ISSN 2689-0852

BIOTECHNOLOGY KIOSK

NOVEMBER 2019

Volume 1, Issue 6

www.biotechkiosk.com

Executive Publishers

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ISSN 2689-0852
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From the Publisher's Desk

Welcome to Biotechnology Kiosk!

We are presenting the 6th issue of Biotechnology Kiosk (BK) to 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 editorials and editors pics on immunotherapy, bacteriology, stem cells, biosynthesis and renewable energy among many other topics. We do hope that you will enjoy reading the articles. These editorials and editors' picks bring news and scholarly perspectives and opinions 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 take this opportunity to wish our readers a very Happy Thanksgiving! We do hope that you will enjoy reading this issue of Biotechnology Kiosk.

Dr. Megha Agrawal and Dr. Shyamasri Biswas

Executive Publishers and Editors

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COLUMNS

Technology & Health Science

By Shripriya Singh, PhD

Contributing Editor

Innovation in electrochemical sensor technology: easing patient lives with hand-held portable diagnostics

In a world where time is money we believe health is a priceless investment. If properly taken care of it can be truly rewarding and help an individual live longer and better. However, disease is inevitable and every human being suffers from one or the other medical condition at some point in life. Hectic work schedules and busy lifestyles leave us with very little time to take care of ourselves and we often ignore our health issues which should ideally be a priority. 'A stitch in time saves nine', the famous idiom aptly applies in context of medical diagnosis. The timely diagnosis of any adverse medical condition or disease is the very first step towards treatment and cure. Several human deaths are caused not because the treatments are unavailable but simply because diseased conditions go undetected and undiagnosed for prolonged periods until a stage is reached where the damage becomes irreversible.

Several physiological conditions in the body such as blood pressure, temperature, pulse rate, heartbeat, glucose levels, etc are good indicators of the health status and their slight deviation from normal can be indicative of a problem. The monitoring of these simple parameters at home is usual practice and helps in detection of minor health issues which may get aggravated if left unattended over time. There are certain abnormal medical conditions and disorders that have no permanent cure but can be managed effectively. Frequent visits to clinics and path-labs are not only practically difficult but cost exhaustive too. In this regard, hand-held electrochemical sensors have played a major role in self-diagnosis and health monitoring. Diabetes is one such major example, where patients are required to monitor their blood glucose levels regularly. Electric glucometers are routinely used by millions of diabetic people across the globe to check their blood sugar levels. Although sensors are popular at-home testing devices in case of diabetes, their use has been limited in case of other conditions.

Diagnostic electrochemical sensors: the limitations in the existing technology

Electrochemical sensors based on enzymatic detection are popular commercial devices which have revolutionized the at-home diagnosis of diabetes. However very few enzymes can serve as clinical biomarkers of disease and thus this enzyme based sensor technology has remained restricted to glucometers. An alternative affinity based detection strategy has been employed which relies on the specific interaction of molecular targets with antibodies or aptamers and shows great potential. No matter how effective the detection strategy may be the success of a technology is housed in its ease of manufacture, sustainable use, durable functioning and its cost effectiveness. Electrochemical sensors fall prey to biofouling which limits the sensing functionality and hinders the commercialization of the technology. Biological fouling or 'biofouling' refers to the growth of plants, algae, small animals or microorganisms on wet surfaces of any mechanical device thus rendering them dysfunctional. Due to direct contact with biological fluids such as blood, sensors are prone to biofouling which not only spoils the detector but also spoils the equipment.

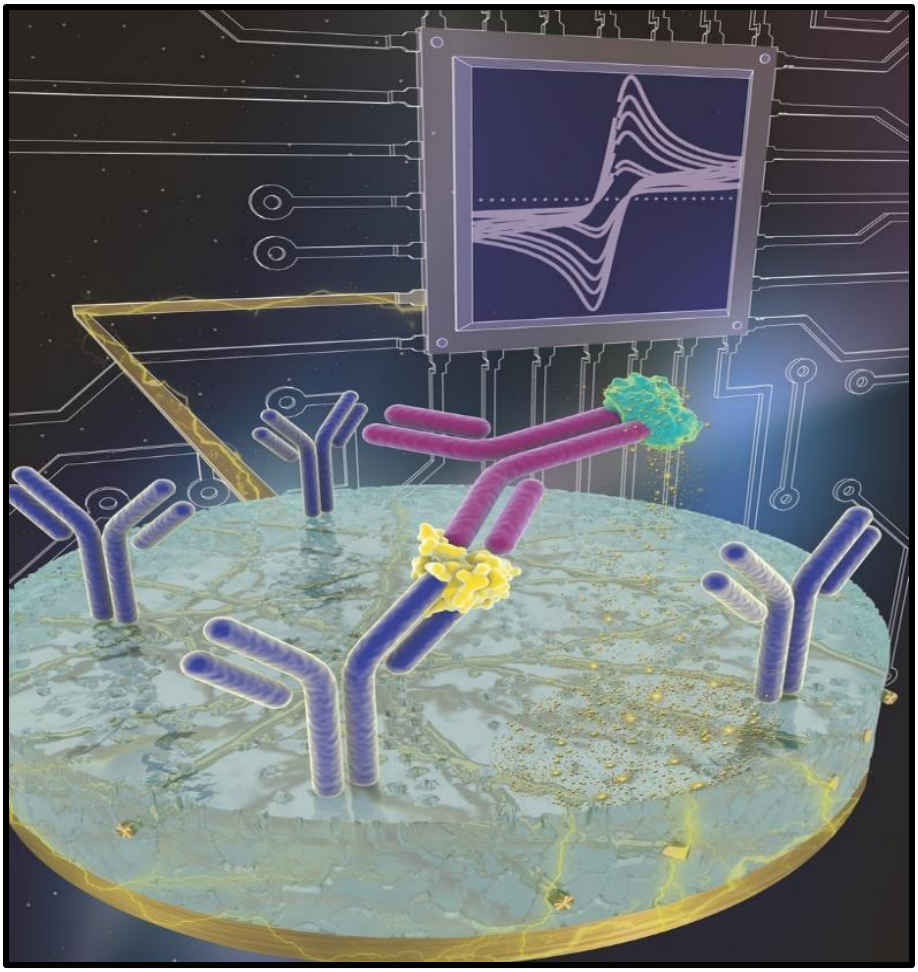

*Figure 1: The diagrammatic representation of the 'eRapid' coating. When probe (purple) and target (yellow) bind together they attract a secondary probe (magenta) that is responsible for the signal generation [**Source:** Wyss Institute at Harvard University].*

Several antifouling coatings and materials have been manufactured from time to time to overcome this problem but have failed in case of medical devices which come in direct contact with biological fluids repeatedly. Although moisture is sufficient to allow the growth of microorganisms, blood, serum, saliva, etc serve as the perfect nutrient medium to culture microbes. The currently available antifouling coatings are not very effective, difficult to mass-manufacture and have consistency and quality issues.

Researchers at the Wyss Institute for Biologically Inspired Engineering at Harvard University have developed a novel diagnostic platform technology known as “eRapid” which enables the creation of handheld electrochemical devices and sensors. These sensors are highly sensitive, can detect multiple disease biomarkers simultaneously, are effective in a variety of biological fluids and require as little as a drop of blood for efficient detection. The technology has been described in the latest issue of Nature Nanotechnology (1).

The knowhow of eRapid technology:

In electrochemical biosensing, the device along with the electrode configuration and the sample conform to a closed ionic and electrical circuit. Thus, off-target substances present in the complex biological fluids bind with the sensor’s metal electrodes non-specifically and decrease the conductivity and sensitivity of the device. Conventional antifouling coatings made of poly (ethylene glycol) self-assembled monolayers (PEG-SAMs) and bovine serum albumin (BSA) also restrict the transfer of electrons and thus hinder the flow of current effectively (2, 3). To overcome the lacunae in the existing technology the research team developed a 3D nanocomposite matrix comprising bovine serum albumin (BSA) cross linked with glutaraldehyde and interlaced with a network of conducting nanomaterials such as gold nanoparticles or carbon nanotubes and gold nanowires. This simple 3D matrix allows electron transfer effectively to the underlying electrode thus improving the efficiency of the device considerably. The BSA matrix owing to its small pore size excludes proteins present in plasma and blood and the weak negative charge of BSA thwarts the strong adhesion of positively charged molecules onto the sensor.

The technology showed potential in terms of reducing the non-specific binding of the off-target substances and superior analytical quality. The newly designed coating has also been proven functionally compatible with the existing bioreceptors and antibodies. The nanomaterial-coated sensors were tested in human plasma and blood serum and it was observed that the signal detection ability was retained up to more than 90% even after storage in biofluids for more than a month. Whereas even the best anti-fouling coatings published earlier failed to protect the sensors for more than an hour after being dipped in biofluids and the devices became completely inactive within a day. This highlights the effectiveness and superiority of the ‘eRapid’ technology (1). The device further passed the functionality test and responded precisely when antibodies were attached to the surface of the coated electrode in a “sandwich assay” and the binding event was converted into a chemical signal. The sensor generated an accurate

electric signal corresponding to the chemical signal that had precipitated onto the electrode surface. The concentration of the target was accurately measured and the device showed superior results when compared with the existing ones so far. The researchers have successfully tested the device against insulin, interleukin 6 (IL6) and glucagon in undiluted human plasma and excellent picogram-per-mL sensitivity was reported. These sensors are reusable, durable and can simply be washed and used a multiple times to detect several biomarkers serially. The ease of manufacture and the low cost has made 'eRapid' a much in demand technology with immense potential. Figure 2 describes the nanocomposite electrode (4).

