ISSN 2689-0852

BIOTECHNOLOGY KIOSK

DECEMBER 2019

The Promising role of Exosomes in Gene Therapy

Volume 1, Issue 7
www.biotechkiosk.com

Executive Publishers

Megha Agrawal, PhD

(Biotechnology)

Publisher and Editor

Expertise:

Neuroscience, Stroke, Pharmacology, Toxicology, Microbiology and Molecular Biology

Email: megha@biotechkiosk.com
meghaagra@gmail.com

Shyamasri Biswas, PhD

(Biotechnology)

Publisher and Editor

Expertise:

Structural Biology, Enzyme Technology, and Protein Crystallography

Email: shyabiswas@biotechkiosk.com
shyabiswas@gmail.com

Editorial, Sales & Circulation Office

160 International Parkway
Suite 100-9, Heathrow
FL-32746, USA
Phone: 386-518-9411
Email: publisher@biotechkiosk.com

www.biotechkiosk.com
ISSN 2689-0852
One stop shop for all things biotech

From the Publisher's Desk

Welcome to Biotechnology Kiosk!

We launched Biotechnology Kiosk (BK) in June, exactly seven months ago. With the 7th issue of Biotechnology Kiosk, we are now concluding the year 2019, and look forward to continue serving our readers in 2020 and beyond. As usual, the regular features include high-end editorials by experts, biotechnology advances around the world and industry news from pharma and biotech sectors.

We are proud to present some metrics to have insights into the accomplishments of BK so far. Since its launch, we have published over 25 scholarly editorials by leading experts in a broad range of areas in biotechnology. Numerous latest exciting discoveries in modern biotechnology R&D in a vast array of cutting edge fields have also been covered by us in the Editor's Picks section of BK. We are proud and honored to have as many as seven distinguished international experts on our editorial board as advisors of BK. In addition, BK has an ISSN now and will soon be a member of the digital object identifier (DOI) worldwide network for cross referencing of the published materials in BK. Our plans for BK for the New Year include partly transforming the magazine into an open access magazine that will publish

peer reviewed articles in biotechnology in special editions while keeping the trade aspects and nature of BK.

We do hope that you will enjoy reading this issue of Biotechnology Kiosk. Please do write to us with your comments and feedback. Your suggestions are always appreciated.

We take this opportunity to wish our readers Happy Holidays!

Dr. Megha Agrawal and Dr. Shyamasri Biswas

Executive Publishers and Editors

Contents

VOLUME 1, ISSUE 7 DECEMBER 2019

COLUMNS

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 carriers 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.

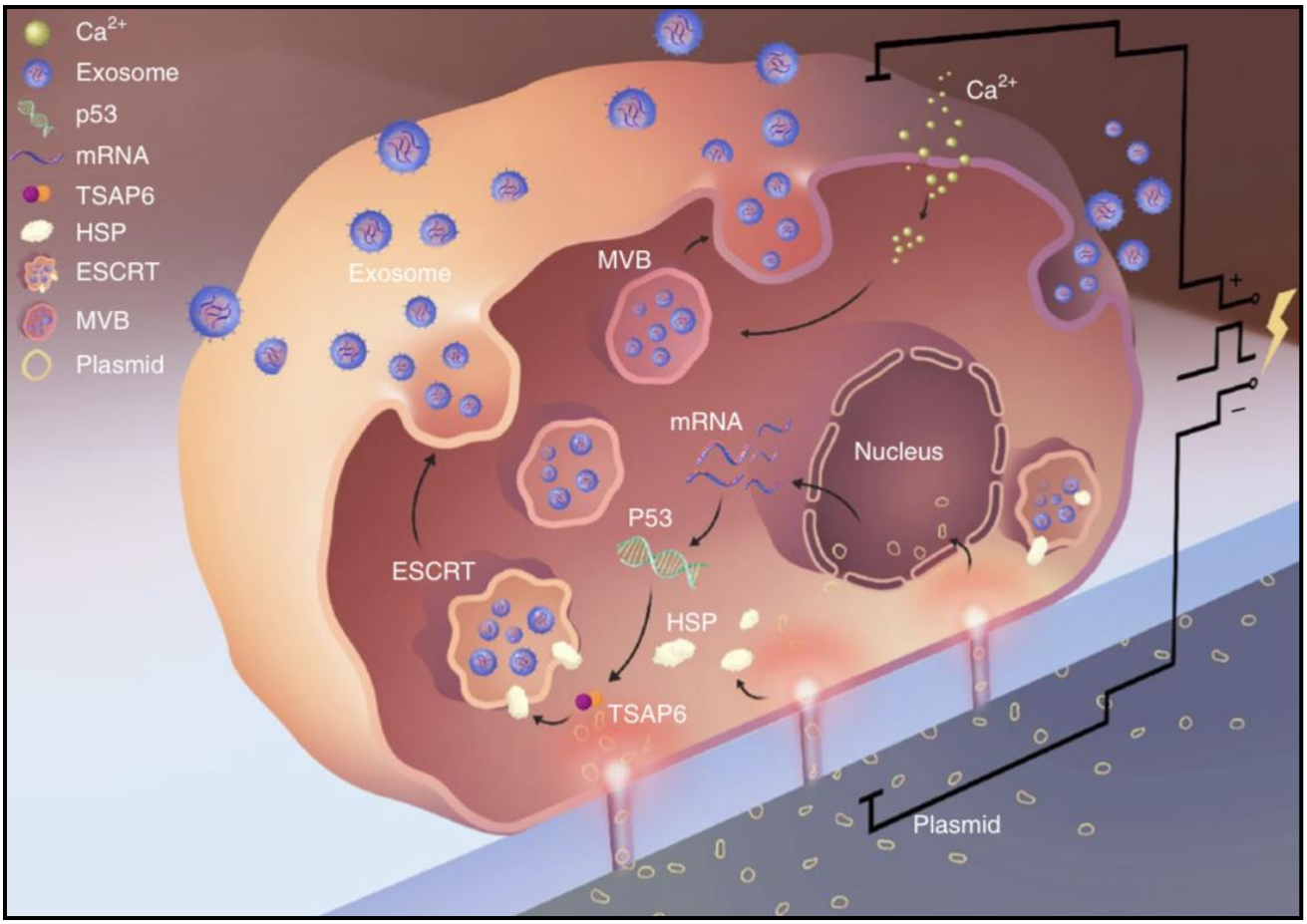

Figure 1: Schematic representation of the proposed mechanism for Cellular Nanoporation (CNP) triggering of exosome release in CNP-transfected cells. [***Source:*** *Nature Biomedical Engineering 2019.*]

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.

