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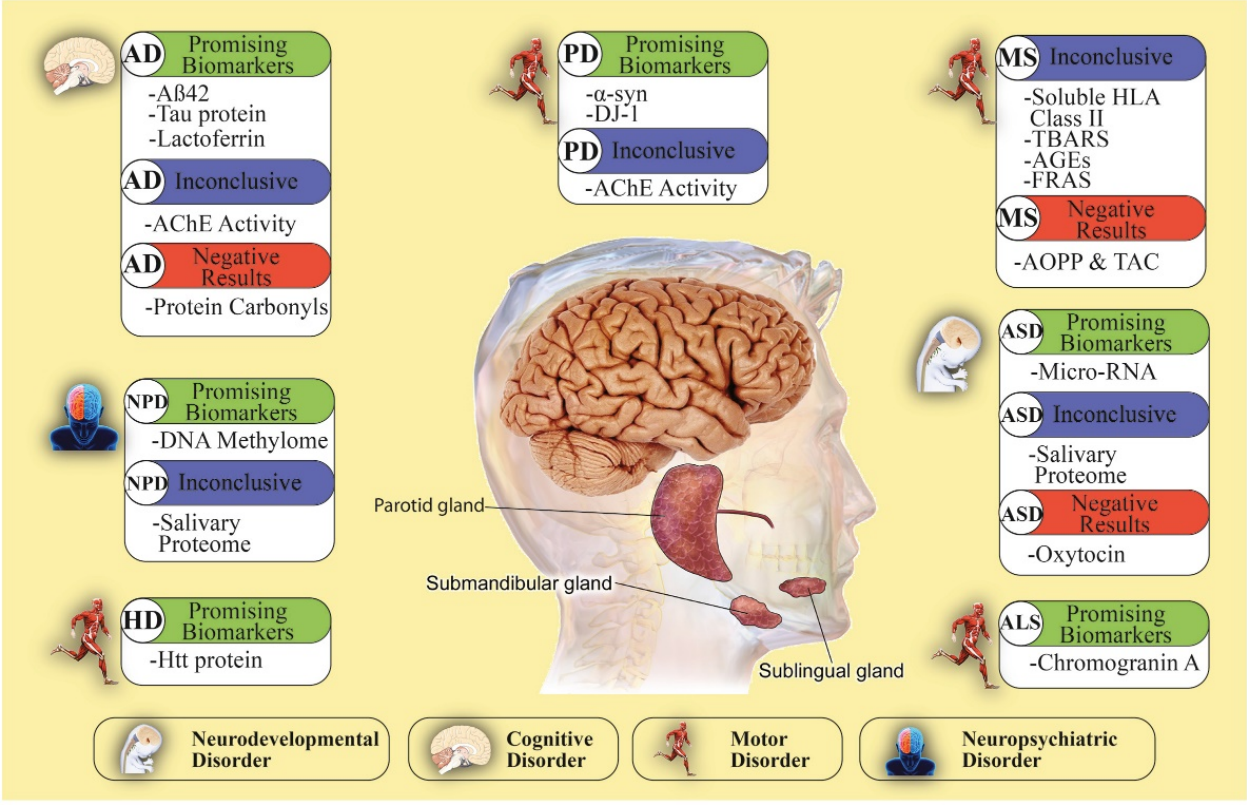

Featured Article

Topic: Molecular Diagnostics

Neurodegenerative disorder detection based on Bodily Fluids.

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From the Publisher's Desk

Welcome to Biotechnology Kiosk!

We are ready with the second issue of Biotechnology Kiosk. This issue includes editorials by experts in the cutting-edge areas of modern biotechnology – neuro, cancer, bio manufacturing and regenerative biotechnology. In addition, the regular sections on editor's pick on several latest biotech R&D innovations and breakthroughs along with industry news are also featured in this issue.

Our goal is to achieve excellence in quality of the contents for Biotechnology Kiosk and we will continue to strive and work tirelessly towards achieving it. We are pleased to report that we have received very enthusiastic support from our readers from different parts of the world. We do hope that we will continue to receive your kind support and patronage that will be the key to make this magazine

a most sought after reading destination for a wide range of readers with diverse backgrounds. We are very encouraged that our online viewers are rapidly increasing and there is a very promising trend of upward subscriptions and followers of the magazine. We now have an editorial board with new distinguished members for the magazine. This list will grow with more board members that will be included in the future.

We will continue to introduce new and innovative sections on news and views on all aspects of Biotechnology including popular features in the coming months. Also, we will go into print editions in the near future and will have both digital and print editions of the magazine to make a wider impact. Please do write to us with your comments and feedback. Your suggestions are always appreciated.

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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Molecular Diagnostics

By Navneeta Kaul, PhD

Contributing Editor

Neurodegenerative disorder detection based on Bodily Fluids

Early diagnosis and detection could limit the progression of several neurodegenerative diseases. However, clinical diagnosis and management have been a daunting challenge, due to the complex nature and manifestations of these diseases. Current diagnostic tests involve using invasive and painful tests including lumbar puncture or genetic analysis. Intense research efforts are underway to seek biomarkers that could allow early and easy detection of the neuro-disorders [1]. In this article, we discuss several promising bodily fluid candidates including Cerebrospinal fluid (CSF), Blood, Urine, Saliva and Tears for early diagnosis of several neurodegenerative disorders.

1. CSF Biomarkers

Examination of CSF biomarkers for their use in various neurodegenerative disorders is an exciting area of research. The clinical use of CSF biomarkers is currently most relevant to the diagnosis of Alzheimer's disease (AD). For AD, Classical markers are T-tau, P-tau and Aβ42. Published evidence demonstrates CSF Aβ42, CSF T-tau and CSF P-tau could be used as screening or diagnostic tools for AD [1] (Figure 1). Elevated levels of CSF Aβ42, and lowered CSF T-tau and CSF P-tau characterize AD. Recent studies show CSF Aβ42/Aβ40 biomarker ratio to be superior to individual constituents for diagnosis of AD with greater sensitivity, specificity, and accuracy [1]. Similarly, CSF T-tau/ Aβ42 ratio could be helpful to predict progression from preclinical to clinical AD. Studies also suggest that different forms of P-tau could have a unique role in identifying the pathology of AD [2]. However, further work is required to analyze the profiles of these CSF biomarkers in patients with AD. In another interesting breakthrough, scientists have identified biomarkers in CSF that are nonspecific to AD pathology. These biomarkers could signify further improvement in the efficacy and specificity for clinical applications. Some

examples include YKL-40, NFL, NSF, markers of neuronal damage and neuronal injury respectively [1, 3]. Intense ongoing efforts are on to identify other potential CSF biomarkers including vascular growth factors, CSF/serum albumin ratios, however further research is required.

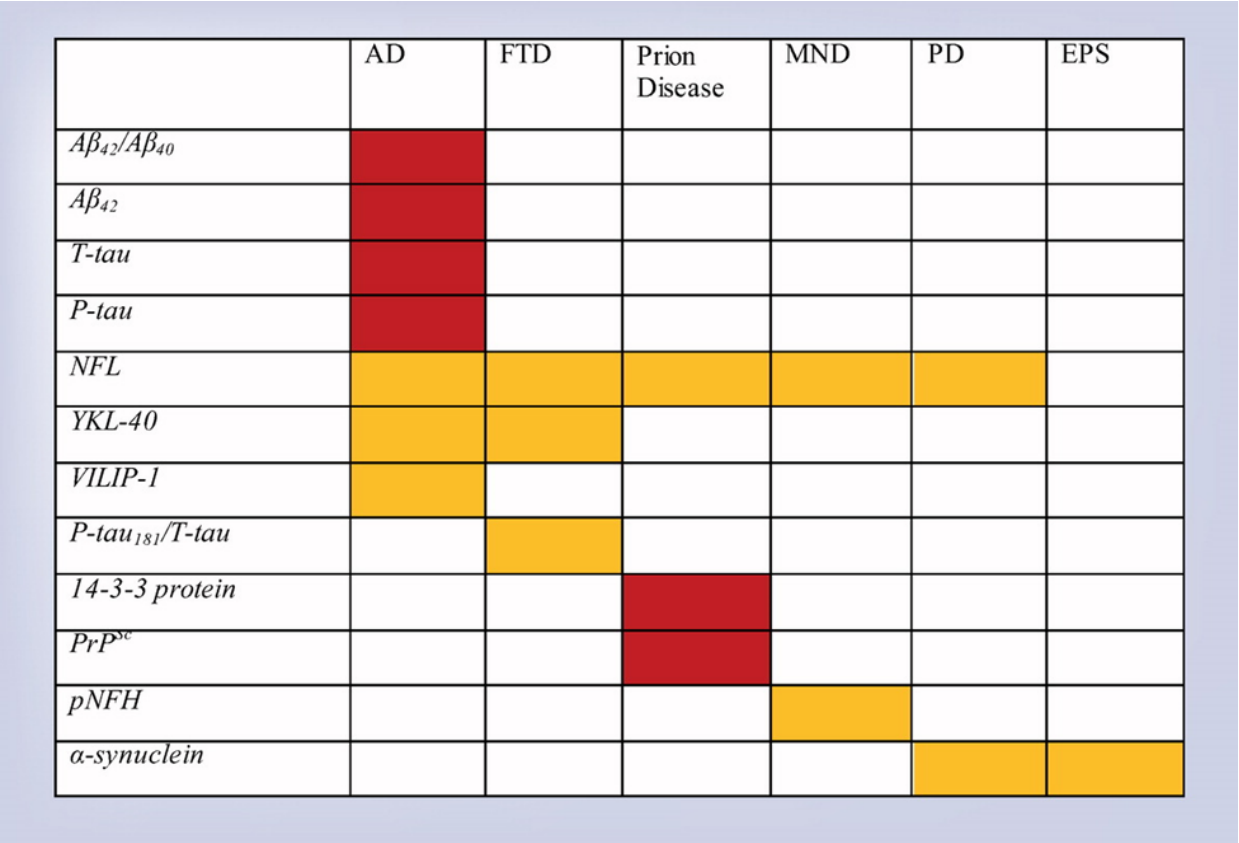

*Figure 1. Cerebrospinal fluid biomarkers in neurodegenerative disorders. Red = useful. Gold = promising, requires further study [**Source:** Future Neurol, 14 (1) 2019].*

It has been tough to find a specific biomarker for Frontotemporal dementia (FTD) due to its multiple clinical features and pathological subtypes. CSF P-tau/T-tau ratio has shown promising results as a useful biomarker for FTD [4]. Studies also suggest that a combination of YKL-40 and NFL could act as valuable CSF biomarkers [1]. Additional studies are required to further assess the applicability of these markers.

Researchers have developed a new technique called read time quaking-induced conversion (RT-QUIC), which is able to detect the presence of pathological prion proteins in CSF [5, 6]. This assay has revealed successful results in the diagnosis of sporadic prion disease, a fatal neurodegenerative disease caused by the formation and propagation of misfolded proteins. Ongoing research is focused on improving the clinical utility of the

traditional CSF marker 14-3-3 by combining with other biomarkers [1]. Again, additional studies are required to understand the clinical application.

CSF biomarkers have also been identified for motor neuron disease. The most promising results have been found with NFL, pNFH, lipids, and the Sonic Hedgehog protein [7, 8]. Studies have also met with some success in identifying some CSF biomarkers for Parkinson's disease (PD) and other extrapyramidal syndromes [1].

