

TABLE OF CONTENTS

ABSTRACT	1
ZUSAMMENFASSUNG	3
TABLE OF CONTENTS.....	5
1 INTRODUCTION	7
Bones - the supportive part of the musculoskeletal system.....	7
1.1 Bone structure.....	7
1.1.1 Mesenchymal stromal cells and osteoblastogenesis.....	8
1.1.2 Extracellular bone matrix – calcium store, source of flexibility, but also mechanical stiffness	10
1.2 Bone physiology and function	10
1.2.1 Processes during bone formation	10
1.2.2 Adaption and renewal of bone tissue	11
1.2.3 Mechanical stimulation as regulator of bone formation and remodeling	12
1.2.4 Oxygen tension in bone – influence in cell differentiation and maintenance	13
1.2.5 Energy metabolism of bone-forming cells.....	14
1.3 Experimental evaluation of bone physiology and function.....	15
1.3.1 <i>In vivo</i> models in the context of bone physiological research	16
1.3.2 <i>In vitro</i> models in the context of bone physiological research	17
2 OBJECTIVES OF THIS WORK	26
3 RESULTS.....	27
3.1 Study design and structure.....	27
3.2 Part I – characterization of primary human osteoblasts	28
3.2.1 Donors can be classified according to their mitochondrial activity and proliferation capacity.....	28
3.2.2 The surface marker profile of hOBs exhibit inter- and intra-donor variability.....	28
3.2.3 Donors can be grouped into low, medium and high/fast osteogenic responders	31
3.3 Part II – establishment of various methods to generate bone-like organoids.....	33
3.3.1 Carrier-free organoids can be fabricated by fusion or self-assembly	33
3.3.2 Carrier-based organoids can be established from commercially available biomaterials	41
3.4 Part III – investigation of the different organoids in the CHIP	42
3.4.1 Carrier-free organoids represent a heterogeneous population	42
3.4.2 Diverse carrier materials affect hOB differentiation and phenotype distinctly.....	48
4 DISCUSSION.....	63
4.1 Primary cells - a reliable basis for <i>in vitro</i> model systems.....	63
4.1.1 What affects data interpretation - inter-donor heterogeneity.....	63
4.1.2 ... and intra-donor heterogeneity	64
4.1.3 (Dis) Advantages of primary cells.....	65
4.2 3D models as suitable <i>in vitro</i> systems to represent human physiology	65
4.2.1 Carrier-free constructs resemble hOB condensation during initial stages of bone formation	66
4.2.2 Carrier-based constructs – ECM as stimulus for cell phenotype retention or differentiation	68
4.2.3 OoC-systems - Does an increase in environmental complexity lead to better results?.....	71
4.3 Glucocorticoids as stimulators of <i>in vitro</i> osteogenesis.....	77
4.3.1 Dexamethasone impairs cell metabolism	78
4.3.2 ...and cell commitment to an osteocytic phenotype.....	78
5 CONCLUSION AND OUTLOOK.....	80
6 METHODS AND MATERIALS.....	82
6.1 Cell culture.....	82

6.1.1	Ethical approval	82
6.1.2	Isolation of primary human osteoblasts.....	82
6.1.3	Passaging and cryopreservation.....	83
6.1.4	Cell thawing and maintenance	84
6.1.5	Characterization of primary human osteoblasts.....	84
6.2	Generation of carrier-free organoids.....	85
6.2.1	Fusion culture.....	85
6.2.2	Micro mass culture.....	86
6.3	Preparation of carrier-based organoids.....	86
6.3.1	Fabrication of hydrogel-based organoids.....	86
6.3.2	Fabrication of scaffold-based organoids.....	86
6.4	Cultivation of hydrogel-based organoids as static cultures.....	86
6.5	Culture in the CHIP.....	87
6.5.1	Microfluidic “on-Chip” technology	87
6.5.2	Cultivation of organoids using the CHIP.....	90
6.6	Sample processing after static culture or in the CHIP	90
6.6.1	Harvesting organoids from micro mass cultures.....	90
6.6.2	Harvesting hydrogel-based organoids.....	91
6.6.3	Harvesting scaffold-based organoids	92
6.6.4	Medium samples	92
6.6.5	Evaluation of cell viability and metabolic activity	92
6.6.6	Quantification of biomolecules.....	93
6.6.7	Evaluation of parameters involved in osteogenic differentiation	95
6.6.8	Histological analysis.....	97
6.6.9	Fluorescence based methods	98
6.6.10	Microscopy.....	100
6.6.11	Statistical analysis	102
REFERENCES		104
SUPPLEMENT		I
LIST OF ABBREVIATIONS.....		VIII
PUBLICATIONS		IX
EIDESSTÄTTLICHE ERKLÄRUNG		X
DANKSAGUNG.....		XI