



Bio-nanotechnology

By Shripriya Singh, PhD

Contributing Editor



Nature's very own nanoparticles are the new gene delivery agents: The promising role of Exosomes in gene therapy

Evolving of the field of therapeutics: From organ therapeutics to molecular therapeutics

The word research spells curiosity, intrigue, and uncertainty and the term medicine spells treatment, healing and cure. Whereas, when the two come together in form of medical research, the term spells change, hope and good health. Research potentially comprises two main phases, first in which we develop an understanding of the subject and delve at the grass root level of the existing problems and the second phase of research deals with solutions and tries to provide answers for the rediscovered problems. In the present times we have successfully transitioned to the second phase where researchers across the globe are focusing on answers and solutions. This aspect of research has brought a tremendous change in the field of medicine where diseases no longer intrigue doctors and practitioners; instead they are tackled with precision and accuracy. Medical advancements have led us systematically from organ therapeutics to organellar

therapeutics to cellular therapeutics and finally to molecular therapeutics. Thereby, we are gradually heading towards an era of precision medicine where the focus is on molecular targets such as specific genes. Gene therapy, genetic engineering and nucleic acid based therapeutics have shown immense potential and hold a promise to abrogate diseased states in their infancy.

The manipulation of genetic machinery of an organism is one of the most interesting aspects of biotechnology and has opened endless avenues for researchers. However, the delivery of any foreign particle such as a drug, a xenobiotic, an antigen or a fragment of nucleic acid is the trickiest part of the job. Every living organism has an efficient natural defense system via which it restricts and resists the entry of any foreign substance. This efficient defense mechanism which is popularly known as the 'immune system' is by far the biggest challenge in the field of medicine. In case of stem cell based therapeutics, regenerative medicine, organ transplantation and even gene therapy, this

major hurdle has to be overcome and resolved. Thus the approach is to use the patients' own tissues and cells for any further treatment.

Bio nanotechnology offers hope in gene therapy

In one of our previous articles we had discussed about nanochips and tissue nano-transfection (TNT), an innovative technology which allows body to regenerate any type of cell through genetic reprogramming. As mentioned earlier the nanochip is a simple yet unique miniscule device designed around the novel concept of tissue nano-transfection. TNT is an electroporation based technique that facilitates the direct delivery of reprogramming factors (DNA) into the cytosol via the application of a focused and highly intense electric field through arrayed nanochannels. The electric field benignly nanoporates the cell membrane and the reprogramming factors are electrophoretically driven in (1). Keeping in pace with this advancing technology we take the story forward and our current article is focused on exosome mediated gene delivery via cellular nanoporation (2).

What is exosome mediated gene delivery - the concept and details of the technology

Exosomes are membrane bound extracellular vesicles (EVs) secreted routinely by all cells of the body. They are considered the smallest organelles possibly present inside a cell and are capable of expelling metabolic byproducts and help in protein clearance. Exosomes are also routinely present in biological fluids such as blood, plasma and even the secretome of

cells cultured in-vitro. Their nano scale size (40–150 nm in diameter) and abundant presence have compelled biologists to consider them as potent tools of biotechnological use. Gene therapy and nucleic acid based therapeutics hold great promise for treating multiple human diseases. However, the inefficient delivery of negatively charged and relatively large molecules into cells and tissues of interest has been a major drawback of the process. Several in vivo gene delivery methods and techniques have been developed and improved over the past years to accomplish precision and efficiency. These techniques comprise synthetic nanocarriers such as polymeric and liposomal nanoparticles and viral vectors. However, these strategies have suffered on grounds of immunogenicity, toxicity and manufacturing costs. The quality control of manufactured products and the high cost incurred have added to the burden further. However, the biggest drawback of the existing techniques has been their inability to deliver the cargo across specific physiological barriers such as the blood-brain barrier (3).

Recently exosomes have emerged as potential carriers for nucleic-acid-based therapeutics (4) and the method takes advantage of these fluid-filled sacs that cells release as a way to communicate with other cells. The scientists used the technique of cellular nanoporation in which they placed around one million donated cells (such as mesenchymal cells collected from human fat) on a nano-engineered silicon chip and used an electrical stimulus to inject synthetic DNA into the donor cells. As a consequence of this DNA force-feeding the cells are compelled to

eject the unwanted substance as part of DNA transcribed messenger RNA and induced to repair the holes that have been induced in their membranes. This serves two main purposes, first the leakage to the cell membrane is fixed and secondly the garbage is dumped outside the cell. In this case as mentioned by the authors the garbage bag is the exosome which carries the drug or the

nucleic acid fragment of interest. The electrical stimulation had an additional effect of a thousand-fold increase of therapeutic genes in a large number of exosomes which were released by the cells. This is a clear sign that the technology is scalable to produce enough nanoparticles required for human use. Figure 1 depicts the proposed mechanism of CNP.

