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Use of High Voltage Amplifier in Electroporation for Transfection Related Medical Applications

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***Abstract*-** Electroporation, a biophysical effect finds immense significance in the fields of medical applications. Used as a method of transfection, it can be employed to find cure for three dangerous and life threatening medical conditions like cancer, decrease in insulin production and autoimmune diseases.

Electrical apparatus used for these treatments are generally based on dc voltage pulses, which due to their inefficiency in producing desired output, have paved ways for electrical apparatus that use ac pulses. Therefore, a high voltage linear amplifier design using vacuum tube valves has been referred to, for the production of the same.

***Index Terms*-** Autoimmune diseases, electroporation, high voltage amplifier, insulin, oncogenes, transfection.

I. Introduction

The process of forcing nucleic acids into cells so as to biologically alter their working is called transfection. This is done by opening transient pores on the cell membrane. Transfection can be carried out by a number of methods like chemical, viral, particle, optical and electroporation.

On application of a high magnitude electric field across a biological cell, the permeability of its outer plasma membrane undergoes a significant increase due to molecular rearrangement, leading to pore formation, thus helping in the passage of large molecules across the cell membrane. This process of applying several hundred volts across millimeters of cell distance, so as to introduce foreign bodies into it, is called electroporation or electropermeabilization.

Apparatus that have been used for medical applications of electroporation, used dc pulses earlier, which proved inefficient as presence of non-conducting capacitive layers in tissues led to the attenuation of dc pulses. DC pulses also being sensitive to the presence of bulk extracellular liquids couldn't detect the presence of cell populations of varying shapes and sizes and thus gave birth to apparatus using ac pulses.

II. HIGH VOLTAGE AMPLIFIER

Since the method of electroporation of human cells using ac pulses, is in a very primitive stage, the ac pulse generating source for such apparatus is difficult to find. The success of such an apparatus lies in its ability to produce ac pulses of varying shapes and its function over specified ranges. Further, since electroporation is being conducted on biological cells, utmost care has to be taken. These considerations led to the reference of the amplifier that has been used, with the following specifications.

Maximum output voltage = 4 kV*p*
Linear Bandwidth (□□3 dB) = 500 Hz – 5 MHz
Amplifier Voltage Gain = 400 V/V (52 dB)
Output Impedance = Less than 100 ohms
Input Impedance = Greater than 1 kilo ohm

The biological cell or variable load in the experiment is substituted by a parallel resistor or capacitor combination, resistance being as low as 1 kilo ohm and capacitance as great as 100 pF. The amplifier provides a 10 ms delay in 1 MHz sine wave, amplitude 3 kV*pk* and a repetition rate of 50 ms up to 10 maximum repetitions.

