An Experimental Comparing Analysis Research on the Nano-Vectors for Drug Delivery and for Gene Therapy

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Abstract:-The purpose of this research is to explore the application of nanotechnology to prepare optimal vectors for drug delivery and for gene therapy. By reviewing relative literature, we found out that nano vectors can be divided into four types: (1) organic high molecular, (2) inorganic mineral clay moutmorillonite, (3) ultrasound, and (4) nanoparticulates. First we review various types of drug delivery systems as vectors and then to compare the differences between the nanovecotrs with other vectors. Finally an experimental comparing analysis is conducted. The findings show that applying nanotechnology will provide preparing nano vectors for both drug delivery and gene therapy another promising implication.

Key-Words: - Nanotechnology, Nanovectors, Biomolecular, Drug delivery, Gene therapy.

1. Background

Nanomedicine is the application of nanotechnology to the prevention and treatment of disease in the human body. Nanomedicine exploration is the biologically motivated discovery and development that will incorporate nanotechnology tools, devices and processes to provide fundamental insights into cellular function and dysfunction, and in turn lead to therapeutic interventions for disease and decrease the drug-resistant impact on Transmission [11]. The World Health Organization estimates that up to 50 million persons worldwide may be infected with drug-resistant strains of tuberculosis *(TB)*. Drug-resistant tuberculosis is produced by the selection of resistant strains in patients who fail to complete chemotherapy with the correct combination of drugs [11]. Nanomedicine is now within the realm of reality starting with nanodiagnostics and drug delivery facilitated by nanobiotechnology. Miniature devices such as nanorobots could carry out integrated diagnosis and therapy by refined and minimally invasive procedures, nanosurgery, as an alternative to crude surgery. Nanotechnology will markedly improve the drug-resistant, implants, gene therapy, and tissue engineering approaches as well.

Gene therapy is an experimental technique that involves introducing genetic material into a

person's cells to treat or prevent disease. In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery. Researchers are testing several approaches to gene therapy, including: (a) Replacing a mutated gene that causes disease with a healthy copy of the gene, (b) Inactivating, or "knocking out," a mutated gene that is functioning improperly, and (c) Introducing a new gene into the body to help fight a disease [19]. It was also demonstrated that through hierarchical clustering on feature importance vectors, hidden relationships among gene ontology (GO) terms can be discovered [8]. Researchers are studying gene therapy for a number of diseases, such as severe combined immuno-deficiencies, hemophilia, Parkinson's disease, cancer and even HIV, through a number of different approaches. [20] Although gene therapy is a promising treatment option for a number of diseases (including inherited disorders, some types of cancer, and certain viral infections), the technique remains risky because the drug remains resistant and is still under study to make sure that it will be safe and effective[11].

Currently an increasing use of nanotechnology by the pharmaceutical and biotechnology industries is anticipated. Nanobiotechnology, an integration of physical sciences, molecular engineering, biology, chemistry and biotechnology holds considerable promise of advances in pharmaceuticals and healthcare [12, 16]. The most important pharmaceutical applications are in drug delivery. Nanotechnology will be applied at all stages of drug development - from formulations for optimal delivery to diagnostic applications in clinical trials. In addition to providing a solution to solubility problems, nanobiotechnology offers an intracellular delivery possibilities and has the potential to provide controlled release devices with autonomous operation guided by the needs [12,16].

2. Objectives

The objective of this paper is to study nanovectors for drug delivery and for gene therapy. From the relative medical literature review, we explore the delivery process, technology method, properties of nanoparticles, and the types of nanovectors for drug delivery and for gene therapy.

Most therapeutic drugs distribute to the whole body, which brings about generally toxicity and poor patient's acceptance of the treatments. Transport across the membrane is one of the key obstacles drug molecules must overcome to effectively function in the cell. Potential drugs should therefore be designed taking into account these specific membrane-transport properties [13]. Although a variety of drug delivery systems as vectors have been designed with their own advantages and limitations, scientists are learning to optimalize drug delivery to enhance the bioavailablility of the drugs towards diseased cells, promoting the required response while minimizing side-effects. Therefore in the field of targeted therapies or in gene therapy how to develop chemically derivatized drugs or drug delivery vectors able to target defined cells by means of specific recognition mechanisms and

also able to overcome biological barriers has become a common goal the scientists try to reach[9,10].