Taken together, more research is required to identify the proteome profile from patients for different combinations or ratios of CSF biomarkers for detecting neurodegenerative diseases. Thorough understanding of CSF biomarkers could help in developing personalized neurology for patients and aid in the clinical applicability in neurodegenerative diseases.

2. Blood

Over the past few years, efforts are on to identify novel blood markers to detect the underlying pathologies of neurodegenerative diseases, including AD, PD, and chronic traumatic encephalopathy (CTE). Blood-based biomarkers would mean less invasive testing, earlier diagnosis, quick and effective treatment [9]. With the advent of recent technologies, including Quanterix's Simoa (single-molecule array), detection of neurological markers in the blood has improved significantly [10]. Quanterix is able to screen patient samples with 72000 microscopic beads coated with a capture antibody specific for the biomarker of interest. The complex is then detected with a fluorescent marker with a high signal-to-noise ratio and increased sensitivity. Quanterix has successfully measured neurofilament (NFL), a biomarker for neuronal damage in blood for concussion, CTE and AD [11]. Another technology, Olink's Proximity Extension Assay (PEA), based on antibody probes linked to DNA tags, can detect about 1000 biomarkers in less than one drop of blood.

Recently, researchers using high-throughput multiplexed Xmap Luminex assay reported a set of eight plasma proteins (BDNF, AGT, IGFBP-2, OPN, cathepsin D, SAP, complement C4, and TTR) for AD diagnosis with high sensitivity and specificity [12]. Some of these proteins are implicated in the pathology of AD for the formation of Aβ fibrils, cell proliferation, and death in previous studies as well. Some other recent studies have identified biomolecules such as creatine, 5-hydroxycytosine, serine, phospholipids, myo-inositol, glutamate, *N*-acetylaspartate, blood dehydroepiandrosterone, vary with the progression of AD [13]. Further research is required to validate the results in a large independent cohort.

Moreover, researchers have also determined elevated levels of the P-tau proteins within neuron-derived exosomes (NDEs), isolated from plasma

[14]. Exosomes are released from neurons and represent a subtype of membrane vesicle for removal of excess proteins, shuttle cargo between cells. Various biomarkers measurable in NDEs including P-tau protein could accurately predict AD progression.

In another breakthrough research, scientists identified RNA blood biomarkers in several independent cohorts to rule out a high rate of misdiagnosis rate between PD and atypical parkinsonian disorders (APD) [15]. The team evaluated the diagnostic potential of nine previously identified RNA biomarkers in 138 blood samples from PD, PSP patients, and healthy controls. The success of these results has encouraged scientists to evaluate the results in a larger cohort and advance the biomarkers into the clinic. With intense ongoing research, detection of neurodegenerative diseases using non-invasive blood-based biomarkers will be useful.

3. Urine

Compared to other body fluids, urine remained mostly neglected for detecting neurodegenerative diseases. However, recent research provides evidence that urine is a promising candidate for effective biomarkers for diagnosis, and monitoring of various neurodegenerative diseases including AD, PD, multiple sclerosis (MScl, and transmissible spongiform encephalopathies (TSEs) [16].

Two recent studies in transgenic mice identified potential biomarkers for AD using different methods. Fukuhara et al analyzed the urinary metabolome of Tg2576 model of transgenic AD mice using NMR and identified 3-Hydroxykynurenine, homogentisate, and tyrosine as biomarkers of AD before the onset of dementia [17]. Moreover, 1-methylnicotinamide, 2-oxoglutarate, citrate, urea, dimethylamine, trigonelline, and trimethylamine were characterized as effective biomarkers of late-stage AD. Another study using liquid chromatography-MS (LC-MS) identified methionine, 5-hydroxyindoleacetic acid, desaminotyrosine, taurine, and N1-acetylspermidine were identified as promising biomarkers in the TgCRND8 model of transgenic mice [18].

Studies indicate that differences exist between the levels of several urinary metabolites between PD patients and healthy control human subjects. These metabolites are associated with fatty acid beta-oxidation, metabolism of phenylalanine, histidine, tyrosine, nucleotide, and tryptophan. The biomarkers include as hydroxylauroylcarnitine, phenylacetic acid, histidine, dihydrocortisol, and acetylserotonin [16]. Similarly, some studies have reported the alterations in urinary proteins in MScl patients versus healthy controls. The disease-related proteins may reflect abnormalities in the central nervous system and could indicate pathological changes in the brain.

Researchers have also identified promising biomarkers using samples

from patients or animal models of TSEs, a group of nervous system disorder associated with the aggregation of prion protein (PrPd). Moreover, preliminary observations report elevated levels of common neurotrophic receptor (p75) in urine samples of ALS patients compared to healthy individuals [19]. The studies indicate that urinary p75 could act as an effective prognostic biomarker of motor neuron degeneration for ALS. Taken together, urinary signatures might open doors to alternative approaches in the diagnosis of various neurodegenerative disorders.

4. Saliva: Concussion diagnosis

It turns out that biomarkers in the saliva could facilitate early diagnosis of a multitude of neurological diseases esp the cases of concussion or neuronal injury [20]. Traditional approaches to detecting neuronal damage rely on the identification of proteins in the blood. However, limited diffusion of these proteins across the blood-brain-barrier leads to a difficult diagnosis of concussion [21]. Therefore, smaller molecules specific to the neuronal injury response, that could easily cross the blood-brain barrier and capable of an easy measurement could provide valuable information to diagnose the disease.

Researchers at the Penn State College of Medicine have identified miRNAs, as ideal candidates for detecting and characterizing concussions [22]. miRNAs are abundant short, non-coding mRNAs that affect the expression of genes in response to different conditions including disease or injury [23]. The researchers predict that the miRNAs might be able to predict the presence and duration of concussions. They collected and examined the saliva from 52 concussion patients for the analysis of the miRNA expression. The team isolated five miRNAs from the patients with the prolonged symptoms of the concussion. Salivary miRNA represents an objective biomarker for diagnosis and management of concussion. Additional follow up studies with larger cohorts have yielded promising results and could be a valuable future point-of-care concussion tool.

Researchers have also detected several proteins implicated in the pathological development of neurological diseases in saliva (Figure 2). For example, a toxic oligomeric form of salivary alpha-Synuclein (α-syn) is higher in patients with PD than in healthy controls. Similarly, both Huntington and amyloid beta peptides, proteins implicated in Huntington's and Alzheimer's disease are capable of being detected in the saliva and could allow easy detection of these diseases [24]. Additional studies are required to verify and implement these early studies for a sensitive, specific, high-throughput, low-cost and portable salivary diagnostics.

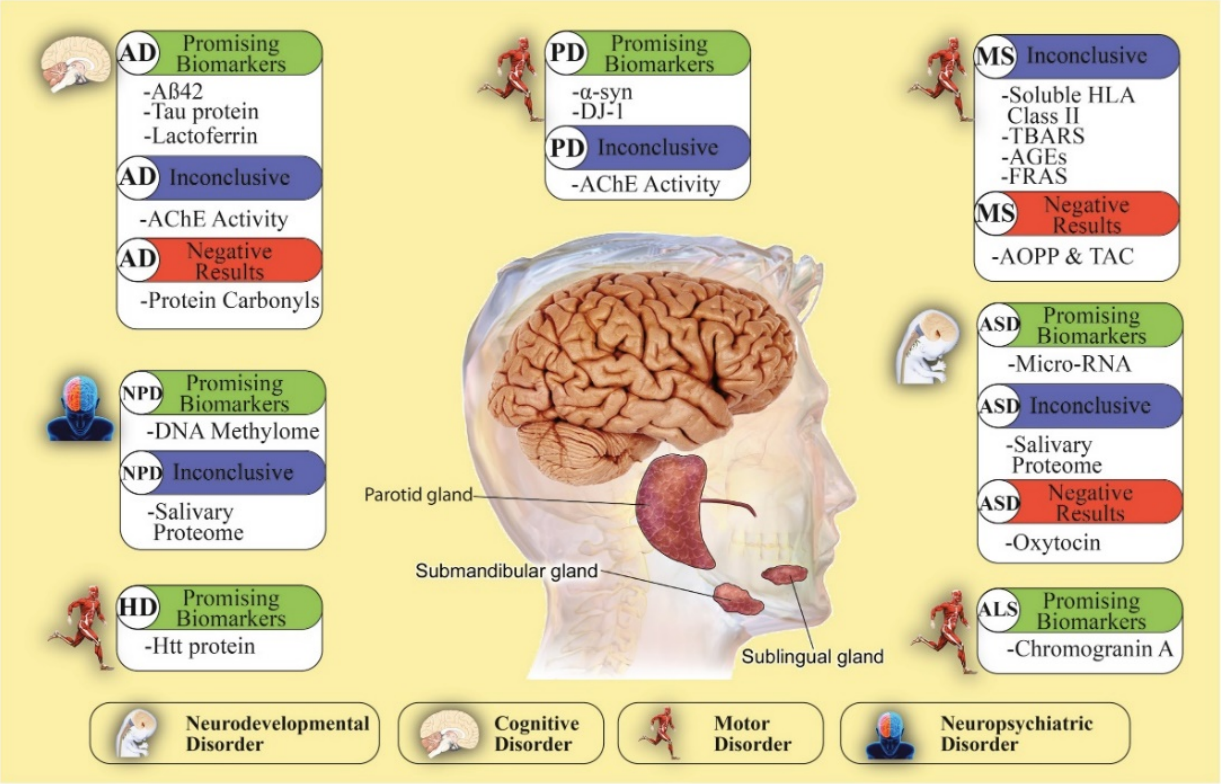

*Figure 2. Salivary biomarkers for the diagnosis and monitoring of neurological diseases [**Source:** Biomedical journal, 41(2), 2018].*

5. Tear fluid: Screening for AD and PD

Screening for AD, Parkinson's disease may become a part of the future eye-exam protocols as a means to improve our understanding and detection of these two debilitating diseases. Research indicates that tear fluid could lead to the characterization of neurodegenerative diseases [25]. Tear fluid, a part of the innate immune system, creates a chemical barrier at the surface of the eye and secrets various antibacterial and immunomodulatory proteins for inhibiting bacterial growth. Changes in the retinal morphology and blood flow in AD could alter the microenvironment of the eye leading to the alterations in the tear proteins (Figure 3).

Alteration in the tear flow rate, total tear protein concentration, and changes in the chemical barrier composition of tears specific to AD have been demonstrated [26]. Combination of 4 tear proteins lipocalin-1, dermacidin, lysozyme C, and lactritin exhibited 81% sensitivity and 77% specificity for AD diagnosis. Several miRNAs, namely hsa-miR-106, -153, -101, -29, -107 are implicated in regulating amyloid production and have been isolated in tear fluid [26]. Therefore, these miRNAs could have a profound influence on the early diagnosis of AD. In another research, a significant increase in the levels of total-tau and Aβ42 were observed in a cohort of 25 patients with AD. However, studies

with larger sample sizes are required to confirm the results.

Tear proteins could also facilitate the diagnosis of PD at different stages of the disease. Researchers from the University of Southern California identified an altered protein composition of basal and reflex tears in patients with PD versus healthy controls. They show that the tear levels of alpha-synuclein may act as a biomarker for PD diagnosis. Moreover, increased TNF-α levels were reported in tears of patients with PD [26].

