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Faculty of Graduate Studies

Development of Three-Dimensional Neuronal Model Systems for Mechanical Stress Application

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Dedication

I humbly dedicate this piece of work to my loving parents (Basim and Jasmin), for their endless guidance and support. To my brothers and sisters who shared their words of advice and encouragement to finish this study.

Acknowledgment

First of all, I would like to express my sincere appreciation to my German Supervisor, PD. Dr. Bernd Hoffmann and his crew in IBI-2 Jülich research center/ Germany and in addition to my Palestinian supervisors Dr. Ashraf Sawaftah (An-Najah National University) and Dr. Feras Al-Battah (Arab American University) for their guidance and encouragement during my master research work and writing. I do not forget the appropriate guidance of Dr. Jella Abraham during my research and thesis writing in Jülich research center Germany.

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الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان:

Development of Three-Dimensional Neuronal Model Systems for Mechanical Stress Application

أقر بأن ما اشتملت عليه الرسالة انما هو جهدي الخاص باستثناء ما تمت الإشارة اليه حيثما ورد، وان هذه الرسالة ككل، او أي جزء منها لم يقدم قبل لنيل أية درجة او لقب علمي أو بحثي لدى أي مؤسسة تعليمية او بحثية أخرى.

Declaration

The work provided in this thesis unless otherwise referenced is the researcher own work and has not been submitted elsewhere for any other degree or qualification.

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Signature: **التوقيع:**

Date: 25/10/2021 **التاريخ:**

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Development of Three-Dimensional Neuronal Model Systems for Mechanical Stress Application

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Abstract

Most of the known biological processes were discovered in two-dimensional (2D) cell culture. An increasing need for an *in vitro* model that mimics the brain's physiological environment to understand neurodegenerative diseases. The human brain is in continuous stretching-release movement due to the normal and repeated heartbeats and blood circulation in our body. This regular stretching-release brain movement changed the size and stretched the neuronal cells. To mimic this size change in the brain, the neuronal cells were cultivated on different scaffolds and stretched on an elastomeric **Polydimethylsiloxane** (PDMS) silicon chamber *in vitro* with different amplitudes. Previous stretch experiments on cortical neurons were applied to two-dimensional (2D) cell cultures testing the neuronal cells' behavior and cytoskeleton due to the mechanical strain applied by the stretcher device. The 2D culture results were attractive to go a step further to come closer to the real brain movement by creating and developing the three-dimensional (3D) cell culture system. The brain movement was mimicked by a stretch apparatus and a mechanical strain was applied to the cortical neurons.

To create a 3D culture stretch experiment, a stretchable 50 kPa PDMS elastomer was needed. The elastomeric chamber was fixed in a specific holder, and primarily isolated rat embryo neuronal cells were cultivated in a 3D scaffold on the PDMS chamber. Lastly, the complete 3D culture was screwed to the stretcher device. In the development process; of a 3D stretch culture, many cultivation scaffolds were studied: three different gel types were used and tested as a scaffold for the study. Naturally synthesized Matrigel, isolated from EngelbrethHolm-Swarm (EHS) mouse tumor was the first scaffold used for our research, in addition to two artificial synthesized gel scaffolds. Matrigel's experiment showed neuronal growth in the used two methods, but it was not stretchable. Hydrogel (VitroGel 3D-RGD) with a modified functional group, and finally a fibrin gels the PVP co-GMA. In this study, three different scaffolds (Matrigel, VitroGel, and PVP co-GMA) had been studied and tested; in addition, the classical ICS-7 PDMS stretch chamber was modified and reconstructed to fit the 3D stretch experiment; and finally, a 3D neuronal stretch method with a stretchable artificial scaffold (PPVP co-GMA) was developed and analyzed with special software and equipment in the lab.

Chapter 1
Introduction

1.1 The human brain

The human brain is responsible for controlling and coordinating the information collected from other body organs and liable for making decisions. The brain is the least understood organ in the human body. It is difficult to access, highly susceptible to damage, and complex in structure and function (Forstmann, Keuken, & Alkemade, 2015). The poor understanding of the human brain is reflected in the lack of effective treatments for various neurological disorders such as Parkinson's, Alzheimer's disease, and motor neuron disorders. To address this research gap, new methods for the culture of human neural (neuronal and glial) lineage cells, particularly *in vitro* three-dimensional (3D) culture, are being developed to more accurately reconstruct the complex in vivo structure and function of the human brain (Murphy, Laslett, O'Brien, & Cameron, 2017).

In a highly simplified view, three main parts are forming the brain, i.e. the cerebrum, cerebellum, and brain stem, as shown in Figure 1.1. The major part of the human brain is the cerebrum, which is divided into two hemispheres (right and left). These two hemispheres are surrounded by the brain's outer layer, which is called the cortex. The cortex is composed of a neuronal network, which is primarily responsible for thinking and body movement. The second part is the cerebellum, which plays a role in balance

and body coordination. The main body functions are controlled in the third part of the brain, which is the brain stem (Nowinski, 2019).

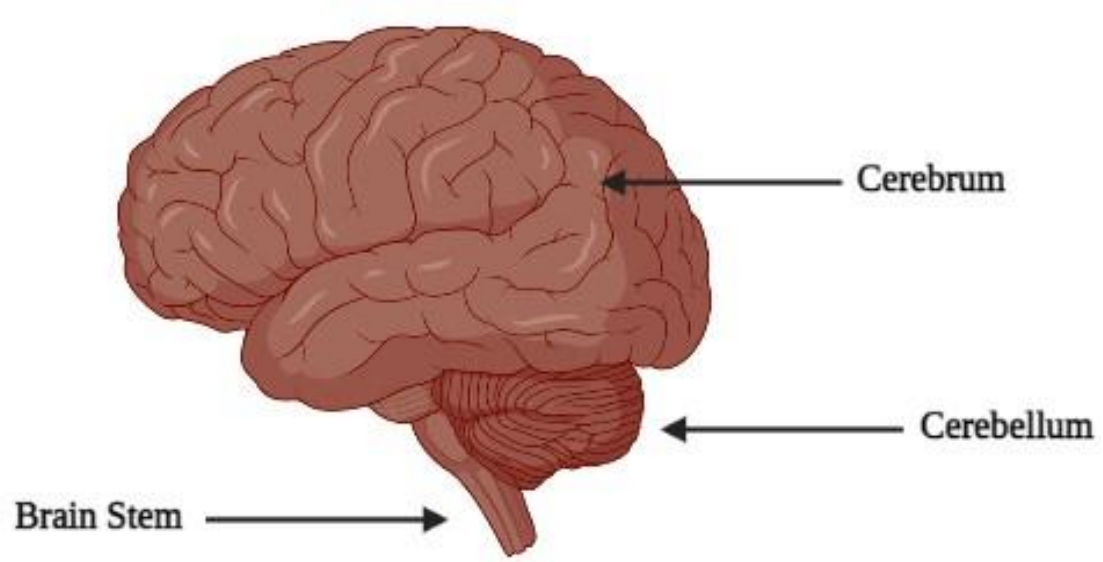

Figure 1. 1: The three main human brain parts. The cerebrum, cerebellum, and brain stem. The cerebrum is the major part of the brain, which is composed of two brain hemispheres (left and right).

The human brain, the spinal cord, and the nerve cells are forming the nervous system. The nervous system is composed of a complex and complicated network of neuronal cells. They are responsible for transferring and receiving messages from the brain and spinal cord to other body parts. This system is composed of two main parts: the first part is the Central Nervous System (CNS), which is called the control center of the body and the largest part of the nervous system. This part consists of the brain and spinal cord that are responsible to coordinate voluntary and involuntary reactions in our bodies. The second part of the nervous system is the Peripheral Nervous System (PNS). This part is capable of sensation and connection of the CNS to other body parts [1] [Figure 1.2; (Gibson & Fambrough, 2018)].

The central nervous system is protected within the brain in the cranial cavity, while the spinal cord is inside the vertebral column (Edition, 2014). The major component of these three brain parts is the neuronal cells. Neurons are the structural and functional building units of the nervous system and brain (Beniaguev, Segev, & London, 2020), which are distributed all over the body and are responsible for human body management and control. The neuronal cells are the basic subunit of the nervous system and are specialized for the response to chemical and physical activity by receiving and transferring cell signals to and from neighborhood cells and tissues (Chung & Chung, 2008). The neuronal cell shape plays a major role in its function that connects to other cells and/or targets with connectors called synapses (Craig, 2003). Cell signals are transferred through these branches and connections (synapses) from one to another cell (Figure 1.3).

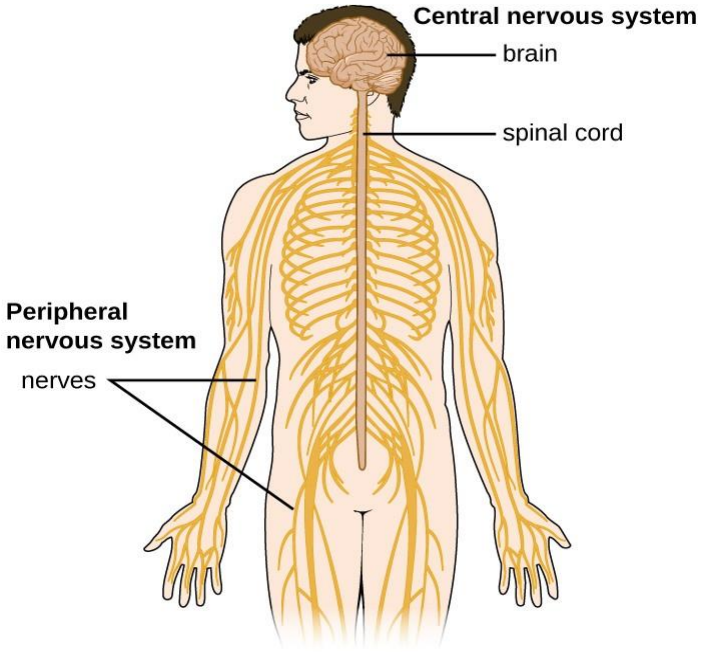

Figure 1. 2: The human central nervous system vs. peripheral nervous system. Adapted from lumen learning anatomy of the nervous system book [2].

Through these synapses, the signal (action potential) is carried from one cell to another releasing a neurotransmitter. The sending cell is called presynaptic, where the signal departures the neuron through the axon terminal to the postsynaptic signal receptor, and enters through the dendrites in the neighbor cell. Between every two neuronal cells, there is a small gap that is separating the axon terminal and the dendrite or tissue signal receptor called the synaptic cleft. Through this space (cleft) the neurotransmitters are transferred from sender to receiver. Along the neuronal axons, there are some shielding's covering the axon arm, these shielding's are called the myelin sheaths they serve as signal speeders. Myeline sheaths are not found in all brain neurons (Micheva et al., 2016).

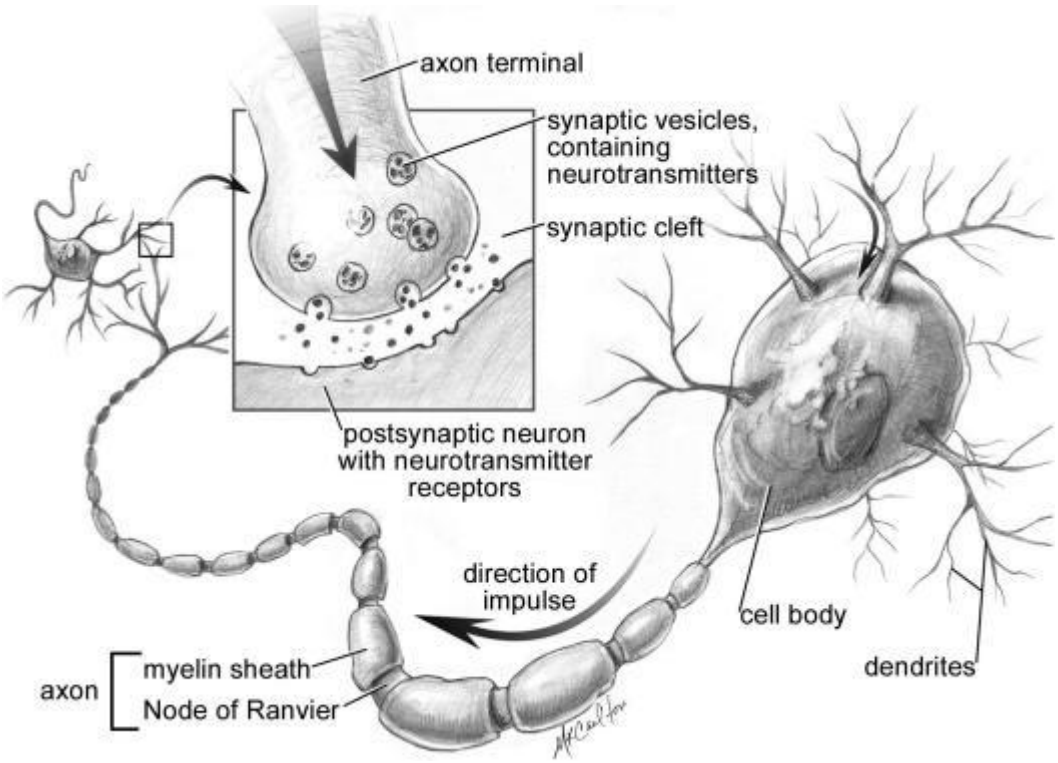

Figure 1. 3: Neuronal cell body structure and cell-cell connection. Signal transfer from one cell to the other cell through the synaptic cleft. Adapt form journal of athletic trainer (Craig, 2003).

Electrochemical signals cross along the neurons to transfer information to and from the brain. The process of signal sending through the neurons has two steps along with the cell, it is called an action potential, and between the cells, it is called the neurotransmitter. The chemical signal (neurotransmitter) is the release of the electrical or chemical signals from the axon into the synaptic cleft, these neurotransmitters bind to their specific receptors in the postsynaptic region (Stufflebeam, 2008) (Carter & Bean, 2009).

The action potential is an electrical impulse that circulates the signal between the neuronal cells in the body, which occurs due to the change in the membrane voltage along the cell axon, which happens by the ion exchange through the cell (Carter & Bean, 2009). All sodium and potassium channels are closed during the rest state. These channels are closed with gates, and these gates are voltage-gated because of the response only to voltage change. Two types of ion exchange channels responsible for charge change are found in neurons the sodium and potassium ion channels.

Recent studies on neuronal biomechanics highlight the considerable role of mechanical forces in neuronal development and synaptic formation. The need for a well-understanding brain development process is related to understanding and treatment of many associated diseases like traumatic brain injury (TBI), Alzheimer's, Parkinson’s disease, and how this complex tissue is regulated.

1.2 The Neuronal cytoskeleton

A neuronal cell structure is composed of a cell body, axon, and dendrites and it is a highly polarized cell (Xiao, Hu, Wei, & Tam, 2016). Cells' shape, polarization, and motility of all types of cells are guided by a filamentous structure that creates the cytoskeleton. The filamentous structure includes the filamentous actin that is composed of G-actin and intermediate filaments (neurofilaments). Three types of protein-polymer are forming the neuronal cytoskeleton: the actin filament (microfilament), microtubules, and intermediate filaments are composed by protein subunit self-association (Figure 1.4)(Malacrida, Meregalli, Rodriguez-Menendez, & Nicolini, 2019). They are nanometers in diameter and are micrometers in length, which allows them to integrate into the intracellular space. These polymers can overcome the mechanical tension, and the compression allows them to give the shape, and the cellular architecture is stabilized, and cell movement occurs. Their long length approves them to move the intracellular components.

In the nervous system, neurofilaments are present and composed of intermediate filament proteins and the cytoskeletal microtubule contains α and β-tubulin heterodimer (Menon & Gupton, 2016). The neuronal cytoskeleton is essential to maintain the cell function since the development and the entire existence of the nervous system (Muñoz-Lasso, Romá-Mateo, Pallardó, & Gonzalez-Cabo, 2020). Many proteins are connected with microfilaments and microtubules, and they are not responsible just for the

structure; they are related also to the dynamic and function (Muñoz-Lasso et al., 2020).

They can connect one filament type with another and form a network to the plasmic membrane. The neuronal cytoskeleton is not a fixed structure, it has the role of maintaining the structural integrity of the cell and promotes axonal growth. It plays an essential role in axonal transport.