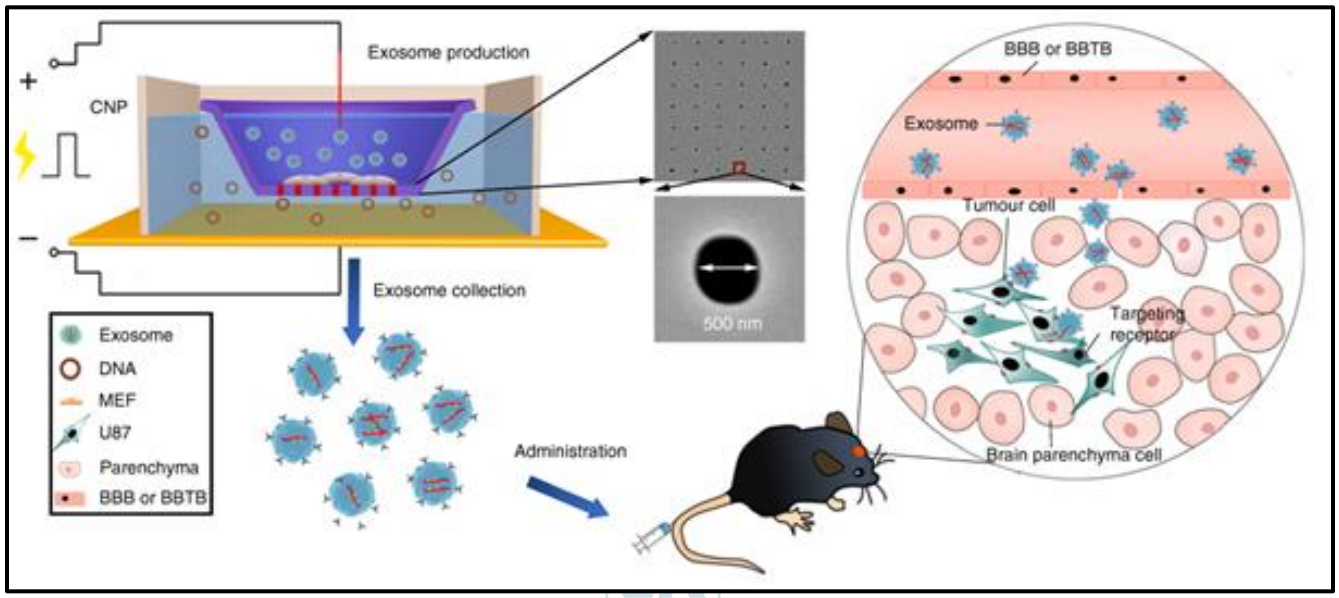

Figure 2: Diagrammatic representation of CNP-generated exosomes for targeted gene delivery. Left: the CNP system comprises a nanochannel array (red rectangles). Plasmid DNA added to the buffer enters attached cells via nanochannels under transient electrical pulses. Attached cells subsequently release 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.

Synthetic Biotechnology

By Peeyush Prasad, MSc

Contributing Editor

Advances in Synthetic Biology to Manipulate Living Systems

The Significance of Synthetic Biology in Living Systems

Imagine a future where you can create anything by manipulating living system and we will reach up to the idea of genetically modified organism. But how about creating new life forms for getting desired things such as life-saving cancer drugs, fuels, sensors, diagnostics, enzymes, storing data and electricity? Here, we are talking about synthetic life which started from few people's bold attempt to understand what life is at its very basic level. Discovery of DNA to discovery of tools to manipulate DNA, we have come very far where we can precisely alter gene by using technology such as CRISPR-Cas9. Idea of synthetic life became reality when Craig Venter reported the design, synthesis and assembly of the 1.08-mega-base pair Mycoplasma mycoides JCVI-syn1.0 genome. Chemically synthesized genome was inserted into the M. capricolum and new M. mycoides was created. They called it synthetic cell as progeny cells contained the new set of proteins coded from transplanted genome [1].

Further, several researches came into light that tried to create or manipulate the organism fundamentally for getting desired products. Synthetic life is usually designed by forward-engineering approach [2] that usually aims to create new molecular function. At the same time there is lack of consensus on what should be called as synthetic life. Is the synthetic life a total new creation of organism from scratch or is it just the alteration in the organism for finding new molecular function such as production of artemisinin in yeast cells. In one of the research, team of scientists has introduced the gene which converts the FPP into artemisinic acid and inhibited the gene which converts the FPP in yeast into ergosterol for producing artemisinin. Artemisinin is produced by plant A. annua (sweet wormwood) [3, 4]. Artificial gene circuit can be used for finding cure for cancer such as development of gene circuits based on adaptive programming. Three components: a sensor for detecting inputs, a processor and an actuator which produces

responses are integrated in artificial multi-gene circuits [5]. These circuits are based on transcriptional networks and regulators (Table 1).

Company	Function	Link
Synthetic Genomics	Working on algal proteins and oil, microbial therapeutics platform (Cmax™), self replicating RNA medicine, growing organs from engineered pig cells and bacteriophage platform for countering bacterial resistance. Company has developed the first automated DNA printer (BioXp).	https://syntheticgenomics.com/
Arzeda	A protein design company which builds its molecules from scratch for performing new function by using deep learning. Working in the field of agriculture, pharmaceuticals and diagnostics, functionalized sweetners and ingredients and advanced chemicals and materials.	https://www.arzeda.com/
Twist Bioscience	Working in the field of medicine, agriculture, industrial chemicals and data storage. Created an innovative silicon-based DNA synthesis platform.	https://www.twistbioscience.com/
Synbicite	Working in the field of medicine, agriculture and biofuels along with others. Provide services such as DNA synthesis, microfluidics, genome editing (CRISPR-Cas9) etc.	http://www.synbicite.com/

Table 1: Companies working in the field of synthetic biology.

Reprogramming of Cells and Bacteria: An Example of Synthetic Biology in Cancer Cells

Cancer cells have multiple signatures and it is important that immune cells must be able to recognize the plethora of variations in the cancer cells for counter measures. Reprogramming of T-cells can be done so that it can recognize the different combination of antigens. For example, use of Synthetic Notch (SynNotch) receptor T cell AND gate [6]. It has been observed that several bacteria specifically accumulate around the tumor therefore these bacteria can be manipulated for delivering specific therapeutics at tumor site [7]. In another

study, researchers have developed the artificial version of E. coli (Syn61) present in stomach which can be used for the synthesis of drugs, catalysts, enzymes etc. In this case 4 million genetic code of Syn61 is synthesized from scratch [8]. Creating eukaryotic organism is another challenge due to complexity of its component. In one of initiative, group of scientists are trying to synthesize the yeast (SC 2.0) genome. The study will provide the information about genome organization, gene content and role of RNA in yeast biology [9]. Other than manipulating the molecular function inside cells, researchers is trying to create the cells from scratch. Synthetic cell is consist of micro-compartments encapsulating biochemical molecules such as DNA, RNA, proteins, ribosomes, enzymes, ATP etc. These cells closely mimic the natural cells [10]. Figure 1 describes the direction of research currently going on in synthetic cell research.

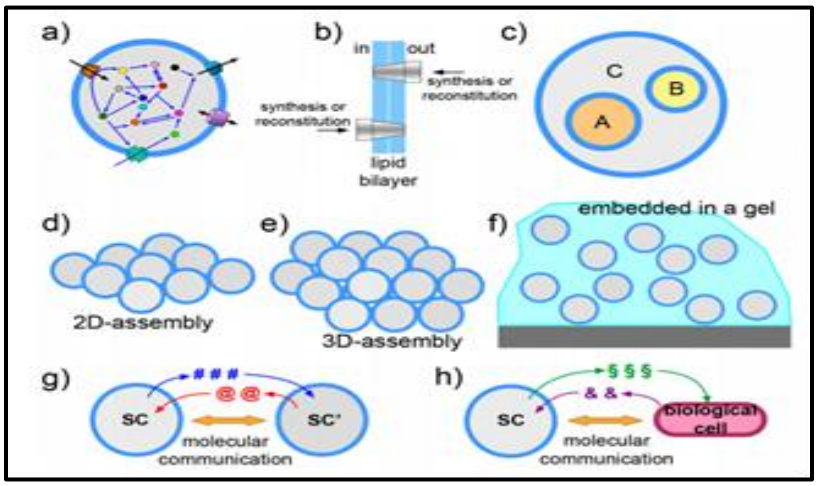

*Figure 1. Schematic drawings representing some of the current directions in SC research. (a) Functionalization of the SC membrane-by-membrane proteins or similar components. Note that orientation, shown in (b), becomes an important issue when dealing with vectorial systems as membrane proteins. (c) The nested multicompartment system, or multivesicular vesicle, also known as "vesosome", is a SC design that allow exploitation of compartmentation, hierarchical levels, chemical gradients across the membranes. (d,e) Assemblies of SCs in two and three dimensions. (f) SCs embedded in biocompatible gel. (g,h) Molecular communication between SCs or between SCs and biological [**Source:** Stano P. Life. 2019].*

Products	Source	Company
Synthetic rubber (BioIsoprene™)	Bacterial fermentation	DuPont and Goodyear
Soybean tire	Soybean oil	Goodyear
Acrylic	Genetically engineered microbe	OPX Biotechnologies
Biofuels	Synthetic algae	Synthetic Genomics

Table 2: Important products from synthetic biology.

