, Secretary, Vice President, Office bearers and Life Members of the National Academy of Biological Sciences, Organizing committee members, Distinguished Scientists, Professors and Faculty members from various Institutions, administrative staff, research scholars, students and friends, representative of press and electronic media, Ladies and Gentlemen! Very good morning to all. It gives me an immense pleasure to deliver 'Prof. S. Kannaiyan Memorial Oration' in the 10th NABS – National Conference on '*Recent Trends in Life Science: Research, Practices and Application for Sustainable Development*' at Bharathiar University today. Thank you very much for inviting and selecting me for the prestigious 'Prof. S. Kannaiyan Memorial Award 2017'.

1.0. Introduction

Modern medicine is the product of our greater understanding of biological processes and it's entirely dependent on technological advancements to uncover and explore the deeper understanding. Nanotechnology takes this enterprise to the sub-microscopic level, with tools, such as nanoparticles, being developed at the sub-cellular level (< 100 nm). Nanomedicine refers to the use of nanostructures for the diagnosis and treatment of medical diseases. The specific route of administration (e.g., oral, intravascular, or intratumoral) is chosen according to which method will most safely and effectively deliver nanostructures to the target organ where they can exert their desired effects. Nanostructures have the potential to play a critical role in the future of medicine by serving as carriers for drugs, genes, and imaging agents that will bind to targets on injured or neoplastic tissue.

The National Nanotechnology Initiative was established in 2001 and since then it has received greater attention worldwide. Organic and inorganic

nanostructures that interface with biological systems have attracted widespread interest in the fields of biology and medicine. For instance, nanoparticles that are novel intravascular or cellular probes are being developed for diagnostic (imaging) and therapeutic (drug/gene delivery) purposes. These agents could play a critical role in the future of medicine, especially in the areas of target-specific drug/gene delivery, early diagnosis, and disease treatment. Nanostructures are well suited for applications in drug delivery, imaging, and early characterization and therapy of brain injuries due to their unique characteristics such as amphiphilic and superparamagnetic.

The application of nanotechnology in medicine, more specifically for targeted drug delivery, is set to spread rapidly and to be adopted in neuroscience and neurological surgery. Currently, many substances are under investigation for drug delivery to the brain - nanoparticles for drug delivery may include biological substances such as albumin, gelatin and phospholipids for liposomes, synthetic substances such as polymers, and solid metal particles.

The cellular toxicity of nanoparticles depends on their composition. Many nanostructures incorporate the MRI contrast agent and heavy metal gadolinium, which is thought to cause nephrogenic systemic fibrosis or nephrogenic fibrosing dermopathy in patients with poor renal function. Exposure of normal tissue to nanoparticle effects may be reduced by altering the technique of nanoparticle delivery. Nanoparticles provide enhanced signal by nature of their inherent properties or attached moieties, distribution of nanoparticles within an organ can be tracked on real-time imaging studies, providing feedback that directs adjustments in infusion rate and volume, which should improve matching of the distribution to the nanoparticles to the distribution of the tumor.

The ability of some nanoparticles to cross the blood–brain barrier may open new avenues for delivery of drugs to the brain. After crossing the blood–brain barrier (BBB), the nanoscale size of the particles promotes transport into the cell and cellular compartments including the nucleus. Nano-immunology, the use of nanoparticles conjugated to antibodies or bound to immune cells, can direct delivery of nanoparticles to tumor markers on target tissues, thus decreasing systemic exposure to nanoparticles. The capability to design better nanovectors will stimulate development of new agents that provide highly targeted nanotherapy to diseased tissue.

Currently, nanovectors and drug delivery systems are interest in worldwide. It describes the potential benefits and safety concerns of nanoparticles and nanomaterials and describes their present and future use in clinical medicine. The extent of application of nanomedicine to the treatment of diseases of the Central Nervous System (CNS) will depend on further advances being made in nanotechnology and image-guided therapy. The ability to design nanoparticles with a variety of unique targeting, imaging, and therapeutic components is well suited to the coming era of personalized medicine (potentially including the use of gene and siRNA therapy).

