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Review Article

In Order To Address Alterations In The Blood Brain Barrier Brought On By Microgravity In An Organ On Chip Model, The Self-Designed Vegf-A Sirna Was Administered Utilising Calcium Phosphate Lipid Nanoparticles (Cap-Lipid Nps) As A Cargo Module. - A Review

Dr Debopriya Ghosh

Department of Physiology, University College of Medical Sciences. Delhi, India

Abstract:

A novel category of microengineered laboratory models called "organs-on-chips" combines some of the best aspects of existing in vivo and in vitro models. The development of blood-brain barrier (BBB) organ-on-chip models has advanced, but there are still obstacles to overcome, which are outlined in this paper. Specialized endothelial cells create the BBB, which divides brain tissue from blood. It offers equilibrium for ideal neuronal activity and shields the brain from potentially hazardous blood-borne substances. Drug development and biological research both benefit from studying BBB function and malfunction. Real-time analysis of (human) cells in an artificial physiological microenvironment is made possible by microfluidic BBBs-on-chips, which, for instance, include tiny geometries, fluid flow, and sensors. The possibility for more accurate microenvironments and the investigation of organ-level functioning is already demonstrated by BBBs-on-chips examples in the literature. The current lack of consistent measurement of variables like barrier permeability and shear stress is a major obstacle in the field of BBB-on-chip development. This restricts the ability to compare the effectiveness of certain BBB-on-chip models directly to that of other models, both new and old. We make suggestions for more model characterisation standards and draw the conclusion that the quickly developing area of BBB-on-chip models has tremendous promise for additional research into BBB biology and medication development.

Keywords: BBBs-on-chips, blood-brain barrier, endothelial cells, microfabrication, microfluidics, organs-on-chips

Introduction:

Microgravity is a major risk factor that, among other risk factors, contributes to the health risks that astronauts face during missions outside the Earth's atmosphere. Recent space missions are primarily concerned with potential short- and long-term spaceflight-induced neurovascular damage and late neurodegeneration1. A tightly regulated intracerebral microenvironment is maintained by the blood-brain barrier (BBB), which is more than just a physical barrier made up of endothelial cells that interact closely with other central nervous system (CNS) cells like neurons, pericytes, and astrocytes through adherent junctions. The Neurovascular Unit (NVU), which is made up of these intricate structures, is widely acknowledged as being crucial for controlling BBB permeability. BBB's distinctive properties prevent paracellular diffusion while permitting tightly regulated receptor-mediated endocytosis of larger molecules. Dysregulation of neurotransmitters and neuroactive agents leads to BBB dysfunction, which is frequently accompanied by neuroinflammation, disruptions in the ionic microenvironment, and fluid volume changes1. Multiple cell types can now be co-cultured in in vitro models to mimic the interactions of neurons thanks to significant recent

