IFSCC 2025 full paper (IFSCC2025-1652)

Development of an innervated skin-on-a-chip to study neurovascular interactions

Lucile Guerber* [1], Laurie Verzeaux[1], Amandine Rovini[2], Elina Casas[3], Robin Siadous[3], Margot Bellenguez[4], Rawan El Amine[4], Jacques Leng[4], François Berthod[5], Joelle Amédée[3], Alexis Desmoulière[2], Elodie Aymard[1], Brigitte Closs[1]

[1]SILAB, Brive, [2]UR 20218 Limoges university, NEurIT, Limoges, [3]Inserm U1026 Université de Bordeaux, BIOTIS, [4]CNRS UMR 5258, Université de Bordeaux, LOF, Bordeaux, France, [5]CHU de Quebec-Laval University research center, LOEX, Quebec, Canada

1. Introduction

Being the largest human sensory organ, skin innervation mainly consists of different types of afferent nerve fibres which spread out beneath the skin surface to sense temperature, touch and pain [1]. It is now well demonstrated that in skin, the success and efficiency of wound healing is dependent on the skin nerve fiber endings and on neovascularization. Close interaction between nerves endings and blood vessels plays an important role under physiopathological conditions [2]. At the nerves level, Schwann cells are also known as key players in human skin homeostasis, secreting multiple crucial bioactive molecules. Yet, tissue bioengineering holds great promise to provide insights into cellular and molecular mechanisms. The generation of a skin model including dermis, a properly differentiated epidermis as well as functional innervation and vasculature still represents a major challenge.

In this context, the aim of this study is to provide a functional innervation with sensory neurons and Schwann cells physically separated from full thickness skin therefore enabling the independant stimulation of each actor. This model will be used for a better understanding of the neurovascular interactions in human skin.

2. Materials and Methods

In order to develop this innervated skin-on-a-chip, the various cell types have to be differentiated and cultured.
First, sensory neurons (iSN) were derived from human induced pluripotent stem cells (hiPSC) according to Guimaraes *et al* [3] (Figure 1a). Results presented in figure 1b to e reveal that generated iSN display the morphological markers of mature sensory neurons. Moreover, they are functional as shown by the substance P release and increased basal firing activity overtime.

