Serum-free generation and cultivation of human PSC-derived endothelial cells in a closed semi-automated cultivation platform.

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Kathrin Godthardt, Claudia Schreiner, Daria Sokoliuk and Sebastian Knöbel
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

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Introduction

The vascular system, coated by endothelial cells (ECs), provides a barrier to tissue and influences blood homeostasis. Among other processes endothelial cells are involved in neovascularization, which is essential for the growth and metastasis of tumors. In this context ECs transport nutrients and remove metabolic waste from tumor cells.
To understand and influence these interactions in more detail as well as to engineer vessels and organ grafts, large amounts of ECs are required. Pluripotent stem cell derived endothelial cells (PSC-ECs) can be produced in unlimited number without ethical concern, under standardized environment and thus provide an optimal source for studying the processes mentioned above.

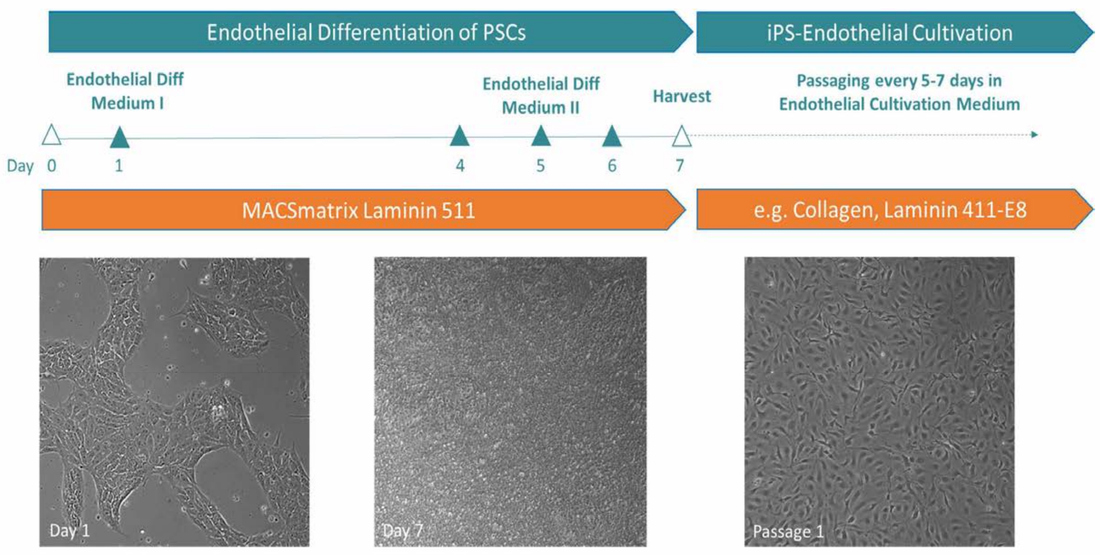
Even though several protocols for endothelial differentiation have been published, the majority of them have to be adjusted for each stem cell clone, e.g., by titration of small molecules and cytokines concentrations, in order to obtain the optimal differentiation efficiency and cell yield. Moreover, lot-to-lot variations of media components also influence the outcome of the differentiation. These protocol optimizations are costly and time consuming. In order to circumvent these limitations, we developed the StemMACS™ EndothelDiff Kit XF, enabling robust and standardized endothelial differentiation. Moreover, we have increased production scale by using a closed and semi-automated system.

Method

1 Endothelial differentiation of PSCs

Endothelial differentiation of PSCs was performed within seven days using subsequently two different serum and xeno-free cell culture media (fig 1). PSCs were seeded in a defined cell concentration as single cells on MACSmatrix Laminin 511 coated culture ware using StemMACS™ iPS-Brew XF. After 24h medium was changed to Endothelial Diff Medium I to support mesodermal induction. At day 4 endothelia differentiation was induced by changing to Endothelial Diff Medium II. At day 7 differentiated cells can be harvested via enzymatic detachment and further cultivated using Endothelial Cultivation Medium XF on collagen coated culture ware. This protocol allows for robust and efficient generation of PSC derived endothelial cells (PSC ECs) within 7 days. A transfer into a 3D based protocol was also successfully tested (data not shown).