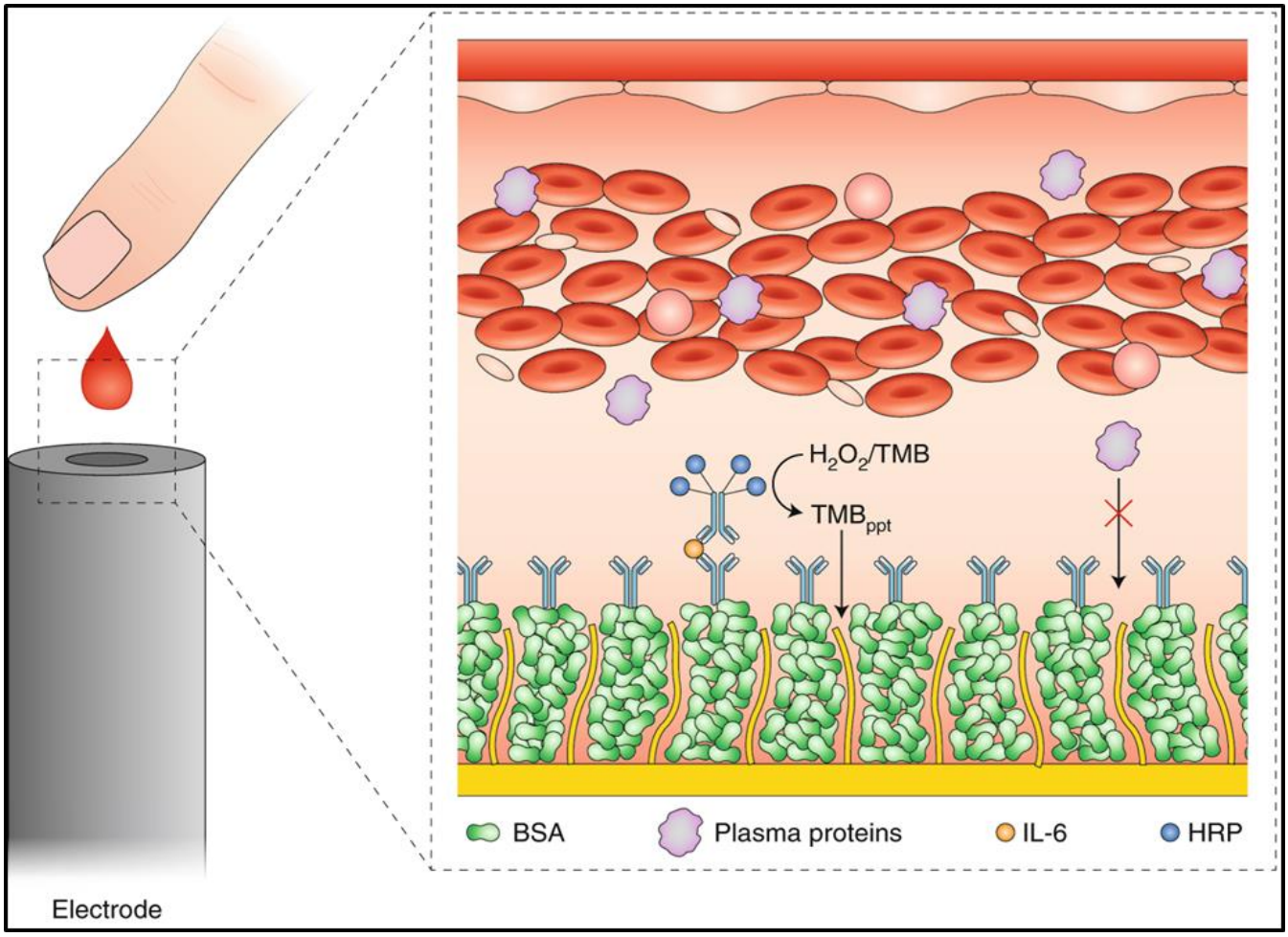

*Figure 2: The nanocomposite electrode [**Source:** Gooding JJ: Nature nanotechnology (2019)].*

The current and future prospects of the technology:

Since its conception the Wyss team has used the technology to successfully detect and test more than a dozen varied biomarkers with different molecular sizes ranging between 100 Dalton to 150,000 Dalton. Further experiments are in progress to optimize the sensitivity, conductivity and the cost of the devices further. This simple innovation in existing technology has paved way for better sensors and medical diagnostic tools which

shall change the current face of self-diagnosis and at-home medical testing. Although in its infancy, ‘eRapid’ is capable in bringing about a revolution in the field of handheld point-of-care diagnostics and can further be used in environmental toxin sensing, implantable medical devices, small molecule detection as well as hospital diagnostics. These portable, multiplexed, innovative diagnostic tools are the next big trend in medicine which is here to stay and change our lives for the better. It is often said that self-medication is harmful but self-diagnosis and personal health monitoring are habits which we need to inculcate so that we are not only aware but also equipped to fight our health issues better. To wrap up we would just like to say take care because self-care is the first step towards living better, healthier and longer.

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Scientific Breakthroughs

By Peeyush Prasad, MSc

Contributing Editor

Breakthrough Research of 21st Century: Shaping Our Future!

Human is ever thinking, inventing and innovating species. Tool making is one of the primary aspects of human life and pondering upon what future holds is basic occupation. From discovering fire for protection from predators and inventing wheel for carrying goods to long distance; human has come very far and now aspire to become multi-planet species. One technology creates the platform for the other technology and ultimately gets integrated in human life in such a way that it looks like another extension of our biological body. How many people thought that one day E=mc2 is going to revolutionize the energy sector and Darwin's theory of evolution will change for forever how we see ourselves. Discovery and invention come from very unlikely sources. Who knew that a Christian monk, while working in the church will find the principle of inheritance which almost challenged the creationism theory? A woman who was silently working in the field of crystallography produced such a wonderful image of DNA that it helped in deciphering the structure of DNA. Field of scientific discovery and inventions are not less adventurous than a thriller movie. Here is my list of breakthrough research of 21st century in biological science and how it is shaping our future.

Human Genome Project

Human Genome Project (HGP) was one of the most ambitious scientific project which came to conclusion in 2003. Several people from government and private industry participated in this project. Major findings of this project were identification of 22,300 protein-coding genes in human and identification of segmental duplication. Findings of human genome project enhanced our understanding of gene manifold. It paves the new way for gene therapy for curing debilitating diseases. Today it helps in understanding several genes which are associated with cancer and diabetes. Figure 1 shows the importance of Human Genome Project. It was covered worldwide on almost every media platform.

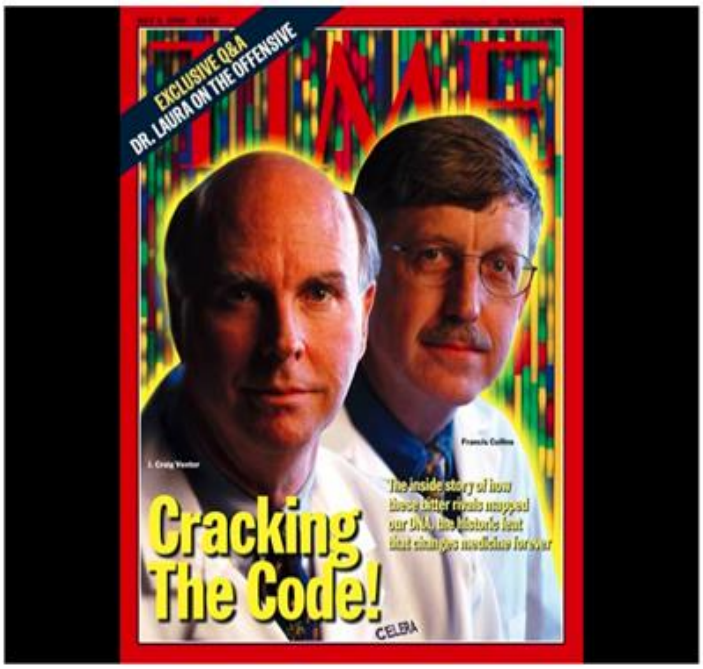

*Figure 1 Human Genome Project was covered almost by every major journal [**Source:** http://content.time.com/time/covers/0,16641,20000703,00.html].*

First Artificial life

At J. Craig Venter Institute (JCVI), a team of 17 researchers assembled and synthesized the 582,970 base pair of bacterial genome (Mycoplasma genitalium JCVI-1.0). They initially wanted to know the minimum set of genes that can sustain life. They chose the Mycobacterium genitalium because it contains the smallest number of genes. But later in 2010, they synthesized the complete genome of M. mycoides from computer record. Further, they inserted the synthesized genome into the DNA removed M. capricolum cell. Interestingly, bacterium started to grow and was named JCVI-syn1.0 or Synthia. Later, after JCVI-syn3.0 was produced that contained 473 genes in which function of 149 genes were not known. JCVI-syn3.0 considered as the first truly synthetic organism. Figure 2 shows the diagram of how first artificial bacterium was designed.

Creation of first artificial organism started a new avenue for exploring designing microorganism which can perform various function from environment cleaning (pollution) to synthesis of new medicine.

Blue Brain Project

Blue Brain project aims to create the digital reconstruction and simulations of the rodent and human brain. It is a Swiss brain research initiative founded by Henry Markram. Project aims to study the brain by using supercomputer-based simulation of brain activity. Several findings came from this project such as deep learning model for early tooth decay detection, how neurons form billions of connection in cortex and how brain finds order amidst chaos [3]. Figure 3 describes one of the outcome of Blue Brain Project. Study has come up with microcircuit model of neocortical region.

