Microfluidics In Drug Delivery Across Blood Brain Barrier (BBB)

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Abstract

To improve the pharmacological activity of therapeutic drugs, the drug delivery system aims to transport them into their desired site of action. The presence of internal barriers in our body is the most challenging thing in drug delivery system. The recent improvement in microfluidics has a great impact in various fields including drug discovery, biology, diagnostics and tissue engineering. Microfluidic systems refer to the control and manipulation of liquids in the range of nano- and pico-litre with appropriate proportions of tunable release profile and reproducible fashion. A well designed in vitro blood brain barrier models must contain structure of ECs, cell-cell interactions, controlled flow and a molecular transportable basal membrane as in BBB in vivo. In recent years, for the construction of microfluidic chips, bio-printing was greatly involved. Polydimethylsiloxane (PDMS), poly methyl methylacrylate (PMMA), the polyethylene terephthalate (PET) and the polytetrafluoroethylene (PTFE) membranes were used as the best chip materials. Initially the classic methods like transwell system was used to create a sandwich like vertical structure in the microfluidic devices. Recently, to overcome problems caused by vertical channels, parallel channels have been designed. The BBB-on-chip model with ECM gels like hydrogels is considered as the best model nowadays. The most commonly used cell sources are hCMEC/D3 and hBMECs which are known to be human brain microvascular endothelial cells and HUVECs. It is necessary to maintain shear stress condition in the microfluidic devices as in natural BBB (4-30 dyne/cm^2 to 1-4 dyne/cm$^{2)}$. Recently, nanocarriers are the most prominent drug carriers with more therapeutic index, less side effects and increased uptake and penetration properties. Commonly, lipid-based nanoparticles, polymeric nanoparticles and inorganic nanoparticles are the widely used nanocarriers. For microfluidic carrier free DDSs include microneedles and micro-reservoirs, the drug is directly applied to the site and the action is quick and effective. The different kinds of microneedles are solid MNs, coated MNs, dissolving MNs, hollow MNs and hydrogel-based MNs. To manufacture a driving system which can achieve the stable drug release is the primary part in microfluidic micro-reservoir system. Hence, microfluidics holds notable assurance in developing the drug delivery system for forthcoming generations. Because of the precise control of fluid, this resourceful technology is emerging as a powerful tool not only for the improvement of drug efficacy and delivery but also for the prediction of preclinical drug carrier testing.

Keywords: Microfluidics, Blood Brain Barrier, Drug delivery system, Neurodegenerative diseases, Microneedles.

I. INTRODUCTION

To improve the pharmacological activity of therapeutic drugs, the drug delivery system aims to transport them into their desired site of action. Recently, on the improvement of drug delivery system numerous drug carriers are being developed for transporting drugs to their target and preventing them from degradation before their respective action mechanism. However, the presence of internal barriers in our body is the most challenging thing in drug delivery system. hypodermic injection, oral administration, inhalation method are the conventional drug delivery methods. They still having the trouble of reaching the target site and crossing the barriers. Auspiciously, arising technologies like microfluidics hold notable assurance in developing the drug delivery system for forthcoming generations. Because of the precise control of fluid, this resourceful technology is emerging as a powerful tool not only for the improvement of drug efficacy and delivery but also for the prediction of preclinical drug carrier testing.

II. BBB

Central nervous system (CNS) which function as the control center of body needs a highly progressed network for the supply of neural signals. The cells that are present at key connection point of brain form some barriers which are involved in regulating the exchange of ions, molecules and cells between blood and brain. The barriers include blood-brain barrier (BBB), blood-CSF barrier and arachnoid barrier. BBB plays a major role in delivering blood to CNS by prevent the crossing of non-selective solutes from blood to CNS. Furthermore, BBB functions as a protective barrier by providing a firm condition for the neural actions. It regulates the composition of ions and shows a less permeability to the hydrophilic nutrients which are essential for the CNS. The properties of BBB is schematically described below which was retrieved from the article by *Chen, Y., & Liu, L. (2012).*