Figure 1

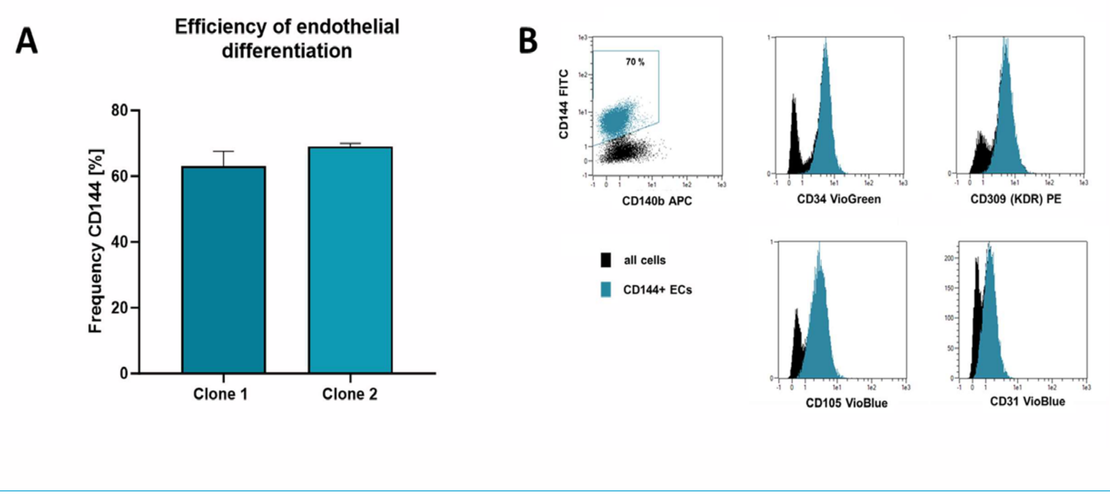


Results

1 Characterization of PSC ECs using flow cytometry

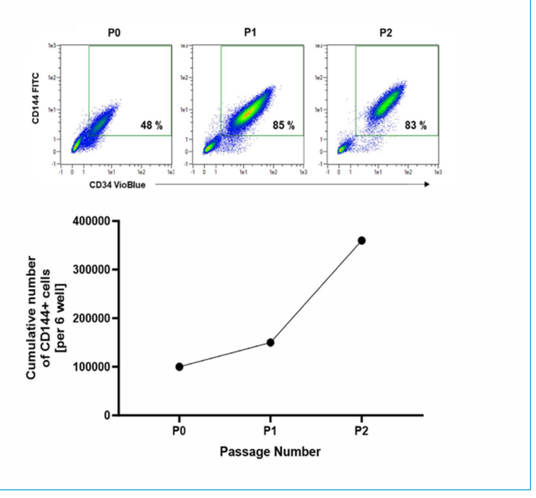
Two PSC clones were differentiated into endothelial cells using the StemMACS™ EndothelDiff Kit XF and yielded in efficiencies up to 70% endothelial cells expressing CD144 (fig 2A). CD144+ PSC derived endothelial cells were further characterized via flow cytometry for expression of CD31, CD34, CD105 and CD309 (KDR, VEGFR2) and lack of smooth muscle cells specific marker CD140b. (fig 2B).

