Ensheathed nerve fibers in human skin help communicate external stimuli

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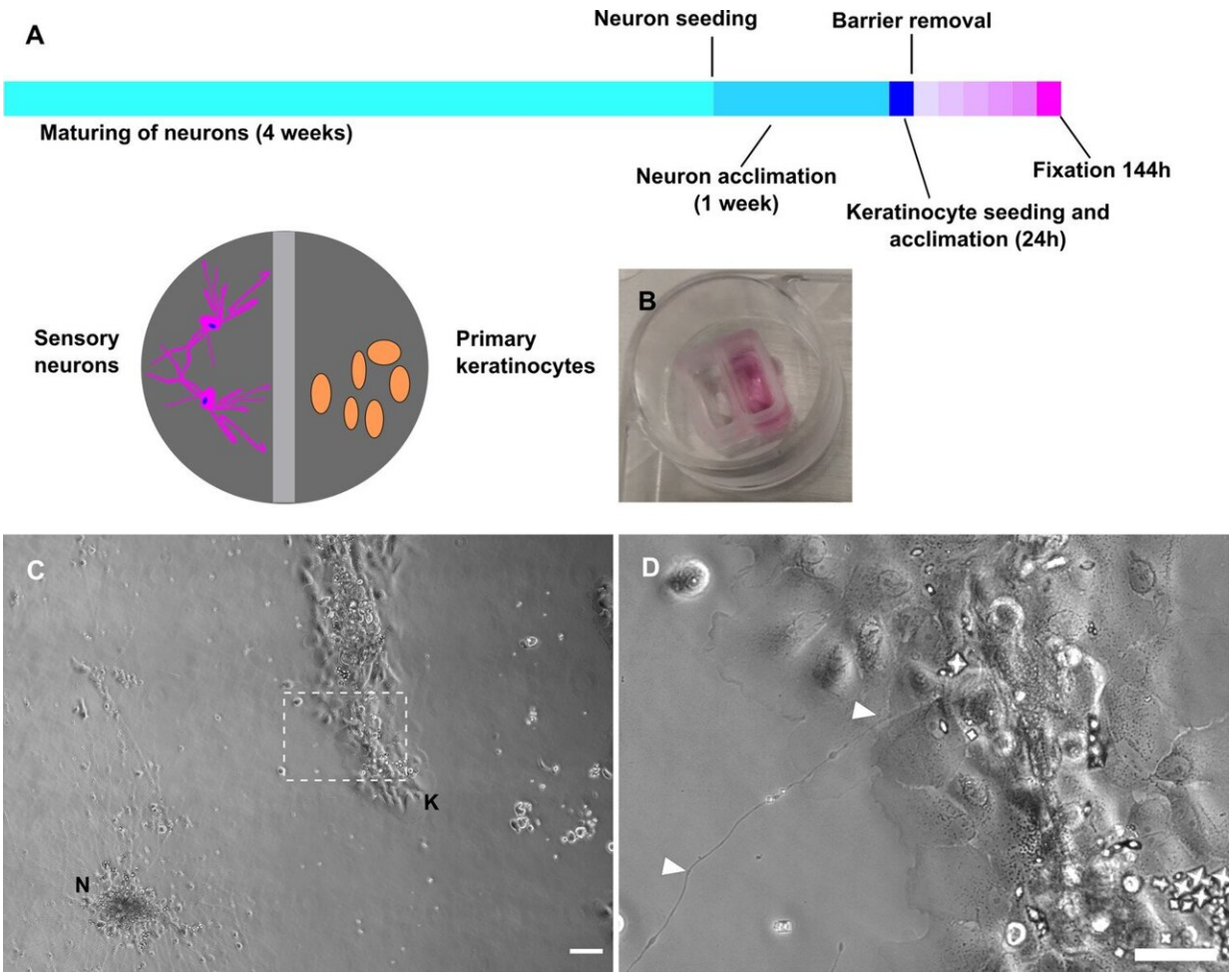

Fully human sensory neuron-keratinocyte co-culture model. (A) Timeline of culturing protocol and compartment scheme. (B) Chamber system. (C) Overview of co-culture after 115 h in conditioned neuronal medium with neuronal cluster [N] and keratinocyte colony [K]. Inset (D) shows a single neurite in contact with keratinocytes (arrowheads). Co-culture kept in conditioned neuronal medium.

