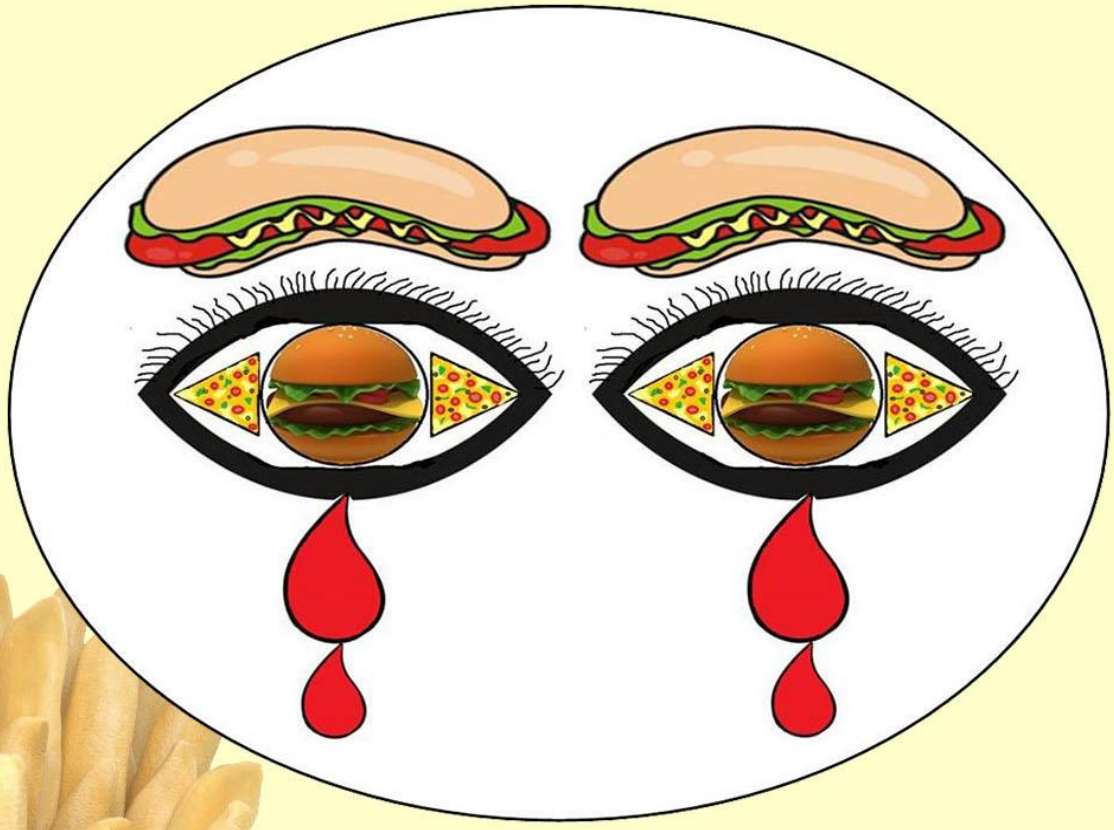
ISSN 2689-0852

BIOTECHNOLOGY KIOSK

October 2019



Can we avoid it for good reasons?

Volume 1, Issue 5
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!

Biotechnology Kiosk (BK) is proud to publish its 5th issue for our readers. The regular features include high-end editorials by experts, biotechnology advances around the world and industry news from pharma and biotech sectors.

In this issue, we present editorial on the irreversible damaging effects of over indulgence of fast food on our health and society. This editorial sheds light on the fast food induced negative impact on health based on hard medical facts and rational. Other editorials by experts cover important areas in bio-robotics, computational biotechnology, medical devices and genetics and agriculture biotechnology.

Our 'Editor's Pics' section covers important areas in genetics and ageing, where we have discussed the role of transcriptional protein 'REST' in regulating molecular cascade related to aging. We have also discussed the surprising role of neutrophil in inflammatory pathogenesis in malaria in immunology and infectious disease. In neuroimaging and bioinformatics, we have presented recent

promising developments in augmented reality app for new visual insight into human biology. These editors' picks bring news about latest discoveries in different areas of biotechnology to our readers. Plus, readers can read biotech and pharma industry news from all over the world. Please do write to us with your comments and feedback. Your suggestions are always appreciated.

We hope that you will enjoy reading this issue of Biotechnology Kiosk.

Dr. Megha Agrawal and Dr. Shyamasri Biswas

Executive Publishers and Editors

CONTENTS

VOLUME 1, ISSUE 5 OCTOBER 2019

COLUMNS

Food and Health Science

By Shripriya Singh, PhD

Contributing Editor

Over Indulgence in Fast Food Comes with a Price: It Can Cost You Your Vision

The Never Ending Temptation of Fast food: Can we avoid it for Good Reasons?

'A platter full of golden fries, a cheese pizza with extra pepperoni toppings, a sumptuous hamburger dripping with molten cheese, a sizzling hot dog and a triple scoop sundae of your favorite flavor'…Did I get you tempted? Well I surely am! Well, do not worry, this is not a party menu; instead, it is a prescription that will surely land you in a doctor's clinic, if not any time soon but eventually. For those who think this is yet another article lecturing you about good health and nutrition, let me bring to your kind attention, we are talking neuroscience here, and to support our arguments, we back it up with potent research evidence. We are familiar with the health implications associated with unhealthy diet; lifestyle issues and so called junk food or fast food, which are the leading causes of obesity, cardiac problems and even cancer. However, more recently the impact of junk food has been explored in relation with the human brain and vision. To clearly define any food which has abnormal high levels of salt, sugar, starch, fat and is processed in a manner which makes it lose its nutritional qualities, qualifies as junk food. These foods are rich in trans-fats and processed carbohydrates, give a feeling of immediate satiation upon consumption but are devoid of any substantial nutrients.

The brain is the master controller of all body functions including hunger and appetite. It is a popular fact that human emotions such as happiness, anxiety, stress and sometimes depression drastically influence our eating habits and food cravings. We often come across the term 'stress buster food' which is actually not entirely incorrect if talking from a neuroscience point of view. However, before we discuss how the brain influences diet and vice versa, we must shed light on a case, which has been making headlines in the past month and obviously for the right reasons.

The Impact of Fast Food on the Health of an Adolescent: A Case Report

An alarming case report recently published in the journal 'Annals of Internal Medicine'

brought to light the story of a teenager who nearly lost his vision due to his junk food addictions. A teenage boy at age 14 started complaining about lethargy and perpetual tiredness, upon consultation with the family practitioner he was diagnosed with macrocytic anemia and low vitamin B12 levels. He was advised against his fussy eating habits and given vitamin B12 injections. Next year at age 15 he developed vision problems and sensorineural hearing loss. However, medical reports, magnetic resonance imaging (MRI) and eye tests were completely normal and the cause went undetected.

Over the next two years his vision deteriorated progressively and at age 17 he was diagnosed with optic neuropathy. His eye test revealed that his vision was 20/200 in both eyes, the threshold for being "legally blind" in the United States. Genetic testing revealed no hereditary links to Leber hereditary optic neuropathy, thus ruling out any possibility of a genetically inherited disorder. Path-lab tests showed weak persisting anemia, low levels of selenium and copper, however iron levels, thyroids functions etc. were normal. His bone mineral density was low and so were the vitamin D levels. The teenager was not addicted to tobacco, alcohol or any kind of drugs which could suitably be held responsible for his condition. However, upon further probing he confessed about his dietary habits and his junk food addictions since his elementary school days. Throughout his growing up years he had routinely hogged on fries, wafers, white bread, processed sausages and ham slices. He further admitted that he had an aversion for certain texture and colors of foods which he perpetually avoided (avoidant restrictive food intake disorder) (1).

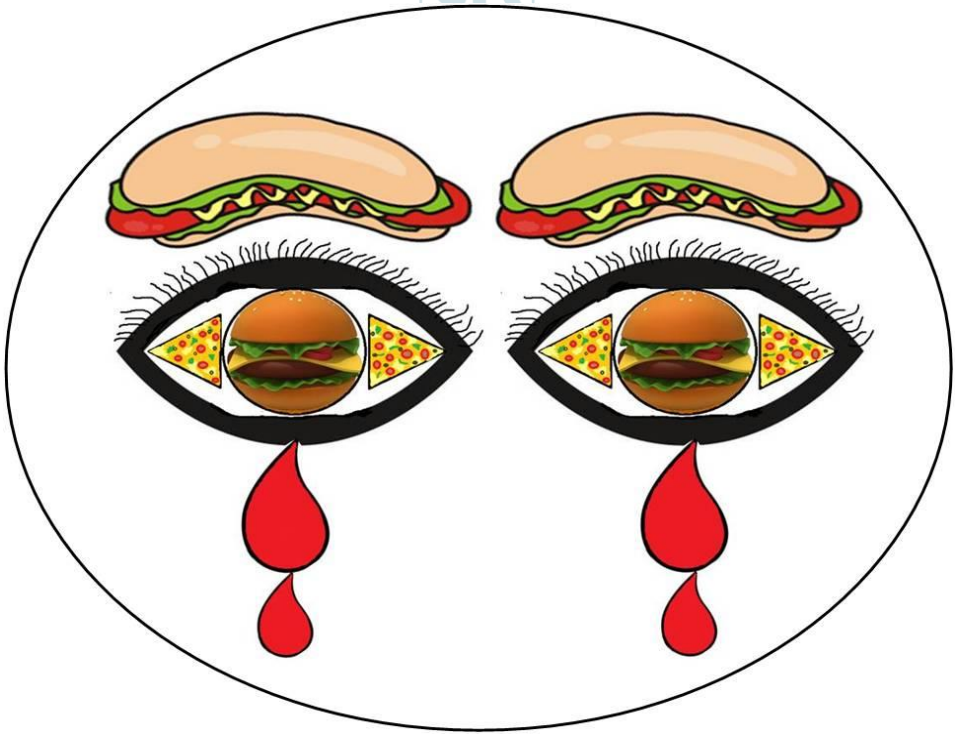


*Figure1: An imaginary visual depiction of junk food taking a toll on optic health. [**Source:** The author has created the image via Paint using Google clip arts.]*

Thus came to light a possible cause which had gone unnoticed for years and now the damage had reached a level which was beyond rescue. Vitamin supplementation improved his dietary deficiencies to some extent and his vision was stabilized but could not be improved or cured any further. This heart rending case of a teenager going virtually blind is not just a medical case being discussed by the fraternity but is a warning to all who underestimate the detrimental effects of junk food.

Nutritional optic neuropathy is usually caused by drugs, malabsorption, smoking/alcoholism combined with poor diet. Dietary reasons alone are not a major cause in developed countries. The mechanism of vitamin B group is well understood and majorly contributed towards the patient's vision impairment while the deficiency of vitamin D probably contributed towards his osteopenia (characterized by low bone density and brittle bones) (1). These were simple nutritional deficiencies which could have easily been rectified, had they not been overlooked for long. This is one of the unusual cases where nutritional deficiency and eating disorder has emerged as the sole cause of nervous system deterioration in the form of vision loss. It may seem a drastic case but has clearly highlighted the possibility of how junk food can severely damage vision and other brain tissues if not controlled timely. Figure 1 is an imaginary visual depiction of junk food taking a toll on optic health.

Human Brain: The trigger or the target of junk food?

The only thing that can distinguish Homo sapiens from any other species in the world is the human brain, one of the smartest organs in the universe. "The mind is a powerful force. It can enslave us or empower us. It can plunge us into the depths of misery or take us to the heights of ecstasy. Learn to use the power wisely. "This famous quote by author David Cuschieri can be aptly applied to the brain in a more biological sense. The brain has an inbuilt reward system which enables us to engage in any pleasurable activity such as gorging on delicious food. Eating junk food helps in the release of a chemical called dopamine, which is a well-known neurotransmitter and hormone associated with happiness and also responsible for fueling addictions. When we consume these so called rewarding foods the central nervous systems (CNS) responds by adapting to the release of happy hormones and in turn makes more dopamine receptors. Therefore, more junk food is required to attain the same level of high or happiness and this triggers what we call junk food cravings. The process is very similar to developing drug addictions. Further the learning and memory forming ability of the CNS rapidly registers the experience of eating salty/sugary and fatty foods as a pleasurable activity and we are tempted visually every time we see our favorite junk dish. This temptation can be resisted by the prefrontal cortex- the major control center in the brain. This area of the brain does not mature until our early 20's, thus underlining the clear cut reason behind the impulsive nature of teenagers. This also explains why children and adolescents are more prone to addictions and these can affect their well-being for a lifetime if not corrected timely. Research findings illustrate how consuming

sugary foods and drinks can alter the brain development. Further junk food is also known to cause hippocampal neuroinflammation which eventually can damage neurons. Hippocampus is the region of the brain which controls the signal for appetite and fullness in coordination with the gut and thus when it is damaged the individual loses his control on appetite and is perpetually hungry (3).