Conclusion

Neurodegenerative diseases are hard to diagnose due to significant clinical overlap and lack of clinical symptoms. However, scientists have made breakthroughs in identifying and isolating several biomarkers from bodily fluids to achieve an early diagnosis to develop a personalized treatment to the patients. With the current advances, investigating biomarkers for neurodegeneration in the bodily fluids would bring us one step closer to find more disease biomarkers and potentially improve the quality of life for patients.

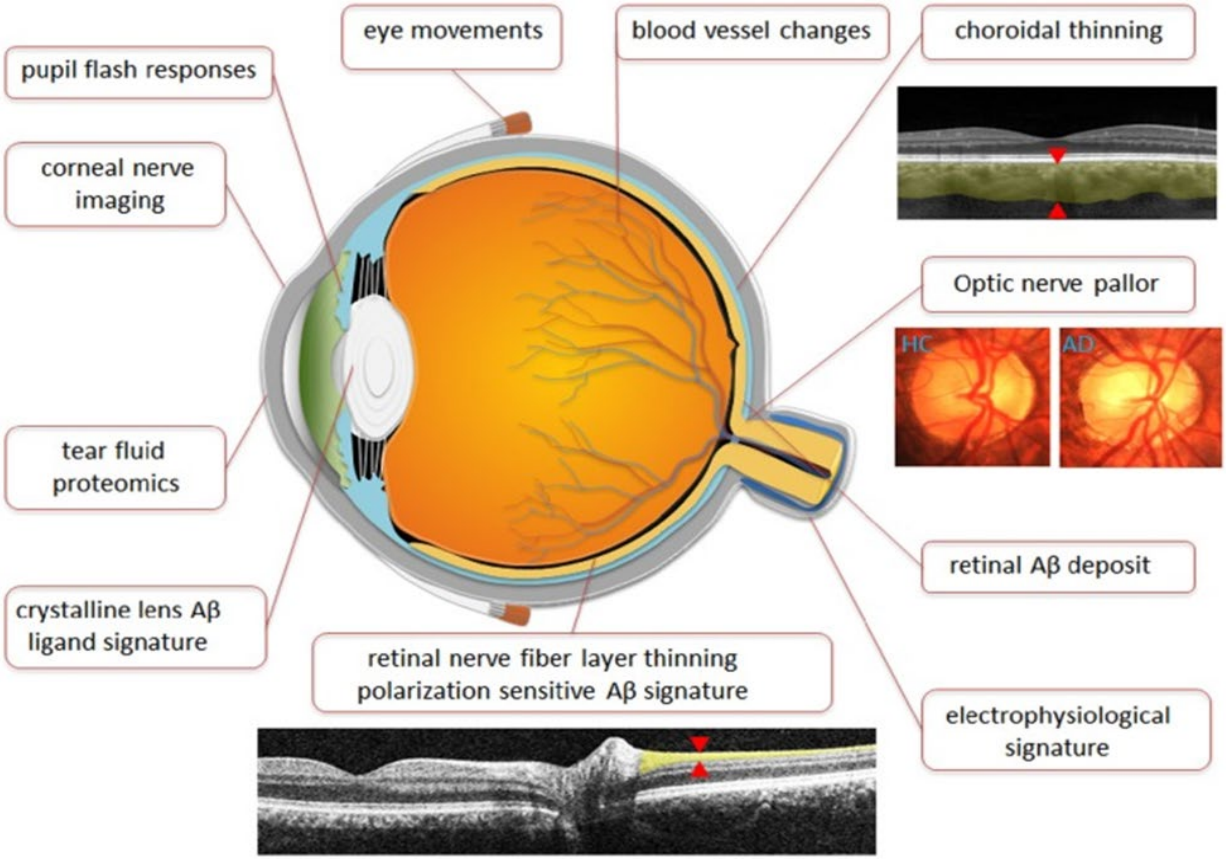

Figure 3. Ocular biomarkers which have shown changes in AD patients as well as potential future ocular biomarkers as marked by location in the eye. [Source: Front.Neurosci. 10, 536.2016]

Author's Biography

Dr. Navneeta Kaul holds a doctorate degree in biology from the University of Denver in August 2018. She works as a scientific consultant in the Biotech industry and is involved in regulatory and clinical affairs consulting. As a researcher, she has experience in biochemical and molecular biology techniques like cloning, PCR, real-time PCR, western blotting, immunoprecipitation, chromatogram analysis, live cell, and fixed cell imaging. She is passionate about communicating new technologies and research advances to a diverse audience. Dr. Kaul can be reached at navneetakaul@gmail.com

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Regenerative Medicine

By Shripriya Singh, PhD
Contributing Editor

Organ healers: Nanochips as the revolution in the field of regenerative medicine

Research and medicine are the two arms of science which have equipped human beings to surpass all other living species on our planet. On one hand research enables us to rediscover, invent and create new technology and on the other hand medicine allows treating, curing and saving lives. When technology amalgamates with medicine it spells nothing less of a miracle. Diseases that seemed untreatable a while ago are curable today and ones that were considered incurable are at least manageable today. The progress made in the world of medicine is undeniable and has changed the current face of human health. However, nature finds its own ways of challenging our existing amenities and resources. In order to combat the upcoming challenges in areas of health and medicine, researchers and medical practitioners are constantly looking for alternative remedies and potential therapies. One such challenge is the problem of organ regeneration and tissue healing.

Medical treatment not only requires the treatment of the diseased state but also involves the restoration of a patient to his/her normal healthy life. In this regard emphasis is laid on not only treatment but also healing. In certain cases of accident, traumatic injury, gangrene or septicemia, limb amputation becomes inevitable and the only means of saving a life. Such incidences not only cripple the patients but also have long lasting debilitating effects on their physical and mental wellbeing.

There has been an upsurge in the field of stem cell biology in the last two decades and it has revolutionized the world of medicine and therapeutics. Stem cell

based research is synonymous with regenerative medicine, organ transplantation therapeutics and drug development. Stem cells are unique in their ability of self-renewal, differentiation and can be directed towards a particular lineage conversion. Therefore it is clear that there is some dormant genetic machinery which can be triggered and activated by a stimulus and thus stem cells are manipulated to differentiate into a tissue or organ-specific lineages. Pioneer work by Yamanaka *et al.* in 2006 further proved that this stem cell differentiation is reversible and by controlling select transcription factors normal adult fibroblasts can be converted back to stem cells, which gave us the concept of induced pluripotent stem cells or iPSCs [1]. Overall the crux of stem cell uniqueness is housed in the stem cell genome. Therefore by manipulating the genetic transcription machinery and molecular switch it is possible for the scientists to decide the fate of a particular cell in the body. This kind of tissue reprogramming offers endless possibilities in the field of tissue regeneration, organ transplantation, limb restoration and healing.

Fast paced, one touch healing devices sounded like science fiction a while ago, but no longer!!!Thanks to the advances made in molecular biology, nanotechnology, stem cell biology and electronics we have a solution that was never earlier imaginable. A breakthrough Nanochip can heal injuries with just a single touch. Researchers at the Ohio State University have developed a novel technology which allows body to regenerate any type of cell through genetic reprogramming and this could lead a medical revolution in how we treat injuries and organ regeneration [2].

What is the Nanochip capable of? The technology and innovation involved:

The nanochip is a simple yet unique miniscule device designed around the novel concept of tissue nano-transfection (TNT). TNT is an electroporation based technique that facilitates the direct delivery of reprogramming factors (DNA) into the cytosol via the application of a focused and highly intense electric field through arrayed nanochannels. The electric field benignly nanoporates the cell membrane and the reprogramming factors are electrophoretically driven in [3, 4]. The process involves placing a finger nail sized nanochip (containing the required genetic material) on the patient's skin and zapping it with an electric current which drives the DNA through the nano channeled device into the skin cells. Thus the patient's own skin tissue acts as a bioreactor and the skin fibroblasts are genetically reprogrammed into specific type of cells that can be used in other parts of the body for grafting and healing purpose. The process of DNA delivery takes less than a second and the nanochip can be removed thereafter. This technique is by far the simplest, non invasive, topical, viral free deterministic tool of *in vivo* gene transfection and cellular reprogramming (Figure 1) [5].

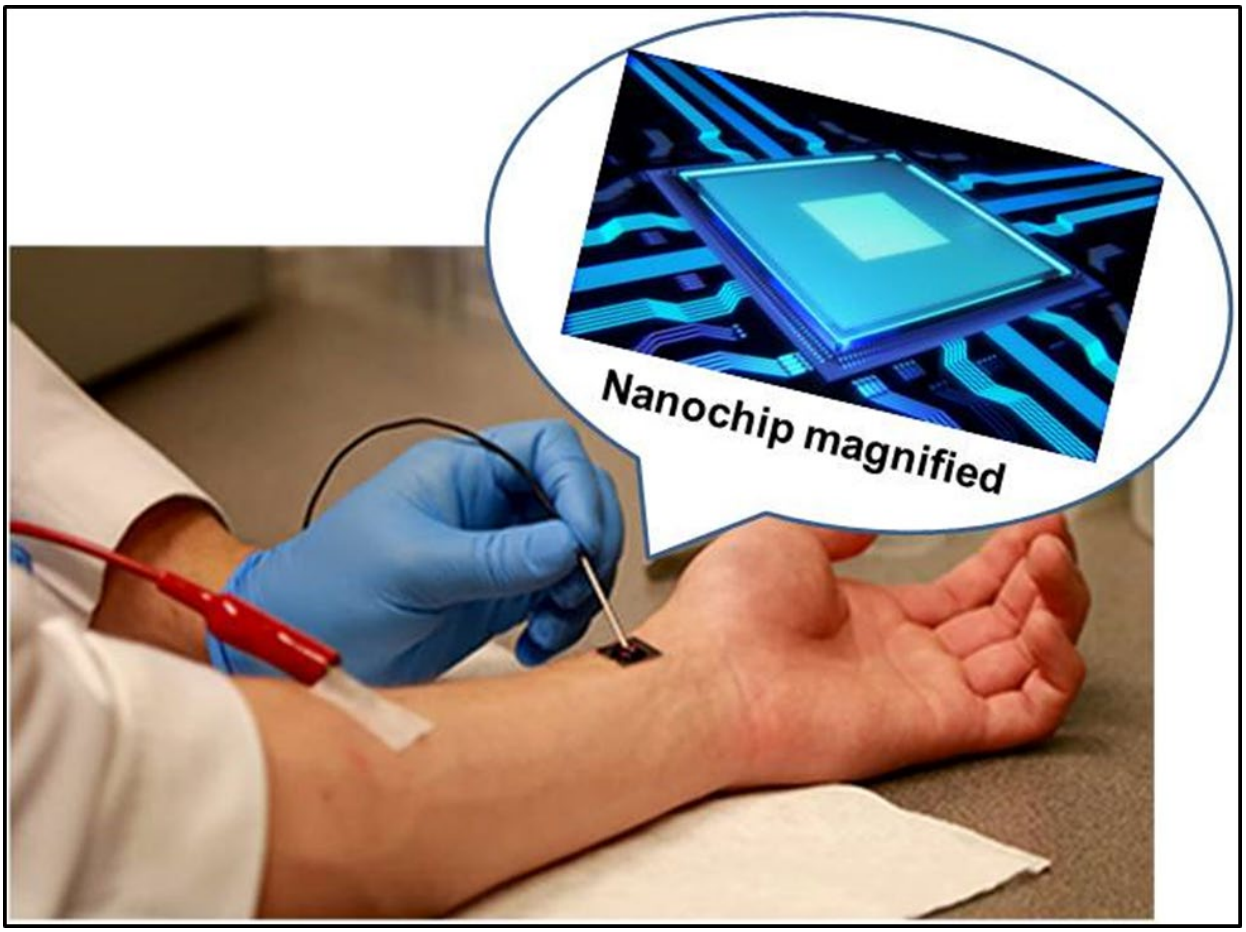

*Figure 1. Concept of the fingernail sized nanochip's direct application on patient skin for cellular reprogramming and tissue healing. [**Source:** Human Regeneration, 2017]*

The technology was initially tested on murine models. In one case the skin cells were successfully reprogrammed into neurons and formed new nerve cells in the legs of brain damaged mice. The induced neurons were then harvested and injected into the mouse brain to help with stroke recovery. Another experiment was conducted on a mouse with a severely injured leg, the skin cells were successfully reprogrammed into vascular endothelial cells and angiogenesis was observed which led to the formation of new blood vessels (Figure 2). The result was a successfully healed limb within two weeks of the application of the nanochip. Thus the technology successfully rescued necrotizing tissue and whole limbs in the murine models of brain damage and injury-induced ischemia [2]. The experiments when duplicated on pigs also furnished fruitful results. The technology is still in its nascent stage and human clinical trials are yet to begin.