Specific functions are related to cytoskeletal proteins to ensure the electrical and chemical transmission between neuronal cells. The neuronal cytoskeleton has to be flexible and dynamic to maintain the functioning circuits of the neurons throughout the organism's life (Muñoz-Lasso et al., 2020).

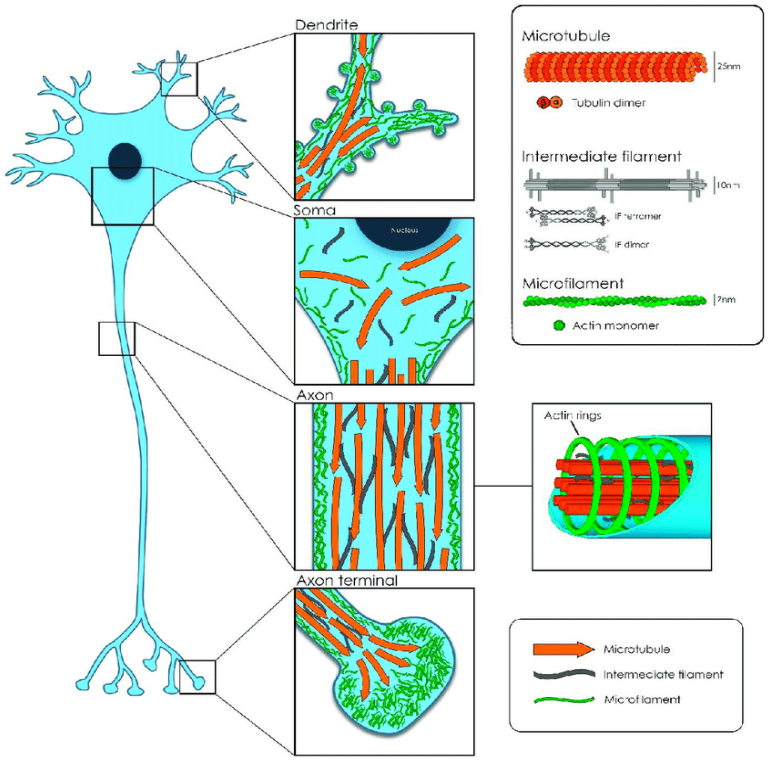

Figure 1. 4: Neuronal cytoskeleton components. The neuronal cytoskeleton consists of three types of bio-polymers actin filament (microfilament) (green), microtubules (red), and intermediate filaments (grey) *(Malacrida et al., 2019)*.

The neuronal cytoskeleton is essential for the cell shape and physiology. Furthermore, it is responsible for the formation of specialized structures like the growth cones, which play a role in the axon elongation and the development of synaptic boutons and dendritic spines [4]. The structural difference in the cytoskeleton affects the cell shape, due to this change the cell signaling and behavior are affected (Stukel & Willits, 2016).

1.3 Traumatic injury in the central nervous system (CNS)

The nervous system is a complex and highly specialized network in our bodies. The central nervous system is composed of the brain and the spinal cord, the brain has a high level of function coordination and the spinal cord serves for the pathway communication between the CNS and PNS. Central nervous system traumatic injury refares to traumatic brain injury (TBI) and spinal cord injury (SCI) (Walter & Zweckberger, 2018). The traumatic brain injury is caused by external physical damage, the result of this damage severity depends on the anatomical location of the injury. Traumatic brain injuries, in the CNS have their unique challenge for clinical and preclinical treatment because of their complex pathophysiology. For traumatic injury patients, specialized centers are responsible for their treatment.

Mechanical loading of the brain is caused by head trauma (Hemphill, Dauth, Yu, Dabiri, & Parker, 2015). TBI occurs due to an external force that affects the brain, which affects brain function. The pathophysiology of this injury is not clear it can be caused by open or closed head injuries. The head injury

can vary from small to large structural changes in the brain (Valente & Fisher, 2011). Neuronal cell damage in traumatic brain injury leads to cytoskeletal rearrangement or damage, microtubule bundle confusion, and protein degradation cascade. Irregular calcium levels lead to the severity of the CNS injury.

1.4 Mechanical strain

To regulate cell and tissue physiology mechanical cues are essential (Boulter, Tissot, Dilly, Pisano, & Féral, 2020). The human tissue's mechanical properties differ depending on the composition, structure, and role (Kim & Choi, 2019). A vital role is played by mechanical stress in the development and maintenance of tissue functionality. The mechanical stimulations regulate different cell functions like proliferation, differentiation, and survival. Cytoskeleton undergoes remodeling in response to mechanical stimuli (Masuda et al., 2007). Environmental, mechanical, and biochemical cues were sensed and integrated by all cells to coordinate tissue development and homeostasis maintenance. A specialized sensor located on the cell membrane receives the stress and force applied on the cell that works as a converter of this signal into a biochemical signal that affects cells' behavior in surviving ECM remodeling, and differentiation (Boccafoschi, Mosca, Ramella, Valente, & Cannas, 2013). All cell types in our body including neurons do forces on their surroundings. To measure and calculate the substrate strain and exerted cell stress, newly developed

procedures and protocols were used for this purpose. A linearly elastic substrate is chosen (Franze, 2013).

Mechanical forces are translated into biochemical signals that affect cell and tissue behavior. The human brain has its appropriate mechanical properties that play an essential role in neural stem cell behavior and tissue development (Barnes, Przybyla, & Weaver, 2017). Cells adapt mechanically to the surrounding forces applied to increase the ECM rigidity and to transmembrane receptor integrins by producing stress-induced response strengthening. By increasing the applied stress level in cells mechanical stiffness increase linearly (Matthews, Overby, Mannix, & Ingber, 2006).

1.5 Stretch experiments

There is a high interest in understanding how cells and tissues respond to mechanical stimuli, but the approaches are limited until now. Many tools and devices were fabricated and invented to mimic the actual cell response to stimuli. Newly some 2D stretch experiments are today arising and applicable in cell culture for measuring cell response to stimuli. Till today the stretch experiments are applied to 2D cultures on different cell and tissue types. Stretching experiments are tools that employ mechanical forces on cells *in vitro,* which mimics the real force applied on cells *in vivo*. These techniques and tools bring us to understand the progression of different diseases that are altered by mechanical cues (Tremblay, Cuerrier, Andrzejewski, O'Brien, & Pelling, 2014).

In our lab, the stretcher device was constructed and developed by Wolfgang Rubner in the ICS-7 Jülich research center for 2D stretch experiments (Faust et al., 2011). Cells respond to cyclic substrate stretch that mimics the body's vital physiological conditions. The adherent cell response to the external forces starting from a random orientation to reorient into a particular angle depends on the applied stretch. At the subcellular level, the cytoskeleton and the stress fibers generate internal contractile forces to reorient into the same angle (Livne, Bouchbinder, & Geiger, 2014).

The first 3D stretch experiments on primary isolated neuronal cells were tested in our study on our house-made stretcher device using an elastomeric PDMS (silicone rubber) chamber. During the study, we developed and reconstructed the PDMS elastomeric chamber to fit the 3D stretch experiment, the chamber modifications and development were needed to build up our 3D system stretcher system. Cyclic stretch experiments were performed on cortical neuronal cells after 24 h of cell cultivation and adherence in the 3D scaffold. The stretch experiment was performed under sterile conditions. A uniaxial stretcher device was utilized to provide strain with different amplitudes and frequencies (Abraham et al., 2018).

Stretch experiments are generally performed with different types of cells and tissues to study different cell issues. At the beginning mechanical stimulus was used and performed in bone remodeling and now it is used and investigated for different and several type of cells (Seriani et al., 2016).

1.6 Traditional two-dimensional (2D) cell culture vs. three-dimensional (3D) cell culture

Two-dimensional (2D) culture in mechanotransduction studies provides basic information on the cellular-material interaction, whereas three-dimensional (3D) culture mimics the actual *in vivo* environment (Stukel & Willits, 2016). The 3D culture is the building of cell culture in the XYZ direction. The three-dimensional (3D) cell culture is not only that the culture is growing in three dimensions, concerning the normal tissue, but the function is also determined by cellular and non-cellular components. The more the cell culture comes closer to the actual conditions, the more the cell culture can mimic the original cell response and behavior. The cell culture showed that cell aggregation in a 3D shape provides a high physiological cell response compared to 2D cell culture is plated on plastic or glassware. The 3D spherical shape of cell aggregation, as a result of cell adherents together and not to cell culture tubes, or due to 3D scaffolds (natural or synthetic gels).

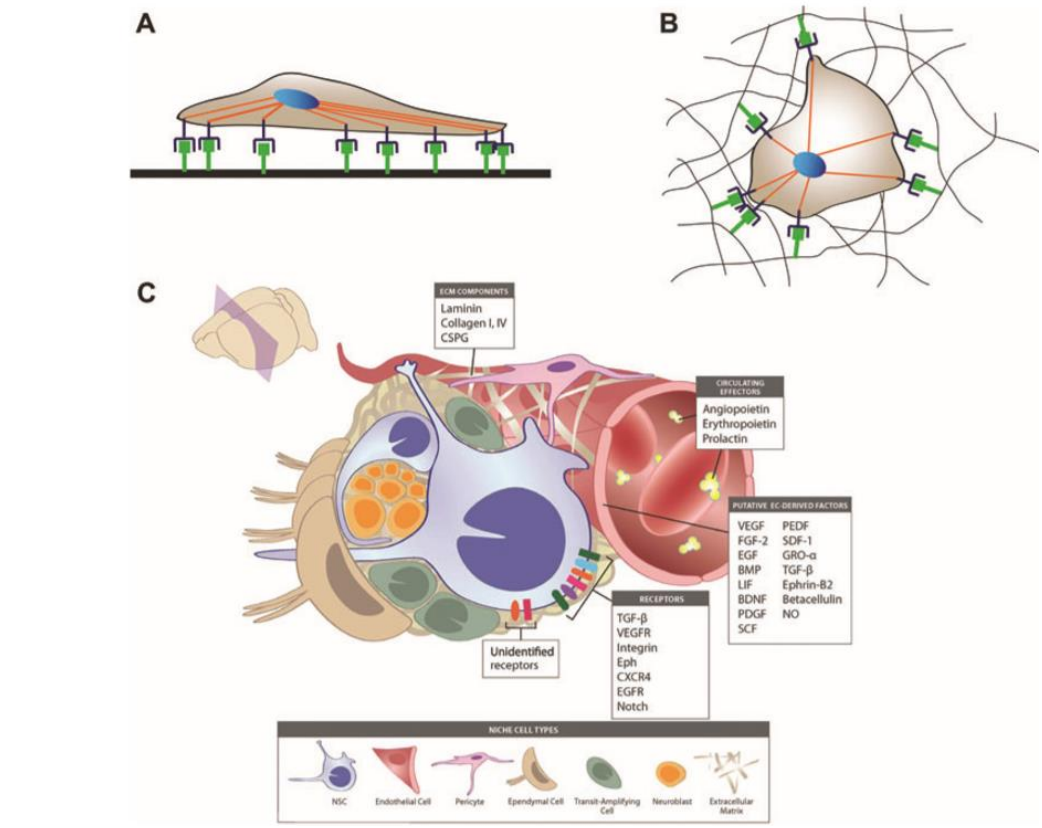

Figure 1. 5: The figure represents a comparison between 2D and 3D cell culture. A represents a 2D culture that is coated with protein, and B is a 3D culture on a scaffold. As shown in the figure, the cell shape is flat in the 2D culture, while in the 3D culture it is taking a spherical shape due to the ECM protein or peptide interaction. The 3D culture is closer to the real environment as shown in C (Stukel & Willits, 2016).

In the two-dimensional culture (2D), cells grow flat only in two dimensions and attach to the culture flask surface (Caliari & Burdick, 2016). Two-dimensional cultures are simple and cheap for experiment replication. Some disadvantages of 2D cultures; it does not mimic the real tissue structure and behavior and the absence of cell-cell and cell-matrix interaction (Meeßen, Graeve, & Offenhäusser). In a three-dimensional (3D) cell culture *in vitro* the cell-cell and cell-ECM interactions mimic's the natural *in vivo* interactions, on the cellular morphology level the shape looks close to the real cell shape (Langhans, 2018) (Edmondson, Broglie, Adcock, & Yang,

2014). The interactions are required for proliferation, cell differentiation, and cell vitality. Cell division and morphology are affected when moving the cell from the tissue to 2D cultures. Cell morphology changes affect cell function, structure, and secretion (Breslin & O'Driscoll, 2013). The lake of the external environment interaction leads to cell response change. Owing to 2D culture disadvantages, an alternative system is needed to mimic the real tissue environment as three-dimensional cell culture (3D) (Kapałczyńska et al., 2018). In addition, neuronal cell culture on 2D cultures receives their information only from the single layer, while neurons in our bodies receive their information from 3D.

Among the first 3D cell cultures was made in soft agar solution by Hamburg and Salmon in the 1970s (Hamburger & Salmon, 1977). Three-dimensional culturing models can be divided into 1) culture in gel-like substances, 2) culture on a scaffold and, 3) suspension cultures on non-adherent plates. The 3D sphere concept is based on building or creating a spherical structure composed of many layers. The sphere structure mimics the physical and biochemical properties of the tissue. Cultivated cells in a 3D structured environment mimic the real cell environment and architecture more accurately than 2D cultures and systems. Three-dimensional (3D) cell cultures keep the cell density constant as in the real tissue. The 3D model system is a perfect intermediate model between 2D cultures and animal models (Kapałczyńska et al., 2018).

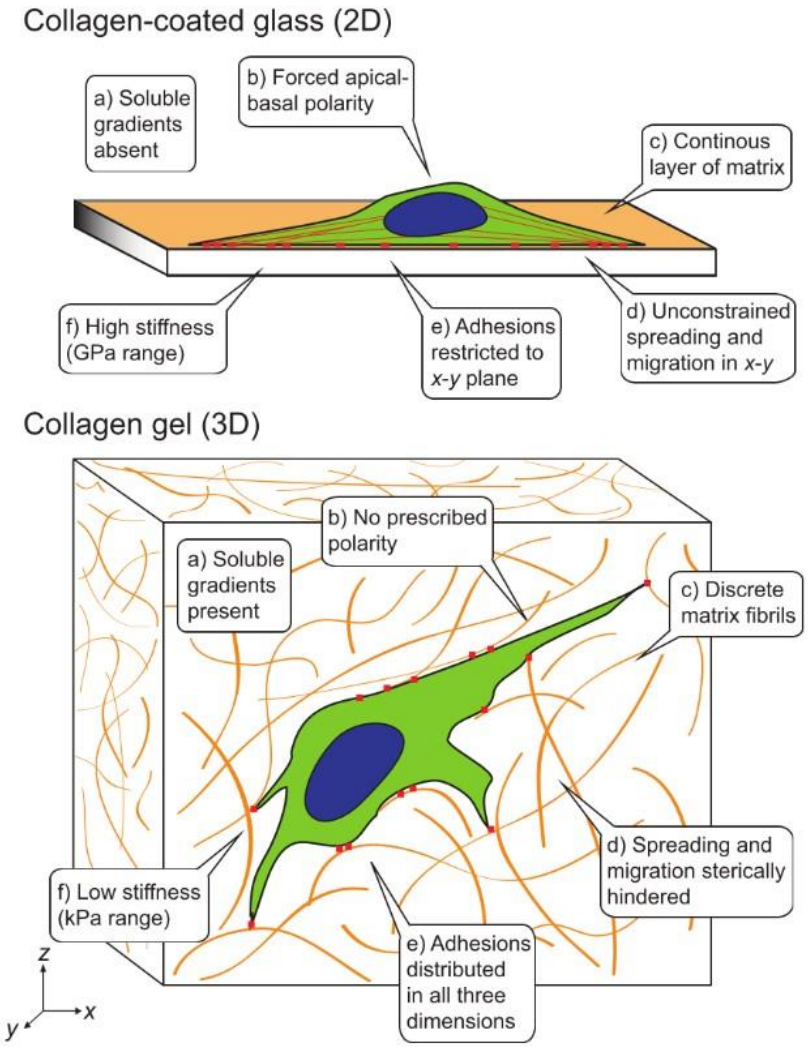

Figure 1. 6: Comparison between 2D cell culture on flat culture vial vs. 3D cell culture on a scaffold from the growth and adhesion direction. The 3D culture shows the growth in an XYZ direction while the 2D culture shows growth only in an XY direction (Baker & Chen, 2012).