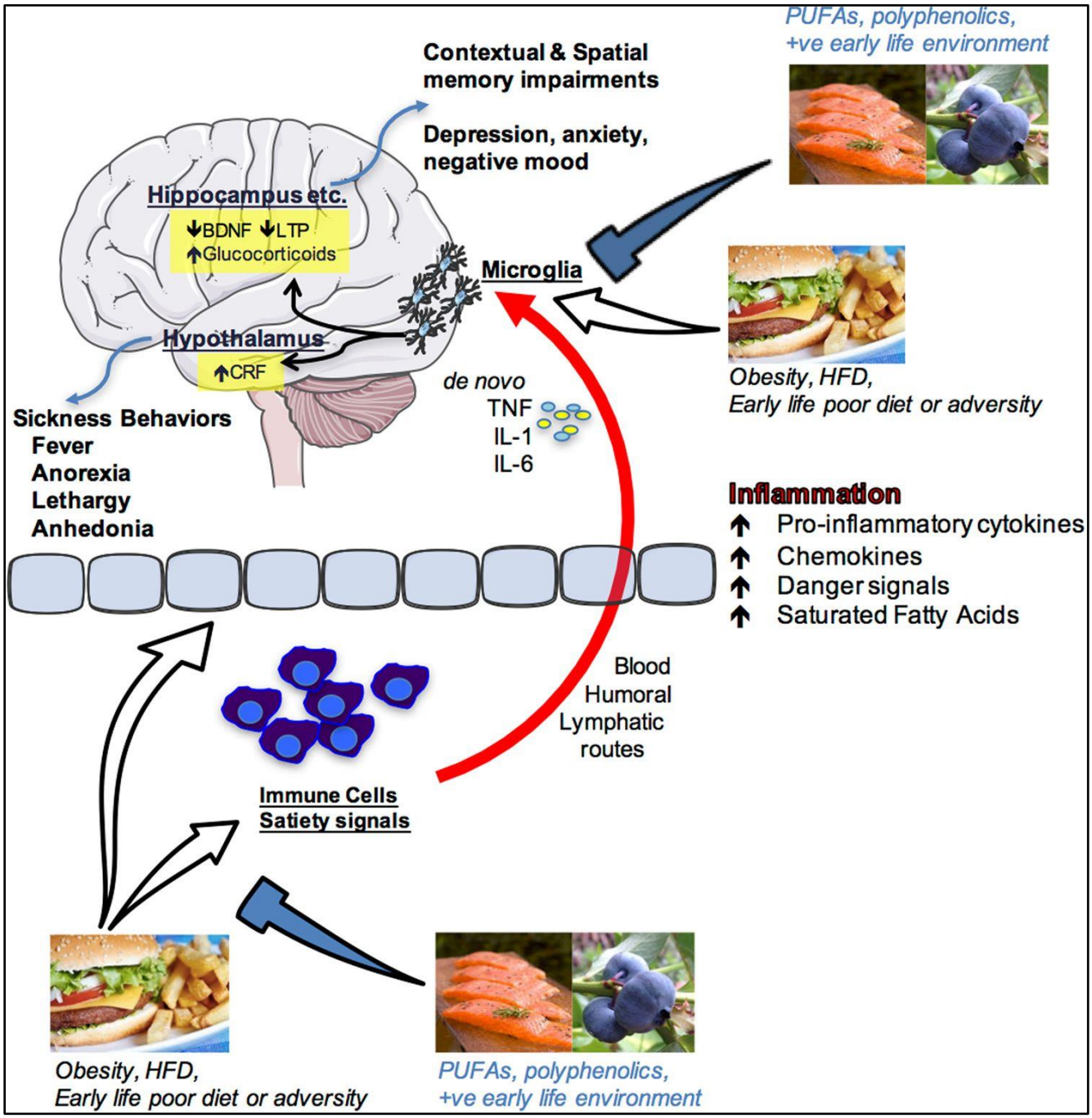


*Figure 2: Schematic depiction of how nutrition influences cognition and emotion. [**Source:** Spencer SJ et al., npj Science of Food, 2017].*

The hippocampus is also the memory centre of the brain and its impairment can further cause CNS disabilities. Junk food is further reported to reduce the neuroplasticity of the brain, which means the neurons are unable to fire and wire properly. This further reduces neurogenesis (the production of new neurons) and leads to gradual neurodegeneration. Thus although the brain sets the trigger for junk food consumption it eventually become the prime target organ of the same. Figure 2 represents the schematic depiction of how nutrition influences cognition and emotion (2).

Conclusion and Food for Thought

This editorial is meant to alert people who mindlessly indulge in eating junk food without realizing the actual irreversible harmful effects it can have on ones vision and brain health. Parents need to be more careful and aware of their children's eating habits and should not ignore any specific unusual patterns of diet because the child may develop a serious mental eating disorder. Growing up children are often fussy and tend to avoid foods of certain colors and shapes. If the pattern goes unchecked it may develop into a psychological dietary disorder which may take ages to be rectified. Proper counseling and timely parental guidance can help in overcoming such problems. Researchers and clinicians can also delve at the grass root level of the mechanism of optic neuropathy in relation with junk food as the sole cause of the disorder. This may give us a complete new perspective on how we can treat and cure such disorders. Thus, if ignorance and indulgence are checked timely and if we can tame our cravings for junk food, we may actually live a lot longer and see the world a lot better, literally!

References

1. Harrison R, Warburton V, Lux A, & Atan D (2019) Blindness caused by a junk food diet. Annals of internal medicine.
2. Spencer SJ, Korosi A, Layé S, Shukitt-Hale B, & Barrientos RM (2017) Food for thought: how nutrition impacts cognition and emotion. npj Science of Food 1(1):7.
3. https://www.rmit.edu.au/news/all-news/2016/sep/five-ways-junk-food-changes-your-brain

Biomedical Devices & Technologies

By Peeyush Prasad, MSc

Contributing Editor

Advances in Medical Devices Offer Exciting Opportunities for Superior Patient Care

The Growth of Global Market for Advanced Medical Devices

When it comes to supporting life, medical devices play huge role. Despite of ambiguous definition, medical device is the system or technology which supports the patient often indirectly. In general, medical device is any instrument or machine that does not achieve its intended goal by pharmacological or metabolic effect but by providing indirect assistance to patient. From MRI machine to robotic arms for surgery all come under the definition of medical device.

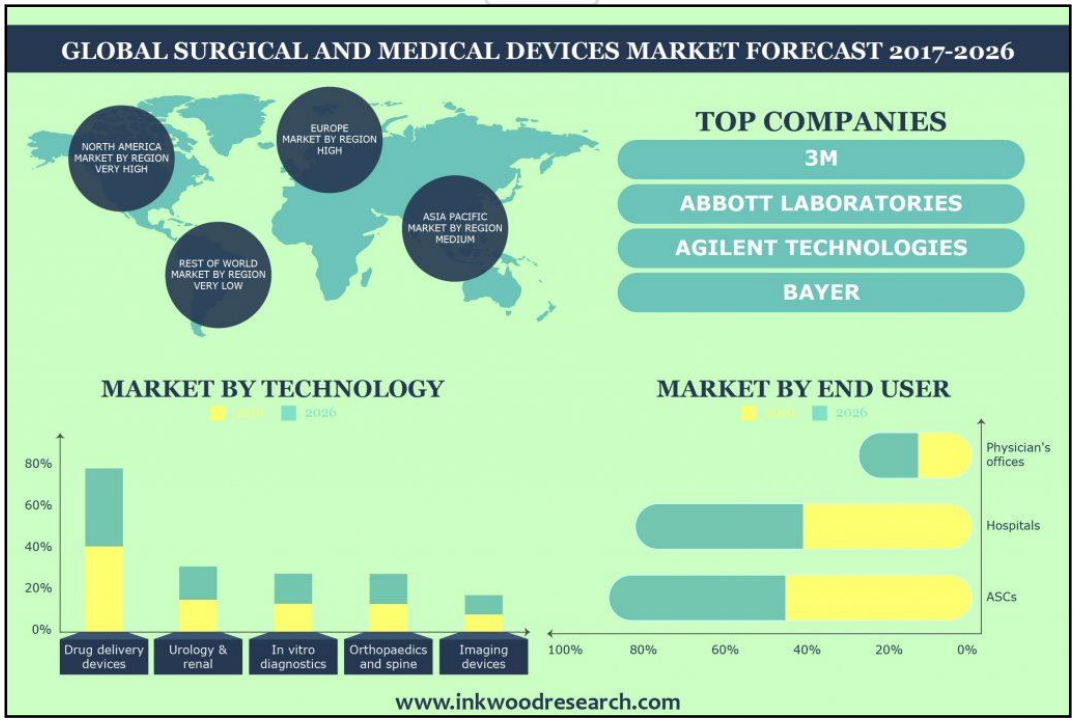


*Figure 1: A forecast of the future surgical and medical devices global market [**Source:** https://www.inkwoodresearch.com/reports/surgical-and-medical-devices-market/].*

Affordability, accessibility, ease of use and want of experts to handle these devices are few of the major limitations in this area. Medical devices are usually used for diagnosis, monitoring, treatment, for supporting anatomical processes and for disinfecting medical devices. Figure 1 shows a forecast of the growth of the medical device market size from 2017 to 2026 (1).

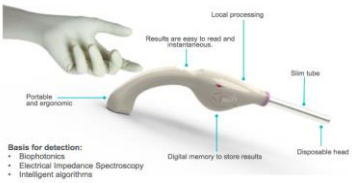
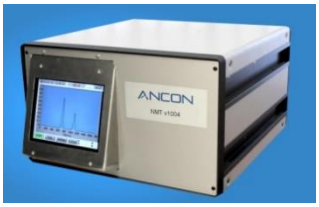
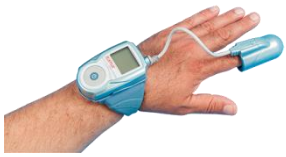
Disease	Device	Product
Cervical Cancer	This is a portable medical device for preventing detecting cancer. Onko Solutions (2).	
Lung Cancer	A product by Ancon for lung cancer early detection (3).	
Diabetes	Abott has developed the Freestyle Lire which a glucose monitoring system for diabetes patients (4).	
ADHD	Monarch eTNS device is made by Neurosigma company. This device is used for treating pediatric attention deficit hyperactivity disorder (ADHD). Device can be used for monotherapy in patients between 7 to 12 years of age (5).	
Sleep apnea	Itamar Medical has made the WatchPAT, a FDA-approved portable diagnostic device for sleep apnea. It uses the finger based physiology and innovative technology for obstructive sleep apnea testing (6).	

Table 1: Innovative medical devices that are used for specific disease.

Some medical devices serve the specific purpose and they are associated with specific disease such as use of dialysis in case of kidney failure. Some of the innovative medical devices for specific disease are mentioned in table 1.

Components in Medical Device Design

Design and development of medical device is a complex process which consists of regulation, specification and application. There are several factors which control the presence of medical device in market. Some of the key factors are usability, regulatory guidelines and affordability. Developer should keep in mind these factors before designing and manufacturing any medical device. Figure 2 outlines few of the important aspects of medical device design (7).

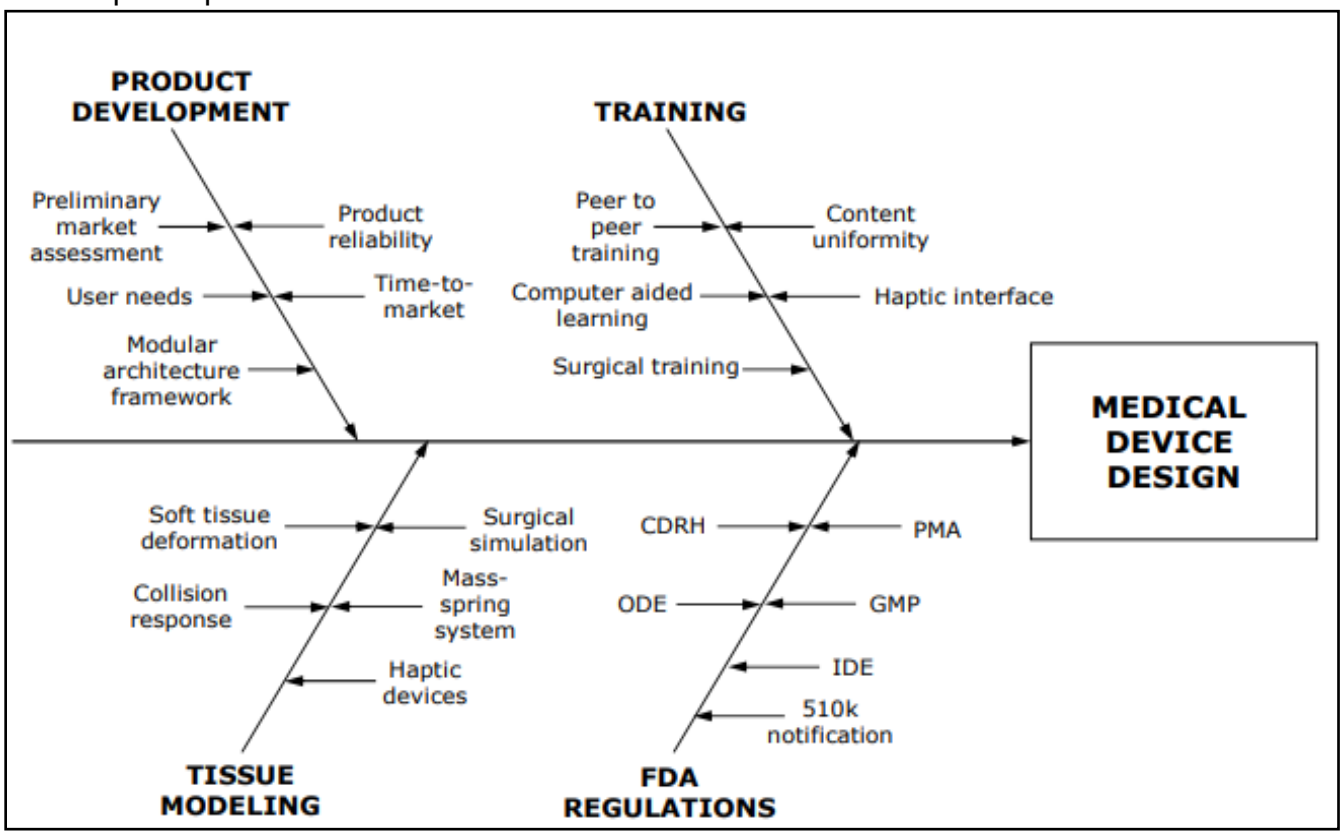


*Figure 2: Key components involved in the medical device design [**Source:** International Journal of Innovation and Scientific Research (2014)].*

Top companies which are working in this field are Stryker, Medtronic, Philips, Cardinal Health, Abott, GE Healthcare and Siemens. Table 2 provides the brief idea about these companies and their product.