*Figure 2. The organ healing potential of the nanochip device. [**Source:** https://www.knobbe.com/news/2017/08/nanochip-device-potential-heal-tissue-and-organs Original article source: Nature nanotechnology, 12(10):974. 2017]*

Once approved, The Walter Reed National Military Medical Center in Bethesda, Maryland, shall run the human trials.

The future prospects

The uniqueness of the technology lies in the fact that it can switch tissue function within the live body in the presence of immune surveillance of the patient itself and can produce autologous cells to rescue tissue damage locally or distally upon harvesting without eliciting any adverse immune responses. The beauty of the technology is envisaged in the fact that it requires no laboratory based procedures and can thus be directly used by medics in a war field, in an emergency room or even the doctor's office directly. If successfully tested in humans the technology holds potential to replace many surgeries, provide limb salvaging, prevent amputations and regenerate organs.

Since the technique can potentially regenerate the damaged brain tissue it could be the next possible therapy for the incurable neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease. The technology is also being looked upon as

the next medical revolution in the field of transplantation therapeutics and organ grafting. The nanochip can rescue the normal functioning of damaged organs and can restore the youthful functioning of aging hearts. The technology is speculated to become an indispensable amenity for military forces of the world where soldiers are in constant need of medical miracles. The technology can be used for treating military wounds and injuries and can be used as an effective treatment for traumatic brain injury and brains damaged by post-traumatic stress disorder. It is not farfetched to believe that the nanochip one day might speed up the healing process of fractured bones, wrecked vertebrae, sprained joints and ruptured spinal discs, thus changing the current face of orthopedics tremendously. Although still in its infancy the TNT based nanochip technology has shown promise and has unlocked limitless avenues in the field of regenerative medicine and organ healing. Thereby we optimistically conclude that if the research stands the test of human clinical trials successfully we are not very far away from achieving a medical and scientific milestone that sound miraculous today but will become a reality of tomorrow.

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Cancer Biotechnology

By Progga Sen, PhD
Contributing Editor

Liquid biopsy- a revolutionary tool in cancer therapy

Precision medicine is a gradually developing field that utilizes specific genetic information of patients to devise treatment methodology. Cancer is no exception, in a majority of the cases, there are genetic mutations involved in the incidence and maintenance of cancer in addition to external/environmental factors. Understanding the underlying genetic aberration(/s) in individual cancer patients can fine-tune their treatment strategies- can bring us one step closer to determining the right therapy for individual patients. Precision medicine is confined because of lack of enough knowledge of the molecular/genetic bases of different types of cancer; even individuals with the same kind of carcinoma may contain distinct, dissimilar gene mutations and dissimilar biomarkers. Current methods of treatments are more like 'one size fits all' and constitute primarily of chemotherapy with certain combinatorial drugs, with some minimal targeted treatment options (precision therapy). Precision medicine is picking up speed at present; however, the standard technique for determining the gene alteration/molecular basis of cancer is tissue biopsy.

Tissue biopsy is the core of determining underlying adverse genetic mutation for cancer incidence and progression, tumor growth monitoring, therapy response, and relapse. It has its shortcomings though- it is invasive, and the analysis takes a long time (several weeks). Since it is an invasive technique, it is somewhat tricky to perform follow-up analyses, cannot be pursued if the tumor is inaccessible, is potentially complicated, and cannot be utilized if cancer has worsened. The common types of tissue biopsy- encompassing surgical biopsy, endoscopic biopsy, bone marrow biopsy, needle biopsy, and skin biopsy- are being relied upon massively for monitoring cancer and devising the correct course of directed therapy. These traditional biopsy techniques also entail the risk of limited information (localized sampling)

regarding tumor heterogeneity- cancer cells are a heterogeneous population, and this dynamicity evolves during every stage of tumor progression towards metastasis. In addition to all these factors, there is an inherent risk of preserved tissue samples- a process that involves chemicals that may result in alteration of biomolecules in the tissue, thereby, adversely affecting actual results.

To overcome these hindrances, a plausible technique that can help in continuous analysis of cancer progression and treatment response, without being affected by the inaccessibility of the tumor, is a liquid biopsy [1]. A method of isolation/extraction of tumor-shed biomolecules, cells, and exosomes from body fluids, and their subsequent characterization to determine the transcriptomic and proteomic profile is known as liquid biopsy. Liquid biopsy is gaining popularity at present as a crucial tool that not only bypasses the problems mentioned above in cancer therapy; it is non-invasive too- one of the most significant advantages. Molecular genomic analyses and immunotherapy can be paired with a liquid biopsy from patients within a much shorter time in comparison to traditional tissue biopsy, as evident from current literature. Different types of body fluids can be utilized as sources of the biopsies- blood, urine, cerebrospinal fluid (CSF) and even saliva; blood is most commonly used among all.

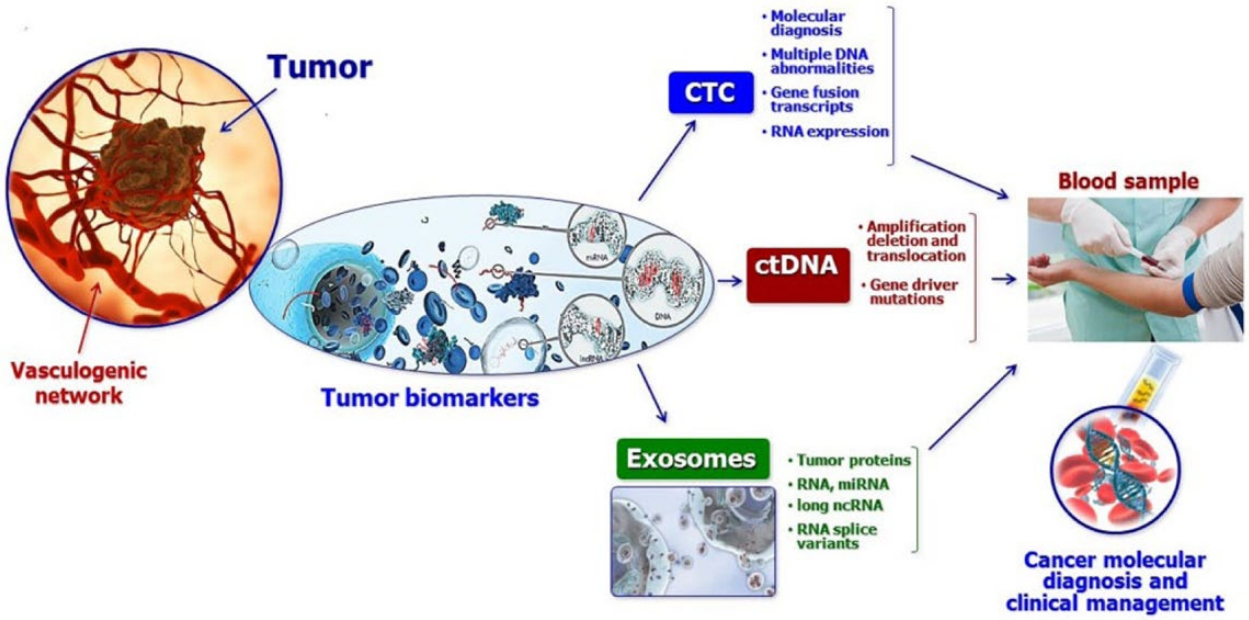

*Figure 1. Types of liquid biopsy samples and their possible applications. CTCs, ctDNA and exosomes are the major types of biomarkers that can be obtained from liquid biopsy from cancer patients' blood, urine, saliva, CSF, etc. [**Source:** Ther. Adv. Med. Oncol. 10, 2018]*

The components that are characterized by blood sample biopsy are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes. These biomarkers are analyzed using myriad techniques to either determine tumor-specific DNA mutations, or cell surface receptors on tumor cells or for understanding the epigenetic cues of cancer progression. Initial investigations were more restricted to determining the number of CTCs that radically increases with tumorigenesis. This technique has further given way to characterization of the biomolecules on and within the cells- DNA and specific proteins/ protein receptors. The continual shedding of cells, DNA, and vesicles by primary and metastatic tumors makes it convenient for the seamless isolation and investigation of these biomarkers. Various techniques have been used to isolate the CTCs from the bloodstream of cancer patients.

Circulating tumor cells (CTCs)

First reported in 1869, these cells generate secondary tumors and therefore, cause metastasis from primary tumor because of their body fluid-mediated mobility. The tumors shed the CTCs because of high turnover of cancer cells. The CTCs possess highly diversified surface proteins/receptors that lead to their heterogeneity at and across different stages of cancer, they are also rare in the bloodstream of patients- requires highly sensitive and specific detection and isolation techniques [1]. Over the years, various research groups have worked towards refining the detection methods of the heterogenous CTC population in tumor-afflicted patient blood samples. Currently, the standard techniques are based on the biological properties of these cells- surface antigens/receptors, or their physical features, including density, size, and inertia. The biological sorting and detection depend on using specific antibodies against specific cancer-type dependent surface proteins, and sometimes, the immunoaffinity is combined with magnetic separation of the antibody bound CTCs [2, 3]. CellSearch, the only FDA-approved CTC detection kit utilizes this immunomagnetic technique and has successfully isolated CTCs (epithelial origin) that are EpCAM+, CD45- (leukocyte marker), CK19+, besides other cell surface antigens. This kit has been successfully used in isolating CTCs in clinical trials for breast cancer, colorectal cancer, and prostate carcinoma. Immunostaining of the CTCs follows their immunomagnetic detection and helps in differentiating the blood cells from the CTCs, thereby enumerating them amongst the leukocytes. Besides the immunomagnetic method, high throughput imaging, leukocyte depletion, density/size (the CTCs are bigger compared to blood cells), and adhesion-based isolation methods are used to detect CTCs. More sophisticated technologies are developing that not only enumerate the CTCs, but also characterize the biomolecules inside them- DNA, RNA, vesicles, and proteins.