1.7 Aim of the study

To come closer to the real brain-like environment, researchers were developing and creating systems to understand how this organ is working and how the connections are formed. The present awareness about biological development is not completely dissolved by two dimensional (2D) culture

systems. The cell-cell and cell-matrix interactions are not represented as they are in the real and three-dimension (3D) cell culture.

The 2D cultures (single layer) lack cell-cell and cell-matrix interactions. The main aim of this study was to establish and develop a 3D system that is mimics better the *in vivo* environment of the real brain. The newly established 3D system should be comparable with previously obtained 2D culture results. Besides, the 3D *in vivo* like model should be tested for the ability to withstand mechanical deformation. A deformable 3D model would allow us to investigate the physiological occurring mechanical strain in neuronal development and disease in more detail. Several *in vitro* models and scaffolds should be tested and investigated for their ability to perform 3D stretch experiments. Finally, one suitable 3D model should be optimized and chosen for stretch experiments. To perform further 3D stretch experiments, an additional aim was to evaluate the model according to the ability of data analysis and optimizing a protocol for data retrieval with the Imaris software (for more details refer to 2.1.11 page 46). Setting up a reproducible and analyzable stretch 3D model was the main aim of the research to study the brain environment closely.

Chapter 2
Materials and Methods

List of materials and equipment's are available in the appendix page 82.

2.1 Cell culture

2.1.1 Elastomeric chamber fabrication

Creating a stretchable three-dimensional (**3D**) system requires an elastomeric chamber to build in the 3D scaffold. This elastomeric chamber is composed of a mixture of two synthetic components, the **Polydimethylsiloxane (PDMS)** and its crosslinker **methyl-hydro siloxane-dimethyl siloxane**. PDMS was mixed with its crosslinker (methyl-hydro siloxane-dimethyl siloxane) in a ratio of 1:40 weight to weight (w/w). The previously mentioned mixing ratio needs to be mixed thoroughly in a plastic mixing cup. The homogenized mixture was degassed in a 5L desiccator to remove the air bubbles, which could disrupt the transparency of the elastomeric chamber. Finally, the degassed mixture is poured into special fabrication molds, and incubated for crosslinking for 16h at 60°C (Figure 2.2). After crosslinking, this mixture proportion gives a deformable substrate with an elastic modulus of 50 kPa (Wisser, Schumm, Mondin, Grothe, & Kaskel, 2015).

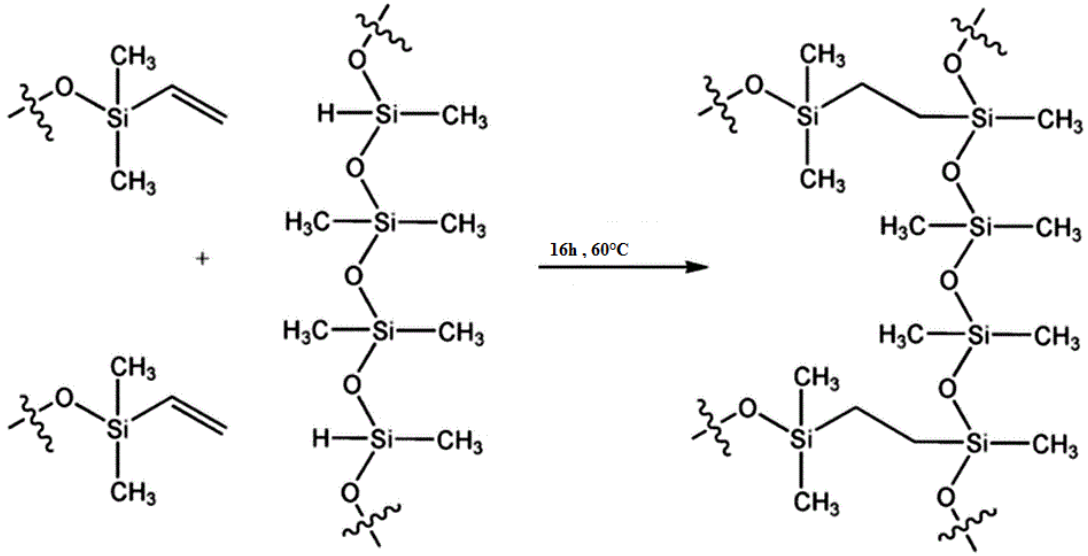

Figure 2. 1: PDMS crosslinking reaction by mixing them and curing it for 16 h at 60 °C (Wisser et al., 2015).

Treated and cured PDMS chambers were screwed out of the mold and sterilized with isopropanol alcohol to prevent contamination during neuronal three-dimensional (3D) cell culture. Sterilized PDMS silicone chambers were fixed in their special metal chamber holders. Then they were placed in the incubator at 37 °C for around 1h to get rid of isopropanol by evaporation. Finally, the sterilized PDMS chambers were used for three-dimensional (3D) system cell cultivation (Figure 2.2).

Figure 2.2: The 50 kPa PDMS fabrication mold chamber steps (Traditional chamber). The chamber cultivation area dimension is 2 cm * 2 cm. Images by Jens Konrad Forschungszentrum Jülich/ ICS-7.

The traditional chamber mold shown in Figure 2.2 is used for two-dimensional (2D) experiments in our institute (ICS-7) to cultivate neuronal cells. In the traditional elastomeric PDMS chamber, 2D stretch experiments were performed. The traditional elastomeric chamber was used and tested for 3D stretch experiments as they have been used in 2D cultures.

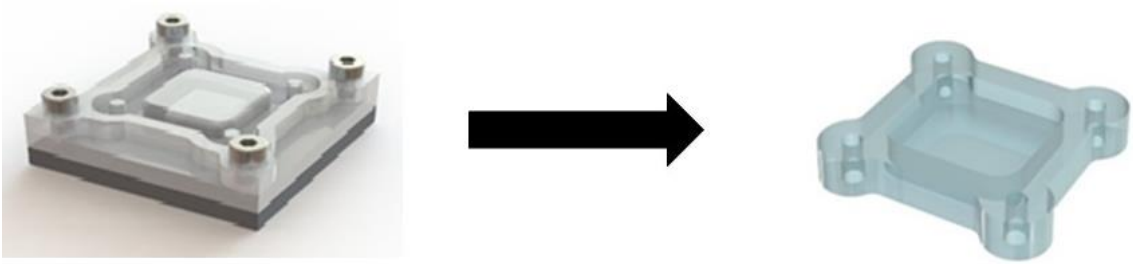

Figure 2. 3: Classical PDMS elastomeric chamber used for stretch experiments in the ICS-7. Images by Jens Konrad FZJ ICS-7.

2.1.2 Primary cortical neuronal cell isolation

Cortical neurons were obtained from pregnant rats (Wistar, Charles River) at (E18- E19) of gestation. Pregnant rats were anesthetized with CO_2 in a large beaker then the embryos were removed surgically from the pregnant rat. The embryos heads were dissected in HBSS Petri dishes under a dissecting microscope, and the cortices were transferred to ice-cold trypsin and incubated for 15 min at 37 °C in the incubator. Then the cortices were transferred to a fresh Neurobasal medium (NB+++) for washing. Neurobasal medium NB is supplemented with B27, GlutaMAX, and Gentamicin. The cortices were washed three times in the NB+++ medium. The dissociated cells were counted using the hematocytometer under the inverted

microscope, up to 7 million neuronal cells were obtained per one embryonic rat head. After that, the cells were seeded within the 3D different scaffolds on glass dishes or/and PDMS elastomeric chambers according to each protocol method. The cortical cell isolation follows the steps, as shown in Figure 2.4.

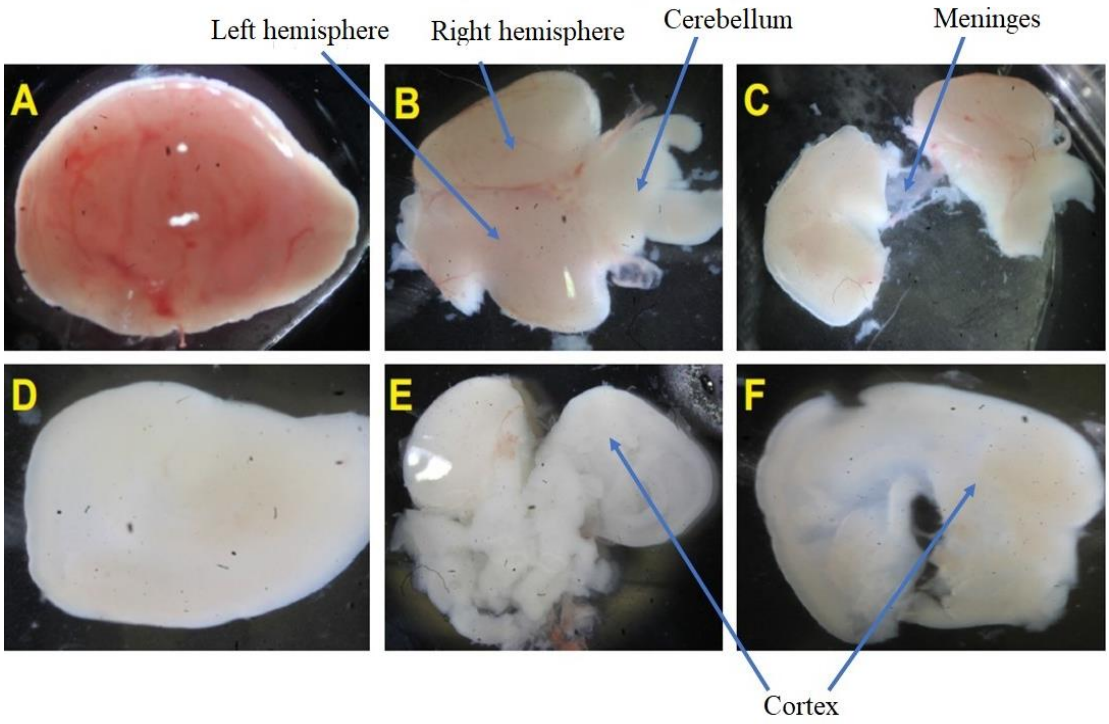

Figure 2. 4: Rat E18 – E19 brain dissection steps and cortex isolation. Brain images under a dissecting microscope in HBSS solution. Images by Mahmoud Ardah/ ICS-7. (A) Rat embryo head. (B) Top view of the rat E18 – E19 brain. The two hemispheres can be seen and the cerebellum. (C) The brain hemispheres are separated and meninges were removed. (D) Brain hemisphere without meninges. (E) Whole hemisphere vs. cortex. (F) Single rat embryo cortex.

The complete dissection process and cell isolation were performed under sterile conditions, using the laminar flow and 80% alcohol to minimize and prevent the contamination possibility. The embryonic head was placed in the frontal direction, to see the brain's hemispheres from the upper side of the head. The embryo's head was dissected in an HBSS solution petri dish

under the stereomicroscope inside the laminar flow. The dissection process was performed using fine-pointed forceps due to the softness of the embryo's brain tissue. Considering the head skin removal and thin skull bones, the brain hemispheres were visible. The brain is covered by a membrane called the dura mater, which was removed smoothly and slowly using fine forceps. After that, the brain was removed from the skull using fine curved forceps. The cerebellum and meninges were separated from the hemispheres. The two hemispheres were separated from each other, and the hippocampus was removed. Lastly, the cortices were transferred to ice-cold trypsin and incubated for 15 min at 37°C for cell dissociation. After the incubation period, the cortices were washed smoothly with neurobasal medium (NB+++) three times. Then the cells were counted using trypan blue and the hematocytometer. Finally, they were seeded in the 3D polymer for each experiment according to the experiment's protocol as described in the coming sections.

2.1.3 Matrigel as a natural product for a 3D scaffold

Matrigel is a gelatinous protein mixture used as a 3D cultivation scaffold that is secreted and isolated from Engelbreth-Holm-Swarm (EHS) mouse tumor. This soluble basement membrane contains several extracellular matrix molecules (Hughes, Postovit, & Lajoie, 2010) (Lee et al., 2015). The isolated natural polymer is rich with ECM proteins (Laminin, Collagen IV, and Entactin), in addition to several growth factors in this product. Matrigel polymerizes at a temperature of 22 °C to 35 °C, which makes it proper for

primary neuronal cell culturing (Corning company). Three-dimensional (3D) cell culture requires an appropriate cultivation scaffold, but the used scaffold should consider the same original conditions but *in vitro* environment. Matrigel was a suitable option that contains most of the features needed from protein contents, brain plasticity and was supposed to mimic the *in vivo* environment. Matrigel was used to build the 3D neuronal cell culture system, it was plated on glass dishes, and a 50 kPa PDMS elastomeric stretch chamber. With this type of gel (scaffold), two different cell cultivation methods were used: the sandwich method and the 3D suspension method. In the following parts, the cultivation methods were described in detail for each manner, presenting the differences and cultivation methods.

2.1.3 A Sandwich method

As its name indicates, it is a multi-layer method that is composed of two gel and two cell layers (Figure 2.5 for method clarification). The two cell layers were separated with a Matrigel layer in between. Through the second gel layer, which separates the two cell layers, we have a cell-cell connection between them. Different Matrigel concentrations were tested and used to apply the sandwich method. Two stock Matrigel concentrations of 10 and 20 mg/ml were studied and used in the research. The sandwich method was applied on glass Petri dishes and 50 kPa elastomeric PDMS chambers. By applying this system on both PDMS chambers or glass, they were kept on

ice to delay the gelatinization and polymerization time during gel layer preparation; an ice block was used for this step for each gel layer addition.

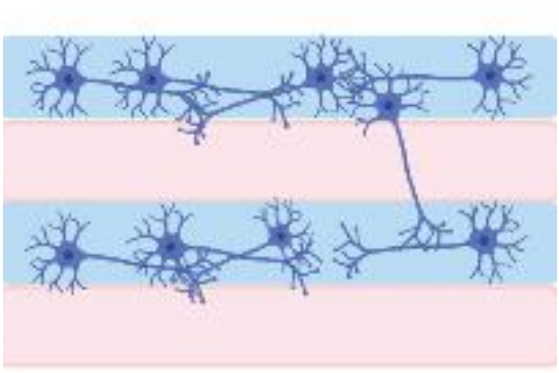

Figure 2. 5: Sandwich method system explanation figure. The pink color indicates the gel layer, and the blue color indicates the neuronal cell layer suspension. The two cell layers were separated by a gel layer, through this gel layer neuronal cells built connections between both cell layers.

Previous to neuronal cell isolation the first Matrigel layer was applied to the culturing chambers a volume of 76 µl of the stock concentration 10 mg/ml or 20 mg/ml were plated on the glass or 50 kPa PDMS elastomeric chambers. The applied layer was incubated for 30 min at 37 °C for polymerization. The neuronal cells were plated and cultured on the polymerized gel layer. Each cell layer is composed of 350.000 cells suspended in 45 µl of pre-warmed NB+++ medium, which gives a cell density of 7.777 cells per 1 mm3. Then they were incubated for 4h at 37 °C for cell adherence. After the incubation period, the second Matrigel layer was added, a 53.2 µl of Matrigel was plated over the first cell layer, and incubated for 30 min at 37 °C for gelatinization (polymerization). The second cell layer is prepared fresh from a stored cortex in the HBSS medium on ice, and freshly isolated cells were seeded for the second layer to avoid

cell death. The same cell number was seeded, 350.000 cells were suspended in 45 µl pre-warmed NB+++ medium, and the culture was incubated for 1-2 h at 37 °C for cell adherence on Matrigel. Finally, after the incubation period and cell adhesion additional 1.5 ml or 500 µl, warm NB+++ medium is added for the glass dish or chamber, respectively.

In the newly developed and reconstructed PDMS chamber, the number of cells and the Matrigel volumes were calculated to fit in the inserted grooves to the PDMS chamber. In the newly developed chamber, 62187 cells were suspended in an 8.48 µl warm NB+++ medium, and this amount of cells was used for each cell layer added in the newly developed and reconstructed elastomeric PDMS chamber having a cell density of 7.333 cells per 1 mm3. For each gel layer, 10.5 µl and 7 µl of Matrigel were used for the first and second layers, respectively. Finally, an additional 500 µl warm NB+++ medium is added. The PDMS elastomeric chambers were incubated for 24h at 37 °C then the experimental protocol was applied to the PDMS chambers like stretching and analyzing. The method was applied to glass and 50 kPa PDMS chambers. The cultivation processes for the newly developed chamber were the same as the traditional chamber.

