Regenerative Medicine

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Contributing Editor

The first Human Stem Cell derived Blood Brain Barrier Organ Chip – The New Age of Precision Neuromedicine

"Precision medicine", is the new lexicon in the field of clinical research and medicine which is being looked upon as one of the most promising and futuristic healthcare solutions. The concept of precision medicine stems from the term "personalized medicine" which has become a popular approach in the field of stem cell therapeutics and regenerative medicine. The word 'personal' evokes a feeling that therapies and treatments are being customized for individuals, which sounds progressive but could lead to a misinterpretation that research is meant only for the rich and the influential. However, the word 'precision' clearly highlights that treatments are being designed for patients on the basis of environmental, genetic and lifestyle factors which implies research shall serve mankind in larger interest. Time and money are the two major deciding factors which hold the potential of transforming a simple research idea into a life transforming technology. Same is the case with "precision medicine" which is like targeted bombing; it is accurate, precise and hits upon a specific target eliminating the root cause of the problem. Stem cell derived 'Organ on Chip' technology has completely revolutionized the field of regenerative medicine and many functional human organs have successfully been recreated. The latest advancement in the field is the recreation of the Blood Brain Barrier (BBB) of the human brain in the form of an organ chip which mimics the disrupted functionality associated with brain disorders. Patient specific induced pluripotent stem cells (iPSCs) were employed for the purpose and thus the research endeavor has carved a niche in the field of "precision Neuromedicine".

Why recreate the Blood Brain Barrier (BBB)?

The Blood Brain Barrier (BBB) is the collective name given to the unique microvasculature of the CNS (central nervous system) and comprises a complex interplay between different cell types such as the endothelial cells of the blood vessels, the pericytes, neurons, astrocytes and the

microglia. The BBB is a structurally and physiologically distinct unit of the brain and adds on to the complexity of the organ which continues to intrigue researchers till date. It regulates the entry of solutes, nutrients, ions, toxins and pathogens and thus plays a crucial role in the CNS homeostasis. The BBB is pivotal in maintaining proper neuronal function, prevents injury, inflammation and safe guards the delicate structures encased within. However, it serves as an obstacle in the route of therapeutics and drugs which cannot be directly delivered into the brain. A disrupted and functionally impaired BBB is a characteristic hallmark of several neurodegenerative diseases and neurological disorders. Thus recreating this complex structure outside the human body will widen the horizon of neuroscience research in health and disease. **Figure 1** depicts the cellular architecture of the blood brain barrier in a normal brain as well as a tumor containing brain (1).

