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Polymer nanoparticles for targeted gene delivery

Salam Massadeh and Manal Alaamery

King Abdulla International Medical Research Center, King Saud Bin Abdulaziz University for Health Sciences, Developmental Medicine Department, King Abdul Aziz Medical City, Ministry of National Guard Health Affairs. P.O Box 22490, Riyadh 11426, KSA.

Outline:

Introduction	2
Gene therapy	3
Methods of Gene Therapy.....	4
<i>Viral vectors used in gene therapy</i>	4
<i>Non Viral gene delivery methods</i>	6
Polymer nanoparticles in gene therapy	7
Conclusions	9
References	10

Introduction

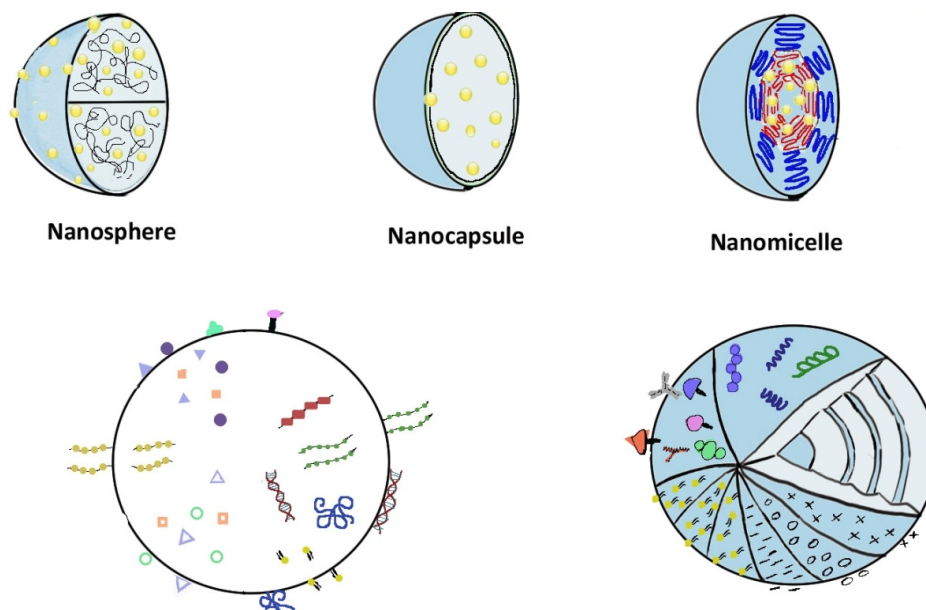
Gene therapy is a medical intervention that uses genes for the treatment or prevention of disease. If the gene of interest is delivered properly to the desired site, then this strategy would allow the direct insertion of a gene into a specific cell. Gene therapy has gained massive researchers' interest because of its potential to be an alternative for surgery and drug treatments. Gene therapy have been applied to replace a mutated gene that causes disease, knocking out mutated genes, and introducing new genes into cells to help fight a disease.

The first attempts of gene therapy were focusing on the treatment of genetic disorders. In 1989, tumor-infiltrating lymphocytes gene transfer was the first application of gene therapy on human. Moreover, patients with SCID (Severe Combined Immunodeficiency Defect) have been treated by gene therapy on the ADA gene in 1990. More recently, gene therapy is used to treat other diseases, such as autosomal dominant disorders, autosomal or X-linked recessive single gene disorders, polygenic disorders, specific cancer diseases, vascular disease, neurodegenerative disorders, and inflammatory conditions. Many methods of gene therapy have been used to treat numerous disorders^{1,2}.

Nanotechnology is one of the key technologies of the 21st century that merges material science and biotechnology; it is currently attracting the attention of many scientists all over the world. This field involves the utilization of biological systems such as cells, cellular components, and proteins, to manufacture efficient nanostructures. Nanotechnology is the new utensil that explores biomolecular structures, functions and properties. Bionanotechnology made it possible to determine structural elements of cells, molecular recognition and drug delivery³⁻²⁶.