advancements in tissue engineering and biomaterials 8,9,10,11. Microfluidic in vitro BBB models have more realistic dimensions and geometries than traditional in vitro BBB models, and they also subject the endothelium to physiological fluid flow, which is expected to enhance BBB modelling. The ability to test the expression of specific markers, such as adherens and tight junction proteins, which can provide information on organ-level function, as well as information about the immediate functionality, such as the permeability of the cell, is what makes "BBBson chips" so important. Additionally, BBBs-on-chips have the potential to directly measure a greater number of BBB functions by incorporating sensors and providing results in real-time. The primary cells of the nervous system, or neurons, are not only morphologically and physiologically diverse, but also rigidly organised to create intricate circuits. Only a small number of specific neuronal types can be replaced thanks to neural stem cells. Large-molecule drugs and the majority of small-molecule drugs cannot cross the blood-brain barrier (BBB) because it exhibits a selective permeability for molecules with a narrow range of molecular weight and lipophilicity. Because it can result in a stable or inducible expression of the therapeutic gene(s) and allow for a nearly specific expression in target cells, gene therapy is becoming a more appealing therapeutic option in this context. Numerous preclinical and clinical studies have amply demonstrated the potential of gene therapy for the treatment of CNS diseases, even though much work needs to be done before it becomes standard practise. The majority of common and sporadic neurodegenerative disorders are directly correlated with gene therapy and BBB dysfunction. As the blood-brain barrier (BBB) plays a critical role in defending the central nervous system (CNS), it is thought that the BBB prevents the delivery of nearly all biologics, including small interfering RNA (siRNA), DNA, and antisense oligonucleotides, as well as more than 90% of small-molecule drugs, into the CNS (ASOs). Nanocarriers offer excellent opportunities to efficiently package and protect the therapeutic agents with subsequent transport across the BBB, avoiding extensive systemic distribution, in particular for CNS drug delivery. Additionally, nanotechnology offers a number of intriguing potential solutions to this issue. Nanoparticles made of calcium phosphate and lipid (CaP-lipid NPs) are consistently effective and satisfactory at delivering DNA. Its structure is based on calcium-phosphate core nanoparticles with cationic lipid shells surrounding them. The therapeutic effects of oligonucleotide-loaded calcium phosphate lipid nanoparticles (CaP-lipid NPs) on blood brain barrier structural changes are the goal of this study's design. The current study was examine the therapeutic potential of oligonucleotide-loaded calcium phosphate lipid nanoparticles as well as the effects of microgravity on the blood-brain barrier (BBB) integrity on the Organ on Chip (OoC) platform (CaP-lipid NPs). Aim of the study is to review into the potential impact of a drug delivery system induced by calcium phosphate lipid nanoparticles (CaP-lipid NPs) on BBB changes brought on by microgravity on Organ on Chip platforms from previous findings.

Organ on Chip Modelling:

The multiplexed chip was have eight parallel channels, each 500 mm wide and 50 mm tall, that branch off of a single inlet and have their own access ports. The simultaneous filling of all channels is made possible by a common inlet, which is positioned exactly the same distance from each channel to ensure an even distribution of flow and cells through the channels. On the other hand, the distinct outlets of eight channels was also make them individually addressable, allowing the simultaneous performance of eight different experimental conditions by drawing liquids through the channels from eight different reservoirs toward the shared access port. Additionally, the channels' width rises successively after each junction to 630 m, 794 m, and reaches 1000 m at the inlet. The fabrication of the microchannels wasdone using conventional soft lithography3. The PDMS prepolymer and curing agent (Sylgard 184 Silicone elastomer kit, Dow Corning) wascast onto a patterned SU-8 mould at a weight ratio of 10:1 to create the microfluidic device. The polymer wasallowed to cure for an entire night at 60 °C before being removed from the mould. The chip was have a 3 mm thickness. In the following step, a biopsy puncher with a 1 mm diameter wasused to punch the inlets and outlets. The PDMS layer was then

be adhered to the glass by being exposed to oxygen plasma for 1 minute at 50 W, Cute, Femto Science. To strengthen the bond between the components, the entire assembly washeated at 60 °C for at least 20 minutes.

Membrane Fabrication:

A delicate, transparent PDMS membrane wascreated in order to replicate barrier models in the two-layer chip. Without the use of additional glue, this membrane can be integrated into the multiplexed chip. The photoresist (PR) spin-coating step wasthe only step required for the fabrication process. The membrane wasreleased from a sacrificial positive PR layer (AZ 9260, Fujifilm, Japan) by submerging it in acetone. To achieve a 10 m thick PR, a Si wafer (525 m thick, Okmetic, Finland) was have a PR deposited on it at 2000 rpm for 60 s. By sandwiching the PDMS membrane between the identical top and bottom pieces, a two-layer device can also be made; in this case, the channel height wasset to 375 m. The three components of the chip wasactivated in oxygen plasma (50 W, Cute, Femto Science)6 for 2 minutes after being punched for all of the inlets and outlets. This was strengthen the bonding. A microscope wasused to align the top compartment with the reversed bottom compartment so that the channels overlap. Individual evaluations are possible for the eight apical channels and the eight corresponding basal channels. The final chip was have a thickness of 6 mm. The two-layered chip was then be baked for 2 minutes at 110 °C on a hot plate. The PR wasdeveloped in an OPD4246 developer for 6 minutes before being rinsed with de-ionized (DI) water to obtain the microcolumns.