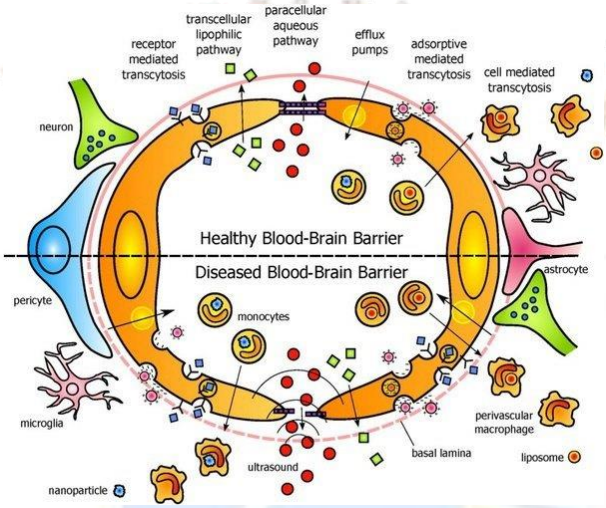

Fig. 1 Properties of blood brain barrier

(1) History of BBB

Paul Ehrlich and Edwin Goldman observed the non-staining of brain and cerebrospinal fluid when the water-soluble drugs are injected into the peripheral circulation. In contrast, the same drugs when injected into subarachnoid space showed the staining of brain and CSF but peripheral tissues remain same. The term blood-brain barrier was first coined by Lewandowsky who studied the barrier between blood and brain in addition to between blood and CSF by observing the penetration of potassium ferrocyanide into brain (1900). Later in 1942, Friedemann showed that the direct transport of dyes into cerebral microvasculature cause staining across the brain.

There were two barriers observed by Broman in 1941: blood-CSF barrier at the choroid plexus and the blood-brain barrier at the cerebral microvasculature. He also argued that the capillary endothelial cells are responsible for the barrier function of brain and not the astrocytic end feet.an electron microscopic cytochemical studies achieved in 1960s by Reese and Karnovsky and in 1970 by Brightman and colleagues put an end to this argument. In 1967, Reese and Karnovsky use horseradish peroxidase to visualize the BBB. Horseradish peroxidase was unsuccessful in reaching brain extracellular fluid when it is administered systemically. But it stained the brain extracellular fluid when it was administered as intracerebroventricular injection into the CSF. The diffusion of horseradish peroxidase takes place at the astrocytic end feet and basement membrane freely but restricted at the tight junction of cerebral endothelial cells. Those components of BBB are clearly shown in the below picture which was taken from the article by *Chen, Y., & Liu, L. (2012).* This showed that zonula occludens of cerebral endothelial cells in brain comprehend the BBB and is the reason for restricted flow of substances from blood and interstitial fluid.

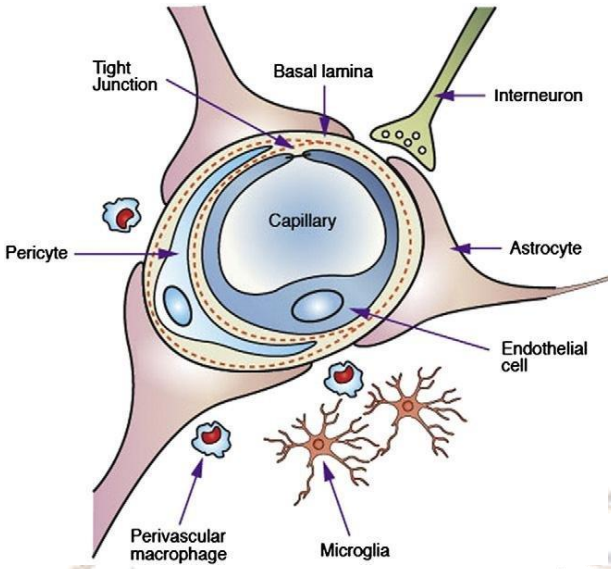

Fig. 2 Components of neurovascular unit

(2) The Uniqueness of BBB

In 1981, Stewart and Wiley performed an experiment relating to the physiology of tight junctions of BBB. In that study, the embryonic brain of quail bird was transplanted into the embryonic gut of chick. Even if the chick gut vessels vascularized quail brain, the microvessels of transplanted quail brain continued to exert the physiological characteristics of BBB by blocking trypan blue dye. Contrarily, if embryonic gut of quail was transplanted into embryonic brain of chick, the microvessels shown leakage to the trypan blue. This showed that the physiological characteristics of BBB was not maintained even if the embryonic gut of quail gets vascularized by the vessels of chick brain. Ultimately, these results assist that the expression of definite set of genes within the capillary endothelium or cofactors from surrounding tissues were responsible for the physiological characteristics of BBB.