Moreover, nanoparticles have been manipulated to perform as specific targets for therapies, as nano-vehicles to deliver certain therapeutic agents (Drugs, genetic material or a combination of both). Additionally, scientists have developed different types of nanoparticles, like carbon nanotubes, silicon oxides, metal oxides, nanocrystals, lipids, polymers, dendrimers, and quantum dots, together with increasing diversity of newly developed materials. These nanomaterials are modified and conjugated to biomolecules, so that they become highly biocompatible and specific targets to certain tissue. In addition, nanoparticles have an improved blood half-life and physiologic behaviour with insignificant side effects, and minimal or no toxicity to healthy tissues in living organisms. The optimal goal of nano drug delivery systems is to develop clinically useful tools for treating diseases in the clinic^{3,15,27-59}.

The field of nanotechnology in gene therapy is very promising and will revolutionize the therapeutics field especially for the treatment of genetic disorders and some types of cancer. It is an advanced translational research area facilitating translation of basic discoveries to the patients. The pharmaceutical industry is now giving a great deal of attention to commercialize new drug delivery systems especially for gene therapy. However, the process of clinical trials and Food and Drug Authorities is time consuming especially when new materials or chemicals are included in the new formulation. Hence, scientists are focusing on the improvement of existing dosage forms through the use of biocompatible biodegradable nanoparticles^{57,60-68}.

**FIGURE 1.1**

Schematic illustration of nanoparticles used for gene therapy

In this chapter we will shed the light on different aspects of gene therapy. We will discuss current and conventional methods of gene therapy. We will also elaborate on the advantages and disadvantages of the most commonly used methods of gene therapy. Additionally, polymer nanoparticles as gene therapy non-viral vectors will be discussed thoroughly, and the recent work in the field of polymer nanoparticles in gene therapy will be reported in this chapter. At the end of this chapter we will give some final remarks and recommendations on the optimal methods of gene delivery.

Gene therapy

Gene therapy is a medical intervention that uses genes for the treatment or prevention of disease. If the gene of interest is delivered properly to the desired site, then this strategy would allow the direct insertion of a gene into a specific cell. Gene therapy has gained massive researchers' interest because of its potential to be an alternative for surgery and drug treatments. Gene therapy has been applied to replace a mutated gene that causes disease, knocking out mutated genes, and introducing new genes into cells to help fight a disease.

In the same vein, gene therapy corrects cellular dysfunction and genetic mutations by delivering genomic materials into specific cells, gene delivery programs functional proteins by modifying the endogenous gene expression to produce a therapeutic effect. The use of messenger ribonucleic acid (mRNA) is widely used in gene transfer based therapies; in such cases a bulky piece of mRNA includes the promoter sequences that activate expression of the gene, the coding Sequences that direct production of a protein, and signaling sequences that direct RNA processing.

Alternatively, another method of gene therapy includes the down regulation/up regulation of a specific cellular gene. This can be achieved by transferring a relatively short piece of genetic material that is complementary to the mRNA. Gene expression can be affected through many blockage translational mechanisms, mRNA processing, or leading to destruction of the mRNA. The initial research interests in the field of gene therapy were on inherited genetic disorders. The first application of gene transfer in human was in tumor-infiltrating lymphocytes, and on immune deficient patients (SCID, Severe Combined Immunodeficiency Defect).

Gene Delivery methods and techniques have evolved heavily over the past few years, which resulted in many promising treatments for a vast number of disorders. There two main types of gene therapy; the Germline gene therapy and the Somatic gene therapy. The germline gene therapy corrects genetic abnormalities by direct manipulation of germline cells without specific targeting, however, this method has its own limitations. The direct germline cells manipulation has not been tested on human subjects for ethical restrictions. Furthermore, the somatic cell modifications have been applied on human subjects and showed promising outcomes. In the somatic gene therapy, genes are introduced to the diploid cells of the patient, where the genetic material is not relocated to its progeny. This kind of treatment can be classified into In Vitro delivery, In situ delivery and In vivo delivery^{69–89}.

