World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Review Article

A review on nanoneedle- A smart nanovector for drug delivery

B.S. Wakure, N.M. Bhatia

Bharati Vidyapeeth's, College of Pharmacy, Kolhapur-416 004, Maharashtra, India

Received: 12-05-2017 / Revised Accepted: 23-06-2017 / Published: 25-06-2017

ABSTRACT

Nanotechnology has become one of the important applicant in biology through the provision of latest nanotechnology tools to probe and manipulate biological processes at the nanoscale (~1 to100 nm), which is the length scale where many fundamental biological processes occur. One-dimensional nanomaterial, such as nanotubes and nanowires, have been used as intracellular biosensors, delivery carriers, and imaging agents. The nanoneedle-based drug delivery provides new possibilities for efficient, specific, and precise introduction of biomolecules into living cells for high-resolution studies of biological processes and it has potential application in solving broad biological complications. The delivery of biomolecules into living cells could provide by a nanoneedle-based delivery system with high spatiotemporal resolution, minimal intrusion and damage. The nanoneedles can act as a carrier for cargo molecules such as peptides, protein, nucleic acids, and drugs. This review provide insight into all nanostructured materials (NSM) and major emphasis set on nanoneedles as a nascent drug delivery vehicle and as a probe to manipulate cellular processes.

Keywords: nanoneedles; nanotubes; ultrathin nanoneedles; cell penetration; intracellular delivery.

Address for Correspondence: B.S. Wakure, Bharati Vidyapeeth's, College of Pharmacy, Kolhapur-416 004, Maharashtra, India; Email: balaji.wakure@gmail.com

How to Cite this Article: B.S. Wakure, N.M. Bhatia. A review on nanoneedle- A smart nanovector for drug delivery. World J Pharm Sci 2017; 5(7): 101-110.

INTRODUCTION

Nanomaterial and Nanotechnology: New terms are introduce often, be it in the world of science or the world in general. These new terms typically represent a trend that is emergent. In the world of science, terms such as genetic engineering, tissue engineering, gene, biotechnology, combinatorial chemistry, stem cells, high throughput screening, nanostructured materials are some examples. Most recently, nanotechnology has become very popular and can be define as a collection of techniques that may use in combination to innovate, produce, characterize, and utilize these materials for dimensions ranging within 100 nanometers [1]. Nanomaterial are having specific and unique properties (such as chemical, physical, magnetic, optical, and electronic) and can acts as models that can provide more in-depth information about the structure activity relationships at nanoscale level and for the guidance in the creation of new structures with enhanced and novel property [2].

One-dimensional nanostructure seen to be important in basic scientific research as well as other technological applications. The one-dimensional nanostructures that have known until date enumerate nanowires, nanotubes, Nano belts, Nano awls and Nano rods. Nanowires, Nanorods and nanotubes have considered the pre-eminent notable one-dimensional structures. Nanoneedles are conical or tubular needles in the nanoscale range, which can be composed of silicon or boron-nitride with a central bore in the size sufficient to allow large molecules, or can be suitable solid needles useful in Raman spectroscopy, in light-emitting diodes (LED) and Laser diode [3].

Recently, Nanotechnology has become one of the important applicants in biology through the provision of latest nanotechnology related instruments and materials to probe and operate biological activity at the nanoscale (approx. 1 to100 nm), which is the length scale where many fundamental biological processes occur [4].

One-dimensional nanomaterial, such as nanotubes and nanowires, have used as nanoneedles and acts as intracellular biosensors, delivery carriers, and imaging agents. In addition, these high aspect ratio nanostructures having needle-like nanoscale geometry and properties such as mechanical and electrical sensitivity have been explored as a bio membrane-penetrating nanoneedles that can control and manipulate biological processes inside cell with minimal intrusiveness and toxicity [4].

Several research groups have prepared the nanoneedle based drug delivery system that uses exterior surface of the nanoneedle for transport of cargo for intracellular delivery. It is important that the cargo is capable of conjugation on the surface of the nanoneedle and can released from the nanoneedle surface once transferred inside the cell. Certain protein molecules that are fluorescently marked can successfully delivered to the cell and by tracking their movement, the distribution and function of a delivered protein can understood by studying various cellular mechanisms such as protein process and delivery, cell growth and cell cycle [4].

Need for Nanoneedles: Common methods of delivery in conventional drug delivery include transdermal, oral, injection and pulmonary, each of these modes has its own qualities and defects. For example, other than direct injection into muscle tissue or vein, cellular layers found in each of the above ways, which acts as a hindrance while conveying the drug within the systemic circulation [5]**.**

Nanoneedles designed with the help of nano-fabrication technology, having nanoscale diameter, can be useful during the delivery of drugs, with the ability to deliver the drug in a localized or painless manner in cell or tissues. Nano carriers can provide more surface area when compared to micrometer-sized carriers and further have the opportunity to increase bioavailability, increase solubility, improve delivery that regulated by time and facilitates entrapped compounds to accurately target largely.

The nanoneedle-based drug delivery system provides new possibilities for efficient, specific, and precise introduction of biomolecules into living cells for high-resolution studies of biological processes that mainly occurs at nano scale length, and it has potential application in solving broad biological questions. The delivery of biomolecules into living cells can provided by a nanoneedle with high spatiotemporal resolution, minimal intrusion and damage. Thus, it become painless and noninvasive means of drug delivery.
Nanofabricated device has variety of advantages that includes easy delivery mechanism, highly precise dosing, and capability to pertain complex release methods, capability for local specific area delivery and enhanced biological drug stability & bioavailability.