Preparation of the Pdms Membrane:

To reduce viscosity, a solution of PDMS prepolymer and a curing agent wasdiluted with hexane at a 2: 5 w/w (PDMS: hexane) ratio. After that, the solution wasspin-coated over the created PR column arrays for one minute at 4000 rpm and baked for at least three hours at 60 °C. The cured PDMS membrane was require a plasma etching process to confirm the pores are in their open state. A reactive-ion etching system (TEtske, Nanolab University of Twente, The Netherlands) wasused for the etching, which was take place at 47 sccm SF6 and 17 sccm O2, 100 W, and 50 mTorr for two minutes. The final membrane was have a 2 percent porosity. Scanning electron microscopy (SEM, HR-SEM, FEI Sirion microscope) can be used to measure the thickness and topology of PDMS membranes at 5 kV acceleration voltage6.

Transfer the pdms membrane to the chip:

The porous PDMS surface on the Si wafer and the top layer of the PDMS device wasbrought into contact with oxygen plasma treatment before being baked at 60 °C for 10 minutes. We was soak the sacrificial PR layer in acetone for 5 minutes to remove it. The bottom part's membrane can be assembled with the top part after receiving an oxygen plasma treatment for 3 minutes 6.

Cell culture for organ on chip:

Human astrocytes (HAc) (Cell Applications, Inc.) wascultured in Astrocyte growth medium containing growth supplement while human cerebral microvascular endothelial cells (hCMEC/D3, Merck Millipore) wascultured in endothelial growth medium (EGM, Cell Applications, Inc.) in collagen I-coated culture flasks (ScienCell Research Laboratories) 5. Every two to three days, the medium needs to be changed, and both cultures need to be kept at 37 °C and 5 percent CO2. The microfluidic chips needed to be exposed to an oxygen plasma (50 W, Cute, Femto Science) before cell seeding in order to make the surface hydrophilic. The following step involves coating the chips with collagen type I (rat tail, Corning) (100 g ml1) for 2 hours at 37 °C after rinsing them with phosphate-buffered saline (PBS; Sigma). The beginning of endothelial cell monoculture was involve adding EGM to the chips. Then, using a single pipetting motion through the common access port, hCMEC/D3 cells waspipetted simultaneously into all eight channels at a density of 6 106 cells per ml (3 104 cells per cm2). Non-adherent cells wasremoved with EGM6 after 1 hour of static incubation at 37 °C and 5 percent CO2. The channels in a two-layer device wasfilled with astrocyte medium to create an endothelial cell/HAc co-culture. First, HAc wasseeded at a concentration of 2 106 cells per ml (7 104 cells per cm2) on the basal side of all eight channels. 6. The chips was then be inverted for 4 hours to allow for the attachment of cells to the membrane's lower side. The chips was then be turned back over, and each access port can

be filled with fresh astrocyte medium using pipette tips. After astrocytes have been cultured for 24 hours, EGM wasadded to the top compartment, and hCMEC/D3 cells wasadded there at a density of 6 106 cells per ml (2 105 cells per cm2). then the cells. Last but not least, morphological observations of the cells inside the chip can be made using phase-contrast and fluorescent imaging (EVOS FL Cell Imaging System, Life Technologies, and LEICA DM IRM HC, air objectives). The following substances waspurchased from Avanti Polar Lipids, Inc.: cholesterol, dioleoylphosphatydic acid (DOPA), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP), 1,2-dipalmitoyl-snglycero-3-phosphoethanolamine-N-(lissaminerhodamine B sulfonyl) (ammonium salt) (LissRd (Alabaster, AL). Without further purification, all other chemicals waspurchased from Sigma-Aldrich (St. Louis, MO). While scrambled siRNA (siScrambled) with sense strand 5′- AUG UAU UGG CCU GUA UUA GdTdT-3′ waspurchased from Dharmacon (Thermoscientific, Lafayette, CO), self-designed VEGF-A siRNA (siVEGF-A) with sense strand 5′- UCC GCA GAC GUG UAA AUG UdTdT-3′ wasdesigned using the webserver We was buy Lipofectamine RNAiMAX from Invitrogen (Carlsbad, CA). Dioleoylphosphatidic acid, 1,2-dioleoyl-3-trimethylammonium-propane chloride salt, and 1,2-distearoryl-sn-glycero-3-phosphoethanolamine-N[methoxy(polyethyleneglycol-2000)] ammonium salt (DSPE-PEG2000) wasacquired from Avanti Polar Lipids (Alabaster, AL). In our lab, DSPE-PEG-AA wascreated as previously described. 16 Without further purification, additional chemicals waspurchased from Sigma-Aldrich (St. Louis, MO).