Figure 2



2 Cultivation of PSC derived endothelial cells

Figure 3

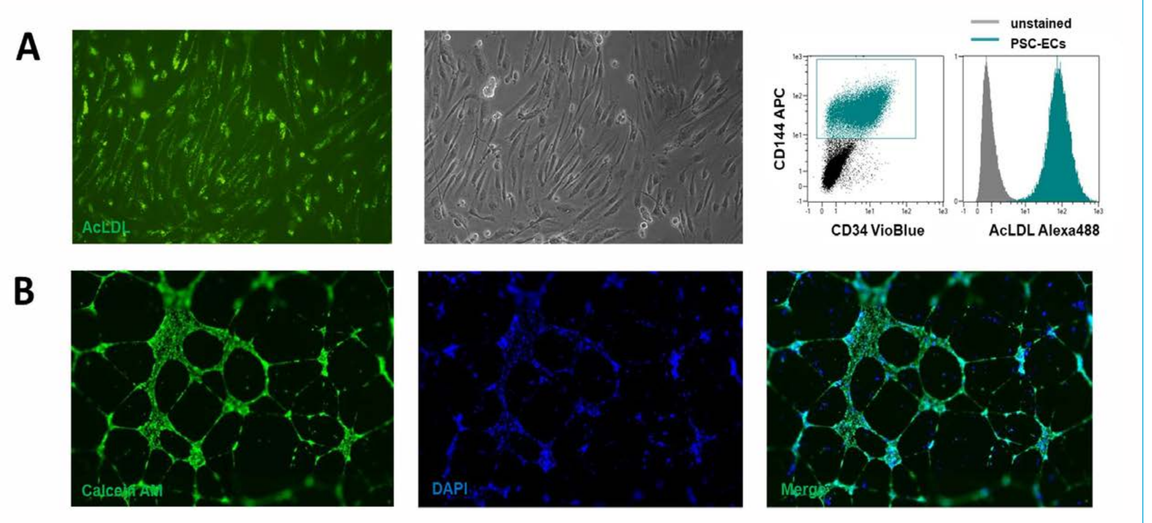


PSC derived endothelial cells were further cultivated for 3 passages using the Endothelial Cultivation Medium XF, which is part of the StemMACS™ EndothelDiff Kit XF. The starting frequency of 48% CD144/CD34 expressing cells was increasing to a purity of more then 80% with a further extension of CD144 and CD34 signal intensity being a hint for endothelial maturation (fig 3).

3 Functional evaluation: LDL uptake and tube formation

To check functionality of PSC derived endothelial cells, uptake of acetylated LDL was shown after seven days of differentiation and further expansion for five days (fig 4A). Tube formation capability was successfully analyzed by plating of Calcein AM dye (green) labeled PSC-ECs on Matrigel for 24h (fig 4B).