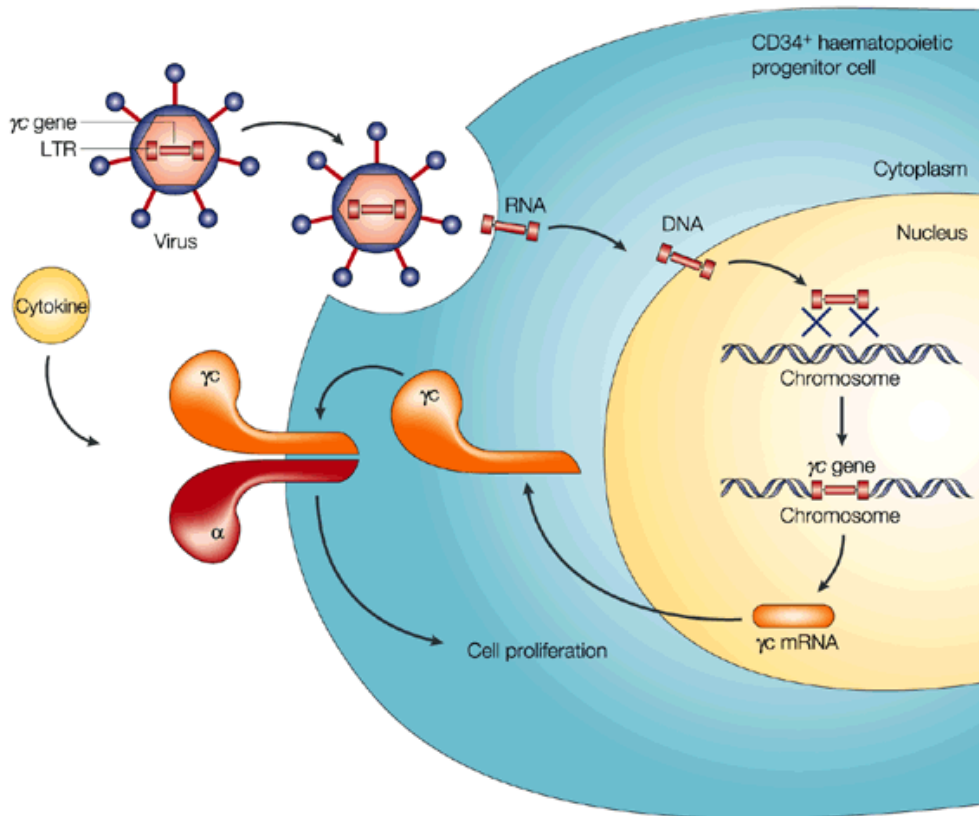
Different gene delivery methods may be used in gene therapy to restore a specific gene function or silencing a special gene. The main aim of gene therapy is to develop a therapy of an appropriate material to repair a mutated gene. Even though gene therapy could be a promising treatment option for a number of diseases, its safety is still negotiable. Therefore, different types of biocompatible vectors have been used to deliver genes intended for gene therapy to overcome the disadvantages encountered with the traditional methods used for genetic material delivery.

Conventional methods of gene delivery

In gene therapy, the genetic material is transferred either through viral or non-viral delivery systems. The most commonly used viral vectors are derivatives from retrovirus, adenovirus, and adeno-associated virus (AAV). When considering gene delivery, three important criteria should be considered. First, the target site (tissue or cells) and its properties and its ability to be transduced. The second issue that should be considered in viral gene delivery, is the permanency of expression required, and lastly the size of the genetic material to be used in gene therapy should also be taken into consideration. In the next section, some of the most commonly used viral vectors will be described briefly.

Viral vectors used in gene therapy

Most of the currently available gene therapy is delivered via viral vectors. The viral vectors used in gene therapy are genetically modified to stop their reproduction which will lead to an enhanced safety. Even though the safety of viral vectors has been improved, they still exhibit many undesirable effects. For instance, viral vectors can induce immunological reactions, prompting the inflammatory system to produce toxins which might lead to mortality. Moreover, viral vectors are used for targeted delivery due to the specific receptors they possess, making it possible for the transfer of transgenes to other particular cells^{90–106}.



Nature Reviews | Immunology

FIGURE 1.2

Schematic illustration of viral gene delivery. The virus binds to the cell, then its followed by cellular internalization of the virus. The viral RNA is retrotranscribed into DNA, to form a preintegration complex, then it recombines within the cell's genome¹⁰⁷

Adeno Virus

Adenoviral vectors are extracted from a vast variety of species; more than 100 different serotypes have been identified. Most humans have been exposed to the adenovirus serotypes 2 and 5, which are mostly used as gene vectors. Furthermore, adenoviruses type 2 and 5 have low specificity to tissues and cells, hence it can transfer genes to a wider range of tissue types. In addition, adenoviruses have the capability to deliver large DNA particles. On the other hand, the use of adenoviruses in gene therapy is limited due to the immunological responses induced in many tissue. Adenoviruses have caused serious side effects in patients, and in some cases have caused death^{95,95,108–110}.