Preparation of oligonucleotide-loaded calcium phosphate lipid nanoparticles (caplipid nps):

We was prepare calcium phosphate lipid nanoparticles (CaP-lipid NPs) in a manner similar to that of Li et al. 13 with a few minor adjustments. First, a water-in-oil microemulsion method wasused to prepare the anionic lipid coating CaP core. In order to create a very well dispersed water-in-oil reverse microemulsion, 300 L of 500 mM CaCl2 wasadded to a 15 mL solution of cyclohexane/Igepal CO-520 (70/30 v/v). 300 mL of 5 mM Na2HPO4 (pH = 9.0) and 200 mL of 20 mg/mL dioleoylphosphatydicacid (DOPA) in chloroform wasdissolved in a further 15 mL of cyclohexane/Igepal solution to create the phosphate phase. The calcium phase was then gradually receive dropwise additions of the phosphate phase. To create the CaP cores, the solution wasstirred for 20 minutes. Divided microemulsions wascreated. Each tube was receive 15 mL of absolute ethanol before being centrifuged in the Heraeus Megafuge X3R at 9,000 g for 30 minutes (Thermo Scientific, USA). The CaP core pellet wascentrifuged three times, washing and rinsing each time with ethanol. The pellet wasreconstituted in 1 mL of chloroform and kept in a tiny glass vial for lipid coating later on. The thin film hydration method wasused to create the final CaP lipid-coated nanoparticles. In a nutshell, 50 mL of 10 mM DOTAP/Cholesterol (1:1) and 50 mL of 3 mM DSPE-PEG-2000 wasadded to 500 mL of CaP cores. LissRdB-DSPE (12.5 L of a 16 mM stock solution) was also be added for cellular uptake studies. The chloroform wasremoved from the solution using a rotary evaporator (Büchi, Switzerland) before the solution is transferred to a flask with a flat bottom. The lipid film was then be rehydrated using a solution of Tris-HCl buffer (1 mL; 5 mM; pH 7.4), which was then be gently sonicated for 5 minutes. The finished CaP-lipid NPs wasbiologically evaluated after being sterile filtered (0.22 m) and stored at 4°C.

Particle characterization:

Malvern Zetasizer ZS (Malvern, CA) wasused to measure the zeta () potential of CaP-lipid NPs, and Malvern Zetasizer APS wasused to determine size by dynamic light scattering (DLS) (Malvern, CA). Particle concentration wasdetermined using NanoSight (LM14, NanoSight, UK). Particles was specifically be diluted to 108–1010 particles/mL (or a 1:500 dilution in Milli-Q water) before being observed under a standard optical microscope with a CCD camera at room temperature. The particle motion wascaptured at 30 frames per second for 60 seconds (20–100 particles per field of view). The NanoSight software was then calculate particle concentration (NTA 2.3, UK). We used transmission electron microscopy to see the morphology of CaPlipid NPs (TEM). The nanoparticle suspension wasdropped onto a

