



Certificate

This is to certify that the thesis titled "*Xurography-based Microfluidic Platform for Mimicking Neuronal Cytoarchitecture and Exploring its Application in Neurodegenerative Disease Research*" submitted by Subhadra Nandi (MP19BB004) to the Indian Institute of Technology Jodhpur for the award of the degree of *Doctor of Philosophy*, is a bonafide record of the research work done by her under my supervision. To the best of my knowledge, the contents of this report, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Date: 26/12/2024

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Declaration

I hereby declare that the work presented in this Thesis titled "*Xurography-based Microfluidic Platform for Mimicking Neuronal Cytoarchitecture and Exploring its Application in Neurodegenerative Disease Research*" submitted to the Indian Institute of Technology Jodhpur in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy, is a bonafide record of the research work carried out under the supervision of **Professor Surajit Ghosh**. The contents of this thesis in full or in parts, have not been submitted to, and will not be submitted by me to, any other Institute or University in India or abroad for the award of any degree or diploma.

Date: 26/12/2024

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Abstract

The brain is an intricate system composed of millions of neural networks. Deciphering its overall dynamics and the underlying significance of several cues that direct neuronal development, the formation of axons, dendrites, and synapses during wiring and re-wiring remains a formidable challenge in developmental and cellular neuroscience. Although traditional *in vitro* macroscopic cell culture techniques are easy to perform, they often fail to mimic the complex phenomenon of brain microenvironments. Recent advancements in microfluidic device-based cell culture technologies have successfully overcome the limitations of conventional cell culture methods, enabling the reconstitution of neural cytoarchitecture through precise spatiotemporal regulation of compartmentalized cell culture microenvironments. These lab-on-chip technologies can aid in elucidating the fundamental principles of brain function and provide innovative platforms for screening neurotherapeutics. This thesis offers a succinct overview of the structural and functional aspects of the human nervous system by the reconstitution of the central and peripheral nervous systems on the chip. It highlights the neurological disorders associated with dysfunctions in both systems. It also reviews the several strategies researchers have adopted to mimic neurogenesis on a chip.

Additionally, we have designed and developed an economical, innovative microfluidic device utilizing a state-of-the-art Xurography technique. Thereafter, we performed on-chip cell culture studies with primary neurons and SH-SY5Y cells to validate the cytocompatibility of the device. Furthermore, we demonstrated the application of the fabricated device as a co-culture model using astrocytes and neurons. The device also served as a drug screening platform for a multi-targeted compound in the differentiated SH-SY5Y cells in the context of Alzheimer's disease. Lastly, we also developed a Ferroptosis model that can be linked to neurodegenerative disorders. In this regard, we also investigated the efficacy of a synthesized small molecule inside our fabricated microfluidic device. All these lead to the conclusion that our simple and cost-effective Xurography-based microfluidic device can open gateways to decipher neuronal events inside the human brain and serve as potential platforms for neurodegenerative disease modelling and screening novel therapeutic agents.

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“The pursuit of a Ph.D. is not solely about achieving a degree; it is an art of constant resilience. Every result that may appear “average” might turn out to be more than sufficient. The essence of a Ph.D. lies in progress, not perfection.” As I prepare to close this remarkable chapter of my life, I reflect on the myriads of people who have played a role in accomplishing this arduous journey. To me, this Ph.D. degree has been much like the creation of a grand tapestry, woven together by threads of support, inspiration, and love. I want to take a moment to acknowledge those who have contributed their unique colours to this intricate design.