The Future Prospects of Synthetic Biology

Development of new technology brings the question of ethics and safety. Autodesk which works in the field of software built its own virus (Phi-X174 bacteriophage). Genome of this virus consisted of 5,386 base pairs of nucleotides. This virus could infect the E. coli but was not harmful for human [11]. But this kind of research often leaves us with the question of how far man can go into his endeavor to intervene with nature. At what point we need to stop and what are the long term consequences of these researches? Life which we see today is outcome of millions of years of evolutionary process where errors often get ample time to adjust in the evolving system but suddenly introducing new organism can be a shock to the system. We have seen how genetically modified crop not only kills the pest against which it is developed but it also harms the other organisms. We are already struggling with multi antibiotic resistant infectious organisms and we have not yet found the drugs for many viral disease. Synthetic life has not yet found the place outside laboratory but accidental introduction of new organism in the outside nature can be very dangerous. Just imagine if synthetic virus as was created by Autodesk will exchange genetic material with other infectious agent. Therefore, it is important to outline guidelines. At the same time we can't deny the fact that there is the need for new source of energy, medicine, agricultural products etc. and for this we need to invest in synthetic life research.

Table 2 mentions the important products which can be derived from research on synthetic life. At research level, challenges are to design the living system from scratch. Understanding what sort of ingredient in what proportion will be required is yet to be explored in detail. Creating a lipid based membrane which can carry several molecules inside is still a very challenging task. Recently researchers used the microfluidics based approach for developing lipid bubbles which is similar to living cell membrane [12]. At last synthetic life research should not be driven by profit but by future need which cannot be solved by existing resource.

References

1. Gibson DG, Glass JI, Lartigue C et. al. Creation of a bacterial cell controlled by a chemically synthesized genome. Science. 2010 Jul 2;329 (5987):52-6. doi: 10.1126/science.
2. Slomovic S, Pardee K, Collins JJ et. al. Synthetic biology devices for in vitro and in vivo diagnostics. Proc Natl Acad Sci U S A. 2015 Nov 24;112 (47):14429-35.
3. Paddon CJ, Keasling JD. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nat Rev Microbiol. 2014 May;12 (5):355-67.
4. https://cosmosmagazine.com/biology/life-2-0-inside-the-synthetic-biology-revolution
5. Benenson Y. Biomolecular computing systems: principles, progress and potential. Nat Rev Genet. 2012 Jun 12;13 (7):455-68.
6. Roybal KT, Rupp LJ, Morsut L et. al. Precision Tumor Recognition by T Cells

With Combinatorial Antigen-Sensing Circuits. Cell. 2016 Feb 11;164 (4):770-9.

7. Wu MR, Jusiak B, Lu TK et. al. Engineering advanced cancer therapies with synthetic biology. Nat Rev Cancer. 2019 Apr;19 (4):187-195.

8.https://www.bbc.com/news/science-environment-48297647

9. http://syntheticyeast.org/

10. Stano P. Is Research on "Synthetic Cells" Moving to the Next Level? Life (Basel). 2018 Dec 26;9(1). pii: E3

11.https://www.vox.com/2014/5/5/11626456/autodesk-builds-its-own-virus-as-the-software-giant-develops-design

12. https://www.nature.com/articles/d41586-018-07289-x

Neuroprostheses

By Megha Agrawal, PhD

Executive Editor

Intra Spinal Micro-Stimulation Implant Technology Shows Promise for Clinical Implementation

Intra Spinal Micro-Stimulation Implant for Neuroprosthesis

Recent breakthroughs in neuroprostheses have accelerated a new wave of biomedical technologies including implantable spinal-cord-neuroprostheses that seek to restore standing and walking after paralysis to augment the human body or restore its lost functions [1]. Researchers have extensively studied these technologies in animal models that have shown tremendous promise. One of the therapeutic targets of these neuroprostheses are neural networks of the spinal cord that have been investigated for a number of applications that include restoring paralyzed limbs and mobility deficits, and promoting targeted plasticity and recovery after neural injury and disease [1-3].

Lately, the intra spinal micro-stimulation (ISMS) implant has emerged as an important procedure of neuroprostheses. ISMS comprises of an array of ultra-fine electrodes that are capable of delivering electrical pulses to the ventral horns of the spinal cord [4]. Researchers have shown that depending on the targeted region within the spinal cord, the application of ISMS can generate functional movements of the lower and upper limbs (lumbosacral and cervical implants), breathing (cervical implant) or bladder function (sacral implant) [1]. Further, studies were conducted on stimulation through an individual intraspinal electrode that demonstrated the possibility to activate motor networks including motoneurons, afferent and propriospinal projections, and associated axons spanning multiple spinal cord segments. In further advances made in ISMS, researchers showed that a small number of implanted electrodes can evoke synergistic muscle contractions, which can produce coordinated movements involving single or multiple joints to perform functional tasks [1].

ISMS in the Lumbar Spinal Cord for Overground Walking

The restoration of hind-limb movements after paralysis is a very important medical problem, and new biomedical solutions are sought to address this issue. To this end, ISMS in the lumbosacral spinal cord studied

in animals has shown great promise (Figure 1) [5].

Researchers showed that hind limb movements triggered by ISMS in cats resulted in significantly more fatigue resistant compared to those obtained by intramuscular electrical stimulation [3, 5]. It was demonstrated that with ISMS implants, animals were able to stand for ~5x longer durations and walk over-ground for ~10x longer distances than animals with intramuscular implants [3].