Company	Device	Company website
Stryker	Image guided therapy, emergency patient transport, surgical suction & technology (temperature management & other equipment).	https://www.stryker.com/us/en/index.html
Cardinal Health	Products related to anesthesia, cardiovascular, infection control, patient monitoring & durable medical equipment.	https://www.cardinalhealth.com/en.html
GE Healthcare	Advanced visualization, anesthesia, diagnostic ECG & computed tomography.	https://www.gehealthcare.in/
Danaher	Diagnostics and mobile equipment.	https://www.danaher.com/
Abbott	Company works on vascular disease, vision and diabetes. Abbott diagnostics provides integrated automation system, assays and informatics.	https://www.abbott.co.in/
Fresenius Medical Care	Developed the technology for hemodialysis. 6008 CARE system and 5008CorDiax machines are used for dialysis. 4008Sclassix is used for hemodialysis.	https://www.freseniusmedicalcare.com/en/home/
Philips	Advanced molecular imaging, diagnostic ECG, Computed tomography, Fluoroscopy and emergency care.	https://www.philips.co.in/healthcare/solutions
DePuySynthes	Works on orthopedics, surgery and interventional solution. Company manufactures the products associated with bone related problems and fluid management.	https://www.jnjmedicaldevices.com/en-US/companies/depuy-synthes

Table 2: Top medical device companies involved in designing and developing the products.

Research and Developments in the Field of Medical Devices

Research and developments are happening at a continuous pace in the field of medical device. In one study, researchers have developed the novel beating heart transapical septal myectomy procedure. This procedure has been shown to reduce the risk associated with surgical myectomy (8). In another study, feasibility of robot-assisted fluoroscopy-guided (RAG) puncture was evaluated by utilizing the novel robotic system for percutaneous renal access with ultra-sound-guided (USG) puncture. Results found that success rates of RAG puncture was 100% and USG puncture was 70.6%. Needle puncture time was found to be 24% shorter than USG puncture. Study concluded that for renal access, RAG puncture shows comparable results and accuracy with respect to USG puncture (9). Tiny microfluidic based medical devices are developed for various purposes such as diagnostics and therapy. Figure 3 shows microfluidic device for point of care viral analysis (10). Design of microfluidic based medical device is one such area which requires lot of attention. Significant growth has been seen in device design companies in these areas, which are also providing regulatory services.

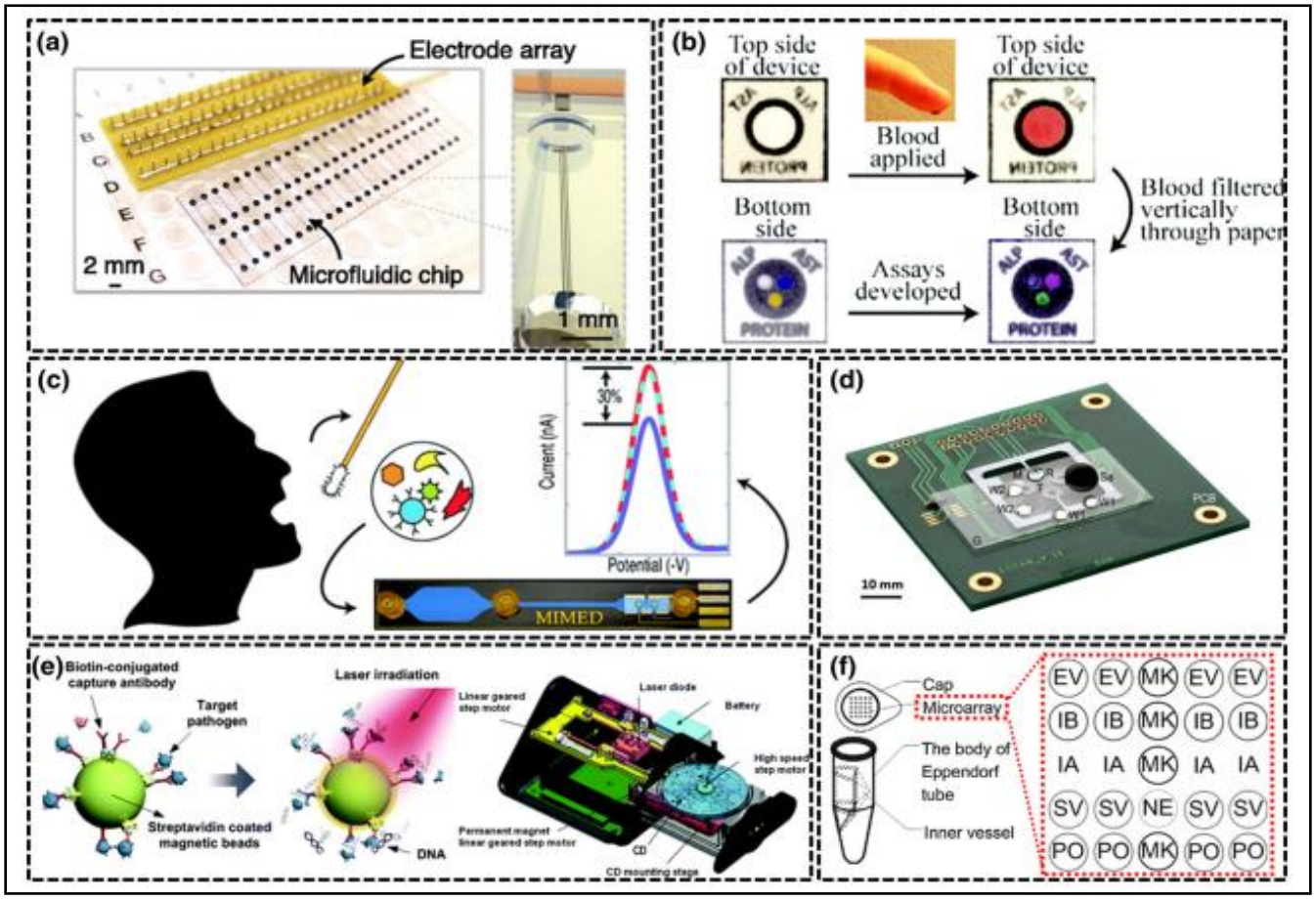


*Figure 3: Microfluidic device based point-of-care viral analysis [**Source:** Yeh YT et al., Ann Biomed Eng. (2014)].*

Future of Medical Device Looks Promising

Future of medical device is promising due to new healthcare challenges. 3D printing and microfluidic device manufacturing is one of the areas which yet be explored extensively. Microfluidic devices are small and complete miniature organ model can be developed on a single chip (Lab on a Chip).

Company	Function
XIMEDICA https://www.ximedica.com/	Leading product development company. Company do research and generate concept and strategy. Also provide services related to process and design validation along with production and commercial support.
JABIL https://www.jabil.com/	Company helps the customer with innovation and technology in the challenging areas such as advanced surgical devices, angiography contrast delivery kits, fluid warming etc.
PLEXUS https://www.plexus.com/en-us/	Plexus works on design and development, new product introduction and manufacturing related to technology such as ultrasound imaging, X-ray imaging and MRI etc.
GLOBAL CENTER FOR MEDICAL INNOVATION https://gcmiatl.com/	Works on early stage product design and biomedical and mechanical engineering along with other services of clinical input and review.
CELESTICA https://www.celestica.com/	Provide services related to supply chain solution (Design and engineering, manufacturing services etc).
ORCHID ORTHOPEDIC SOLUTIONS http://www.orchid-ortho.com/	Works on product design, development and prototyping.

Table 3: Some of the leading medical device design companies involved in developing advanced biomedical technologies and devices for next generation applications.

Although, these devices do not directly assist patients but they can be hugely successful in finding biomarkers and personalized therapeutic target. Table 3 mentions few of the important company involved in design and regulatory services for medical devices. Design groups of the future can be classified into four groups which may vary in size and scope. These groups are: a) Independent design engineers b) Design bureaus c) Design departments of medical device contract manufacturers and d) Design

departments of non-medical device contract manufacturers which focus on digitization and integration with consumers (11).

Concluding Remarks

We have come a long way in developing and making use of medical device. New innovations and research will pave the way for better device which can alleviate the pain of patients and provide a superior healthcare in the future.

References for Further Reading

1. https://www.inkwoodresearch.com/reports/surgical-and-medical-devices-market/
2. https://innovationmatchmx.com/announcement/portable-medical-device-to-detect-and-prevent-cervical-cancer-in-women/
3. https://lungdiseasenews.com/2014/12/08/ancon-medical-seeks-funding-for-revolutionary-lung-cancer-early-detection-screening-device/
4. https://www.medicaldevice-network.com/projects/freestyle-libre/
5. http://www.neurosigma.com/
6. https://hitconsultant.net/2017/09/29/40279/#.XbM61ugzbIU
7. Tamsin M and Bach C. The Design of Medical Devices. International Journal of Innovation and Scientific Research. ISSN 2351-8014 Vol. 1 No. 2 May 2014, pp. 127-134
8. Fang J, Wang R, Liu H, Su Yet. al., Transapical septal myectomy in the beating heart via a minimally invasive approach: a feasibility study in swine. Interact CardiovascThorac Surg. 2019 Oct 23. pii: ivz249.
9. Taguchi K, Hamamoto S, Okada A et. al., Robot-assisted fluoroscopy- vs ultrasound-guided renal access for nephrolithotomy: A phantom model bench-top study. J Endourol. 2019 Oct 23.
10. Yeh YT, Nisic M, Yu Xet. al., Point-of-care microdevices for blood plasma analysis in viral infectious diseases. Ann Biomed Eng. 2014 Nov;42(11):2333-43.
11. https://www.mpo-mag.com/issues/2018-11-01/view_columns/the-future-of-medical-device-design-companies/

Genetics & Agriculture Biotechnology

By Progga Sen, PhD

Contributing Editor

Managing next generation crops by genome editing using the precise snipping tool- CRISPR/Cas9

CRISPR-Cas9 systems

A widely used gene editing tool in Molecular Biology, CRISPR-Cas9 or clustered regularly interspaced short palindromic repeat (CRISPR)- associated protein 9 (Cas9) was first discovered by scientists in the fermentation industry in bacteria. This technique has been used successfully on different organisms, including bacteria, fungi, plants and mammals. Bacteria use this sophisticated defense system against invaders, including phages or even foreign plasmid DNA. Certain fragments of the foreign DNA are stored as memory by inserting into the CRISPR-repeat spacer array within the bacterial chromosome (host DNA) to thwart future attacks. Discovery of this uniquely advanced adaptive immune response led to the exploration of non-homologous repair and homologous repair mechanisms in target cells, which eventually resulted in the advent of the CRISPR-Cas9 gene-editing technique- a highly precise and popular tool in the field of Molecular Biology (1).

The CRISPR-Cas9 system constitutes of the mature CRISPR RNA (crRNA) that contains a spacer sequence (complements the foreign sequence) at its 5' end a repeat sequence at the 3' end. This crRNA forms a stable complex with the Cas9 nucleases, and this complex functions in interrogating and destroying invading DNA targets (1, 2, 3). A short 2-5 bp sequence, located on the invading DNA near the target site (PAM sequence), plays an important role in identification and destruction of this foreign invading target DNA. During the complex formation, an additional trans-activating crRNA (tracrRNA) pairs with the crRNA repeat sequence to generate a dual RNA hybrid, this hybrid RNA structure assists the Cas9 to cleave a DNA molecule that contains the complementary target DNA and the protospacer-adjacent motif (PAM sequence). Cas9 nuclease has two essential functional domains: RuvC-like domain and HNH domain; each domain is responsible for cutting one DNA strand of the double-stranded target DNA. The nicks are then

repaired by either the non-homologous end-joining (NHEJ) methods, often leading to small insertions or deletions, or a highly targeted homologous DNA repair, which offers the opportunity of introducing or removing specific gene sequences in the host genome.