2.1 Nanoparticles

What is *nanotechnology*? Most definitions of Nanotechnology revolve around the study and control of phenomena and materials at length scales below 100 nm and quite often they make a comparison with a human hair, which is about 80,000 nm wide. And what are Nanoparticles? Nanoparticles are often defined as particles of less than 100nm in diameter. Nanoparticles are also classified to be particles less than 100nm in diameter that exhibit new or enhanced size-dependent properties compared with larger particles of the same material.[21] Nanoparticles can be classified into three catagories: (1) combustion-derived nanoparticles (like diesel soot), (2) engineered nanoparticles like carbon nanotubes, and (3) naturally occurring nanoparticles from volcanic eruptions, atmospheric chemistry etc. Titanium dioxide, alumina, zinc oxide, carbon black, and carbon nanotubes, and "nano-C_{60}" are tipical nanoparticles. For example, titanium dioxide and zinc oxide become transparent at the nanoscale, however are able to absorb and reflect UV light, and have found application in sunscreens. Because of their larger surface area per unit mass, nanoparticles seem to have some different properties from larger particles.

2.2 Types of Delivery Vectors

Before exploring the delivery vectors in gene therapy, we will first review various drug delivery vectors. Numerous biodegradable vectors have been used for drug delivery, including liposomes, microspheres made of the biodegrable poly (lactic-co-glycolic) acid, albumin microspheres, synthetic polymers, peptoid Fluo-{6,6,6,6,6,6}-NH delivery [13], protein-DNA complexes, protein conjugates, and erythrocytes [11,12]. Apart from micron drug delivery vectors, over the past few decades, there has been extensive interest in developing sub-micron drug delivery vectors, such as nanoparticles.

Currently nanoparticles have served as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules[6,11]. They have been used in vivo systemically or locally to protect the drug entity in the systemic circulation, restrict access of the drug to the targeted sites and to deliver the drug at a controlled and sustained rate to the targeted cells. Various polymers have been used in the formulation of nanoparticles for drug delivery research [2, 4], particularly biodegradable polymeric nanoparticles, such as liposomes, micelles, nanospheres, nanoparticles and nanocapsules. They have been applied to drug targeting, cancer chemotherapy [3], intracellular antibiotherapy[14], gene therapy [4], ocular and oral drug delivery[3] , oral drug delivery[4,16], etc.

By using different preparing methods, nanospheres, nanoparticles and nanocapsules can be obtained. Nanocapsules are one of the sub-micron drug delivery vectors in which the drug is confined to a cavity surrounded by a

unique polymer membrane, while nanospheres are matrix vectors in which the drug is physically and uniformly dispersed [15].

Nanoparticulate drug vectors include a class of particles made of polymers or lipids that — because of their size and chemical composition — permit systemic and local treatment. Synthetic polymers offer an almost infinite array of chemical composition and structure combinations. Polyesters, including polylactide (PLA) derivatives and polyepsiloncaprolactone (PCL), polyalkylcyanoacrylate (PACA) and corresponding copolymers with polyethylene glycol (PEG), polysaccharides and polyethylenimine, are a few of the polymers with the requirements that make them useful as nanoparticulate drug carriers[3]. Depending on the materials of preparation, nanoparticles have been prepared most frequently by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers [10]. The applications of nanoparticulate delivery systems include: for tumor targeting; for oral delivery of peptides and proteins; for targeting of nanoparticles to epithelial cells in the GI tract using ligands; for gene delivery; and for drug delivery into the brain, etc.

Unlike organic drug vectors, in 1980 Camazano & Sanchez and Vicenteu utilized montmorillonite (MMT), one of clay minerals, as drug carrier in which drugs are intercalated into layers to control the drug release under different sites. In 2002 Jian & Lin and Lee utilized pillared-montmorillonite to develop a new carrier for sulfasalazine (SAZ) for the sustained release purpose. Such a complex of SAZ-pillared-MMT, will exhibit a high durability as adduct, because its SAZ may be released slowly from pillared-Mont molecular interlayer [7].

2.3 Vectors in Gene Therapy

In recent years, gene therapy is opening up a possibility for treating some incurable diseases. Gene therapy covers a broad range of applications, from gene replacement and knockdown for genetic or acquired diseases such as cancer, to vaccination, each with different requirements for gene delivery. In most gene therapy, a "correct copy" or "wild type" gene is provided or inserted into the genome; therefore a carrier called a vector must be used to deliver the therapeutic gene to the patient's target cells. Therefore in gene therapy, an optimal gene delivery vector plays a very important role. Currently, the most common vectors in gene therapy involve three types: (i) viral methods, such as viruses, retroviruses, adenoviruses, adeno-associated viruses. These viral vectors show great promise for the development of "magic bullet" gene therapy. ; (ii) non-viral methods, such as naked DNA, oligonucleotides, and lipoplexes and polyplexes, and (iii) hybrid methods, such as virosomes and dendrimers[17]. Both viral vectors and synthetic liposomes have limitations and risks, including complexity of production, limited packaging capacity, and

unfavorable immunological features.