DRUG DELIVERY SYSTEMS

A drug delivery system is one that referred to as the process through which a drug administered in order to achieve its therapeutic benefits intended for animals or humans. There are drug delivery vehicles such as micro and nanoparticles, soluble polymers that are composed of biodegradable natural and synthetic polymers, micelles, cells, microcapsules, and liposomes that have developed more recently.

Drug delivery systems most often are a replication of the existing body mechanisms such as the manner in

which the body processes hormones like insulin and cortisol. Polymers that are sensitive to specific stimuli such as change in pH, temperature or exposure to light achieve this particular mimic of the mechanism and used in the drug delivery.

Recently, nanotechnology has become the most beneficial for many sectors in healthcare and other scientific fields. Scientists especially for drug targeting and delivery have recognized the benefits of nanotechnology. Various techniques and theories can used with a combination of polymer science, bioconjugate chemistry, molecular biology and pharmaceutics. Other integrations such as nanotechnologists, biologists, biochemical and biophysical engineers also constitute for the development of new drug delivery in the last decade. Therefore, from this current review all these components integrated on the same platform.

DRUG DELIVERY VEHICLES

Drug delivery vehicles are substances that serve as mechanisms to improve the delivery and the effectiveness of drug(s). The aim of the development of drug delivery vehicles is to load the optimum drug and to achieve release properties, long shelf life and low toxicity. Some of the recently developed nanostructured drug delivery vehicles briefed here.

Nanostructured material (NSMs): Nanomaterial are hollow or solid structure, with diameter in the 1-1000 nm range, and can be filled or loaded with drugs and detection agents. Targeted drug delivery can achieved by attaching targeting moieties on the surface of nanomaterial, to make it useful. The nanomaterial include nanoparticles, lipid core micelles, polymeric micelles, biodegradable polymeric nanoparticles, liposomes, nanopores, nanospheres, nanocapsules, nanotubes, nanowires, nanoshells, nanocantilever, dendrimers, gold nanoparticles, protein nanoparticles, Buckminster fullerene (commonly called Bucky balls) and quantum dots. In particular, all the nanomaterial related discussions identified by the user's convenience as 'nanoparticles'. However, nanoparticles include only solid entities.

Nanoparticles: Nanoparticles have either amorphous or crystalline solid property. Generally, they have made up of single component and exist in the size of about 5 - 200 nm. They have ability to absorb and (or) encapsulate drugs, so protecting them against chemical and enzymatic degradation. In relation to their role in controlled drug delivery, they have emerged as potential drug delivery systems [6]. They have particularly employed in targeting particular tissues or organs as carriers of DNA in gene-therapy, and in their ability to deliver proteins, peptides, and genes through oral route. Efforts have made for applications of protein nanoparticles in drug delivery [7].

Super paramagnetic iron oxide nanoparticles (SPIONs) were generally produce by reduction of the amount of iron salts under the alkaline conditions. They have assured to be promising drug delivery vehicles for some biomedical applications like targeted drug delivery and imaging, hyperthermia, magneto-transfections, gene therapy [8,9], stem cell tracing, molecular or cellular tracking, magnetic separation technology (e.g. rapid DNA sequencing), and detection of liver and lymph node metastases. In modern development, SPION have used successfully in order to quickly detect inflammatory diseases, cancer, diabetes and atherosclerosis [9]. Currently, polyethylene glycol polyhedral oligo silsesquioxane (PEG-POSS) amphiphilic nanoparticles investigated to encapsulate insulin as new drug delivery vehicles. The research of insulin release have shown that PEG-POSS nanoparticles have used to protect insulin inside at gastric pH for two hrs.' and it was released at intestinal pH (pH 6 - 7), which is necessary for its absorption and activation [10].

Nanowires: Nanowires are nano-scale sensing wires. Nanowires can coated with molecules such as antibodies to bind to proteins of choice and transmit their information through sensing electrodes to the computers [11]. They have applications in the early detection of breast and ovarian malignancies. In the frequently emerging area of Nano neurosciences, nanowires drug delivery using Titanium dioxide (TiO2)-related nanowires (50 - 60 nm) labeled with drugs have found to improve the neuroprotective efficacy of drugs in spinal cord injury following trauma [12,13].

Combining nanowire-coated beads of silicon (e.g., nanowire coatings), with epithelial physiology, has significantly increased bio adhesion (about 100 times), in mucosal conditions [14]. Recently, novel hybrid nanomaterial such as iron nanowires being developed to improve high thermal performance hyperthermia therapy [15].

Nanoshells: Nanoshells are composite nanoparticles consisting hollow silica core (SiO2, semiconductor) surrounded by a gold shell (Au). Silica-Gold (SiO2-Au) nanoshells are special because their peak extinctions is very easily tunable in wider areas of wavelengths, especially in the near the infrared zone (800 - 1200 nm) of spectrum. Light in this region is transmit through tissue with relatively little attenuation due to absorption. In addition, at their peak extinction coefficient, the SiO2-Au nanoshells convert light from their radiation to heat energy, which in turn causes local rise in temperature. This makes nanoshell hot; consequently, the heat kills the cancer cell. Thus, to develop a photo thermal

modulated drug delivery system, some groups of scientists have fabricated a nontoxic hydrogel polymer network encapsulating silica gold (SiO2-Au) nanoshells.