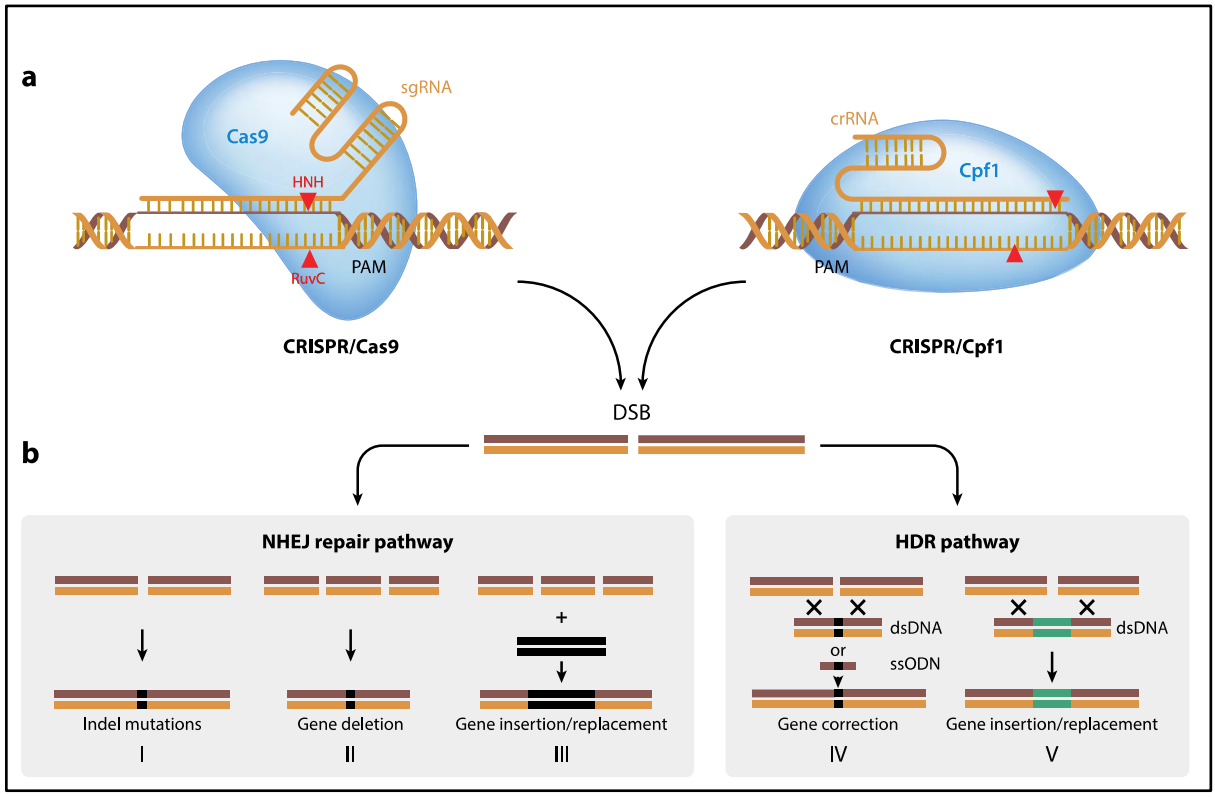


Figure 1. The CRISPR/Cas9 system. a. The Cas9 nuclease binds to the single-guide RNA (sgRNA) and identifying the target DNA sequence, it utilizes its HNH domain and RuvC-like domain to nick either strand of the target DNA. b. The nicks can be repaired either by non-homologous end joining (NHEJ) or homologous DNA repair (HDR). ***[Source:*** *Kunling Chen, et al., Annual Review of Plant Biology, 2019].*

In application, scientists have gravitated towards synthesis of a synthetic guide RNA (sgRNA) molecule of 20 bp, combining the crRNA and the tracrRNA, and the Cas9 protein. There are two major groups of the CRISPR-Cas9 systems: class I (types I, III and IV) form big functional complexes of proteins with the RNA, whereas class II (type II, putative types V and VI) constitutes a single RNA-guided nuclease.

CRISPR-Cas9 genome editing for superior crop management

There are several advantages of the CRISPR-Cas9 gene editing system (second generation gene editing system) over the first-generation gene editing machineries- the TALENs (transcription activator-like effector nucleases) and the ZFNs (zinc-finger nucleases) (4, 5). Both of these technologies are protein-dependent and involve time-consuming and tedious procedures to reach optimum target specificity.

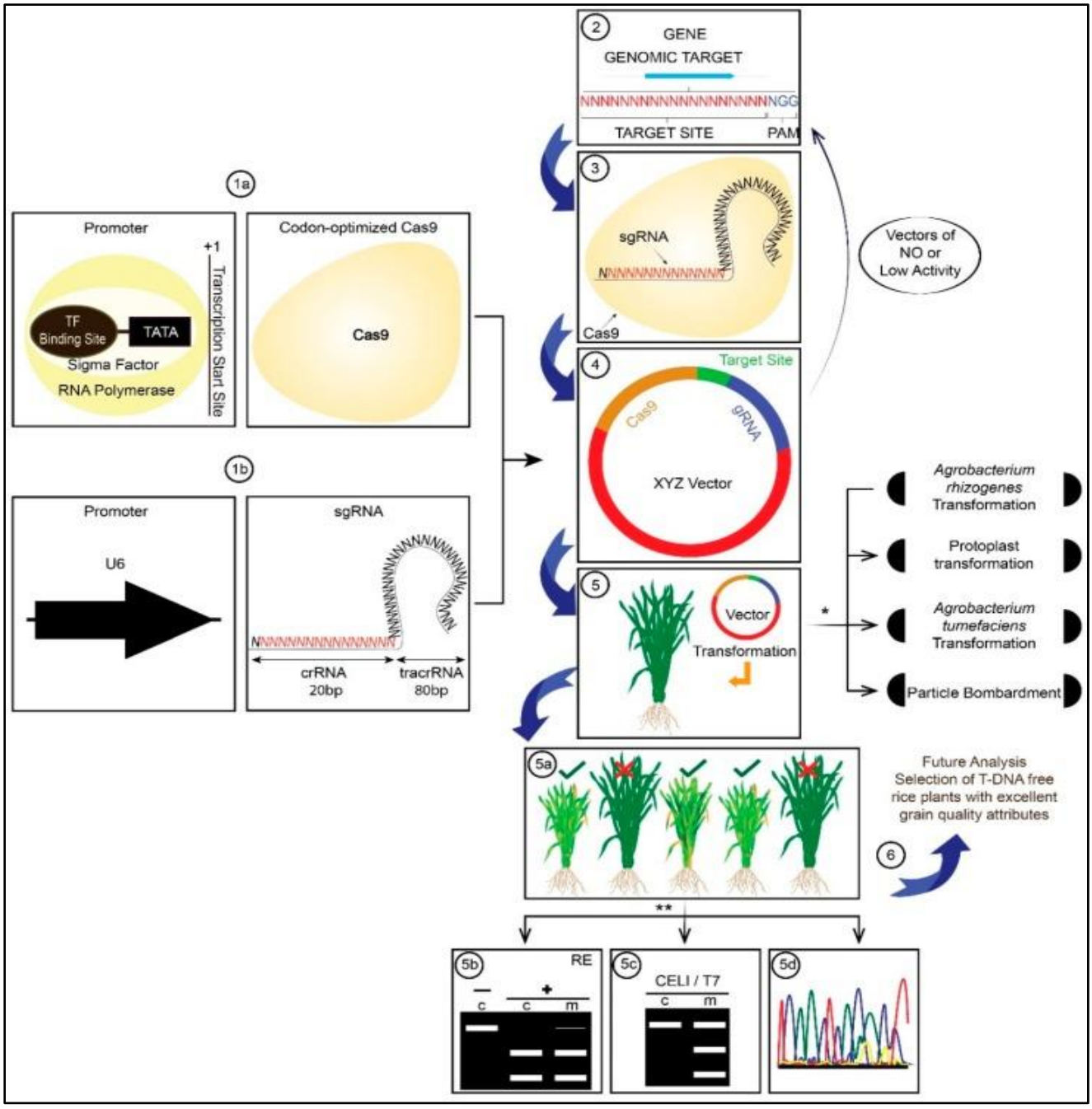


*Figure 2. Flow chart of the steps involved in CRISPR/Cas9-mediated genome editing in plants. The engineered editing complex consists of a single-guide RNA (sgRNA) and the Cas9 endonuclease. The fused crRNA and tracrRNA (trans-activating crRNA) form the stem-loop structure that binds the Cas9 protein. Vector encoding the Cas9 and the sgRNA is transferred to the target plant (rice, maize, wheat, tomato, cotton, etc.) via one of the techniques including protoplast transformation, Agrobacterium-dependent transformation, or particle bombardment. Analyses of the successful gene editing are done on plants selected and screened on the basis of phenotypic changes. [**Source:** Sajid Fiaz, et al., International Journal of Molecular Sciences, 2019].*

CRISPR-Cas9, on the other hand, is a versatile genome editing technique that functions at high efficiency, presents low off-target effects, and offers simplistic synthesis of the sgRNA for the process. Multiplexing by editing multiple loci simultaneously by the Cas9 can be achieved by introducing different targeting sgRNA molecules. Such efforts often lead to major chromosomal alterations in the target genome.

CRISPR-Cas9-dependent genome editing has wide implications in crop management. It presents before us a favorable route for fighting world's hunger. Subtle alterations can result in drastic improvements at several fronts, including resistance to biotic and abiotic stress, stacking favorable mutations, increased yield, high quality (improved nutritional profile), and crops requiring fewer inputs (5).

With a projected population of over 9 billion by 2050, food shortage is inevitable if we fail to increase yield substantially. Genetically modified crops (GM crops) have given the much-needed hope but complex safety concerns (for health and environment) have restricted their application. Instead, subtle alterations produced in the genome by gene editing machinery like CRISPR-Cas9 has revolutionized the crop management extensively. CRISPR-Cas9 is used extensively in both groups of plants- monocots and dicots, including grains, fruits and vegetables.

Monocots

Rice: the major area of focus for this staple crop is stress resistance- abiotic and biotic stress, high yield, and better quality of grains (5, 6, 7). This crop is a favorite among scientists because of the high prevalence of PAM sequence in its genome- 1 in every 10 bp. Scientists have performed knockout experiments to generate successful bacterial blight (OsSWEET13) and blast (OsERF922) disease resistant varieties. Various other findings indicate successful identification and alteration of targets that regulate draught resistance, cold resistance, potassium deficiency tolerance, and glutinous rice grain- to mention a few. Grain quality improvement is key for rice and successful application of CRISPR-Cas9 system has utilized various techniques for transformation, including particle bombardment, protoplast transformation, and Agrobacterium-mediated (A. rhizogenes, A. tumefaciens) gene transfer. Restriction digestion, next-generation sequencing and various confirmation methods are utilized to analyze and proof the successful generation and propagation of the desired traits. In addition to gene knockouts, successful point mutations, deletions, and knockins have played crucial roles in obtaining superior varieties of rice. In addition, CRISPR-Cas9 has been used to modulate transcriptional regulation, thereby imparting favorable characteristics in in rice. Furthermore, forward genetic screens have helped identify important mutations for the purpose of stacking favorable traits. This process also serves well for traits that often require the participation of multiple gene products. Several such quantitative gene loci (or QTLs) are mapped in crop improvement programs and have aided in improving rice grain quality- size and appearance (GS2, GS3,

GS5, Gn1a, GS9, TGW6), nutritional aspects (Wx, SBE1, SBEIIb, ISA1, FLO2, BADH2, FLO5), stress tolerance, and edibility. All these studies have made crucial progress in identifying and modifying contributing characteristics for the survival of the rice crops and improving nutritional output of the grains; however, much needs to be addressed given the wide backgrounds/varieties of rice to provide a common set of applicable gene variations for use.

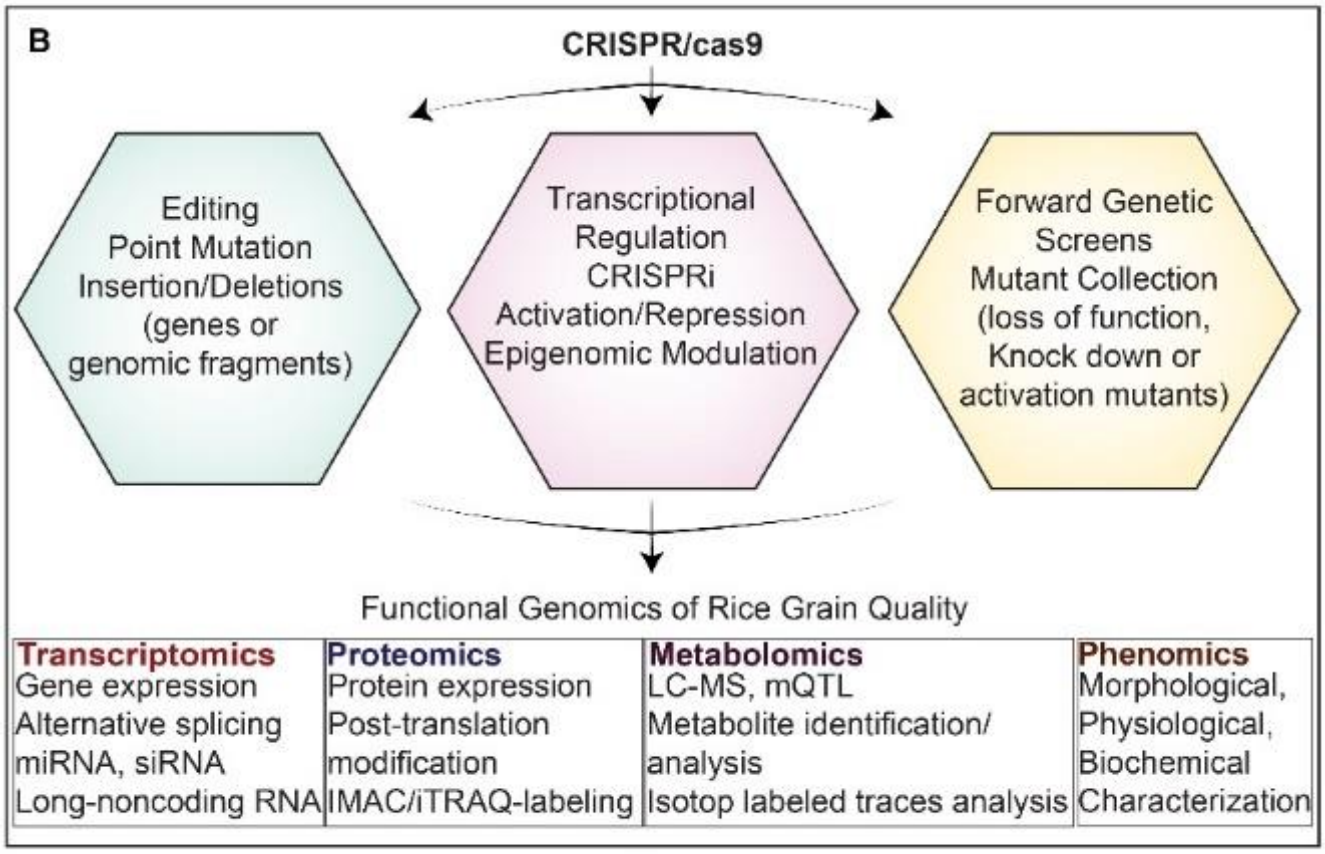


*Figure 3. Application of CRISPR/Cas9 in improving rice grain quality. Introduction of point mutations, deletions, or insertions via CRISPR/Cas9 can cause gene editing via generating point mutations, deletions or insertions, modulate expression by transcriptional or epigenomic regulation (activation/repression), or even create forward genetic screens for studying the molecular basis of rice grain quality. [**Source:** Sajid Fiaz, et al., International Journal of Molecular Sciences, 2019].*