CTC-iChip, that uses a microfluidic platform approach and combines both negative and positive selection of the CTCs. Driven by three principles of isolation- blood sample debulking using lateral displacement, inertial focusing of the debulked nucleated cells, and positive or negative selection of CTCs by immunomagnetic detection or depletion of the white blood cells (WBCs) respectively, this technique overcomes certain crucial shortcomings of CellSearch- higher number of detected CTCs and ease of downstream expression (protein and RNA) analysis. A clinical study on prostate cancer analyzed the mRNA expression pattern to determine the cause of cancer progression and drug resistance among castration-resistant prostate cancer (CRPC), using this latter technology. The level of DNA, RNA, and protein expression can serve as rich sources of information about the disease stage, recurrence, overall survival of the patients, and drug efficacy. There are several other advanced CTC isolation technologies available commercially now, and almost all of them provide a good range of downstream analysis of the isolated CTCs, encompassing the specific marker protein upregulation/downregulation and point mutation study.

Circulating tumor nucleic acids (ctNAs)

Constituting of tumor-derived DNA and RNA, these biomarkers are an essential category of cancer prognosis factors [2, 4, 5]. The tumor cfDNA fragments have an average length of about 150 bp, constitute of only about 0.1% to 10% of the total cfDNA in circulation, and have a shorter lifespan in comparison to normal apoptotic/necrotic cell-shed cDNA. These DNA molecules are believed to have metastatizing potential, as reported in several studies, including colorectal cancer and colon cancer. Like CTCs, an increase in the number/amount of these tumor-shed cfDNA signifies poor prognosis and thereby poor overall survival. Most of the pre-clinical and clinical studies currently use blood as a source of ctDNA for extraction and analysis. Isolation of the tumor cfDNA is based on the following principles- magnetic separation, electrical force, column-based affinity, fluorescent probes, or filtration [6]. Of late, nanotechnological advancements have aided in isolation of pure ctDNA from the blood plasma of breast and lung cancer patients. Scientists and clinicians use either these nano-tech methods or available commercial kits and can investigate the whole genome analyses by omic approaches; they also study specific genes for their expression levels, mutations and epigenetic signatures like methylation pattern. The various methodologies include RNA-Seq, NGS technologies, digital PCR (dPCR), peptide-nucleic acid-locked clamp PCR (PNA-LNA), allele-specific PCR, and others; all of these techniques have their advantages and limitations, however, combining multiple methodologies provides a more comprehensive picture

of the tumor microenvironment [7]. Sorensen et al. used the tumor cfDNA isolated from plasma to investigate specific drug-resistant mutations in the EGFR gene by allele-specific PCR, Oxnard et al. utilized digital PCR to isolate the exon 19 deletion patients of the advanced NSCLC, and Sozzi compared the cfDNA levels in NSCLC patients with healthy individuals using the plasma-derived cfDNA fragments. So, similar to the CTCs, the ctDNA is an exhaustive source of information that is pushing cancer therapy, disease monitoring, remission, and/recurrence to new heights. In spite of providing a wealth of knowledge to help the clinicians and medical professionals, tumor cfDNA extraction has an inherent variation in extraction amount among different samples; the investigation on finding the known mutations in various cancers cast a shadow on the versatility of the cfDNA application too- it lacks knowledge about possible new mutations associated with the cancer because of narrow target region. Therefore, further refining of techniques is underway to improve uniformity of extraction and a broader gene interrogation region has been added to focus on a genome-wide scale to generate a vast repertoire of the associated mutations (digital karyotyping and personalized analysis of rearranged ends/PARE). Newman et al. investigated plasma obtained tumor cfDNA for sets of mutations on a genome-wide scale-variations that are common in NSCLC. Though comprised of small sample size, there was a perfect correlation of cfDNA result with stage II, III, and IV patients.

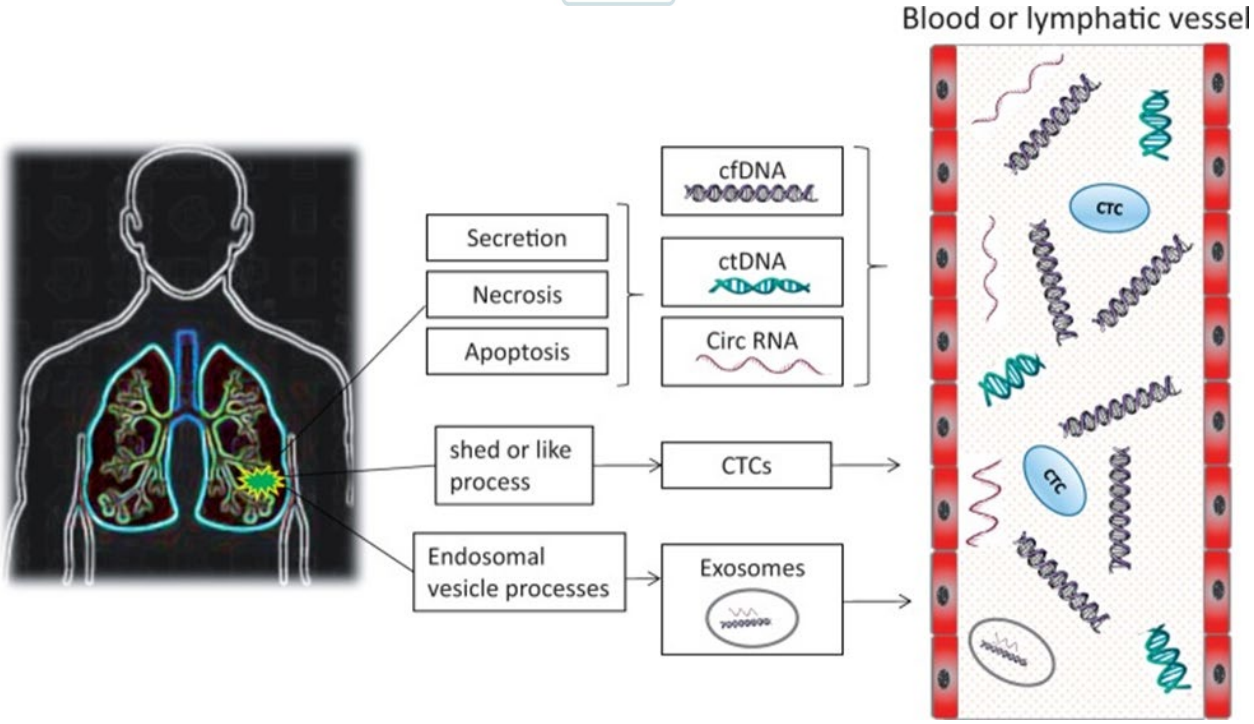

Figure 2. Sources of ctDNA. Healthy cells, necrotic or apoptotic cells can shed cfDNA. The cfDNA can also secreted passively into the bodyfluids. However, tumor cells shed cfDNA (called ctDNA) in circulation at a much higher rate than normal, as a result of incomplete processing and rapid tumor cell turnover. Exosomes also serve as a source of ctDNA in cancer patients. ***[Source:*** *Experimental Biology and Medicine,* 243(3):262-271, *2018]*

As biomarkers, microRNAs(miRNAs) and long non-coding RNAs (lncRNAs) are pivotal to cancer progression, since tumor growth and spread require upregulation of oncogenes and gene expression machinery, while simultaneously downregulating tumor-suppressing genes such as p53 [2]. In addition to the circulated RNA in the body fluids, exosomes can carry the RNAs to a destination- a secondary or tertiary site of the tumor. The lncRNAs are crucial for regulating gene expression: mRNA splicing and stability. miRNAs regulate the expression of about 30% of human genes (protein-coding genes) and there are reports of non-exosomal miRNA in the bloodstream that become stabilized by binding RNA-binding proteins or high-density lipoproteins. Therefore, these non-coding RNAs are a promising biomarker for understanding tumor growth as well as treatment outcome.

Exosomes

A vital mode for intercellular communication between cells, the exosomes carry biomolecules including membrane and non-membrane proteins, microRNAs, long non-coding RNAs, single or double-stranded DNAs, and lipids [1, 6]. These players are emerging as key drivers in chemo-resistance and they mediate tumor angiogenesis, proliferation, and metastasis. Abundantly found in patient body fluids like plasma, saliva, ascites, and urine, the exosomes are known to contain a conserved set of the membrane and cell surface proteins, namely CD9, CD81, and CD63, though studies from different groups show cell-type or tissue-type specific protein content of exosomes. Traditional methods of extraction involving size-dependent filtration has given way to a more sophisticated methodology- the centrifugal microfluidic platform (microfluidic centrifugal nanoparticles separation and isolation or μCENSE) that reduces the total time of exosome extraction considerably. Immunocapture, with antibodies against CD63 or CD4 are also in use to extract exosomes from human sera that can be successfully utilized for downstream analyses.

Further technological upgrades have now added the use of electrical field in exosome isolation. Simultaneous detection, enrichment, and investigations are now possible by commercially available ‘on-chip’ that combines immunocapture and electrochemical signaling. Various investigators, including Doldan et al., Wang et al., and Ko et al. have refined this advanced technique further by enhancing the sensitivity of the detection system by

several folds. For analyses of the contained biomolecules, the isolated exosomes are tested for the presence of marker proteins; genome-wide analyses are also performed to obtain a bigger picture of the tumor spread and/therapy response in patients.

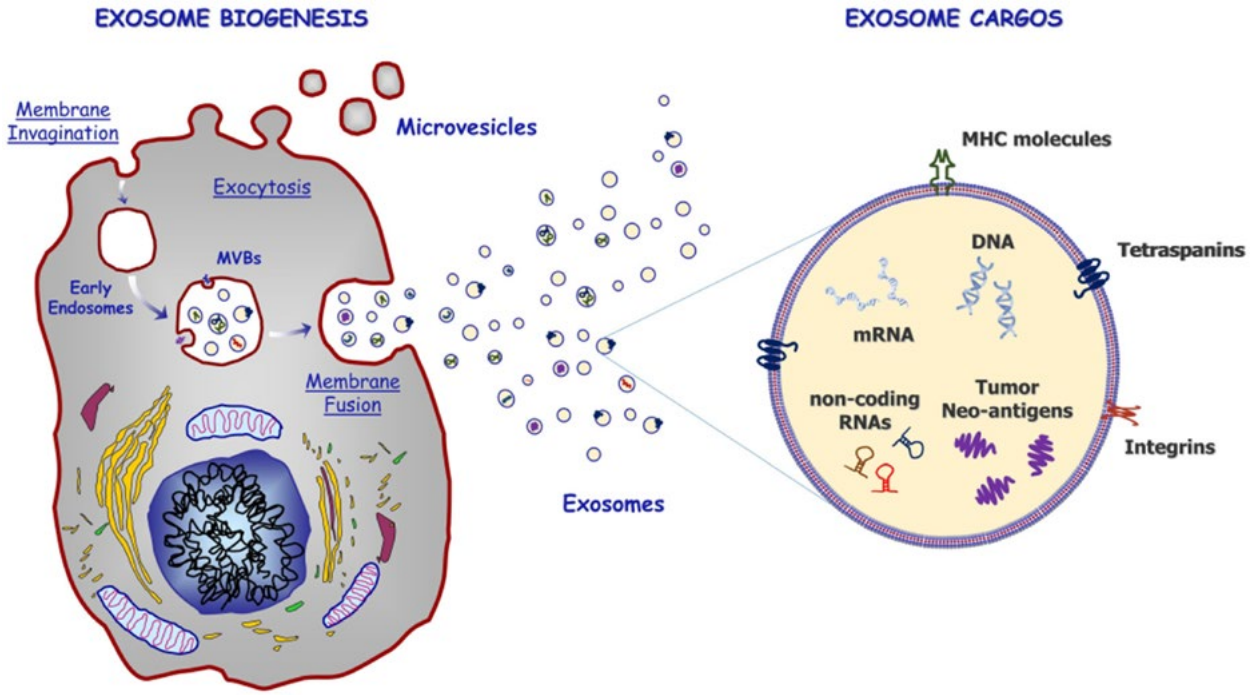

*Figure 3. Exosome biogenesis and content. Originating as an inward invagination of the plasma membrane, the exosomes are the end-products of the recycling endosomal pathway. They can be a rich source of various tumor-associated biomolecules- miRNA, ctDNA, mRNA, etc. [**Source:** Ther. Adv. Med. Oncol. 10, 2018]*

It is thrilling to be able to study cancer- a debilitating and fatal disease- at the ease of a blood draw or collecting a urine sample from the patient [8, 9]. This remarkable advancement is a step closer to devising patient need-specific treatment strategies, something highly relevant in cancer therapy. Constant evolution of cancer markers throughout its growth and presence or absence of similar markers in different patients with the same cancer type, are two of the biggest hindrances on the path of recovery from cancer. Affordability, accessibility, shorter turnover time of detection, and analyses are all contributory to the central idea of personalized precision medicine. Though the liquid biopsy technologies prevalent at present are substantially informative, they still have to be combined with established tumor detection and analytical procedures that include imaging and tissue biopsy; the sensitivity and specificity can also be concerns, depending on the biomarker in question. Therefore, it is imperative that clinicians and pre-clinical researchers invest their resources extensively to

improve this hallmark tumor monitoring method- liquid biopsy- to develop it as a comprehensive tool for cancer detection and monitoring technology soon.