2.1.3 B Three-dimension (3D) Suspension method

The 3D Suspension method differs from the sandwich method in the cell location. The three-dimension (3D) suspension is supposed to fill the gap between two-dimensional (2D) cultures and animal systems (Febles, Ferrie,

& Fang, 2014). In the first method, the cells were on the gel surface, while in the second method, the cells are embedded in the Matrigel itself. In this system, the cells are distributed randomly within the gel components, as illustrated in figure 2.6. The cells were suspended in NB+++ medium and mixed with Matrigel. The number of cells is constant for this system at all; 3 Million cells were suspended in different NB+++ volumes depending on the final Matrigel concentration. Different Matrigel concentrations were tested and used for this study (5,6,10, and 12 mg/ml of Matrigel). The values in table 2.1 are for the 13 mm pre-drilled Petri dish and the traditional PDMS chamber. Due to the gelatinization and polymerization temperature of the Matrigel, the elastomeric PDMS chamber and glass are prepared on ice to delay the gelatinization time.

Figure 2. 6: The 3D suspension method explanation. The neuronal cells (Blue) are randomly distributed inside the Matrigel in purple color.

Table 2. 1: The 3D suspension method Matrigel stock and final concentration for 13 mm pre-drilled Petri dish glass and PDMS traditional elastomeric chamber.

Matrigel stock conc.	Cell suspension volume [µl] NB+++	Matrigel volume [µl]	Matrigel final conc.
20 mg/ml	60	90	12 mg/ml
	75	75	10 mg/ml
10 mg/ml	60	90	6 mg/ml
	75	75	5 mg/ml

To keep the experimental conditions constant in both PDMS chambers (traditional and reconstructed) all component volumes were recalculated to fit in the inserted wells. The total volume of the inserted wells was 17.5 µl each. Only 350.000 neuronal cells were needed instead of three Million cells for the 3D suspension method. The cells were suspended in a warm NB+++ medium with different volumes regarding the final concentration of matrigel, as shown below in Table 2.2. In this method, we have a cell density of 20.000 cells per 1 mm^3 in both chambers. These cells were distributed randomly within the gel scaffold.

Table 2. 2: Matrigel volumes for stock and final concentration for the newly reconstructed PDMS elastomeric chamber.

Matrigel stock conc.	Cell suspension volume [µl] NB^{+++}	Matrigel volume [µl]	Matrigel final conc.
20 mg/ml	7	10.5	12 mg/ml
	8.75	8.75	10 mg/ml
10 mg/ml	7	10.5	6 mg/ml
	8.75	8.75	5 mg/ml

2.1.4 Synthetic scaffold for three-dimension (3D) cell cultivation in Polyvinylpyrrolidone (PVP) copolymerized with Glycidyl methacrylate (GMA)

An artificial synthesized water-soluble polymer for three-dimension (3D) cell cultivation was produced by Deutsches Wollforschungs institute Aachen (DWI Aachen) [5]. Due to its content of fibrinogen and thrombin, it is classified as fibrin gel. Fibrin gels are a mixture of fibrinogen and thrombin, which is formed from peripheral blood that is found in wound regions for repairing the injured regions. Fibrin formation occurs during the coagulation process of fibrinogen cleavage by thrombin to fibrin monomers, and they polymerize to form a three-dimensional (3D) polymer (Ju, Janmey, McCormick, Sawyer, & Flanagan, 2007). It is not only used for therapeutical uses but is also investigated as a biodegradable material in biomedical applications like tissue engineering and drug delivery. Hydrophilic cross-linked fibrils contain fibrin gels that are suitable for three-dimensional (3D) cell culture. The PVP chemical structure is shown in figure 2.7 below.

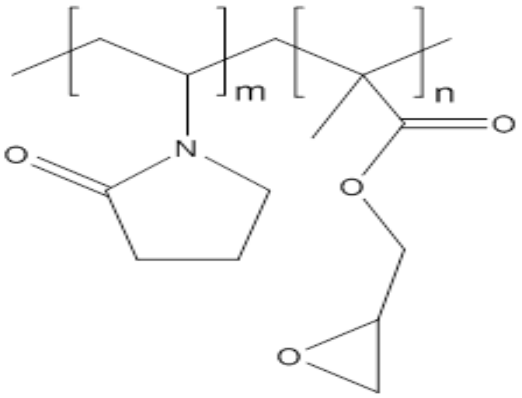

Figure 2. 7: The chemical formula of Polyvinylpyrrolidone (PVP) copolymerized with Glycidyl methacrylate (GMA) PVP co-GMA. This chemically synthesized polymer produced by DWI Aachen.

Fibrin gels components assemble rapidly by a modified polycondensation reaction, unlike the extracellular matrices and basement membrane components which assemble in a slow ordered manner directed by the cell that secret them (Janmey, Winer, & Weisel, 2009).

In the study, the primary isolated cell suspension was mixed with the artificially synthesized **PVP** polymer. Additionally, fibrinogen and thrombin were added to the cell suspension. The complete mixture was plated and cultivated in the newly reconstructed PDMS elastomeric chamber. After that, the PDMS chamber was incubated for 10 to 15 min at 37°C for polymerization. Then, an additional 500 µl of pre-warmed Neurobasal medium (NB+++) supplemented with Tranexamic acid, GlutaMAX, N2 supplement, and fetal bovine serum (FBS) was added to the chambers and incubated at 37°C for 24h. Culturing medium was supplemented with tranexamic acid to avoid fibrinolysis (Shakur et al., 2016). The NB+++ medium was supplemented with N2 supplements to

provide optimal growth conditions for neuronal cells (Dhara & Stice, 2008). Finally, after 24 h of incubation, the chamber was ready for stretching periods at different amplitudes and analysis. With an LSM 880 microscope, the results were analyzed and observed.

2.1.5 Synthetic scaffold for three-dimension (3D) cell cultivation in VitroGel 3D-RGD

This gel is an artificial polymer synthesized by a company, which mimics the natural extracellular matrix (ECM) [3]. VitroGel 3D-RGD type is a modified polymer with integrin-binding ligand (RGD), the modified polymer is responsible for cell adhesion and cell-matrix interaction (Haruna & Huang). VitroGel polymer was tested with four different concentrations regarding the producer's protocol on the 3D suspension method. The gel was diluted with 0.5x PBS and mixed gently to be homogenous (The wellbioscience). Finally, (200.000 or 350.000) cells were suspended in NB+++ medium and mixed with the gel mixture, then they were plated on glass dishes or PDMS elastomeric chambers. The producer's protocol is suitable for large cultivation areas. To keep the gel concentrations constant in the newly reconstructed PDMS elastomeric chamber, the producer's protocol volumes were adapted (Table 2.3).

Table 2.3: VitroGel mixing protocol from producer company was adjusted to fit our newly reconstructed PDMS chamber (The wellbioscience).

Dilution	Vitrogel [µl]	0.5X PBS [µl]	NB+++ [µl]
1:0	18	0	4.5
1:1	9	9	4.5
1:2	10.8	21.64	8.13
1:3	4.5	13.5	4.5

2.1.6 Cell Stretching of cortical neuronal cells

For this purpose, a special uniaxial mechanical stretcher device was developed in the ICS-7 by Wolfgang Rubner to mimic brain movement. The stretcher device is composed of the elastomeric PDMS chamber fixed in a special metal chamber holder, and the stretcher device is connected to the PC for parameter control. The elastomeric chamber was equipped in a horizontal state in a pre-stretch position (1.5 mm stretched) to prevent the drop of the bottom of the chamber. The cells were cultivated in their 3D scaffold (gel) on the elastomeric PDMS chamber. After cultivating the 3D culturing system in the chambers, they were incubated for 24h at 37 °C. By the end of the incubation time, the complete holder (holder and elastomeric chamber) was fixed and screwed into the uniaxial stretch apparatus. The stretcher device is driven by a motor that moves uniaxially, as shown in figure 2.8. This device is connected to a PC where the stretching parameters were controlled manually in the software. To apply strain on the sample, the holder's frame should be removed by starting the device, the mechanical strain was applied with different amplitudes and parameters (for optimizing

the system). Table 2.4 shows the different parameters used in the elastomeric chambers.

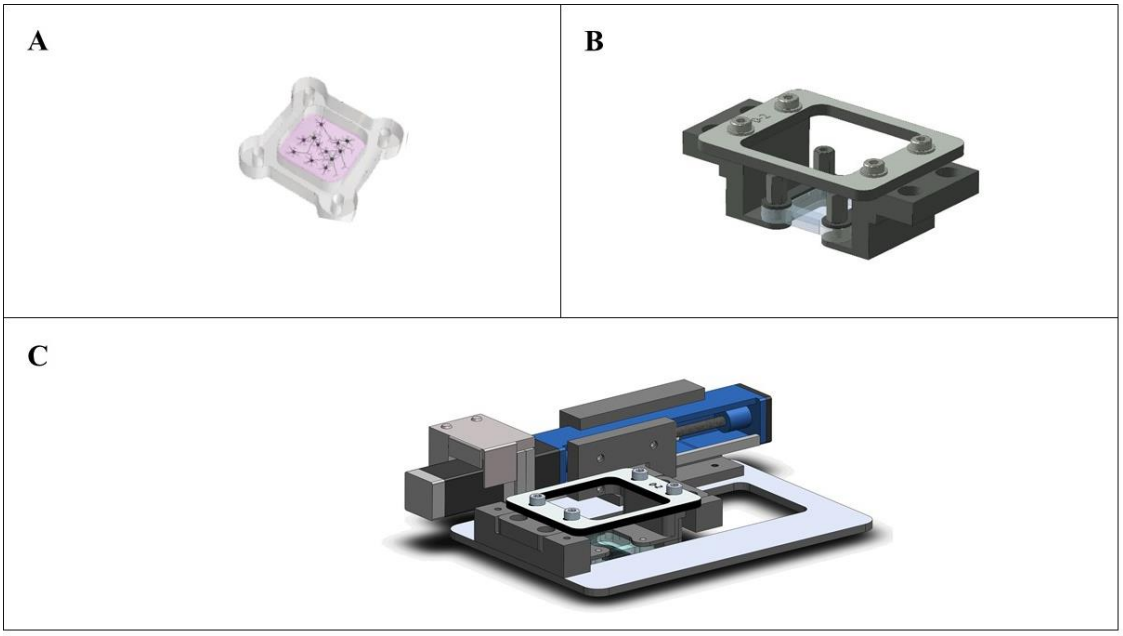

Figure 2. 8: Uniaxial stretcher device set developed by Wolfgang Rubner, ICS-7. A) PDMS chambers 1:40 where the cells were cultivated and stretched. B) Chamber holder where the chamber is screwed/ fixed in. C)Stretch apparatus where the holder with chamber was built-in to be stretched. Images by Jens Konrad FZJ ICS-7.

Table 2. 4: Stretch parameters on natural and synthetic 3D polymers with different frequencies.

Stretch percent	Amplitude [mm]	Frequency [mHz]	Speed [mm/s]	Pause [s]
7%	1.505	100	0.401	1.250
15%	3.225	100	0.860	1.250
30%	6.45	100	1.720	1.250
7%	1.505	300	1.204	0.417
15%	3.225	300	2.580	0.417
30%	6.45	300	5.160	0.417

The stretcher parameters were forming a sinusoidal-like curve by repeating the wave speed and amplitude continuously regarding the entered parameters, as shown in the above table. The sine curve in the experiment

represents the heartbeat in our bodies, which causes stretching and release in the brain cells.

Depending on experiment conditions, stretch periods of 1h, 4h, 24h, and one week were used and applied. After the stretch cycles were finished, the stretcher device was stopped in the release position. To visualize the stretched and un-stretched cortical neurons within a 3D culture scaffold, the PDMS chambers were stained using: Calcein staining or immunocytochemistry stain. These two staining methods were used to observe the results of the samples before and after the stretch. The following sections describe each staining method in detail.

2.1.7 Calcein Staining

For neuronal cell analysis, cell staining was obtained for this step. Calcein stain is used for the analysis with a fluorescent dye with a specific wavelength of 495/515 nm. Calcein stain can distinguish live cells from dead cells. Calcein stain is found in two forms Calcein-AM (Calcein-AM, AM: acetoxymethyl), the non-fluorescent form of the stain before passing the cell membrane (figure 2.9), and Calcein is the fluorescent form after passing the cells membrane. The calcium affine binding site in the chemical structure is covered with the acetoxymethyl group that allows the dye to penetrate through the cell membrane without being damaged. When the stain penetrates the cell membrane, the non-fluorescent calcein-AM is converted to fluorescent calcein by cell esterase. Regarding the dissociation of the

acetoxymethyl group, calcein binds to calcium ions in the alive cell and appears in a fluorescent green color upon adequate illumination.

To stain the neuronal cells, the calcein stock (Calcein-AM) was diluted in a normal NB+++ medium, and then the stain was added to the sample and incubated for 15 min at 37°C inside the incubator. After the stain incubation period, the sample was washed for 5 min with a normal NB+++ medium to remove the excess of calcein from the sample. Finally, 500 µl of the imaging medium was added to the sample, and the medium was supplemented with 10 mM of HEPES. Diluted HEPES was added to the imaging medium for sample observation under the upright microscope with a 40x objective lens.

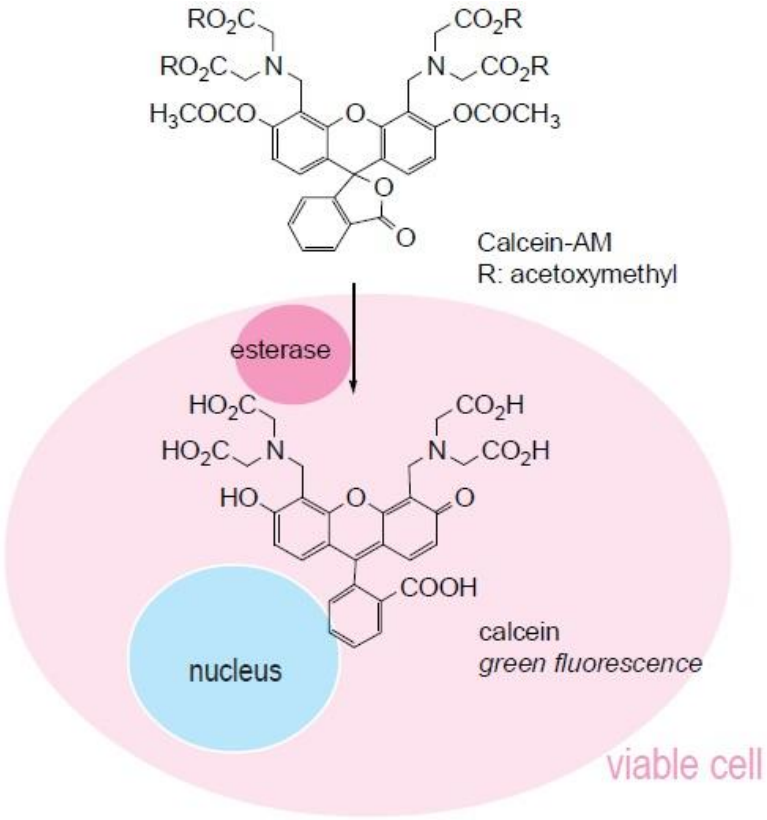

Figure 2. 9: Cell Esterase converts Calcein-AM to Calcein, pathway inside alive cell. Image adapted from Dojindo. Living and dead cell staining, 2016 [6].

2.1.8 Immunocytochemistry stain

A staining method is used for cell screening. It uses special antibodies to determine specific proteins or antigens in cells based on antigen-antibody interaction. This technique uses two types of antibodies primary and secondary antibodies. Primary antibodies are unconjugated antibodies, while the secondary antibodies are conjugated to a fluorophore, which absorbs light in a certain wavelength and emits it in different ranges. Primary antibodies bind directly to the target protein. Primary antibodies were incubated and washed with skim milk powder. Secondary labeled antibodies were added to the sample, which detects the primary antibody attached to the target. The secondary antibodies are directed against the immunoglobulin class or subclass of the primary antibody's species (Figure 2.10). Finally, the sample is observed under the confocal microscope (LSM 880).