2.0. Nanoscale platforms

2.1. Polymeric nanoparticles

Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometer-size promotes effective permeation through cell membranes and stability in the blood stream. Polymers are being developed to create delivery systems with excellent drug and protein loading and release properties, a long shelf life, and little toxicity. Amphiphilic block or graft

copolymers assemble spontaneously into polymeric nanoconjugates in aqueous solution. Polymers, such as poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactic glycolic acid (PLGA), poly(ε-caprolactone), polyglutamic acid, and polymalic acid, and their copolymers have been the most extensively used. Due to the proven biocompatibility, these polymers have been used in surgery for the past three decades. Further, several biodegradable polymers are suitable for preparing nano-sized particles for drug delivery applications. The degradation drug release rate of these polymers can be controlled by adjusting their molecular mass and, in the case of copolymers, their composition and microstructure.

2.2. Polymeric nanoconjugates

Polymeric nanoconjugates are highly innovative technologies in nanomedicine which are generally synthesized around a polymer with pendant functional groups like –OH, –COOH, or –NH_2. These agents bear numerous functional groups that are available for covalent binding to a variety of biochemically active groups, which direct them to malignant tumors where they can deliver functional drugs acting on several tumor targets (e.g., mRNA and/or protein). Nanoconjugates that carry more than one functional group provide the capability to simultaneously inhibit several tumor pathways, deliver optimal drug concentrations to the site of treatment, and reduce adverse effects on healthy tissue.

2.3. Micelles

Micelles are amphiphilic spherical structures composed of a hydrophobic core and a hydrophilic shell. The hydrophilic shell stabilizes the micelle in an aqueous environment for intravenous delivery and the hydrophobic core stores a payload of drug for therapy. Due to their nanoscale dimensions (diameter less than 50 nm) and their hydrophilic shell, polymeric micelles resist elimination by the

reticuloendothelial system, which increases their circulation time and ability to deliver drug to the target.

Polymeric micelles are highly stable *in vitro* and *in vivo*, are very biocompatible, and can dissolve a broad variety of poorly soluble pharmaceuticals; several types of drug-loaded micelles are currently being tested in preclinical and clinical trials. Among polymeric micelles, a special group is formed by lipid-core micelles, i.e., micelles formed by conjugates of soluble copolymers with lipids (such as polyethylene glycol-phosphatidyl ethanolamine conjugate). Tumor may be targeted with micelles by exploiting the enhanced permeability and retention (EPR) effect, by making micelles of stimuli-responsive amphiphilic block copolymers, or by attaching specific targeting ligand molecules to the micelle surface.

2.4. Peptides and proteins

Peptides and proteins have better defined chemical compositions and molecular weights than most nanomaterials. Large-scale production of peptides and proteins has become routine in industry. Discovery of novel peptides and proteins sets in motion an industrial effort to rapidly produce suitable quantities of pharmaceutical-grade product, which is suitable for rapid biological and pharmaceutical testing and eventual clinical application. Peptides interact non-specifically with cell membrane components and specifically with various cellular receptors. Peptides that specifically interact with certain receptors overexpressed by cancer cells have been successfully developed as targeting molecules for drug delivery and *in vivo* imaging.

2.5. Metal complexes

The use of metals as contrast agents is currently being investigated. Iron oxide-based nanoparticle MRI contrast agents have in particular shown great promise. Iron oxide-based nanoparticles are super-paramagnetic, having a magnetic moment that can be changed by ambient thermal energy. Superparamagnetic iron oxide contrast agents either form the core of magnetic nanoparticles that have a polymeric coating or are more homogeneously integrated into polymeric nanoparticles. Gold nanoparticles (AuNPs) have also been used for cancer cell imaging and targeting. AuNPs are very attractive nanoscale agents as they are biocompatible, may naturally emit radiation, and have high surface reactivity.

2.6. Superparamagnetic iron oxide nanoparticles

Imaging techniques that can selectively image proliferating cells *in vivo* can provide critically important insights into tumor growth rate, degree of tumor angiogenesis, effectiveness of treatment, and vigor of normal cells. Researchers have recently demonstrated the use of superparamagnetic iron oxide (SPIO) nanoparticles to image neovasculature in glioma animal models and to image stem cells *in vivo* and *in vitro*.