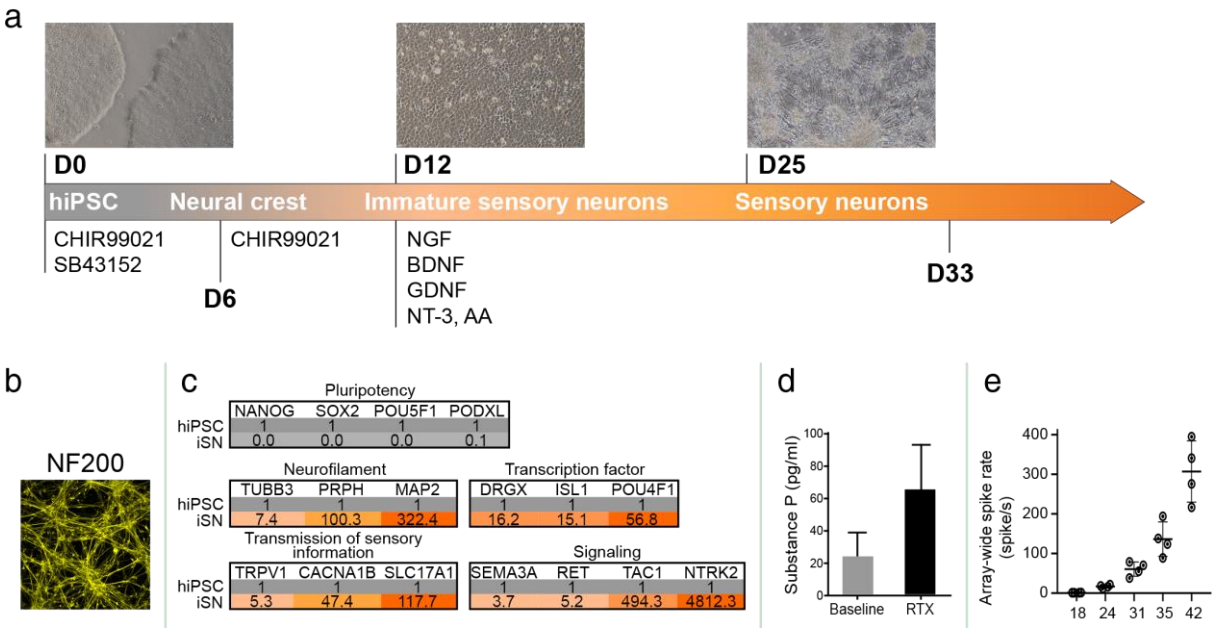

Figure 1. Phenotypical and functional characterization of sensory neurons.
(a) Schematic representation of hiPSC differentiation into iSN; (b) Morphological immunostaining of iSN after 35 days of culture (NF200 in yellow); (c) mRNA fold change in iSN after 28 days of culture compared to hiPSC; (d) Substance P release measurement in the supernatant of D18 iSN after 45min incubation with 300nM resiniferatoxin (RTX); (e) Basal electrical activity of the iSN measured during the differentiation process.

Schwann cells (iSC) were also derived from hiPSC according to Powell and Phillips [4] (Figure 2a). Results presented in figure 2b and c reveal that generated iSC display typical markers of mature Schwann cells.

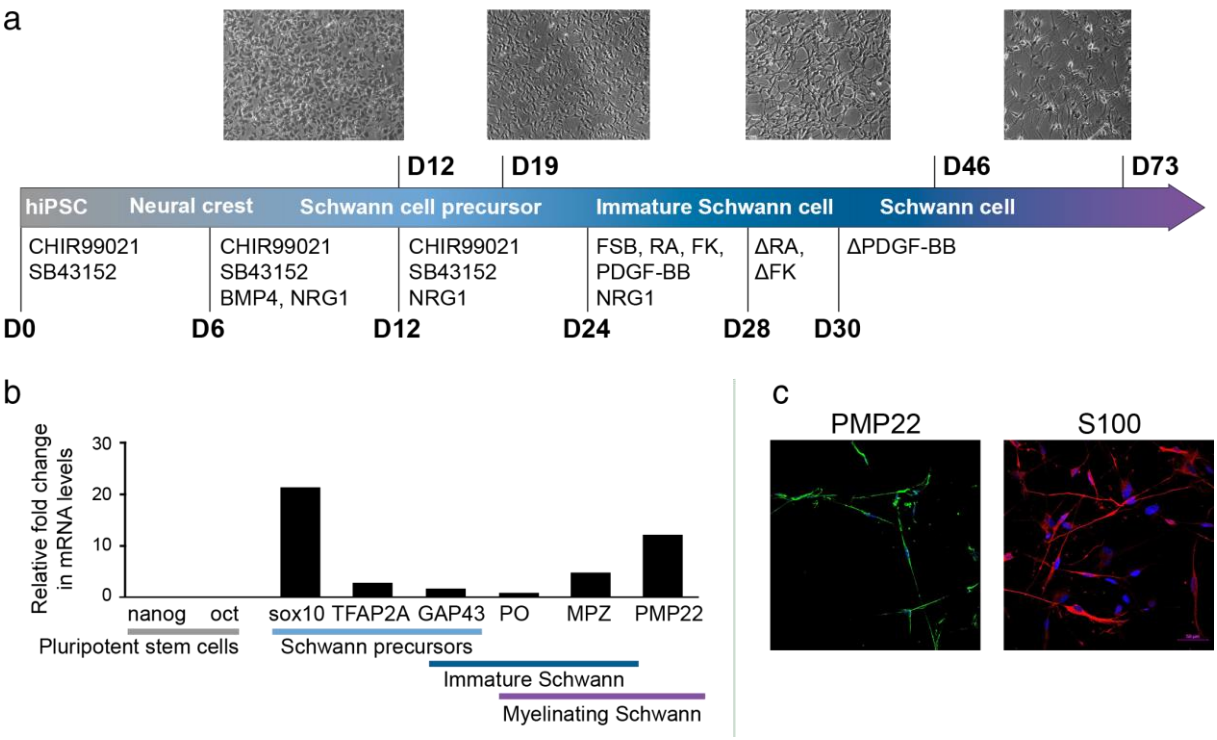

Figure 2. Phenotypical and functional characterization of Schwann cells. (a) Schematic representation of hiPSC differentiation into iSC; (b) Relative fold change in iSC marker mRNA levels in iSC after 21 days; (c) Morphological markers immunostaining in iSC after 66 days of culture (PMP22 in green, S100 in red and DAPI in blue).