Figure 4

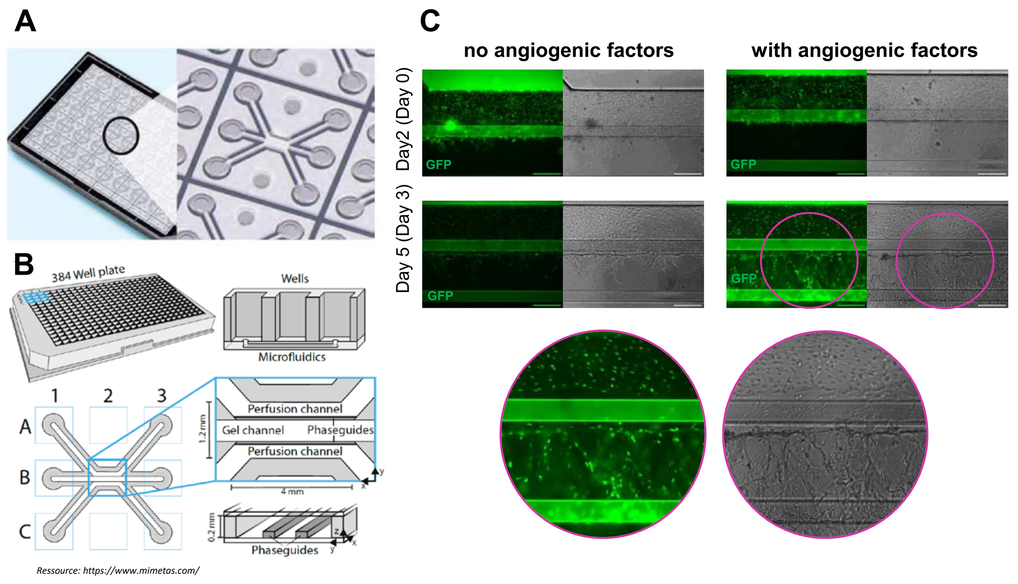


4 Functional evaluation: Angiogenic sprouting

Angiogenic sprouting was achieved by culturing PSC-ECs within the microfluidic OrganoPate® 3-lane from Mimetas (fig 5A). Each chip consists of three channels: one 'gel' channel for gel patterning, and two adjacent channels (fig 5B). The gel channel was filled with collagen-1. Previously differentiated PSC-ECs were grown in the top channel of each chip and formed an endothelial vessels under perfusion by placing the OrganoPlate on a rocker platform within two to three days. Addition of angiogenic factors in the bottom channel induces the directed formation of angiogenic sprouts within another three days (fig 5C). To visualize cells a GFP expressing PSC line was used for differentiation into endothelial cells.

Angiogenic factors: 50 ng/mL VEGF + 0.5 nM, Sphingosine-1-Phosphate (S1P) + 2 ng/mL phorbol 12-myristate 13-acetate (PMA).

Figure 5



5 Large scale differentiation of PSC derived endothelial cells using the closed and automated CliniMACS Prodigy system

The CliniMACS Prodigy® provides a range of ports for connecting bags containing buffers, media, reagents, and cellular material. Various tubing sets allow for a multitude of applications. For this cultivation process, we chose the tubing set CliniMACS Prodigy TS 730 (fig 6A). Bags can be connected in a sterile manner prior to the installation procedure or later via sterile welding. Previously expanded PSCs in single cell solution were provided in the application bag (Starting cell bag: PSC) of the tubing set and transferred automatically into the precoated (Laminin 521) CCU using the flexible CliniMACS Prodigy Adherent Cell Culture System. All liquid handling steps, i.e., inoculation, washing of cells, medium exchange, and cell harvest were performed closed and automated.

Figure 6 A

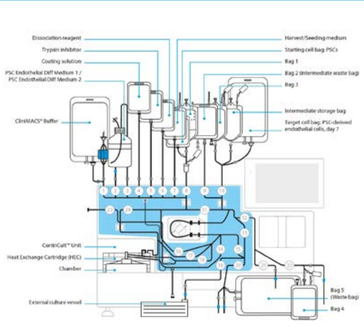
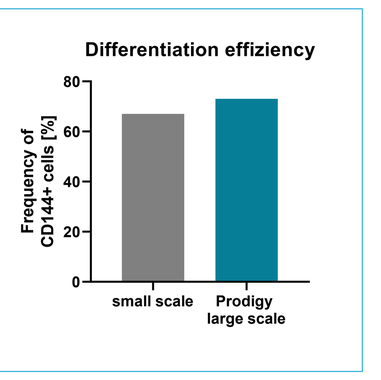


Figure 6 B



PSC endothelial differentiation medium was used to show closed and automated differentiation of PSCs within the CliniMACS Prodigy Adherent Cell Culture System (CCU, 100 cm^2) compared to manual small scale (12 well, 3.5 cm^2). In more detail PSCs were seeded into precoated CCU and the differentiation process was performed as mentioned in figure 1. After seven days of culture PSC ECs were harvested and a differentiation efficiency of 70% was achieved for Prodigy large scale via flow cytometry based CD144 expression. The small scale differentiation revealed comparable results (fig 6B).

Conclusion

- Efficient and robust differentiation and cultivation of PSCs into endothelial cells can be achieved by using the optimized StemMACS™ EndothelDiff Kit XF within 7 days.
- Besides the optimized 2D workflow, a transfer into a 3D based protocol is possible.
- PSC derived endothelial cells reveal standard vascular specific marker expression as well as functional properties.
- The PSC endothelial differentiation and cultivation media formulation used in this process is xeno-free.
- Closed and automated differentiation within the CliniMACS Prodigy Adherent Cell Culture System and manual scale using standard tissue culture vessels led to comparable results.

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