Wheat: the primary focus of utilizing this versatile gene-editing technique in wheat is for developing resistance to powdery mildew disease (6). The TaMLO locus has been targeted for the purpose, the targeted knockout by CRISPR-Cas9 was achieved by particle bombardment in embryo technique. Other abiotic resistance characteristics have been tested in wheat by performing gene editing on TaDREB2 and TaERF3 genes for draught resistance (8). Protoplast transfection was done to achieve the alterations in target genes and about 70% efficiency was observed. Validation is

performed mostly by employing a combination of T7 endonuclease assay, restriction enzyme assay, and Sanger sequencing. Recently, Wang et al. 2018 have attempted to target multiple genes for multiplexing CRISPR-Cas9 gene editing, including TaMLO, TaGW2 (negative regulator in gene quality traits), and TaLpx-1 (disease resistance) (9). Three sgRNAs were used and a polycistronic cassette was generated for the purpose. Agrobacterium-mediated transformation was validated by next generation sequencing. This study provides a promising baseline for multiplexing CRISPR-Cas9 in wheat.

Maize: another staple crop that has gained much attention because of its high content of phytic acid. Phytic acid constitutes about 70% of the grain- considered undigestible by monogastric animals and an environmental pollutant, and scientists have reported that knocking out ZmIPK, ZmIPK1A, and ZmARP4- all involved in phytic acid synthesis- have alleviated the problem substantially (combining TALENs and CRISPR-Cas9 and obtaining comparable results) (5, 6). t-RNA based multiplex gene editing was achieved by Qi et al by generating polycistronic cassette targeting three transfection factors RPL, PPR and lncRNA, whereas simplex editing of MADS, MYBR and AP2 yielded commendable efficiency of about 100%. For stress tolerance, scientists have targeted ARGOS gene- identification of novel allelic variants for increasing draught tolerance have successfully helped generate high-yielding draught-resistant maize. There are plenty of other monocots that have been experimented on for improving yield and quality of grains, and to impart tolerance/resistance to abiotic and biotic stress.

In addition to monocots, a large number of dicots have been tested for genetic trait improvement, using CRISPR-Cas9.

Dicots

Soybean: it is an essential crop, it provides oil and protein for human consumption, and feed for animals. Because of successful application in model dicot Arabidopsis thaliana, several genes have been identified that can be plausible gene editing targets (5, 6, 10). One such gene is the GmFT2a, an integrator in the photoperiod flowering pathway. Though extremely valuable, soybean's sensitivity to seasonal changes to day light limits (photoperiod sensitivity) restricts its geographical expanse of cultivation. CRISPR-Cas9-dependent targeted frameshift mutations (1-bp insertions or short deletions), introduced via Agrobacterium-mediated transformation, in this GmFT2a gene resulted in late flowering, irrespective of long or short day. This widened the prospect of growing soybean at varied climate and geographic regions. Another group targeted the small RNA-directed RNA silencing pathway targets, GmDrb2a and GmDrb2b, both loci were successfully edited by multiple sgRNA constructed in the same vector. Scientists have also generated ALS (required for branched chain amino acid synthesis) gene mutants by site-directed mutagenesis using CRISPR-Cas9 system in soybean to develop herbicide resistant varieties.

Cotton: in cotton, transient and stable transformations (using Agrobacterium) have been successfully tested by scientists, they

have targeted the GhCla1 (chloropastos alterados 1) and GhVP (vacuolar pyrophosphatase) (11). Agrobacterium-mediated transformation of the shoot apex resulted in a successful editing of the two target genes- mostly characterized by deletions with one insertion. Scientists have successfully edited the gene for lateral root formation in cotton (12). Two sgRNAs, specific for the two orthologous cotton arginase gene (GhARG), were generated, stable transformation across generations was achieved via Agrobacterium-dependent transformation. This CRISPR-mediated gene editing greatly improved lateral root formation in cotton under high and low nitric oxide conditions, rendering this essential fiber-yielding crop suitable to grow at variable soil conditions.

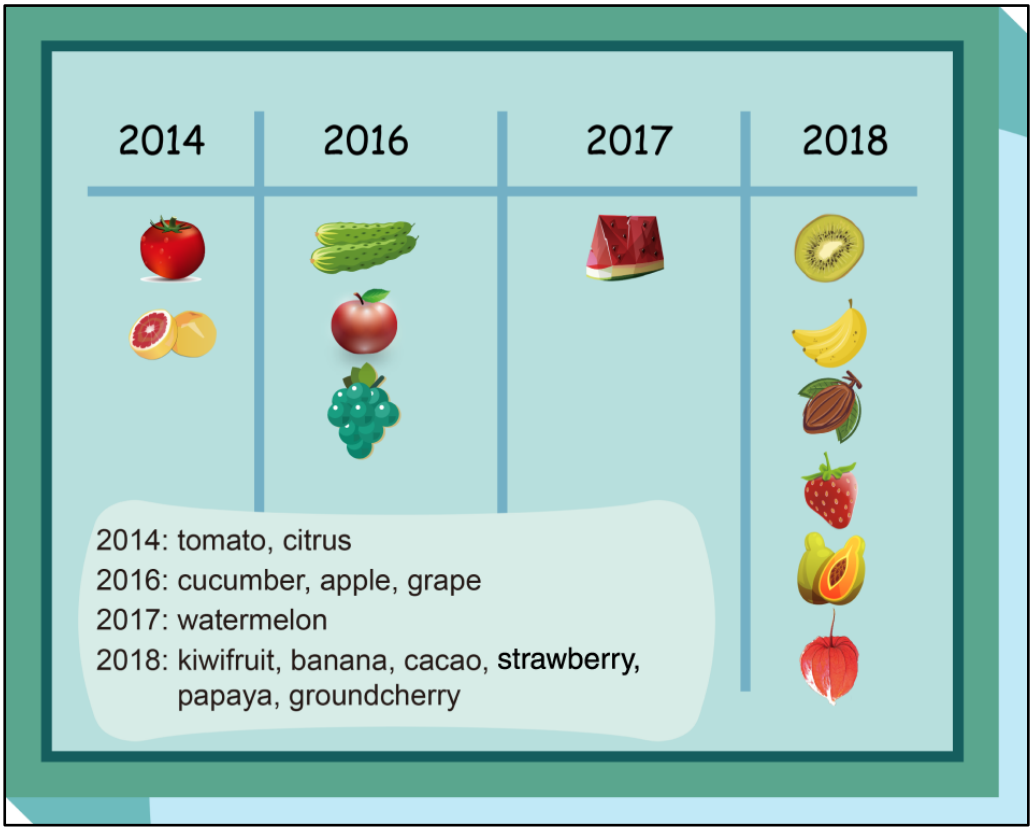


*Figure 4. Timeline for introduction of CRISPR/Cas9 in fruit crops. Initially starting with tomato, this precise genetic editing has a wide application in different fruit crops, as shown above. Most often multiple traits are targeted simultaneously by a single vector constituting of several sgRNAs for various target genes. [**Source:** Tian Wang, et al., Horticulture Research, 2019].*

Tomato: it is a fruit crop that has undergone substantial editing for improving its fruit quality, biotic and abiotic stress resistance, and domestication (13). CRISPR-Cas9 has helped in identifying crucial genes, like DCL2, that render the plant resistant to several different types of viruses, including potato X virus, tomato mosaic virus, and potato mosaic virus. CRISPR-dependent inactivation of DMR6 has resulted in

resistance towards several fungal pathogens too. MLO1 loss-of-function mutation protects tomato plants from mildew disease-causing fungus. Scientists have also addressed the bacterial speck disease of tomato by generating a C-terminal end-lacking mutated JAZ2 repressor. CRISPR-Cas9 has helped identify the gene important for rendering resistance to pre- and post-harvest infection by gray mold disease- knockout of MAPK3 makes tomato plants susceptible to the causal pathogen. In addition to mold disease resistance, MAPK3 also reinforces draught response in tomato crop. Tomato is a cold sensitive plant, and CBF1 was found to protect from cold stress by CRISPR-mediated mutagenesis. The various parameters addressed by scientists to improve tomato fruit quality are- fruit size, color, and texture. Scientists have successfully used CRISPR-Cas9 technology to target CLV3, LC- QTLs (quantitative trait locus) controlling the tomato size to generate large fruits. Several targets including locule number, color and texture have been altered by identification of either individual genes or QTLs. Scientists have been able to use CRISPR-Cas9 to generate variation in fruit colors. Inactivation of RIN or DML by CRISPR also has shown promising results with increasing tomato shelf-life, a major problem with this crop. CRISPR-Cas9 has also aided in producing tomato with high bioactive compounds, including GABA, anthocyanine, malate, and lycopene. Often crops have their wild counterparts that may exhibit favorable characteristics. Abiotic stress may play a significant role in deciding the outcome of the crop. The height of this fruit crop can be controlled by mutating the GAI gene- helps to withstand windy environment. Falling fruits can be a real problem if the crop has to be marketed, and scientists have found a way around- using CRISPR-Cas9, they have identified and tweaked MBP21 gene that regulates the formation of jointed stem/branch. Loss-of-function mutation has enabled the scientists to obtain plants with jointless phenotype. Similarly, scientists are relentlessly characterizing genes in the wild varieties of various fruit crops including tomato- the genes can be useful for increasing their economic value (14). Often, researchers are taking multiple genes to construct the CRISPR-Cas9 sgRNA cassette and using a single cassette to target several loci for enhancing crop productivity.

In addition to tomato, there are several other fruit crops that have been targeted for gene editing, including cucumber, watermelon, banana, grape, apple, strawberry, and kiwifruit. The sole motivation of using CRISPRP-Cas9 is to ensure high yield, less maintenance, and better quality. Given the scope and urgency, CRISPR-Cas9 has a bright future in crop improvement and addressing world hunger. Careful observation of valuable traits in wild relatives of cultivated crops can provide us with the much-needed tools to forward the cause of improved crop development and management. The biggest advantage of this technology is, unlike transgenic plants, it does not pose an ethical issue, and this is key to expedited and rational endeavors towards fulfilling the ultimate goals.

Conclusion

A major aspect of marketing and consuming gene-edited crops is public awareness and

acceptance. Though we have high hopes with this super-efficient editing system, we have to remember that there is still much work to do for fine-tuning the CRISPR-Cas9 technology for widening its application and scope, such as improving its off-target effects. Another point of concern is the chromatin state of the target region; according to certain studies, it may play important role in affecting CRISPR-mediated gene editing. Therefore, in-depth studies in deciphering the detailed mechanism of identification and editing of the target by the CRISPR-Cas9 complex can demystify these problems. Overall, with CRISPR-Cas9 we can hope to obtain answers to several difficult questions plaguing the growing population.

References

1. Jennifer A Doudna and Emmanuelle Charpentier. The new frontier in genome engineering with CRISPR-Cas9, Science, 2014.
2. Haifeng Wang et al. CRISPR/Cas9 in genome editing and beyond. Annual Review of Biochemistry, 2016.
3. Deborah M Thurtle- Schmidt and Te-Wen Lo. Molecular Biology at the cutting edge: a review on CRISPR/Cas9 gene editing for undergraduates. Biochemistry and Molecular Biology Education. 2018.
4. Tariq Shah et al. Genome editing in plants: advancing crop transformation and overview of tools. Plant Physiology and Biochemistry. 2018.
5. Deepa Jaganathan et al. CRISPR for crop improvement: an update review. Frontiers in Plant Science. 2018.
6. Xingliang Ma et al. CRISPR/Cas9 platforms for genome editing in plants: developments and applications. Molecular Plant. 2016.
7. Sajid Faiz et al. Applications of CRISPR/Cas9 system for rice grain quality improvement: perspectives and opportunities. International Journal of Molecular Sciences. 2019.
8. Dongjin Kim et al. CRISPR/Cas9 genome editing in wheat. Functional and Integrative Genomics. 2018.
9. Wei Wang et al. Transgenerational CRISPR-Cas9 activity facilitates multiplex gene editing in allopolyploid wheat. The CRISPR Journal. 2018.
10. Yupeng Cai et al. CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soya bean. Plant Biotechnology Journal. 2018.
11. Xiugui Chen et al. Targeted mutagenesis in cotton (Gossypium hirsutum L.) using the CRISPR/Cas9 system. Scientific Reports. 2017.
12. Yanling Wang et al. Increased lateral root formation by CRISPR/Cas9-mediated editing of arginase genes in cotton. Science China. Life Sciences. 2017.
13. Tian Wang et al. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. Horticulture Research. 2019.
14. Kunling Chen, et al. CRISPR/Cas9 genome editing and precision plant breeding in agriculture. Annual Review of Plant Biology. 2019.