Human dermal fibroblasts (HDF) and endothelial cells (HDMEC) were isolated from human tissues (abdominal and mammary plasties, facelift) (Figure 3a). Then, HDF and HDMEC were co-cultured. Results presented in figure 3b and c reveal that the co-culture conditions allow the obtention of a complex an mature pseudo-vascular network.

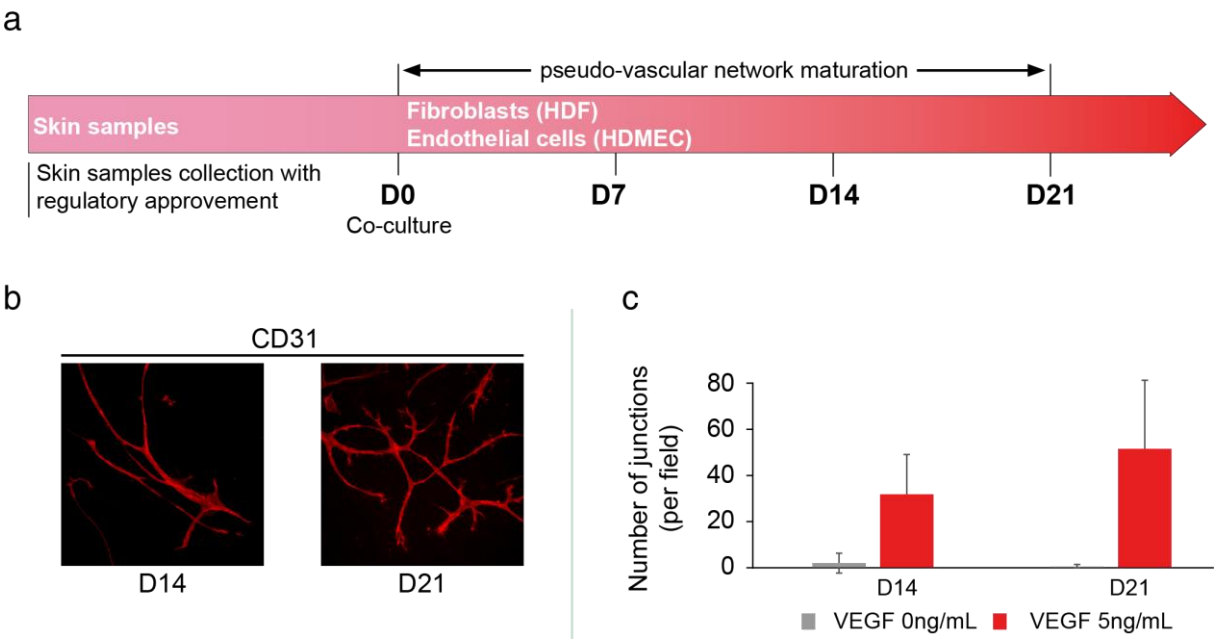

Figure 3. Co-culture of human dermal fibroblasts and endothelial cells. (a) Schematic representation of HDF and HDMEC culture; (b) Immunostaining of HDMEC and HDF after 14 and 21 days of culture in co-culture medium supplemented with 5ng/mL VEGF (CD31 in red); (c) Quantification of the number of vascular junctions after 14 and 21 days of culture with or without addition of VEGF at 5ng/mL.

3. Results

1. Study of iSN conditioned medium on HDMEC/HDF co-culture

In order to determine the impact of iSN on the pseudo vascular network, the iSN conditioned media was added on the HDMEC/HDF co-culture.