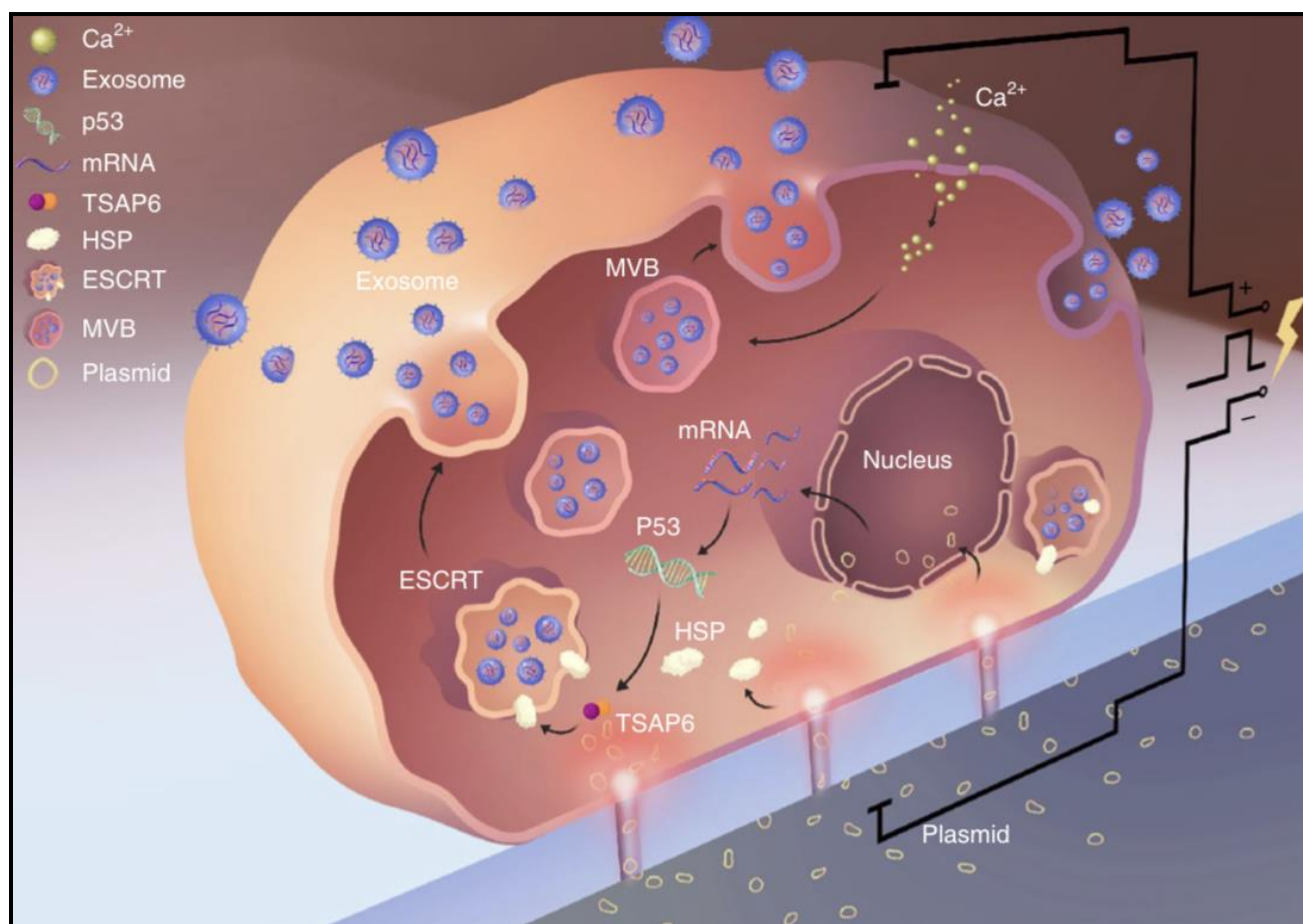


Figure 1: Schematic representation of the proposed mechanism for Cellular Nanoporation (CNP) triggering of exosome release in CNP-transfected cells. [Source: Nature Biomedical Engineering 2019.]

The knowledge of the exact molecular target and the exact gene responsible are crucial for the success of gene therapies. To test the efficacy of the above mentioned technique scientists chose to test the results on glioma

brain tumors of mice by delivering a cancer-suppressor gene called PTEN. Mutations in the PTEN gene interfere with the suppression role and thus allow cancer cells to proliferate and grow unchecked. Gliomas represent

about eighty percent of malignant brain tumors in humans and thus were a target of interest. The results clearly showed that labeled exosomes carrying the gene of interest not only travelled to the brain tumors accurately but slowed their growth as compared to the test substances used as

control. Further these gene carrying exosomes restored the tumor suppressor function, inhibited the growth and spread of malignant tissues and increased overall survival. Figure 2 depicts the overall technique and its application in mice gliomas.

