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Research Paper

Application of Micro/Nano Hydrogels for Gene Transfers

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Abstract

Today, the biggest limitation in gene therapy is the lack of appropriate vectors for gene transfer to cells and this causes dullness Process of Clinical treatments in this method. According to what was said, research on Making Vector systems is necessary. In developing different methods of gene therapy for human, choosing a safe and controllable vector with high performance to deliver the therapeutic gene to target cells, is the most key step of the process of designing and carrying out gene therapy. With the advances in nanotechnology and Nano-hydrogels, a new window of the knowledge of gene therapy is opened. The small size of Nano-hydrogels, have create new research opportunities for researchers specially in gene-therapy field and it have been promising in the treatment of many diseases, especially cancer and genetic disorders. However, to prove successful results of the investigation, more clinical trials are needed. Researches are so wide in the field of using Nano-hydrogels in Biomedical and Provide a comprehensive picture of this research is not an easy task. In this research have been tried to deal with the major research areas in the field of Nano-hydrogels used in gene therapy, as much as possible. Investigations showed that in the gene therapy, Nano-hydrogels as Non-viral vectors don't have many of Viral vectors problems.

Keywords: Nano-Hydrogels; Gene Therapy; Genetic Diseases; Non-Viral Vectors

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1. Introduction

Genes are heredity biological units and determinant of most biological traits such as color of eye and hair as well as other subtle features such as the ability of blood to carrying oxygen. Complex properties such as physical ability can be caused by the interaction of several different genes, along with environmental effects. The genes are in the chromosomes inside the cells and they have been formed from deoxyribonucleic acid (DNA) that is a type of biological molecule [1].

Progress in field of familiarity with the genes and using changes on them has provided this possibility for scientists, to change the genetic material to dealing or preventing disease. Gene therapy is entrance a gene and its production to the cells of patient for treatment or decrease of speed of the progress of genetic diseases. In fact, in gene therapy, doctors try to force body to do new work to that has previously not been able to do it by creating changes in the human genetic structure. One of the problems of diseases with genetic fountain is that for their treatment, should be change the cells of body that signs of disease have been appeared on them and correct them. Access to all this cells or significant number of them requires

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creation of necessary introductions for this work. Since the diseases with genetic origin, the cause of producing disease is modified genetic codes that defect caused by that almost found in all cells, the treatment of this group of these patients is along with many problems and limitations [2].

According to what was said gene therapy is a technique for correcting defective genes that are responsible for disease. Researchers may be applying one of several approaches available to correct defective genes. A normal gene may be planted into a nonspecific place into the genome to replace one an unemployed gene. This method is a common approach. Another approach is to replace abnormal genes through "homologous recombination". The abnormal gene could be repair through "selective reverse mutation" that causes the gene to restore normal functioning. Set of a specific gene (the extent to which a gene is turned on and off) can also be changed. In most gene therapy studies, gene "natural" enters into the genome to be replacing pathogenic genes "abnormal" [2].

Genetic material conveyed to cells of patient's body with a variety of methods, including changes in protein available expression in the cell, produce proteins toxic, producing enzymes activating pro-drugs [3], turn off oncogenes by a variety of sirna and other multiple ways, apply their therapeutic effects [4]. Identification of therapeutic gene and transfer that to target cells with high efficiency is necessary for successful performing of gene therapy process [5]. Gene therapy is generally done ex-vivo and in-vivo. In vitro method cells is taken from the patient and the desired gene enters in the outside environment of body to this cells and after that the modified cells are returned to the patient's body. In the other method, gene therapy is done in in-vivo, in this way, that gene should enter in a way to the body and after reaching the cell core, to do its therapeutic function [6]. To entering treating gene into patient target cells, a vector molecule that called a vector is used. In the development of multiple gene therapy methods for human, choice of safe vector and controllable with high efficiency for transfer therapeutic gene to target cells, is considered as most key stage of designing process and performing gene therapy [7].

With the progress in Nanotechnology and making Nanohydrogels, a new window opened up of knowledge gene therapy. Small size of Nano hydrogels has provided new research opportunities for researchers, especially in the field of gene therapy and they have been promised in treatment of many diseases, especially cancer and genetic diseases. However, for proving successful results many of the researches are needed to more experiments clinical. Researches are widely used in the field of Nano hydrogels in biomedical and it's not easy to presenting a comprehensive image of this researches. In the present research, we have tried as much as possible to be addressed the major research areas in the field of using Nanohydrogels in gene therapy.