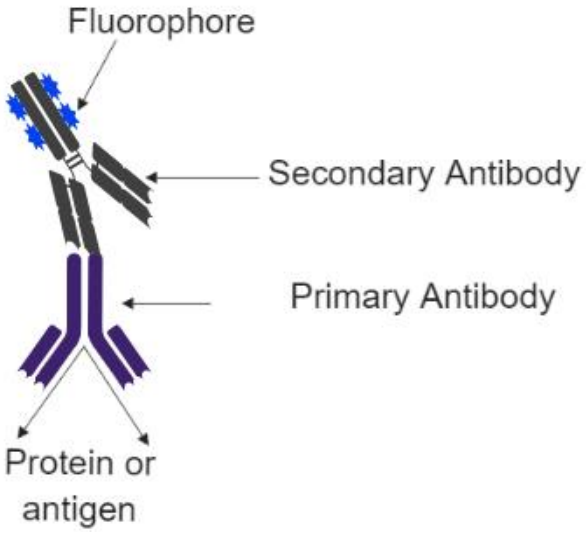

Figure 2. 10: Immunocytochemistry mechanism. Primary and secondary antibodies bind together to the target and the fluorophore.

2.1.9 Sample fixation and preparation for LSM

To observe a stretched sample in PDMS chambers, the stretcher device is stopped in a relaxation (release) position. The sample was fixed with 3.7% paraformaldehyde (PFA) solution diluted in 1X CB buffer and incubated for 10 min at 37 °C. The sample was washed with 30 mM glycine 1X CB solution, and the chamber was washed 3 times with 1X CB to remove the excess of PFA. The chamber was treated with 0.05% Triton X 100 diluted in 1X CB for 10 min for cell membrane permeabilization. After the treatment with Triton X 100, the chamber was washed 3 times with 1X CB buffer and blocked with 5% skim milk powder diluted in 1X CB buffer for 1h at room temperature to block unspecific antibody binding. Anti-MAP2 antibody and Anti-Tubulin Antibody clone YL 1/2 primary antibodies are prepared in 1% to skim milk powder in a dilution ratio of 1:500 and incubated for 2h while shaking. Primary antibodies excess is removed, then the chamber was washed 3 times with 1% milk powder. The chambers were treated with Anti-Rat IgG (H&L) Alexa Fluro 647 and Anti-Rabbit IgG (H&L) Alexa Fluro 488 as a secondary antibody. They were diluted in 1% skim milk powder in the ratio of 1:500 and incubated for 1h in dark (covered) with shaking. After the shaking period, secondary antibodies excess is removed, and the chamber was washed 3 times with 1X CB, and finally with H2O. Before covering the chamber, it was mounted on an object slide, which serves as a sample holder for the confocal microscope. Both wells are covered with 40 µl of the mixture of Fluoromount mounting media and 15 mM DABCO (1,4-

diazabicyclo [2.2.2] octane), then covered with a coverslip Ø = 15 mm. The sample is settled for drying for the next day. After the fixation and mounting process, the chamber walls are removed, and the sample was examined upside down on confocal LSM 710 or LSM 880. Secondary antibodies Alexa Fluro 647 and Alexa Fluro 488 were excited with a helium-neon laser at 633 nm and an argon-ion laser, respectively. In one microscope scan, two fluorophores were detected by the mean beam filter MBS 488/543/633.

2.1.10 Microscopy

Different types of microscopes were used for different purposes as follow:

Table 2. 5: Each type of microscope was used for special purposes as mentioned in the table above.

Microscope type	Company	Lenses	Camera	Purpose
Dissecting / stereo microscope	ZEISS stemi 508 microscope	10x ocular lens		Neuronal cell isolation
Inverted microscope	ZEISS Axio Vrt.A1	5x, 10x,20x objective. 10x ocular lens.	AxioCam ICm1	Cell counting and observation during cultivation time
Upright microscope	ZEISS Axio Imager.M2	40x objective	Axio 503 mono	Live cell imaging and stained samples
LSM 880 confocal microscope	ZEISS LSM 880	20x air objective		Observing fixed sample stained with antibodies

2.1.11 Imaris imaging processing software

A computer software used for 3D and large microscopic data analysis. This software provides many functions for the segmentation and interpretation of the 3D data. The data were imported to the program, and different program tools were used and applied. Advanced visualization and analysis methods were supported with this software. This program allows the data vision in a 3D perspective. With the obtained results from Imaris software, we can compare both data from the original and derived from the software together. Different tools from distance measurement and neurite length can be applied to the data using this software.

Chapter 3

Results

To understand one of the most complex organs in our body, how does it work, and how to overcome and deal with its diseases, it is essential to develop an *in vitro* brain-like model (Frimat et al., 2015). Researchers started to study the brain neuronal network *in vitro* in typical 2D cultures, but 2D cultures do not mimic the actual brain environment. To come closer to the original brain environment and brain-behavior 2D cultures were not enough to fill the study gap, so there should be an alternative culturing system.

We, therefore, aimed to create and develop a three-dimensional (3D) cell culture system. Several materials and natural or synthesized gels were used for this purpose. The used gels and scaffolds will be discussed in detail in the coming sections.

3.1 Three dimensional (3D) cell culture

The commonly used cell culture is the two-dimensional (2D) cultures. Recent research on three-dimensional (3D) cultures gained popularity (Kapałczyńska et al., 2018). Creating a three-dimensional (3D) culture is extremely sensitive and requires appropriate culturing matrixes (scaffolds) for building the systems used in the study. The step after chamber fabrication and cell isolation is the cultivation of primary isolated cortical neurons in a 3D environment. To mimic the brain-like environment, the used scaffolds

should include suitable and essential growth conditions close to the real brain environment. Two-dimensional (2D) cell cultures do not mimic and do not provide the physiology and morphology of the real brain environment, whereas the 3D cell culture provides the required and needed environment. The cell-cell and cell-matrix interaction in 2D cultures are not represented as they were in the reality. These mentioned interactions mimic the natural environment *in vivo* (Edmondson et al., 2014; Neto, Levkin, & Mano, 2018).

Two types of gels are used in the study a natural and artificial synthesized to build the 3D culture. According to the experiment's protocols, the cells were seeded and mixed with the gels in different concentrations and ratios used for each method. Depending on the culturing system, as illustrated in the following sections.

3.2 Neuronal cell culture in a 3D system and stretch experiments using Matrigel as a natural scaffold.

Two different cultivation systems were tested using the naturally secreted and isolated gel, the 3D suspension, and the sandwich method. Both neuronal cell cultivation methods were plated on the classical PDMS elastomeric chamber. Both cultivation methods used different Matrigel concentrations as described and shown in the following sections.

3.2A The 3D Suspension method

In this system, the cells were distributed randomly within the Matrigel in all directions, a droplet-like system (sphere-like) (Figure 2.6 for illustration). The difference in Matrigel concentrations affected cell growth and distribution in the gel. Clearly visible is cell aggregation with increasing Matrigel concentrations (12 mg/ml). In contrast, the homogeneous distribution was seen for lower Matrigel concentrations, (5 and 6 mg /ml). Different Matrigel concentrations were tested for the 3D suspension method. Only the four optimized concentrations were suitable and stable on PDMS elastomeric chamber and glass dishes, but in an un-stretched position (5,6,10, and 12 mg/ml) as shown in figure 3.1.

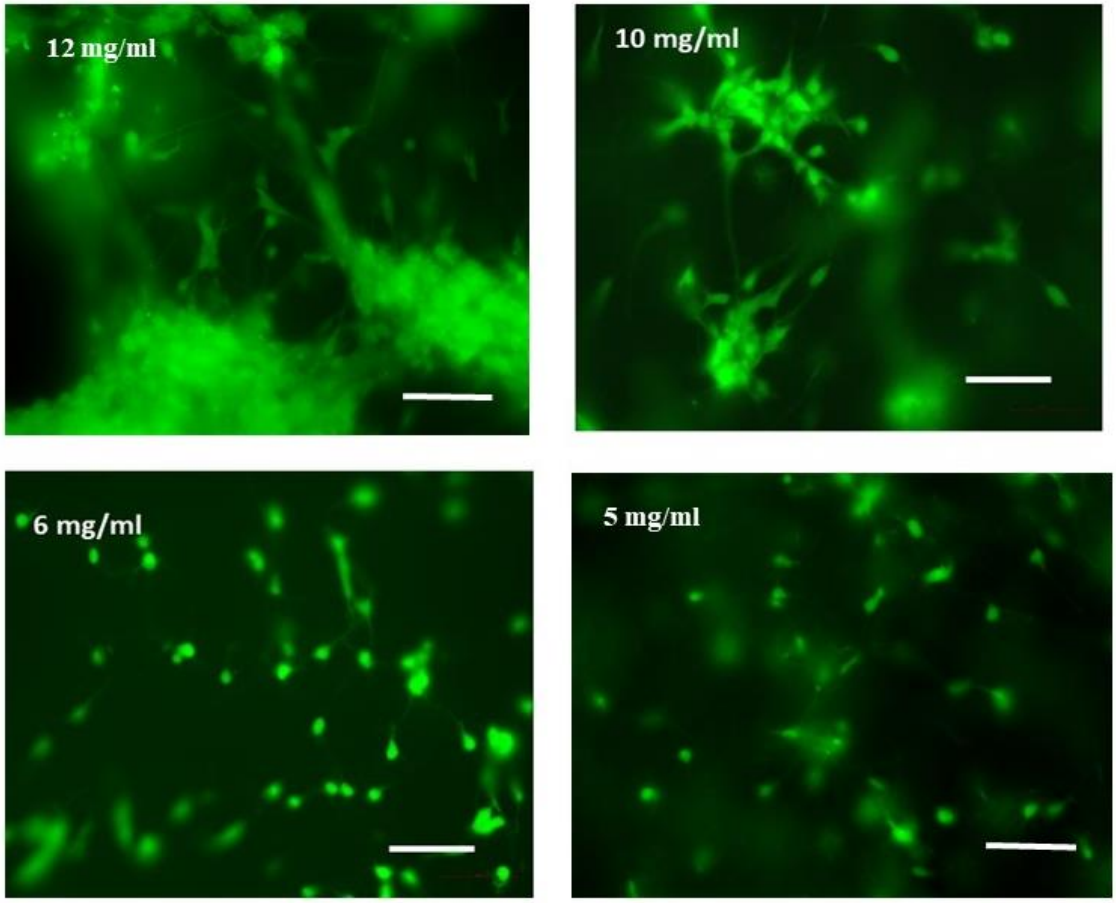

Figure 3. 1: Four different optimized Matrigel concentrations (12, 10, 6, 5 mg/ml) respectively were used in the 3D suspension method as shown. The above-shown figure represents an un-stretched classical PDMS chamber. The higher the Matrigel concentration, the more the cells are clustered together and harder to distribute within the Matrigel. The PDMS chamber was stained with calcein and observed under the upright microscope with a 40X objective lens. The figure scale bar was 20 µm.

In the above-shown figures, cortical neurons were plated and cultivated in the classical PDMS chamber. Cortical neurons grow in a 3D sphere-like environment and form neurites in all Matrigel concentrations and all directions. Neuronal cells clump and cluster together in high Matrigel concentrations (12 mg/ml) and well cell distribution in low Matrigel concentrations (5 and 6 mg/ml). High protein concentrations are found in high Matrigel concentrations. This high protein content prevents the neurons

from homogeneous distribution and cell growth. In low matrigel concentrations (5 and 6 mg/ml), cortical neurons are well distributed equally in the 3D suspension method and form neurites in all directions because of low protein concentrations and amount in low gel concentration.

3.2B Sandwich method

In addition to the 3D suspension method, we had another cultivation and culturing method for the 3D system the Sandwich method is studied. This method differs from the 3D Suspension method as it is composed of two gel layers and two neuronal cell layers, as shown in Figure 2.5. Thus, neuronal cells were distributed in a horizontal direction on each matrigel layer in contrast to the 3D Suspension method. This method is not close to reality compared to the 3D Suspension method from the culture shape. Two gel concentrations were studied (10 mg/ml and 20 mg/ml) and plated on the classical elastomeric PDMS chamber.

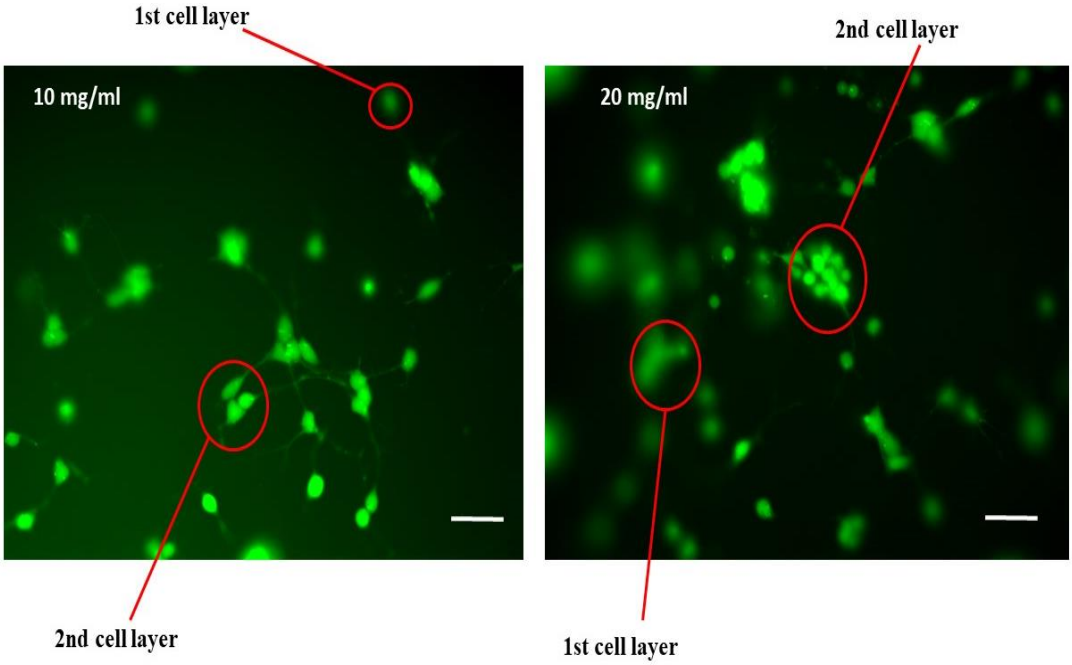

Figure 3. 2: Sandwich method cultivated in Matrigel with two different optimized concentrations (10, 20 mg/ml). This method was plated on the classical PDMS elastomeric chamber in an unstretched position. Neuronal cells were observed only on the upper cell layer. Cells were stained with calcein and observed under the upright microscope with a 40X objective lens. The figure scale bar is 20 µm.

Two cell layers were observed by staining the culture with calcein, but the second Matrigel layer in between cannot be recognized clearly. The top cell layer cells were clear to identify, and the beneath neuronal cell layer is blurry. As shown in the figures above, the first cortical neuronal cell layer cannot be identified clearly because of the gel and cell layers above.

In both systems, the 3D suspension and sandwich method, the neuronal cells were cultivated in Matrigel on 50 kPa classical PDMS chambers (Figure 3.3). The cultivation of neuronal cells in Matrigel was optimized at different gel concentrations, and the neuronal cells survived within these different concentrations in both methods.

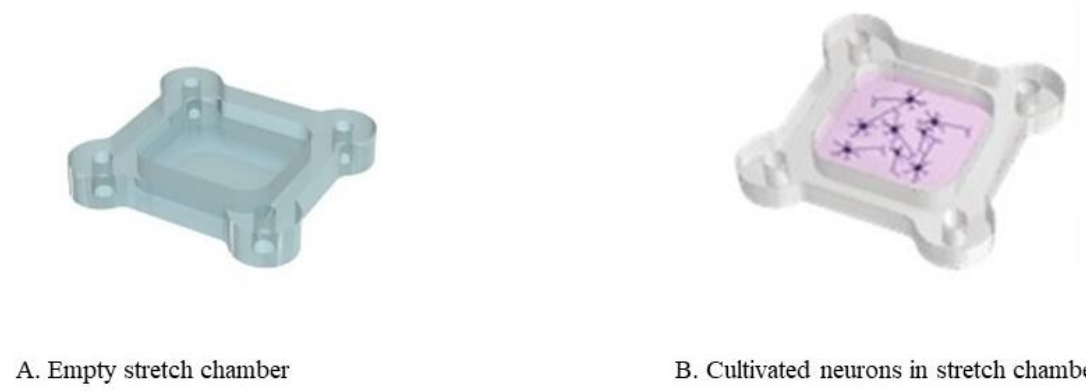

Figure 3. 3: Traditional (classical) 50 kPa PDMS chamber, empty chamber vs. cultured chamber. Image by Jens Konrad (ICS-7).