The hydrogel coatings can slow down through stimulation of strong Plasmon resonance of silica-gold nanoshells due to light exposure; this excitation of nanoshells gives a better source of heat to the system. The photo thermally modulated drug delivery for methylene blue, insulin and lysozyme has obtained by the irradiation of drag-loaded nanoshell-composite hydrogel, which showed that the drug release was dependent on the molecular weight of the therapeutic molecule. Recently, these nanoparticles have updated as photo thermally responsive drug delivery devices that respond to temperature above physiological temperature and undergo structural changes [16-19].

Nanotubes: Nanotubes are hollow cylindrical tubes made up of carbon atoms, with cross-sectional dimensions in the nanometer range, and lengths can extended over a thousand times their diameters. They can be filled and sealed with drugs and thus can provide potential drug delivery tools. Nowadays, Aluminum oxide nanotubes found to been fabricated and appears to have many beneficial properties as drug carrier for regulated release of drug(s) [20]. Medical implants have therapeutic surface coatings made up of nanotubes. Nanotubes have testified as high-specificity sensors of antibody signatures of autoimmune disease [21] and of single-nucleotide polymorphisms (SNPs) [11,22].

Nanobots: Nanobots are nanotubes admitted along with anticancer drug(s) and release drug(s) only close to location of tumor in response to pH stimulus, since the pH of tumor and inflamed places are quite acidic (pH 6.5) when compared to normal tissues (pH 7.4) [23].

Nanorods: Nanorods are needle like solid nanostructured material and composed of silicon or boron-nitride. They are useful in light emitting diodes (LED), and laser diodes. They have the potential to use as drug delivery vehicles.

Nano cantilevers: Nano cantilevers are consisting of flexible beams of carbon atoms. Like the lines of the diving boards, their beams have anchored with one end. The beams of Nano cantilever deflect when the biomolecules of interest bind. The deflections can found by a laser light or by creating visual shifts in the physical properties of beams such as resonant-vibration frequencies. Therefore, these bio-molecular sensors have a large amount of "multiplex" functionality that is, to find a large number of different molecular species at the same time. In this way, nano cantilevers (like microscopic cantilevers) have developed for serum and tissue proteomics-based cancer diagnostics, prognostics and diagnostic-efficiency monitoring [11,24,25].

Nanopores: Nanopores are small holes designed in the particle. The pores are so small that even DNA molecules can pass through them as one strand at a time, allowing accurate and specific DNA sequencing. Drug manufacturers can design nanopores into the surface of a drug capsule, which are only slightly larger than the drug's molecular size, therefore regulating the rate of diffusion of drug in the body. Currently, nanoporous systems engineered to mimic natural filtration systems, actively developed for use in smart implantable drug delivery systems, bio artificial organs, and other novel nano-enabled medical devices. Synthetic nanoporous element have many effective biological and medical applications including sorting, sensing, isolating, and releasing biological molecules [26]. Researcher have applied nanopores with mesoporous thin silica films as anticancer drug carriers and attained mechanistic insights into the feasibility of using such films for drug delivery at the target-site [27].

Bucky balls (Fullerene): Bucky ball (fullerene) is the common name given to the recently discovered molecule, which called the buckminsterfullerene [28]. Bucky ball showed that as soccer ball-like structure of over 60 carbon atoms. Like other molecules, which used as cancer drug delivery vehicles, the fullerenes do not break down into the body and they excreted intact. This characteristic helps for some cancer treatment and compounds that affects the healthy cells. For example, the fullerenes drug delivery particles with radioactive atoms will allow the entire elimination of the radiation from the body after treatments [29]. Bucky balls and other fullerenes due to their chemistry and their atypical hollow, cage-shaped architect make them very stable and can tolerate very high temperatures and pressure. Bucky ball is the only molecule build by a single element to give a hollow spheroid, which provides the efficiency for filling it, and using it as novel drug delivery systems. It could find worldwide application in future materials and applications like: a) drug delivery vehicles for antitumor drugs or cancer therapy, b) to cure allergy, c) as inhibitor of HIV, and d) as powerful anti-oxidant, and e) in ultra-hard coating in the military services.

Gold Nanoparticles: Gold nanoparticles are consisted of gold (Au) solid core. Scientists are using gold nanoparticles to develop ultrasensitive detection systems for DNA diagnostics, biosensors and protein markers associated with many forms of cancer, including breast and prostate cancer [30]. Colloidal gold nanoparticles represent a completely novel technology in the field of particle-based tumor-

targeted drug delivery. Recently, gold nanoparticles has used successfully for the targeted delivery of tumor necrosis factor, which used to treat a solid tumor growing in mice [31].

Quantum Dots: Quantum Dots are the miniscule semiconductor nanoparticles coated with inactive, nontoxic polymer. The core material can chose depending on the emission wavelength range to target. Targeting molecules can fixed to the coating and they serve as sign posts of certain types of cells or molecules in the body because they emit different wavelengths of radiations depending upon the type of Cadmium used in their cores.

Based on emission wavelength, various types of Cadmium have used, such as Cadmium sulfide (CdS) for ultra violet to blue, Cadmium selinide (CdSe) for most of the visible spectrum and Cadmium telluride (CdTe) for far-infra red and near infrared. Quantum dot bio conjugates with targeting antibodies have used to recognize molecular signatures including ERBB2 [11,32,33]. Quantum Dots are very stable under the complex biological environment that makes them suitable for advanced molecular and cellular imaging, drug delivery and for highly sensitive bioassays and diagnostics [34-37]. Advances in the quantum dot technology can provide a better knowledge of molecular events in tumor cells and early detection of cancer.