Bio Robotics

By Sathyanarayanan Sridhar, PhD

Contributing Editor

Micro-Robotics Can Be a Game Changer in Analyzing and Manipulating Single Cells for Next Generation Life Sciences

Biomedical Micro-Robotic Systems

A robot is a pre-programmed device, which can elicit a specific response either upon manual stimulation or autonomously. The field of robotics offers a number of applications including remote controlled toys and humanoid robots, to name a few. Robotics based approaches are making tremendous impact in today's medicine and biomedical technologies. In particular, surgical robots are becoming more common enabling high precision and minimally invasive surgeries. All these robotics-based approaches follow technologies at the macro-structure level. Lately, researchers have focused on developing technologies based on micro-robots. These micro-robots have been demonstrated to substitute a regular house cleaner, desktop printer, bridge inspector, micro-scallop that can swim through eyeballs, drug delivery scaffold and can also take the role of bees to help in pollination (1) (Figure 1). While there have been significant advances in micro-robotics, the research area involving use of a micro-robot interacting with cells and tissues at a molecular level is relatively unexplored. Recent studies have shed light on the ability of micro-robots to operate in physiological fluids and manipulate cellular and sub-cellular constituents (2-4). Especially, it addresses the important application of manipulating cellular constituents including single cell manipulation and analysis by employing micro-robots.

The challenges of Single Cell Analysis

It is well known that even a primary population isolated from the same tissue does not possess similar characteristics and this heterogeneity is the hallmark of cell biology. Identical clonal populations have been shown to deviate in their genetic expression in response to external stimuli over generations of cell divisions. Thus, a generalized characteristic of a group can obscure difference between individuals in it. The aggregate and average approach used

to characterize cellular function is unable to probe rich information available from study of single cells (5).

On the other hand, studies made at single cell level are not subject to the averaging effect characteristic of bulk phase population scale methods and they can offer a level of discrete observation that is unavailable with traditional biological methods. Single cell analysis provides an approach to identify the differences between the individuals in it. For example, we can take single cells as an ideal model to (i) detect disease, (ii) test medicines, (iii) develop drugs, (iv) deeper understanding of cellular functionalities and behaviors, (v) to yield new insights into signaling pathway mechanisms and the biochemical basis for cellular function, and (vi) for intercellular signaling (5).

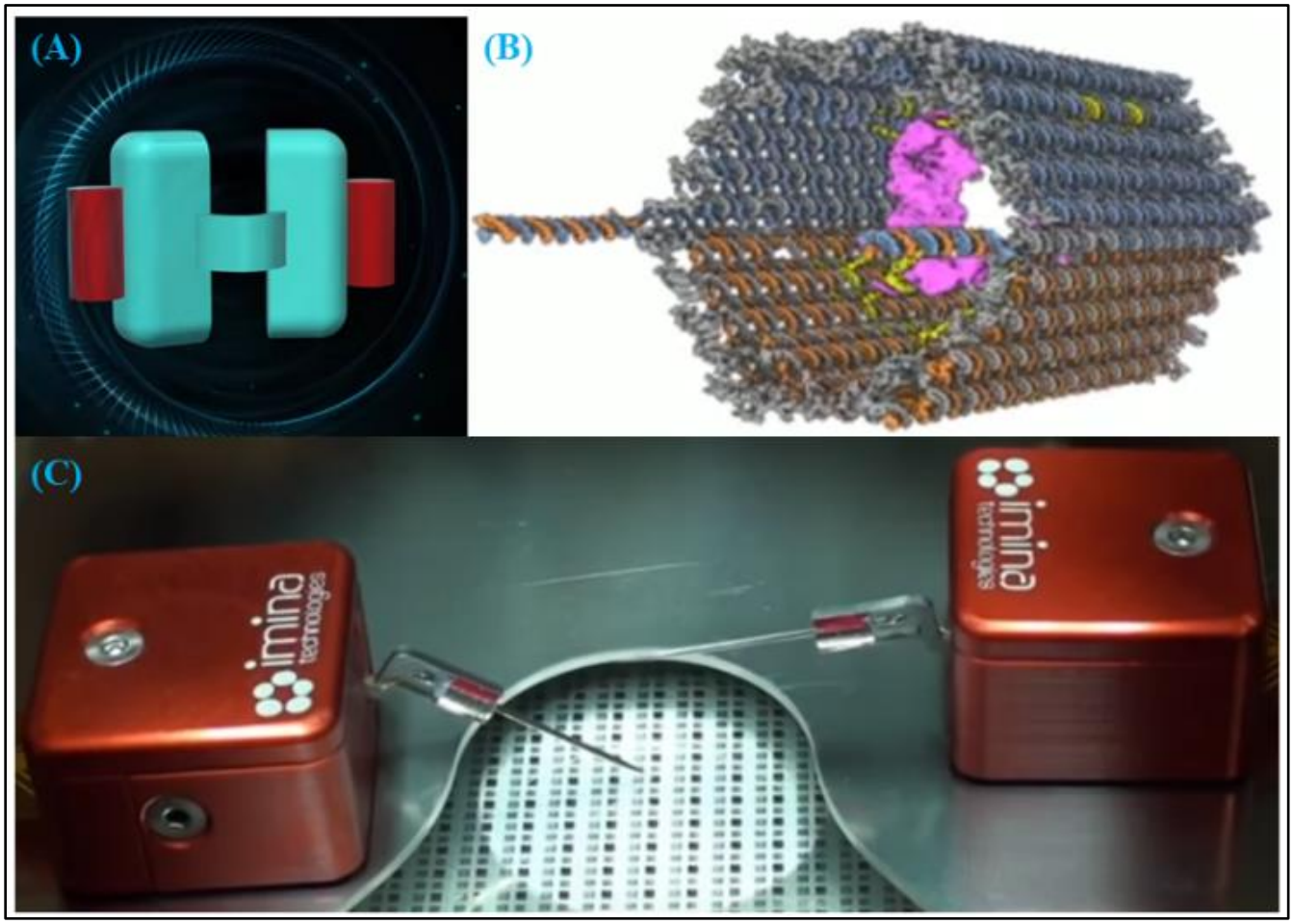


*Figure 1: Biomedical micro-robotic systems. (A) Robotic micro-scallops (Source: Nature Communications, 2014 and IEEE Spectrum 2014); (B) DNA nano-robot (Source: https://wyss.harvard.edu/media-post/dna-nanorobot-cell-targeted-payload-delivering/); (C) Imia Mibot cell manipulation system [**Source:** https://www.youtube.com/watch?v=o3 IhGZY0TCE].*

Therefore, nowadays techniques for single cell analysis have attracted increasing interests in cell biology and life science. Some of the well-known single cell analysis

related applications are (i) cell injection; (ii) cell characterization; (iii) cell positioning; (iv) 3D cell assembly. Cell injection and cell characterization require breaking into a single cell structure while cell positioning and 3D cell assembly simply involve moving cells to a specific, desired position in a given space (5).

To perform single cell analysis, the analysis platform should have both accurate observation and manipulation abilities. For observation, various effective microscopes such as optical microscope, atomic force microscope (AFM) and environmental scanning electron microscope (ESEM) have been developed (5). Individual cell analysis is therefore, challenging and micro-robotic manipulations offer hope in single cell analysis. We will discuss these aspects next in the following section.

Micro-Robotic Manipulators for Individual Cell Analysis

Manually manipulating single cells is tedious, time-consuming and requires special skills. These manually operated tools even when used by skilled technicians, the throughput is limited. Last two decades have witnessed the emergence of micro-robotic manipulation devices that could facilitate single cell analysis. There are two types of micro-robotic manipulators. They are (i) Wired micro-robotic manipulator; (ii) Wireless micro-robotic manipulator that we will describe next.

(a) Wired Micro-Robotic Manipulator

Wired micro-manipulators are macroscale tools with microscopic end attachments that can trap, hold, and inject single cell. Their actuation is mainly based on mechatronic principles such as hydraulic motor, electric motor, electrostatic actuator and piezoactuator. In this manipulation system, the micro-robot either controls the stage in which the cells are placed (or) the ends attachments which interact with the cells (Figure 2). Some of these commonly used micro-manipulator actions and steps are summarized below (5).

Micropipette injection: Micropipette injection has been widely used owing to its low toxicity and high delivering efficiency. It normally refers to the process of using a glass micropipette to insert into or suck out substances from a single living cells. In this process, two key procedures (i) how to move pipette to the cell precisely and (ii) how to penetrate cell membrane safely determine the success of and efficiency. At such a small scale, it becomes very inefficient and ineffective to perform the injection task by hand directly. Therefore, precise methods based on micronanorobotic manipulation systems are widely used. Two main types of injection systems are autonomous embryo injection system by glass micropipette and AFM cantilever based nanoinjection. In the autonomous embryo injection system, the single cell is handled by a pipette straw and the microinjector is assembled on the micromanipulator. The position of the micropipette can be controlled precisely by micromanipulator with a positioning resolution higher than 2 μm and more than 3 degrees of freedom. Nanoinjections based on AFM cantilever nanoprobe are also proposed recently in which a smaller injector with nm size is used to penetrate cell and deliver the material into cell. The usage of

robotic technology including image processing and force feedback could improve traditional injection system greatly (5).

Micropipette Aspiration: It is the traditional technique for cell-cell adhesion study. In this method, 2 aspiration pipettes are assembled on 2 micromanipulators. Pipettes are used to handle and separate 2 single cells driven by micromanipulator. In this process, cell-cell adhesion strength calculated from the suction pressure applied through pipette (5).

AFM: Atomic force microscope (AFM) is another powerful system for single cell adhesion characterization, which enables to measure the cell substrate (or) cell-cell adhesion force. One common method is to put cell on the AFM sample stage which is taken as the micromanipulator. Then cell is moved against the AFM cantilever driven by the stage and the adhesion strength can be measured from the deflection of the AFM cantilever (5).

ESEM: Researchers have used environmental scanning electron microscope (ESEM) for the nanorobotic manipulator stage to control the movement of the nanoneedle with nanoscale resolution inside an ESEM chamber which allows characterization of cell surface (5).

Microgripper: Microtool such as micropipette and microgripper are assembled on the microrobotic manipulation system. The microtool has the capability to handle and release the cell and the microrobot can control the position of microtool precisely. To improve accuracy and reproducibility, force feedback and vision feedback are also employed in the cell positioning process. There is a limitation of microtool that involves difficulties to contact with the cell during manipulation process (5).

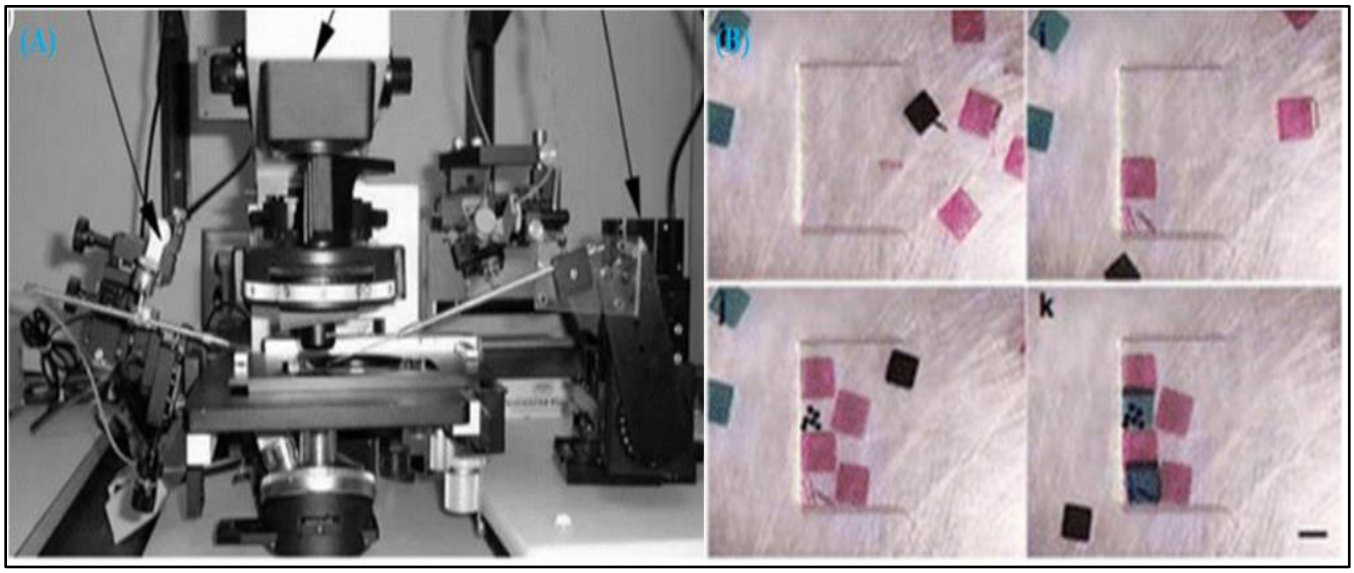


*Figure 2: Wired micro-robotic manipulation system: (A) Autonomous embryo injection system by glass micropipette; (B) 3D Cell assembly by micro-robot [**Source**: Shen Y & Fukuda T, Robotics and Biomimetics (2014)].*

(b) Wireless Micro-Robotic Manipulator

Wireless micro-robotic manipulators can be further categorized into non-contact and contact wireless micro-robotic manipulator. These manipulators work based on the wireless actuation principles. The commonly used wireless actuation techniques include optical tweezers, dielectrophoresis (DEP) and magnetic driving. Wireless actuation is driven by physical fields. Thus, they can manipulate the object in a narrow space with high positioning accuracy. Controllability and repeatability in wireless micro-robotics are big challenges since it is difficult to build an exact physical model to control the statues of the small objects in the field. Force generated by these methods is usually smaller than mechatronic actuation due to the physical principle. Here, we describe different types of wireless micro-robotic manipulators.