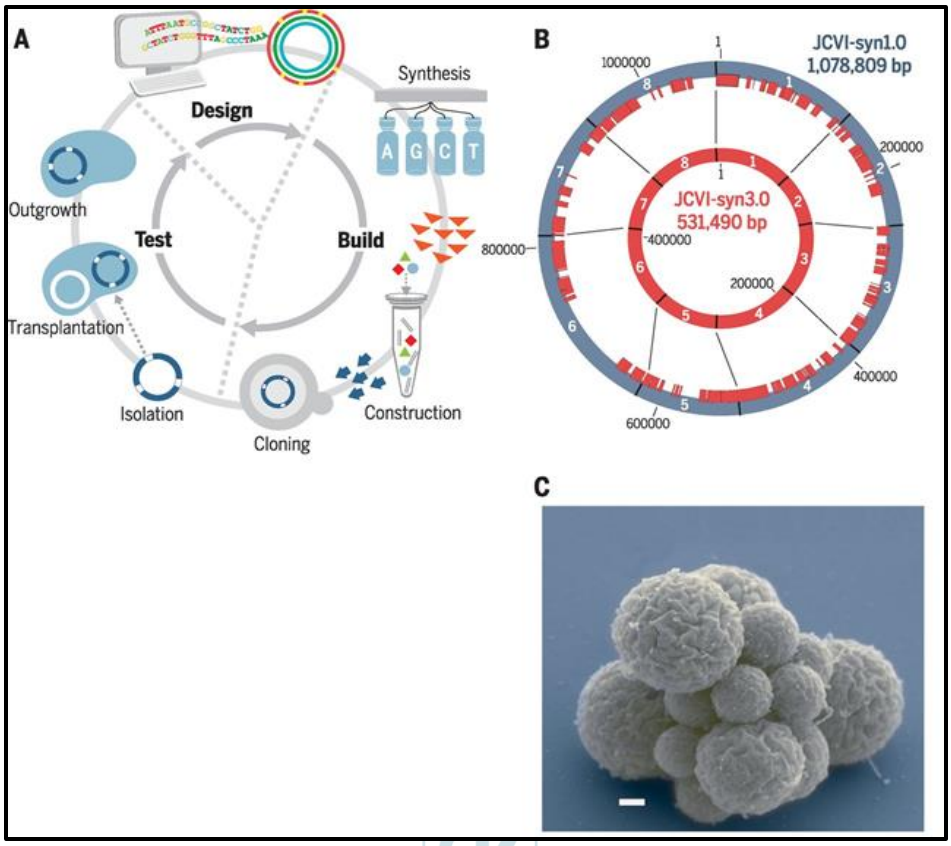

*Figure 2: Four design-build-test cycles produced JCVI-syn3.0. The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (B) Comparison of JCVI-syn1.0 (outer blue circle) with JCVI-syn3.0 (inner red circle), showing the division of each into eight segments. The red bars inside the outer circle indicate regions that are retained in JCVI-syn3.0. (C) A cluster of JCVI-syn3.0 cells, showing spherical structures of varying sizes (scale bar, 200 nm). [**Source:** Hutchison CA III et. al., Science. 2016]*

3D Printed Organ

3D printing of organ is a revolution in the field of biological engineering for rapid manufacturing of artificial organs with high level of anatomical precision. This technology uses the layer-by-layer 3D printing method in which biolinks or biomaterials are deposited for creating 3D tissues used for regenerative medicine for organ transplant. Organovo Inc. has created the 3D printed human liver tissue particle and showed successful implantation in the mice. Future of 3D printing looks very promising with diverse application from transplantation to drug discovery. In the same company researchers have developed the bioprinted 3D intestinal tissue for ADME/Tox modeling (absorption, distribution, metabolism and excretion) [4]. Figure 4 schematically describe the development of bio printed 3D model for ADME/Tox evaluation.

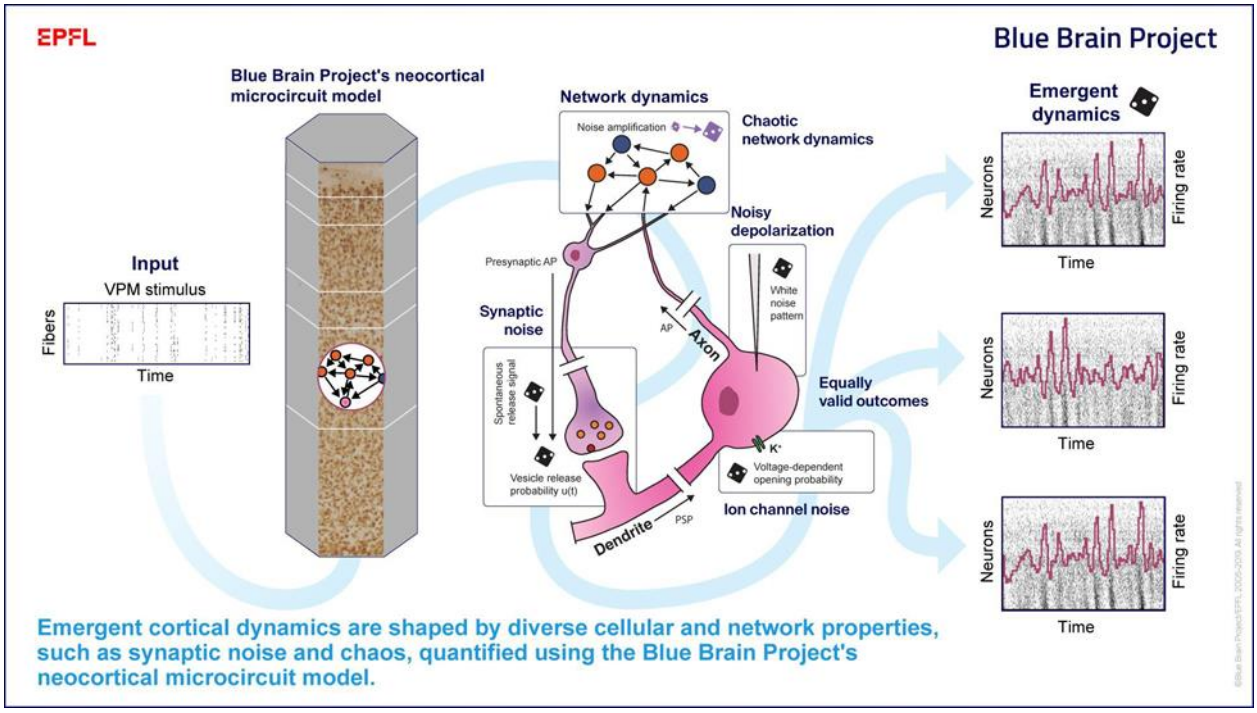

*Figure 3: Microcircuit model of neocortical region [**Source:** https://actu.epfl.ch/image/82042/original/1920x1080.jpg]*

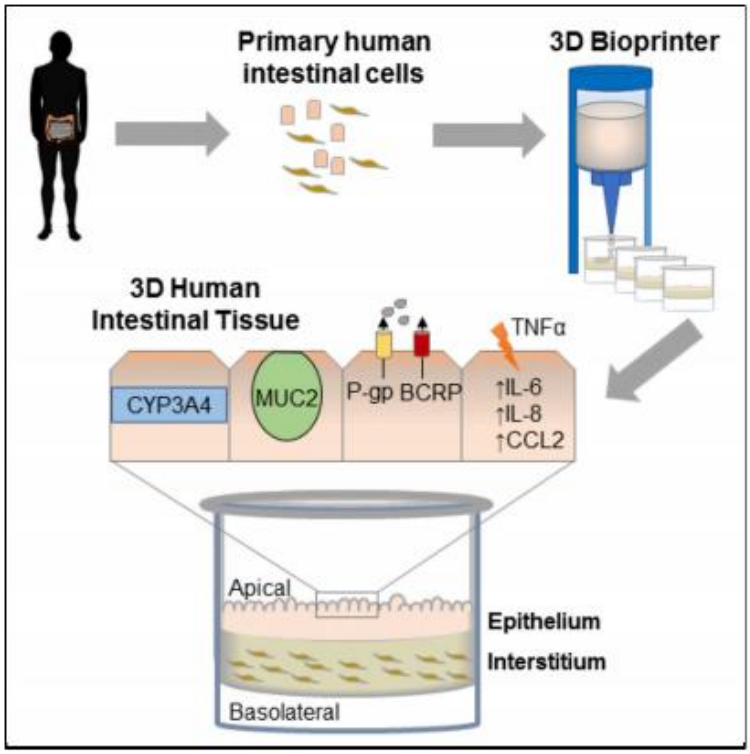

*Figure 4: Schematic diagram of bioprinted 3D intestinal tissue for ADME/Tox modeling [**Source:** Madden et. al., iscience, 2018]*

Immunotherapy

In modern age, cancer has become a big menace and millions of people die every year. Late diagnosis and therapy failure are two major factors which need to be addressed while solving the problem of cancer. Usually, immune cells of the host try to fight the cancer but subsequently cancer cell hijack the tumor microenvironment and made the immune cells non-functional.