Carbon nanotubes (CNT): Carbon nanotubes have hollow nanostructures consist of carbon elements only. Carbon nanotubes have covalently or noncovalently transform to promote good circulation within the body. These changes can increase or decrease their circulation time in the body. CNT can be functionalized with bioactive peptides, proteins, nucleic acids and drugs, and used to deliver their cargos to cells and organs. The programmed CNT display non-significant toxicity and are non-immunogenic, therefore, they have better efficiency as drug delivery vehicles and have appeared as a potent nanovector for transporting and translocating therapeutic molecules [38-40]. Researchers have applied CNT to deliver active agents and drugs in the animals. Carbon nanotubes have developed to target and destroy tumor cells [41].

Nanorobots: Nanorobots have the ability to treat, cure and diagnose diseases. They describe the next generation diagnostic tools [42-44]. Coming era is most likely to see its usefulness in treating and diagnosing certain diseases.

Dendrimers: Dendrimers are synthetic nanoparticles (nanovectors) that are about 5 to 10 nm in diameter. They have central core that made up of polymers. Dendrimers provide drug molecules a greater water solubility, bioavailability, and biocompatibility. They are hyper branched (like trees) and monodisperse with three-dimensional molecules with defined molecular weight and large number of functional groups in which drugs molecules can attached. They have well-known host-guest entrapment properties. Due to the presence of various functional groups (sites) in dendrimer, other molecules as polyethylene glycol can attached. Dendrimer attached polyethylene glycol does not allow the body's immune system to be known it as foreign, so the decomposition process of "Drug loaded Dendrimer" is slowing down. This interesting nanovector holds significant promise for cancer diagnosis and treatment.

Recently, scientists have fashioned dendrimers into sophisticated anticancer machines carrying five molecules as five different tools: a) a molecule designed to bind to cancer cells, b) second molecule exhibit fluorescence on detecting genetic mutations, c) a third molecule to support for imaging tumor shape using X-rays, d) a fourth were carrying drugs and released on demand, and e) a fifth that were sending the signal when cancerous cells are eventually dead. Therefore, Dendrimer has a significant assurance as delivery vehicles for drugs, especially non-steroid anti-inflammatory, anti-microbial or anti-viral and effective anti-cancer drugs [45,46].

Nanoneedles: Nanoneedles are hollow, tubular or conical needles in the nanometer size range. They are composed of nontoxic, biodegradable and eco-friendly components like silicon or boron-nitrate. Nanoneedles allow the passage of molecules through its central bore of sufficient size. They can mounted in an array and can used to transport any drug or vaccination antigen, directly through the cellular wall, in the cytoplasm or at the very center of the cell. Arrays can pressed on the skin. This may decrease the volume of antigen needed to guide adequate immunity and making multiple immune directions. This system found effective for insulin and diclofenac delivery in animal models [47]. Due to non-invasive penetration mechanism, nanoneedles avoid the sensation of pain during drug or vaccine delivery. This reduces vaccine or drug degradation at the same time and increases bioavailability.

Nanoneedles serve as a powerful new tool for quantitative examination of biological processes in cell nucleus or cytoplasm; and for studying the biophysical properties at the molecular levels in living cells. Combined with molecular targeting approaches using quantum dots and magnetic nanoparticles as molecular probes, the nanoneedle drug delivery method can enable simultaneous monitoring and manipulation of individual molecules [48,49]. In the future, nanoneedles can be applied in the form of electrochemical probes and as an optical biosensor to study cellular environments.

Ultrafine Nanoneedles: In this context, ultrafine needles [50] provide less invasive means for molecular delivery, manipulating cells, and transmitting genes to living cells using atomic force microscope [51]. The covalent bonding and affinity binding can used to immobilize DNA on the surface of nanoneedles. This method enables accurate displacement and low invasiveness.

Recent studies have shown that chemically modified carbon nanotubes acts as nanoneedles and can easily pass through biological barriers and penetrating a wide range of cell types [39,40,52]. This researcher reveals the efficacy of systemized carbon nanotubes as a new configuration of direct drug delivery [53]. These systemized nanotubes able to act as cell-penetrating substances and can act as nanoneedles that pierce plasma membranes and translocate directly into cytoplasm without causing any cell damage and with the advantage of readily excreted. The nanotubes have structural advantages in which they are very thin but are too long, to offer a large surface area to which the necessary drug can bound. The large surface area allows the amount of drug loaded on nanotubes to regulate. Current researches directed to the study of improved efficiency of drug delivery and drug targeting [54], superior release profiles, and the study of reversible associations for the intracellular release of the drug [55].

Oncology therapy, on the other hand, involves synthetic nanoneedles [56], which are highly custom-made ion channels that control the entire molecular movement across the cell membrane, targeting specific diseased cells [57]. Recently, using quantum chemical calculations electronic and theoretical properties of ultrathin carbon [58] and nitrogen [59] needle-like and tube-like nanostructures, which are tighter than the smallest single wall nanotubes, have studied. However, although the preparation of this structure is not relatively simple [60], the objective of study was to interpret the geometry and stability of the family of the packed carbon nanoneedles (CNNS) using the Quantum Chemistry Computational Modeling Method.