2. Research Methodology

Present article in terms of goal is functional and method was Descriptive – Analysis Due to the nature. In order to collect information, the library method and check out books and articles on the topic and note taking tool was used.

3. Gene-Therapy

Today, the biggest limitation in gene therapy is the lack of appropriate vectors for gene transfer to cells and this causes dullness Process of Clinical treatments in this method. Therapist gene faces several obstacles, from the moment of entry into the body until it reaches the target cell nucleus, but Naked Gene is not able to overcome these obstacles because of its nature, because on one hand it will be attacked by Nucleases and will destroy, in other hand negative charge density and lack of hydrophilic gene prevent it from entering the cell. So gene should be shipped with the help of special transport until the reach to the site of action. According to what was said, researches on making Vector systems seems to be necessary and Studies and several reports have been published in this area [2].

3.1. The Limitations and Problems of Gene Transfer

A gene vector to carry the genes of a solution (in a vial) to reach the cell nucleus must pass several obstacles inside and outside cells. Viruses have the ability to overcome all obstacles in the way of gene transfer because of their nature, but Non-viral vectors generally don't have the ability to overcome Cellular obstacles and Structures. Understanding the cellular barriers and Non-viral vectors limitations are very to overcome them in better design of this vector [2]. Figure 2 shows the way that non-viral vector passes to deliver the gene to the cell nucleus [8].

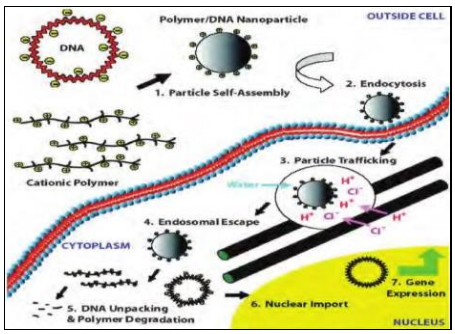

Figure1. Non-Viral Gene Delivery Mechanism [8]

3.1.1. Extracellular Barriers

Extracellular barriers includes a series of physicochemical challenges Such as DNA binding and compression and Maintaining the resulting complex in solution in one hand and challenges in vivo environment Including stable in the bloodstream, the influence of vessel walls and surrounding texture and binding to target cells from the other side.

➢ Packing DNA

Gene vectors bind to DNA molecules and make them to compact nanoparticles. These nanoparticles are able to keep away the DNA molecules from the reach of nucleases Enzymes. Unprotected plasmid DNA, In the presence of DNase enzymes completely destroyed only in a few minutes but the structure of the nanoparticle vector is stable for a long time. This small and compact structure is the result of Electrostatic interactions between the DNA heads of phosphate scaffold and the Positive charges in the vector. Compression process and the formation of nanoparticles, is a progressive reaction with entropy that will be done completely spontaneous during mixing ingredients of vector with plasmid DNA [2].

➢ Serum Stability

Nucleic acid molecules (DNA, sirna, mirna, …) in the situations that they enter vivo circulation will be destroy by nucleases in serum (blood plasma without clotting factors, in other words, "pure blood"), Formation of a complex between nucleic acid molecules and vector gene directory, this molecule on from harm and prevent its degradation by nucleases protected. On the other hand, the resulting complex stability in serum depend on vector structure and toward the negatively charge DNA to vector positively charge. Neutral complexes in physiological salt concentrations immediately constitute a large gathering that basically do not have the ability to transfer gene and even can cause create embolism in the lung, are toxic too. In opposite, complexes by positively charge are stable and do not foundation gathering. However recent studies have shown that the aggregation formation in complexes of positively charge may happen by over time. On the other hand adsorption of albumin and other proteins toxic to the negatively charge complexes is also lead to accumulations of more and more complexes and in ultimately quickly removed them from circulation by the immune system. Hydrophilic polymers connection such as polyethylene glycol (PEG), hydroxypropyl Mtaakrylamyd (HPMA), oligosaccharides and sugars by complexes are leading to decrease gatherings formation and the more stable them. These compounds prevent the creation of space around the complex particles are cause decrease by Interactions between particles complex on one hand and decrease interactions between the complex particles with serum proteins (such as albumin) on the other side [2].