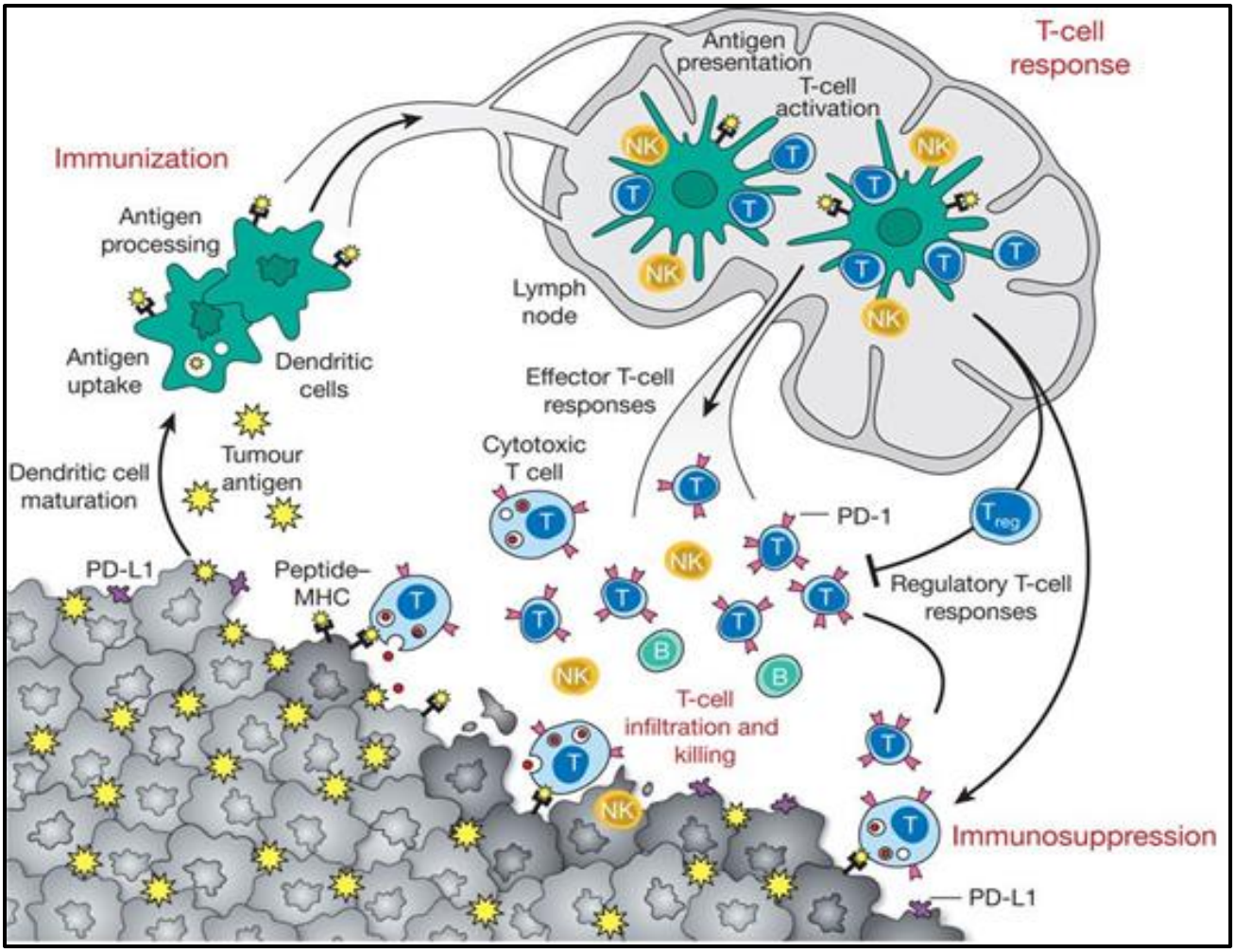

*Figure 5: Generation & regulation of antitumor immunity [**Source:** Mellman et. al., nature, 2011].*

One example of this is how PDL1 secreted from the cancer cell interact with PD-1 receptor present on the T-cells and made it dysfunctional. Immunotherapy aims to identify and block these checkpoints present on immune cells to counter inhibitory effect of cancer cells. Several types of immunotherapy are available such as monoclonal antibodies, non-specific immunotherapies, oncolytic virus therapy, T-cell therapy and cancer vaccine. Some of the immune check point inhibitors are Ipilimumab, Nivolumab and Pembrolizumab. Figure 5 describes the how antitumor immunity is generated and regulated [5].

Artificial intelligence (AI) and healthcare

Pattern recognizing ability of AI makes it very interesting tool for understanding the complex disease such as cancer. From the analysis of high-throughput data to analysis of images of pathological tissues, AI can revolutionize the diagnosis and therapy. AI can help in integrating and overlapping data from different sources and can help in designing therapy by using machine learning. Companies are working mainly in the field of cancer for diagnosis purpose. Artificial intelligence can bring the personalized medicine to practical reality. In Figure 6 some major area in which AI can be very helpful is mentioned [6].

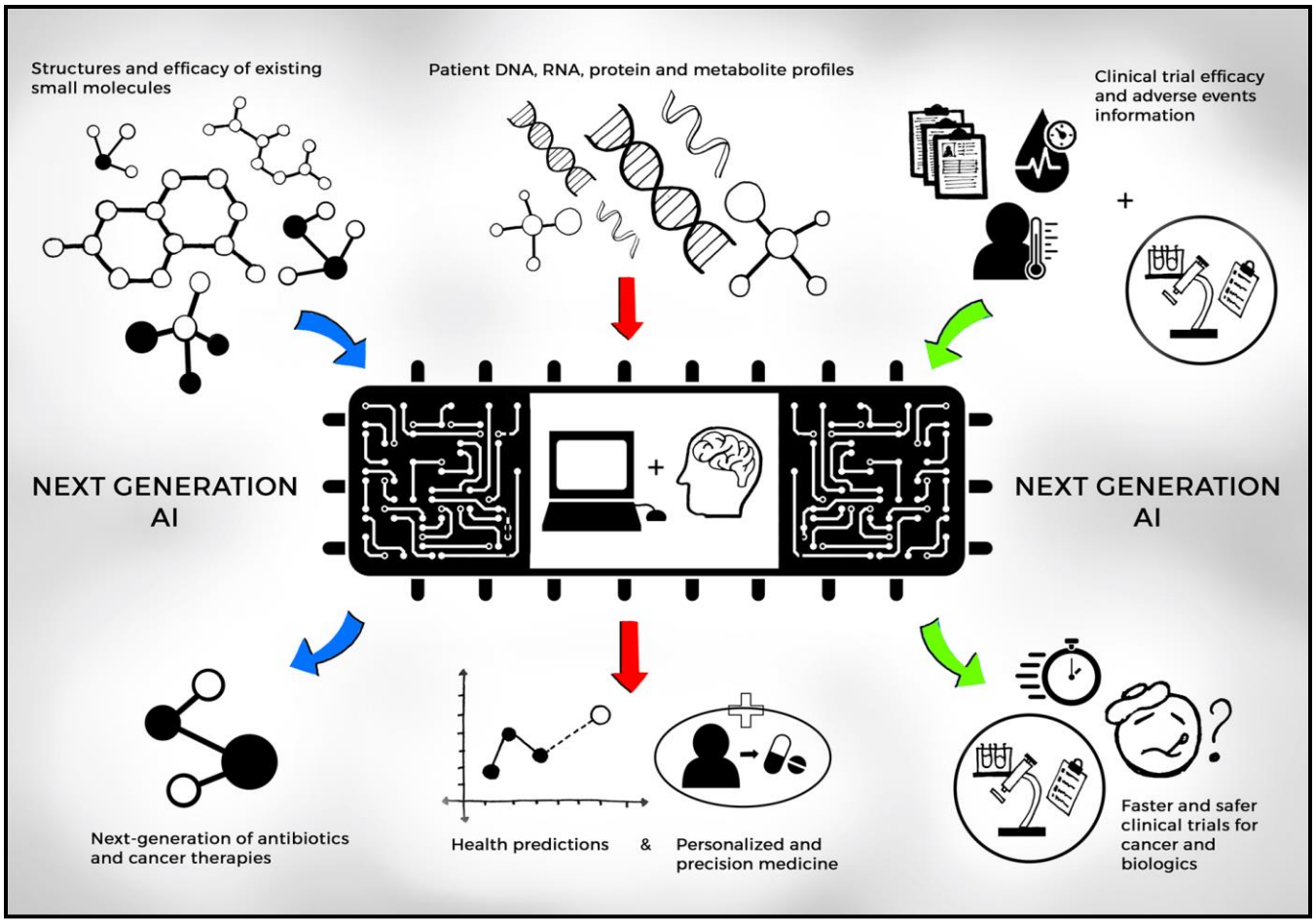

*Figure 6: AI has huge potential in healthcare from finding new drug molecules to analyzing diagnostics images [**Source:** https://www.pratiks.info/research/mit/novel-ethical-and-explainable-artificial-intelligence-based-digital-medicines-and-treatments].*

Genome editing (CRISPR-Cas9)

Genome editing is the technology which has the ability to change an organism's DNA. With the help of genome editing, genetic material can be added, altered and removed from the genome. Several genome editing tools have been developed such as TALEN and CRISPR-Cas9. In gnome editing tool, CRSPR-Cas9 has attracted special attention due to its ability to alter gene very precisely. Figure 7 shows the basic application and mechanism of CRISPR-Cas9 based genome

editing [7]. This technology can be used for gene silencing, gene editing, homology directed repair process and transient gene silencing. CRISPR-Cas9 can be used for treating diseases such as cancer, blood disorders, blindness, cystic fibrosis and AIDS.

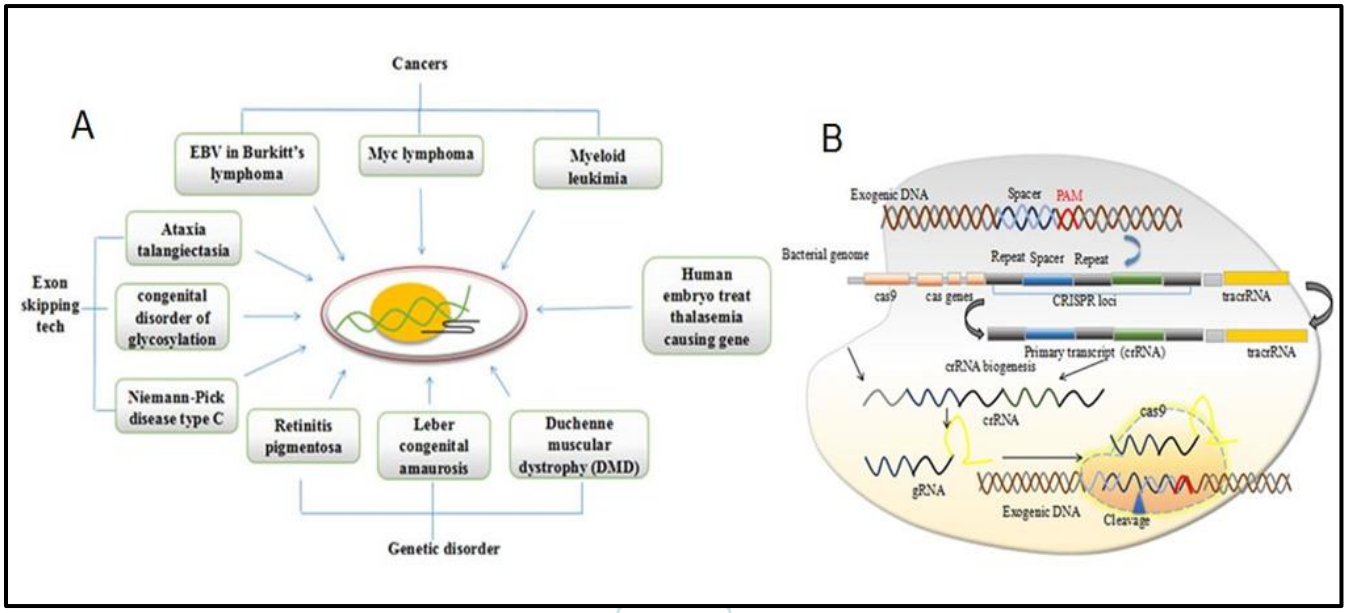

*Figure 7: A) CRISPR-Cas9 can be redesigned to cure mutations causing cancers and genetic diseases B) The CRISPR-Cas9 mechanism [**Source:** Khan FA et. al., Oncotarget, 2016].*