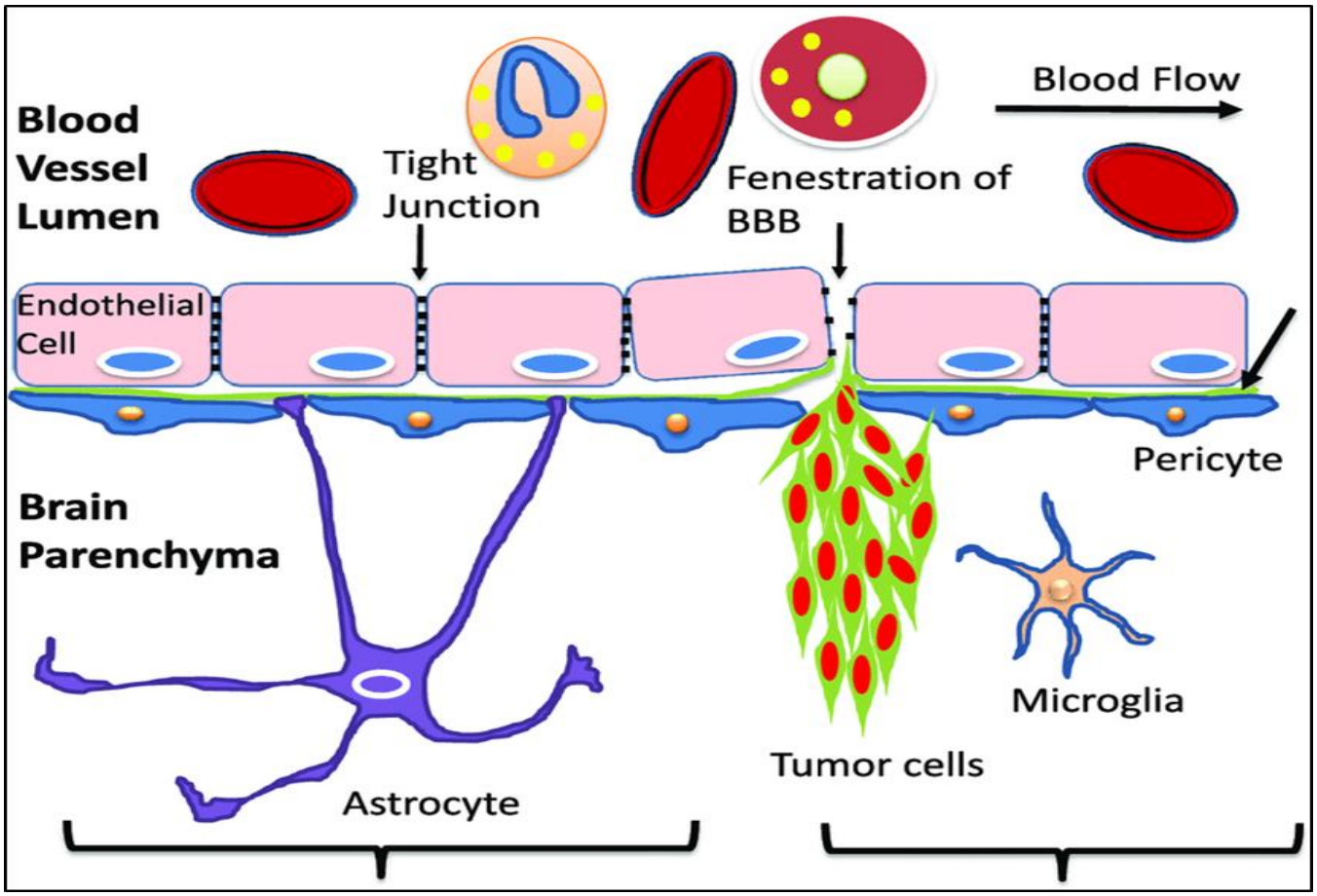

*Figure 1: The cellular architecture of the blood brain barrier in a normal as well as a tumor brain. [**Source:** Advanced drug delivery reviews, 2017].*

The technical knowhow underlying the Human 'Blood-Brain Barrier Organ on Chip'

Several attempts have been made to recapitulate the complex physiology of the human brain within a laboratory but it is

evident that we need to assemble the various structures of the brain one at a time in order to complete the big jigsaw puzzle called the 'Human brain'. The BBB has been a lucrative target for recreation and met success earlier however, this time researchers from Ben-Gurion University of the Negev (BGU) and Cedars-Sinai Medical Center in Los Angeles have jointly replicated a patient's BBB creating a human BBB chip. They have for the first time employed patient specific induced pluripotent stem cells (iPSCs) for the purpose, which can be used as a potent tool to develop precision medicine and design novel approaches to research upon CNS disorders (2).

Induced pluripotent stem cells (iPSCs) are the normal somatic cells of a body which can be genetically reprogrammed into functional stem cells which can then be directed to convert into any specific cell type or lineage. These somatic cells can be derived from the skin, blood or any normal healthy tissue of the body. For this study patients' blood samples were used to obtain functional iPSCs. Further induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial like cells (iBMECs), neurons and astrocytes were cultured and then planted inside 'organ chips' so as to mimic the physiological microenvironment and the natural mechanical forces that the cells experience in-vivo. The organ chips were procured from Emulate, Inc. in Boston, which is the commercial manufacturer of the 'Organs-on-Chips' technology.