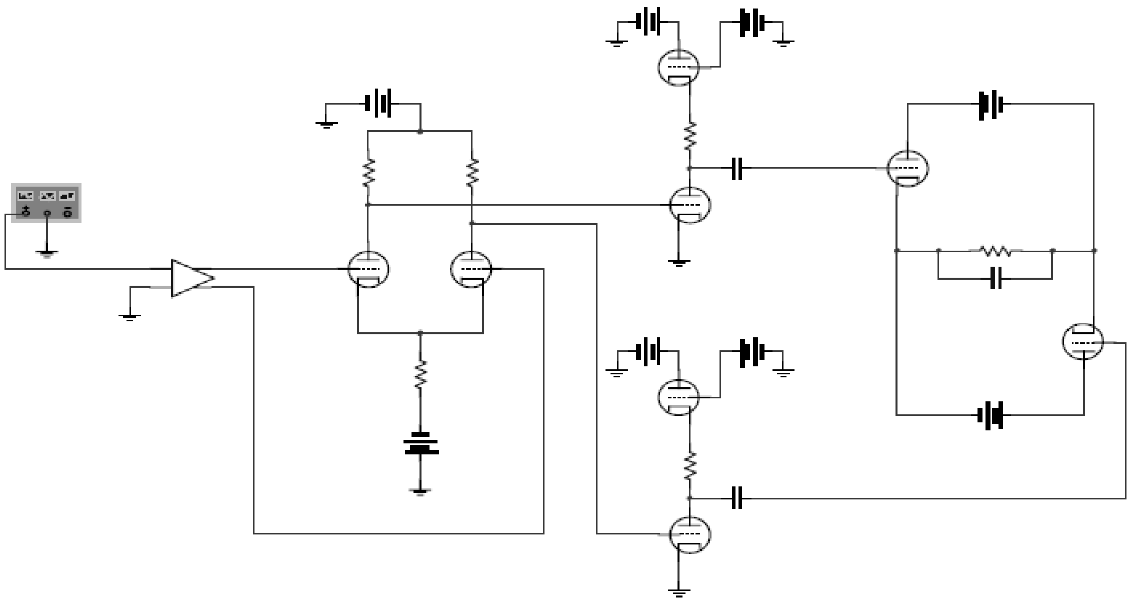

Fig 1: Proposed High Voltage Amplifier

III. AMPLIFIER DESIGN

High frequency and wide linear bandwidth combinations producing high voltage output are realized by using vacuum tubes which have to meet output specifications that demand impedance to be less than 100 ohms. A modified circlotron buffer is used in the output stage which provides low output impedance and thus eliminates the need of an expensive component which is an impedance matching transformer.

The proposed amplifier in figure 1 consists of a number of stages in which the first stage i.e the input stage is a differential signal producing stage(to be utilized by the second stage),where an op-amp buffer is used. In the second stage,a differential triode valve produces half the voltage gain. A series of push pull amplifiers constitute the third stage and provide the rest of the gain. The final stage has the modified circlotron as discussed above.

IV. TRANSFECTION

Once the biological load or cell has been electro orated by the above method, the pores formed in the cell membrane give easy passage to the molecules to go inside the cell. This deliberate process of introducing foreign materials especially nucleic acids into the cells is called transfection. Another term for transfection is transformation since genetic material inside the cells are transformed and genetically modified. In transfection, the material to be deposited inside is mixed with a cationic lipid, thus producing liposomes that fuse to the cell membrane and deposit their cargo inside. The general structure of a synthetic cationic lipid has been shown in fig 2.

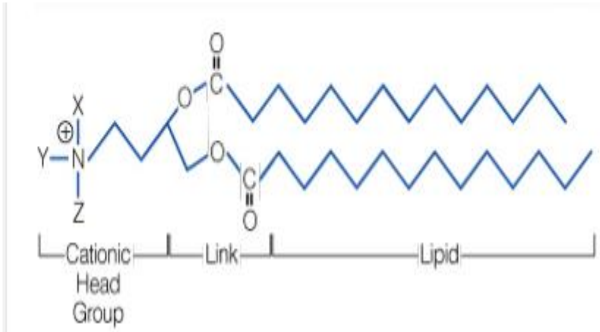

Fig 2: General structure of a cationic lipid

Various methods that are employed under transfection are (Fig 3 shows the related diagram):

1. Chemical methods:
   a. Use of calcium phosphate
   b. Use of highly branched organic compounds
   c. Lipo fection
   d. Use of cationic polymers
2. Non chemical methods
   a. Electroporation
   b. Sono poration
   c. Optical transfection
   d. Protoplast transfusion
3. Particle based methods:
   a. Use of gene gun
   b. Magneto fection
   c. Impale fection
4. Viral methods
5. Hybrid methods