The Cancer Genome Atlas (TCGA)

The Cancer Genome Atlas (TCGA) has changed our understanding of cancer and changing the way it is being treated in clinical set up. The program has deepened our understanding of cancer by molecular characterization. Molecularly, it has characterized the 20,000 primary cancers and matched normal samples spanning 33 cancer types [8]. The project sampled 500 patients and used techniques such as gene expression profiling, copy number variation profiling, SNP genotyping, genome wide DNA methylation, exon sequencing and microRNA profiling. The project has analyzed 11,000 tumors from 33 most prevalent forms of cancer and reclassified the tumor type on the basis of molecular subtypes. Figure 8 describes the major findings of TCGA in which they showed that genomic analysis can demarcate the cancer types and subtypes [9].

New technology is causing the explosion of new information and further this new information will be used to develop something more for making human life easy. Research in basic and applied science will keep on happening and it will provide more insight about us. There are several areas such as machine-human interface, easy and cost effective diagnostics platform and effective integration of healthy data in disease data, which need to be explored in depth.

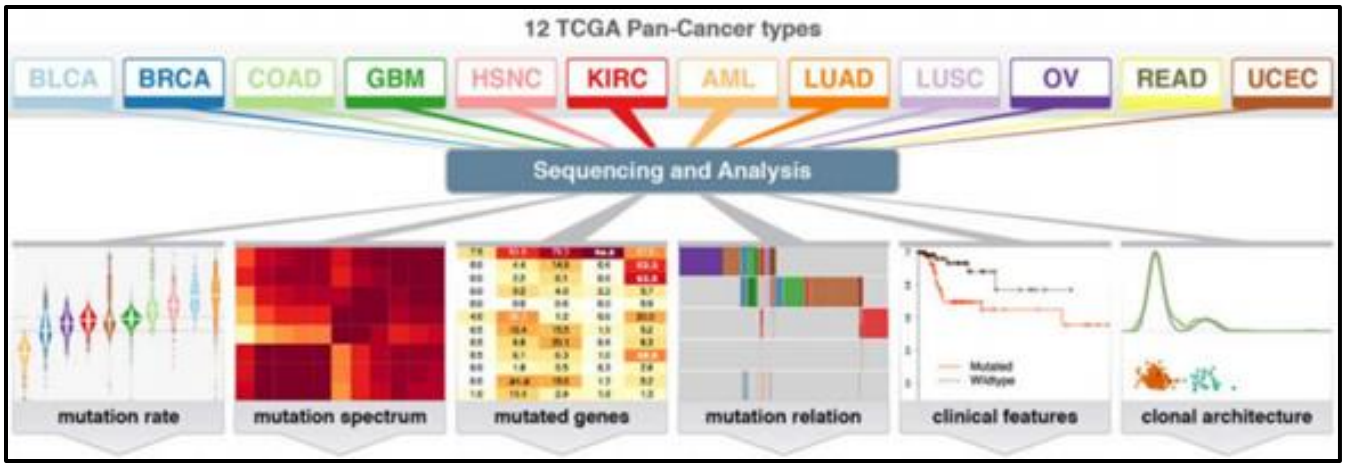

*Figure 8: Genomic analysis of multiple cancer types (aka "Pan-Cancer analysis) highlight commonalities and differences among various types and subtypes [**Source:** https://www.genome.gov/Pages/About/NACHGR/September2017AgendaDocuments/Hutter_TCGA.pdf].*

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Infectious Disease

By Shyamasri Biswas, PhD

Executive Editor

Innovative Therapeutic Technologies to Fight against Drug-Resistant *Pseudomonas aeruginosa* Infections

Pseudomonas aeruginosa (P. aeruginosa) is a ubiquitous Gram-negative bacterium. It belongs to the family Pseudomonadaceae that is known to survive in a wide range of environments. *P. aeruginosa* exhibits a relatively large genome (5.5–7 Mbp) compared to other sequenced bacteria such as *Bacillus subtilis* (4.2 Mbp), *Escherichia coli* (4.6 Mbp) and *Mycobacterium tuberculosis* (4.4 Mbp). Further, it encodes a large proportion of regulatory enzymes important for metabolism, transportation and efflux of organic compounds [1-4]. P. aeruginosa is known to be an opportunistic pathogen, which causes morbidity and mortality in cystic fibrosis (CF) patients and immunocompromised individuals. It is therefore, of paramount clinical importance to eradicate *P. aeruginosa*. However, it is a significant challenge to eliminate this highly harmful pathogen due to its remarkable capacity to resist antibiotics (Figure 1) [1].

Clinical studies have shown that the lung of a CF patient provides a conducive environment for bacterial growth and colonization. Thus, it is believed that *P. aeruginosa* is the predominant pathogen causing CF lung infections [1]. Further, researchers have shown that chronic infection with *P. aeruginosa* is recalcitrant to antibiotic treatment that subsequently results in declined pulmonary functions and ultimately to mortality in CF patients [5]. In addition, it has been shown that over 5% of infectious exacerbations in patients with chronic obstructive pulmonary disease (COPD) are caused by *P. aeruginosa* that has been associated with increased mortality of these patients [6].

New Antipseudomonal Antibiotics and Therapeutic Strategies for Treatment of P. aeruginosa Infections

The rise of multidrug-resistant strains of *P. aeruginosa* poses a major therapeutic challenge for the applications of conventional antibiotic therapies against infections [7]. Hence, there have been growing demands for the discovery and development of alternative therapeutic strategies including new antipseudomonal antibiotics that present

novel avenues against *P. aeruginosa* infections. Most of the studies that have reported several innovative therapeutic technologies with demonstrated effectiveness in fighting against drug - resistant *P. aeruginosa* strains are in the pre-clinical state of studies [1].

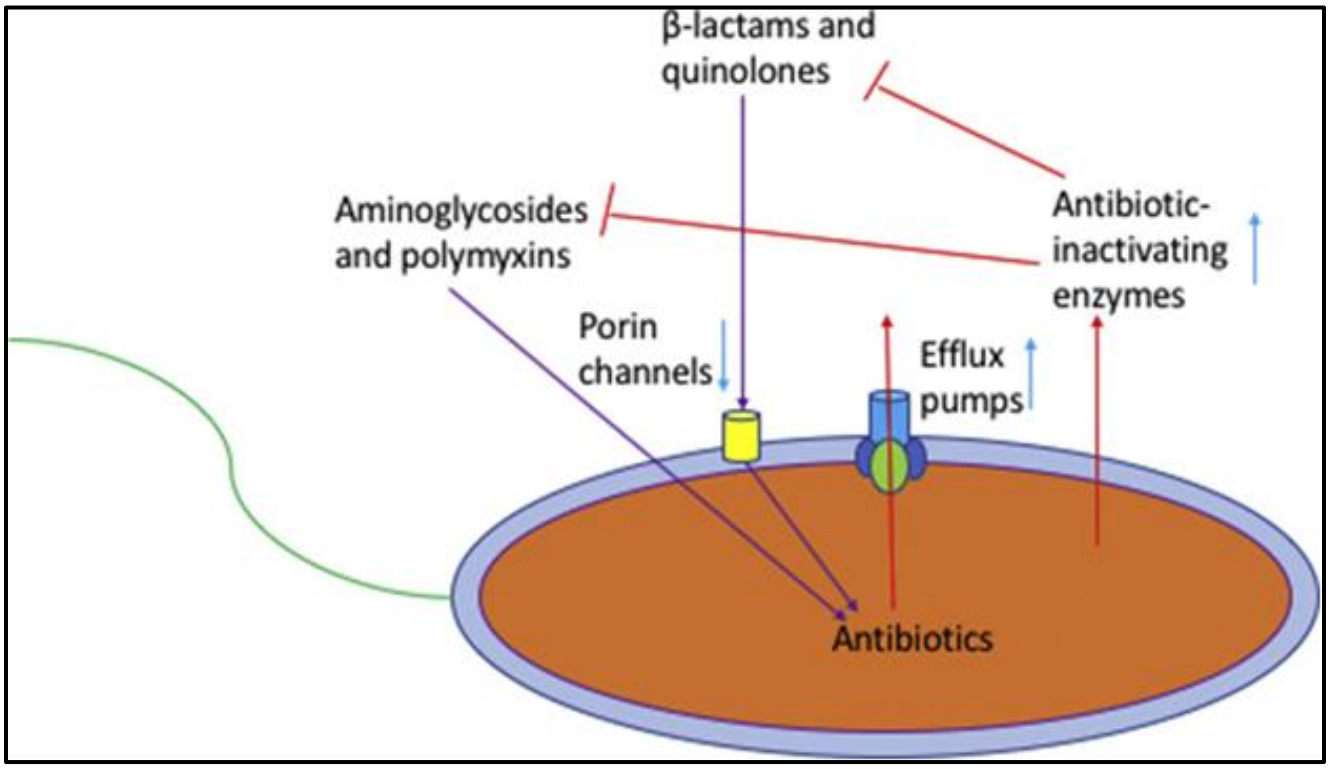

*Figure 1: A schematic representation of the underlying mechanisms showing intrinsic antibiotic resistance in P. aeruginosa that include restricted outer-membrane permeability, efflux systems that pump antibiotics out of the cells and production of antibiotic-inactivating enzymes. Further, β-lactams and quinolones penetrate cell membranes through porin channels. By interacting with P. aeruginosa lipopolysaccharides on the outer membrane, aminoglycosides and polymyxins promote their own uptake [**Source:** Biotechnology Advances, 37 (2019)].*

The use of different antibiotic combinations along with development of new antibiotics constitute a major part of current therapeutic strategic options for *P. aeruginosa* treatment [8]. The new antibiotics that are in the process of getting into clinical trials have shown their effectiveness in killing P. aeruginosa pathogens. These new antibiotics have also demonstrated a lower frequency of resistance development compared to existing antibiotics. This is due to their novel modes of therapeutic action, efficient drug delivery including inhaled antibiotics and also resistance to modification by bacterial enzymes [1, 7]. New alternative therapeutic approaches include quorum sensing inhibitors, phages, probiotics, anti-microbial peptides, vaccine antigens and antimicrobial nanoparticles (Figure 2) [1]. Researchers have shown that these novel therapeutic strategies can act either alone or in combination with conventional therapies to combat *P. aeruginosa* infections [1, 7, 8].