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List of Symbols

<i>Symbol</i>	<i>Description</i>
μ	Micro
nm	Nanometer
ρ	Fluid Density
v	Velocity Field
p	Pressure drops across the channel
μ	Fluid Viscosity
f	Volume forces
Re	Reynold's number
L	Characteristic length scale of the system
c	Molecule concentration
D	Diffusion coefficient
Ra	Average Roughness
KCl	Potassium chloride
μ	Micrometer
Au	Gold
Cu	Copper
Fe	Iron
MeOH	Methanol
Tyr	Tyrosine
Trp	Tryptophan

List of Abbreviations

<i>Abbreviation</i>	<i>Full form</i>
Ph.D.	Doctor of Philosophy
MEMS	Microelectromechanical Systems
OoC	Organs-on-a-Chip
LOC	Lab-on-a-Chip
MPS	Microphysiological Systems
NMR	Nuclear Magnetic Resonance
HPLC	High-Performance Liquid Chromatography
GPC	Gas-Phase Chromatography
DARPA	Defence Advanced Research Projects Agency of the USA
μFD	Microfluidic Device
2D	Two-dimensional
3D	Three-dimensional
CAD	Computer-Aided Design
Si	Silicon
PDMS	Polydimethylsiloxane
FDM	Fused Deposition Modelling
SL	Stereolithography
UV	Ultraviolet
MJM	Multi Jet Modelling
DLW	Direct Femtosecond Laser Writing
2PP	Two-Photon Polymerization
POC	Point-of-Care
MB-AgNP	Magnetic Bead-Silver Nanoparticles
ECM	Extracellular-Matrix
iPSCs	Induced Pluripotent Stem Cells
BBB	Blood-Brain Barrier
AD	Alzheimer's Disease
Ca ²⁺	Calcium ion
TH	Tyrosine Hydroxylase
NSCs	Neural stem cells
CNS	Central Nervous System
DRG	Dorsal Root Ganglion
pMEAs	planar Microelectrode Arrays
SHH	Sonic Hedgehog
DA	Dopamine
DN	Dopaminergic neurons
SNc	Substantial Nigra pars compacta
VTA	Ventral tegmental area
ELISA	Enzyme-Linked Immunosorbent Assay
PD	Parkinson's Disease
hBMVECs	Human Brain Microvascular Endothelial Cells
ADAM10	A Disintegrating and Metalloprotease 10
AChR	Acetylcholine Receptor
HUVEC	Human Umbilical Vein Endothelial Cells
SCG	Superior Cervical Ganglion and
VMs	Ventricular Myocytes
NGF	Nerve Growth Factor

UMNs	Upper Motor Neurons
LMNs	Lower Motor Neurons
GDNF	Glial Cell-Derived Neurotrophic Factor
ALS	Amyotrophic Lateral Sclerosis
SMA	Spinal Muscular Atrophy
BoC	Brain-on-Chip
PMMA	Poly (methyl methacrylate)
PC	Polycarbonate
PI	Polyimide
PVC	Poly Vinyl Chloride
Si	Silicon
iNeurons	iPSC-derived neurons
FEA	Finite Element Analysis
EF	Electric Field
ESCs	Embryonic Stem Cells
EBs	Embryoid Bodies
ECM	Extracellular Matrix
NPCs	Neural Progenitor Cells
NSA	Neurosphere Assay
μ CDC	Microfluidic-Cell Dissociation Chip
SCs	Stem Cells
hNSCs	human NSCs
VMs	Ventricular Myocytes
Tpm	Tropomyosin
NT-3	Neurotrophin-3
SARAPE	Survey, Anchor, Wrap, and Expand
LGICs	Ligand-Gated Ion Channels
μ LP	Microfluidic Local Perfusion
MBP	Myelin Basic Protein
ChR2	Channelrhodopsin 2
TBI	Traumatic Brain Injury
SCI	Spinal Cord Injury
PPAR γ	Peroxisome Proliferator-Activated Receptor Gamma
NDD	Neurodegenerative Disease
MCE	Microfluidic Capillary Electrophoresis
mQCM	Miniature Quartz Crystal Microbalance
α Syn	alpha-Synuclein
A β	Amyloid Beta
CSPG	Chondroitin Sulfate Proteoglycans
PEGDA	Poly(ethylene) glycol diacrylate
AI	Artificial Intelligence
CANDY	Communicative Astrocyte-Neuron DYnamics
MeHg	Methylmercury
CAD	Computer-Aided Design
PDL	Poly-D-Lysine
IAEC	Institutional Animal Ethics Committee
CPCSEA	Committee for Control & Supervision of Experiments on Animals
HBSS	Hanks' Balanced Salt solution
FBS	Fetal Bovine Serum
BDNF	Brain-Derived Neurotrophic Factor
RA	Retinoic Acid

