

3D BLOOD VESSELS-ON-A-CHIP FOR ATHEROSCLEROSIS MODELLING

Heleen H.T. Middelkamp^{1,2}, Albert van den Berg^{1,2} and Andries D. van der meer³

¹*BIOS lab on a chip group, University of Twente, Enschede, the Netherlands*

²*Max Planck – University of Twente Center for Complex Fluid Dynamics, Enschede, the Netherlands*

³*Applied stem cell technologies group, University of Twente, Enschede, the Netherlands*

ABSTRACT

More than 25% of all deaths worldwide have a direct vascular cause, with coronary artery disease and stroke being two leading causes of death. These two diseases are the direct result of the acute rupture of atherosclerotic plaques in the arterial wall that contain dysfunctional tissue, fats and extracellular matrix. The disease processes in early atherosclerosis are hard to study due to the lack of realistic *in vitro* models. In this paper we present a 3D blood vessel-on-a-chip model for early atherosclerosis, which includes vascular tissue, cholesterol and immune cells in a patterned extracellular matrix. For the first time, we demonstrate that human iPSC-derived vascular cells take up oxidized LDL ('bad cholesterol') in a 3D microfluidic vessel on chip.

KEYWORDS: blood vessel, organ on chip, atherosclerosis

INTRODUCTION

More than 25% of all deaths worldwide have a direct vascular cause, with coronary artery disease (CAD) and stroke (respectively 9.4 and 5.7 million deaths) being the two leading causes of death in 2016[1]. These vascular diseases are the result of atherosclerotic plaques in large arteries, which take many years to develop as a result of chronic inflammation, lipid deposition, oxidative stress, and apoptosis, proliferation and senescence in the vascular tissue, followed by acute arterial thrombosis and cardiac or cerebral ischemia.[2], [3]

In the European Union alone 906.200 animals were used for cardiovascular research in 2011[4]. An issue with animal models is their lack of human physiology, they cannot be used to study early onset of an atherosclerotic plaque and most importantly, there is no mouse model which can mimic patient-specific diseases and drug responses for future use in personalized medicine.

Atherosclerosis is a complicated process which starts with endothelial injury for example by exposure to irritating substances such as smoking, high blood pressure or diabetes. This activated endothelial layer then takes up and deposits Oxidative Low Density Lipoprotein (Ox-LDL). The innate immune cells, such as monocytes, are recruited to the inflammatory site and migrate through the endothelial layer, after which they differentiate into macrophages and ingest the Ox-LDL [5], [6]. The end stage of the process is when the accumulation of these lipid-laden macrophages turn into foam cells, which is the key pathological feature of an atherosclerotic plaque[6]. The exact order and mechanism of the early onset of an atherosclerotic plaque is hard to determine *in vivo* and currently no *in vitro* models available which contain all these different components in a physiologically relevant co-culture system.

We therefore present our 3D blood vessel-on chip model, which can be used as a tool to study the early stages of atherosclerosis and drug development. Human induced pluripotent stem cell derived endothelial cells (iPSC-EC) were used to produce a 3D endothelial layer. The blood vessel can either be used to culture macrophages that are pre-loaded with lipids or, more realistically, to expose the cells to fluorescently labelled Ox-LDL *in situ*.

EXPERIMENTAL

Polydimethylsiloxane (PDMS) chips were prepared using a micro milled Poly(methyl methacrylate) (PMMA) mold. (figure 1A¹) 3D blood vessels were created in a 5mg/ml rat tail collagen hydrogel using viscous finger patterning [7], [8]. (figure 1A²⁻³). iPSC-ECs were seeded in the lumen at 5 million cells/ml to form a monolayer and kept under bidirectional flow on a rocking table. (figure 1B¹).

Endothelial cells (well plate and 3D blood vessel) were incubated overnight with fluorescently labelled Ox-LDL (figure 1B²,1C¹). After cells are lipid laden fluorescently labelled monocytes were added to observe lipid transfer (figure 1C²⁻³).

RESULTS AND DISCUSSION

Well plate and blood vessel experiments show the possibility of loading endothelial cells with Ox-LDL (figure 1B²,1C¹⁻²). Well plate experiments furthermore show the ability of endothelial cells transferring the LDL to

monocytes (figure 1C²⁻³). Next steps will include adding monocytes to LDL laden 3D blood vessels, to observe trans endothelial migration and LDL uptake of monocytes.

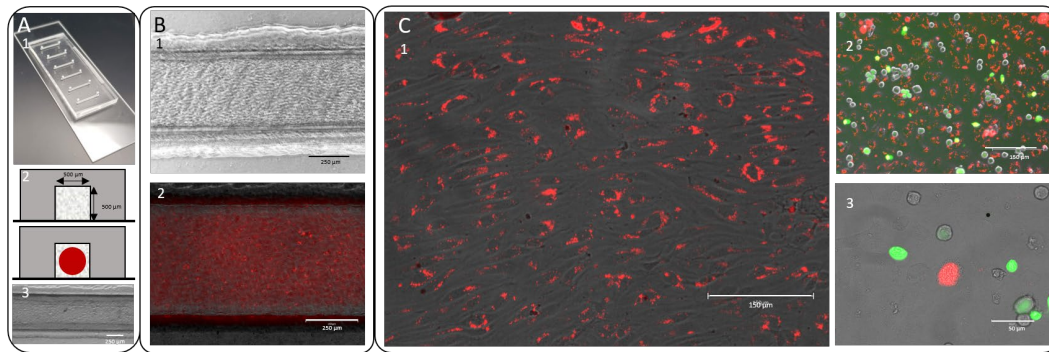


Figure 1: 3D blood vessel-on-chip for early atherosclerosis. a) The chip contains multiple microfluidic channels that are filled with collagen and then subjected to viscous finger patterning. b) hiPSC-derived endothelial cells are cultured in the lumen and form a 3D monolayer that can be loaded with lipids (red). c) Monocytes (green) can also be loaded in well plate by lipid (red) transfer from endothelial cells to monocytes.

CONCLUSION

A 3D blood vessel model to mimic early atherosclerosis was created. This is the first microfluidic iPSC-EC blood vessel model which shows the uptake of Ox-LDL. Next steps will be to include monocytes in the 3D blood vessel model before and after exposing the vessel to Ox-LDL. This model can then be used to help create a higher understanding of the mechanics of early atherosclerosis as well as personalized drug testing to prevent atherosclerotic plaque formation.

ACKNOWLEDGEMENTS

This work was supported by the Netherlands Organ-on-Chip Initiative, an NWO Gravitation project (024.003.001) funded by the Ministry of Education, Culture and Science of the government of the Netherlands. Kim Vermeul is acknowledged for differentiating the iPSC-EC needed for this project. Mees de Graaf is acknowledged for his help in first creating the viscous finger patterned blood vessels.

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CONTACT

* H.H.T. Middelkamp, +3153- 489 7893, h.h.t.middelkamp@utwente.nl