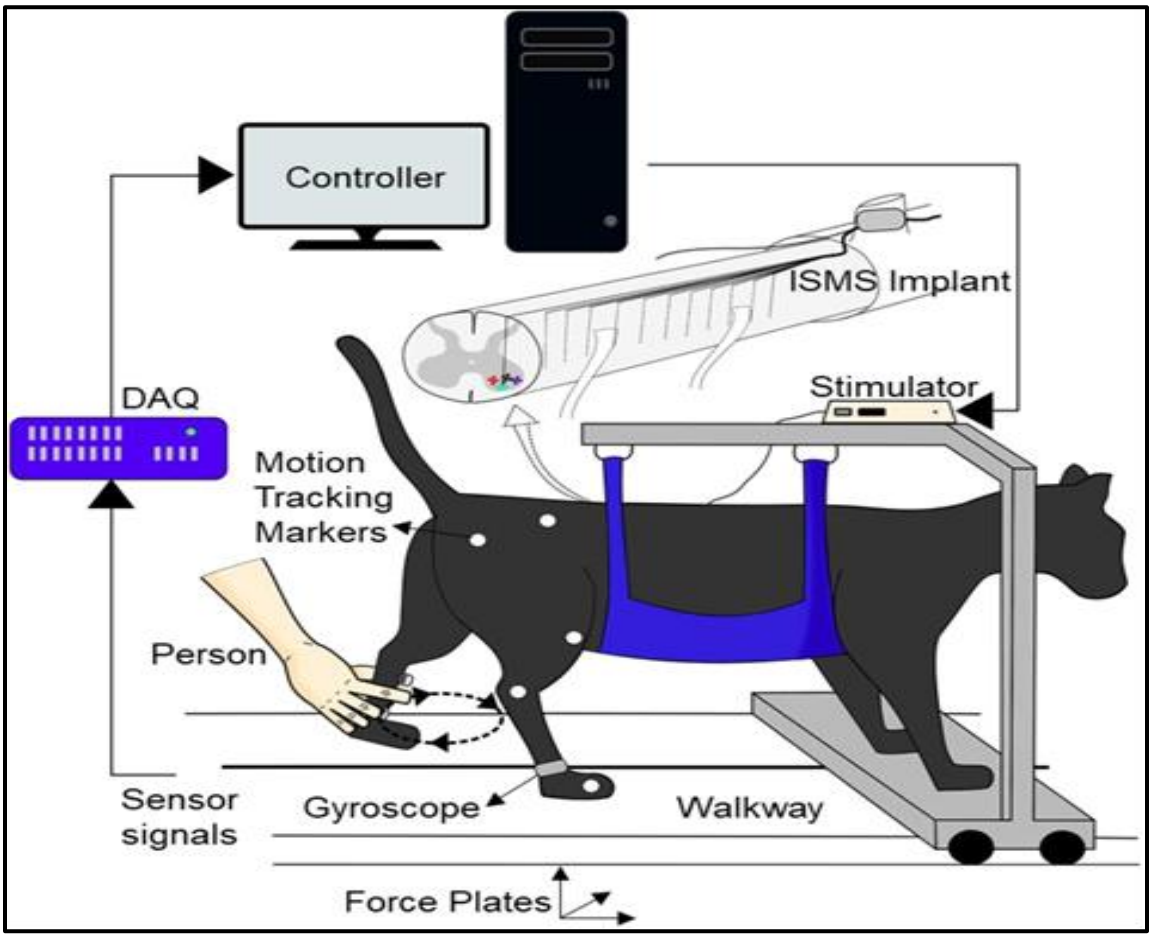

*Figure 1: Illustrations showing the experimental setup for over-ground walking. The process involves sensor signals that come from force plates under the walkway and a gyroscope placed on the tarsals from both hind-limbs. These signals are then converted to digital signals by the data acquisition device (DAQ) and steamed into a Matlab program where an algorithm can control the stimulation to the spinal cord to allow right-hind limb move to the opposite phase of the walking cycle [**Source:** bioRxiv, (2019)].*

Transitioning ISMS from Pre-Clinical World to Clinical One

Transition of ISMS to full clinical implementation is a critically important task that requires understanding about the functional organization of the motor networks to be targeted in the lumbar spinal cord of humans [1]. The existing information regarding the anatomical organization of the motoneuronal cell bodies in the human

lumbar spinal cord that innervate the leg muscles (i.e., anatomical map) is not sufficient to reveal the functional organization and connectivity of various motoneuronal pools (i.e., functional map). Further, the required stimulation amplitudes for their activation are also not known [1].

These steps are critical to design and realize a clinical translation path of ISMS as effective neuroprostheses. To this end, researchers recently investigated the organization of the neural networks targeted by these implants in a non-human primate, the macaque monkey. They presented the hypotheses of their study based on preservation of similar functional organization of motor networks in non-human primates (Figure 2) [1].

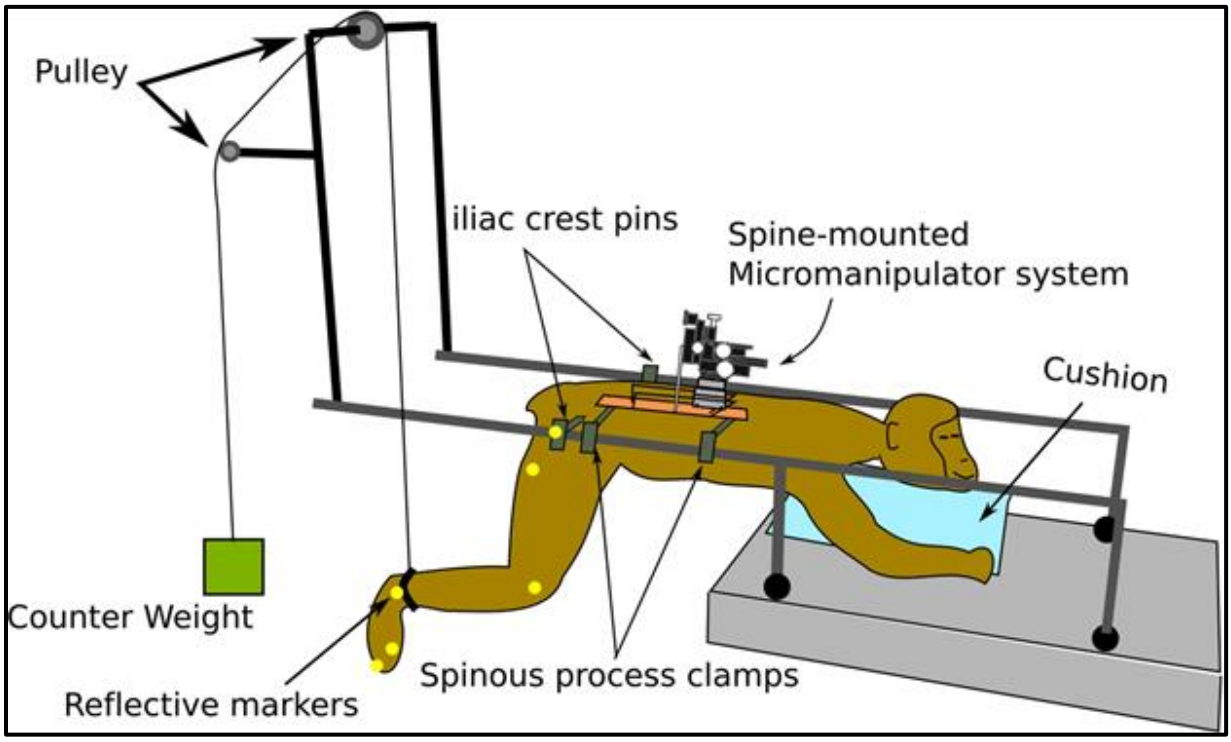

*Figure 2: Experimental setup showing the concepts of functional mapping of the lumbosacral spinal cord in non-human primates [**Source:** Scientific Reports, (2019)].*

Concluding Remarks

Research advances made in spinal-cord neuroprostheses have shown tremendous promise for clinically improving lower limb mobility after spinal cord injury (SCI). For example, researchers have shown that by targeting the motor networks in the ventral horns of the spinal cord, ISMS can specifically result in various coordinated leg movements that are necessary for functional tasks such as walking over-ground. In this regard, preclinical studies have indicated the potential of ISMS to produce beneficial outcomes of standing and walking even after a chronic complete SCI. Therefore, it is believed that ISMS has the potential to restore walking for people with more severe SCIs than may be possible with any other existing procedure such as epidural stimulation. Further, to achieve the goal of clinical implementation of ISMS implants, the

knowledge of the functional organization of the motor inter-neuronal networks in the lumbosacral spinal cord of humans is critical. This involves the knowledge of exact location of the spinal cord to place the implant for successful targeting of the leg movements that are required for functional standing and walking. This will enable the optimized design of the implant. Future technical design considerations are expected to include the number of microelectrodes in the array, spacing between the microelectrodes, and targeting depth and length of the microelectrodes. We anticipate that further R&D advances in specifications of the clinical microelectrode and stimulator would pave the way to safely deliver the current intensities that are required for producing the pre-requisite functional movements.