Adeno associated viral vectors

Adeno-associated vectors (AAV) are considered safer than adenoviral vectors, because of the lack of pathogenicity and replication. In human, AAVs are able to integrate into a specific site on chromosome 19 with no noticeable expression in vivo. AAVs have been successfully used in the treatment of some diseases, such as CF, hemophilia B, Leber congenital amaurosis, and AAT (Alpha-1 antitrypsin) deficiency. The main drawback of this type of gene delivery is their restricted transgene capacity (up to 4.8 kb)^{111,112}.

Helper-dependent adenoviral vector

The Helper-dependent adenoviral vector (HdAd), consists of two vectors; the helper, which contains all the viral genes required for replication but has genetic defect in the packaging domain. The other vector comprises the ends of the viral genome, therapeutic gene sequences, and the normal packaging recognition signal. The HdAd vector is an optimised version of the adenovirus. Therefore, many of the disadvantages encountered with the first-generation adenovirus has been overcome. the packaging capacity has been improved, no immunogenicity, and reduced toxicity. A more developed form of the adenovirus is the Hybrid adenoviral vectors; The Hybrid adenoviral vectors are a hybrid between adenovirus and retrovirus that shows improved features and high stability^{112–115}.

Retroviral vectors

Retroviral vectors have an advantage over types of viral vectors, they have the ability to pass through the nuclear pores of mitotic cells, hence it is capable to transfect dividing cells making them prime candidates for in situ and ex vivo treatments. Retrovirus is the most common viral vectors used for gene delivery especially in germline and somatic gene therapy^{90,97,98,104,105}.

Lentiviruses

Lentiviruses have the ability to integrate with non-dividing cells which gives them unique features over retroviruses. Lentiviruses are a subclass of retroviruses, they have the capacity to deliver 8 kb of sequence. They have high-efficiency infection of dividing and nondividing cells, they also have high stability expression of a transgene, low immunogenicity, and the capacity to transfer larger transgenes. Plus, lentiviruses are extensively used for *ex vivo* gene transfer in central nervous system with no significant undesired effects. Lentiviruses have been applied in the treatment of neurological disorders, like, Alzheimer, Huntington's disease, lysosomal storage diseases, and spinal injury^{70,99,100,102,103}.

Non Viral gene delivery methods

The nonviral gene delivery include cationic liposomes and polymers, or physical methods, such as gene gun, electroporation, particle bombardment, ultrasound utilization, and magnetofection.

Naked DNA

This technique although widely investigated, its efficiency is low compared with other methods of

gene delivery. And it is only suitable for specific applications. Naked DNA transfer is limited to some cells like cardiac muscles, skeletal muscle, skin where small genes are injected directly into the cells^{116,117}.

DNA particle bombardment by gene gun

This method has been developed to replace the naked DNA delivery. In this technique, gold micro beads are attached with plasmid DNA and then targeted through a gas pressure gun where the genetic material penetrates into the target tissue cells^{118–127}.

Electroporation

In this method the DNA is incorporated into the cells through an electrical current. Electroporation can be applied in vivo on different types of tissue, and it has been used in cancer treatment. The major drawback of technique is the requirement of surgery to insert the electrodes into internal tissue, and the damage that may be caused by the high voltage, as it may harm organs^{128–133}.

Other non-viral gene delivery methods

The hydrodynamic gene transfer has shown promising results in vivo, it is an efficient and uncomplicated process for intracellular delivery of genetic material^{134–138}. Another non-viral gene delivery method, is the Ultrasound. Ultrasound facilitates the internalization of DNA particles by making nanopores in the membrane, the applications of this method is limited due to its low efficiency¹³⁹. Magnetofection, is a gene delivery technique where a magnetic field is applied to concentrate Iron Oxide particles containing nucleic acid desired target. In this way, the magnetic force allows a rapid concentration of the entire applied vector dose onto cells^{140–145}.