Author's Biography

Dr. Progga Sen is a postdoctoral research fellow at the Stanford University School of Medicine, working at the Veterans Affairs Health Care System Palo Alto. She focuses on understanding the mechanism of substrate-mediated regulation of long-chain fatty acyl-CoA synthetase family member 4 (ACSL4), her research has implications in liver disorders including non-alcoholic fatty liver disease (NAFLD) and non-alcoholic hepatosteatosis (NASH). Besides research, she has a strong inclination towards writing engaging blogs and articles. Recently, she published her blog on the award-winning blog website SCOPE (published by Stanford School of Medicine). Progga completed her Ph.D. at the Wayne State University from the Department of Biology and has presented her research at several annual symposia and research retreats during her Ph.D. Her keen interest in science communication and education has been reinforced by her appointment as a Graduate Teaching Assistant. She intends to work towards improving human health and quality of life. To that end, she is leveraging her extensive academic background and strong communication skills to successfully convey scientific discoveries and advancement tailored for intended target audience. She can be reached at progga.sen@gmail.com

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Bio-manufacturing in Medical Devices

By Sathyanarayanan Sridhar, PhD
Contributing Editor

The Promise of Additive Manufacturing in Biotechnology: A New Era of Biomanufacturing in the Medical Device Industry

The latest advances in biomanufacturing are creating new exciting avenues in research and developments in modern biotechnology for applications in next generation implantable organs and medical device industry. 3D printing or additive manufacturing (AM) is one such initiative in biomanufacturing. AM comprises of a versatile, growing array of technologies for generating 2D or 3D materials that are synthesized layer-by-layer, which deposits, fuses or builds layers of materials [1]. AM has come a long way from being a prototype builder to a thriving manufacturing technique that has the potential to take over the future medical device industry (Figure 1). Currently, there are many US Food and Drug Administration 'FDA' approved 3D printed medical devices including surgical instrumentations (e.g., guides to assist with proper surgical placement of a device), implants (e.g., cranial plates or hip joints), and external prostheses (e.g., hands) [2]. To this end, industrial and lab scientists are working together to push the envelope to develop fully functional organs with all necessary tissues and subcellular entities embedded in it. Few of them have reported success in building a prototype [3, 4].

Recently, researchers reported the first 3D printed heart with vascularized tissues and chambers. In this work, researchers demonstrated manufacturing of the artificial organ with human cells and patient-specific biomaterials containing sugar and proteins as bio inks. It was mentioned that this 3D printed heart had similar biochemical, mechanical and topographical properties of the patient's own tissues (Figure 2) [1]. However, the 3D printed heart has yet to overcome the limitations of pumping and functioning mechanically like a normal heart [3]. In a related work, another research group reported prototype fully functional heart. The prototyping phase of organ 3D

printing was reported with the potential to transition to clinical trials and practical applications with further research and developments of such 3D printed heart. Researchers are working to address the challenges that are associated with the development, manufacturing, sterility and packaging and regulatory approval.

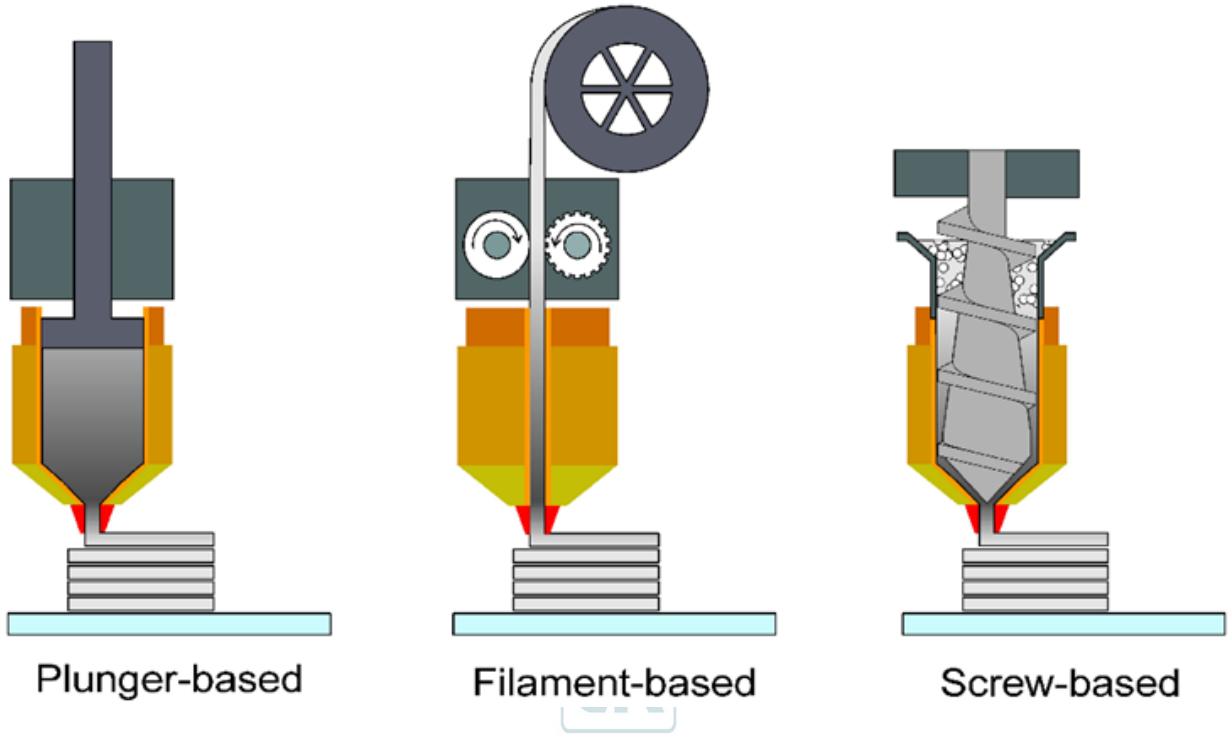

*Figure 1. The Additive Manufacturing Process. (**Source:** Materials* 11(5), 840, 2018*].*

Despite the challenges, AM based biomanufacturing is believed to impact the field of biotechnology quite significantly in the future. The main reason is that there are no major regulations that are required in general, in adapting 3D printing for biotechnological applications. We will describe here the major applications for AM in this field that are related to bioprinting of complex cellular co-culture constructs, advanced bioprocess engineering and development of task-specific AM-enabled microfluidic systems [5].

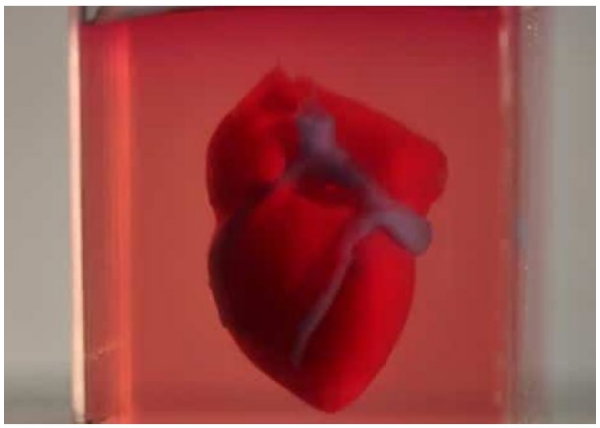

*Figure 2. 3D printed heart by additive manufacturing [**Source:** Advanced Science 2019].*

3D Bioprinting for Printed Tissues and Organs and a Whole Host of New Applications in Modern Biotechnology

Bioprinting involves controlled deposition of acellular functional scaffolds incorporating biological components or deposition of cell-laden constructs recapitulating *in-vivo* process. In addition to the development of printable tissues and organs, there are a number of other possible applications of 3D printing that include(i) printed tissue mimics that can function as disease models, (ii) acellular constructs functioning as structured scaffolding for bone grafting materials, (iii) development of high throughput assays and drug discoveries, (iv) pattern drugs and factors toward the development of printable medicines, (v) bioactive coatings, (vi) affinity membranes and (vi) high throughput screenings. A recent research has reported a technique known as 'Green Bioprinting' in which patterned human/algal co-culture scaffolds are fabricated by multichannel- plotting. This co-culture has been suggested to serve as the basis for new therapeutic approach to overcome the lack of oxygen in human cell-lines during *in-vitro* tissue engineering. This technique could also be extended to plants *in-vitro* cultures for cascaded bioprocesses using the metabolic properties of different cell types, or to establish model systems for studying biofilms or symbiotically living species [5, 6].

Additive Manufacturing Brings Hope in Advanced Bioprocess Engineering

AM can play an important role in the bioprocess engineering. The latest developments are summarized here. Mainly, there has been several studies, which have reported possibilities of making multifunctional bioreactors through different 3D printing techniques. Selective laser sintering (SLS) fabricated microwell plates made from polyamide, which could cultivate *S. cerevisiae* and adherent/suspended human cells. A smart 3D designed shake flask caps known as "shake flask pH controller unit" (SFC) was fabricated by SLS technique. The SFC included materials-integrated channels, membranes and valves as well as controlling electronics and piezoelectric micropumps, to close the control loop by pumping sodium hydroxide (NaOH) or hydrochloric acid (HCl) into the culture broth. The SFC was successfully used in a controlled shake flask cultivation of E. coli K12, during which it maintained a narrow pH range in the culture medium. A novel rocking bioreactor for fermentative hydrogen production that features internal AM-fabricated porous cartridges made from polylactic acid with REPRAP 3D printing platform. The cartridges were filled with immobilized microbial communities and were designed to increase the cells' exposure to the culture medium by acting as baffles and facilitating mixing. The tailor-made reactor system exhibited a higher peak hydrogen production than a conventional production system. A modular reactor setup consisting of porous monolithic enzyme carriers made from VeroClear RGD810 (PolyJet®) and

an AM-fabricated housing with integrated fluid distributors. The 3D-fabrication of porous columns and monolithic structures could be also useful as an analytical system in biotechnology. The MicrOLED-PBR is a miniaturized flat-panel airlift-PBR (FPA-PBR) with an overall volume of 15 mL and several useful features. The FPA-PBR was made from polyamide using the SLS technology and includes a sensor board containing optical elements (LEDs, photodiodes and control electronics) for non-invasively monitoring cell-specific process parameters such as the cell density and chlorophyll fluorescence. The MicrOLED-PBR enables rapid strain screening and small-scale bioprocess development for photobiotechnological processes. This represents the first step towards a new generation of tailor-made AM fabricated bioreactors [5].