NANONEEDLE ARRAYS AND INTRACELLULAR DRUG DELIVERY

Inspired by the success of using micro needle arrays for drug delivery, researcher reported using a nanoneedle arrays to mechanically disrupt cell membranes for intracellular delivery. Transdermal and intracellular delivery has one similar concept. For transdermal delivery, the outmost layer of skin, stratum corneum restrict the diffusion of most materials, molecules, genes and drugs to the skin. For intracellular delivery, the cell membrane plays a similar role, so micro needles can used to pierce the stratum corneum in order to achieve transdermal delivery, and one should be able to use a same approach for intracellular delivery.

However, if micro needles applied to cells, because of their large structural geometry, the cells might destroyed easily. Therefore, the best method designed for the delivery to interior of cells is nanoneedles. To allow the nanoneedles with an extremely small diameter to have enough mechanical strength to disrupt the cell membranes, diamond, the hardest material in nature, used to produce the nanoneedle patch. This diamond nanoneedle array fabricated by bias-assisted reactive ion etching (RIE) of a silicon substrate with a pre-deposited diamond film. Against the time consuming culture of cells on silicon nanowires substrates, in this case a suspension, having a large number of cells rapidly applied to diamond nanoneedles. During the process, it expected that the cell membranes would collapsed so a high-throughput intracellular delivery would attained. A luminescent iridium (III) polypyridine complex introduced to the cell suspension. This creates it like a luminescent complex and is significant because it indicates negligible nuclear uptake [61]. The result indicates that nanoneedle-treated cells exhibit great fluorescence in the cytoplasm of the cells whereas the untreated group provides less signal. After their fluorescence intensities in the cytoplasm have assessed, representatives of two groups compare the fluorescence signal in the nucleus of the cells with the same value; it is quite easy to find that there is a much stronger fluorescence in the nuclei of the nanoneedle-treated cells.

Because the fluorescent probe is not able to enter the cell nuclei by diffusion, it is appropriate to conclude that nanoneedle treatment has played an important role in direct delivery to the nucleus. This novel technology of using nanoneedle arrays for intracellular delivery has many superior advantages compared to prior approaches. Nanoneedle based drug delivery expected to be convenient, highly efficient, high-throughput, universal, safe and cost-effective, easy way to distribute potential high-throughput delivery of genes, drugs and fluorescent probes inside the cells.

FEATURES OF THE NANONEEDLE-BASED INTRACELLULAR DELIVERY

The intracellular delivery using a cell membrane-penetrating nanoneedle that have many unique features that may allow new strategies for biological experiments inside living cells. Without a clear loss to the cell, the nanoneedles can deliver a discrete, smaller number of molecules in the target area of the single targeted cell at desirable time.

Cellular Delivery methods: The nanoneedle can deliver cargo into living cells with spatial and

temporal precision. The ability of nanoneedle to capture targeted areas in cells allows the direct delivery of cargo into target areas or compartments inside cells, but it cannot be definitely attainable by traditional delivery methods. It was evident that the nanoneedle-based method can selectively deliver quantum dots into the cytoplasm or the nucleus of living HeLa cells [62]. This ability may effectively pertain spatially resolved experiments inside living cells (e.g., inside the nucleus).

Since the cargo is released inside the cells from the nanoneedle, the spatial resolution of the delivery is usually determined by the size of nanoneedle fixed in a cell (≤ 100 nm in diameter and ≤ 4 μm in length) and the spatial accuracy of the manipulator (e.g., the nanoscale resolution of 1 nm, attainable with the piezoelectric manipulator, such as AFM mechanism). The delivery can also done with high temporal precision (for example at sensible times throughout the cell cycle); the temporal resolution of approx.15 to 30 minutes is attainable, for example, when the reductive cleavage of disulfide bonds applied as a release mechanism. At present, in electrochemical delivery approach, the release of the attached cargo can obtained by applying a pulse of a small electrical capacity to the nanoneedle. Such delivery systems may make significant improvements in temporal resolution up to a few seconds. Such high- precision delivery can allow short-term scalability of cellular process, such as signal transduction and protein transfer inside the cells.

Nanoneedle can dispense a discrete, small number of molecules into cells. For example, the nanoneedle-based method can uniquely deliver well-dispersed single quantum dots into cells [62]; this capability may potentially allow the use of the delivered quantum dots for molecular imaging inside living cells.

The nanoneedles unique ability to deliver only a small number of nanoprobes can reduce interference of nanoprobes (e.g. quantum dots and magnetic nanoparticles), with proposed experiments in living cells, and the effect of such nanoprobes on cellular physiology [62].

The way by which nanoneedle affects cellular function and viability is important in any living cell experiments. Most studies exposed that the entry of nanoneedle in a living cell does not impair cell viability or membrane integrity. For example, the cell viability tests, using the trypan blue exclusion assay, the Calcein AM assay, and the Annexin V-FITC or propidium iodide assay, or the monitoring of the cell propagation using nanoneedles having diameter less than 100 nm indicates that mammalian cells, such as HeLa cells, mouse embryonic stem cells and human embryonic kidney cells (HEK 293), were feasible for the duration of the experiments [62-64]. Cell viability analyzed with nanoneedles with different diameters, applying the 4,6-diamido-2-phenylindole (DAPI) exclusion assay for human epidermal melanocytes, HEK 293, and breast cancer cells (MCF-7), also advised that regulating nanoneedles with diameter less than 400 nm inside cells does not affect the cell viability for at least one hour [65]. However, by what method does nanoneedles affect the cellular process and functions would achieved by examinations that are more detailed.