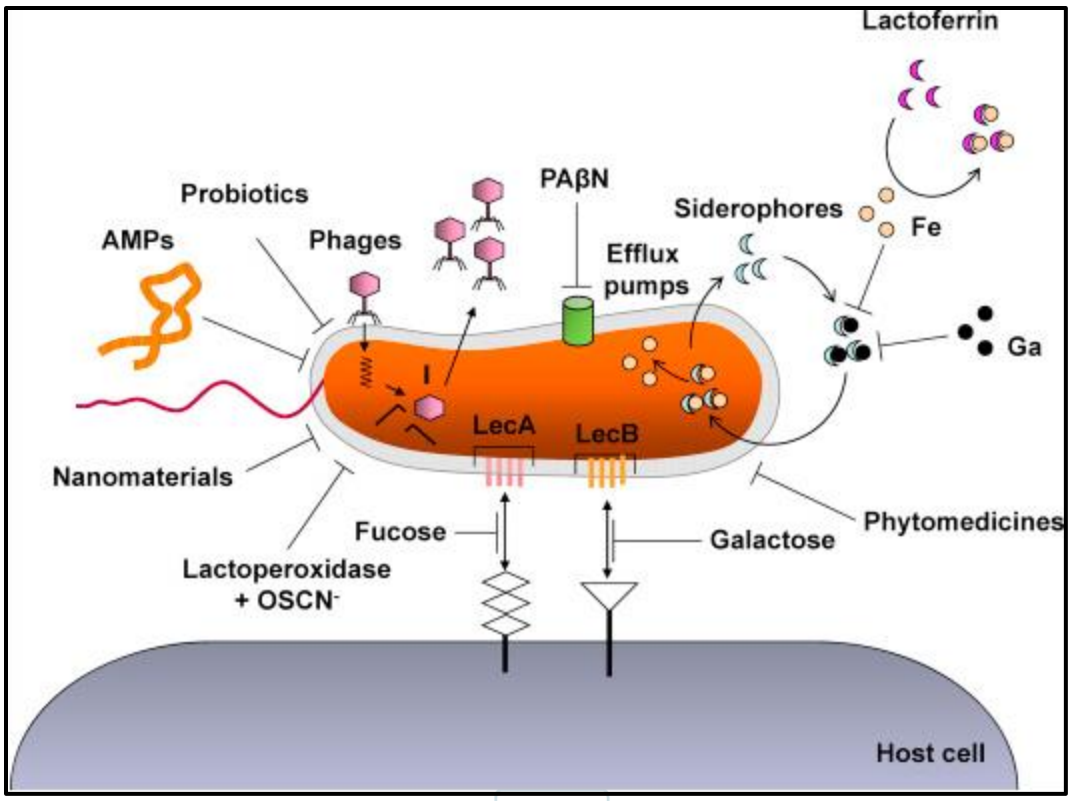

Figure 2: Various novel therapeutic strategies to combat P. aeruginosa *infections [**Source:** Int J Med Microbiol, 306 (2016)].*

Concluding Remarks

It is a significant challenge to treat P. aeruginosa infections that continues to be a serious tough task in the field of bacteria and infectious disease. The current efforts have focused on developing new antibiotics and novel therapeutic strategies that are based on alternative methodologies to tackle the antibiotic resistance problem of *P. aeruginosa*. There has been significant progress made in this direction especially developing novel modes of action, and resistance to modification by bacterial enzymes along with improvements to drug delivery efficiency. However, most of these newer approaches are in the pre-clinical stage and we anticipate that it might take some time before these innovative therapeutic technologies enter into clinical studies for practical applications in humans. We further anticipate that development of new antimicrobial agents and alternative strategies for prevention and treatment of *P. aeruginosa* infections will continue to evolve and shape for potential major breakthroughs and discoveries in therapeutics for the effective elimination of this bacteria.

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Regenerative Medicine

By Megha Agrawal, PhD

Executive Editor

Treating Genetic Disease by Antigen-Specific Induction of Immune Tolerance in Humans

Antigen-Specific Induction

The therapeutic efforts over the past several years for the prevention or reversing of autoimmune, allergic or anti-protein drug responses and transplant rejection saw the development of monoclonal antibodies, fusion proteins, and regulatory T cell therapies. These biological drugs helped expand substantially the toolkit of suppression immunotherapy and enabled more targeted treatments than traditional immune suppressive drugs [1]. However, these drugs are limited for their wide-scale use in the consumer sector due to the reason that they are very expensive in the first place. In addition, to perform the manipulations, specialized centers are often required for patient care. Antigen-specific induction of immune tolerance in humans has been suggested a viable alternative therapeutics. It is believed that antigen-specific regimens could significantly benefit the treatment of autoimmune diseases, where some of the target antigens are known [1]. For example, in the treatment of genetic diseases using protein replacement therapies, the widespread problem encountered is the formation of anti-drug antibodies (ADA). Antigen-specific tolerance induction could overcome this problem and would be far more desirable than general immune suppression. This is due to the fact that antigen-specific induction could enable avoiding risks of infection and side effects of immune suppressive drugs. Researchers have shown potential pathway toward such a protocol that involves the introduction of the target antigen via a route, which leads to tolerogenic antigen presentation [1]. To this end, the available pre-clinical data, have shown that oral delivery of protein antigens may induce immune tolerance [2-4]. This approach was recently successfully demonstrated in the prevention of peanut allergy through regular ingestion of peanut-containing foods in infants [5].

Oral Immunotherapy Offers Hope to Prevent Life-Threatening Food Allergies: A New Concept of Antigens in Plant Cells

Oral immunotherapy (OIT) has been employed to prevent life-threatening food

allergies [1]. The clinical application of OTT has been demonstrated with high rates of success in desensitization of the allergic response. OIT also showed effectiveness in some cases that involved long lasting tolerance and termed sustained unresponsiveness [6]. A plant based product is currently considered the front-runner for first FDA approval for an orally delivered for peanut allergy [7]. Advanced pre-clinical development of this next generation of drugs is underway to develop the ability to orally administer specific auto-, allo-, and therapeutic antigens that are based on transgenic plants (Figure 1) [1].

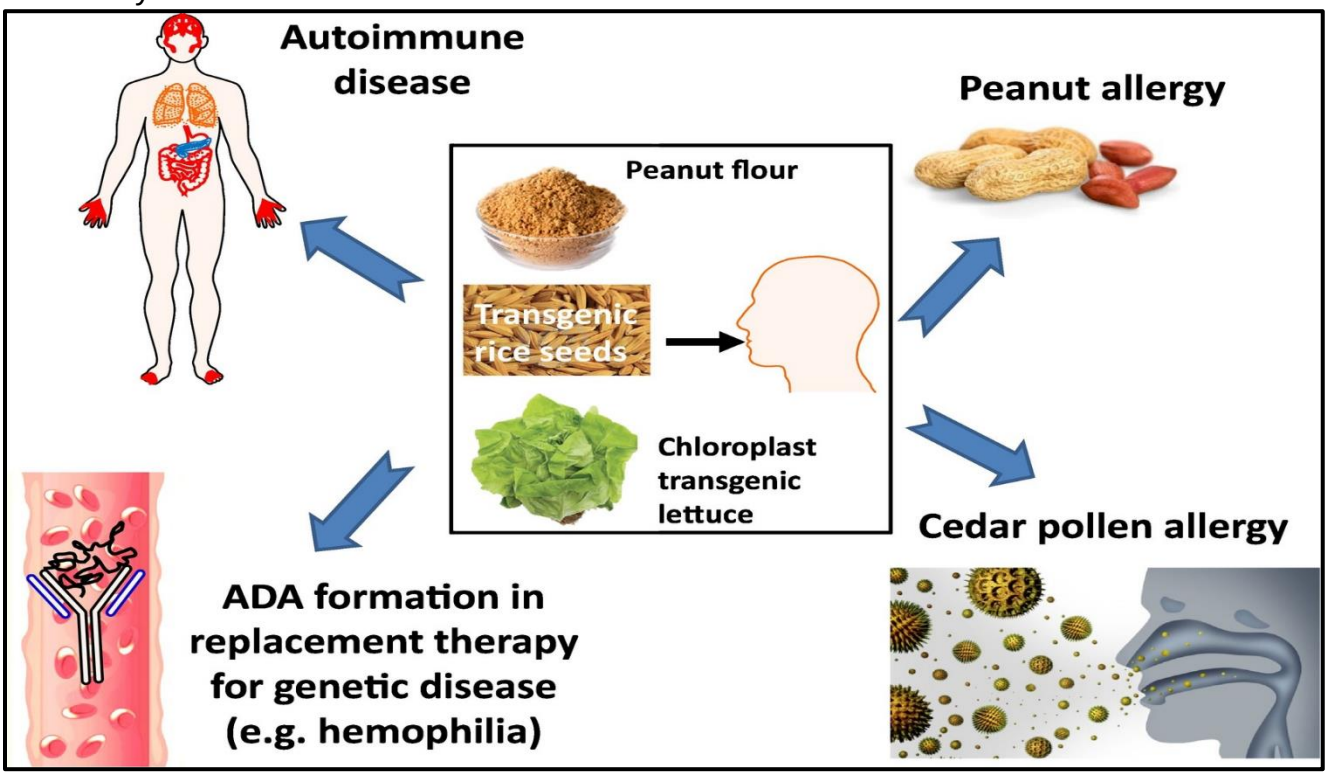

*Figure 1: Schematic illustrations showing specific examples of oral immune modulatory therapy using plant cells. Antigens bioencapsulated in plant cells are derived from leaves, nuts, or seeds that can be orally delivered to promote tolerance to autoantigens, allergens, and therapeutic proteins. These antigens can be expressed in transgenic plants and can be employed to treat genetic disease [**Source:** Biotechnology Advances 37 (2019)].*

The major clinical advantage of the oral tolerance method is that it does not require custom design of antigens for specific major histocompatibility complex (MHC) molecules unlike in the case of peptide-based methods. It also does not require genetic manipulations of host cells that are required for example, in gene therapy. However, the current state-of-the-art of translation of this method poses technological limitation due to the reason that it involves substantial costs of production of large amounts of antigen for oral delivery. Another reason is the inefficiency of delivery to the gut immune system due to antigen digestion in the stomach by acids/enzymes that create blockage of antigen absorption by gut epithelium [1].