Non-contact wireless micro-robotic manipulator

The direct manipulation of the physical fields used in the wireless actuation technique comprises the non-contact micro-robotic manipulator. Optical tweezers (OT), dielectrophoresis (DEP) and opto-electronic tweezers (OET) are the three widely known non-contact manipulation techniques.

OT: It is a technique that uses a highly focused laser beam to hold and manipulate a single cell. Fakuda et al., developed a microrobotic manipulation system based on the controlling of the light path of the optical tweezers. This study demonstrated the ability of optical tweezers to manipulate small objects in 3D flexibility such as microbead and single cell. Drawback of optical tweezers is that the force generated by laser is usually limited to nano-newton which is not large enough to drive the non-suspending cells.

DEP: It is a phenomenon by which a force is exerted on a dielectric particle when it is subjected to a non-uniform electric field. It is widely used for cell trapping and sorting, especially for cell analysis in microchip. Strength of force strongly depends on medium and particles' electrical properties, on the particles' shape and size, as well as on the frequency of electric field. One of the challenges is to fabricate nanoscale electrodes which affects manipulation accuracy.

OET: This modality does not necessitate fabrication of electrodes and overcomes the drawback of DEP. Projected optical images are employed to grab tiny particles. Light, first creates virtual electrodes on the substrate. Then the image in conjunction with an externally applied electrical bias creates the localized DEP trap in illuminated areas.

Contact wireless micro-robotic manipulator

In contact manipulation methods, the wireless actuation physical field initially actuates a microstructure which in turn interacts with the cellular constituents to effect the manipulation. The dimensions of these microstructures are comparable to those of target cells. These micro-robots are microstructures that can physically manipulate objects and the physical method of manipulation is programmable and operates in parallel. The significant challenges include scaling of the robot sizes and automation and geometry of the existing

system. Some of the physical methods of manipulation are described here.

Magnetic micro-robots: These are aligned by magnetic fields and pulled by field gradients. An oscillating out of plane magnetic field which induces a stick/slip mechanism that enhances control of the robot. These microstructures are composed of iron-oxide embedded in a polymer. They are similar in density to the working fluid. Thus, very small magnetic forces are required for movement.

Hydrogel/Gas bubble micro-robots: In this technique cavitation bubbles act as an actuation mechanism to drive hydrogel structures. Laser induced cavitation bubbles are used to drive a hydrogel structure made of Poly (ethylene glycol) diacrylate (PEGDA), a biocompatible polymer. The advantages include: (i) optically controllability; (ii) programmable for automated operation; (iii) insensitiveness to the chemistry (or) electrical conductivity of cell culture.

OET based micro-robots: OET depends on light to control DEP rather than relying on photonic forces. OET light patterns are generated by consumer grade optical projectors which are suitable for parallel manipulation of multiple micro-robots. In this technique, OET primarily manipulates the micro-robot structures which are controlled to perform the cell manipulation (**Figure 3**).

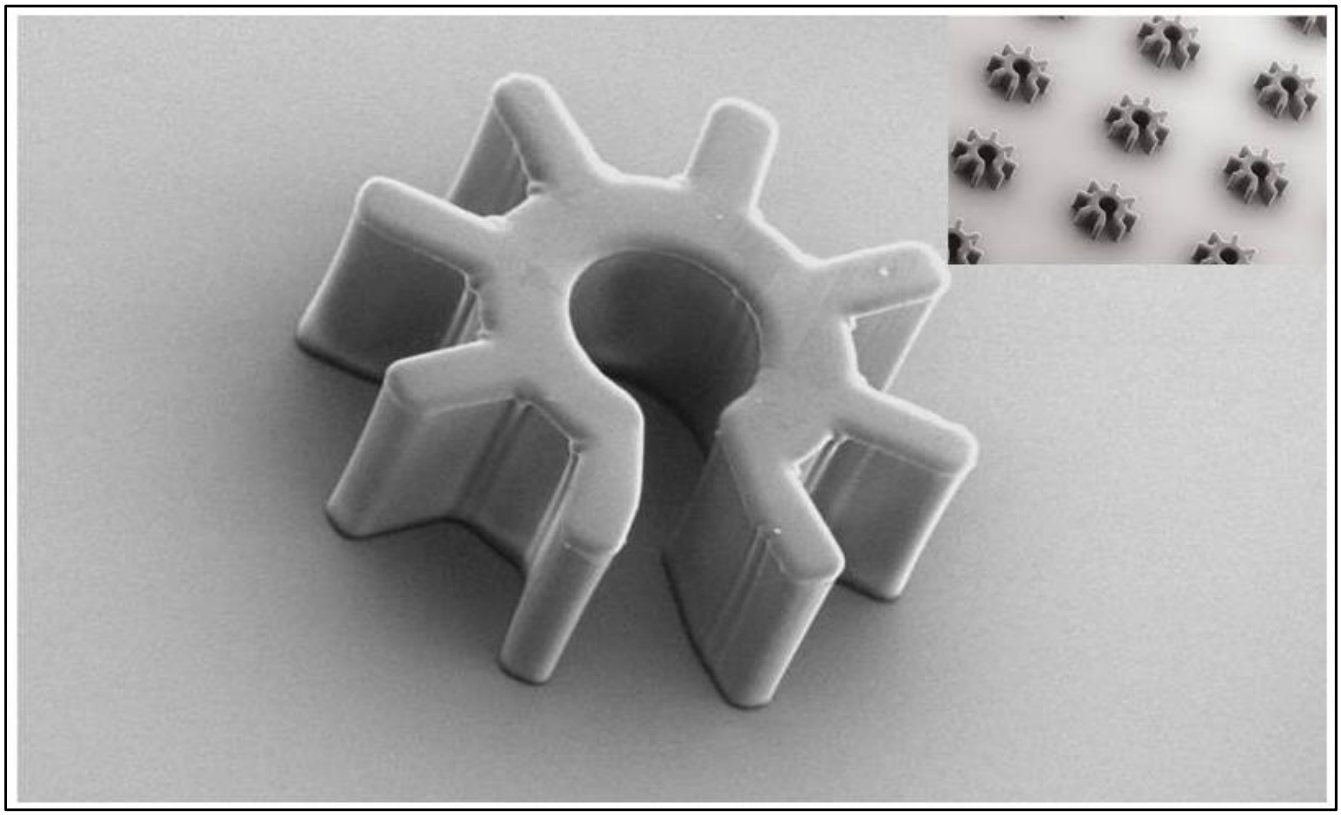


*Figure 3. A fabricated OET based micro-robot structure [**Source**: Zhang S et al., Proc. Natl. Acad. Sci. (2019)].*

Conclusion

Advances in micro-robots are making great strides in developing single cell manipulation techniques. The current research focus is to develop the ability of micro-robotic manipulation of cells that can accurately actuate at the micron and sub-micron scales. Currently, these micro-robotic are in the preliminary research phase. Going forward, the important challenge will be transitioning these technologies into commercially viable products that can benefit the society. However, in view of already demonstrated performance of the micro-robotic systems and their ability to participate in 3D assemble and sorting of cells, we anticipate that avenues for commercialization should happen soon. Micro-robots have the potential to take over 3D printing of organs in the future.

References

1. Tian Qiu, et al., Swimming by reciprocal motion at low Reynolds number, Nature Communications, **5**, Article number: 5119 (2014).
2. Evan Ackerman, Robotic Micro-Scallops Can Swim Through Your Eyeballs, IEEE Spectrum, November 4 (2014), https://spectrum.ieee.org/automaton/robotics/medical-robots/robotic-microscallops-can-swim-through-your-eyeballs.
3. Sakar MS, Steager EB, Cowley A, Kumar V, Pappas GJ. Wireless manipulation of single cells using magnetic microtransporters, IEEE International Conference on Robotics and Automation, (2011); DOI: 10.1109/ICRA.2011.5980100.
4. Hu W, Ishii KS, Fan Q, Ohta AT. Hydrogel microrobots actuated by optically generated vapour bubbles. Lab Chip. Oct 7;12(19):3821-6 (2012).
5. Zhang S, Scott EY, Singh J, Chen Y, Zhang Y, Elsayed M, Chamberlain MD, Shakiba N, Adams K, Yu S, Morshead CM, Zandstra PW, Wheeler AR. The optoelectronic microrobot: A versatile toolbox for micromanipulation, Proc Natl Acad Sci. Jul 23;116 (30):14823-14828 (2019).
6. Shen Y, Fukuda T. State of the art: micro-nanorobotic manipulation in single cell analysis, Robotics and Biomimetics, 1, Article 21 (2014); DOI: https://doi.org/10.1186/s40638-014-0021-4.

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

Genetics and Ageing

The transcriptional protein plays key role in regulating molecular cascade related to aging

Despite significant advances made in the research of ageing and how do we age and how the ageing process can be regulated, the mechanisms that extend lifespan in humans are still elusive. Especially, the question - why only a minority of humans live to centenarians has fascinated researchers over the years. Previous research indicated that signals emanating from the nervous system could be potent modulators of longevity in humans. The latest research has shown that overall neural excitation can potentially be a key determinant of lifespan.

Genetic scientists at Harvard Medical School in the United States have shown for the first time the key role of the brain's neural activity in human aging and life span. The study, published Oct. 16 in the journal Nature (Regulation of lifespan by neural excitation and REST, Nature, 2019 DOI: 10.1038/s41586-019-1647-8), reports findings from human brains, mice and worms that suggests an unexpected link between the nervous system and aging, which shows that excessive activity in the brain could reduce life spans, while suppressing over activity in the brain could potentially extend life. Some of the molecular players in this effect are also characterized in this study that reveals connection to a well-known regulator of lifespan, which is the hormone insulin or insulin-like growth factor 1 (IGF1). The researchers report that the downregulated genes can be targets of the transcriptional regulator protein REST, which is a general regulator of genes involved in neuronal excitation and synaptic function. This research reveals that upregulated transcription factor REST in humans suppresses neural excitation, which can then extend longevity. The researchers demonstrated that reducing or blocking REST or its equivalent in the animal models led to higher neural activity and earlier deaths, and enhancing REST did the opposite. In other words, REST-deficient mice exhibited increased cortical activity and neuronal excitability during ageing. It revealed that an elevated neural excitation resulted due to the loss-of-function mutations in the C. elegans REST orthologue genes spr-3 and spr-4 that subsequently reduced the lifespan of long-lived daf-2 mutants.

Interestingly, it was observed that human centenarians had significantly more REST in the nuclei of their brain cells compared to the people who died in their 70s or 80s. This finding is quite exciting as it opens the doors of possibility that being able to activate REST could potentially reduce excitatory neural activity and thus slow down aging in humans and control the longevity.

Regarding neural activity in humans, it can have variation because such activity might have both genetic and environmental causes, and therapeutic interventions might be advantageous. For example, certain drugs that target REST, or certain behaviors, such as meditation could be administered that could potentially extend life span by modulating neural activity. Additionally, this

also opens up avenues for the design of new therapies for medical conditions that involve neural hyper activity, such as Alzheimer's disease and bipolar disorder that could potentially lead to discoveries of new drugs to combat neurological disorders.

Immunology & Infectious Disease

The surprising revealing role of neutrophil extracellular traps in inflammatory pathogenesis in malaria

Malaria is considered one of the world's deadliest infectious diseases, which is caused by protozoan parasites. These parasites invade red blood cells and trigger systemic neutrophil activation. Plasmodium falciparum is believed to be the most important and virulent species, which causes more than 200 million malaria episodes and close to 500,000 deaths annually worldwide. Previous research has shown that malaria can trigger a range of pathological manifestations that include mild nonspecific symptoms, fever, and mild anemia to organ failure, acidosis, coma, and death. More severe cases of the disease are accompanied by tissue damage, coma, prostration, respiratory distress, metabolic acidosis, renal failure, liver damage, and severe anemia to name few. The mechanism for these medical complications that result from malaria infection has remained poorly understood so far.