2.7. Endohedral metallofullerene nanoparticles

Endohedral metallofullerenes have attracted considerable interest over the past decade. These particles are able to transfer electrons from encapsulated metal atoms to the fullerene cage. Metal fullerene cages solubilize metallic agents and prepare them for use in MRI applications.

3.0. Carbon nano platforms

3.1. Carbon nanotubes

Carbon nanotubes (CNTs) can be fabricated into biodegradable nanostructures (cylindrical buckytubes) which are currently being used for nanomedicine and tissue engineering studies as nanovectors. They are synthesized by rolling sheets of carbon into hollow tubes that are single-walled (0.4 to 2 nm diameter), double-walled (1 to 3.5 nm diameter), or multi-walled (2 to 100 nm diameter). CNT size and composition must be carefully controlled to promote intracellular delivery of these nanotubes and to prevent immune reaction to them.

3.2. Fullerenes

Fullerenes are a family of carbon allotropic compounds that were predicted to exist in 1970 and officially discovered in 1985. The most common form is C_{60}. Their landmark discovery has since led to synthesis of fullerene derivatives, such as C_{61}-butylic acid. Hydrated C(60) fullerene protects against damage from X-ray irradiation (7 Gy) *in vitro* and *in vivo* in mice by reducing the formation of reactive oxygen species (ROS). Research continues into ways to increase the solubility of fullerenes and to investigate the toxicity of fullerenes and their derived compounds.

3.3. Nanodiamonds

Compared to other carbon nanomaterials, nanodiamonds (NDs) are attractive agents for use in biological and medical applications largely due to their greater biocompatibility, stable photoluminescence, ease of purification, commercial availability, and minimal cytotoxicity. Nanodiamonds can be functionalized and conjugated to a variety of molecules for the purpose of cell labeling and drug delivery. Adding certain functional groups to NDs can improve their solubility,

direct them to specific binding sites on target cells and tissues, and reduce their effects on normal tissues.

3.4. Dendrimers

Dendrimers are one of the most versatile and extensively studied nanostructure carrier systems. Dendrimers are highly complex molecules with a core, branches, and end groups. Dendrimers have been studied extensively for targeting and delivery of therapeutic agents for cancer and of contrast agents for magnetic resonance imaging. Biocompatible dendrimers have been used as delivery systems for potent drugs, such as cisplatin and doxorubicin, in cancer treatment. The surface chemistry of these materials can be modified relatively easily to include ligands (molecules that attach to cell surface receptors) that can be used to target the dendrimer to tumor tissue.

4.0. Applications

4.1. Cancer diagnosis and treatment

Scientists have focused on improving nanoscale drug delivery systems, such as liposomes, gelatin nanoparticles, polymeric nanoconjugates, and micelles, and on the development of new nanoscale platforms (e.g., quantum dots, nanoshells, gold nanoparticles, paramagnetic nanoparticles and carbon nanotubes). The overarching goal of this research is to deliver imaging probes and therapeutics in high concentration to the tumor. Novel diagnostic and therapeutic agents for use in oncology will be created using nanotechnology.

Immunoassays that detect the presence of tumor markers are one application of nanotechnology in oncology. A sensitive and specific immunoassay for the detection of human alpha-fetoprotein (AFP), a tumor marker seen with

hepatocellular carcinoma, has been developed that uses Ag/SiO_2 core-shell nanoparticles, which are embedded with rhodamine B isothiocyanate dye molecules as Raman tags. Silica-coated magnetic nanoparticles with modified amino groups make up the immobilization matrix and separation tool. The novel nanostructure Raman tags have a very high stability compared to traditional tags and the use of the silica-coated magnetic nanoparticles as an immobilization matrix and separation tool is simpler than traditional techniques. This strategy resulted in detection of human AFP at concentrations as low as 11.5 pg/mL.