Researchers have shown some recent breakthrough inventions that have addressed these limitations by expression of antigens or protein drugs in plant cells. These expressed antigens in plant cells can be protected from stomach acids or enzymes through bioencapsulation. The process involve commensal bacteria that degrades plant cell wall and releases antigens in the gut lumen [1]. Subsequently, transmucosal carriers are fused to antigens that deliver them across the gut epithelium and to the immune system through ubiquitous binding sites [8-10].

Oral Tolerance Induction Using Plant Cells

The process involving use of plant cells is often termed as green bioreactors, which is now rapidly becoming a promising approach for production and delivery of biopharmaceutical proteins [1]. Researchers have shown this approach very successful in the case of treatment for genetic disease, where oral tolerance induction to the therapeutic protein has been shown to suppress formation of anti-drug antibodies. This enables the administration of replacement therapy that can correct the genetic disease [1]. Current ongoing research suggests that several other protein antigens made in plant cells are in clinical development. These plant cell-made proteins are advantageous as they are protected in the stomach from acids and enzymes after their oral delivery because of bioencapsulation within the plant cell wall. They are eventually released to the immune system upon digestion by gut microbes [1].

Researchers have also shown that the utilization of fusion protein technologies can facilitate delivery to the immune system. In such a process, the oral tolerance induction done at low antigen doses can result in efficient induction of FoxP3+ and latency-associated peptide (LAP)+ regulatory T cells, which enables to express immune suppressive cytokines such as IL-10. LAP and IL-10 expression. They all represent potential biomarkers for plant-based oral tolerance (Figure 2) [1].

Concluding Remarks

Oral tolerance by antigen-specific induction from plant cells is an emerging immunotherapy that can potentially prevent or reverse autoimmune, allergic, or anti-protein drug responses. This approach is shown to be advantageous over other biological drugs due to the fact that it offers non-invasive and antigen-specific therapeutics. To this end, a number of successes have recently been demonstrated by researchers that have shown the applicability of this approach for food allergies employing crude forms of peanut antigens in pre-clinical trials. We anticipate further research and developments happening in this field in the near future that would enable transition of oral tolerance to the successful practical applications in the treatment of autoimmune diseases. The future strategies could combine with other immune modulatory strategies, such as monoclonal antibody therapy. The plant-based method offers distinct advantages including substantially more cost effective and also blocking antibody formation in replacement therapies for inherited protein deficiencies.

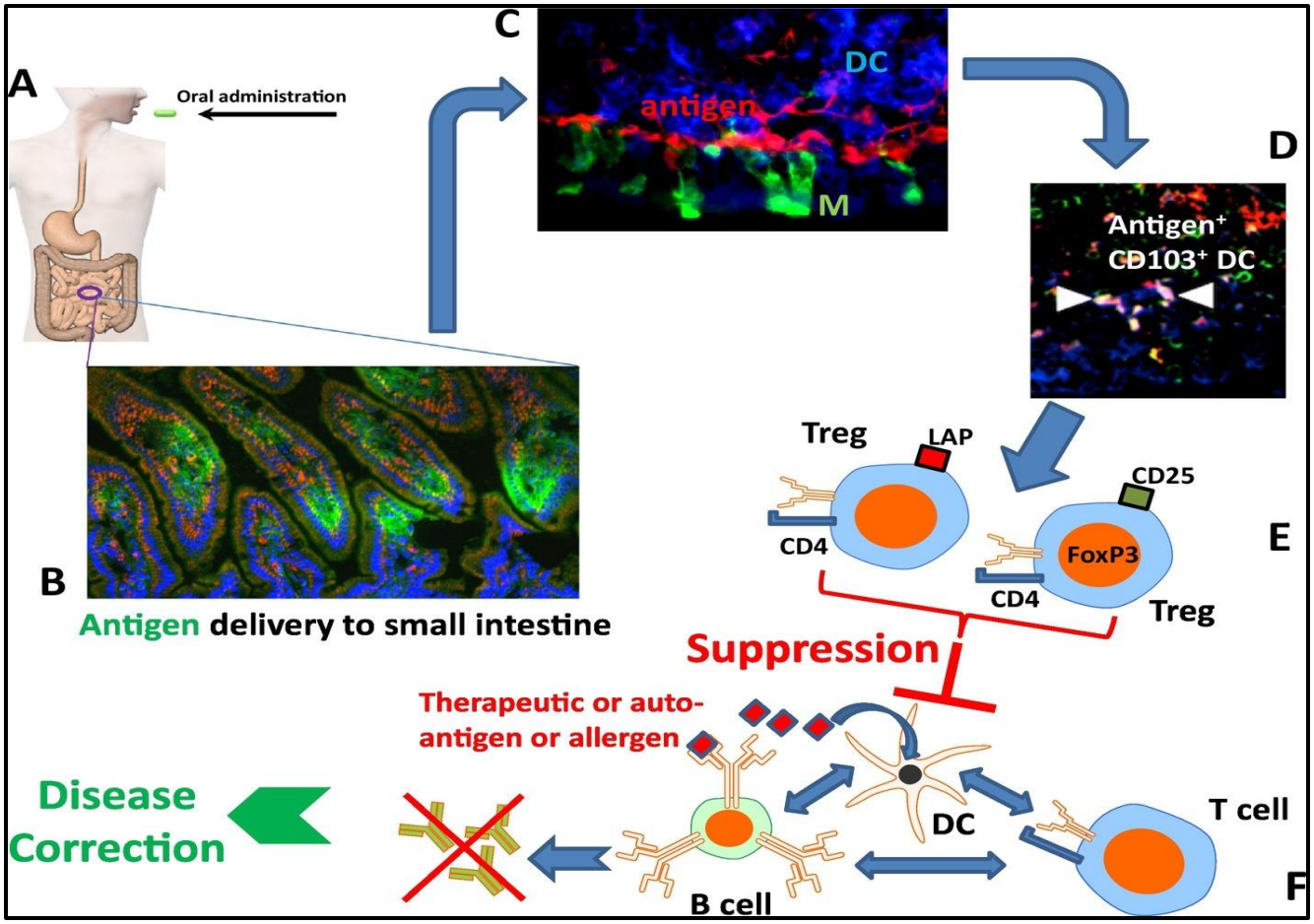

*Figure 2: A schematic representation showing the concept of oral tolerance induction using plant cells. (A) Transgenic plant cells are shown expressing the specific antigen that are orally delivered. (B) Subsequently, the antigen (shown in green) is translocated to the gut-associated immune system upon release in the small intestine. (C) Antigen (shown in red) then accumulates in areas rich in to dendritic cells (DCs, blue). M cells are also shown (green) in the picture. (D) Some of the antigen is used by tolerogenic CD103+ DCs. (E) Antigen-specific regulatory T cells (CD4+CD25+FoxP3+ and CD4+CD25−FoxP3−LAP+ T cells) are induced. (F) Induced Treg suppress B and T cell responses against the antigen that result in elimination of autoimmune or allergic responses. Further, oral tolerance induction to the therapeutic protein can suppress formation of anti-drug antibodies that allows the administration of replacement therapy, which can correct the genetic disease [**Source:** Biotechnology Advances 37 (2019)].*

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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
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Biosynthesis and Renewable Energy

Biological enzymes as more environmentally friendly energy sources

Hydrogenases are biological enzymes that are known as nature's machinery for making and burning hydrogen gas. There is an intense interest in the biosynthesis of these enzymes' active sites to explore these materials in the field of renewable energy.

The current state-of-the-art that produces hydrogen use a very complex industrial process that eventually restricts its commercial viability to the global renewable energy market. To overcome this challenge, scientists are now pushing the envelope to biologically synthesize hydrogen, which is considered far more efficient than the current human-made process.

In a new study, chemical researchers in the US demonstrated a novel route to recreate nature's most efficient machinery for generating hydrogen gas based on the enzyme [FeFe]-hydrogenase (HydA1) containing a unique 6-iron cofactor, the H-cluster. This research of major significance in the field of biosynthesis enabled renewable energy was recently reported in the journal PNAS (The binuclear cluster of [FeFe] hydrogenase is formed with sulfur donated by cysteine of an [Fe(Cys)(CO)2(CN)] organometallic precursor, Proceedings of the National Academy of Sciences, 2019; 116 (42): 20850 DOI: 10.1073/pnas.1913324116). This new study focuses on the iron-iron variety in the hydrogenase because it is considered to do the job faster. They reported the in-vitro assembly of the H-cluster in the absence of HydG that was functionally replaced by adding a synthetic [Fe(Cys)(CO)2(CN)] carrier in the maturation reaction. The carrier then allowed HydG-free maturation to HydA1, whose activity overlapped that of the native enzyme. This demonstration of synthetic surrogate approach has the potential to strengthen existing biochemical strategies that can greatly facilitate the understanding of pathways involved in the assembly of the H-cluster for hydrogen production.

