



Table of contents

List of Tables	iv
List of Figures	v
List of Abbreviations	viii
1 Introduction	1
1.1 Strategies for Drug Discovery	1
1.2 The Central Nervous System	3
1.2.1 Neurons and Synapses	3
1.2.2 Alzheimer's disease – an example of neurological disorders	7
1.3 Organ mimicking systems	9
1.3.1 <i>In vitro</i> Neuronal Networks	9
1.3.2 2D vs. 3D and Encapsulation	10
1.4 Extracellular Matrix Mimics	11
1.4.1 Hydrogels	12
1.4.2 Gelatin methacryloyl – a semi-synthetic polymer	13
2 Results and Discussion	15
2.1 Gelatin Methacryloyl Synthesis	15
2.2 Gelatin Methacryloyl Quality.....	21
2.2.1 Nuclear Magnetic Resonance Spectroscopy.....	21
2.2.2 TNBSA	26
2.3 Cultured Biological Neuronal Networks	32
2.3.1 3D Neuronal Tissue Engineering Optimization.....	32
2.4 Engineered 3D Neuronal Networks	38
2.4.1 Live-dead analysis	38



Table of Contents

2.4.2 Immunohistochemistry	44
2.4.2.1 Axon and dendrite length development over time	46
2.4.2.2 Synaptic vesicle quantification	50
2.4.2.3 The neuronal network	54
2.4.3 Calcium activity	61
2.4.3.1 Neuro-Pharmacological activation	62
2.4.3.2 Signal amplitude	69
2.4.3.3 Signal frequency	73
2.5 μ 3DVasc 2.0.....	78
2.5.1 Cultivation of co-cultures in the μ 3DVasc 2.0.....	82
3 Materials and Methods	87
3.1 Materials.....	87
3.1.1 Antibodies	87
3.1.1.1 Primary Antibodies.....	87
3.1.1.2 Secondary Antibodies	87
3.1.1 Cells	87
3.1.2 Materials.....	87
3.1.3 Equipment.....	89
3.1.4 Software.....	91
3.2 Methods.....	91
3.2.1 Gelatin methacryloyl synthesis.....	91
3.2.1.1 ¹ H-Nuclear magnetic resonance spectroscopy	92
3.2.1.2 TNBSA.....	93
3.2.1.3 TNBSA analysis	94



Table of Contents

3.2.2 Neuronal hydrogel formation	94
3.2.2.1 Neuronal extraction.....	94
3.2.2.2 Preparing GelMA pre-solution.....	95
3.2.2.3 Setup of aperture	95
3.2.2.4 Photocrosslinking.....	96
3.2.2.5 Primary Cell culture.....	97
3.2.3 Mature neuronal networks.....	98
3.2.3.1 Viability of neurons in 3D	98
3.2.3.2 Immunocytochemistry	99
3.2.3.3 Image analysis	99
3.2.3.4 Statistical analysis.....	100
3.2.3.5 Calcium activity	101
3.2.3.6 Calcium activity quantification	101
3.2.4 μ 3DVasc 2.0.....	102
3.2.4.1 Fabrication	102
3.2.4.2 Handling of the μ 3DVasc 2.0 chip.....	104
3.2.4.3 Coating of the microchannels.....	104
3.2.4.4 Cultivation of an endothelial layer	104
4 Appendix.....	107
4.1 ^1H -NMR spectra	107
4.2 Length of neurites and number of synapses.....	112
4.3 Frequency and Amplitude of action potentials.....	113
5 References.....	115