➢ Targeting Gene Directory Vector

If the gene directory is done with treat cancer target and the treatment process is along by killing cancer cells with the purpose of carrying the gene directory will be of great importance. Vectors gene directory are not able to enter targeted to a specific cells type lonely. These vectors with connectivity targeting components, in addition to increase the amount of vector-gene complex entry into the cells gains the ability to select and enter targeted to a specific cells type in the target tissue. Binding receptor proteins to the cell membranes such as epidermal growth factor (EGF) and glycoside components such as Plateau are good candidates for targeting gene delivery vectors. These targeted parts suppliers, vector complex entrance - are possible the gene into the cell by mechanism of endocytosis depended on receptor. The success of targeting strategy depends on the variety of factors including the type of chemical bond between the vector and ligand (targeting the same segment), the strength of the bond between the ligand and receptor and finally the number of ligands in each complex vector-gene. More of connections between the vector and ligand are the type of covalent. Due to this point is very important that the covalent bond between the vector and ligand do not lead to disorder in interaction between ligand- receivers; thus, efficient targeting needs to optimize all parameters that affect the binding to the cell surface [9].

3.1.2. Intracellular Barriers -

Cellular uptake:

At the cellular level, the first barrier on the way of complex polymer / DNA is plasma membrane (cell membrane). There are several routes to enter to the cell that contains Phagocytosis, pinocytosis, Endocytosis by Clathrin-Mediated, Endocytosis by Caveolae mediated and Endocytosis without clathrin and Caveolae.

Endocytosis or endocytosis is an active process (needs energy) in which the cell take molecules or objects into his own. Sometimes endocytosis molecules are very important but for some reason such as large magnitude or polarity cannot easily cross of cell membrane. Endocytosis can be classified to three types of phagocytosis, Pinocytosis and receptormediated endocytosis. Phagocytosis or phagocytosis and pinocytosis or Pinocytosis, both are mechanisms of endocytosis, which of course are different from each other. In the

phagocytic one cell can devour other cell. This process is usually occurring when the other cell has been under the pressure, damaged, worn out, become cancerous, or has been infected by virus or is an alien. Cells pull large polar molecules, proteins, sugars, and so on inside through hydrophobic cell membrane with the process of endocytosis. This process is performed by energy consumption. Cell are required to perform many tasks such as recycling cell surface receptor, entering extracellular molecules to the cell, as well as inclusion and decomposition of various pathogenic factors around the cell.

In general, phagocytosis means entering the solid particles, extracellular and larger such as bacteria from environmental surroundings and sometimes are saying it "eating cell"; While Pinocytosis means entering extracellular fluids with its dissolved molecules from an environmental surroundings and is sometimes called "cell drinking". Figure 2 illustrates processes of phagocytosis and Pinocytosis.

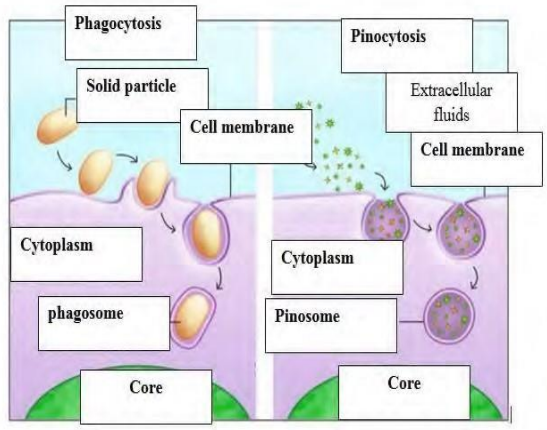

Figure 2. Schematic representation of the processes of phagocytosis and Pinocytosis

The size of the complex polymer / DNA effects on the cellular uptake of the various routes. . The optimal size of the non-purposeful cationic complex vector / DNA in order to gene transfer is between 70 and 90 nanometers. In addition to the non-purposeful complexes vector / DNA, several polyplexes are designed aimed to entering to the specific cells through endocytosis by receptors mediated. For this purpose, various types of ligands were used in polyplexes. As soon as the connection of receptor to the system surface, polyplexes are entering to the cell through endocytosis by clathrin-mediated and endocytosis by Caveolae mediated. Figure 3- illustrates an example of an endocytosis by receptor mediated.