Polymer nanoparticles in gene therapy

A major limitation of gene therapy is the exposure of genetic material to nucleases, which hinders this kind of therapy to achieve its desired therapeutic effect. Using the conventional methods of direct gene delivery or vector based delivery, a number of obstacles stands in the way of localizing the nucleic acids into the cell nucleus. Even though gene therapy could be a promising treatment option for a number of diseases, its safety is still negotiable. Therefore, different types of biocompatible nanoparticles have been used to deliver genes intended for gene therapy to overcome the disadvantages encountered with the traditional methods used for genetic material delivery.

In fact, viral vectors exhibit major safety issues like antigenicity, off site targeting, and inflammation. An optimal gene delivery system is one that guarantees the delivery of the genetic material to the target site with high specificity and high efficiency; with minimal side effects. Nanoparticles (NPs) are nonviral gene delivery systems. Their unique nano structure provides them with properties that allows the incorporation of genetic materials and drugs. Plus, the surface of the NPs could be modified with different functional groups to allow efficient penetration and specific targeting^{3,17,50–53,146–157}.

Polymer nanoparticles (PNPs) deliver genes or therapeutic proteins including drugs which can either be dissolved or encapsulated within them forming a nanoparticle and a nanocapsule

respectively. PNPs can also deliver proteins to the targeted cells by entrapping them within its structure forming a nanosphere. The delivered therapeutic proteins or drugs act by altering defective proteins or genes in the patient's cells. The size of the polymer nanoparticle could be tuned to enable these drugs and therapeutic protein to fit in. PNPs, like all nanoparticles are capable of regaining their size once inside the cell through the physiological change in pH^{13,57,60-63,157-167}.

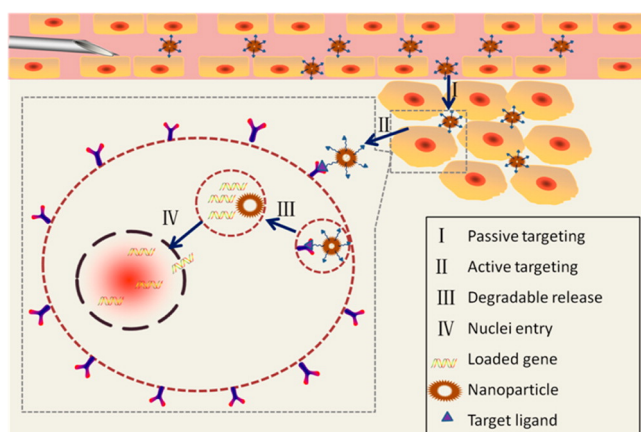


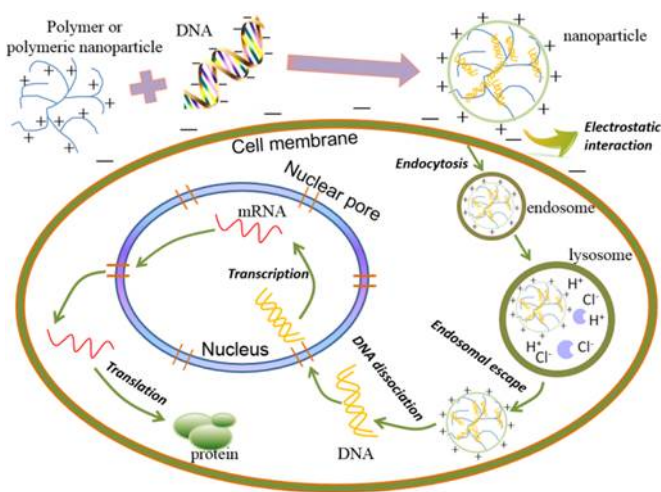
FIGURE 1.3

Mechanism of the delivery of genes using NPs⁵⁸

PNPs have been utilized in drug delivery, where they have shown high biocompatibility and high encapsulation capacity. They are great candidates for gene delivery, because they are highly stable and they offer controlled release of active ingredients. Also, PNPs can be used for targeted delivery by surface modification, and they allow the delivery of combined active materials. PNPs are synthesized from non-toxic biodegradable, biocompatible polymers like, Chitosan, cyclodextrin, polyethyleneimine (PEI), poly(lactic-co-glycolic) acid (PLGA), and dendrimers.

