

List of contents

<b>Zusammenfassung</b> .....	1
<b>Abstract</b> .....	2
<b>1. Introduction</b> .....	3
<b>1.1. Hepatic Cell Functions in Homeostasis</b> .....	3
<b>1.1.1. Hepatocytes</b> .....	3
<b>1.1.2. Hepatic Stellate Cells</b> .....	5
<b>1.1.3. Liver Sinusoidal Endothelial Cells</b> .....	5
<b>1.1.4. Hepatic Macrophage Populations</b> .....	6
<b>1.2. Hepatocellular Functions in Liver Injury</b> .....	8
<b>1.2.1. Acetaminophen Hepatotoxicity</b> .....	8
<b>1.2.2. Non-Alcoholic Fatty Liver Disease</b> .....	10
<b>1.3. Modelling of Liver Injury In Vitro</b> .....	11
<b>1.4. Current Technologies for Culturing Hepatic Cells</b> .....	11
<b>1.5. Current Technologies for Modelling Liver Injury In Vitro</b> .....	13
<b>1.6. Aim of the Study</b> .....	15
<b>2. Material and Methods</b> .....	16
<b>2.1. Material</b> .....	16
<b>2.2. Mouse Strains</b> .....	25
<b>2.3. MOTiF Biochips</b> .....	25
<b>2.4. Handling of MOTiF Biochips</b> .....	26
<b>2.5. Biochip Sterilization and Coating with Collagen</b> .....	26
<b>2.6. PET Membrane Sterilization and Coating with Collagen</b> .....	26
<b>2.7. Preparation of Cell Isolation Solutions</b> .....	27
<b>2.8. Isolation of Primary Murine Liver Cells</b> .....	27
<b>2.9. Isolation of Bone Marrow-Derived Monocytes</b> .....	30
<b>2.10. Seeding of Primary Hepatic Cells</b> .....	32

2.10.1. Seeding of Primary Hepatic Cells into Biochips .....	32
2.10.2. Seeding of Primary Hepatic Cells onto Isolated PET Membranes.....	32
2.11. Connection of Biochips to a Microfluidic System.....	33
2.12. Extraction of Cell Culture Supernatants from Biochips .....	34
2.13. Calcein and PI Staining .....	35
2.14. Immunofluorescence Staining of Cultured Cells .....	35
2.15. Acetaminophen Intoxication .....	36
2.16. N-Acetylcysteine Treatment .....	36
2.17. Stimulation of Hepatocytes with Free Fatty Acids .....	36
2.18. Lanifibranor Treatment .....	37
2.19. AdipoRed Assay .....	37
2.20. Measurement of Serum Transaminase Levels and Lactate Dehydrogenase Activity in Cell Culture Supernatants .....	37
2.21. Phagocytotic Assay .....	38
2.22. RNA Isolation from Isolated Murine Cells .....	38
2.23. cDNA Synthesis from Isolated RNA .....	39
2.24. Real-Time Quantitative PCR .....	39
2.25. RNA Sequencing of Freshly Isolated Cells .....	40
2.26. Mass Spectrometry Protein Analysis from Biochip Material .....	41
2.27. Albumin ELISA.....	41
2.28. Statistical Analysis .....	43
3. Results.....	44
3.1. Isolated Primary Murine Hepatic Cells Grow on Collagen- Coated PET Membranes .....	44
3.2. Introduction of Primary Murine Hepatic Cells into the Liver Biochip System.....	46
3.3. Primary Murine Liver Cells Express Key Markers of Functionality in Biochip Co-Culture .....	49
3.4. Development of In Vitro Liver Damage Models for Acute	

	<b>Acetaminophen-Induced Liver Damage and Liver Steatosis .....</b>	<b>54</b>
<b>3.5.</b>	<b>Phenotypical Characterization of Freshly Isolated Murine KCs and Bone Marrow Monocytes .....</b>	<b>58</b>
<b>3.6.</b>	<b>The Influence of KCs and Monocytes on Liver Damage In Vitro .....</b>	<b>62</b>
<b>4.</b>	<b>Discussion .....</b>	<b>69</b>
<b>4.1.</b>	<b>Choosing the MOTiF Biochip as a Cell Culture Model for Investigating Liver Diseases In Vitro .....</b>	<b>69</b>
<b>4.2.</b>	<b>Investigation of Basic Cell Culture Conditions for the Co-Culture of Primary Hepatic Cells on Biochip Membranes .....</b>	<b>71</b>
<b>4.3.</b>	<b>Morphology and Flow Tolerance of Primary Hepatic Cells in Biochip Culture .....</b>	<b>72</b>
<b>4.4.</b>	<b>Metabolic Activities and Function of Primary Hepatic Cells in Biochip Culture .....</b>	<b>74</b>
<b>4.5.</b>	<b>The Capacity of Mimicking Prototypic Liver Injuries by Biochip-Cultured Primary Hepatic Cells .....</b>	<b>78</b>
<b>4.6.</b>	<b>The Contribution of KCs and Monocytes to Liver Disease Progression In Vitro .....</b>	<b>81</b>
<b>4.7.</b>	<b>Summary and Conclusion .....</b>	<b>86</b>
<b>4.8.</b>	<b>Outlook .....</b>	<b>89</b>
<b>5.</b>	<b>Literature .....</b>	<b>90</b>
<b>6.</b>	<b>Publications .....</b>	<b>98</b>
<b>7.</b>	<b>Eidesstattliche Erklärung .....</b>	<b>100</b>
<b>8.</b>	<b>Acknowledgement .....</b>	<b>103</b>
<b>9.</b>	<b>Curriculum Vitae .....</b>	<b>104</b>