References

1. Amirali Toossi, Dirk G. Everaert, Steve I. Perlmutter & Vivian K. Mushahwar, Functional organization of motor networks in the lumbosacral spinal cord of non-human primates**,** *Scientific Reports*, **9**, Article number: 13539 (2019).

2. Mushahwar, V. K., Jacobs, P. L., Normann, R. A., Triolo, R. J. & Kleitman, N. New functional electrical stimulation approaches to standing and walking. *J. Neural Eng.* **4**, S181 (2007).

3. Tator, C. H., Minassian, K. & Mushahwar, V. K. Spinal cord stimulation: therapeutic benefits and movement generation after spinal cord injury. *Handb. Clin. Neurol.* **109**, 283–296 (2012).

4. Bamford, J. A., Lebel, R. M., Parseyan, K. & Mushahwar, V. K. The fabrication, implantation and stability of intraspinal microwire arrays in the spinal cord of cat and rat. *Trans. Neural Syst. Rehabil. Eng*. (2016).

5. Ashley N Dalrymple, David A Roszko, Richard S Sutton, Vivian K Mushahwar, Pavlovian Control of Intraspinal Microstimulation to Produce Over-Ground Walking, *bioRxiv,* (2019), doi: https://doi.org/10.1101/785741.

Regenerative Medicine

By Shyamasri Biswas, PhD

Executive Editor

The Emerging Potentials of Exosome-Based Drug Delivery

Intercellular Communication through Exosome Amplification: Reprogramming of Cells

Exosomes are ultra-small (50-150 nm) special membranous bio-vesicles. They are released from living cells into surrounding body fluids upon fusion of multi-vesicular bodies (MVB) and the plasma membrane [1]. These extracellular vesicles are known to be functional vehicles and carry cell-specific cargos of proteins, lipids, nucleic acids and genetic materials that can be selectively taken up by neighboring or distant cells that are located far from their release points. Subsequently, the recipient cells are reprogrammed through the triggered bioactivities of exosomes [2].

Studies have indicated that the bioactive molecules contained in exosomes could impact target cells by direct stimulation of target cells via surface-bound ligands and transferring of activated receptors to recipient cells. The subsequent epigenetic reprogramming of recipient cells can happen via the delivery of functional proteins, lipids, and RNAs (Figure 1) [2]. As Figure 1 schematically illustrates, the exosome amplification can potentially enable communication of parental cells with specific proximal or distant target cells [2]. In this way, exosomes represent an intercellular communication mode that can be implemented in many cellular processes including immune response, signal transduction and antigen presentation [2].

The regulated formation of exosomes, specific makeup of their cargo and cell-targeting specificity show high potential of exosomes for their use as next generation non-invasive diagnostic biomarkers, as well as therapeutic carriers [1, 2].

Therapeutic Ability of Exosomes

Exosomes' therapeutic and clinical application potentials have attracted a lot of attention, lately. One area of application that is being considered is cancer diagnosis and therapy. Studies have suggested that tumor-derived exosomes or TDEx regulate tumor microenvironment and assist in distant metastasis [1]. Researchers have shown the possibility of employing molecular markers on exosomes or TDEx that can be an

effective method as liquid biopsy indicator for cancer diagnosis and prognostic monitoring [1, 3].

Exosomes are also known for their ability to cross cytoplasmic membrane and the blood/brain barrier. This ability of exosomes can be leveraged for therapeutic delivery of drug molecules. Therefore, a great deal of current research has focused on using the potential of exosomes being a drug carrier due to exosomes' natural material transportation properties, intrinsic long-term circulatory capability, and excellent biocompatibility that are considered suitable to deliver a variety of chemicals, proteins, nucleic acids and gene therapeutic agents [1, 2]. To this end, the modified exosomes were shown to treat pancreatic cancer by delivering siRNA, and the CD47 protein on the exosomes prevented them from being phagocytosed by immune cells. This makes the exosomes more efficient than synthetic analogiliposomes. Researchers utilized the targeting effect of exosomes, and leveraged it to apply drugs such as adriamycin directly to tumor cells that limited the toxicity to normal cells [3].

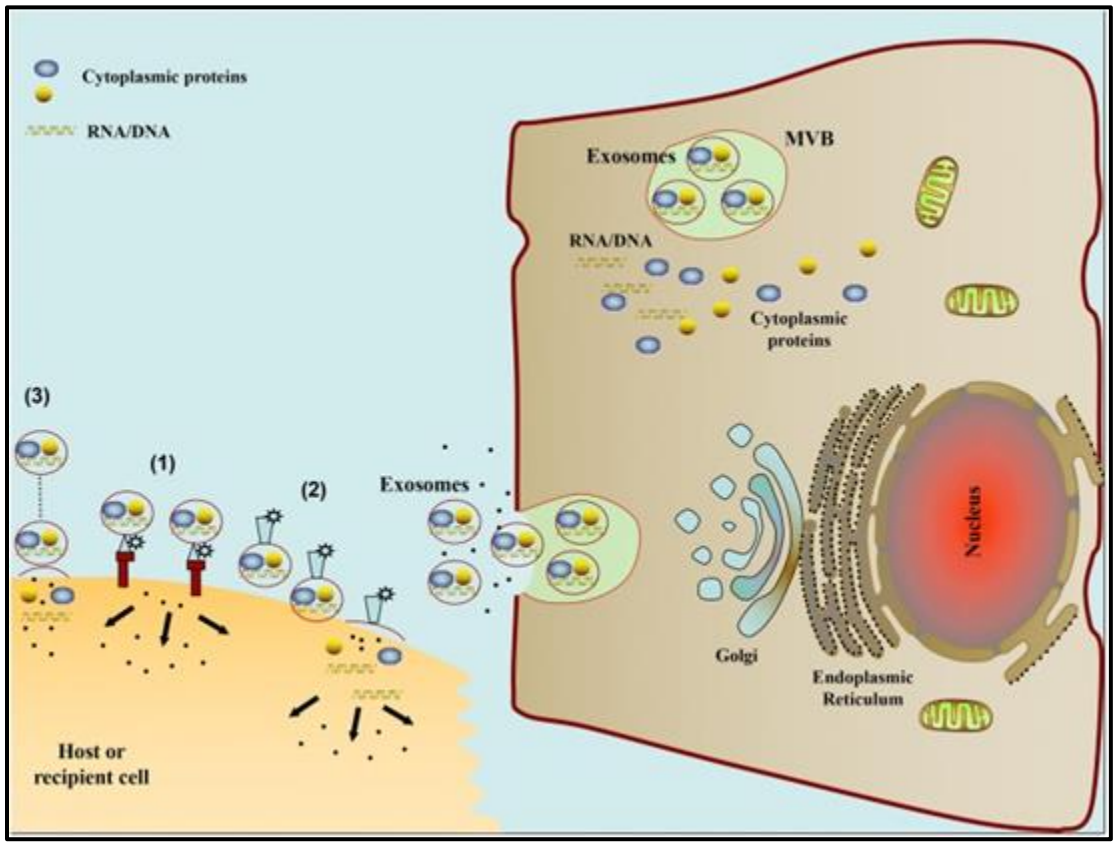

*Figure 1: The schematic illustration showing intercellular communication involved in exosome mediated process. (1) Direct surface-bound ligands by the recipient cells. (2) Transfer of activated receptors to recipient cells. (3) Epigenetic reprogramming of recipient cells [**Source:** Cell Biosci. 2019].*

Further, studies were conducted on the cellular immunity, angiogenesis, and regenerative effects in cell therapy mediated by mesenchymal stem cells (MSCs) and some other cells that were implemented by the exosomes released from these cells [4, 5]. The emerging field of exosome therapy has shown great application potential from oncology to regenerative medicine [1].