Applications of Nanoneedle Based Intracellular Delivery: Although it is exciting to go for a functionalized nanoneedle in the form of a new gene or protein delivery system for the study of cells, more remains to be discovered to use such an elegant yet powerful system for the study of various cellular processes. In this section, we highlight some initial efforts and potential applications with the functionalized nanoneedle for intracellular delivery.

Polypeptide or Protein delivery for detection of protein transportation, stability and degradation: The functionalized nanoneedle-based delivery can used to deliver polypeptides and proteins directly to specific cellular compartments. Although the direct delivery of polypeptides and proteins should be attainable by modifying the surface functionalization of the nanoneedle for attaching protein molecules to it [62,63]. Alternatively, it can used to take advantage of physical and chemical properties in a particular cellular environment, so that proteins can be absorbed on the needle and released into cellular environments.

Creating various functional needle surfaces for conjugation of different polypeptides or proteins on nanoneedles is a challenge. Due to the complexities of handling large proteins in vitro, it would be another additional challenge for conjugating large proteins on the nanoneedle for delivery.

Upon successful delivery of a few protein molecules with fluorescent markers into a cell, the movement of the delivered proteins can potentially tracked to understand the protein distribution and functions at different cellular processes such as cell cycle, cell growth, and protein processes and delivery. Furthermore, the activity of the delivered protein can tracked with live cell imaging through identification of the substrate production or transformations in biophysical and chemical characters, including ion concentrations. Moreover, the stability of delivered proteins can detected inside of the cells. This is particular useful to check protein stability of signaling molecules which can undergo rapid degradation under stimulation of hormones and growth factors.

DNA/RNA molecular delivery for gene appearance: Gene delivery pertains highly beneficial for cellular studies; especially, high efficiency delivery of exogenous genes into numerous primary cultures as well as stem cells remains a challenge.

As mentioned earlier, Nakarmura and colleagues [65,66], has shown the direct delivery of DNA at the nuclei of various types of mammalian cells, including human mensenchamal stem cells. The improvement in the releasing methods expected that the direct delivery of plasmid DNA into the nucleus of individual cells could force to a premier achievement rate of expression at the single-cell level in comparison to other nonviral gene delivery methods. Due to the high spatial resolution, the functionalized nanoneedle can applied to deliver exogenous genes into small cellular sections, such as axonal terminals or dendritic spines and their particular gene expression systems are not dependent from genes present in the neuron cell body (soma).

Small signaling molecules for amplification and transduction: One of the advantage of the functionalized nanoneedle-based delivery is that it can deliver a small number of molecules into cells. The signaling molecules have the ability to amplify signaling cascade to mammalian cells; however, the quantifying signaling amplification is very complex. While various theoretical methods has been applied to understand quantification of signaling amplification, most experimental approaches are rudimentary, and yield little information on quantification of any specific cellular events in native environment. Using functionalized nanoneedles based delivery, one can detect delivery of small diffusible signaling molecules like second messenger cyclic AMP, cyclic GMP, inositol phosphates and diacylglycerol, and monitor how second messengers can trigger the amplification of signaling in a particular cellular setting.

CONCLUSION

Development of new drug delivery technologies has also hastened through progress in nanotechnologies, which is imperative to convert biological possibility into medical actuality. The drug delivery systems based on biofunctionalised nanoneedle have become innovative methods for the delivery of biomolecules into cellular membranes with high spatio-temporal resolutions. Biofunctionalised nanoneedle-based drug delivery systems also possess the advantages of delivering few molecules with high precision in terms of temporal and spatial precision thereby enabling cellular level studies involving the examination of cell growth, cell cycle, potential of membrane and redox environment, amplification and signaling transduction and expression of genes. Hence, the applicability of nanoneedle is evident for cellular studies.

Further developments in the field of drug delivery using nanoneedles could facilitated for the delivery of biomolecules from proteins and nucleic acids to very small diffusible molecules. Moreover, developments may also combine nanoneedle drug delivery with other techniques such as patch clamping and live cell imaging to widen the scope of nanoneedles to high-end techniques. The vital goal thus remains to increase the stability of a product in a biological environment to regulate the release of bioactive materials and improve the processes such as drug loading, transport, targeting, release and interaction with biological barriers. Therefore, increasing biocompatibility thereby stability can considered as one of the impending works concerning the drug delivery using nanomaterial structures.

Functionalized nanotubes or nanoneedles entered the terrain of biological research only few years ago. However, they are displaying a great deal of promise especially when used in developing cancer therapeutics. Their biological activity can be accompanying with their capacity to interfere with processes and functions at molecular level.

In present review, we have summarized how nanoneedles as new class of nanomaterial can influence molecular or cellular delivery of proteins. The probing and manipulation at cellular level could possible using such nascent drug delivery module. However, the activities and competencies of nanoneedles to interact with biological matter is growing, many new possibilities anticipated in the near future.

REFERENCES

[1]. Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. *Curr Mol Med* 2006; 6 (6):651-63.

[2]. Gonsalves E, Halberstadt R, Laurencin T. & Nair S. *Biomedical Nanostructures.* John Wiley & Sons: *Hoboken*. New Jersey, 2007.