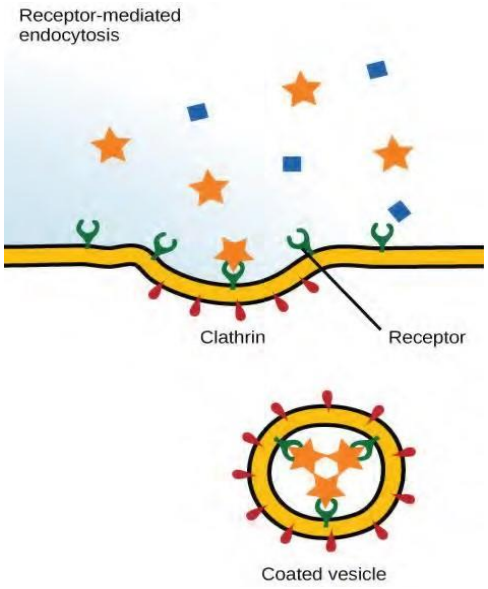

Figure 3. Endocytosis by receptor mediated

While cationic systems vector / DNA, are often successful in laboratory conditions; these systems in physiological conditions are usually suffer barriers due to the presence of various instabilities. Interaction with plasma proteins (Opsonization) in the significantly form is barrier to cellular uptake and increases the possibility of accumulation of vectors and also elimination of them by RES. In addition, physiological salt found in the bloodstream, usually causes exacerbated the accumulation of cationic polyplexes leading to artery blockage.

➢ Endo-Lysosomal Escape :

After cellular uptake, the route of intracellular of system depends on the way of entering it to the cell. At the present time, most studies have been conducted on the route of endocytosis by clathrina mediated. By using the route of endocytosis by clathrina mediated, polyplexes can be surrounded within the endosomal vesicles and pass the route of endo-lysosomal. An important issue for some intracellular vectors, especially nucleic acids is escape from endosomal and lysosomal destruction because of their acidic environment (PH 5-6/2).

➢ Mobility in The Cytoplasm and Entering to The Nucleus:

Unlike sirna that the place of its activity is into the cytoplasm, DNA must be delivered to the nucleus to begin gene expression. As soon as the release in the cytoplasm, Polyplexes must overcome the barriers exist in Cytosol. In the this section there are strings which have a dense network structure and prevent intrusion of large molecules such as naked DNA. Vectors compresses DNA as much fine particles

help to its displacements into the cell. In addition, there are many nucleotide enzymes within environment of Cytosol that are ready for destruction of not protected nucleic acids. Cationic vectors can protect DNA from destruction like this in the cytoplasm.

4. Hydrogels and Nanohydrogels

Common and wrong explanation in the polymer science is the use of term "gel" or "hydrogel" as synonymous. Although polymeric networks of gel and hydrogel may be similar in terms of chemical, but they are different in terms of mechanical. Dorithy Jordan Lioyd has been said rightly about gels “colloidal state, gel, is state that diagnosis of it is simpler than defining it." In terms of technical, gels are semi-solid systems made up of a small amount of distributed solid in the a relatively large amount of liquid that their solid-like properties are more than their liquid-like properties. Sometimes by the prefix "hydro", hydrogels are described as aqueous gels. In fact, the hydrogels are made of cross linking networks of hydrophilic polymers. They are insoluble in water but have the ability to absorb large quantities of water and swollen, while retain their structure. Gels are polymeric networks that swell to reach equilibrium and adding more fluid would lead to the dissolution of polymeric network.

