

# Contents

<b>1 Introduction .....</b>	<b>1</b>
1.1 Stem cells .....	1
1.2 Yamanaka factors .....	2
1.3 Generation methods and starting material of human iPSCs .....	3
1.4 Characterization of human iPSCs.....	4
1.5 Culture medium and cultivation of human iPSCs .....	5
1.6 Induced pluripotent stem cell banking .....	6
1.7 Differentiation into mature cell types and organ models .....	6
1.7.1 Hepatocytes and liver models .....	8
1.7.2 Intestine model.....	10
1.7.3 Kidney model .....	11
1.7.4 Cardiomyocytes .....	12
1.7.5 Blood vessels.....	13
1.7.6 Mesenchymal stromal cells .....	14
1.8 The drug testing dilemma.....	15
1.9 Microphysiological systems .....	16
1.9.1 Single-organ systems.....	17
1.9.2 Multi-organ systems .....	17
1.10 Microphysiological systems in combination with stem cells .....	19
1.11 Aim of this study and objectives .....	20
<b>2 Material and Methods .....</b>	<b>21</b>
2.1 Adult fibroblasts isolation from hair biopsies.....	21
2.2 Isolation of peripheral blood mononuclear cells .....	22
2.3 Cultivation of iPSCs.....	23
2.3.1 Maintenance of iPSCs.....	23
2.3.2 Matrigel coating of cell culture plates.....	24
2.3.3 Passaging of iPSCs.....	24
2.3.4 Thawing of iPSCs.....	25
2.3.5 Freezing of iPSCs .....	26
2.3.6 Banking of iPSCs .....	26
2.4 Differentiation of iPSCs .....	26
2.4.1 Differentiation into definitive endoderm.....	27
2.4.2 Differentiation into hepatocytes and liver spheroids formation .....	27
2.4.3 Differentiation into intestinal organoids and cell culture insert seeding .....	28
2.4.4 Differentiation into mesenchymal stromal cells .....	31

2.4.5	Differentiation into renal organoids .....	31
2.4.6	Differentiation into cardiomyocytes in a 2D monolayer .....	32
2.4.7	Differentiation into cardiomyocytes in spheroids in ULA plates .....	32
2.4.8	Differentiation into cardiomyocytes in 3D spheroids in Erlenmeyer flask ...	33
2.4.9	Differentiation into endothelial cells .....	34
2.4.10	Seeding of the 2-Organ-Chip with iPSC-derived endothelial cells.....	34
2.5	Chip-based co-cultures .....	35
2.6	Analyses .....	36
2.6.1	RNA isolation and qPCR .....	36
2.6.2	Immunohistochemistry .....	38
2.6.3	F-actin staining.....	40
2.7	Live cell staining .....	40
2.7.1	Calcein AM staining in the chip circulation.....	40
2.7.2	Acetylated low-density lipoprotein staining .....	40
2.8	Tube-forming assay of iPSC-derived endothelial cells.....	40
2.9	RNA sequencing .....	41
2.10	Flow cytometry .....	41
2.11	Microscopy .....	43
2.12	List of materials .....	43
<b>3</b>	<b>Results .....</b>	<b>46</b>
3.1	iPSC characterization.....	46
3.2	Definitive endoderm differentiation .....	50
3.3	Hepatocyte differentiation in monolayer.....	52
3.3.1	3D liver model development .....	55
3.4	Intestinal organoid differentiation.....	63
3.4.1	Intestinal barrier model development.....	70
3.4.2	Cavity seeding with single cells of intestinal organoids .....	74
3.5	Mesenchymal stromal cell differentiation .....	77
3.6	Renal organoid differentiation.....	79
3.7	Cardiomyocyte differentiation .....	82
3.8	Endothelial cell differentiation.....	86
3.9	ADME-MOC co-culture experiment .....	88
<b>4</b>	<b>Discussion and Outlook .....</b>	<b>97</b>
4.1	iPSC characterization and banking.....	97
4.2	Definitive endoderm differentiation .....	97
4.3	Liver model development .....	98
4.4	Intestinal model development.....	101

4.5	Mesenchymal stromal cell differentiation .....	104
4.6	Kidney model development .....	105
4.7	Cardiomyocyte differentiation .....	108
4.8	Endothelial cell differentiation .....	109
4.9	Co-culture of four iPSC-derived models in the ADME-MOC .....	110
<b>5</b>	<b>Perspective .....</b>	<b>115</b>
5.1	The Human-on-a-Chip.....	115
5.2	Transformation into Patient-on-a-Chip readout systems.....	116
5.3	Upscaling of tissue generation .....	116
	<b>Zusammenfassung .....</b>	<b>I</b>
	<b>List of Abbreviations .....</b>	<b>III</b>
	<b>List of Figures .....</b>	<b>VII</b>
	<b>List of Tables.....</b>	<b>IX</b>
	<b>References.....</b>	<b>X</b>
	<b>Contribution to Publications.....</b>	<b>XX</b>
	<b>Acknowledgment .....</b>	<b>XXI</b>
	<b>Eidesstattliche Erklärung .....</b>	<b>XXIII</b>