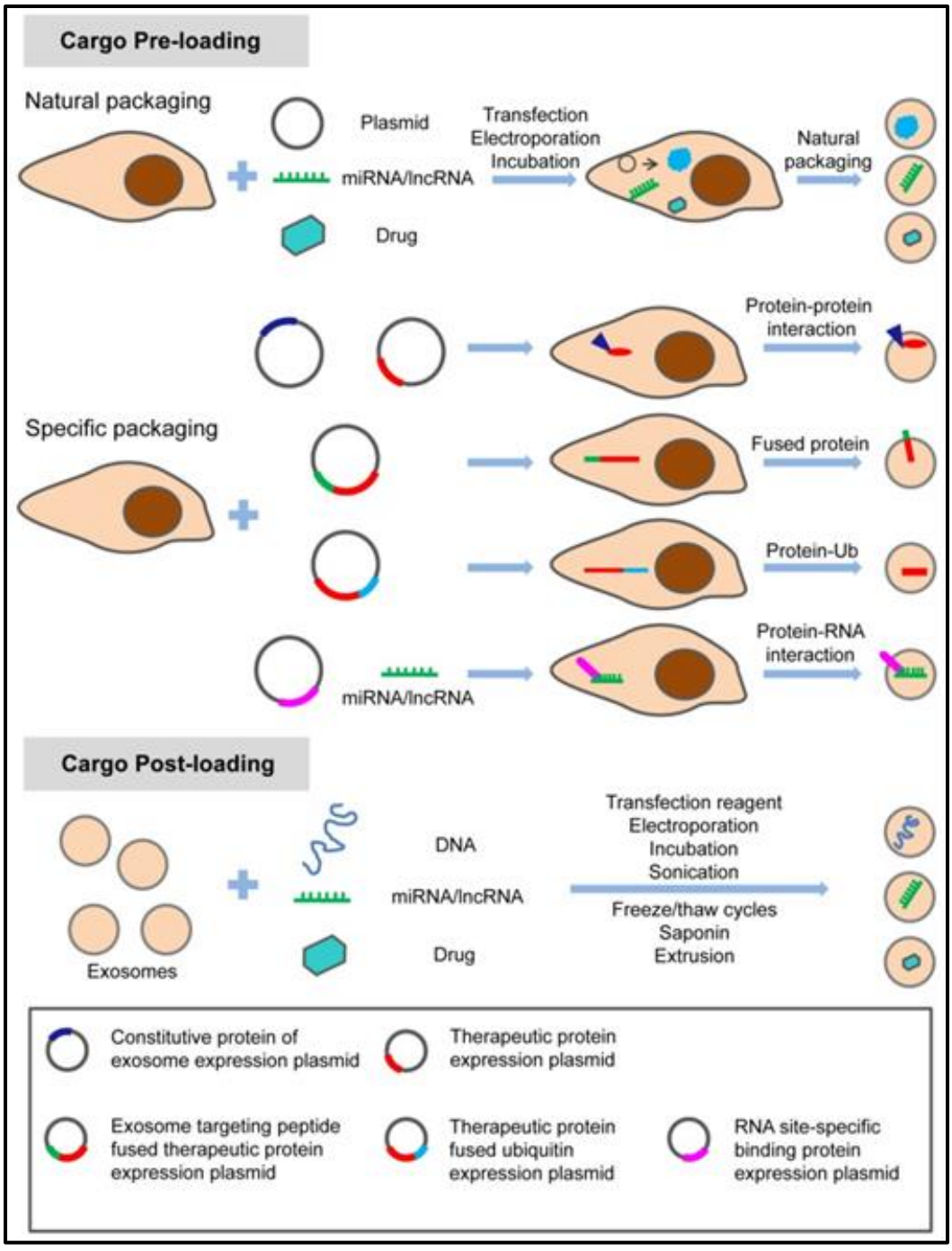

*Figure 2: Various methods that are employed for loading specific proteins, nucleic acids and small molecular drugs into exosomes [**Source:** Theranostics (2019)].*

Exosomes Loaded with Small Molecule Drugs

Exosomes can be employed as carriers of chemotherapy drugs. Researchers have developed methods of loading small molecule drugs into exosomes that include direct mixing, incubation, ultrasonic treatment, and eddy oscillation [1]. In addition to these methods, several other different ways of loading catalase into exosomes were demonstrated *ex-vivo* such as incubation, saponin permeabilization, freeze and thaw, sonication, and extrusion that were employed to treat Parkinson's disease. These methods have been demonstrated for their use to load exosomes with small molecule drugs as well.

The current state-of-the-art employs most of these passive loading methods for small molecule drugs that are loaded into exosomes. However, major drawbacks of these methods and approaches are the resulting loss and degradation of exosomes from multiple purification steps that are used in these methods. Also, a prolonged *in-vitro* treatment and the inherent physicochemical properties of drug molecules can affect the bioactivity and degrade the stability of exosomes. In view of these shortcomings, current research has focused on viable alternative methods that would enable the stability of exosomes.

Figure 1 summarizes the methods for loading the specific treating molecules (proteins, nucleic acids and small chemicals) into exosomes [1].

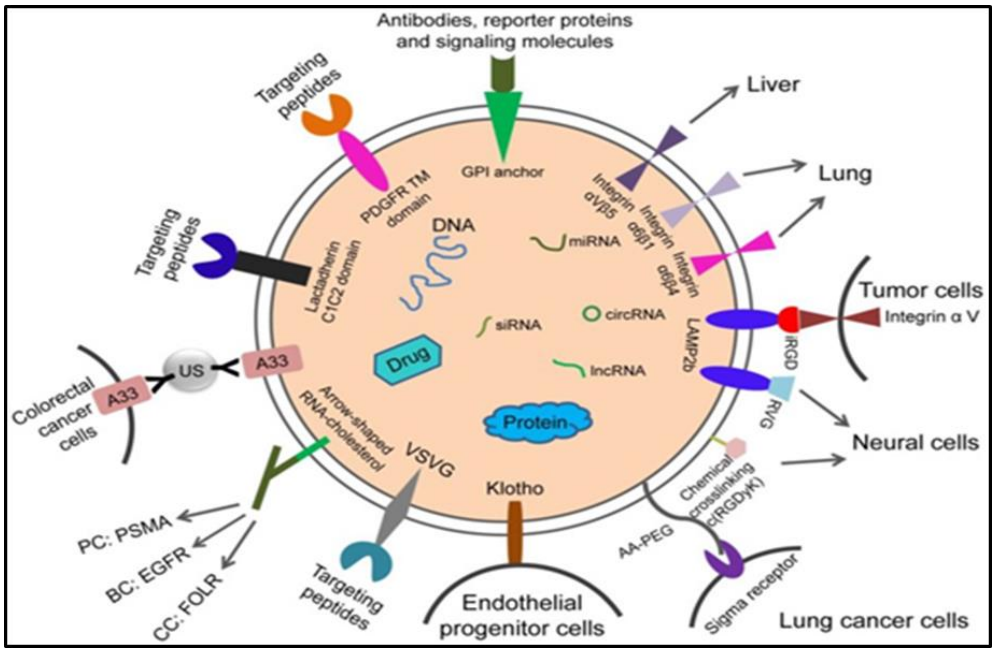

*Figure 3: A schematic presentation showing design strategies for therapeutic exosome targeting (AA-PEG: aminoethylanisamide-polyethylene glycol; GPI: glycosylphos-phatidylinositol; RVG: rabies virus glycoprotein; VSVG: vesicular stomatitis virus glycoprotein; A33Ab-US: the surface-carboxyl superparamagnetic iron oxide nanoparticles coated with A33 antibody, to bind to A33-positive exosomes; iRGD: an α V integrin-specific internalizing peptide; PDGFR TM domain: PDGFR transmembrane domain) [**Source:** Theranostics (2019)].*

Future Design Strategies for Therapeutic Exosome Targeting

Figure 3 shows a number of design strategies that are currently being developed for loading and targeting to realize the therapeutic potential of exosomes [1]. In the application of therapeutic exosomes, future designs will focus on accurately targeting a specific type of cells including tumor cells or a certain type of tissue (e.g., brain tissue) as opposed to broad distribution of exosomes to the liver, kidney and spleen. In view of this, researchers are focusing on to improving the targeting of exosomes through the study of exosome donor cells and the modification of surface molecules on exosomes [1].