copper grid with a carbon coating of 200 mesh and stained with phosphotungstic acid at a rate of 1%. (PTA). The stained suspension wasdried at room temperature and then examined under a transmission electron microscope after being washed with Milli-Q water (TEM; Gantan, Inc.). A JEOL 2010 instrument running at 200 kV wasused to conduct the TEM. To improve diffraction contrast, an objective aperture wasinserted into bright field images that are being captured. Using a Gatan Orius camera and Gatan's Digital Micrograph software, images wascaptured. The final VEGF-A siRNA (siVEGF-A) loaded CaP-lipid NPs wasdissolved in an equal volume of lysis buffer (2 mM EDTA and 0.05 percent Triton X-100 in water). Oligonucleotide loading was then be determined using the propidium iodide (PI) staining method. Fluorescence (RFLU) wasassessed using a FLUO star plate reader (BMG Labtech, Germany) and PI (1 L; 1 g/L) in each 100 L sample. The reader's excitation and emission wavelengths are 540 nm and 620 nm, respectively. The interpolation of RFLU from a VEGF-A siRNA (siVEGF-A) standard curve was then be used to calculate the amount of VEGF-A siRNA (siVEGF-A) loaded into CaP-lipid NPs. By measuring the change in particle size (number peak area) over time as measured by DLS, the stability of VEGF-A siRNA (siVEGF-A) -loaded CaP-lipid NPs wasevaluated. In a nutshell, 100 L of a sterile, 4°C-incubated VEGF-A siRNA (siVEGF-A) CaP-lipid NP suspension wasused. At the following time intervals: 0, 4, 8, 12, 16, 20, 24, and 28 days, the peak area of the particles wasmeasured. The particles wasdiluted 10 times (v/v) in PBS (pH 7.4, 6.5, or 5) for pH-responsive studies, and they wasincubated for 2 and 4 hours at 37°C with constant shaking at 200 RPM. DLS was note the change in particle size (number peak area). To ensure that the CaP-NPs maintain their size when diluted in complete DMEM media supplemented with 10% FCS, the corresponding anti-dilution ability would also be found.

In Vitro Cellular Uptake Of Oligonucleotide Loaded Cap-Nps:

To track cellular uptake over time, hCMEC/D3 Cells was then be incubated for an additional 30, 60, or 90 min at 37°C. A Leica TCS SPII laser scanning confocal microscope wasused to image the cells after they have been washed in chilled 1 PBS and the media has been removed (Heidelberg, Germany). By using Image J to calculate the mean fluorescence per cell from five images for each treatment, internalisation wasquantified (Schneider et al., 2012). This indicates a minimum of 100 cells were examined for each treatment. LysoTracker Green DND-26 (Life Technologies, Mulgrave, VIC) wasused to stain for acidic compartments, lysosomes, and endosomes to confirm the mechanism of particle uptake. Prior to adding LissRdB-DSPE CaP-lipid, hCMEC/D3 cells wasincubated with Lysotracker Green DND-26 for 30 min at 37°C. Microgravity Exposure: Materials International Space Station Experiment science carrier module (MISSE MSC) wasused for the microgravity exposure for this experiment. The duration of the exposure was90 days. The experiment module wasmounted under the exposure deck in the MSC volume (for the requirement of vacuum, temperature cycles and ionizing radiation exposure). Power requirement for the experiment was75 W and input DC voltage requirement wasof 12 V. Standard mounting fixtures wasrequired with the dimension of 3 sets of 2”X1”X0.3”, 3 sets of 1.5”X1”X0.3” and 2 sets of 3”X1.5”X0.3” fabricated mounting along with respective exposed window. The sensor data wascollected from the temperature, radiation and humidity sensor. The downlink of data requirement wasRS -422. Viability and Metabolic activity of hCMEC/D3 i.e. oxidative damage, apoptotic cells expression, blood brain barrier integrity and most importantly evaluation of cell adhesion molecule wasperformed by live-dead staining. Mitochondrial Activity: MTS assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay; Promega, Madison, WI, USA) wasperformed to analyse mitochondrial function. Measurements of Caspase 3/7 Activity: The Caspase-Glo 3/7 Assay System (Promega) was used to measure the activity of caspases 3 and 7 in cells treated with the use of simulated microgravity. Chip fabrication validation can be performed by finite element modelling of fluid flow, selective addressing of the each channels and immunofluorescence. Barrier permeability and blood brain barrier integrity across the endothelial monolayer wasassessed (Permeability assessment) on day 5 after cell seeding by using a fluorescence microscope (LEICA DM IRM HC). The FITC-dextran (Sigma-Aldrich) at two

different molecular weights (4 kDa and 20 kDa) wasused as a paracellular permeability marker. Finally, the average permeability coefficients of eight channels wascalculated. Statistical Analysis would be Two-way ANOVA determined statistical significance with Tukey's post-hoc test within groups following a normal distribution. Differences within p values ≤ 0.05 were assumed to be statistically significant. The results wasanalysed with the Microsoft Office Excel 2019 and Octave mathematical analysing software.