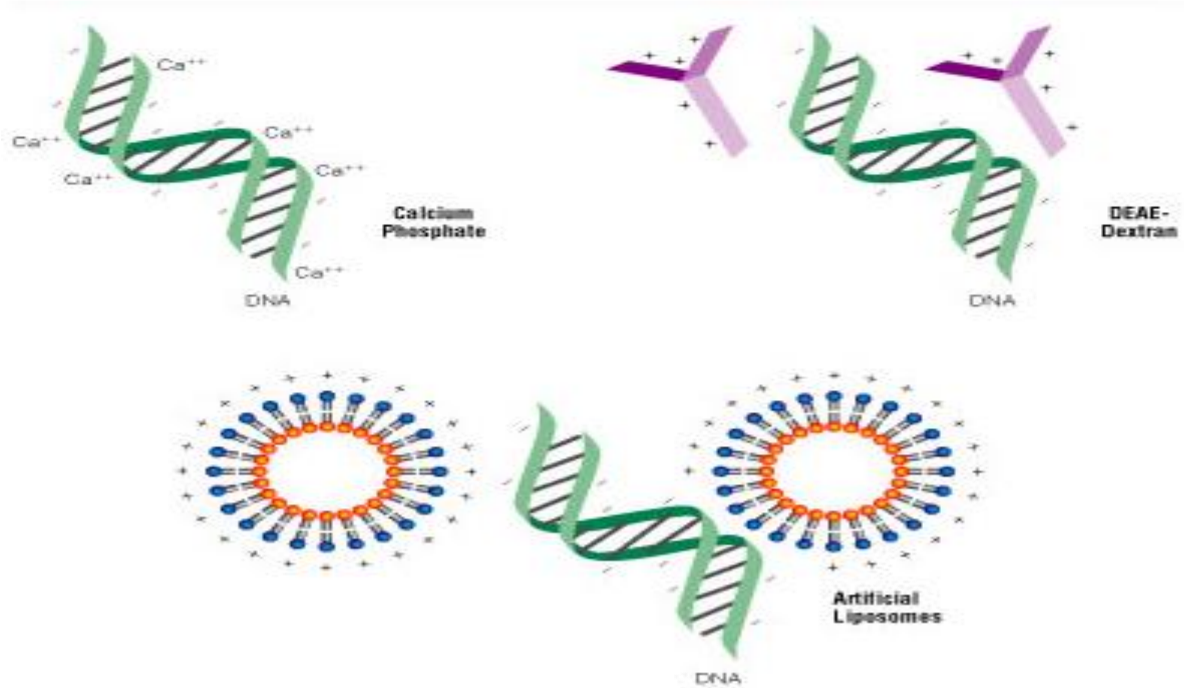

Fig 2: various transfection technologies.

Thus on transfection of nucleic acid materials into the cell, they affect the genes of the nucleus, help in gene therapy, protein metabolism and mutation of cancer cells etc. In this paper, we have studied the effect of transfection carried out by electroporation, for finding methods, which can help in finding cure for cancer, decrease in insulin levels and autoimmune diseases. Let us now see some of the uses of this method.

V. EFFECT ON ONCOGENES

All cells have nuclei that contain genes. The genes that trigger cancer are called oncogenes.

1. **Transfection of DNA to detect the presence of oncogenes**: Laboratory tested oncogenes are similar to RAS genes. DNA transfection has been found to be a very promising method of detecting oncogenes and experimental tumor. It has thus led to the knowledge of several oncogenes. To generate recombination events, fragments of random genes are litigated together inside transfected cells. Proto-oncogenes are activated by fusion events like gene fusions, gene truncation, enhancer activation etc. These proto-oncogenes were earlier absent in cancer cells. Thus, to detect oncogenes, presence of putative oncogenes has to be demonstrated. Thus, by the above process, we can detect the presence of oncogenes and take precautions to prevent it from mutating further.
2. **Transfection to stop oncogenes from mutating:** Even though much research hasn't been done in this direction, but if we transfect some material into the cells, which react with the mutating oncogenes and stop the replication process, spread of cancer can be stopped at a very early stage.
3. **Effect of oncogene transfection:** To increase the growth related properties of heterohybridomas cell line in humans and mice, expression plasmids containing oncogenes v-src, c-Ha-ras are transfected by electroporation into them.

VI. EFFECT ON INSULIN PRODUCTION

Insulin is produced in the pancreas of the human body. When the production of insulin goes down, a person suffers from diabetes. Thus, we have suggested transfection as a method to inject material into pancreatic cells that can help in keeping insulin production on.

For the treatment of type 1 diabetes, beta-cell replacement therapy has proved inefficient due to the availability of limited supply of islet tissues. Thus, a retrovirus vector pLNCX can be used to transfer human insulin into bone marrow stem cells (hMSCs). These transfected hMSCs were found to secrete insulin for more than 3 weeks.

Thus transfection of insulin cells into the bone marrow can help in keeping the production of insulin on and thus prevent type 1 diabetes.

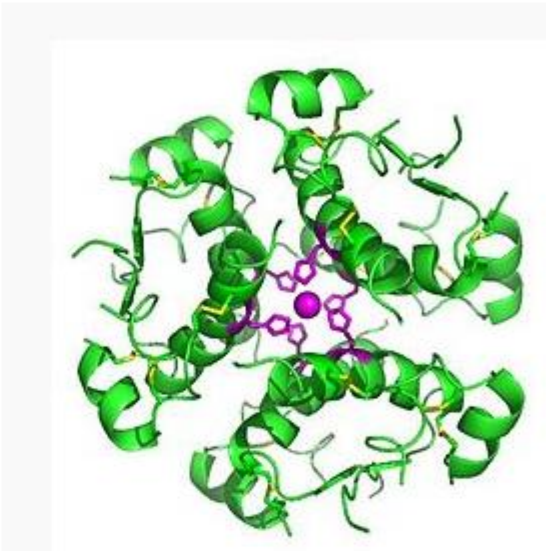

Fig 3: computer generated insulin

VII. EFFECT ON AUTOIMMUNE DISEASES

When the body immune system loses its ability to differentiate between foreign objects and the cells present in the body, it attacks both alike, thus harming the cells of ones own body. This is basically treated with immunosuppression i.e., decreases the immune response. Another condition related to this is inflammation. By activating anti-inflammatory genes or suppressing inflammatory genes, we can find a cure for these diseases.