3.3 Matrigel is a naturally suitable 3D culture system but a non-stretchable scaffold.

Matrigel provides a suitable neuronal cell growth and cell culture environment. Neuronal cell growth and branch formation occurred during the first 24h of cell cultivation in the scaffold. Both cultivation methods the sandwich and the 3D suspension were used and tested on Matrigel. These two cultivation methods were applied on glass dishes and classical PDMS elastomeric chamber. The scaffold (Matrigel) detached from the classical PDMS chamber during medium change, and even without any stretch force on the elastomeric classical PDMS chamber. Due to the shear force that occurred in the chamber gel detaches from the classical elastomeric PDMS chamber. The classical PDMS elastomeric chamber was cross-linked with different substances like glutarate and different Matrigel concentrations to prevent the detachment of Matrigel culture from the elastomeric PDMS chamber. With or without cross-linking the PDMS chambers Matrigel

detached from the elastomer even without applying any force on the chamber.

Newly developed and reconstructed elastomeric PDMS chambers (developed and reconstructed by Mahmoud Ardah) were designed to reduce the shear force applied by the medium change and stretch movement in the classical PDMS chambers. The new PDMS chambers walls work as Z-direction supporters for the complete cell culture.

Neuronal cells were cultivated in the newly developed PDMS elastomeric chambers, so matrigel detachment during medium change was observed again. By applying even small amplitudes on the culture Matrigel detachment was observed beginning from the small, inserted grooves wall as shown in (Figure 3.4).

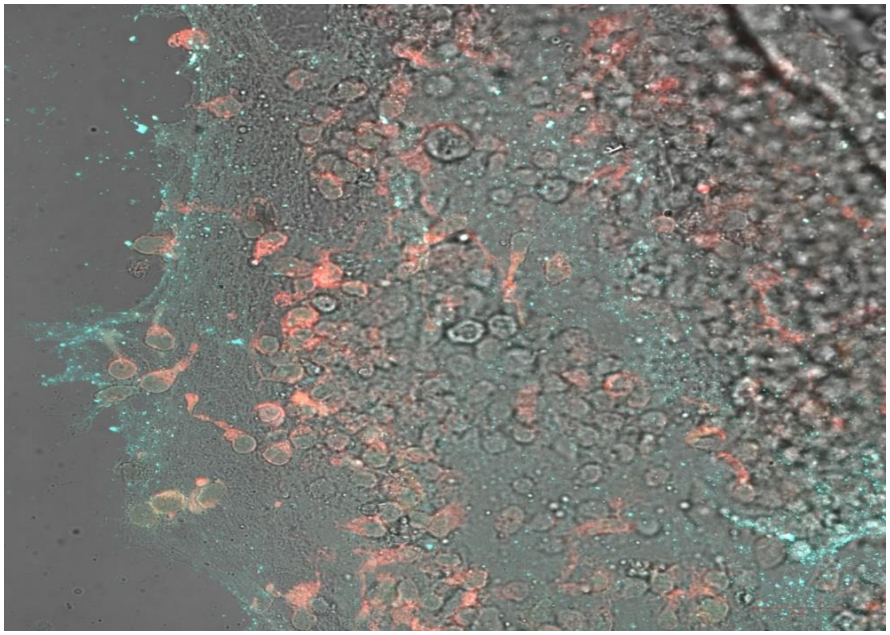

Figure 3. 4: Neuronal cells cultivated on Matrigel in the newly developed and reconstructed PDMS elastomeric chamber. Matrigel detachment from the inserted grooves wall. Chamber stained with MAP2 in green, anti-tubulin in red, and NFH in blue. A 40x objective LSM 880 image.

3.4 Classical PDMS chamber reconstruction and development

The classical PDMS elastomeric chamber design fits 2D stretch and cultivation experiments, the chamber was tested and used for 3D neuronal cultivation and stretch experiments. The 3D cultivation and stretch experiments in the classical PDMS elastomeric chamber were not successful at all. The different types of scaffolds detached from the classical elastomeric PDMS chamber. There was a need for an alternative and new elastomeric chamber design that fits the 3D stretch and cultivation experiment. The newly reconstructed chamber had the same external dimension as the classical chamber with small, inserted wells. The reconstruction idea came from that the 3D culture should have Z-direction supporters, and the newly reconstructed elastomeric chamber was designed to have these supporters. The elastomeric chamber modification and reconstruction were performed by Mahmoud Ardah. The newly developed and reconstructed elastomeric chamber had small, inserted wells with 5 mm *5 mm * 0.7 mm dimensions. The wells measurement was obtained using a 40x and 63x objective lens of an upright microscope to determine the actual working distance in the chamber. The figure 3.5 below shows the reconstruction and modification carried out to the classical elastomeric chamber.

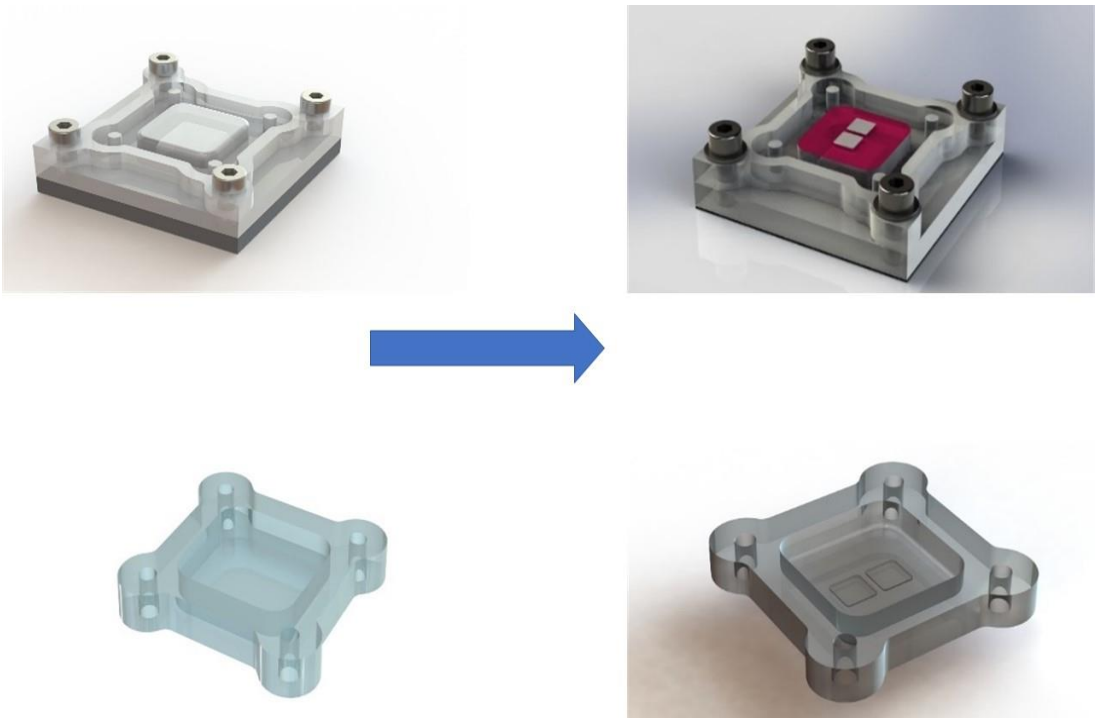

Figure 3. 5: Classical elastomeric chamber vs. newly developed and reconstructed elastomeric chamber. The external chamber dimensions were constant as the classical and the inserted wells dimensions were 5 mm* 5 mm*0.7 mm. reconstruction and modifications were performed by Mahmoud Ardah. The reconstruction were of the PDMS elastomeric chamber was performed and designed to fit 3D experiments. Images by Jens Konrad FZJ ICS-7.

3.5 VitroGel 3D-RGD as a synthesized polymer.

An artificial chemically synthesized polymer classified as hydrogels that closely mimics the endogenous physiological microenvironment for *in vitro* cell cultivation. Different concentrations of VitroGel were tested for cell growth, cultivation, and stability on both PDMS elastomeric chambers (classical and newly developed). Cells were cultivated in the VitroGel 3D-RGD model and were tested in the 3D suspension method in different gel concentrations. This type of hydrogel was not suitable for neuronal primary cell cultivation in addition to its instability in PDMS elastomeric chambers. Polymer detachment from the PDMS chambers was observed during the first

24h of cultivation, even in the newly reconstructed PDMS chambers. The cell suspension was mixed with 0.5x PBS and enough VitroGel, which gave different concentrations regarding the company's cultivation protocol (Figure 3.6).

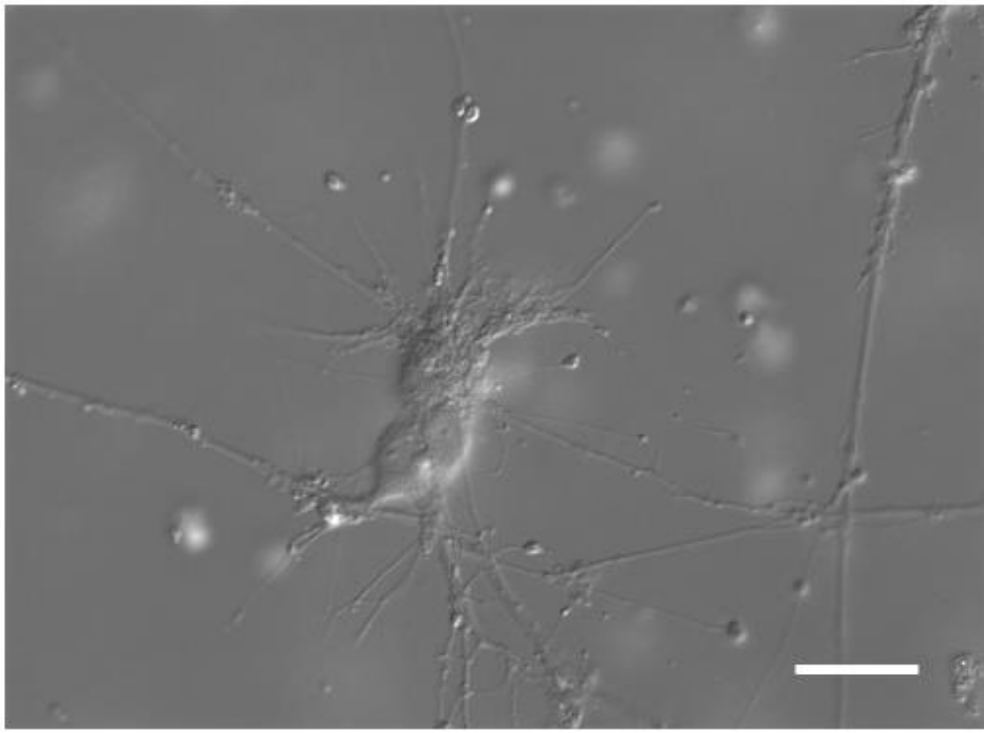

Figure 3. 6: An un-stretched sample of neuronal cells cultivated in VitroGel 3D-RGD, neuronal cells, and some cell branching can be observed after 24h of incubation at 37°C. Cells were stained with calcein and observed under the upright microscope with a 40x objective lens with transmission light (TL). Figure Scale bar 30µm.

Figure 3.6 illustrated above shows the primary neuronal cell growth in VitroGel 3D- RGD forming branches in an unstretched position. Primary neurons grow slowly on VitroGel 3D- RGD, but the polymer detaches from the 50 kPa PDMS elastomeric chambers by the medium change even without stretching like the Matrigel.

3.6 PVP co-GMA a synthesized stretchable 3D scaffold

A preliminary test was performed on the third type of cultivation polymers. This type of fibrin gel the PVP co-GMA was designed by the DWI Aachen Institute to fit the three-dimensional (3D) cultures. According to its sticky "glue-like" property, it was tested separately without neuronal cells on a PDMS elastomeric chamber for stretch experiments with different parameters. After 24h of incubation, a stretch experiment was applied with different parameters on the chamber to test the PVP co-GMA gel stability on the PDMS elastomeric chamber during stretch experiments (Figure 3.6).

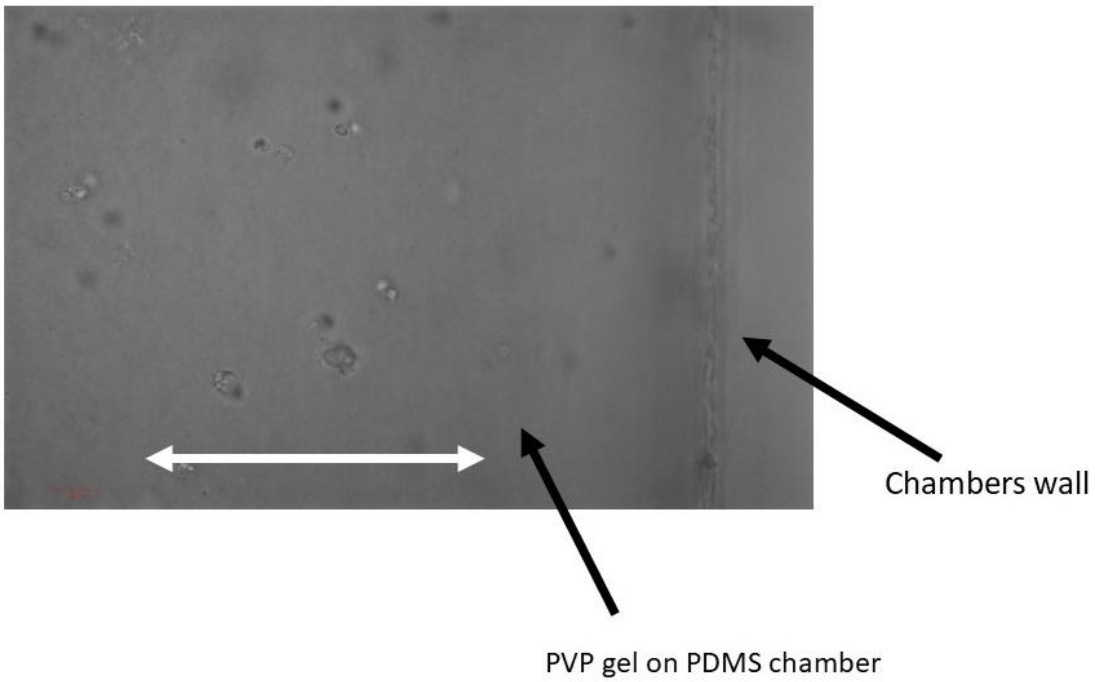

Figure 3. 7: PVP co-GMA polymer plated without neuronal cells in a newly developed PDMS elastomeric chamber. The chamber was stretched for 1hr at a frequency of 300 mHz and an amplitude of 15%. The white arrow shows the stretch direction which was applied to the newly reconstructed PDMS chamber. No detachment was observed during stretch and chamber medium change.

The figure above 3.7 represents the preliminary test experiment of PVP co-GMA gel stability on PDMS elastomeric chambers without neuronal cells. The experiments showed that the synthesized polymer was stable on PDMS chambers during stretch experiments and chamber medium change.

Fibrin concentration plays a major role in neuronal cell growth in this type of cultivation polymers. High Fibrin concentrations lead to low neuronal cell growth rate due to crowded contents of fibrin and thrombin mixture in PVP polymer (Figure 3.7).