Viruses are the most common type of vectors used in gene therapy, involving a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Viral vector have natural host cell populations that they infect most efficiently. Retroviruses have limited natural host cell ranges, and although adenovirus and adeno-associated virus are able to infect a relatively broader range of cells efficiently, some cell types are refractory to infection by these viruses as well. Attachment to and entry into a susceptible cell is mediated by the protein envelope on the surface of a virus. Retroviruses and adeno-associated viruses have a single protein coating their membrane, while adenoviruses are coated with both an envelope protein and fibers that extend away from the surface of the virus. Retroviruses are the first vectors used in gene therapy. [20] These vectors show great promise for the development of "magic bullet" gene therapy

Non-viral vectors, on the other hand, with simple large scale production and low host immunogenicity being just two, show certain advantages over viral methods and have good therapeutic potentials. To improve the delivery of the new DNA into the cell, new moleculars, lipoplexes and polyplexes, have been created that have the ability of transfection efficiencies similar to those of viruses and of protecting the DNA from undesirable degradation during the transfection process. The most common use of lipoplexes, complexes of liposome with DNA, has been in gene transfer into cancer cells. Recent studies have shown lipoplexes to be useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis. Polyplexes, on the other hand, are complexes of polymers with DNA. The major difference between polyplexes and lipoplexes is that polyplexes cannot release their DNA load into the cytoplasm. [20].

Due to every method of gene delivery having drawbacks, some hybrid methods have been developed that combine two or more techniques, such as dendrimers that can be specially constructed to carry a DNA or RNA payload that transfects cells at a high efficiency with little or no toxicity [17].

Gold nanoparticle (Au NP) is again utilized in a research in 2008 July. Yu Cheng and Clemens Burda, researchers at Case Western Reserve University, have developed a technique that has the potential to deliver cancer-fighting drugs to diseased areas within hours, as opposed to the two days it currently takes for existing delivery systems. The system uses gold nanoparticle (Au NP) vectors to deliver photodynamic therapy (PDT) drugs through the bloodstream to cancerous sites since gold is chemically inert with a versatile surface chemistry, large surface-to-volume ratio and variable size and shape, and nontoxic to the human body. The diameter of an Au NP is only 5 nm and the addition of PEG ligands expands the total diameter to 32 nm, larger than some other nanoparticles currently in use, but still small enough to pass unencumbered through the

bloodstream. Looking like a "Hair Ball", each Au NP is coated with polyethylene glycol (PEG) ligands.

In a 2009's study, the researchers review the prospects for nonviral biological delivery vehicles as gene therapy vectors with focus on their unique evolved biological properties and respective limitations and potential applications. The potential of these nonviral biological entities to act as clinical gene therapy delivery vehicles has already been shown in clinical trials using bacteria-mediated gene transfer and with sufficient development. These entities will complement the established delivery techniques for gene therapy applications [18].

3. Method

Nowadays new vectors for Gene Therapy have been proposed through the integration of nanoparticles with virus or ultrasound or high molecular under the competitive medical environment. Many researchers study the relative topics by means of case study method or exploratory method, but lack of the support of the real experimental data. Based on nanoparticles, traditional drug delivery vectors, and traditional vectors for gene therapy, nanovectors for drug delivery and for gene therapy were investigated in this paper. Then, we analyzed the differences among the organic high molecular, inorganic mineral clay montmorillonite, ultrasound and nanoparticulate vectors.

Method of secondary data analysis was used here to complete the experimental analysis design. The real experimental data are taken from our literature review by using the induction. The data in "Paclitaxel-Functionalized Gold Nanoparticles", by Jacob D. Gibson, et al. (2007), "Biophysical Characterization of an Integrin-Targeted Lipopolyex Gene Delivery Vector" by P. A, Shamlou., et al. (2007), "Biological Gene Delivery Vehicles: Beyond Viral Vectors, Molecular Therapy" by Y, Seow., et al. (2009), and the date in the studies by Yang-chuang Chang (2005), Yuh-herng Lin (2006), Chang & Shieh & Chang and Chew., (2005), and C. Montemaguo, (2000), are used as the main experimental data to support our experimental analysis. The induction and conduction are utilized.