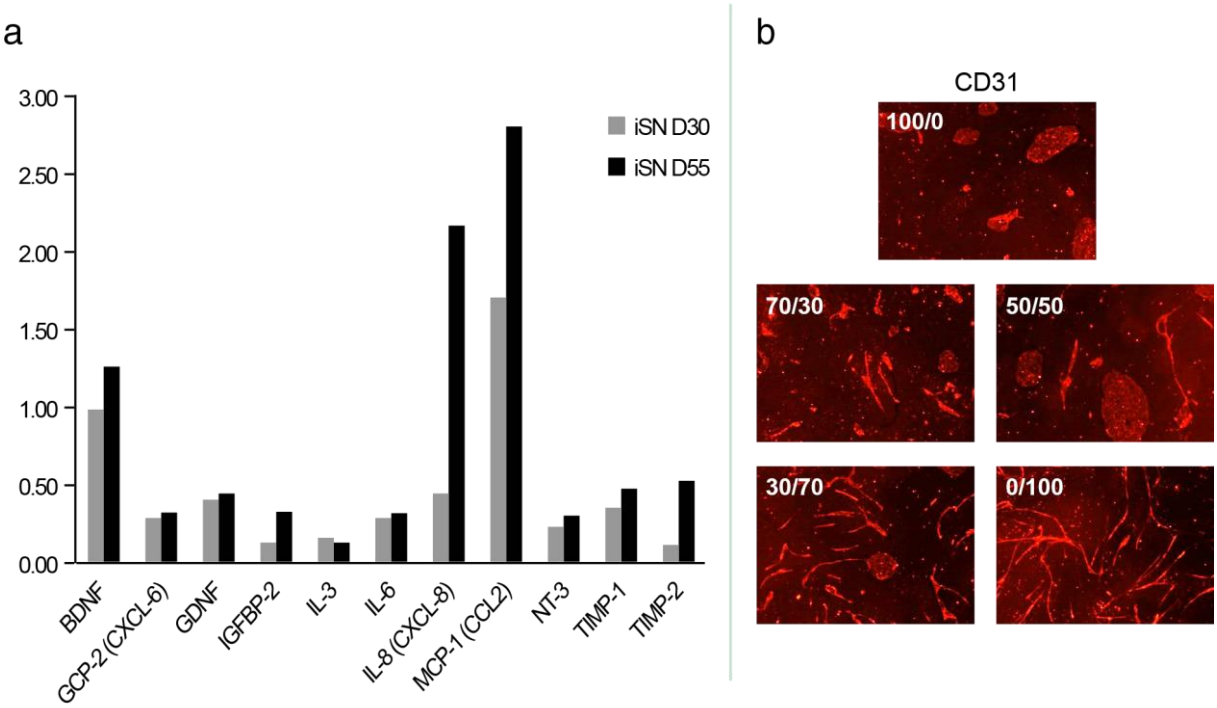

Figure 4. Study of the impact of iSN secretome on the pseudo-vascular network. (a) Angiogenesis cytokine array conducted on mature iSN supernatant after 30 and 55 days of culture; (b) Immunostaining of HDMEC/HDF cultured in co-culture medium / mature iSN conditioned medium at various ratio (CD31 in red).

Results presented in figure 4a and b reveal that mature sensory neurons secrete pro-angiogenic factors enabling the formation of a pseudo-vascular network.

2. Development of a microfluidic device

This microfluidic device was developed by incubating HDMEC and HDF co-culture at the middle and iSN and iSC in lateral compartments (Figure 5a). Then, the cutaneous neurovascular interplay was investigated in the microfluidic device. Results presented in figure 5b to e reveal that this homemade microfluidic model can be used to visualize and study the interplay between the neuronal compartment and microvascular cells.