4.1. Classification of Hydrogels

According to the nature of the bounds between the molecules in the polymeric network connection, hydrogels can be divided into two groups: Chemical and physical hydrogels. In the physical hydrogels network are held together by molecular nodes or secondary forces include ionic forces, hydrogen bonds or hydrophobic forces. Physical hydrogels are not homogeneous, because molecule nodes cluster or being bonded hydrophobic zones or ionic can create a heterogeneous. The free end of a chain or ring chain also causes transient disadvantages of network in physical gels. Hydrogels are called permanent or chemical gels, when the networks are cross linking as covalent. There are very different macromolecule structures for physical and chemical hydrogels. They include the following: crosslinking networks or tied of linear homopolymers, linear copolymers, cluster or bond, polyion-ion- multivalent ion, polyion-polyion or hydrogen bond complexes, stabilized hydrophilic networks by hydrophobic areas and Interpenetrating Polymer Network or physical mixtures. Hydrogels may contain different physically forms including solid molded forms (such as soft contact lenses) fields of compressed powder (such as tablets or capsules to digest food), nanoparticles (such as vectors of bio-sticky for wound treatment), coating (such as implants level, tablets or capsules), membranes or sheets (as a tank in an adhesive of liberation of transcutaneous).

4.2. swelling of hydrogels

The rate pf water in Hydrogels can determine solvent exudation or nutrients to the inside and solute exudation or cellular products to the out of gel, when a dry hydrogel began to absorb the water, the first water molecules entering to the field solvate the most polar and hydrophilic groups of hydrogen and leading to the creation of the primary bond water. By hydrolysis of polar groups, the hydrogel will swells and hydrophobic groups are exposed interaction by water molecules that lead to the formation of Hydrophobically Bound water or secondary water. Usually for convenience, primary and secondary bond water, are called total bound water. After the locations of polar and hydrophilic are interacted with molecules of bond water, the network traps more water due to osmotic motive force of its chains. This additional swelling is in the opposite direction with cross linked and physical and leads to create a Retraction force in the network. So hydrogel will reach to an equilibrium swelling level. Additional swelling water that traps after saturation of ionic groups, polar and hydrophobic or bond water in the hydrogel, is called free water or Bulk water and it is assumed that it fills the space between network chains or space inside the large holes. With swelling of hydrogel, if network or cross linking be degradable, hydrogel will began to fragmentation and dissolution by speed dependent on its composition.

4.2.1. Factors Affecting Swelling of Hydrogels

Proportion of a cross linking is one of the most important factors that effect on the swelling of hydrogels. The proportion of cross linking is defined as the proportion of moles of cross linking factors to the moles of repeating unit of the polymer. Hydrogels with more cross linked have denser buildings, and in comparison with similar hydrogels with lower cross linking proportion will have lower swelling. Chemical structure of the polymer can also have an impact on the amount of swelling of hydrogel. Hydrogels containing hydrophilic groups in comparison by hydrogels contain hydrophobic groups have a higher amount of swelling. Hydrophobic groups are wrinkled in contact with water, so their contact surface with the water molecules will be minimized and they will have a lower amount of swelling. Swelling of hydrogels sensitive to the environment can be affected by certain motives and swelling of temperature-sensitive hydrogels varies with variations in ambient temperature.

5. A Review on Conducted Researches

In the following some researches on the field of using nano-hydrogels in the gene therapy applications will be discussed.

5.1. Gene Therapy by Nanoparticle Sensitive to Magneto

Magnetic force is known as one of the best options for external excitation of drug and gene delivery systems, because compared to other physical motives such as light radiation, ultrasonic and electric field has no physical interaction with body. Another advantage is that the magnetic material reacts as soon as excitation by an external field.

Magnetic transfection is a method for controlled gene delivery. Mah & et al [14], in 2009 by connecting nanoparticles to the Non-viral vector, reported the first case of magnetic transfection. A new nanocomposite of silica-magnetite (Fe3O4-SBA-15) that its level was covered with small chains of complex Polyatylin-imin of DNA (PEI-DNA). When an external magnetic field was applied to this composite, leads to 15 percent more transfection.

Lu and Hernandez Reported that the nanoparticles placed inside the carbon nanotubes magnetic (msns) can be used to trap nucleotides within the porosities of these nanoparticles and by functionalization, we can change them to a system sensitive to magnetic fields. At these nanoparticles sensitive to motives, warheads are made iron oxide that trap genes into the porosities matrix. These warheads are connected to a string of DNA and another string connected to the silica membrane porosities with porosities with a diameter of 2 to 50 nanometers (mesoporous). Due to the spiral structure of DNA, the created structure is maintained and as a result under normal conditions, no gene will release. By applying a magnetic field, the temperature of these system increases, the bonds created by broken DNA and genes or molecules are released from the matrix. This sensitive mechanism to motives operates like a switch-off key.