In a recent research of major significance, researchers at the Max Planck Institute for Infection Biology in Berlin, Germany discovered a possible mechanism behind these complications that occur as a result of malaria. They published their research in the journal Science Immunology (Neutrophil extracellular traps drive inflammatory pathogenesis in malaria, Science Immunology, 2019; 4 (40): eaaw0336 DOI: 10.1126/sciimmunol.aaw0336). This study reveals that the malaria parasite triggers an immune reaction in the bloodstream that is intended for local defense. However, in the event of systematic act of escalating immune response, it can damage the patient's own tissue as well. Researchers show that the negative effect of immune response is due to the involvement of a highly abundant white blood cell type, which is called the neutrophil. This is a surprising finding, because in the normal circumstances, the main role of neutrophils is to identify and destroy harmful microorganisms that invade our body.

In their previous research by the same group at the Max Planck Institute for Infection Biology, they had discovered a special defense mechanism of neutrophils. Neutrophils were shown to react to pathogen contact by disintegrating and giving up their own cell and nuclear membrane and subsequently forming a network-like DNA structures. These structures are termed as neutrophil extracellular traps or NETs. They showed in their previous research the ability of NETs to trap and kill microbes. However, their latest research has revealed that these NETs can also attack the body's own tissue

in the event of an escalating immune response that can happen when the human body immune system fights against malaria virus. They showed that NETs could cause organ damage in malaria. It is therefore recommended by the researchers that neutrophils are only activated locally and for a limited duration.

In an animal model, researchers demonstrated that a high concentration of NETs in the blood promotes the attachment of infected red blood cells to vessel walls. Additionally, they also accelerate the recruitment of neutrophils. These two factors were considered important causes of organ damage in the infected animals. These findings advance our fundamental understanding of how to effectively treat malaria induced life threatening complications including liver and kidney failure, pulmonary edema and brain swelling that can lead to the death of the patient.

Computational Biotechnology

Computation based identification of bioactive molecules and microbial genes

Over the past years, microbiologists have generated a number of large databases of microbe DNA. The reason is that a number of microbes are capable to produce molecules that can protect their host. This makes them promising candidates for applications in therapeutic drugs. However, it is quite challenging to identify the potential drug candidate molecules and isolate the microbes that produce them due to reason that microbes tend to die quickly if removed individually for study.

Ribosomally synthesized and post-translationally modified peptides (RiPPs) belong to an important class of natural microbial products that contain antibiotics and a variety of other bioactive compounds that make them suitable drug candidate molecules. The existing methods that are employed for discovery of RiPPs include combining genome mining and computational mass spectrometry. However, such methods have shortcomings to discover specific classes of RiPPs from small datasets along with also difficulty in handling unknown post-translational modifications.

Computational biologists in the USA have overcome this challenge by devising a software tool that can be employed to identify bioactive molecules and the microbial genes that produce them. The researchers have shown the effectiveness of their method that enables quick evaluation of possible antibiotics and other therapeutic agents.

The researchers reported their findings including their discovery of seven previously unknown molecules of biological interest from various environments like the human gut, the deep ocean and the International Space Station in the journal Cell Systems (MetaMiner: A Scalable Peptidogenomics Approach for Discovery of Ribosomal Peptide Natural Products with Blind Modifications from Microbial Communities, Cell Systems, 2019; DOI: 10.1016/j.cels.2019.09.004). Researchers

were able to identify 31 known RiPPs and seven previously unknown RiPPs fairly quickly in about two weeks by using MetaMiner that enabled to search millions of molecular product spectra and compare them to the gene clusters in eight datasets.

Food Biotechnology

Growing muscle cells on edible fibers for lab-grown meat

Skeletal muscle and fat tissue make animal meat that usually consists of long, thin fibers as evidenced in the grain of a steak or shredded pork or chicken. Lab-grown meat or bioengineered meat requires reproducing these fibers with real texture and taste. Food scientists and technologists believe that lab-grown or cultured meat could revolutionize food production by having realistic meat products that may eventually be produced without the need to raise and slaughter animals. This would enable establishing a global food industry and chain that is greener, more sustainable, more ethical alternative to large-scale meat production. However, to achieve such goals is quite challenging, as producing bioengineered or cultured meat that mimic the texture and consistency of animal meat in terms of taste and feel is a tough hurdle to overcome.

To develop cultured meat for practical applications, a number of technical challenges need to be overcome. These include the formulation of a scaffold material to support cells and the development of cell lines that are compatible to cultivation for consumption at large commercial scales.

In a recent study published in Nature Science of Food (Muscle tissue engineering in fibrous gelatin: implications for meat analogs, npj Science of Food, 2019 DOI: 10.1038/s41538-019-0054-8), researchers in the USA demonstrated grown rabbit and cow muscles cells on edible gelatin scaffolds that mimicked the texture and consistency of meat. The researchers then followed an innovative approach to seed the fibers with rabbit and cow muscle cells that were anchored to the gelatin. The fibers eventually grew in long, thin structures, similar to real meat.

This research makes important advances in realizing the potential of full lab-grown meat. However, big challenges remain to be overcome that include muscle and fat cell maturation in-vitro. It is anticipated that this research will pave the way for further development of approaches that will be based on a combination of advanced stem cell sources, serum-free culture media formulations, edible scaffolds along with possibility of combining bioreactor culture methods.

Neuroimaging and Bioinformatics

Augmented reality app leads to new visual insight into human biology

Augmented reality technology can potentially replace the current methodologies adopted by the instructors and students for scientific and academic presentations interact with presentations based on virtual 3D models of scientific objects, for example, biological macromolecules. Such a technology could be leveraged to neuroimage the brain 3D structure. The 3D model can provide a detailed insight into where these arteries are situated within the context of important structures in the brain that would be more attractive to students and presenters alike.

To this end, scientists in the USA have launched a smartphone application that uses augmented reality to add 3D models, fly-throughs and other data to enrich science communication materials such as posters, publications and presentation materials.

A brief news article on the exciting development of this app is recently published on the web (https://news.usc.edu/161786/augmented-reality-app-usc-scientific-data-3d-models/). The app is called Schol-AR, and users of this augmented reality app can simply point their smartphone cameras focusing on a supported image. The app then pulls up hidden interactive content for the viewers. Viewing brain image through the Schol-AR app can give an enhanced and rich experience as the app enables to view the size, shape and positioning of blood vessels as they relate to the brain structures they supply. Further, these vessels can manually be enlarged, rotated and explored in various angles that generate a much more realistic picture of the underlying biology emerges.

The scientists who developed this app envision that this will transform our thinking and presenting visualizing scientific data that will change the way how findings are communicated well beyond the field of neuroscience.

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

Biotech and Pharma Industry Roundup

GlaxoSmithKline's PARP inhibitor Zejula gets US FDA approval

The U.S. Food and Drug Administration (FDA) recently approved GlaxoSmithKline's PARP inhibitor Zejula. This drug has been approved for wider use in some cancers. This latest approval from the FDA enables Zejula widening the use of PARP inhibitors beyond the narrow scope that was initially thought by the company. It expanded the scopes of Zejula's (niraparib) approved treatments by including previously treated patients with advanced ovarian, fallopian tube, or primary peritoneal cancer patients. This new incorporation will be used in those patients who have already been treated with multiple prior chemotherapy regimens. This also includes patients whose cancer is associated with homologous recombination deficiency (HRD) positive status that is defined by either a deleterious or suspected deleterious *BRCA* mutation, or genomic instability. Also, to be included in the new treatment regime, it must have progressed more than six months after response to the last platinum-based chemotherapy [Source: https://www.biospace.com/].

AbbVie to strategically collaborate with the Cystic Fibrosis Foundation

The US based AbbVie recently announced a planned strategic collaboration with the Cystic Fibrosis Foundation. Under this strategic alliance, AbbVie will develop a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator compound that the Foundation licensed to AbbVie. Cystic fibrosis (CF) is a complex progressive genetic disease and that eventually leads to persistent lung infections. The mutations in the CFTR gene causes CF, which in turn, causes the CFTR protein to behave abnormally. In the event of CFTR not functioning correctly, chloride, whose function is to attract water, fails to move to the cell surface. This results in thick and sticky mucus in various organs, particularly in the lungs. To overcome this issue, it is believed that modulator therapies can present the most transformative treatment advance in CF. This strategic alliance opens up the potential to identify new therapeutic options for people with CF [Source: https://www.biospace.com/].

Allergan's Botox approved to treat lower limb spasticity in pediatric patients

Ireland-based Allergan's Botox has been approved by the U.S. FDA to treat patients that are aged between 2 to 17 with lower limb spasticity. Spasticity corresponds to a debilitating neurological condition that involves muscle stiffness. This stiffness leads to tight muscles in the upper and lower limbs that can interfere with movement. The company hopes that the Botox can bring some relief to the patients [Source: https://www.biospace.com/].

In a joint partnership, NIH and Gates Foundation commit $200M to cure HIV and sickle cell diseases

In a major joint scientific partnership between corporation and federal funding agency to solve challenging medical problems, the National Institutes of Health (NIH) and the Bill & Melinda Gates Foundation have come together under a same roof. Each party has committed $100 million for research and developments to advance cures for HIV and sickle cell disease. The goal of this joint venture is to enable testing *in-vivo* gene-based treatments for the diseases in humans within next 10 years. The ultimately aim of this effort is to make the treatment available at a globally affordable cost to the affected population.

The plan that is jointly agreed by the NIH and Gates Foundation includes the research program on sickle cell disease that is added to the agenda. Sickle cell is different from HIV in many aspects due to the reason that unlike HIV, sickle cell disease is a genetic one and not an infectious. However, the inclusion of sickle cell to the agenda shows that the NIH sees important overlaps between the two diseases. The same gene-based strategies in both diseases will be pursued by the NIH and Gates Foundation. The shared strategies were listed by NIH including vector tropism and efficiency, gene targeting and *in-vivo* delivery [Source: https://www.fiercebiotech.com/].

Invizius raises $3.57 million for protein coating

Spun out from the University of Edinburgh in 2017, the Scottish startup, Invizius has raised £2.75 million, or about $3.57 million. The funds will be used to develop the system that can battle the potential harms and risks of cardiovascular disease linked to dialysis procedures. To this end, Invizius is developing a protein-laden solution which is sticky and that coats the inside of a dialysis machine before every time it is used [source: https://www.fiercebiotech.com/].

GTX Medical and NeuroRecovery to merge to jointly develop spinal cord implant

The Netherlands and Switzerland-based GTX Medical—formerly known as G-Therapeutics and NeuroRecovery Technologies, out of San Juan Capistrano, California, USA are two companies focused on restoring movement and the ability to walk in patients with paralysis and spinal cord injuries. They have now announced plans to merge and combine their neuromodulation expertise and conduct joint research and developments [source: https://www.fiercebiotech.com/].

Veracyte to launch nasal-swab lung cancer test

The noninvasive nasal-swab test developed by Veracyte is poised to enter the market in 2021 for lung cancer test. It is believed that this particular breakthrough in diagnosis could speed up treatment for high-risk patients. This could also help low-risk patients avoid invasive diagnostic procedures such as tissue biopsy [source: https://www.fiercebiotech.com/].

Novartis' Zolgensma sales show early promise

The Swiss pharma Novartis' gene therapy for spinal muscular atrophy has shown huge

early promise of success. The sales of Zolgensma exceeded early forecasts, with about 100 infants treated in the drug's first full quarter of commercial availability in the United States that earned the company $160 million [Source: https://www.biopharmadive.com/).

Clinuvel's drug to treat skin disease gets FDA approval

Australian drugmaker company, Clinuvel recently got FDA approval for a drug that is designed to treat a rare skin disease. This disease causes painful sensitivity to light. The drug Scenesse is approved to sell in the United States to treat patients suffering from erythropoietic protoporphyria, which is an inherited disorder that results in intense skin pain and skin thickening [Source: https://www.biopharmadive.com/)].

Zealand Pharma acquires Encycle Therapeutics Inc, including a peptide therapeutics platform and library

The Danish biotech company Zealand Pharma A/S will acquire all outstanding shares in Encycle Therapeutics Inc., and all its intellectual property. This includes methods for generation of nacellins, drug-like cyclic peptides that target protein-protein interactions. Also, the acquisition involves merging Encycle Therapeutics' preclinical candidate ET3764 with Zealand Pharma. ET3764 is used to treat pathogenesis of inflammatory bowel disease (IBD). It is an oral, membrane-permeable peptide macrocycle that targets integrin alpha-4-beta-7. Experts believe that it might be superior to Takeda's market-approved injected alpha4-beta-7 inhibitor vedolizumab because it is not immunogenic, and orally bioavailable [Source: https://european-biotechnology.com/].

PFF acquires stake in Autolus Therapeutics

Prague-based PPF Group has acquired a 19.2% stake in London-headquartered Autolus Therapeutics plc, which is a developer of T cell cancer therapies. With the investment into Autolus Therapeutics, PPF Group N.V. is expected to significantly increase its commitment to the cancer therapy sector [Source: https://european-biotechnology.com/].

Polpharma partners with Sandoz

Poland based Polpharma Biologics SA has partnered up with Novartis subsidiary Bavaria, Germany based Sandoz AG. This partnership targets marketing of natalizumab biosimilar. The global commercialization agreement between these two biotech companies involves Polpharma Biologics' critical role that will focus on the development, manufacturing and supply of the biosimilar, which is currently in Phase III clinical development for the treatment of relapsing-remitting multiple sclerosis. This important commercial agreement is expected to reach a significant milestone in Polpharma Biologic's ongoing commitment to producing more affordable, high-quality biopharmaceuticals for patients worldwide [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