(3) Peripheral vs Cerebrovascular Endothelial cells

The fenestrations which are responsible for the free exchange of water and solutes with the extracellular fluid in the peripheral endothelial microvessels are not found in the cerebrovascular endothelial cells. Further, the pinocytotic vesicles also absent in microvessels of brain endothelial cells. The presence of many mitochondria in those cells represent its metabolic barrier property in addition to that physical barrier property. The anatomy of cerebral capillary is depicted below which was retrieved from the article by *Sood. A et al (2022)*

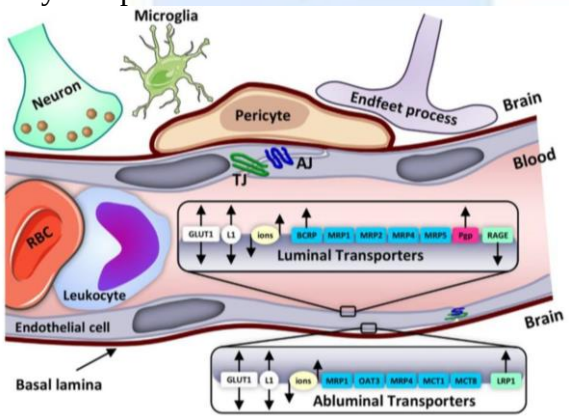

Fig. 3 Cerebral Capillary

(4) Interference of BBB in drug delivery to CNS

The various methods which are enhancing the drug delivery to brain are of great interest. It is due to the inefficacy of existing interventions to deliver and sustain the drug at therapeutic site within brain. The failure of CNS drugs is not due to the impotency of drug but the way in which the delivery system of drug is to be designed. Since there are many frustations in the drug delivery system, it is considered as a challenge for drug designing to CNS diseases.

The BBB is the major problem that shows complexity in the drug delivery to CNS. The transport mechanism across the BBB is depicted and explained below which was taken from the article by *Chen, Y., & Liu, L. (2012).* Hence for designing the most efficacy drug for CNS diseases it is important to characterize BBB properties.

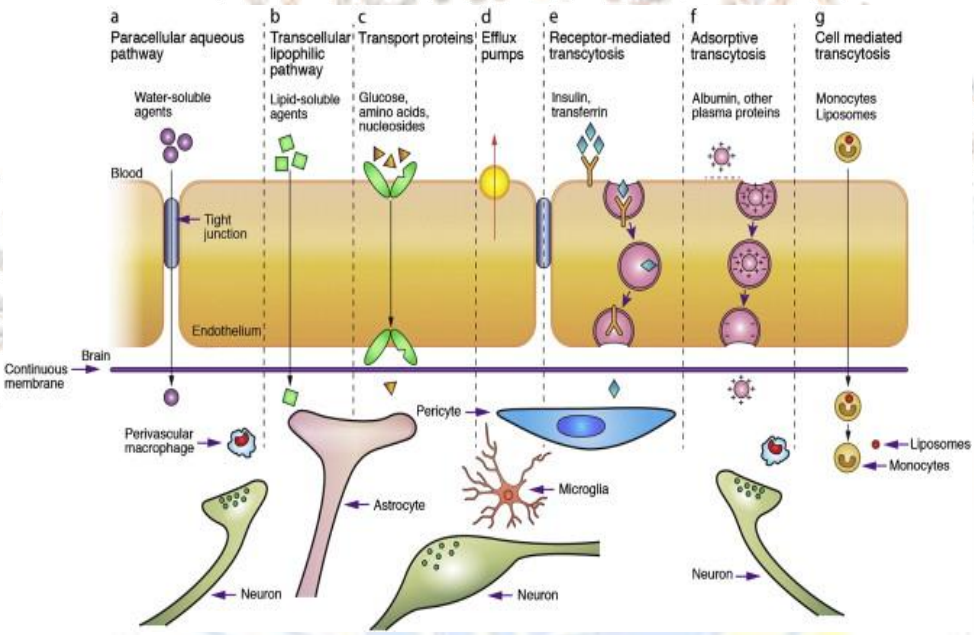

Fig. 4 Transport routes across the blood–brain barrier. Pathways "a" to "f" are commonly for solute molecules; and the route "g" involves monocytes, macrophages and other immune cells and can be used for any drugs or drugs incorporated liposomes or nanoparticles.