5.2. Gene Therapy by Nanoparticle Sensitive to Ultrasonic

Two main advantages of ultrasonic means energy concentration and the depth of good effect, raise this method as a convenient method for gene delivery. There are many commercial Contrast US Agent such as Optison, sonovue, and Levovist that can be used to monitor the delivery of therapeutic genes while applying ultrasound by low-intensity. Low-intensity of ultrasonic prevents damage to the tissues and organs of the body. When the vector reached to the the target tissue, we can apply high-intensity ultrasonic that leads to creating a vacuum in the microbubbles gas core and release of DNA to the target cells.

He and his colleagues [17], were used sirna delivery method to improve in the treatment of yolk sac cancer in the laboratory conditions and to the collapse of the microbubbles used ultrasonic. They suggested that due to negatively charged of DNA, cationic lipid microbubbles are a good option for gene delivery. Atger and his colleagues [18], indicated the capability of ultrasonic to increase the gene expression and efficiency of transfection by using cationic liposomes. Other reports also indicate the use of ultrasonic in the treatment of cardiovascular, such as preventing of re-blockage and improvement of gene delivery vascular, reducing hardening of the tissues of the kidney in the chronic kidney disease, transfer the luciferase gene to the tissues around the mouth that lead to high gene expression for genes placed in the muscle cells of gum tissues.

5.3. Gene Therapy with Nanoparticles Sensitive To PH

Chemical-ionization groups include acid groups (proton donor) and base (proton receivers) weak (such as carboxylic acids and amine) are used respectively in anionic and cationic polymers sensitive to PH. These systems sensitive to PH have advantages such as better cellular uptake, more efficient gene delivery (for example pdna and sirna), easier rupture of endosomal membranes by the effect of sponge proton (instability Adozomy through osmotic swelling), restore the surface charge of Nano vectors and finally the release of the vector in low PH of tumor tissue (extracellular). Chitosan nanoparticles sensitive to PH, this polymer has been created a perfect option for Non-viral gene delivery and with features such as creating low cytotoxicity and transfection and high gene expression. Figure 4, indicates a variety of nanostructures based on chitosan that are used in biomedical.

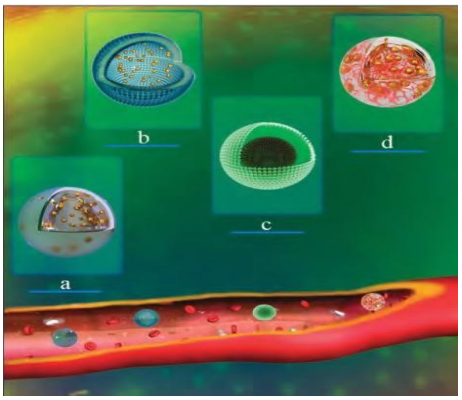

Figure 4. The types of chitosan Nanostructure used in biomedical a) nanospheres b) vesicles c) micelle d) Nanogel

Peptides are also considered as safe and efficient nano-vectors in Non-viral gene delivery to the cells in that of course in their structural design have been inspired of the structure of viral systems of gene transfer. Peptide vectors have advantages such as the ability to penetrate the cell membrane, endosomal fusion (in the low PH) and delivered to the core. These vectors do not have some adverse effects of viral vectors such as inflammation and irritation, resistance of the immune system and cause cancer or even death. Acid-Labile Linker that react to small changes of PH, also can be considered a new categories of nanoparticles with high sensitivity to PH. Covalent bonds of acid- Labile in acidic environments, such as tumor tissue and cell components, including lysosomes (PH 5/4-5), the primary endosomes (PH 6-5/6) and the next endosomes (6-5 PH 5-6) are rapidly hydrolysis. Polymers that have such connectors (like Stal / Ketal, hydrazine, imine groups and etc.) Are stable at physiological PH of the body while by reducing PH and hydrolysis of their bonds, genes will find the capacity of release. These broken able bonds can be placed in the main chain of polymer or lateral chains. Acid-Labile Linker in nanostructures have been indicates significant advantages in gene delivery such as biocompatibility, biodegradability, high delivery efficiency, efficient gene transfer with resistance against good serum and cytotoxicity. The use of nano-systems includes bonds sensitive to PH hydrazine provides capability of simultaneous delivery of drugs such as DOX with sirna to the tumor cells. Figure 5 is a nanoparticle with branch structure of dendrites that contains Hyparyn (a blood-thinning drug) bounded with DOX by a hydrazone acid- Labile bond that its surface has negatively charge. This nanoparticle indicated more quickly release in PH 5 than PH 7 that were aligned by a significant destruction of cancer cells 4T1 (related to breast cancer) and without significant adverse effects.