4. Results of this research

4.1. Experimental Comparing Analysis

The vectors explored in this article include: (1) organic high molecular, (2) inorganic mineral clay moutmorillonite, (3) ultrasound, and (4) nanoparticulates. On the basis of existing literature, inclusive of Sprockel and Prapaitraku., (1990); Watts & Daivs., and Malia., (1990); Ogawa., (1990); Jian & Lin and Lee., (2002): Tacibana and Taichibana, (1995); Barratt., G.M., (2000); Shinka., (2002); Ahn. &Choi, and Cho., (2004); Yang-chuang Chang (2005); Ren-yil Lee (2000); Yuh-herng Lin (2006); Shyang-yuh Liaw (2004); Chang & Shieh & Chang and Chew., (2005); C. Montemaguo, (2000), we inference out the estimated values in the Table 1. It's a secondary comparing data value. Based on these estimated values, we first do the comparison and analysis to find out the implications of relative factors, and then compared the properties of these vectors.

Table 1 shows that the encapsulation efficiency of all the vectors reaches up to 90%;

therefore, in the future, 90% encapsulation efficiency has to be the lowest criterion. As for releasing efficiency, since inorganic vectors have the problem of initial burst release, they are the most unsteady ones. To have steady release efficiency has to be the common goal to reach. Because of different preparing conditions, the drug loadings of all the vectors are rather different. Steady drug loading also has to be the common goal to reach. As for product efficiency, all the vectors reach up to 50%; therefore, in the future, 70% should be the lowest criterion. Among all the vectors, only nanoparticulates have the properties of in vivo detectors gene diagnosing, non-surgery, and non-oral administation. Finally, all the vectors face the problem associated with toxicity. How to deal with the toxicity problems associated with nanovectors have to be researched in the future, and currently there is a lack of sufficient data in this field.

Table 1. Experimental Comparing Analysis

Vectors: / Factors	organic high molecular	inorganic mineral clay montmorillonite	ultrasound	nanoparticulates
Encapsulation efficiency	>90%	10~80%(AVG:42%)	50%~90%	70%~98%
Burst speed rate	60%~14%	initial burst (100~70%)	<70%	84%~69%
Experimental drug loading	98%~86% (2%~14%)	90%~57%	No data	57%~20%
Production rate	40%~68.94%	>50%	>50%	70%~75%
Virus burst rate	√	√	√	≠
Long term vector	√	√	√	√
vivo detector	×	×	×	√
gene diagnosing	×	×	×	√
Acid melting	√	√	√	√
Resistant Bacteria	√	√	√	√
Zhao removed disease	×	×	×	√

Note: 1. "√": means is yes; "≠": a little; "×": is no.

Data sources: We inference out the estimated value on Table 1 based on the secondary data analysis method from reference 1, 2, 3, 4, 5, 17, 18, 20.

4.2 Findings and Discussion

Depending on the materials or the methods of preparation, and the mechanisms or the surface size of the particles, the drug delivery vectors can be categorized into virus or non-virus, organic or inorganic, micron or sub-micron, particles or nanoparticles, biodegradable or non-biodegradable, polymers or non-polymers, including bacteria, virus, liposomes, microspheres made of the biodegrable poly (lactic-co-glycolic) acid, albumin microspheres, synthetic polymers, protein-DNA complexes, protein conjugates, and erythrocytes. They, in other sense, can be divided into four types: (1) organic high molecular, (2) inorganic mineral clay montmorillonite; (3) ultrasound, and (4) nanoparticulates. Among them, nanoparticulates are the most potential one. Particle size and size distribution are the most important characteristics of nanoparticulate delivery vectors. They influence the in vivo distribution, biological fate, toxicity and the targeting ability of nanovectors. In addition, they can also influence the drug loading, drug release and stability of nanoparticles.

The review in this article shows that nanovectors have great potentials, inclusive of high encapsulation efficiency, high drug loading, high drug release, and high stability. However, they do have limitations, such as difficult physical handling in liquid and dry forms, limited drug loading, burst release, potentially blocking arteries and veins or even blocking the kidneys, triggering sensitization reactions, and some biophysical barriers, such as increased osmotic pressures in malignant cancer tissues. These practical problems have to be overcome before nanoparticle drug carriers can be used clinically or made commercially available.

5. Conclusions

Nanotechnology holds tremendous promise for various cancer treatments. Specifically, nanovectors holds great potential for the delivery of precisely targeted medical procedures that will minimize collateral tissue damage—to a far greater degree than current cancer therapies. To make drug delivery system more optimal, greater understanding of the different mechanisms of biological interactions, nanoparticle engineering, and toxicity associated with nanovectors, is still required. Further improvements are needed in order to turn the concept of nanoparticle technology into a realistic practical application. In the future the following work of this research will be focused on analyzing Nano-vectors correlation in expression pattern based on feature importance vector and various microarray analyses, hoping to provide some implications for the Nano-vectors development.

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