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A REVIEW: GENE GUN A MICRO PROJECTILE APPROACH FOR DELIVERY OF MICRO PARTICLES IN HUMAN

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Abstract: Gene is a segment of DNA having specific base sequences that direct the synthesis of proteins. Gene transfer includes the transfer of genetic material DNA to the cell. The alteration in the base sequences of DNA can change or alter the protein synthesis which causes disease condition, and this can be overcome by manipulation of gene. Gene transfer is applicable in treatment of vital diseases by replacing absent or defective gene in heritable disorder. Gene gun is till the dates are under the line of investigation for gene transfer, immunomodulation and vaccination. The present review highlights on design, working and application of gene gun in treatment of vital diseases by transferring micro particles.

Keywords: Gene, Gene transfer, DNA, Gene gun, Micro particles, Heritable disorder.

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INTRODUCTION

Genes are working subunit of deoxyribo nucleic acid (DNA), DNA which is about 3.4 nm in diameter is composed of four base pairs sequences carrying information for directing synthesis of specific polypeptide chains (proteins), which is essential for regulation of cellular functions. Any change in these base pair sequences leads to diseases condition which can be corrected by gene manipulation or gene transfer. Genetic engineering has also lead to advances in the field of medicine and developed a biolistic particles delivery technology or particles bombardment technology known as Gene gun technology, it transfers the gene into the cells to cure the genetically effected diseases gene transfer simply means transfer of genetic material DNA into the cells. Gene gun transfers the genes directly not only into the mammalian cells *in-vitro* but also into tissues of living animals. This technology involves the propelling of DNA coated onto microscopic gold or titanium particles (bullets) at high velocity to transfer into the living tissues. The high velocity acceleration is may be provided by compressed gas, acceleration is necessary force to punture the cell membrane and to deliver the genetic material into the cells of living tissues.

Advantages:

1. Gene gun is not depend on receptor or biochemical factor on cell surfaces.
2. Fastest method of gene transfer.
3. Walled intact cells can be penetrated.
4. Literally firing of DNA into target cells are possible.
5. Micro and nanoparticles is possible to deliver into living cells
6. Nanoparticles avoids tissue damage when delivered by gene gun^{1,2}

Disadvantages:

1. Expensive technology
3. The jet of high speed compressed Helium from gene gun barrel is highly damaging.
4. The gene gun has the limitation of poor aiming, and reproducibility.
5. Possibility to tissue damage³

History of Gene gun: The concept of gene gun was introduced in 1980s, when the researchers study on delivery of genetic material into organism. It was invented by Jhon Sanford, Ed Wolf and Nelson Allen at Cornell university. It delivered particles coated with marker gene firstly study was carried on Onions, but it was also applied in human and animals ⁴

PRINCIPLE AND DESIGN OF GENE GUN: The principle of gene gun device includes firing of DNA coated particles into the target tissues without damage in order to deliver genetic material to living cells. The several designs of gene gun are described previously using helium gas with macro carriers for accelerating DNA coated micro carriers. The early gene gun device includes surge tanks and explosive agents, in later design surge tanks is removed and device is improved with using helium propellants ²



Fig. 1. Hand Held Gene gun

Working of Gene gun: Gene gun fires DNA microparticles to petridish filled with desired cells, the released DNA enters into the cells. The genetic markers also inserted with DNA microparticles to know genetic material has entered the cells successfully sometime the uncoated particles are to be fired on solution which pick up the DNA from solution and take into the cells. The gene gun consist of two stainless steel chambers connected to a vaccum pump. The bullet or DNA coated micropellets of tiny gold or tungsten particles which delivers the DNA are put onto plastic disk at is inside the gene gun(Fig.2). The helium gas propells the plastic microcarrier or whereas macrocarrier in some devices. Propelling creates shock waves which accelerate the high density DNA coated micropellets and allows to pass through stopping screen to reach the target tissues. Stop screen obstruct passage of plastic microcarriers or macrocarriers. The gold particles are favoured to use because of better uniformity than tungsten, as tungsten is toxic to cells ^{2,4}

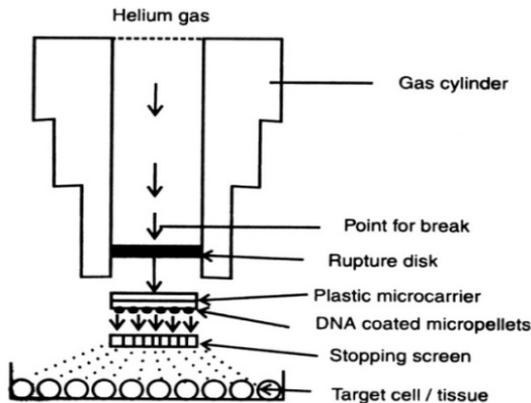


Fig. 2. Working of gene gun

APPLICATIONS OF GENE GUN :

1) Disease condition :