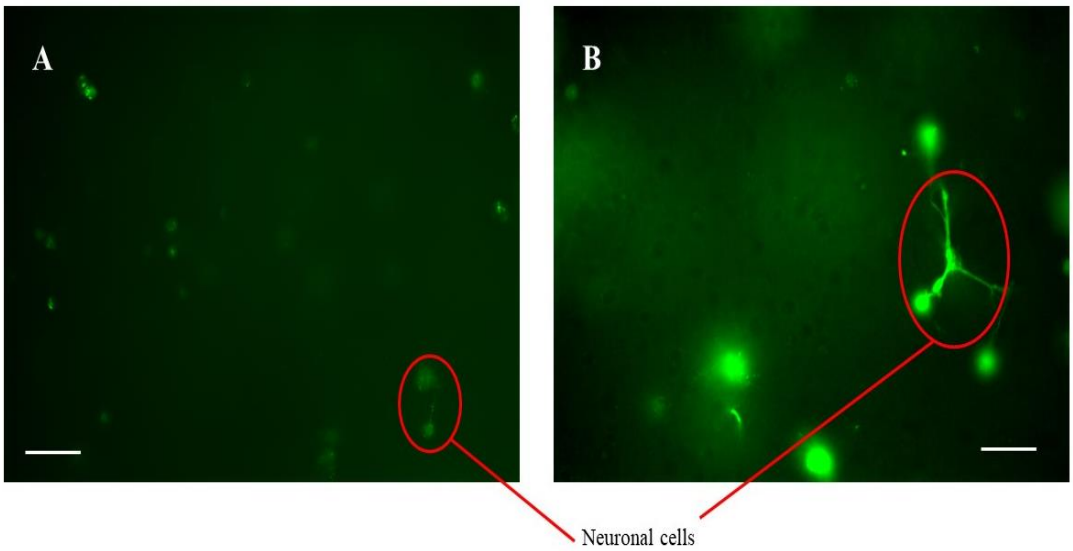

Figure 3. 8: Primary isolated neuronal cells cultivated in PVP co-GMA with high fibrin concentration (16.3 mg/ml). Slow neuronal cell growth due to high fibrinogen concentration. Cells were stained with calcein and observed under the upright microscope with a 40x objective lens. The figure scale bar is 20 µm. A) Represents primary neuronal cells after 24h of cultivation and incubated in 37°C. B) Neuronal cells after 1 week of cultivation.

Different fibrinogen concentrations were tested and changed to get the optimal concentration for neuronal cell growth and cultivation. Neuronal cells do not grow in any fibrinogen concentration, because of the dense fibrinogen thrombin interaction which prevents smooth cell growth. As tested before the neuronal cell grows in PVP co-GMA gel the sample shows to be suitable for 3D cell cultivation and stretch experiment model as shown in the figure below (Figure 3.8).

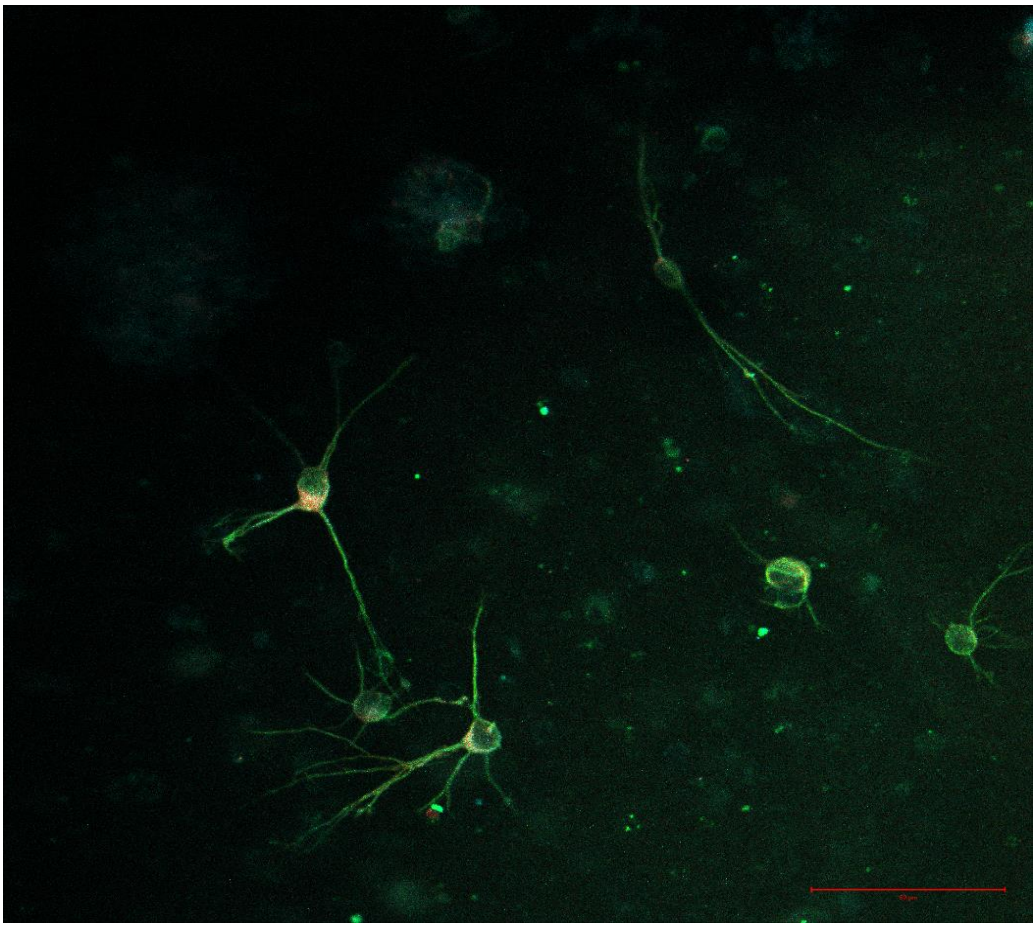

Figure 3. 9: Primary neuronal cells cultivated in PVP high fibrinogen concentration (16.3 mg/ml). Neuronal cells were stained and treated with MAP2 (green), NFH (blue), and Tubulin (red). The sample was observed under the LSM 880 with an air objective 20x. The figure scale bar is 50 µm.

The different fibrinogen concentrations were tested varied from high to low concentrations. Low fibrinogen concentration was more suitable than high fibrinogen concentration for primary neuronal cell cultivation in PVP modified gel. Slow neuronal cell growth in high fibrinogen concentration 16.3 mg/ml compared to lower fibrinogen concentration faster neuronal cell growth and branch formation (Figure 3.10).

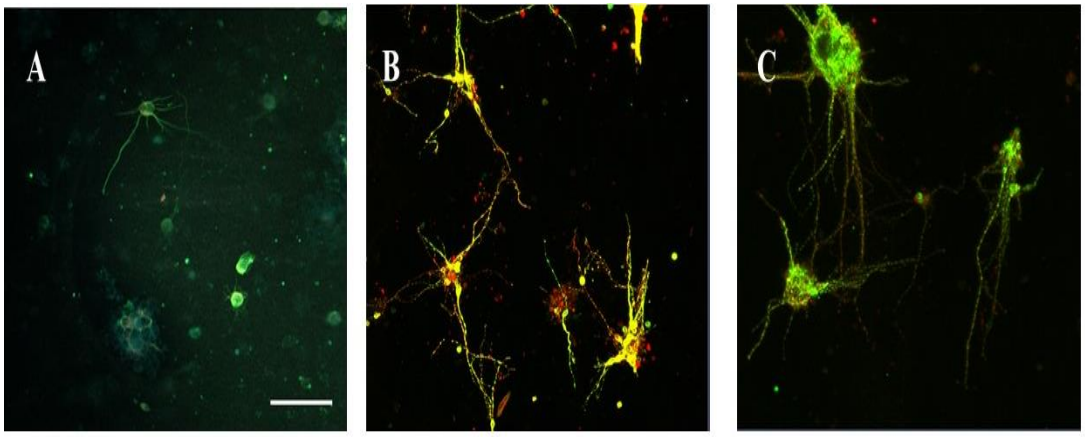

Figure 3. 10: Different fibrinogen concentrations were tested for primary neuronal cell cultivation in PVP co-GMA gel. Neuronal cells were stained with MAP2 in green and tubulin in red staining. A) High fibrinogen concentration 16.3 mg/ml low and slow neuronal cell growth. B) Moderate fibrinogen concentration of 8.16 mg/ml was acceptable for neuronal cell growth. C) Low fibrinogen concentration of 4.03 mg/ml favorable for neuronal cell growth. The figure scale bar is 50 µm.

PVP co-GMA was mixed with primary isolated neuronal cells and plated on the 50 kPa PDMS elastomeric chambers. Neither detachment nor tear-off from the PDMS chambers was observed during the medium change, even by applying stretch on the elastomeric chambers. PVP co-GMA gel was a stretchable and analyzable polymer. Due to its stability on the PDMS elastomeric chambers stretch experiments were applied to them with an amplitude of 300 mHz and 15% of stretch for 24 h. Two different fibrinogen concentrations were used with these conditions, after the stretch period the chambers were stained with MAP2 in green and tubulin stain in red (Figure 3.10 a&b).

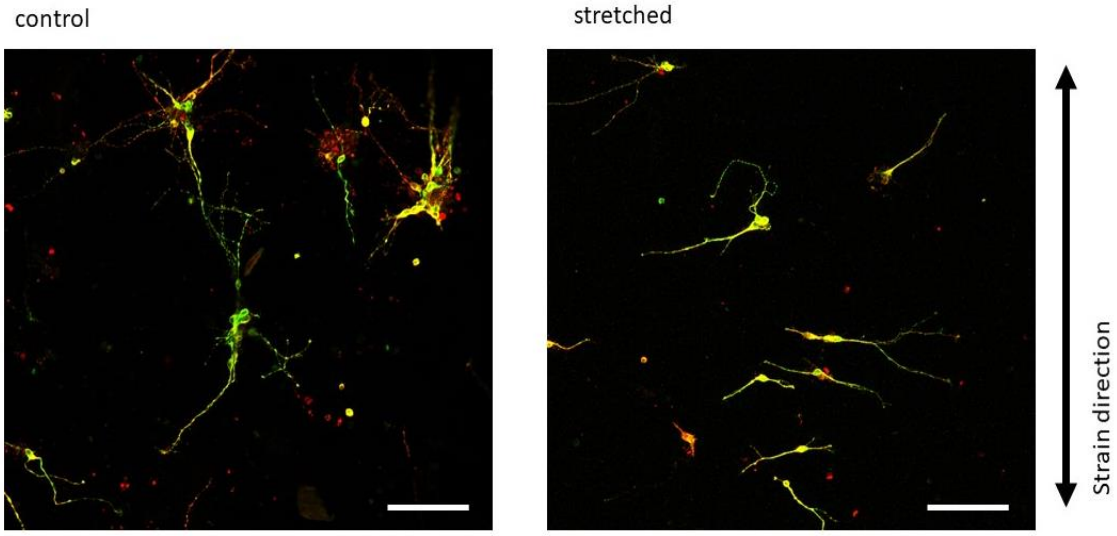

Figure 3.11. 1: Primary neuronal cells cultivated in PVP co-GMA with a moderate amount of fibrinogen concentration 8.16 mg/ml. Neuronal cells were stained with MAP2 in green and tubulin in red. Neuronal cells under 15% strain for 24 h with a frequency of 300 mHz The figure scale bar is 50 µm.

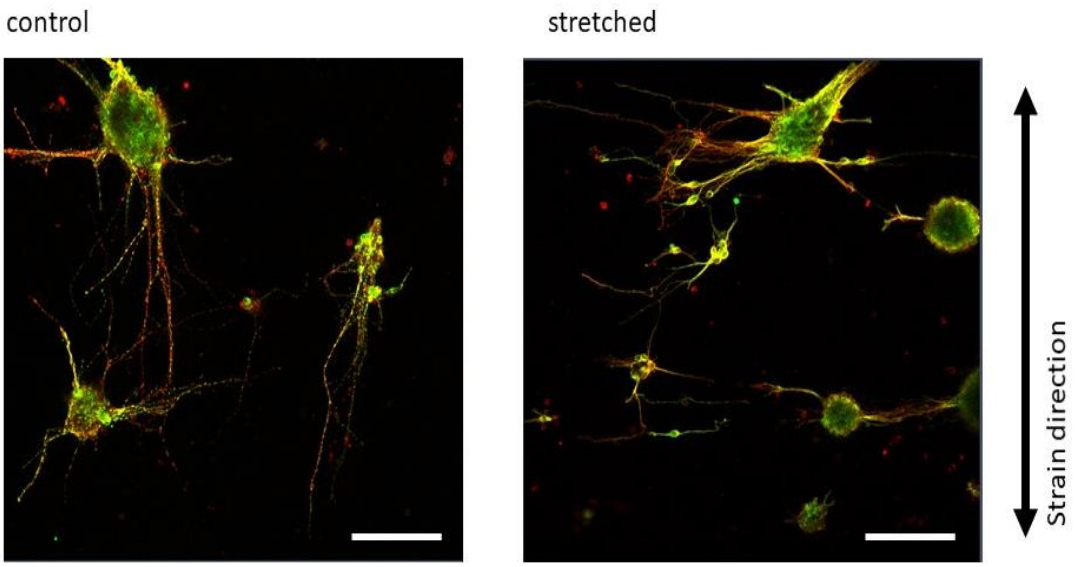

Figure 3.11. 2: Primary neuronal cells cultivated in PVP co-GMA gel with a low amount of fibrinogen 4.03 mg/ml. Neuronal cells were stained with MAP2 in green and tubulin in red. Neuronal cells under 15% strain for 24 h with a frequency of 300 mHz The figure scale bar is 50 µm.

As seen from the previous images the neuronal cell grows in 3D culture in an opposite direction to the strain direction to reduce the force applied on the cells from the stretch. Unstretched neuronal cells remain randomly growing and distributed in all directions of the polymer as shown in the above images.

Chapter 4
Discussion and Conclusion

The movement from 2D cultures *in vitro* to 3D cultures *in vitro* is a quantum leap in the research world. In the 3D research, we are coming closer to the actual tissue environment, especially the brain. The 3D cell cultures demonstrate the practical and real application for the in vivo studies (Ravi, Paramesh, Kaviya, Anuradha, & Solomon, 2015). The brain is among the tissues that are in continuous movement regarding the heartbeats. This brain movement is simulated in *in vitro* environment by using a house-made stretcher device (Faust et al., 2011). Cortical isolated neuronal cells were stretched on PDMS elastomeric chambers cultivated in their 3D scaffold. The used 3D scaffold was either naturally isolated or chemically synthesized. The 3D cell cultures represent the actual cell-cell and cell-matrix interactions; these interactions are required for normal cell behavior to feel or behave as in the brain, unlike 2D cultures. Two-dimension cell cultures are low in cost, unlike the 3D culture that requires many possibilities to build the culture (Kapałczyńska et al., 2018) (Neto et al., 2018). Owing to the 2D reality shortness and disadvantages, there was a necessary aim and need to create and develop a system that touches the reality in the research field. The three-dimension cell culture mimics the actual brain environment. The 3D culture is an intermediate model between 2D cultures and animal models, this intermediate model (3D) can be used for neurological disorders including traumatic brain injury, Alzheimer, and

Parkinson's diseases (Febles et al., 2014). The 3D cell culture's importance lies in the treatment of brain trauma, injuries, and brain stroke and aided in pre-clinical therapies (Benton, George, Kleinman, & Arnaoutova, 2009). Different types of 3D scaffolds were tested and stretched in our house-made stretcher, among these gels (scaffolds) naturally, and chemically synthesized polymers were studied.

Naturally synthesized scaffold contains the proteins and molecules found in the brain tissue that is responsible for cell growth, unlike the chemically synthesized polymers growth factors. Moreover, proteins must be added to the culture (Benton et al., 2009). Each of the polymers has its advantages and disadvantages among each other as described in the paragraphs below.

4.1 Matrigel non-suitable three-dimensional (3D) system stretch scaffold

Matrigel provided an applicable neuronal cell growth due to the richness of essential proteins and ECM molecules. The cultivation environment was close to the actual brain environment. Matrigel allowed the neuronal cells to grow and form neurites, as shown in the results part in the previous sections. Both cultivation systems, 3D suspension, and sandwich methods were suitable for cell growth, but they were not appropriate for stretch experiments.

Matrigel advantages provide applicable 3D neuronal cell growth conditions and environment. Otherwise, Matrigel had a considerable disadvantage in

our study; it detaches from our 50 kPa elastomeric PDMS chamber. High Matrigel concentrations prevent equal neuronal cell distribution in the cultivation scaffold, it avoids neurite formation and smooth cell growth. High gel concentrations are rich and dense with the internal components that determine cell growth and neurite formation. Low Matrigel concentrations allow equal or homogeneous neuronal cell distribution that allows the cells to form neurites smoothly. Cell growth and neurite formations depend on the internal components of the scaffold (Matrigel) from proteins and other molecules. Matrigel is suitable for neuronal cell culture without applying stretch experiments.