This new development may pave the way to help the hydrogen fuel industry to go to the next level to take up larger role in the global push toward more environmentally friendly energy sources.

Stem Cells Biotechnology

Discovery of tendon stem cells could revolutionize injury recovery without surgery Tendons are connective tissue that tether muscles to bones. The function of tendons are vital to our body as they improve our stability and facilitate the transfer of force that allows us to move. However, tendons are also known to be particularly susceptible to injury and bodily damage, and once they are injured, they do

not fully recover. This is a serious medical condition as it may result in limited mobility and may require long-term pain management or even surgery to recover from the injury. It is believed that fibrous scars disrupt the tissue structure of the tendon that creates hindrances in the recovery of injured tendons.

Stem cells have been shown to be associated with nearly every type of tissue. It has been also shown that stem cells have the ability to self-renew that can create a pool from which newly differentiated cell types can form to support a specific tissue function. For example, it can be understood from the functions of muscle stem cells that can differentiate into muscle cells. However, despite all breakthroughs in stem cells, up until now, stem cells for the tendon were unknown.

A recent new study has discovered tendon stem cells that could be a game-changer in the treatment of tendon injuries that would not require a surgery. A team of researchers in the US recently discovered tendon stem cells and in a significant finding they showed tendon stem as part of a competitive system with precursors of fibrous scars. This explains exactly why tendon healing is such a challenge. Their research was published in Nature Cell Biology (A Tppp3 Pdgfra tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis, Nature Cell Biology, 2019; DOI: 10.1038/s41556-019-0417-z). This new study that shows the existence of tendon stem cells could potentially pave the way for new therapeutics that could be harnessed to improve tendon healing and even to avoid surgery.

Fungi in Biotechnology

A library of fungi can help to search for new medical drugs

New therapeutic compounds are constantly needed in the clinic for various health reasons. These include ageing and corresponding illnesses, and also resistance to existing drugs among many other health related issues. Fungi offer a lot of promise as an excellent source of these kinds of compounds. An example is lovastatin, which is a compound produced by the fungus *Aspergillus terreus* and that is used as a cholesterol lowering drug. However, up until now, the vast medical potentials of fungi have remained underexplored.

To address this issue, a team of researchers in the Netherlands built an enormous library of products derived from more than ten thousand fungi that could help discover new drugs. Researchers set up the library and screened it for biologically active compounds, and subsequently tested the biological activity of these fungal products. They first employed zebrafish embryos in their study. The selection of zebrafish embryos was due to their ability to allow the analysis of effects on many cell types at the same time, in a working body. Additionally, zebrafish are known to be physiologically very similar to humans. In the study, the researchers found various known compounds, among which the

cholesterol lowering drug lovastatin. This library of fungal products offers huge potential to search for new drugs. This research was published in the scientific journal Scientific Reports (A new perspective on fungal metabolites: identification of bioactive compounds from fungi using zebrafish embryogenesis as read-out, Scientific Reports, 2019; 9 (1) DOI: 10.1038/s41598-019-54127-9).

Plant Biotechnology

A new discovery in photosynthesis could help achieve higher yields and urgent food security needs

Photosynthesis is a critically important process in plants that serves the foundation of life on Earth. It enables the production of food, and supplying oxygen and energy, which sustain the biosphere and human civilization. It is believed that cytochrome b6f is the beating heart of photosynthesis that plays a critical role in regulating photosynthetic efficiency. It has been previously shown that by manipulating the levels of this complex, bigger and better plants can be grown.

A recent discovery by a team of researchers in the UK could lead to photosynthesis being 'redesigned' that would enable to achieve higher yields and meet urgent food security needs. The study was published in the journal Nature (Cryo-EM structure of the spinach cytochrome b6 f complex at 3.6 Å resolution, Nature, 2019; DOI: 10.1038/s41586-019-1746-6) that revealed the structure of cytochrome b6f as the protein complex and that was found to significantly influence plant growth via photosynthesis. Researchers employed the high-resolution structural model determined by single-particle cryo-electron microscopy that revealed new details of the additional role of cytochrome b6f as a sensor that can tune photosynthetic efficiency in response to ever-changing environmental conditions. This study showed that the response mechanism can protect the plant from damage during exposure to harsh conditions such as drought or excess light.

This research of major significance provides important new insights into how cytochrome b6f can utilize the electrical current passing through it to power up a proton battery. Subsequently, this stored energy can be utilized to make ATP, the vital energy mechanism of living cells. Researchers envision that ultimately this reaction can provide the energy that plants need to convert carbon dioxide into the carbohydrates and biomass, which can potentially sustain the global food chain. With this new insights, it is hoped that the revealed structure could help pave the way to rationally redesign photosynthesis in crop plants. This would enable to achieve the higher yields that are needed to sustain a projected global population of 9-10 billion by 2050.

Pathogens and Immune Cells

Shortage of magnesium can stop pathogen growth

When pathogens invade the cells in the immune system or infect an organism, the defense system of the human body immediately responds to the attack and starts fighting the bacteria. Some invading bacteria can escape the patrolling immune cells and start replicating inside host cells.

Researchers in the Switzerland demonstrated how a cellular pump can keep such invading pathogens in check. Researchers showed that the pump can cause a magnesium shortage, which can in turn restrict bacterial growth in the host cells. They reported their research in Science (Host resistance factor SLC11A1 restricts Salmonella growth through magnesium deprivation, Science, 2019 DOI: 10.1126/science.aax7898). This research shows that as magnesium is a key component of many metabolic enzymes, a shortage of magnesium can reduce bacterial metabolism and growth. This study could lead to new therapeutics to better manage infections that are considered a race between the host and the pathogen. New drugs that can be developed based on the concept demonstrated in this study would make it even harder for the bacteria to obtain magnesium. This could slow down the pathogens even more. This subsequently would provide the host a decisive advantage in defeating the infection.

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

Biotech and Pharma Industry Roundup

Sanofi's Toujeo got FDA approval to treat childhood type 1 Diabetes

The U.S. Food and Drug Administration (FDA) recently approved an expanded indication for Sanofi's Toujeo (insulin glargine injection) for blood sugar control in adult and pediatric patients who are ages six years and older. Insulin glargine injection developed by Sanofi is a long-acting, manufactured insulin. The advantage of this insulin is that the injection holds three times as much insulin in 1ml as a standard 100 units/ml insulin [Source: https://www.biospace.com/].

TauRx's Alzheimer's Drug Shows promise in large clinical trials

Aberdeen, Scotland and Singapore based TauRx recently published unexpected results from a pharmacokinetic analysis of the drug hydromethylthionine (LMTM) in Alzheimer's disease. This research was published in the Journal of Alzheimer's Disease. The drug called Hydromethylthionine is a world health organization (WHO) approved non-proprietary name for a drug previously called LMTM by TauRx. It is known that two proteins that accumulate in the brain of Alzheimer's patients are beta-amyloid and tau, where beta-amyloid occurs earlier in the disease and tau appears later in the disease. The functions of the drug by TauRx allow blocking abnormal accumulation of the tau protein in the brain. In the published research, TauRx analyzed the relationship between various factors including treatment doses, blood levels and pharmacological activity of the drug on the brain in more than 1,000 patients with mild-to-moderate Alzheimer's disease. This was a part of two Phase III global clinical trials. The drug demonstrated concentration-dependent effects on cognitive decline and brain atrophy [Source: https://www.biospace.com/].

Recipharm announces commercial manufacturing

The contract development and manufacturing organization, Recipharm recently announced to the ongoing large-scale commercial manufacturing of RedHill Biopharma's drug, Talicia® [Source: https://www.biospace.com/].

Novartis buys Medicines Co.

Novartis is set to buy cholesterol drug developer The Medicines Company for $9.7 billion. This acquisition is aimed to reshape the Swiss pharmaceutical company around newer technologies and higher-margin prescription therapies. The acquiring of Medicines Co. will give Novartis control of a near-to-market heart drug, called inclisiran [Source: https://www.biopharmadive.com/).

Lilly to invest $400M in Indianapolis manufacturing facilities

Eli Lilly is set to invest $400 million in manufacturing facilities in its hometown of

Indianapolis. This will create 100 new jobs. This plan of expansion of existing facilities will involve production of insulin, more capacity for the company's other diabetes treatments, and initial capital investments for future medicines [Source: https://www.biopharmadive.com/)].

France opens up access for wide use of Vertex's Orkambi

In a major decision, the French government recently authorized full reimbursement for Vertex Pharmaceuticals' cystic fibrosis drug Orkambi. This decision is expected to open up its use to an estimated 2,500 to 3,000 patients. France's decision comes after the decisions taken by the United Kingdom, which last month authorized reimbursement for all of Vertex's cystic fibrosis drugs [Source: https://www.biopharmadive.com/)].

€7.34m granted to Swiss based biotech MaxiVAX to develop cancer vaccine

Private clinical-stage biotech company MaxiVAX recently announced that it has secured a European Commission grant of €2,785,000, within the framework Horizon 2020 EIC Accelerator Program. In addition, the company also announced that it successfully closed a Series B2 round of funding for €4.55m. The Geneva-based biotech is focused on developing personalized anti-cancer vaccination [Source: https://european-biotechnology.com/].

Azeria Therapeutics granted €37.3m in Series B financing

Azeria Therapeutics Ltd, which is a Cambridge, UK based pharma has announced that it has raised a £32m (€37.3m) in a Series B financing. Azeria announced that it will use the funding to develop its preclinical FOXA1 inhibitor to Phase I clinical safety testing. FOXA1 is known for its functions in curbing the tumor growth, its progression and maintenance of oestrogen receptor positive luminal breast cancer. This is an area of significant need that patients need, where approximately 30% of patients progress to late stage endocrine resistant disease [Source: https://european-biotechnology.com/].

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