[3]. Ajayan PM. Nanotubes from Carbon. *Chem Rev* 1999; 99 (7):1787-1800.

[4]. Ali-Boucetta et al. Multiwalled carbon nanotube doxorubicin supramolecular complexes for cancer therapeutics. *Chemical Communications 2008;* 28 (4):459-61.

[5]. Andersen PH, Maibach HI. Skin irritation in man: a comparative bioengineering study using improved reflectance spectroscopy. *Contact Dermatitis 1995;* 33(5):315-22.

[6]. Reddy PD, Swarnalatha D. Recent Advances in Novel Drug Delivery Systems. *International Journal of PharmTech Research* 2010; 2(3):2025-27.

[7]. Jahanshahi M, Babaei Z. Protein nanoparticle: A unique system as drug delivery vehicles. *African Journal of Biotechnology 2008;* 7(25):4926-34.

[8]. Dobson J. Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. *Gene Therapy 2006;* 13(4):283-287.

[9]. Scherer F et al. Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. *Gene Therapy 2002;* 9(2):102-9.

[10]. Kim KO et al. Self-Assembled Core-Shell Poly (ethylene glycol)-POSS Nanocarriers for Drug Delivery. *Journal of Biomaterials and Nanobiotechnology 2001;* 2(3):201-6.

[11]. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nature Reviews Cancer* 2005; 5(3):161-71.

[12]. Sharma HS et al. Nanoparticles influence pathophysiology of spinal cord injury and repair. In: *Progress in Brain Research 2009; 180:154*-80.

[13]. Sharma HS et al. Nano wired-Drug Delivery Enhances Neuroprotective Efficacy of Compounds and Reduces Spinal Cord Edema Formation and Improves Functional Outcome Following Spinal Cord Injury in the Rat. In: *Acta Neurochir Supplement* 2010; 343-50.

[14]. Fischer KE et al. Biomimetic nanowire coatings for next generation adhesive drug delivery systems. *Nano Letters*; 2009; 9(2):716-20.

[15]. Liou HW et al. Synthesis and Characterization of Novel Hybrid Poly (methyl methacrylate)/Iron Nanowires for Potential Hyperthermia Therapy. *Journal of Biomaterials and Nanobiotechnology 2010;* 01(01):50–60.

[16]. Bikram M et al. Temperature-sensitive hydrogels with SiO2–Au nanoshells for controlled drug delivery. *Journal of Controlled Release 2007;* 123(3):219-27.

[17]. Bob I. Nanoparticle Drug Delivery Systems. *Innovations in Pharmaceutical Technology 2007;* 24(1):58–62.

[18]. Hughes GA. Nanostructure-mediated drug delivery. *Nanomedicine: Nanotechnology, Biology and Medicine 2005;* 1(1):22–30.

[19]. Villiers MM, Lvov YM. Nanoshells for Drug Delivery. *Nanotechnologies for the Life Sciences 2007*.

[20]. Noh K et al. A New Nano-Platform for Drug Release via Nanotubular Aluminum Oxide. *Journal of Biomaterials and Nanobiotechnology 2011;* 02(03):226-33.

[21]. Chen RJ, Hongjie D. Noncovalent Functionalization of Carbon Nanotubes for Highly Specific Electronic Biosensors. In: *Proceedings of National Academy of Sciences*. USA 2003; 4984-89.

[22]. Woolley AT et al. Direct haplotyping of kilobase-size DNA using carbon nanotube probes. *Nature Biotechnology 2000;* 18(7):760-63.

[23]. Yarin AL. Nanofibers, nanofluidics, nanoparticles and nanobots for drug and protein delivery systems. *Scientia Pharmaceutica 2010;* 78(3):542-42.

[24]. Chen H et al. Microelectronic DNA Assay for the Detection of BRCA1 Gene Mutations. *Biomedical Microdevices 2004;* 6(1):55–60.

[25]. Sengupta S, Sasisekharan R. Exploiting nanotechnology to target cancer. *British Journal of Cancer* 2007; 96(9):1315-19.

[26]. Adiga SP et al. Nanoporous membranes for medical and biological applications. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology 2009;* 1(5):568-81.

[27]. Lebold T et al. Nanostructured Silica Materials as Drug-Delivery Systems for Doxorubicin: Single Molecule and Cellular Studies. *Nano Letters 2009;* 9(8):2877-83.

[28]. Nagda D et al. Bucky balls: A novel drug delivery system. *Journal of Chemical and Pharmaceutical Research 2010;* 2(2):240-48.

[29]. Chan WCW. *Bio-Applications of Nanoparticles*. Landes Bioscience: Austin, USA, 2007.

[30]. Mirkin CA et al. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature 1996;* 382(6592):607-9.

[31]. Paciotti GF et al. Colloidal Gold: A Novel Nanoparticle Vector for Tumor Directed Drug Delivery. *Drug Delivery 2004*; 11(3):169-83.

[32]. Jaiswal JK et al. Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nature Biotechnology 2002*; 21(1):47-51.

[33]. Wu X et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nature Biotechnology 2002;* 21(1):41-6.

[34]. Gao X, Nie S. Molecular profiling of single cells and tissue specimens with quantum dots. *Trends in Biotechnology 2003;*21(9):371-73.

[35]. Jovin TM. Quantum dots finally come of age. *Nature Biotechnology 2003;* 21(1):32-3.