Concluding Remarks

Studies have shown that exosomes can play a crucial role in both physiological and pathological processes including the capacity of exosomes to load a wide range of content including lipids, RNAs, and proteins to signal specific recipient cells or tissues. This makes them a promising diagnostic biomarker and therapeutic tool for treatment of cancers and other complex pathologies and offer exciting new possibilities in drug delivery. The early studies indicate the great clinical potential and value of exosomes due to their strong biocompatibility, offering a new frontier in drug delivery. We anticipate a rapid growth in research and developments in the future especially in the area of large scale production of exosomes for clinical use.

References

1. Chunying Liu and Changqing Su, Design strategies and application progress of therapeutic exosomes, Theranostics. 2019; 9(4): 1015–1028.
2. Yuan Zhang, Yunfeng Liu, Haiying Liu, and Wai Ho Tang, Exosomes: biogenesis, biologic function and clinical potential, Cell Biosci. 2019; 9: 19.
3. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA. et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature. 2017;546:498–503.
4. Baglio SR, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. Front Physiol. 2012;3:359.
5. Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM. et al. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. Cardiovasc Res. 2014;103:530–41.

Biotechnology Advances around the World

Editor's Picks

Every issue of Biotechnology Kiosk presents select latest research news picked by the executive editors on significant research breakthroughs in different areas of biotechnology around the world. The aim is to promote further R&D in all of these cutting edge areas of biotechnology. The editors have compiled and included the following innovations and breakthroughs to highlight the recent biotechnology advances.

Dr. Megha Agrawal

Executive Editor

Dr. Shyamasri Biswas

Executive Editor

Marine Biotechnology

Revealing the structure of the microbial coated plastic marine debris

The world's ocean is vulnerable to pollution caused by millions of tons of tiny pieces of dumped microplastics in the ocean. These plastic marine debris (PMD) can be ingested by even the smallest marine animals that can potentially threaten their survival. It is believed that PMD can seriously affect spatial scales of life from microbes to whales.

These marine microplastics or PMDs upon entering the aquatic environment get quickly coated with a layer of organic and inorganic substances. Subsequently, a rapid colonization of bacterial and eukaryotic (micro) organisms happens that results in a thin biofilm coating of bacteria and other microbes, which is called the plastisphere. While there have been studies on the impacts of PMD on marine animals, the interactions between PMD and microscopic life and especially microbes are not sufficiently explored. To address this issue, a collaborative team of researchers in the US and Netherlands used an innovative microscopy method to reveal the structure of the microbial communities coating microplastic samples from a variety of ocean sites. Their results were recently published in Molecular Ecology Resources (Spatial structure in the "Plastisphere": Molecular resources for imaging microscopic communities on plastic marine debris, Molecular Ecology Resources, 2019; DOI: 10.1111/1755-0998.13119).

This study involved a combinatorial approach consisting of labelling and spectral imaging based on fluorescence in-situ hybridization (CLASI-FISH), method and using confocal microscopy to study Plastisphere communities. Researchers created a probe set that included three existing phylogenetic probes (targeting all Bacteria, Alpha-, and Gammaproteobacteria) and four specially designed probes (targeting Bacteroidetes, Vibrionaceae, Rhodobacteraceae and Alteromonadaceae). These were then labelled with a total of seven fluorophores and validated the probe set using pure cultures.

Researchers envision that future applications of this study could pave the way to visualize specific groups of microbes to investigate real-time their interactions with the substrate surface. This includes possible polymer hydrolysers that contribute to PMD degradation.

Environmental Biotechnology

Reducing air pollution can have dramatic health benefits

In the world today, poor air quality or ambient air pollution including household air pollution is a serious environmental risk to human health that can affect almost every organ in the body and result in morbidity and mortality. In a comprehensive study, an international team of bio and medical researchers from the US, Mexico, UK, Germany, Korea and South Africa looked at the health benefits of pollution reduction at different levels of

interventions. They published their paper in Annals of the American Thoracic Society (Health Benefits of Air Pollution Reduction, Annals of the American Thoracic Society, 2019; 16 (12): 1478 DOI: 10.1513/AnnalsATS.201907-538CME). In their study, they investigated the impact of reductions in air pollution on human health that showed fast and dramatic impacts on health-outcomes, as well as decreases in all-cause morbidity. This important study showed that reducing pollution at its source can have a rapid and substantial impact on health. For example, researchers demonstrated that imposing a ban on smoking for week yielded a 13 percent drop in all-cause mortality, a 26 percent reduction in ischemic heart disease, a 32 percent reduction in stroke, and a 38 percent reduction in chronic obstructive pulmonary disease. Some of the significant health benefits could be achieved within a few weeks that included disappearance of shortness of breath, cough, phlegm, and sore throat. Other socio-health beneficial impacts included reduction of school absenteeism, clinic visits, hospitalizations, premature births, cardiovascular illness and death.

Researchers showed that these interventions are cost-effective and can be implemented and reducing factors causing air pollution and climate change can have strong health benefits.

Virology

Breakthrough in solving structure of key pneumonia virus enzyme

It is known that respiratory syncytial virus (RSV) and human metapneumovirus (HMPV) cause severe respiratory diseases in humans. Past studies have shown that RSV and HMPV are two interlinked viruses that cause severe and life-threatening respiratory diseases such as pneumonia and bronchiolitis mostly in premature babies and infants, and also these viruses affect the elderly, and anyone with a weak immune system. However, despite clinical advances, there is no effective vaccine or an antiviral therapy that exists to control or eliminate RSV or HMPV infections.

HMPV and RSV control the cell's internal mechanisms to make copies of themselves when they infect human cells. Special proteins are released by the virus that starts the process of replication. Subsequently, distinct protein complexes are created from the interaction of these viruses with each other.

In a new research, a team of molecular and structural biologists in Singapore have reported the structure of one of its key components and proposed a potential new route to disabling respiratory syncytial virus (RSV) and human metapneumovirus (HMPV). Their research was published in Nature (Structure of the human metapneumovirus polymerase phosphoprotein complex, Nature, 2019; DOI: 10.1038/s41586-019-1759-1). Researchers revealed the detailed structural knowledge that can pave the way to develop inhibitors to disrupt the enzymatic activities of HPMV L:P protein and potentially block infection by the virus. They envision their study would open up new therapeutic pathways to help

researchers in pharma and academia to design much needed therapies for treating severe viral infections. It is hoped that inhibitors developed against HPMV could also work against a broad spectrum of viruses involved in respiratory diseases.

Tropical Medicine

Genetic modification of malaria carrying mosquitos

It is known that the transmission of malaria in most of sub-Saharan Africa is carried out by the primary mosquito vector Anopheles gambiae. Past studies have shown that the resistance in Anopheles gambiae mosquitoes to members of all 4 major classes (pyrethroids, carbamates, organochlorines, and organophosphates) of public health insecticides can seriously affect malaria control programs.

Discovering the underlying molecular basis is critical to overcome this issue. To this end, studies have shown an increase in expression of detoxifying enzymes associated with insecticide resistance. However, the knowledge about their direct functional validation in An. gambiae is still not adequate. It is therefore, believed that understanding the mechanisms by which mosquitoes evolve resistance is essential that will enable the design and implementation of mitigation strategies along with the evaluation of new classes of insecticides.