Opportunities & Conclusion:

In several physiological systems, prolonged microgravity has been proven to accelerate the onset and progression of illness, including fast bone loss (1-2% monthly loss of bone mineral density), cardiovascular deterioration, and loss of skeletal muscle mass and strength. These alterations resemble ageing and chronic human illnesses that exist on Earth. The ability to detect illness onset and development as well as test potential therapies more quickly and effectively than in normal labs may be made possible by doing experiments in microgravity. Other researchers may find the microgravity setting advantageous for promoting the growth of complex tissues made up of several cell types. In the lack of buoyancy and density-driven convection, which cause cells to sink to the bottom of culture containers and prevent cell and tissue culture in microgravity, For deployment on the International Space Station National Laboratories, the MPS system must undergo the adaptation shown in Figure 1. (a) Nortis Bio's Kidney MPS platform, which it created and sold. (b) A typical laboratory perfusion system for 40 single channel chips, based on a syringe pump and incubator. (b) The demands for environmental control and perfusion system miniaturisation. Microphysiological Systems, or MPS. instead of distributing in an anatomically accurate way, aggregate based on size and density. Cells in microgravity, on the other hand, have a tendency to self-assemble gradually and aggregate based on cell-to-cell contact and innate physiologic affinity. 10 A 3D microfluidic tissue chip and a microgravity environment can be combined in a special way to reveal novel insights into disease models and therapeutic targets. For instance, the creation of a biomimetic kidney organ-oid has been the subject of much research. The pathophysiology of both acute and chronic kidney disorders is difficult to replicate in vitro due to the anatomic complexity and interactions between at least 26 cell types in the kidneys, as well as the gradual and insidious progression of many kidney diseases (occurring over years to decades). Although current kidney organoids made from induced pluripotent stem cells are made up of a variety of cell types, they still retain a foetal phenotype and might not be entirely suitable for research on kidney disease in ageing adults. For conditions like polycystic kidney disease, diabetic kidney disease, and chronic kidney disease of unknown aetiology, a truly biomimetic organoid with correctly polarised and physiologically self-assembled cell types developed in microgravity may be used to identify initiating events, biomarkers of disease progression, and therapeutic targets. It has only recently been discovered how to examine ageing and altered organ function at the cellular/molecular level in microgravity using 3D microphysiological devices. BBB research has a lot of potential to advance with the introduction of BBBs-on-chips. The benefits of both in vivo and in vitro models are merged in microfluidic systems, where organ-on-chip technologies allow for the study of organ-level function while still being reliable, repeatable, and simple to assess like in vitro models. Numerous papers on BBBs-on-chips have already been published in the literature, showing cutting-edge techniques and encouraging outcomes. The advantages of using microfluidics for BBB research applications are already demonstrated by these instances. Organ-on-chip technologies also offer versatility in the creation of microenvironments, control over those settings, and readout techniques. This makes it possible to create a variety of BBB-on-chip models, each of which may respond to a particular research topic. Though it is advantageous to have some consistency and agreement across researchers, comparison and validation of the BBB-on-chip models may be compared and validated more quickly. Four areas of this review are highlighted. Any BBB model should have a readout for BBB permeability. The barrier permeability should be provided as universal numbers, such as permeability coefficients, to allow comparison between platforms.