4.2. Nanoconjugate concept for cancer therapy

Mechanisms of multiple drug resistance and impact of normal tissues ie., non-specific target are the major drawbacks for the cancer chemotherapy. Nanoconjugates can surmount these drawbacks of classical chemotherapy because they can be designed for (i) sustained release of drug, (ii) passive enhanced permeability (EPR) effect-based targeting of macromolecules to tumor tissue, (iii) ligand-based targeting of cell surface antigens and modules active in endosomal uptake and membrane disruption, (iv) drug release into the cytoplasm, and (v) protection from enzymatic degradation. Polymers as platforms for delivering agents into tumor cells have increasingly gained importance because they are unaffected by the multidrug resistance (MDR) effect, have minimal immunogenicity and are able to maintain effectiveness with each cycle of tumor treatment.

4.3. Blood–brain barrier

Drug delivery to the brain continues to be one of the most significant challenges of modern neuromedicine. For drugs to reverse pathologic changes in the CNS, they must be able to traverse the nearly impervious blood–brain barrier

(BBB). Unlike other capillaries in the body, the capillaries of the BBB are extraordinarily selective in permeability; only hydrophobic, non-toxic, and uncharged molecules can pass through the BBB along a diffusion gradient. A nanoparticle drug complex could be effective against CNS disease if it could pass through the BBB, find the CNS lesion, target tumor cells specifically, and release a payload of therapeutic agent without altering the vital functions of the CNS. To deliver molecules across the BBB, a few invasive and non-invasive methods have been developed and studied, but their clinical effectiveness has not exceeded that of current treatment methods. These methods include lipidization, temporary alteration of the BBB, invasive delivery, convection-enhanced delivery (CED), and active/facilitated cell transport.

Nanotechnology may offer solutions to CNS drug delivery problems because (i) the size of the molecular cargo and the carrying complex can easily be controlled and optimized for drug delivery to the CNS; (ii) nanoscale technologies offer specificity to site of action, creating drug targeting that is precise enough to avoid damage to the delicate CNS structures; and (iii) the requirement of lipid solubility can be circumvented by using microemulsions of nanoparticle complexes in oil that can cross the BBB.

Nanotechnology may also have a role in improving delivery of boron to brain tumors and in enhancing the effectiveness of neutron capture therapy. Boron neutron capture therapy (BNCT) involves the initiation of nuclear reactions in the presence of boron-10 and free low-energy thermal neutrons. Boron-10 captures these neutrons to yield high linear energy transfer (LET) alpha particles and recoiling lithium-7 nuclei, which, in turn, kill tumor cells. Neutron capture therapy currently depends on the use of the low molecular weight agents, sodium borocaptate and boronophenylalanine; however, significant research is being

conducted with higher molecular weight compounds, including nanoscale ones. Studies exposing cells to boron-containing nanovehicles have shown promising effects, including more specific targeting of tumor cells, reduced toxicity to healthy cells, and significant permeation through the BBB. Both vesicular endocytosis and facilitated transporter binding are potential mechanisms for translocation of nanoparticles through the BBB. The additional mechanisms for entry of nanoparticles into the CNS and to evaluate if nanoparticles will elicit CNS toxicity deserve further investigation.

4.4. Detection of tumor angiogenesis

Angiogenesis is required for the growth of solid tumors and antagonism of angiogenesis can slow tumor growth. Nanotechnology is being used to identify mechanisms of tumor angiogenesis. To explore the contribution of endothelial precursor cells to the neovascularization of certain tumors, endothelial precursor cells (EPC) of hematopoietic stem cell origin were labeled with FDA-approved dextran-coated superparamagnetic iron oxide (SPIO) nanoparticles. The labeled cells were injected intravenously into mice and then monitored by MRI for migration and incorporation into growing tumor. This method non-invasively detected early migration and incorporation of EPC into tumor vasculature with high spatial resolution. The findings on MRI were verified by findings on histology of EPC within tumor vasculature and of differentiation of EPC into endothelial cells. This study suggests that nanoparticle-loaded EPC might be used clinically to detect sites of tumor angiogenesis.

4.5. Nano-imaging

Unlike current imaging methods, nano-imaging has grown due to its potential to detect and diagnose cancer and other human diseases even at an earlier stage.