III. MICROFLUIDIC SYSTEM

The recent improvement in microfluidics has a great impact in various fields including drug discovery, biology, diagnostics and tissue engineering. Microfluidic systems refer to the control and manipulation of liquids in the range of nano- and pico-litre. Microfluidics overcome the crisis that arise from the conventional methods for fabricating drug carriers, in the way of reducing quantity of expensive drugs for fabrication and encapsulation. Further, microfluidics enables generating a sustained release profile for a drug which is one of the issue in conventional method. Hence, this system facilitates the fabrication of complex drug carriers with appropriate proportions of tunable release profile and reproducible fashion.

Micro-technologies put an end to the traditional pain causing delivery methods by fabricating microneedles or needleless parenteral administration. Nowadays this system focuses on transdermal administration to upgrade patients' standard of life. Recent development in micro-electro-mechanical systems (MEMS)technology had a great role in the creation of microfluidic devices to mimic biological microenvironments in vitro sciences. A microfluidic model which was retrieved from the article by *Sood. A et al (2022*) was picturized below.

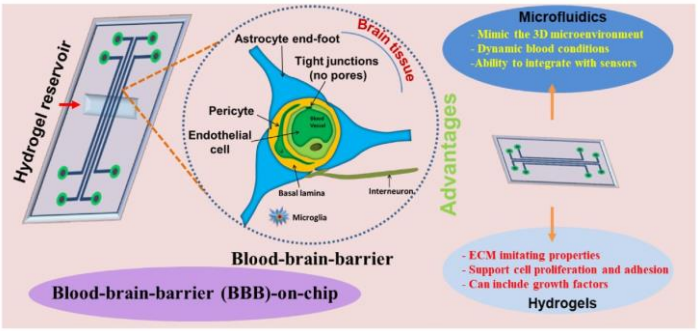

Fig. 5 Overview of the hydrogel-based BBB microfluidic model

IV. MICROFLUIDIC DEVICE

A well designed in vitro BBB models must contain structure of ECs, cell-cell interactions, controlled flow and a molecular transportable basal membrane as in BBB in vivo. In recent years, for the construction of microfluidic chips, bio-printing was greatly involved.

(1) Chip materials

Polydimethylsiloxane (PDMS) is the most generally used chip material. PDMS, a polymeric organosilicon compound, is a good material for fabricating biomedical devices due to its biocompatibility, easily shaped and cost-effective features. The other features of PDMS are non-toxic, non-flammable, optically transparent, gas and water permeable. Since it is optically transparent, the photography and observation of fluid flow and cell behaviours were made clear. Besides PDMS can be made into channels with different 3D structure due to its flexibility. The wet nature of PDMS surface can be easily adjusted with silanized modification. Eventhough the PDMS was considered to be the best material for microfluidic devices, the hydrophobic nature of it results in the coating of other substances.it may damage the expected cell behaviors. To master these limitations, poly methyl methylacrylate (PMMA) chips were designed. PMMA, a transparent thermoplastic chip which has better optical transmission, higher chemical stability, good compatibility with organic solvents and better bioavailability than PDMS. But these thermoplastic materials are not easily fabricated into complex micro- and nanostructures.

(2) Design and construction

Initially the classic methods like transwell system was used to create a sandwich like vertical structure in the microfluidic devices. The separation between the channels were made by the porous membrane. Generally, a barrier of EC's is cultured in the upper layer and to mimic cell-cell interactions other cells were cultured in the bottom layer. This type of vertical models was convenient for fabrication but due to the effect of gravity, the cells were settled in the bottom of channel. This makes less interaction between EC barrier and other BBB cells. And the observation of cell behaviors and other movements in the channels were difficult in case of vertical model. The polyethylene terephthalate (PET) and the polytetrafluoroethylene (PTFE) membranes were used as the best chip material for this model.

Recently, to overcome problems caused by vertical channels, parallel channels have been designed. In these models, the usage of extra membranes are avoided and thus the photography and observation of cell behaviors were made easy. Here, the membrane is replaced by PDMS. Because of the flexible nature of PDMS, making of stable thin membrane structure by photolithography seems challenging.in addition to that, the construction of tubular vessel like structure with shear stress as it is in brain is a complicated process. These constructed tubes were further cocultured with astrocytes in presence of a collagen matrix.