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
GFAP	Glial Fibrillary Acidic protein
TUJ1	beta III tubulin
NFTs	Neurofibrillary Tangles
APP	Amyloid Precursor Protein
ROS	Reactive Oxygen Species
DCM	Dichloromethane
HFIP	1,1,1,3,3,3-Hexafluoroisopropanol
HEPES	2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid
PIPES	4-Piperazinediethanesulfonic acid
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
ThT	Thioflavin T
BDNF	Brain-derived Neurotrophic Factor
RA	Retinoic Acid
DCFDA	2',7'-dichlorofluorescein diacetate
PBS	Phosphate-buffered saline
DIPEA	Diisopropylethylamine
FBS	Fetal Bovine Serum
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
FITC	Fluorescein isothiocyanate
TRITC	Tetramethylrhodamine isothiocyanate
ANOVA	Analysis of Variance
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
MMP	Mitochondrial Membrane Potential

List of Publications

Publications From Thesis Work:

1. **Nandi, S.#**; Ghosh, S.#; Adak, A.#, Roy, R., Bera, A., Ghosh, S.*, **2025**. Crafting Precision: Design and Fabrication of Xurography-Driven Microfluidic Platform for Neuronal Co-Culture Exploration and Targeted Drug Screening. *ACS Chem. Neurosci.* (Revision Submitted)
2. **Nandi, S.#**, Ghosh, S.#, Garg, S. and Ghosh, S.*, **2024**. Unveiling the Human Brain on a Chip: An Odyssey to Reconstitute Neuronal Ensembles and Explore Plausible Applications in Neuroscience. *ACS Chem. Neurosci.* 15, 21, 3828–3847.
3. **Nandi, S.**, Ghosh, S., Garg, S., Sarkar, A., Ghosh, S.*, **2022**. Brain on a-Chip. *Microfluidics and Multi Organs on Chip, Springer*, 475–493.
4. Mukherjee, N., **Nandi, S.**, Ghosh, S., Garg, S. and Ghosh, S.*, **2019**. Three-dimensional microfluidic platform with neural organoids: model system for unraveling synapses. *ACS Chem. Neurosci.*, 11(2), 101-102.

Publications Beyond Thesis Work:

1. Roy, R., Khan, J., Pradhan, K., Nayak, P., Sarkar, A., **Nandi, S.**, Ghosh, S., Ram, H. and Ghosh, S.*, **2022**. Short peptoid evolved from the key hydrophobic stretch of amyloid- β 42 peptide serves as a potent therapeutic lead of Alzheimer's disease. *ACS Chem. Neurosci.* 14(2), 246-260.
2. Mukherjee, N., **Nandi, S.**, Garg, S., Ghosh, S., Ghosh, S., Samat, R. and Ghosh, S.*, **2020**. Targeting chondroitin sulfate proteoglycans: an emerging therapeutic strategy to treat CNS injury. *ACS Chem. Neurosci.*, 11(3), 231-232.
3. Roy, R., Khatua, M., Ghosh, S., **Nandi, S.**, Ram, H. And Ghosh, S.*, **2025**. Endogenous Pigment Mimicking Engineered Nanovesicle Targets Extrasynaptic NMDA Receptors Against Ca^{2+} Mediated Excitotoxicity in Alzheimer's Disease. *ACS Appl. Mater. Interfaces*. (Under Peer Review)

Publication under Communication:

Equal Contribution

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