4.2 VitroGel 3D-RGD cultivation scaffold

VitroGel 3D-RGD is a chemically synthesized polymer that had a longer polymerization time than Matrigel and PVP co-GMA but was not appropriate for primary isolated neuronal cell cultivation. As with Matrigel, VitroGel detaches from PDMS elastomeric chamber by cell cultivation and the medium change. This type of polymer; was not applicable for primary neuronal cell cultivation and was even not suited for 3D neuronal cell culture in our research. The chemically synthesized polymer was not suitable for cortical neuron cultivation, but this type is applicable for other cell-type cultivation. A considerable disadvantage of this type of gel is the detachment of the PDMS elastomeric chamber during stretch cycles and medium change. Due to this disadvantage of VitroGel, it was not more tested in our study for neuronal cultivation and stretch experiments.

4.3 PVP copolymerized with GMA (PVP co-GMA) the 3D artificially synthesized stretchable scaffold

This type of polymer is an artificially synthesized one it has an advantage among the other gels previously mentioned so that it does not detach from our 50 kPa PDMS elastomeric chambers like the other natural and synthesized polymers. This polymer is classified as fibrin gel due to its content of fibrinogen and thrombin. Due to its fibrinogen content, the neuronal cell growth is controlled by the component concentration, high fibrinogen concentrations lead to slow neuronal cell growth due to the small space inside the gel between the contents of the polymer in contrast to low fibrinogen concentration where the polymer is less crowded and high cell growth and neurite formation. The development of a 3D stretch experiment with this type of polymer was successful as the cultivation of primary neuronal cells in the suspension method on our newly developed 50 kPa PDMS elastomeric chambers was stretched without polymer detachments. The result of the experiment was stained with MAP2 and tubulin stain and observed under the LSM 880 microscope. The results showed neuronal cell reorientation perpendicular (opposite direction) to the stretch direction. Primary isolated neuronal cells grow in a perpendicular (opposite) to stretch direction even under low amplitudes. The obtained results were analyzed using a computational software called IMARIS.

Additional experiments are required on the stretchable optimized fibrin gel (scaffold) concentrations and system. Two-dimension experiments and

studies regarding structural and functional aspects can be performed in the optimized three-dimension (3D) stretchable cell culture.

Chapter 5
Future perspectives

To overcome the organ environment difficulties, the experiments should be close enough to the actual organ environment. In our study, to investigate a suitable 3D stretch model, the neuronal cells were seeded on a suitable scaffold on a stretchable silicone PDMS elastomer. This elastomer is fixed on a house-made stretcher device to apply force and strain to the culture sample. More stretch experiments will be needed to study the synthesized artificial polymer **PVP co-GMA**, in addition to short and long-term cell cultivation. Three-dimensional (**3D**) cell stretch experiments with different amplitudes will be studied in more and specific details. The synthesized polymer was optimized on two suitable fibrinogen concentrations, and these concentrations were examined in our research and study. The tested fibrinogen concentrations were advisable for neuronal cell growth, as shown in the results part. Further experiments are needed on the optimized fibrinogen concentrations. The applied researches on two-dimensional (**2D**) neuronal cell cultures could be practiced with the newly developed three-dimensional (**3D**) systems and the optimized fibrinogen concentrations. The achieved results from the 3D system culture could be compared with the previous 2D experiment results. Cytoskeletal and functionality stretch experiments of neuronal cells will be studied in more detail. Neuronal cell morphology and growth behavior can be studied in detail in a 3D environment. In the newly developed three-dimensional (**3D**) system life

cell imaging can be performed to study neuronal synchronization. Additionally, extreme amplitudes can be performed on the newly reconstructed PDMS elastomeric chambers using the optimized PVP co-GMA polymer.

Finally, with the newly developed 3D system we come to a step closer to the actual brain environment. This step leads us to overcome some brain illnesses like Alzheimer's and Parkinson's diseases and go a step further with brain trauma researches.

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Appendices

Consumable Materials

CONSUMABELS	COMPANY
CELL CULTURE DISH 35 X 10 MM	Greiner Bio-one , Frickenhausen, Germany
COVER SLIP Ø 12 MM #1	Menzel, Braunschweig, Germany
COVER SLIP Ø 15 MM #0	Menzel, Braunschweig, Germany
COVER SLIP Ø 18 MM #1	Menzel, Braunschweig, Germany
COVER SLIP Ø 25 MM #0	Menzel, Braunschweig, Germany
IMMERSIONS OIL 518 F	Carl Zeiss, Jena, Germany
PARAFILM	VWR, Darmstadt, Germany
PETRI DISH 3.5 CM WITH PRE-DRILLED 2.5 CM^2 HOLES	Cell E&G, San Diego, USA
PIPETTE TIPS (10 µL, 200 µL, 1250 µL)	StarLab, Hamburg, Germany
PIPETTE (5ML, 10ML, 25ML, AND 50ML)	Eppendorf, wesseling/ Berzdorf, Germany
EPPENDORF TUBE (0.5 ML, 1.5 ML, 2 ML)	Eppendorf, Wesseling/Berzdorf, Germany
FALCONE TUBE (15ML, 50ML)	Greiner Bio-one, Frickenhausen, Germany
MICROSCOPE SLIDES, PLAIN 26 MM X 76 MM	Thermo Fisher Scientific, Massachusetts, USA
WHATMAN, LENS CLEANING TISSUE 100 X 150 MM	GE Healthcare, Freiburg, Germany
PTRI DISH 9 CM	Greiner Bio-one, Frickenhausen, Germany
PDMS MIXING CUP	StarLab, Hamburg, Germany

Instruments

Instrument	Company
Centrifuge 5415-D	Eppendorf, Wesseling/Berzdorf, Germany
Centrifuge MEGAFUGE 8R	Thermo Fisher Scientific, Massachusetts, USA
Clean bench HeraSafe	Heraeus, Osterode, Germany
Desiccator (5 L)	Duran Group GmbH, Wertheim/Main, Germany
Laser Scanning Microscope (LSM) 710	Carl Zeiss, Jena, Germany

Laser Scanning Microscope (LSM) 880	Carl Zeiss, Jena, Germany
Microscope Axiovert Imager-M2	Carl Zeiss, Jena, Germany
Microscope Axiovert Vert.A1	Carl Zeiss, Jena, Germany
Stereomicroscope stemi 508	Carl Zeiss, Jena, Germany
Motor-driven stretch apparatus	ICS-7, Forschungszentrum Jülich, Jülich, Germany
Water bath WNB-22	Memmert, Schwabach, Germany
Objective LDA-Plan Ph1 5x air (NA 0.15)	Carl Zeiss, Jena, Germany
Objective LDA-Plan Ph1 10x air (NA 0.25)	Carl Zeiss, Jena, Germany
Objective LDA-Plan Ph1 20x air (NA 0.35)	Carl Zeiss, Jena, Germany
Objective W N-Apochromat 40x DIC (NA 1.0)	Carl Zeiss, Jena, Germany
Hematocytometer	Labor Optik, Lancing, United Kingdom (UK)
CO_2 Incubator	Thermo Fisher Scientific, Massachusetts, USA
Block heater SBH 130D	Stuart, Staffordshire, UK
Micropipette (10µl, 20µl, 200 µl, 1000 µl)	Eppendorf, Wesseling/Berzdorf, Germany
Pipetteboy	Accu-jet brand, Essex, UK

Software

Software Name	**Company**
Image J	Wayne Rasband, U.S. National Institutes of Health, Bethesda, MD, USA
Zen black 2012	Carl Zeiss, Jena, Germany
Zen blue 2012	Carl Zeiss, Jena, Germany
Imaris	Oxford Instruments, Bitplane

Chemicals

Chemicals	Company
BAMBANKER	Wacko Chemicals, Osaka, Japan
B-27 Supplement (50X) serum-free	Thermo Fisher Scientific, Massachusetts, USA
4-(2-hydroxyethyl)-1piperazineethanesulfonic acid (HEPES)	Sigma, Taufkirchen, Germany
Ethanol, absolute	Merck, Darmstadt, Germany
Fetal Bovine Serum (FBS)	Thermo Fisher Scientific, Massachusetts, USA
Fluoromount Aqueous Mounting Medium	Sigma, Taufkirchen, Germany
Formaldehyde 37%	Merck, Darmstadt, Germany
Gentamicin (50 mg/ml)	Sigma, Taufkirchen, Germany
GlutaMAX Supplement (100X)	Thermo Fisher Scientific, Massachusetts, USA
Glycine	Sigma, Taufkirchen, Germany
Hank's Balanced Salt Solution (HBSS)	Thermo Fisher Scientific, Massachusetts, USA
Hibernate-A Medium	Thermo Fisher Scientific, Massachusetts, USA
Hibernate-E Medium	Thermo Fisher Scientific, Massachusetts, USA
Isopropanol (2-propanol)	Merck, Darmstadt, Germany
Neurobasal Medium (1X) (NB)	Thermo Fisher Scientific, Massachusetts, USA
Phosphate-Buffered Saline (PBS) pH 7.2	Thermo Fisher Scientific, Massachusetts, USA
Skim milk powder	Sigma, Taufkirchen, Germany
Sylgard® 184 silicone elastomer kit (PDMS)	Dow Corning, Wiesbaden, Germany
Trypsin-EDTA, 0.5% trypsin 0.2% EDTA	Sigma, Taufkirchen, Germany
Triton-X-100	Sigma, Taufkirchen, Germany
Matrigel	Corning, Bedford, England

Human Fibrinogen	MILAN ANALYTICA AG, Rheinfeld, Switzerland
VitroGel 3D-RGD	The well Bioscience, USA
Human Thrombin	Sigma, Taufkirchen, Germany
Polyvinylpyrrolidone (PVP) copolymerized with Glycidilmethaacrylat (GMA)	Deutsches Wollforschungsinstitute Aachen DWI, Aachen, Germany
1,4-DIAZABICYCLO[2.2.2]OCTANE (DABCO)	Sigma, Taufkirchen, Germany
Tranexamic acid	Carinopharm, Elze, Germany
Ethylene gycol tetra-acetic acid (EGTA)	Sigma, Taufkirchen, Germany
MES (2(N-Morpholino) ethanesulfonic acid)	Sigma, Taufkirchen, Germany
Magnesium chloride (MgCl2)	Sigma, Taufkirchen, Germany
Neurobasal Medium (1X) phenol-free	Thermo Fisher Scientific, Massachusetts, USA
Trypan Blue solution	Sigma, Taufkirchen, Germany

Media and Buffers

Cytoskeleton buffer (1x CB), pH 6.1

Component	**Concentration**
EGTA	5 mM
Glucose	5 mM
MES (2(N-Morpholino)Ethansulfonacid)	1.95 g/L
MgCl2	10 mM
NaCl	150 mM
Streptomycin	1.72 mM

Neurobasal medium +++

Components	**Concentration**	**Volume**
Neurobasal Medium	1 X	48.6 ml
B-27 Supplements	1 X	1 ml

GlutaMAX Supplement	4 X	500 µl
Gentamicin	50 µg	50 µl

3D culture media

Components	Concentration	Volume
Neurobasal Medium	20 ml	20 ml
B-27 Supplements	1 X	1 ml
N-2 supplement	100 X	200 µl
Fetal Bovine Serum		200 µl
GlutaMAX Supplement	4 X	400 µl
Gentamicin	50 µg	20 µl
Tranexamic acid	100 mg/ml	1080 µl

Imaging medium

Components	Concentration	Volume
Neurobasal Medium phenol free	1 X	9.72 ml
B-27 Supplements	1 X	200 µl
GlutaMAX Supplement	4 X	100 µl
Gentamicin	50 µg	10 µl
HEPES	1 M	100 µl

Antibodies

Primary antibodies

Antibody	Host species	Company
Anti-MAP2 antibody	Rabbit	Abcam, Cambridge, UK
Anti-Tubulin Antibody clone YL $\frac{1}{2}$	Rat	Merck, Darmstadt, Germany

Secondary antibodies

Antibody	Host species	Company
Anti-Rat IgG (H&L) Secondary Antibody Alexa Fluro 647	Chicken	Thermo Fisher Scientific, Massachusetts, USA
Anti-Rabbit IgG (H&L) Secondary Antibody Alexa Fluro 488	Chicken	Thermo Fisher Scientific, Massachusetts, USA

جامعة النجاح الوطنية

كلية الدراسات العليا

تطوير نموذج ثلاثي الأبعاد لتطبيق الاجهاد الميكانيكي على الخلايا العصبية

اعداد

محمود عارضة

اشراف

د. أشرف صوافطة

د. فراس البطة

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الاحياء من كلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين

2021

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الملخص

معظم العمليات البيولوجية (الحيوية) المعروفة في زراعه الخلايا تم اكتشافها في حاضنات ثنائيه الابعاد (2D) .تزايد الحاجة إلى نموذج في المختبر يحاكي البيئة الفسيولوجية للدماغ لفهم الأمراض التنكسية العصبية. ان دماغ الإنسان في حالة حركة مستمرة في التمدد و التقلص للخلايا العصبية ويعود ذلك الى نبضات القلب الطبيعية والمتكررة والدورة الدموية في جسم الانسان. إن هذه الحركة المنتظمة المستمرة للدماغ تعمل على تغيير حجم الخلايا العصبية بالإضافة الى تمددها وتقلصها. لمحاكاة هذا التغيير في حجم الدماغ ، تم زراعة الخلايا العصبية في المختبر على سقالات مختلفة وتمتد على فالب سيليكون مرن مصنوع من بولي دايميثيل سيلوكسان(PDMS) **Polydimethylsiloxae** بسعات و ترددات مختلفة. تم تطبيق تجارب التمدد السابقة على الخلايا العصبية القشرية المزروعة في حاضنات ثنائية الابعاد (2D) لاختبار سلوك الخلايا العصبية والهيكل الخلوي بسبب الإجهاد الميكانيكي المطبق عليها بواسطة جهاز شد. كانت نتائج الحاضنات ثنائية الأبعاد جذابة للمضي قدمًا خطوة أخرى للاقتراب من حركة الدماغ الحقيقية من خلال إنشاء وتطوير نظام حاضنات الخلايا ثلاثي الأبعاد (3D). تم محاكاة حركة الدماغ بواسطة جهاز تمدد وتم تطبيق إجهاد ميكانيكي على الخلايا العصبية القشرية.

لبناء وإنشاء تجربة تمتد ثلاثية الأبعاد ، كانت هناك حاجة إلى المطاط الصناعي القابل للمط 50 كيلو باسكال .PDMS تم تثبيت المطاط المرن في حامل محدد وخاص ، وزُرعت الخلايا العصبية لجنين الفأر المعزول في سقالة ثلاثية الأبعاد في حاضنه .PDMS أخيرًا ، تم تثبيت الحاضنة ثلاثية الأبعاد الكاملة بجهاز الشد. اثناء عملية التطوير ؛ من حاضنة التمدد ثلاثية الأبعاد ، تمت دراسة

العديد من سقالات الزراعة: تم استخدام ثلاثة أنواع مختلفة من الهلام واختبارها كسقالة للبحث. كانت مادة Matrigel المُصنّعة بشكل طبيعي والمعزولة من ورم الفئران EngelbrethHolm-Swarm (EHS)السقالة الأولى التي تم استخدامها في بحثنا ، بالإضافة إلى سقالتين هلاميتين اصطناعية. أظهرت تجربة Matrigel نمو الخلايا العصبية في الطريقتين المستخدمتين، لكنها لم تكن قابلة للتمدد و الشد. تم استخدام هيدروجيل (VitroGel 3D-RGD) مع مجموعة وظيفية معدلة، وأخيراً مادة هلامية مصنوعه من الفيبرين .PVP co-GMA في هذه الدراسة ، تمت دراسة واختبار ثلاث سقالات مختلفة (Matrigel و VitroGel و (PVP co-GMA ، بالإضافة إلى أنه تم تعديل حاضنة التمدد ICS-7 PDMS وإعادة بنائها لتناسب تجربة التمدد ثلاثية الأبعاد. أخيرًا ، تم تطوير طريقة شد الخلايا العصبية ثلاثية الأبعاد مع سقالة صناعية قابلة للمط (PVP co-GMA) وتحليلها باستخدام برامج ومعدات خاصة في المختبر