Additive Manufacturing Assisted 3D Printed Microfluidic Devices in Biotechnology

Microfluidic devices are miniaturized structure, which facilitated the analyses of fluid in the microliter (μL) to milliliter (mL) range. They were used as lab-on-a-chip and micro total analysis system (μTAS) with multiple application in the field of biomedical engineering, biotechnology, environmental sensing and analytical techniques. Fabrication of microfluidic systems has been performed by lithography and soft lithography techniques using glass and silicone as the materials of interest in building these systems. However, this particular fabrication procedure is time consuming and expensive. AM can be employed to enable the microfluidic based fabrication technology to overcome the shortcomings in three different ways. They are as follows: (i) In general, soft lithography technique involves development of a mold which is later used to make the microfluidic system of choice. AM has the capability to eliminate the need for the mold preparation as it can create a structure with micro-channels. Not to forget the fact that AM itself can be employed to fabricate molds with more precision compared to traditional manufacturing techniques. (ii) AM-assisted 3D printed microfluidic process allows one-time fabrication capability to complete the whole structure of the system over a single manufacturing process and an equipment. However, the exception is with the lithographic techniques where additional steps are required, for example, an extra step is required for the development of molds in the process of soft lithography. (iii) AM-assisted process also allows the possibility of including device specific active components such as pumps and microlense, and passive components such as droplet generators, microcapillary emulsion generators, functional microfluidic valves, chip interconnectors or flow meters [5].

Researchers developed an immunomagnetic flow assay based on antibody-functionalized magnetic nanoparticles for capturing and quantifying Salmonella bacteria in water samples and E. coli from milk,

respectively (Figure 3). Separately, a Czech group used the FDM/FFF process with ABS or polylactic acid to fabricate a number of similar bio-analytical microfluidic devices for detecting the influenza virus, methicillin-resistant Staphylococcus aureus, and nucleobases in hydrolyzed samples. Recently, Dolomite Microfluidics launched the Fluidic Factory, which became the first commercial available AM device for fabricating tailor-made microfluidics from a cyclic olefin co-polymer.[5]

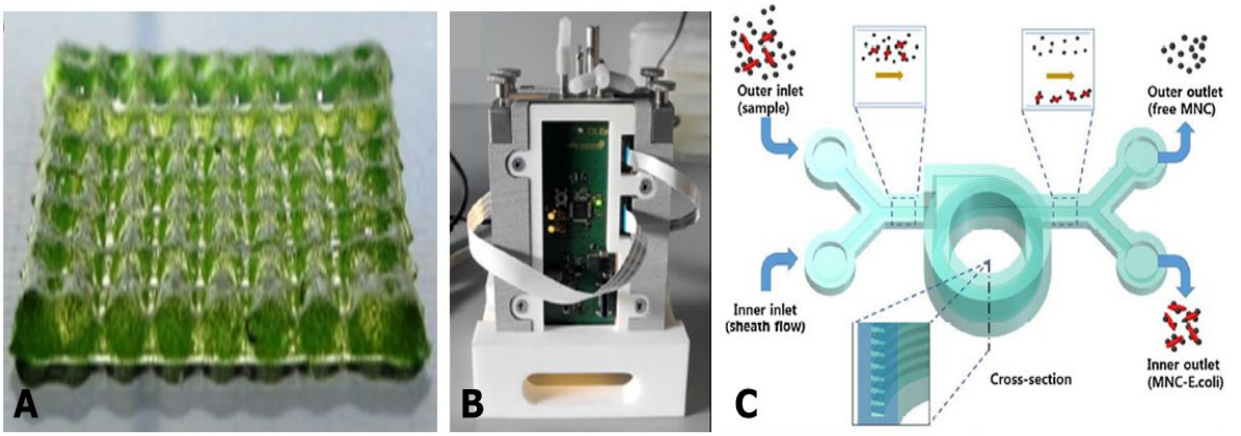

*Figure 3. AM-assisted microfluidic biomanufacturing (A-C): (A) Photosynthetic active cell clusters are visible within the 3D extruded hydrogel environment; (B) MicrOLED-PBR with combined electronic and structural design; (C) 3D-designed microfluidic device for capturing bacteria by inertial focusing (MNC = magnet nanoparticle clusters) [**Source:** N Biotechnol. 2017].*

Conclusion

This article gave a brief overview into the possibilities of leveraging AM for biotechnological applications in developing next generation medical devices and printed organs. Even though most of these reported applications are still in their research phase, commercialization of these techniques will be significantly easier and it is believed that organs can be developed with lesser risk with respect to their end use. The ability of AM to cut down fabrication cost, labor, develop multifunctional microfluidic systems and bioreactors, and create complicated co-cultures will be invaluable in providing a new impetus to the field of biotechnology in the future.

Author's Biography

Dr. Sathyanarayanan Sridhar holds a PhD in Bioengineering from the University of Texas at Dallas. He has more than five years of experience in the medical devices space. As a part of his PhD dissertation, he has developed novel testing protocols to understand the material performance of commercial titanium and zirconia dental implants. In addition to his research expertise, he is a

passionate science communicator. He has authored/co-authored a number of peer-reviewed publications and a book chapter. He has been invited to present his research outcomes at prestigious international conferences organized by National Association of Corrosion Engineers (NACE), Society for Biomaterials (SFB), International Association of Dental Research (IADR) and American Society for Microbiology (ASM). He runs a newsletter titled "Medtech Outtakes" which provides snippets of recent trends in the medical device industry. Currently, he is part of a "Medical Innovation Fellowship" program offered by WORLDiscoveries at the University of Western Ontario in Canada. In his free time, he loves to hit the trails and enjoys learning music. Dr. Sridhar can be reached at sathya.sridhar1389@gmail.com

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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.

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Executive Editor

Dr. Shyamasri Biswas
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Computational Biotechnology

Artificial designer protein for smart cell therapies

In a breakthrough discovery, researchers have designed an artificial protein on a computer and then synthesized in the laboratory. The artificial protein that has unprecedented potential can be used to build advanced biological circuits by employing transformative biotechnology tools inside living cells. These breakthrough biological circuits have exceptional abilities that can be leveraged to transform ordinary cells into super cells with smart functions. A research team of bioengineers in the US described this designer protein, formally known as the latching orthogonal cage-key protein, or LOCKR or more specifically, degronLOCKR, in a pair of back to back papers published July 24 in the journal Nature *(**De novo design of bioactive protein switches**.* Nature*, 2019; DOI: 10.1038/s41586-019-1432-8; **Modular and tunable biological feedback control using a de novo protein switch**.* Nature*, 2019; DOI: 10.1038/s41586-019-1425-7).*

The smart cells can act like tiny autonomous robots which, the researchers believe that in the future, may be used to detect damage and disease. These cells can also help deliver safe therapeutic help that is administered at just the right time and in very precise doses. Medicines cannot be effective or may be harmful, if it is administered not at the right time and with too large or too small an amount. These smart cells fulfil this demand and open a whole host of new possible applications including programming the cells that can be employed to treat a wide range of debilitating conditions for which safe and effective therapeutics are not yet available.

For example, degronLOCKR-based smart cells could treat a variety of diseases and ailments including traumatic brain injury (TBI). This can be achieved by transforming a patient's own cells into smart cells by installing degronLOCKR-based circuits that are designed to sense inflammation and modulate the activity of the immune system. To be able to keep inflammation well within the narrow therapeutic zone is crucial in treating TBI, and it is believed that these engineered cells can perform the tasks. In addition to TBI, smart cells could also be deployed in a number currently untreatable diseases that include cancers that are resistant to the latest drugs and cell therapies and autoimmune diseases for which no effective therapies are yet available. Researchers behind this remarkable discovery foresee the enormous possibilities and opportunities of this

designer protein and sophisticated biological circuits that could initiate a whole new generation smart, precise and robust live cell therapies with unprecedented functions.

Environmental Biotechnology

Extinction of Elephants could lead to increased carbon dioxide levels in the atmosphere

It is known that during photosynthesis, trees and plants consume carbon dioxide that gets removed from the atmosphere. It is therefore believed that plants can be very helpful in storing carbon emissions to battle global warming. In a remarkable research observation that can have far reaching impacts on our ecological system, forest elephants are believed to control and engineer the ecosystem and preserve the helpful plants of the entire central African forests. However, the real danger is that their decline toward extinction could have serious effects on mitigating carbon pollution in the atmosphere. This could have significant implications for climate and conservation policies.

Forest elephants are considered one of the last remaining megaherbivores in the world. These giant creatures shape and preserve their environment on multiple fronts. They act as forest bulldozers and serve as seed dispersers. These Elephants create trails and clearings as they eat over a vast varieties of species of fruits, trample bushes and knock over trees. Thus, their ecological impact is enormous that affects tree populations and subsequently carbon levels in the forest.

In a joint study, researchers from Italy and France recently published a paper on July 15, 2019 in Nature Geoscience *(Carbon stocks in central African forests enhanced by elephant disturbance,* Nature Geoscience*, 2019; DOI: 10.1038/s41561-019-0395-6).* Their comprehensive study revealed that feeding habits of elephant populations in central African forests promote the growth of slow-growing trees than fast growing species which are the preferred foods of elephants. The slow growing trees with high wood density are a much preferred scenario as these trees actually sequester more carbon from the atmosphere than any other fast growing species. This implies that without the forest elephants will likely result in an increase in the abundance of fast growing tree species at the expense of slow growing species that will reduce the ability of carbon capture.

Discovery of new cell that could repair damaged hearts

The human heart unlike other organs is limited in its capacity to repair itself after a damage or injury. This is the reason why heart disease is the leading cause of death in the world. Cardiology researchers never thought before to explore the possibility of employing cells just outside the heart to participate in healing and repair of hearts after injury.

Now, there is a hope to repair damaged hearts. Researchers at the University of Calgary in Canada have discovered a previously unidentified cell population in the pericardial fluid found inside the sac around the heart. This is a major discovery that could potentially lead to new treatments for patients with injured hearts. The discovered cell was found in the pericardial fluid (sac around the heart) of a mouse with heart injury. The innovation of this research lies in the fact that the same cells were also found within the human pericardium of people with injured hearts. Thus, this gives hopes that the repair cells could be promising for a new therapy for patients with heart disease. This research was published July 16, 2019 in the journal, Immunity *(Gata6+ Pericardial Cavity Macrophages Relocate to the Injured Heart and Prevent Cardiac Fibrosis.* Immunity*, 2019; 51 (1): 131. DOI: http://dx.doi.org/10.1016/j.immuni.2019.06.010).*

This research addressed the contribution of resident cavity macrophages near the heart. Macrophages are a type of white blood cells of the immune system. It has been studied that these cells play an important role in structural cardiac remodeling and myocardial infarction (MI) induced transition to heart failure. In order to gain new insights in these cells, researchers investigated the contribution of resident cavity macrophages located in the pericardial space adjacent to the site of heart injury and found that disruption of the pericardial cavity accelerated maladaptive post-MI cardiac remodeling. Significantly, $Gata6^{+}$ macrophages cells were found in mouse pericardial fluid that were believed to contribute to the reparative immune response. Researchers then stumbled into a major finding that showed that $Gata6^{+}$ macrophages were present in human pericardial fluid. This essentially implies that the reparative function that was observed in mouse can also be relevant in human heart disease.