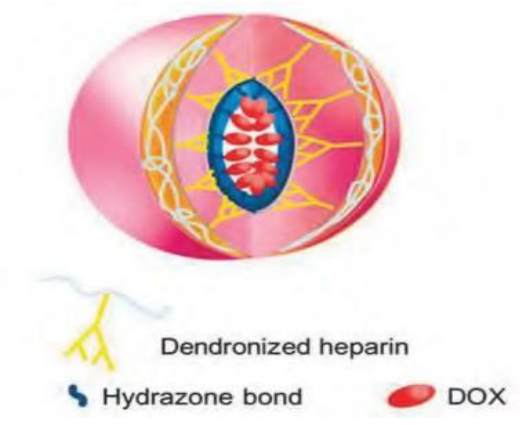

Figure 5. nanoparticles containing dendriworms complex heparin

In the discussion of drug and gene delivery together, the issue of the time interval between the start of the function of genes (24 to 72 hours for transcription and translation of genes or to prevent the expression of the protein) and start the drug activity should be fully considered. . As a suggestion for future research in this field, this issue may be solved with a new class of nanoparticles that have ability to release sequentially (stage) genes and drugs in optimal intervals. In order to use of internal motives (like enzyme activity and PH) and external motives (such as temperature or light) can be the release solution of drug and gel individually. By entering nanoparticles to the cells and endosomal parts, genes are released by internal motives and their function ends within 24 to 72 hours. Then, by applying an external motive, drug release takes place in the next stage.

Recent researches on gene delivery systems indicate that viral and Non-viral vectors, plays an important role in the ultimate success of gene therapy, so the way of behavior of vectors in vitro and in inside the body is very important to reduce the failure rate in clinical trials. In fact, understanding the mechanisms that leads to the determination of transfection efficiency can significantly increase the success chance of gene therapy applications. For example, Stopak and his colleagues [22,23], assessed liposome-based vectors in clinical trials for the treatment of cancer. The product of them (Allovectin-7, a complex lipid / plasmid) containing one molecule of DNA was clear that the complex Microgolobolin was carrying HLA-B7 / β2. They proved that there is a logical metamorphic process of complex formation, intracellular delivery of them and ultimately achieve the successful results in patients with metastatic melanoma in both phase 1 and 2 of clinical studies. Nevertheless in the phase 3 of trial, 375 patients were collected from 100 medical centers and Allovectin-7 was tested on them; The obtained results indicate the failure of this product in the impact on patients, thus clinical trials failed and program stopped.

6. Conclusion

In gene therapy, nano-hydrogels as Non-viral vectors, do not have a lot of problems of viral vectors and due to their small size and ability to reform, they have ability to cross many intracellular and extracellular boundaries in order to have an efficient gene delivery. Chitosan and alginate are most applied natural polymers and polyethylene alcohol, polyethylene oxide and polyethylene imine, are most applied synthetic polymers in the preparation of nanostructured hydrogels. . The most important fields of use of nano-hydrogels can be considered in cancer therapy, insulin delivery, and protein and gene delivery. Smart

nanohydrogels or sensitive to motive, have attracted most attention in various researches, for example, gene therapy with nanoparticle sensitive to magnetism, because of the lack of physical or chemical interaction and gene therapy with ultrasound because of the energy concentration and depth of good effect is one of the best methods of gene therapy with foreign stimulate. Gene therapy with nanoparticle sensitive to PH also, due to the acidity of tumor tissues, is one of the best ways to attack tumor cells that is place in the category of gene therapy by internal stimulate.

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