Alzheimers disease : The accumulation of large number of activated microglia and astrocytes with small number of T cell, both the microglia and astrocytes have been shown to generate the β amyloid protein ($A\beta$) one of the main pathologic feature of alzheimers disease, induction of a humoral response is beneficial for alzihmers disease patients. The high concentration of anti amyloid beta antibodies may prevent the accumulation of toxic forms of amyloid beta peptide prevents the progression of alzheimers disease. An alzheimer DNA epitope vaccine (DepVac) delivered intradermally by gene gun a needle free ballistic device induce potent titter of anti amyloid β antibodies with strong cellular response against forign Th epitope, PADRE. The functional activity of the anti amyloid β antibodies analysed by binding of anti-sera to amyloid beta plaques in brain tissues of alzheimers patient⁵

Pome disease or Glycogen storage disease type II (GSD II) : GSD II is the autosomal recessive metabolic disorder in which the muscle and nerve cells throught the body are damages. In this disease the glycogen is accumulated in the lysosome due to lysosomal deficiency of acid alpha glycosidase or lysosomal acid maltase, Martnik et al evaluate particle bombardment as delivery system for thrrapy of GSD II and utilized the vector carrying cytomegalovirus (CMV) promoter linked to human lysosomal acid maltase cDNA, Helicos gene gun delivering gold particles coated with lysosomal acid maltase expression plasmid shows gene transfer with human GSD II cell line fibroblast and lymphoid as well as *ex-vivo* with an adult peripheral blood cells. All cell types shows an increase in human lysosomal maltase activity with reverse muscle weakness⁶

Schistosomiasis: Schistosomiasis disease caused by schistosoma species, gene gun delivery of DNA vaccine based on thioredoxin glutathione reductase of schistosoma japonicum (SjTGR) shows the significant developed specific anti SjTGR antibodies in animals high level of these antibody may contribute to protective effect against schistosomiasis⁷

Hepatitis B: The helios gene gun delivers the DNA plasmid that encodes the hepatitis B surface antigen (HbsAg)⁸

Cancer: The cytotoxic T cell immunity against various cancer disease is increase with the gene based immunization with transgenic DNA vector expressing tumor associated antigen (TAA), cytokinins. The particle mediated gene gun technology for gene transfer in various mammalian tissues and it has been shown to induce both humoral and cell mediated immune response⁹

Malaria: After the bite of infected mosquito malarial parasite firstly target the liver and then enters the blood expressed the serine repeat antigen (SERA) of plasmodium falciparum is a use in human malarial vaccine. Belperron et al used the gene gun for delivery of SERA expressing plasmid DNA vaccine and observed the immune response with significantly high serum antibody. gene gun immunization with SERA plasmid DNA was immunoglobulin (G1)¹⁰

2) Immunomodulation: Stimulation of the immune response to weakly immunogenic tumors, achieved by various cytokines eg. Granulocyte, gene gun delivery of IL-12 cDNA in murine models showed that local expression of IL-12 in epidermal cells near tumor cells resulted in regression of underlying tumors and inhibition of systemic metastasis, resulting in prolonged survival of test mice, immune stimulation by gene gun techniques can result in local as well as systemic anti-cancer effects.

3) Genetic Vaccination: Genes are introduced into the body using the gene gun with the purpose of eliciting an immune response to the proteins expressed by the delivered gene. This method of vaccination may be safer than other methods because only foreign DNA is introduced and not foreign proteins or killed vaccines.

4) Suicide Gene Therapy: A gene that expresses a toxic protein but has tumor specific promoters is introduced to tumor cells. When the protein is expressed the tumor cell dies. The protein is only toxic to tumor cells because the specific promoters needed for expression are only produced in tumor cells¹¹

5) Particulate delivery:

Delivery of Nanostructured loaded anti cancer drugs: Mitoxantrone dihydrochloride (MTX), an antitumour drug has significant effectiveness against metastatic breast cancer when loaded within the nano structured porous silicon particle which are fabricated by electrochemical etching of silicon wafers. These MTX nano structured particles are injected into a gene gun with a continuous stream of high speed helium in a capillary tube, it was shown to be effective for delivery of MTX into target breast cancer cells and exhibits sustained release over several days with cytotoxic action¹²

Nanoparticulate delivery of gene: The DNA loaded in 40 nm diameter projectile nanoparticles are transfected to human embryonic kidney HEK293 cells by gene gun. The particles are bombarded at 50 psi at a distance 1 cm. Use of nanoparticles shows less cell damage with efficient biolistic transfection as compared to microparticles¹³

Liposome mediated gene delivery: Liposomes are artificially prepared vesicles composed of lipid bilayer, it can be used as a vehicle for successful delivery of plasmid DNA coding for the lac Z gene to mouse skin cells by gene gun, to cultivate hair growing skin cells. Without liposome code lac Z gene applied to skin tissue did not appear in hair follicles¹⁴

CONCLUSIONS:

As the gene gun is a fastest method of gene transfer and has distinct advantages over the other gene transfer systems. It has a significant importance in treatment of genetically affected diseases like cancer, Alzheimer, effectively transfer DNA coated micro particle in animals and humans.

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