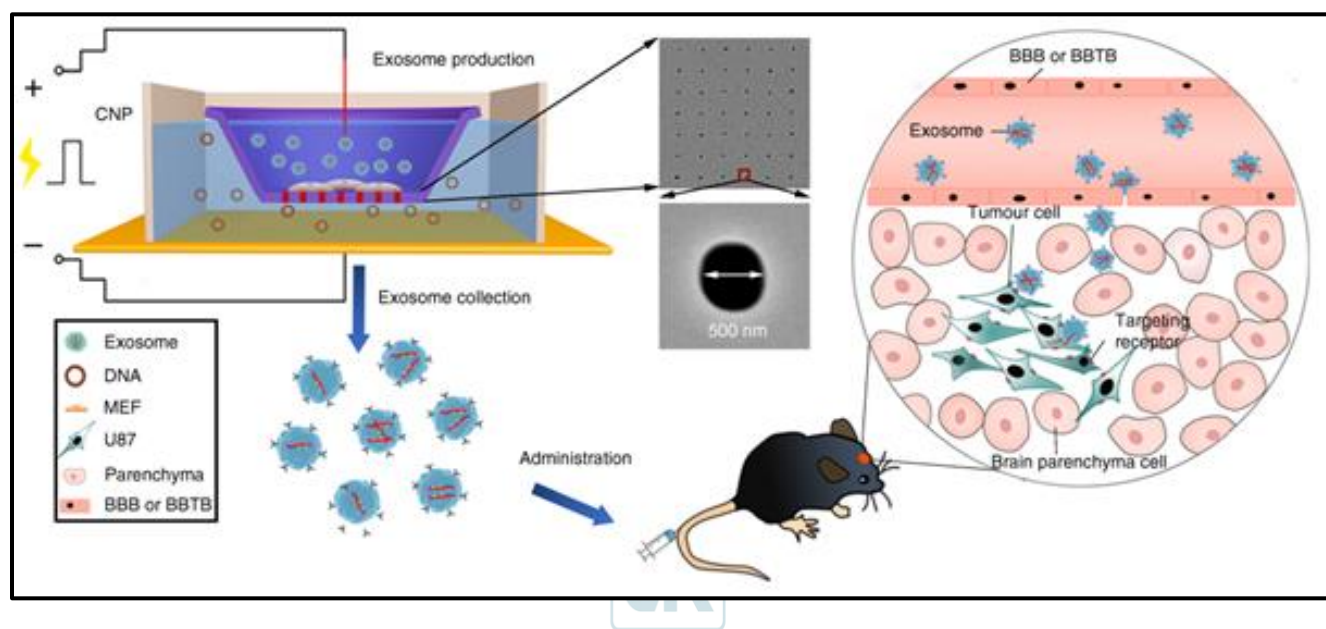


Figure 2: Diagrammatic representation of CNP-generated exosomes for targeted gene delivery. Left: the CNP system comprises a nanochannel array (red rectangles). Plasmid DNA added to the buffer enters attached cells via nanochannels under transient electrical pulses. Attached cells subsequently release large quantities of exosomes containing transcribed mRNA that can be collected for tumor-targeted delivery through the blood–brain barrier (BBB) or blood–brain tumor barrier (BBTB) (right). [Source: Nature Biomedical Engineering 2019.]

The current and future aspects of the exosome based nano-carriers

Exosomes are superior to any artificially made nano-carriers as they are non toxic, biocompatible and do not evoke an immune response in the patients. They express transmembrane and membrane-anchored proteins which prolong their circulation in blood and help them in transport. These proteins further facilitate the cellular uptake of

encapsulated exosomal contents and helps in tissue-directed delivery of genes. The only drawback was the large scale production and purification of exosomes which have been overcome by the technique of cellular nanoporation (CNP) discussed in this article. Thus CNP helps in injecting of the nucleic acid (gene of interest) into the cell and simultaneously produces a large number of exosomes (50 fold more than normal)

carrying the same genetic transcript. The biggest advantage of these exosome based carrier is that they can efficiently cross the blood brain barrier and thus will provide endless opportunities for treating neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Since the study was published in December the authors have fondly termed their innovative method as a small Christmas gift for the medical and research fraternity. The exosomes are like nature induced parcels that will carry the healing gift (gene or drug) inside and will efficiently deliver to the molecular target of interest. They are being referred to as "Mother Nature-induced therapeutic nanoparticles". A small modification in tissue nanotransfection (TNT) has led us towards cellular nanoporation. This small innovative technology has once again proven how smoothly we are progressing towards achieving goals that seemed miraculous a while ago. This technique holds immense potential as it is a simple, efficient and cost friendly method via which we can deliver drugs of interest and genes required for treating and repairing medical issues at a very nascent stage. This is a new gene therapy technique where the

body's own natural process has been tapped and manipulated to serve as a drug delivery system. The breakthrough research has transformed human cells into mass producers of miniscule nanoparticles full of genetic material that could possibly cure or even reverse disease conditions.

References

1. Gallego-Perez D, et al. (2017) Topical tissue nano-transfection mediates non-viral stroma reprogramming and rescue. *Nature nanotechnology* 12(10):974.
2. Yang Z, et al. (2019) Large-scale generation of functional mRNA-encapsulating exosomes via cellular nanoporation. *Nature Biomedical Engineering*:1-15.
3. Singh A, et al. (2016) Multifunctional photonics nanoparticles for crossing the blood-brain barrier and effecting optically trackable brain theranostics. *Advanced functional materials* 26(39):7057-7066.
4. Andaloussi SE, Mäger I, Breakefield XO, & Wood MJ (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. *Nature reviews Drug discovery* 12(5):347-357.