4.6. Nanoneurosurgery

The ability to track stem cells has the potential to revolutionize nanoneurosurgery. Implementation of this strategy will require (i) design of functional nanoparticles that are personalized to cellular markers and genes, (ii) bundling of these functional nanoparticles into a suitable delivery vector, (iii) incorporation of nanoparticles *ex vivo* into stem cells, and (iv) injection of stem cells into the cell-deficient brain region that requires repair. Once the cells are tagged, their position and their effect on the underlying disease can be evaluated by imaging on an ongoing basis. Because this work could provide a method to monitor and control stem cell therapy for degenerative diseases of the CNS, there is great need for further investigation in this area that could pave the way for future clinical trials.

4.7. Lymph-node MRI

Staging of many types of cancers establishes a prognosis and directs cancer treatment. Accurate staging requires detection of the presence, or confirmation of the absence, of lymph node metastases. MRI provides soft tissue images with excellent resolution but is relatively insensitive to identification of early involvement of lymph nodes by metastatic cells. High-resolution MRI with lymphotropic superparamagnetic nanoparticles has the potential to be adopted broadly for clinical use for the identification of occult lymph node metastasis.

5.0. Conclusion

Scientists are striving to design ways to tailor the characteristics of nanomolecular and cellular agents and to restrict their effects to specific organs and sites. **Nano-neuro-immunotherapy** is a developing field based on development of nanoparticles that bind to a target with immune specificity and that deliver their drug payload there. This highly focused drug delivery and therapy technique contrasts sharply with current drug therapies that distribute their effects equally to areas of normal function and disease, the so-called shotgun approach, which results in systemic exposure and toxicity. Another emerging strategy, **nanoparticle-augmented cell therapy**, provides a method to simultaneously provide treatment and imaging at the site of brain disease. These strategies have the potential to reduce the morbidity and mortality of diseases of the central nervous system and to improve the quality of life of patients.

In the near future, nanomedicine will participate in the development of personalized medicine. Patients will undergo treatment tailored to their unique genetic makeup. Developments in the fields of pharmacogenomics, nutrigenomics, and ecogenomics will assume increasing importance. Neurosurgeons will be able to consider the individualized gene and brain maps (anatomic, functional, proteomic) of a patient and choose the treatment that should be safest and most effective. Using techniques of image guided therapy and nanoneurosurgery, neurosurgeons will be able to detect, confirm, and treat brain injury with nanostructures. Surgeons will inoculate cells with various types of nanostructures that carry a regimen of drugs, which can be released and act at different steps of a biochemical pathway. In the case of brain tumors, therapeutic cells will be delivered into tumors, where they will distribute their nanoparticles into tumor cells and release drugs to selectively eliminate tumor cells. The imaging contrast

produced by therapeutic nanoparticles will permit imaging to monitor the progress of treatment for each patient.

Nanovectors, nanostructures, nanoplatforms, and nanoscale objects hold the potential to bring about less invasive and more selective treatment of brain tumors and other CNS diseases. Reaching this potential will require more research and the development of nanovectors that are less toxic, more versatile, and more biodegradable that current ones. Poor water solubility of some nanoplatforms must be overcome before they can be utilized in the development of nanodrugs. Many groups have functionalized very stable nanoplatforms such as CNT and gold nanoparticles in order to achieve solubility. Others have designed soluble nanoplatforms such as poly(malic acid) nanoconjugates, which contain various antibodies and oligonucleotides for multitargeted drug delivery. A new generation of nanovectors could incorporate multi-functional compounds and allow multistage, complex delivery of therapeutic compounds and augmented cellular therapies. The fields of nanomedicine, image-guided drug delivery and therapy, and gene therapy will inevitably converge further and to enable personalized medicine and targeted disease therapy. Advances in each field will drive the development of synergistic, more effective, and less toxic therapies for presently incurable neoplastic and non-neoplastic diseases of the CNS.

To improve efficiency in moving nano-technology from the laboratory to the clinic, the creation of a central science, technology, medicine and law–healthcare policy (STML) hub/center that fosters and coordinates collaborative efforts across all institutions while creating policies, which could encourage such interaction. The central hub would encourage cross-disciplinary research that focuses on specific nanotechnology/nanomedicine initiatives. The creation of the central hub and its efforts will cultivate a spirit of partnership between industry, government,

universities, and foundations that will translate innovations in nanotechnology into medicine, where they can be developed and implemented as powerful therapeutics for neurological and other disorders.

Thank You.

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