PNPs have facilitated the development of new treatment methods with improved efficacy for treating diseases which had once been viewed as incurable like genetic, immunological and neural disorders. In some cases, the delivered genes act by enhancing the functions of the cells. Polymer nanoparticles are used to overcome the various challenges that have been encountered in using gene therapy. Some genes have relatively long base sequences which make it difficult for them to be delivered to the desired sites. To fit into the target cell, the DNA must be condensed into the nanostructures, to permit their internalization within the cells. In some cases, gene silencing may also arise as the target cells may act against the delivered genes^{14,58,59,64-66,169-182}.

Putnam et al, have demonstrated that using polycations such as polylysine can overcome the DNA size barrier as it "can condense DNA into toroidal nanostructures" to sizes less than 150 nm which can be internalized within the cell. Researchers have also identified various ways in overcoming the challenge of separation of the DNA from the carrier. Using nanoparticles to conjugate the DNA, researchers have developed an effective way to ensure that the genes are delivered to the targeted cells⁸¹.

**FIGURE 1.4**

Schematic illustration of the internalization of PEI polymer nanoparticles loaded with DNA¹⁶⁸

Mohammedi et al. have synthesized DNA-Chitosan nanoparticles to deliver DNA to the Lung Epithelial cells¹⁷⁴. Also, in 2014 Tang et al. have utilized chitosan based (PNPs) Trimethylated chitosan has been synthesized as gene delivery systems, TMC-g-PCL/DNA polyplexes have shown high uptake efficiency than PEI/DNA polyplexes³¹⁸³. Plus, Das et al have utilized PEI based nanoparticles to deliver siRNA to STAT3 in lung cancer, in vitro and in vivo⁵¹. Other research groups have also synthesized chitosan as the main targeting nanoparticles for siRNA delivery to treat different diseases like, lung cancer, ovarian cancer, pancreatic cancer and hepatocellular carcinoma^{3,53,57,63,159,177,180,184}. In 2015, Bishop et al. have utilized polymer coated gold nanoparticles for DNA and siRNA delivery, where this type of inorganic nanoparticles have shown good results in gene silencing¹⁵⁴. Colombo et al have synthesized hybrid lipid-polymer nanoparticles for siRNA delivering⁵⁵. While, other up to date studies have shown the improved cancer treatments obtained with co delivery^{52,150,156,158,162,164,175}.

Conclusions

Many gene delivery methods have seen the light over the past three decades. The gene delivery systems are either viral or nonviral delivery systems. These gene delivery methods exhibit side effects and have their own limitations, hence, some of the methods mentioned in this chapter have not yet had clinical applications. Yet, some of the gene delivery systems have showed great potential when studied in vitro and in vivo and show promising results to be further investigated on specific cells and tissues. Non viral delivery systems are still in a juvenile stage of research, more *in vivo* studies are required in this field. Major improvements on the currently available systems; refining the extracellular targeting and delivery, improving the intracellular delivery, and minimizing toxicity and side effects on human body.

Polymer nanoparticles have led to an enhanced development of gene therapy different diseases in the past years. The rise of many biocompatible materials led to the development of gene therapy systems that will revolutionize the field of gene therapy. NPs can be great alternative of the conventional viral and nonviral gene delivery methods. The gene targeting using biocompatible NPs

will definitely result in an enhanced patient treatment of various diseases and disorders. Moreover, the use of polymer nanoparticles in gene delivery have shown to have less undesirable effects and better targeting. The synthesis methods of PNPs, the polymers used, and surface functionalization should all be taken into account to get the therapeutic effectiveness of a therapeutic NP.

Clinical evaluations are extremely significant and are not yet widely investigated. The current outcomes are inadequate to make a final opinion regarding the effectiveness of NP based gene therapy. Therefore, toxicity studies in vitro and in vivo are needed so that researchers can translate this advanced basic research to the bedside. In addition, toxicological studies “Nanotoxicology” has focused on the safety of nanoparticles based therapies, however, only few studies have been reported so far.

In conclusion, the realization of PNPs gene therapy still needs further proof of concept. Moving from the lab to the clinic has not yet been achieved. In the future, research in this area still requires in depth studies that involve functional assays. The nanomaterial should be designed and characterized; then, the routes of administration of the PNPs gene therapies should be confirmed and finally, the synthesis methods should be streamlined in order for the formulations to be replicated at the industrial level.

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