Calcitonin gene-related peptide (CGRP) shows prominent anti-inflammatory actions. On examination, it has been found that CGRP-transfected dendritic cells (DC) prevent the development of Experimental Autoimmune Optic neuritis (EAON) and experimental autoimmune encephalomyelitis (EAE). Due to autoimmune syndrome, normal optic nerves were infected, leading to optic neuritis.

It has also been shown that transfection works better than other methods of introducing these compounds into the cells. Thus transfection of cgrp can help in finding a cure for autoimmune diseases.

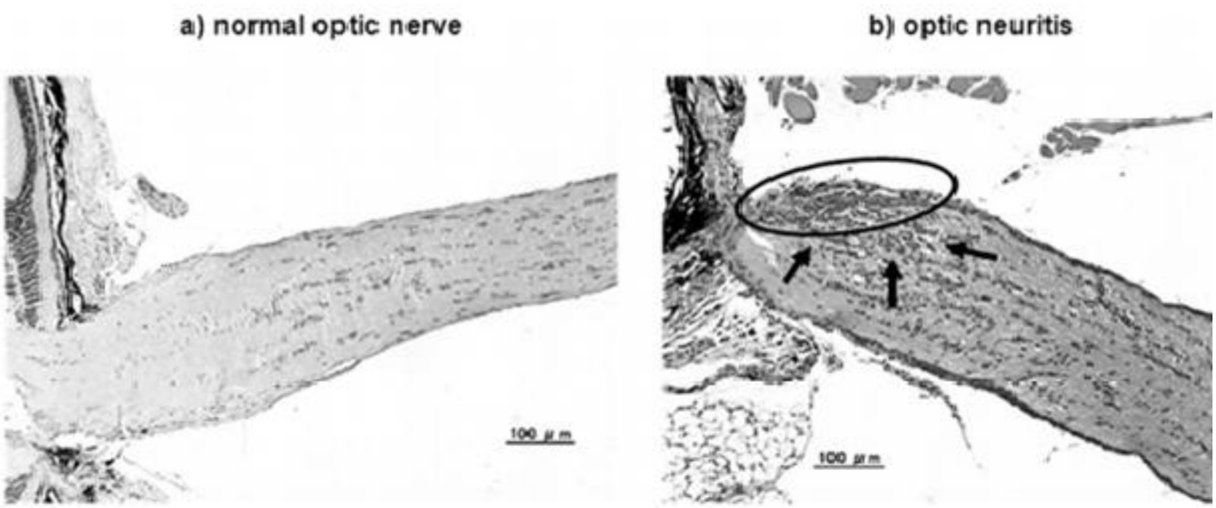

Fig 4: Normal optic nerves and optic nerves with optic neutris disorder

VIII. CONCLUSION

We can thus say that by applying methods of electroporation, to be carried out by the use of high voltage amplifier, transfection can be carried out in human cells. This can eventually help in finding a cure , bringing awareness or doing some research work in the field of transfection to treat cancer, diabetes, autoimmune diseases, HIV aids etc.

REFERENCES

[1] Alteration of oestradiol metabolism in myc oncogene-transfected mouse mammary epithelial cells.

N. T. Telang, F. Arcuri, H. L. Bradlow, M.P. Osborne, and L. Castagnetta, O. M. Granata

[2] Detection and identification of activated oncogenes in spontaneously occurring benign and malignant hepatocellular tumors of the B6C3F1 mouse (Ha-ras/non-ras/hepatocellular adenoma/hepatocellular carcinoma)

Steven H. Reynolds T, Shari J. Stowers, Robert R. Maronpott, Marshall W. Anderson, and Stuart A. Aaronson.

[3] The effects of oncogene transfection on growth and antibody production of human-mouse heterohybridomas.

Otman Hohenwarter, Andrea Waltenberger, Christine Schmatz, Hermann Katinger

[4] Suppression of Murine Experimental Autoimmune Optic Neuritis by Mature Dendritic Cells Transfected with Calcitonin Gene–Related Peptide Gene

Ryusaku Matsuda, Chiharu Nishiyama, Yoshihiko Usui, Takeshi Kezuka

[5] Transfected insulin-like growth factor II modulates the mitogenic response of rat thyrocytes in culture.

Veneziani BM, Di Marino C, Salvatore P, Villone G, Perrotti N, Frunzio R, Tramontano D

Dpt. di Medicina Sperimentale e Clinica, Università di Reggio Calabria, Catanzaro, Italy

[6] Human bone marrow mesenchymal stem cells transfected with human insulin genes can secrete insulin stably.

Lu Y, Wang Z, Zhu M.

[7] Depatment of General Surgery, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China, 2226001.

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