In a breakthrough in this area, researchers in the UK recently published research results on genetically modified malaria carrying mosquitoes that demonstrated the role of particular genes in conferring insecticide resistance (Functional genetic validation of key genes conferring insecticide resistance in the major African malaria vector, Anopheles gambiae, Proceedings of the National Academy of Sciences, 2019; 201914633 DOI: 10.1073/pnas.1914633116).

Researchers showed for the first time characterization of three genes (Cyp6m2, Cyp6p3 and Gste2) associated with insecticide resistance. This was accomplished by their direct overproduction in genetically modified Anopheles gambiae. This significant breakthrough has revealed that increased production of just these three genes can cause the mosquitoes to become resistant to all four classes of public health insecticides that are currently being used in malaria control. This study validate particular genes as excellent markers for resistance that can be employed as tools to monitor the problem through molecular testing.

Alternative Medicine

Positive effects of drinking tea on the brain structure

The main health benefits of tea come primarily from its constituents including catechin, L-theanine and caffeine. Among these constituents, catechin has been reported to be beneficial to cognitive health that has shown enhancements in memory recognition and memory performance in both animal and human studies. In majority of these studies, more focus has been given on understanding neuropsychological measures

and studies on neuroimaging measures, especially for interregional connections are required to be done to better understand the mechanisms of health benefits of tea as an alternative form of medicine.

In a recent study, researchers in Singapore and China revealed that regular tea drinkers can have better organized brain regions. They compared with non-tea drinkers and reported noticeable health benefits associated with healthy cognitive function from drinking tea. This discovery was made the researchers after examining neuroimaging data of 36 older adults and they published their research in Aging (Habitual tea drinking modulates brain efficiency: evidence from brain connectivity evaluation, Aging, 2019; 11 (11): 3876 DOI: 10.18632/aging.102023)

The results of this study showed the first evidence of positive contribution of tea drinking to brain structure, and suggested that drinking tea regularly could potentially impart a protective effect against age-related decline in the organization of brain structure. This research shows the importance of further work in this area as cognitive performance and brain organization are closely related. This would help better understand how important cognitive functions work such as emerging of memory from brain circuits, and how the possible interventions can take place to better preserve cognition during the ageing process.

Compiled and Edited by Dr. Megha Agrawal and Dr. Shyamasri Biswas.

Biotech and Pharma Industry Roundup

AstraZeneca and Daiichi Sankyo present positive data of breast cancer drug trastuzumab deruxtecan

In a ray of hope for breast cancer patients, AstraZeneca and Daiichi Sankyo presented positive data of trastuzumab deruxtecan (DS-8201) from their Phase II single-arm DESTINY-Breast01 trial in HER2-positive metastatic breast cancer patients. These patients received two or more previous HER-2 targeted regimens. The objective response rate (ORR), was found to be 60.9% with the drug alone [Source: https://www.biospace.com/].

Merck's inhibitor Keytruda (pembrolizumab) shows promise in the treatment of lung cancer patients

Merck reported brand new findings at the European Society for Medical Oncology (ESMO) Immuno-Oncology Congress 2019 from its Phase III KEYNOTE-042 Study that showed improvements in overall survival, progression-free survival and overall response rate in patients treated with Keytruda as a monotherapy. The promising findings of Keytruda showed the reported benefits as a first-line treatment for patients that were affected with metastatic nonsquamous non-small cell lung cancer (NSCLC) and whose tumors expressed PD-L1 regardless of KRAS mutation. The data on Keytruda demonstrated significant upward benefits that included reduced risk of death by 58% in patients with any KRAS mutation and by 72% in patients with the KRAS G12C mutation compared to chemotherapy [Source: https://www.biospace.com/].

Alvogen to acquire the product Gralise® (gabapentin) from Assertio Therapeutics

Assertio Therapeutics, Inc., ("Assertio") will transfer all responsibilities associated with the product Gralise® (gabapentin) to Alvogen expectedly in January 2020, subject to regulatory approval. This announcement was made recently. To acquire the product, Alvogen will pay Assertio a total value of $127.5 million [Source: https://www.biospace.com/].

Eleven biopharma companies filed for bankruptcy (chapter 11) in 2019

Chapter 11 filings are considered rare in the biotech and pharma industries. However, a high number of drug makers including Purdue Pharma, Achaogen and Pernix Therapeutics filed for chapter 11 so far in this year than in any year since at least 2011. The reasons are legal liabilities that loomed large with several drug makers that felt the weight of thousands of opioids lawsuits. On the other hand, prominent antibiotics makers

Achaogen and Aradigm struggled in 2019 due to apparent commercial roadblocks. Despite the FDA approval, antimicrobial drugs had apparently a market failure according to industry experts [Source: https://www.biopharmadive.com/).

Sanofi plans to stop diabetes, heart research and $2.2B in cost cutting going forward

Sanofi's new CEO, Paul Hudson plans to shake up the French pharma giant in a recent announcement. As a future plan, Sanofi will stop all research in the disease areas involving diabetes and cardiovascular programs, and reduce costs by 2 billion euros, or about $2.2 billion, by 2022. These savings are expected to come from spending less on expenses such as travel, consultants and training costs, and reduced investments in deprioritized businesses including diabetes and cardiovascular, and manufacturing improvements [Source: https://www.biopharmadive.com/)].

The British pharma AstraZeneca is growing

AstraZeneca showed a robust performance in 2019. For example, its oncology business led by its versatile products such as Tagrisso, Imfinzi and Lynparza matched the sales of its strong cholesterol-lowering statin pill Crestor at its peak that came out to be more than $6 billion [Source: https://www.biopharmadive.com/)].

Merck diversifies its cancer drug pipeline through a $2.7 billion acquisition of biotech firm ArQule

Merck & Co. recently announced its plans to diversify its cancer drug pipeline through a $2.7 billion acquisition of ArQule. [Source: https://www.biopharmadive.com/)].

Madrid-based RNA specialist Bioncotech Therapeutics enters into a Phase II clinical trial collaboration with a MSD subsidiary

The clinical collaboration between Bioncotech Therapeutics and Merck Sharpe & Dohme's aims at providing clincial Phase II evidence that' RNA–based cancer lead BO-112 can improve the efficacy of PD1 checkpoint inhibitor pembrolizumab in partients with tadvanced-stage solid tumors with liver metastases [Source: https://european-biotechnology.com/].

Pierre Fabre to amend license agreement with Puma Biotechnology

In a recent announcement, Pierre Fabre SA and Puma Biotechnology, Inc. have agreed to broaden the geographic coverage of their license agreement on commercialization of Nerlynx® (neratinib). The amended and extended agreement allows Pierre Fabre to not only market the breast cancer recurrence blocker Nerlynx® (neratinib) within Europe and part of Africa but also in the Middle East and several African territories [Source: https://european-biotechnology.com/].

Advertising rates for the magazine:

Top Banner..... 728 pixels wide x 90 pixels high (includes URL link)

Medium Banner.....400 pixels x 90 pixels (includes URL link)

Please supply animated banners as gifs. Static banners may be supplied as gifs, PNGs or JPEGs. All banner files, static or animated, should be kept to 75k or below.

Top Page Banner Position: $600 per month

Middle Page Banner Position: $350 per month

Side and Bottom Page Banner Position: $250 per month

General AD Rates for the Magazine

	Per month rates
Full Page	$1000
Half Page	$700
1/4 th Page	$500

For all production related questions or sending your ads, please e-mail or call our production department: E-mail: sales@biotechkiosk.com; Phone: 386-518-9411