



Universiteit  
Leiden  
The Netherlands

## Force sensing and transmission in human induced pluripotent stem-cell-derived pericytes

Iendaltseva, O.

### Citation

Iendaltseva, O. (2022, November 15). *Force sensing and transmission in human induced pluripotent stem-cell-derived pericytes*. *Casimir PhD Series*. Retrieved from <https://hdl.handle.net/1887/3485923>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3485923>

**Note:** To cite this publication please use the final published version (if applicable).

# Force sensing and transmission in human induced pluripotent stem-cell-derived pericytes

PROEFSCHRIFT

ter verkrijging van  
de graad van doctor aan de Universiteit Leiden,  
op gezag van rector magnificus prof.dr.ir. H.Bijl,  
volgens besluit van het college voor promoties  
te verdedigen op dinsdag 15 november 2022  
klokke 16.15 uur

door

Olga Iendaltseva

geboren te Kharkiv, Oekraïne  
in 1990

Promotores: Prof. dr. T. Schmidt  
Prof. dr. E.H.J. Danen

Promotiecommissie: Prof. dr. E. M. Hol (University Medical Center Utrecht)  
Dr. V. V. Orlova (Leiden University Medical Center)  
Prof. dr. E. R. Eliel  
Prof. dr. B. van de Water  
Prof. dr. J. Aarts

©2022 Olga Iendaltseva. All rights reserved.

Cover: Kindly offered artwork of Michael Antonov referring to figure 2.3c of this thesis.

Casimir PhD Series, Delft-Leiden, 2022-29

ISBN 978-90-8593-539-1

An electronic version of this thesis can be found at

<https://openaccess.leidenuniv.nl>

Het onderzoek beschreven in dit proefschrift is onderdeel van het wetenschappelijke programma van de Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO).

For Iryna, Alexandr, Pavel, Michael and Leo



---

# CONTENTS

---

<b>1</b>	<b>Introduction</b>	<b>1</b>
1.1	Pericytes and their functions . . . . .	2
1.1.1	Morphology of pericytes and location in blood vessels . . . . .	2
1.1.2	Identification of pericytes . . . . .	2
1.1.3	Pericyte abundance and functions . . . . .	3
1.2	Pericytes and mechanobiology . . . . .	4
1.3	Pericytes and pathology . . . . .	7
1.4	Outline of this thesis . . . . .	8
<b>2</b>	<b>Towards capillary basement membrane <i>in vitro</i> modeling</b>	<b>15</b>
2.1	Introduction . . . . .	16
2.2	Methods . . . . .	20
2.2.1	Generation of PDMS flat substrates . . . . .	20
2.2.2	Patterning of PDMS flat substrates . . . . .	20
2.2.3	Generation of hPAA hydrogel . . . . .	22
2.2.4	Generation of PDMS gel . . . . .	22
2.2.5	hPAA hydrogel and PDMS gel patterning . . . . .	22
2.2.6	hPAA hydrogel and PDMS gel imaging . . . . .	23
2.3	Results . . . . .	24
2.3.1	PDMS surface micropatterning . . . . .	24
2.3.2	PDMS gels micropatterning . . . . .	25
2.3.3	hPAA gels micropatterning . . . . .	27
2.4	Discussion . . . . .	29
2.5	Appendix . . . . .	31
<b>3</b>	<b>FN patches as anchoring points for force sensing and transmission in hiPSC-derived PCs</b>	<b>39</b>
3.1	Introduction . . . . .	41
3.2	Methods . . . . .	42

## CONTENTS

---

3.2.1	Cell culture . . . . .	42
3.2.2	Immunostaining . . . . .	43
3.2.3	PDMS surface patterning with FN and LM . . . . .	43
3.2.4	PDMS micropillar array preparation . . . . .	44
3.2.5	Hydroxy-PAAm gel preparation . . . . .	44
3.2.6	Microscopy . . . . .	45
3.2.7	Image analysis . . . . .	45
3.2.8	Statistical analysis . . . . .	46
3.3	Results . . . . .	46
3.3.1	Preferred binding of PCs to FN patches on multilayered substrates. . . . .	46
3.3.2	Highest PC spreading is accompanied by lowest force application on FN substrates of intermediate stiffness. . . . .	49
3.3.3	A switch in PC cytoskeletal organization on stiff substrates. . . . .	52
3.3.4	Suppression of PC spreading on 2D patterned FN substrates of high and low stiffness . . . . .	54
3.4	Discussion . . . . .	59
3.5	Appendix . . . . .	63
<b>4</b>	<b>Insights into the regulation of <math>\alpha</math>-SMA expression in pericytes</b>	<b>75</b>
4.1	Introduction . . . . .	77
4.2	Methods . . . . .	79
4.2.1	Cell culture . . . . .	79
4.2.2	Immunostaining . . . . .	79
4.2.3	Microscopy . . . . .	79
4.2.4	Image analysis . . . . .	80
4.2.5	Statistical analysis . . . . .	82
4.3	Results . . . . .	82
4.3.1	$\alpha$ -SMA expression and recruitment to stress fibers quantified by orientation analysis. . . . .	82
4.3.2	Changes in $\alpha$ -SMA recruitment to stress fibers of PCs driven by growth factors. . . . .	85
4.3.3	Inhibition of the time dependent increase in the $\alpha$ -SMA expression and recruitment to stress fibers in PCs on patterned FN substrates. . . . .	89
4.4	Discussion . . . . .	96

<b>5</b>	<b>Pericyte forces in <i>in vitro</i> Hypoxia and Ischemia conditions</b>	<b>103</b>
5.1	Introduction . . . . .	105
5.2	Methods . . . . .	107
5.2.1	Cell culture . . . . .	107
5.2.2	PDMS micropillar array preparation . . . . .	108
5.2.3	Immunostaining . . . . .	108
5.2.4	Microscopy . . . . .	109
5.2.5	Image analysis . . . . .	109
5.2.6	Preparation of flow channels with encapsulated PDMS micropillar arrays . . . . .	109
5.2.7	Seeding cells on top of PDMS micropillar arrays inside flow channels . . . . .	110
5.3	Results . . . . .	111
5.3.1	Pericyte force generation and spreading in starvation, Hypoxia and Ischemia conditions . . . . .	111
5.3.2	Micropillar array technology does not allow fast liquid exchange in the area with cells . . . . .	113
5.3.3	Design of a microfluidic channel to track cellular response in rapidly changing conditions . . . . .	114
5.3.4	Channel preparation . . . . .	115
5.3.5	Fluid flow in a microchannel with pillars . . . . .	119
5.4	Discussion . . . . .	122
5.5	Apendix . . . . .	126
<b>General Summary and Discussion</b>		<b>131</b>
<b>Samenvatting</b>		<b>138</b>
<b>Publications</b>		<b>143</b>
<b>Curriculum Vitae</b>		<b>144</b>



**CONTENTS**

---