To briefly describe, organ chips are micro devices made up of flexible PDMS (polydimethylsiloxane) elastomers. The chip employed for the study comprised two micro-channels of the size 131 mm and 130.2 mm that were aligned in parallel. The two channels representing the brain and blood side were further separated by a porous flexible PDMS membrane which was laminin coated and provided a porosity of 2% over a surface area of 0.171cm^2. The brain side was coated with laminin and the blood side with a mixture of fibronectin and collagen. These biopolymers help in replicating the structural complexity of the human BBB. In order to function like an actual human blood brain barrier the device was connected with a microfluidics system which mimics human blood flow and a Human Emulation System which recreates the exact microenvironment needed for physiological functionality (3). **Figure 2** shows the schematic representation of the BBB organ chip showing the cellular components as well the biopolymer coated micro device (2).

Morphological, functional and physiological parameters were assessed to validate the organ chip model. Morphologically the iBMECs expressed the entire set of cellular markers specific to the vasculature of the brain and the chip exhibited trans endothelial electrical resistance (TEER) which is a physiological characteristic of the BBB (4). When the vascular lumen was perfused with blood, the neural cells were protected from plasma-induced toxicity by the micro engineered capillary wall system. The chip further demonstrated functional blood-to-brain permeability and accurately predicted the transport feasibility of drugs and pharmacologics in case of CNS disorders. Further when patient derived iPSCs were used to create the BBB chip the barrier

replicated the disease specific malfunctions. In this case iPSCs of individuals suffering from Allan-Herndon-Dudley syndrome (an unusual congenital neurological disorder) and Huntington's disease (a neurodegenerative disorder) were employed and the Chip showed the exact lack of disease specific transporters and disrupted barrier integrity as present in the patients. Thus the BBB organ chip tested positive on all molecular, structural and functional parameters and was validated as a functionally sound model which is by far the closest possible mimic of the human blood brain barrier system.