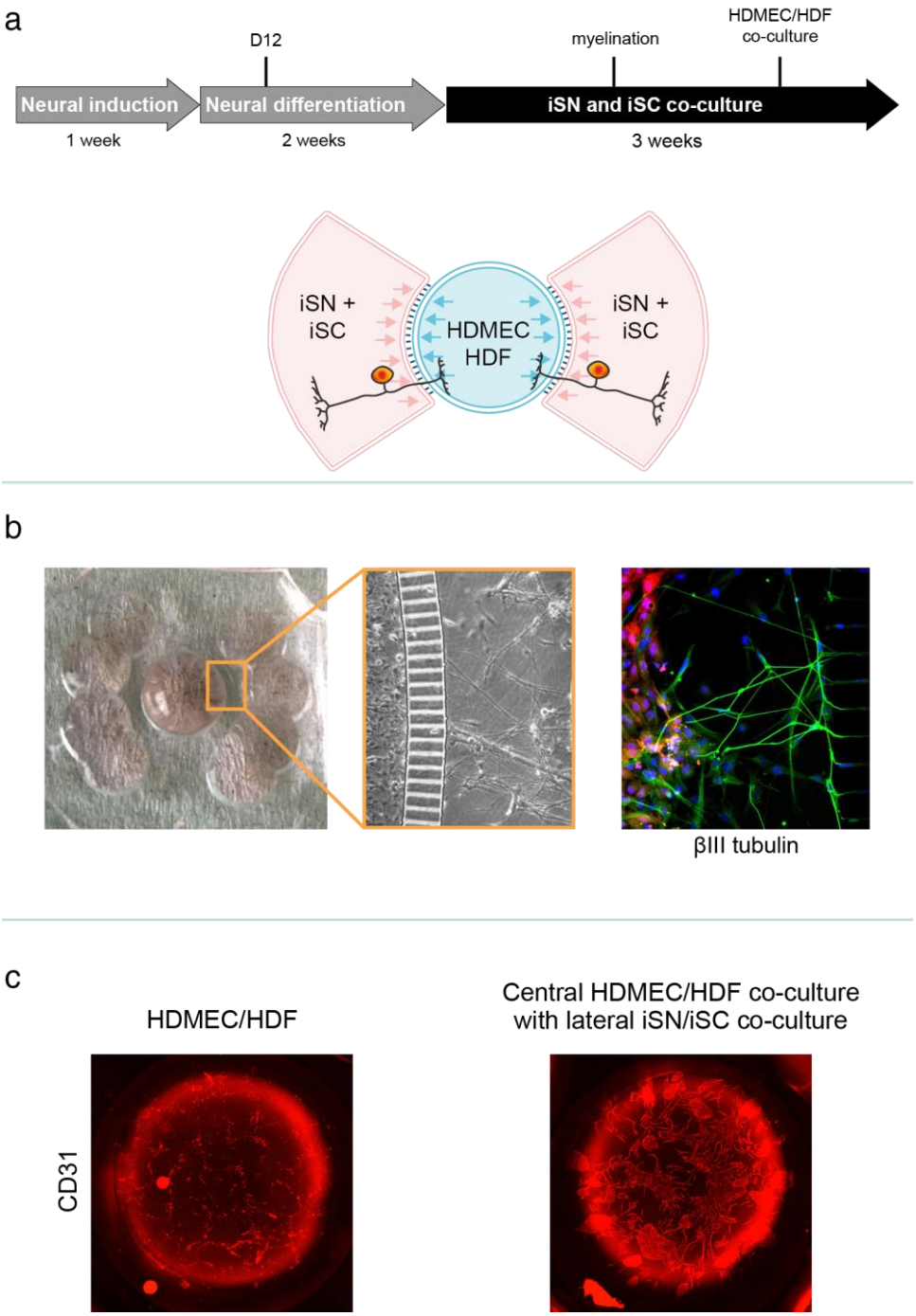

Figure 5. Investigation of cutaneous neurovascular interplay in microfluidic device. (a) Schematic representation of the microfluidic chips; (b) Picture of the microfluidic chips, phase contrast imaging and immunostaning of sensory neurons (βIII tubulin in green), fibroblasts and endothelial cells (in red) cultured in the microfluidic device (DAPI in blue); (c) Immunostaining of HDMEC/HDF co-culture laterally innervated by iSN and iSC in microfluidic device (CD31 in red).

4. Discussion

This study reports the development of a new model consisting of a human quadriculture of sensory neurons, Schwann cells, normal fibroblasts and dermal microvascular endothelial cells co-cultured in an in-house designed microfluidic chips mimicking the normal skin innervation. From both conditioned media and microfluidic chips, results demonstrate the effects of the neural actors on the morphological organization of the endothelial compartment. Further work is needed to better apprehend the functional and morphological rearrangement of the vascular network upon neuronal physiological and pathological conditions.

5. Conclusion

These innovative co-culture chips could therefore be used to investigate the impact of innervation in normal and pathological 3D skin equivalent models as well as to investigate active ingredients mechanism of action to alleviate innervation-dependent cutaneous diseases.

6. References

[1]. Ashrafi M. *et al*., The role of neuromediators and innervation in cutaneous wound healing, *Acta. Derm. Venerol*., 2016, 96(5): 587-94.
[2]. Malheiro A. *et al.,* peripheral neurovascular link: an overview of interactions and *in vitro* models, *Trends Endocrinol. Metab.,* 2021, 32(8):623-638.
[3]. Guimaraes M *el al*., Generation of iPSC-Derived Human Peripheral Sensory Neurons Releasing Substance P Elicited by TRPV1 Agonists, *Front. Mol. Neurosci.,* 2018, 22:11:277.
[4]. Powell R. and Phillips JB. Engineered tissues made from human iPSC-Derived schwann cells for investigating peripheral nerve regeneration *in vitro*. *Methods Mol Biol.,* 2021, 2269:245-254.