The BBB-on-chip model with ECM gels is considered as the best model nowadays. This model has the similar characteristics of basal membrane as in the in vivo model. Hydrogels are mostly used for designing a ECM barrier in BBB devices. Hydrogels play the role of scaffolds in cell culture models by providing a 3D culture environment. The bulk hydrogels with better porosity can furnish the essential permeable surface for the molecules and keep up the ECs to form barrier. For fabricating hydrogels- soft lithography, extrusion-based

bioprinting, light-based 3D bioprinting and laser-based photopatterning methods are used in microfluidics. It can be further cultured with astrocytes, pericytes and neurons. The EC layers can be produced on the surface of gels. This hydrogels-based models have been used to scrutinize cell spreading, tumor penetration and angiogenesis. The construction methods of BBB-on-a-chip was depicted in the picture shown below which was taken from the article, *Wang, Y. I., et al (2017)*

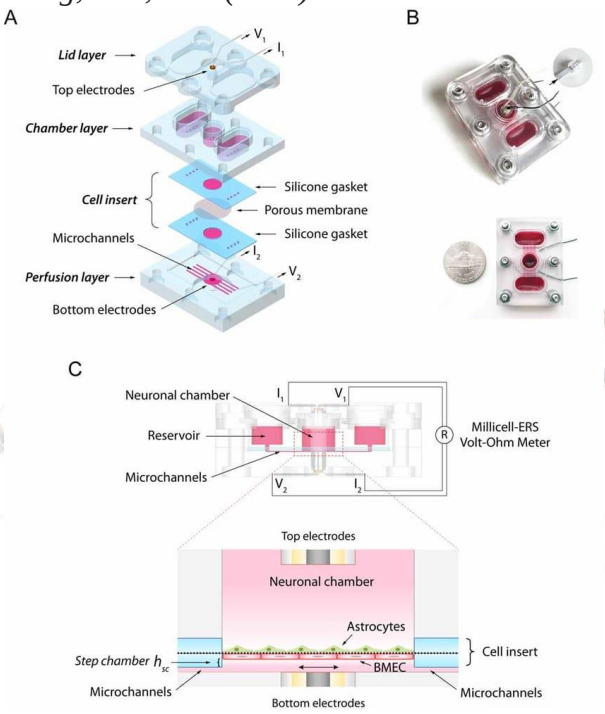

Fig. 6 Design of the BBB-on-a-Chip. (A) Schematic exploded view of the microfluidic platform. (B) The assembled device, with or without the lid. In order to visualize the microfluidic device (microchannels, neuronal chambers, reservoirs), a red dye was used. (C) Side view showing the fluid pathway, electrode wiring, and the BBB co-cultural orientation.

(3) Porous membrane

The membranes are used to separate the tight junction made by luminal and abluminal layers. To generate porous membranes, polycarbonate (PC), polyester (PE), polyethylene terephthalate and polytetrafluoroethylene were used.

(4) Cell source

As mentioned before, microvascular ECs are the major reason for maintaining physical barrier between blood and brain apart from astrocytes and pericytes. The complexity in isolation of cells, high expense and accessibility of human cell source needs replacement for fabricating in vitro BBB models.

The best replacement for human ECs are the immortalized cell lines. The most commonly used cell sources are hCMEC/D3 and hBMECs which are known to be human brain microvascular endothelial cells and HUVECs.

The usage of immortalized cell lines for BBB models may affect the stability of tight junctions. And also getting enough number of primary cells for model development is difficult. Hence the human pluripotent stem cells, hpsc -ECs are the efficient cell sources for fabricating in vitro BBB models.

(5) Shear stress

Under fluid flow condition, the characteristics of cells gets different from static condition. Hence it is necessary to maintain shear stress condition in the microfluidic devices as in natural BBB. The range of physiological shear stresses in the venous circulation is about 4-30 dyne/cm^2 to 1-4 dyne/cm^2.

IV. MICROFLUIDICS IN NEURODEGENERATIVE DISEASES

For the examination of CNS axon propagation, dopaminergic neurons, fabrication of neurovascular unit-on-a-chip, studies related to neural stem cells, BBB function, Alzheimer's disease, brain tumors and neurotransmitter function, microfluidic system is greatly involved.

