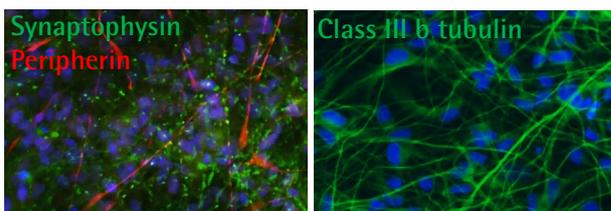
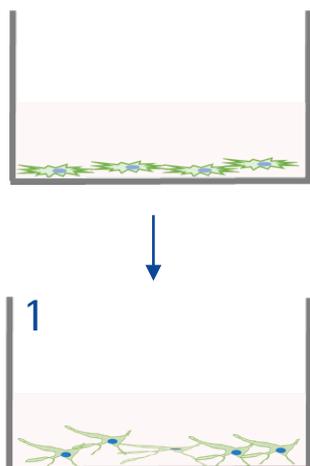
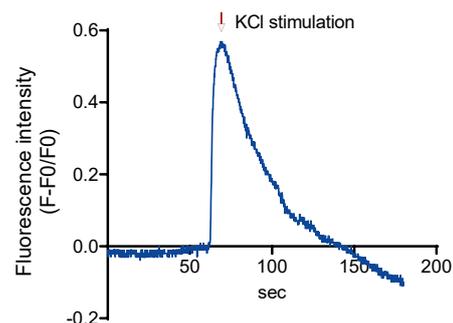


# A pre-clinical assay for testing the effects of compounds/drugs on innervated skin *ex vivo*

## 1. Differentiation of human iPSC derived neural stem cells *in vitro*

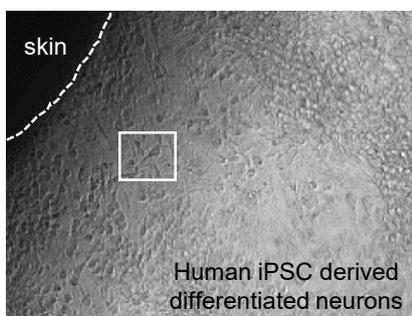
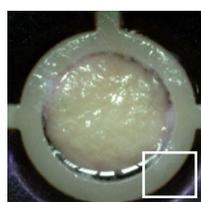
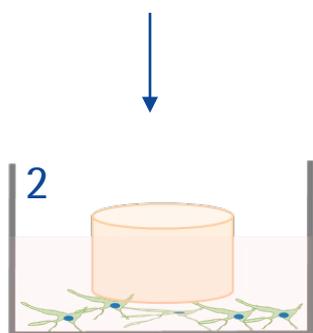


Human iPSC derived neural stem cells start to express **class III  $\beta$ -tubulin** which is associated with neuronal maturation (Sainath and Gallo, Cell Tissue Res 2015), **peripherin** which is a peripheral nervous system neuronal marker (Yuan et al., J Neurosci. 2012), and **synaptophysin** which is a marker of mature neurons (Kwon et al., Neuron. 2011)

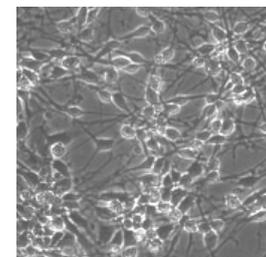


Differentiated human iPSC derived neural stem cells are functional. They release calcium in response to a depolarizing potassium chloride (KCl) stimulus. (response of  $n = 1$  neuron is shown)

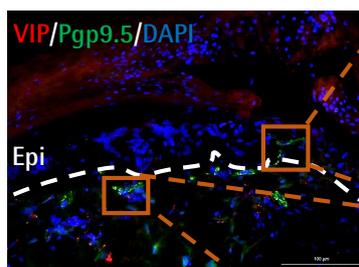
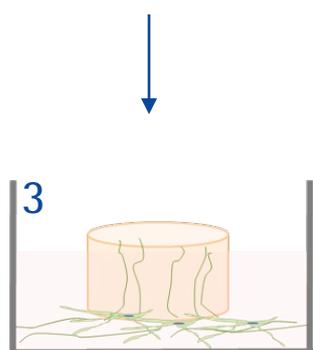
## 2. Initiation co-culture of human skin with human differentiated iPSC derived neural stem cells *ex vivo*



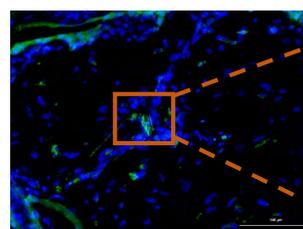
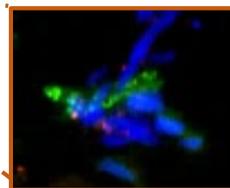
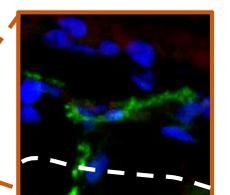
Topical application possible



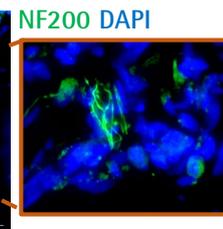
## 3. Model ready to use: Human skin punch is fully re-innervated



nerve fibers expressing the pan neuronal marker **PGP9.5** and the sensory neuronal marker **VIP** are reaching into the epidermis



Myelinated nerve fibers expressing the neurofilament marker **NF200** in the dermis



This novel assay can be utilized for dissecting and manipulating the bi-directional communication between defined skin and hair follicle cell populations and (sensory) human nerve fibers under stringently controlled *ex vivo* conditions, or for testing cosmeceuticals or drugs that target the cross-talk between human skin and hair follicles and cutaneous nerve fibers.

Relevant for e.g. sensitive skin, itch, atopic dermatitis, psoriasis

Keren et al., *Sci Adv.* 2022; Cheret et al., *JID* 2022

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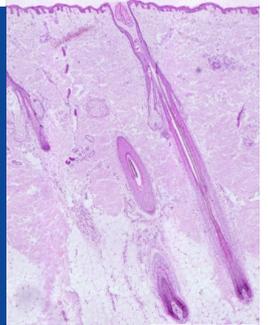


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