Additionally, TEER is a simple and non-invasive way to gauge how tight a barrier is. TEER levels may also be compared between devices if they are accurately recorded and computed. Although human cells are more useful for human BBB research, animal sources of brain microvascular endothelial cells are more frequently available. iPS cells have the potential to be a more widely available source of relevant cells that are also appropriate for customised treatment. Finally, physiologically comparable shear stress may be applied to the endothelial cells inside BBBs-on-chips. In order to obtain mainly homogeneous shear stress across the cell barrier, suitable channel designs are necessary. In addition to the factors for BBBs-on-chips design and protocol optimization that were addressed in this research, there are other microenvironment factors that will gain from a more uniform approach. The selection of chip materials and geometries, as well as the inclusion of biological agents in the microenvironment, are a few of these. To achieve this, it is advantageous to have interdisciplinary teams create BBBs-on-chips so that both biological and engineering considerations are taken into account. 6 The widespread use of microfluidic BBBs-on-chips also necessitates low-cost manufacturing, simple operation, and the capability of parallelized, high-throughput models. We are optimistic that the fast developing area of BBB-on-chip models will soon have a significant influence on biological research and medication development. It should be possible to identify new therapeutic targets for the prevention and treatment of many chronic health conditions using tissue chips in the unique microgravity environment. It should also be possible to gain new insights into the onset and course of sickness.

References:

[1] A. Kaneko, Y. Sankai, PLoS One 2014, 9, e102703.

[2] A. M. Taylor, D. C. Dieterich, H. T. Ito, S. A. Kim, E. M. Schuman, Neuroendocrinology 2010, 66, 57.

[3] Banerjee, R, Tyagi, P, Li, S and Huang, L (2004). Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. Int J Cancer 112: 693–700.

[4] Cashman, N. R., Durham, H. D., Blusztajn, J. K., Oda, K., Tabira, T., Antel, J. P., et al. (1992). Neuroblastoma x spinal cord (NSC) hybrid cell lines resemble developing motor neurons. Dev. Dyn. 194, 209–221. doi: 10.1002/aja.1001940306

[5] Chen, Z.-Y.; Guo, S.; Li, B.-B.; Jiang, N.; Li, A.; Yan, H.-F.; Yang, H.-M.; Zhou, J.-L.; Li, C.-L.; Cui, Y. BioMed Res. Int. 2019, 2019, 1–17.

[6] Chu MQ, Liu GJ. Preparation and characterization of hydroxyapatite/liposome coreshell nanocomposites. Nanotechnology. 2005; 16:1208–1212.

[7] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, Annu. Rev. Biomed. Eng., 2001, 3, 335–373.

[8] H. Le-The, M. Tibbe, J. Loessberg-Zahl, M. P. Do Carmo, M. Van Der Helm, J. Bomer, A. Van Den Berg, A. Leferink, L. Segerink and J. Eijkel, Nanoscale, 2018, 10, 7711–7718.

[9] H. Yuan, M. W. Gaber, T. McColgan, M. D. Naimark, M. F. Kiani and T. E. Merchant, Brain Res., 2003, 969, 59–69.

[10] J. Park, S. Kim, S. I. Park, Y. Choe, J. Li, A. Han, J Neurosci. Methods, 2014, 221, 166.

[11] K. Zografos, R. W. Barber, D. R. Emerson and M. S. N. Oliveira, Microfluid. Nanofluid., 2015, 19, 737–749.

[12] M. Zakharova, M.A.Palma do Carmo, Lab Chip, 2020, 20, 3132-3143.

[13] Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9:671. doi: 10.1038/nmeth.2089

[14] Xiao W. Mao1 | Nina C. Nishiyama1 | Stephanie D. Byrum2,3 | Seta Stanbouly1 The FASEB Journal. 2020;34:15516–15530.

[15] Y. H. Kim, S. H. Choi, C. D'Avanzo, M. Hebisch, C. Sliwinski, E. Bylykbashi, K. J. Washicosky, J. B. Klee, O. Brüstle, R. E. Tanzi, Y. Kim, Nat. Protoc. 2015, 10, 985.

[16] Yuan, B, Latek, R, Hossbach, M, Tuschl, T and Lewitter, F (2004). siRNA selection server: an automated siRNA oligonucleotide prediction server. Nucleic Acids Res 32: W130–W134.