(1) Drug delivery carriers on microfluidic chips

Recently, nanocarriers are the most prominent drug carriers with more therapeutic index, less side effects and increased uptake and penetration properties. Microfluidics, due to its excellent power to operate nanolitre flows, has been widely applied in the synthesis of nanoparticles as well as nano-particle based drug delivery system. Commonly, lipid-based nanoparticles, polymeric nanoparticles and inorganic nanoparticles are the widely used nanocarriers.

(2) Carrier free drug delivery using microfluidic chip

In the case of nanocarriers, a large quantity of expensive drugs may be used and there are many hurdles in fabricating nanocarriers. For microfluidic carrier free DDSs, the drug is directly applied to the site and the action is quick and effective. There are two categories in carrier free drug delivery: drug delivery based on microneedles and drug delivery based on micro-reservoirs.

a) Microneedles (MNs)

Stratum corneum (SC), a barrier to drug molecules is easily crossed by the MNs. Moreover, through MNs, patients can administer drugs by themselves. The length of MNs is in the range of 10µm to 1mm.due to their short size they can achieve a painless delivery to the patients. The improper degradation of drugs through metabolic pathway in conventional oral administration is avoided in percutaneous administration by MNs. The different kinds of microneedles are solid MNs, coated MNs, dissolving MNs, hollow MNs and hydrogel-based MNs. The mechanism of different types of microneedles are illustrated below which was retrieved from the article, *Kim, Y. C., et al (2012)*

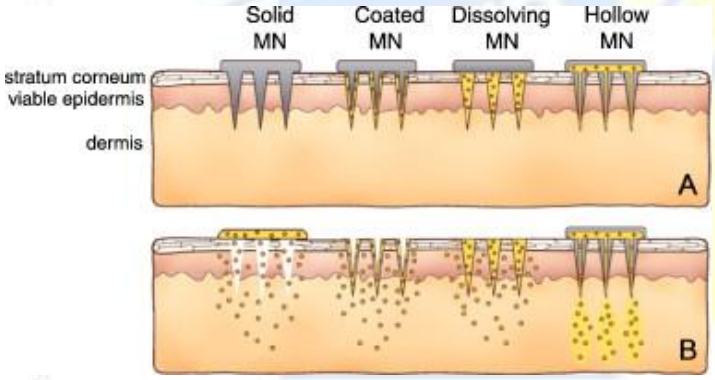

Fig. 7 Types of microneedles

b) Micro-reservoir system (MRS)

One or more reservoirs of drug are present in the micro-reservoir system to release the drug in controlled manner as shown in the picture which was retrieved from *Sahu. M et al (2017)*. The various delivery methods such as zero-order, pulsatile and on-demand dosing methods are used in micro-reservoir system. The efficient and accurate drug delivery is achieved by micro-reservoir system fabricated by microfluidic technology. To manufacture a driving system which can achieve the stable drug release is the primary part in microfluidic micro-reservoir system. There are two different motion mechanisms in micro-reservoir such as active and passive mode.

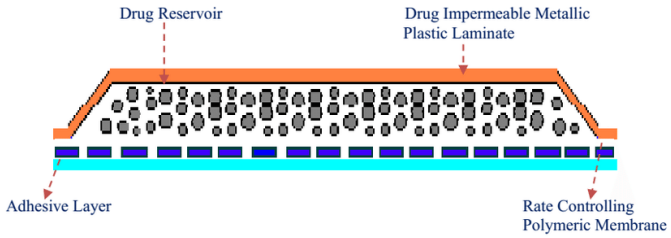

Fig. 8 Schematic representation of micro-reservoir system

V. CONCLUSION

There were many systems which play a huge part in the study of drug delivery principle across blood brain barrier. But the microenvironment of neural tissues is not completely regenerated in those methods. The pathophysiology and etiology of neurodegenerative diseases are not recapitulate in cell cultures and animal models. These drawbacks drive researches to follow the potential method like microfluidics to study neural problems. Nowadays microfluidics was emerging as a prominent tool in the study of brain related disorders due to the controlled monitoring of cells. In the study of drug delivery, high-throughput screening was achieved by the use of microfluidic technique. Hence microfluidics is an advanced and novel method in the study of neurodegenerative diseases which have its role in medical investigation and clinical studies.

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