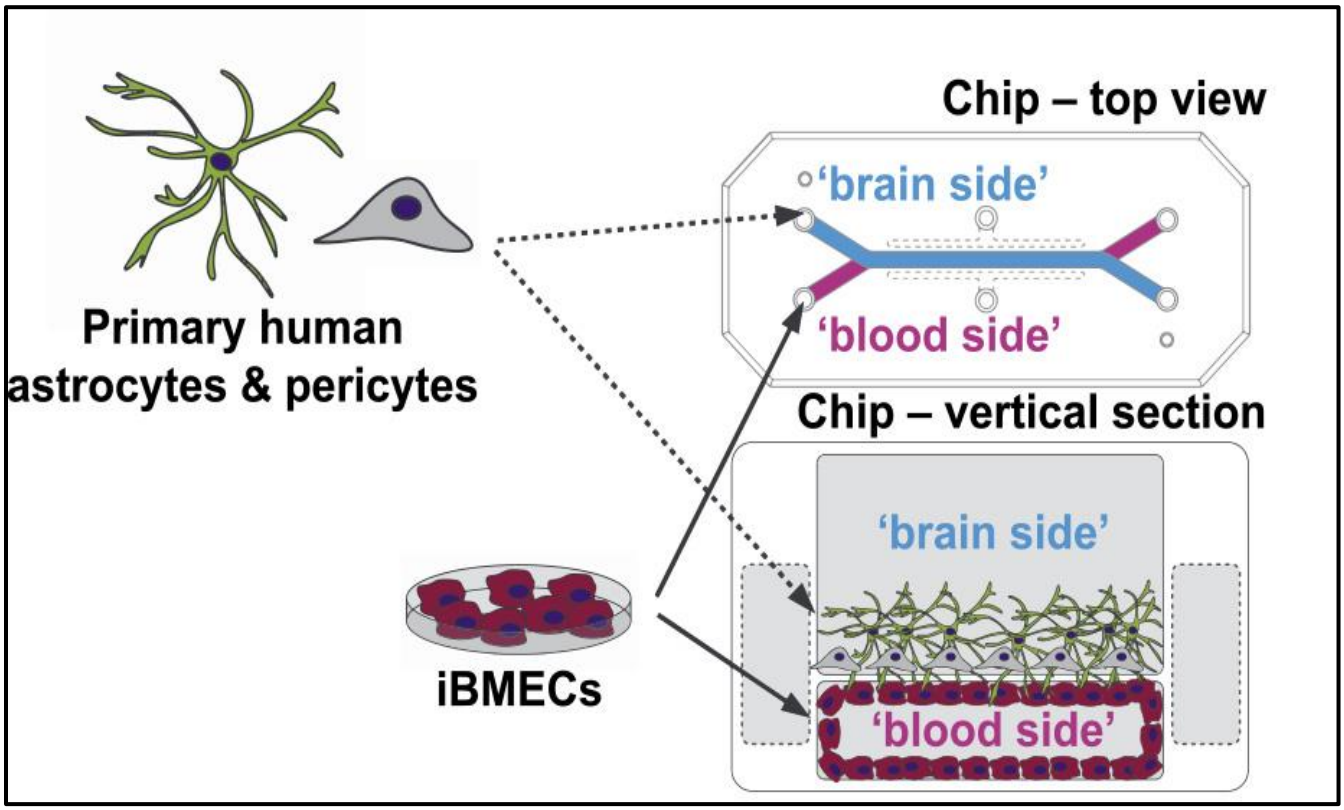

*Figure 2: Schematic representation of the 'BBB organ chip' showing the cellular components as well the biopolymer coated micro device. The iBMECs were seeded on the blood side and primary human pericytes and astrocytes were seeded on the brain side. [**Source:** Cell stem cell, 2019].*

The current and future prospects of the technology

The BBB chip is a first of its kind human testing model system which blurs the boundary between *in vivo*, *in vitro* and *in silico* approaches. It is a perfect combination of innovative stem cell science, software design, instrumentation and microfluidics technology. 'Organ on chip' models are promising tools that can play a pivotal role in basic research, diagnosis and treatment. The brain is one of the most complex organs of the body and so are the implications associated with it.