This discovery of a new cell that can help repair injured heart muscle could lead to new pathways for therapies that offers hope for patients worldwide who suffer from heart disease.

Understanding the pathophysiology of early stage Parkinson's disease

Obtaining new insights in the pathophysiology of early stage neurological disease such as Parkinson's disease 'PD' is vital for better diagnosis and prognosis and possible cure of the disease. To this end, researchers have focused on their studies related to the changes in the function and microbiome of the upper and lower gastrointestinal tract that have been reported in PD that actually precede the pathology in the central nervous system. However, most studies conducted so far have examined merely fecal microbiome profiles and patients with PD especially at the advanced states of disease that do not shed enough lights on the pathophysiology of early stage PD, which is believed to be the key for combating PD.

Researchers from SUNY Upstate Medical University, Syracuse, New York in the United States addressed this issue in a new significant research published June 27 of this year *(The oral microbiome of early stage Parkinson's disease and its relationship with functional measures of motor and non-motor function, PLoS ONE 14(6): e0218252. https://doi.org/10.1371/journal.pone.021 8252)*. In their research, they identified sensitive and specific biomarkers of changes in the oral microbiome of early stage PD through shotgun metatranscriptomic profiling.

Further, researchers conducted parallel study on the evidence for changes in human salivary mRNAs with the microbial analysis that revealed significant changes in a set of 9 host mRNAs. These were then mapped to various brain functions that showed important correlation with some of the significantly changed microbial taxa.

Researchers studied the correlations between microbiota and functional measures, including those reflecting cognition, motor, balance and sensory changes, PD balance, and disease duration. Specifically, the early stage PD subjects showed significant changes in several indices of motor, cognitive and sensory function. This very significant discovery made in the pathophysiology diagnosis of early stage PD opens the possibility of using oral microbiome that may represent a highly-accessible and informative microenvironment to understand the pathophysiology of early stage PD.

Genomics

Combating malaria becomes more challenging as multidrug-resistant malaria rapidly spreading in Asia

In a significant breakthrough, researchers from the Wellcome Sanger Institute, University of Oxford, UK and Mahidol University in Bangkok, Thailand have employed genomic surveillance that has revealed that malaria resistance to two first-line antimalarial drugs has spread rapidly from Cambodia to neighboring countries in Southeast Asia. In a major discovery, researchers have found that descendants of one multi-drug resistant malaria strain are replacing the local parasite populations in Vietnam, Laos and northeastern Thailand. The enhanced resistance is attributed to the resistant strain that is considered to pick up additional new genetic changes causing the resistance to antimalarial drugs even further.

Plasmodium parasites that spread through mosquito bites cause Malaria in humans. According to estimates by the World Health Organization on malaria infected patients and fatalities in Sub-Saharan Africa, nearly 220 million people were infected in 2017, causing at least 400,000 deaths, with children under the age of five placed at most risk category. Malaria needs to be detected at an early stage for an effective treatment and elimination of the disease, which can be fatal, if left undiagnosed and untreated. This most up-to-date and comprehensive whole genome study in Southeast Asia revealing that parasites are becoming resistant to antimalarial drugs, show that there are additional challenges in the malaria elimination efforts that need to be addressed urgently with new strategies. The study, published 22nd July in The Lancet Infectious Diseases *(Evolution and expansion of multidrug-resistant malaria in Southeast Asia: a genomic epidemiology study, The Lancet Infectious Diseases*, 2019; DOI: *10.1016/S1473-3099(19)30392-5),* shows the significance of having public health malaria control strategies in place that can be achieved by a continuous genomic surveillance. This is especially important to maintain the status of the global efforts to eliminate malaria. An early detection and prevention of the spread of resistance to drugs is the key for malaria elimination.

Medical Biotechnology

The potential role of high levels of estrogen hormones in developing autism

Scientist from the University of Cambridge, U.K. have validated an earlier theory on autism that identifies a link between exposure to high levels of oestrogen sex hormones in the womb and the likelihood of developing autism. Their important research findings were most recently published on July 29, 2019 in Molecular Psychiatry (*Foetal oestrogens and autism, Molecular Psychiatry*, *2019; DOI: 10.1038/s41380-019-0454-9*). They observed that predictability of likelihood of developing autism was much higher with high levels of prenatal oestrogens than with the same high levels of prenatal androgens (such as testosterone). They attributed the elevated hormones that could be coming from the mother, the baby or the placenta and showed that these hormones may likely interact with genetic factors to affect the developing foetal brain. This finding is noteworthy because the detailed role of oestrogens in autism is studied for the first time and how these hormones could contribute to foetal brain development.

Food Biotechnology

Ultra-processed and generally unhealthy packaged food supply

Today's consumer's total calorie consumption mostly comes from packaged and unpackaged foods and beverages that are usually bought from the stores. It is well recognized that this food and beverage supply plays a key role in the development of many complex and chronic diseases that include obesity and cardiovascular disease, just to name a few.

This prompted a team of researchers from Northwestern University in the US and the University of New South Wales in Australia to conduct a collaborative study on the healthfulness of the US packaged food and beverages. They reported that consumers in the US are overexposed to products that are high in energy, saturated fat, sugar and salt due to the ultra-processed packaged food supply. This study was published on July 24, 2019 in the journal Nutrients *(The Healthfulness of the US Packaged Food and Beverage Supply: A Cross-Sectional Study. Nutrients*, *2019; 11 (8): 1704, DOI: 10.3390/nu11081704)*.

This important report is timely as it educates consumers, researchers and policymakers on the downside of the overconsumption of ultra-processed packaged food. In this regard, food and

beverage manufacturers have a major role to play in not only creating a healthier food environment, but also through health promotion efforts to improve population diets. This report therefore, encourages policy makers to regulate food manufacturers so that they reformulate or replace unhealthy products with the healthier foods. Further as a remedial action, the authors of the report suggested government's action that may be needed to improve the healthfulness of the packaged food and beverage supply.

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

Several pharma companies fighting infectious diseases brought on by the climate change

A recent Morgan Stanley research note predicted that global warming will bring an increased prevalence of several deadly tropical diseases in the future. In this regard, the healthcare sector needs to be prepared with new vaccines. This analysis projects that depending on the pace and severity of global warming, between 383 million and 725 million more people may be exposed to Zika, dengue and other tropical diseases by 2050. Vaccines worth roughly 50 billion USD to as much as 170 billion USD will be needed to fight those diseases. According to the Morgan Stanley analysis report, global pharma companies such as Sanofi and GlaxoSmithKline are best positioned to help fight the diseases. In addition, several other major pharma companies including Moderna, Takeda Pharmaceutical, Merck, Johnson & Johnson's Janssen Pharmaceuticals, and Pfizer are also expected to play a big role in supplying vaccines. (Source: https://markets.businessinsider.com/).

Eli Lilly's key breast cancer drug Verzenio gets boosted by survival benefit in a phase 3 study

After nearly two years of the first U.S. approval of the breast cancer drug Verzenio, Eli Lilly recently produced interim data from a Phase 3 study that showed proof of a greater survival benefit among women with metastatic disease. This important data enhances the prospects for Verzenio for effective cancer treatment that is expected to play in helping women with breast cancer live longer. This study by Lilly called MONARCH-2. The company plans to read out full results of overall survival in 2020. However, a pre-planned interim data disclosure by Lilly demonstrated a statistically significant survival benefit and that was reported as definitive. The trial for Phase 3 study involved an enrollment of 669 pre- and postmenopausal women that were previously treated. The patients were administered with Verzenio that was given together with an older endocrine therapy, fulvestrant, against administration of fulvestrant alone. To broaden its oncology portfolio, Eli Lilly bought Loxo Oncology and its portfolio of targeted cancer drugs for $8 billion in January this year (source: https://www.biopharmadive.com/).

Exact Sciences to acquire Genomic Health to create a bigger single diagnostic company

In a major merger, Exact Sciences has moved to acquire Genomic Health. This merger is based on the plans to bring their cancer screening and diagnostic tests up through a combined commercial organization of about 1,000 employees, including sales, marketing and reimbursement staff. This merger of the two diagnostic companies is based on a $2.8 billion deal that is split between $1.1 billion in cash and $1.7 billion in stock. This acquiring of Genomic health is expected to create a single diagnostics company with at least $1.6 billion in annual revenue by the end of 2020. Exact Sciences' main revenue earner is Cologuard at-home fecal DNA test for colorectal cancer. Combining this product of Exact Sciences with Genomic Health's Oncotype DX assays for breast and prostate cancer can create a bigger footprint in the diagnostic areana, and the company leaders hope to see a commercial presence in more than 90 countries with a gross profit of $1.2 billion next year. The acquiring will also include merging the R&D teams of the two companies, with the goal of identifying biomarkers across the top 15 deadliest cancers (source: https://www.fiercebiotech.com/).

Merck's measles vaccine sales jumps upward in the second quarter

Pharmaceutical giant Merck reported that high consumer demand for its measles vaccines propelled their sales of measles vaccine in its second quarter. Merck's second-quarter earnings report showed that the sales of children's vaccines including the company's MMR vaccine, for measles, mumps and rubella, jumped 58% year over year to $675 million. Merck has the monopoly as a supplier of measles vaccines in the US. The strong growth in vaccine sales was attributed to this year's measles outbreak in the US [source: https://www.cnbc.com/).

Plant protection specialist AgroSavfe raises substantial financing to develop green products

European plant protection specialist company Agrosavfe raises financing to the tune of 35 million euros to push the development, registration and commercial scale production of its biofungicides and bio-insecticide. Agrosavfe specializes in protein-based biocontrols, which promise a more sustainable future in agriculture that has the potential to mitigate the concerns of pesticides that have come onto the radar of critical consumers. Agrosavfe's plant protection products seem to have good future prospects. They plan to launch their products in the US in 2022 (source: https://european-biotechnology.com/).

Italian pharma Zambon heading to acquire respiratory company Breath Therapeutics in the US

The Italian pharma Zambon is set to acquire respiratory company Breath Therapeutics for a deal set at 500 million euros. This acquisition is designed to strengthen its respiratory pipeline and get access to the US market. This is the largest deal, Zambon SpA has ever landed. In this deal, Zambon will pay €140m upfront to Breath Therapeutics BV, which is subsequently eligible to receive further €360m in milestones. Zambon will develop the eFlow nebulizer technology that evaluates a novel,

liposomal formulation of Cyclosporine A (isL-CsA-i) in a pivotal Phase III trial, in the US and the EU. This therapeutics is considered very promising to treat Bronchiolitis Obliterans Syndrome (BOS), an orphan inflammatory respiratory disease. It has been estimated that BOS affects 30,000 patients and leads to death with two years of the first diagnosis, if left untreated. Zambon's expansion into the US aims at maximizing Zambon's commercial reach and attract investors (https://european-biotechnology.com/).

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