[36]. Qi L, GAO X. Emerging application of quantum dots for drug delivery and therapy. *Expert Opinion on Drug Delivery 2008;* 5(3):263–67.

[37]. Vashist SK et al. Review of Quantum Dot Technologies for Cancer Detection and Treatment. *Journal of Nanotechnology 2006;* 2:1-14.

[38]. Bianco A et al. Applications of carbon nanotubes in drug delivery. *Current Opinion in Chemical Biology 2005; 9*(6):674–9.

[39]. Klumpp C et al. Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 2006; 1758 (3): 404-12.

[40]. Lacerda L et al. Carbon nanotubes as nanomedicines: From toxicology to pharmacology☆. *Advanced Drug Delivery Reviews 2006;* 58(14):1460-70.

[41]. Shi Kam NW et al. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proceedings of the National Academy of Sciences 2005;* 102(33):11600-05.

[42]. Cavalcanti A. *Nanorobot for drug delivery and diagnosis*, Nanotech Web 2007.

[43]. Lou M, Jonckheere E. Magnetically levitated nano-robots: an application to visualization of nerve cells injuries. *Studies in health technology and informatics* 2007; 125:310-2.

[44]. Rastogi A et al. Characterization of nanoporous surfaces as templates for drug delivery devices. *The AAPS journal 2009;* 11(4):758-61.

[45]. Cheng Y. Pharmaceutical applications of dendrimers: promising nanocarriers for drug delivery. *Frontiers in Bioscience 2008;* 13(13):1447-71.

[46]. Samad A et al. Dendrimers: A Class of Polymers in the Nanotechnology for the Delivery of Active Pharmaceuticals. *Current Pharmaceutical Design 2009;* 15(25): 2958-69.

[47]. In-Pharma Technologist. *Nano needles make injections painless:* William Reed Business Media. http://www.in-pharmatechnologist.com/Ingredients/Nano-needles-make-injections-painless (Accessed August 30, 2016).

[48]. Lewcock A. *Carbon nanoneedles for drug delivery*: In-Pharma Technologist. http://www.in-pharmatechnologist.com/Ingredients/Carbon-nanoneedles-for-drug-delivery (Accessed August 30, 2016).

[49]. Vieru T. *Nanoneedles May Change the Face of Medicine*: Softpedia. http://news.softpedia.com/news/Nanoneedles-May-Change-the-Face-of-Medicine-110439.shtml (Accessed August 30, 2016).
[50]. Bradley D. Getting the nano-needle. *Drug Discovery Today 1997; 2*(11):452.
[51]. Obataya I et al. Direct Insertion of Proteins into a Living Cell Using an Atomic Force Microscope with a Nanoneedle. *NanoBiotechnology 2005;* 1(4):347-52.
[52]. Kostarelos K et al. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. *Nature Nanotechnology 2007;* 2(2):108-13.
[53]. Prato M et al. Functionalized Carbon Nanotubes in Drug Design and Discovery. *Accounts of Chemical Research 2008; 41*(1):60-8.
[54]. Molema G, Meijer DKF. *Drug Targeting: Organ-Specific Strategies*. Methods and Principles in Medicinal Chemistry. G Molema, DKF Meijer, Eds; Weinheim, FRG: Wiley-VCH Verlag GmbH 2001.
[55]. Lin CC, Metters AT. Hydrogels in controlled release formulations: Network design and mathematical modeling. *Advanced Drug Delivery Reviews 2006;* 58(12-13): 1379-1408.
[56]. Portney NG, Ozkan M. Nano-oncology: drug delivery, imaging, and sensing. *Analytical and Bioanalytical Chemistry 2006;* 384(3):620-30.
[57]. Hara C et al. An efficient gene delivery system into cells using a nano-size silicon needle. *Neuroscience Research 2007; 5*8:S242.
[58]. Wang JL, Mezey PG. The Electronic Structures and Properties of Open-Ended and Capped Carbon Nanoneedles. *Journal of Chemical Information and Modeling 2006;* 46(2):801-7.
[59]. Wang JL et al. Stability and Electronic Properties of Nitrogen Nanoneedles and Nanotubes. *Journal of Chemical Information and Modeling 2006;* 46(5):1965-71.
[60]. Wang X et al. Engineering nanomaterial surfaces for biomedical applications. *Exp Biol Med 2009;* 234(10):1128-39.
[61]. Alonso M et al. Aromaticity changes along the reaction coordinate connecting the cyclobutadiene dimer to cubane and the benzene dimer to hexaprismane. *Structural Chemistry 2007;* 18(6):773-83.
[62]. Stephens DJ, Pepperkok R. The many ways to cross the plasma membrane. *Proceedings of the National Academy of Sciences of the United States of America 2001;* 98(8):4295-98.
[63]. Na S et al. Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proceedings of the National Academy of Sciences of the United States of America 2008;* 105(18):6626-31.
[64]. Chen X et al. A cell nanoinjector based on carbon nanotubes. *Proceedings of the National Academy of Sciences of the United States of America 2007;* 104(20):8218-22.
[65]. Kim W et al. Interfacing silicon nanowires with mammalian cells. *Journal of the American Chemical Society 2007*; 129(23):7228-9.
[66]. Han SW et al. High-efficiency DNA injection into a single human mesenchymal stem cell using a nanoneedle and atomic force microscopy. *Nanomedicine : nanotechnology, biology, and medicine 2008;* 4(3):215-25.