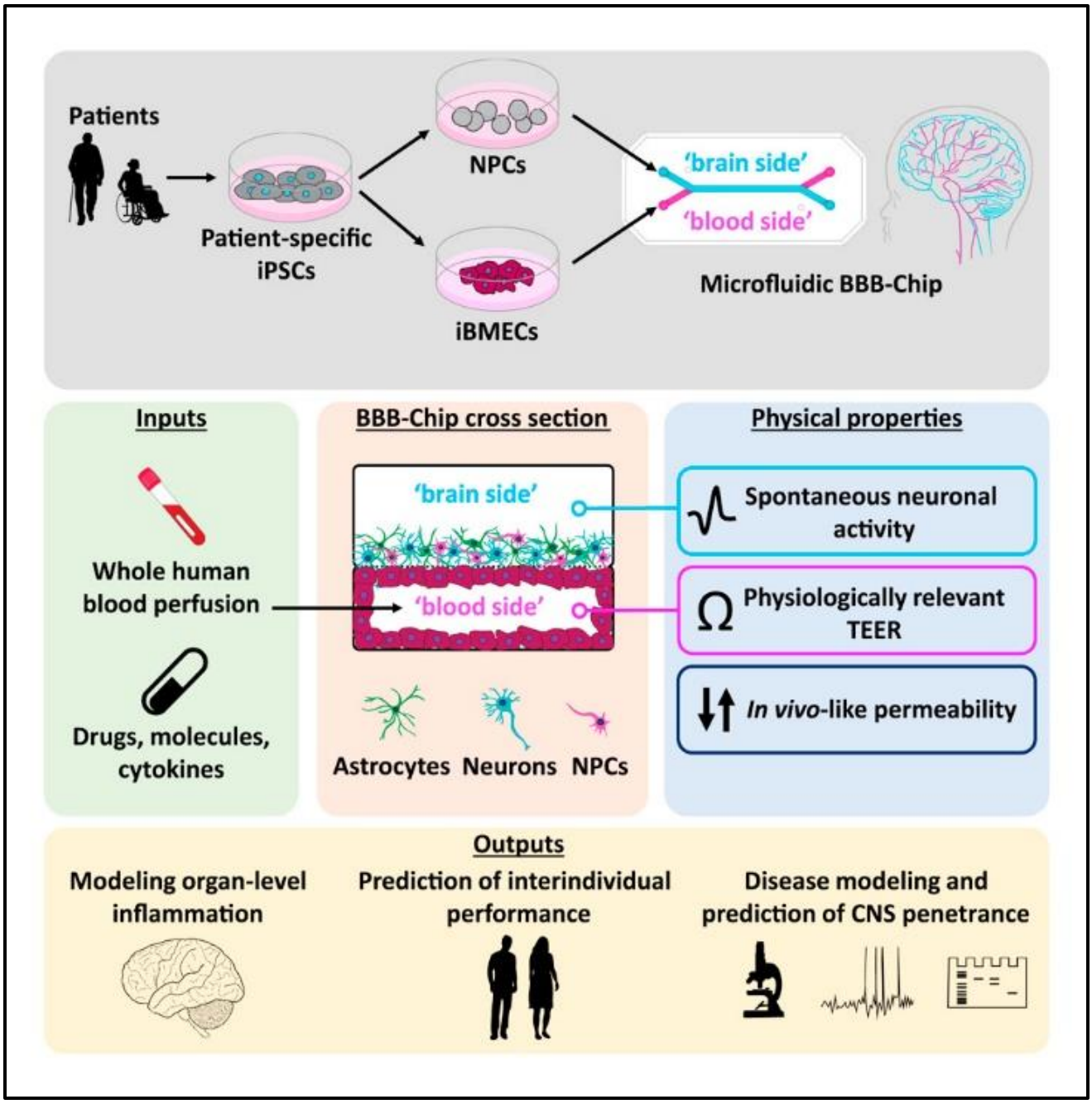

*Figure 3: A complete summary of the 'BBB organ chip' depicting the technical knowhow involved, the underlying scientific concept as well as the applications of the device. [**Source:** Cell stem cell, 2019]*

A major problem being that any form of CNS disorder cannot be diagnosed directly via path-lab tests as reflective markers of the brain are not present in the blood (5). Neurodegenerative diseases as well as neurological disorders spell doom, uncertainty, hefty financial strain and helplessness. Even a lay man is aware of the complications associated with brain related disorders. 'Every Human being is unique',

this cliché probably derives its origin from biology as it highlights the fact that humans are genetically, phenotypically and environmentally diverse. Treatments are common but how a patient responds to it will depend on its genetics, immune system, environment and lifestyle. Therefore it is improbable to administer the same set of pharmacologics/drugs to every patient which might just lead to a toxic overload of drugs without any respite. Targeted therapy and precision medicine take the patients' individual parameters into account and thus enables us to customize treatments which shall be effective and devoid of deleterious side effects. **Figure 3** is the conceptual summary of the 'BBB organ chip' and clearly depicts the current and future applications of the technology (2).

Customizing treatments for patients is an age old tradition where the medical practitioners usually asks patients regarding allergies and dietary habits before a treatment but technology has taken this to another level altogether. The 'BBB organ chip' derived from patient iPSCs is a novel tool which can help in predictive diagnostics, screening the efficacy of drugs, patient specific pharmacokinetics, identification of potent molecular targets and thus focused treatment strategies. This technology has opened new exciting possibilities in the field of neuroscience research and medicine as it is cost effective, rapid, reproducible and also potentially applicable for humans. With new technological innovations and unprecedented research endeavors we are gradually heading towards an era of futuristic "precision neuromedicine" which shall completely redefine the way we currently look at and deal with the human neurological disorders and neurodegenerative diseases.

References

1. Ljubimova JY, et al. (2017) Covalent nano delivery systems for selective imaging and treatment of brain tumors. *Advanced drug delivery reviews,* 113:177-200.
2. Vatine GD, et al. (2019) Human iPSC-Derived Blood-Brain Barrier Chips Enable Disease Modeling and Personalized Medicine Applications. *Cell stem cell*, 24(6):995-1005. e1006.
3. Jain A, et al. (2018) Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. *Clinical pharmacology & therapeutics,* 103(2):332-340.
4. Lippmann ES, Al-Ahmad A, Azarin SM, Palecek SP, & Shusta EV (2014) A retinoic acid-enhanced, multicellular human blood-brain barrier model derived from stem cell sources. *Scientific reports,* 4:4160.
5. Singh S, et al. (2015) Stem Cells in Neurotoxicology/Developmental Neurotoxicology: Current Scenario and Future Prospects. *Molecular neurobiology*: 1-12.