Scale bars: 100 μm (C), magnified inset: 50 μm (D). Credit: *eLife* (2024). DOI: 10.7554/eLife.77761

Researchers have used new imaging techniques to explore the function and structures of the neuro-cutaneous unit, which is the connection between the nervous system and the skin.

The findings, published today (Jan. 16) in *eLife*, suggest that nerve fibers in the neuro-cutaneous unit tunnel through a type of skin cell called keratinocytes, in a process called ensheathment. Keratinocytes' main function is to produce the protein keratin to form a protective barrier for the skin, but the study lends support to the recently established idea that these cells also play a role as sensors and transmitters of external sensations by tightly interacting with intraepidermal nerve fibers (IENF).

The results may change our understanding of how the body processes external stimuli. They could also open new avenues for treating patients with small fiber pathology, a painful condition caused by damage or dysfunction in the small fibers of the nervous system.

Traditionally, it has been thought that external stimuli acting on the body are sensed and transmitted to the central nervous system exclusively by specific nerve cells called peripheral sensory neurons. However, recent research has suggested that epidermal keratinocytes may also play a role in communicating information about external stimuli.

In animal models, such as fruit flies, it has been shown that the axons of sensory neurons are ensheathed by epidermal cells similar to keratinocytes, which helps spread signals of external stimuli. The interactions between keratinocytes and nerve fibers in human skin are hard to identify, due to the small size of IENF and the density of tissue

in the epidermis.

"Scientists have recently demonstrated the ensheathment of nerve fibers by keratinocytes in human skin, using confocal microscopy imaging," explains lead author Christoph Erbacher, a postdoctoral researcher at the Department of Neurology, University Hospital of Würzburg, Germany. "However, direct and systematic information on ensheathment of human IENF is scarce, and the precise structure and molecular processes that occur remain obscure."

Erbacher and colleagues aimed to study the contact zones between sensory neurons and keratinocytes in human skin. They took samples from three healthy volunteers, which were quickly frozen at high pressures and treated with a fixative solution. Using a correlative light and electron microscopy approach via super-resolution array tomography, they captured highly detailed images of the samples.

Their analysis confirmed that, in all three samples, many IENFs were ensheathed by keratinocytes. They also observed clusters of hexamer 43 (Cx43), a protein complex that creates gap junction channels. These channels enable cell-to-cell communication by allowing the passage of small molecules, such as ATP.

In addition, they validated their findings by identifying ensheathment in a thicker section of skin. To do this, they took skin tissue samples from four patients with small fiber neuropathy and five healthy people, and used expansion microscopy to create large, detailed images. This allowed them to trace IENFs throughout the epidermis, and they confirmed that IENFs were ensheathed by keratinocytes throughout the samples.

Next, the team created a 2D co-culture model of sensory neurons and keratinocytes to simulate the cellular interactions between the two cell types. At first, sensory neurons and keratinocytes were bedded into

separate chambers and formed clusters. When the researchers removed the barrier between the two cultures, they observed the neurons actively growing toward the keratinocytes, establishing contact and becoming ensheathed.

They also noticed that non-ensheathed neurons frequently passed in close proximity to and over keratinocytes. At these passing sites, there were clusters of Cx43—hinting at the possibility of cell-to-cell communication between keratinocytes and neurons.

The team then used calcium ion imaging to see if this communication was taking place. They discovered that spontaneous calcium ion peaks in keratinocytes preceded a calcium ion peak in the neurons. This suggests that cell-to-cell signaling occurs between keratinocytes and neurons in the human neuro-cutaneous unit.

The authors caution that their study is limited by a small cohort, due to the necessity of handling large super-resolution data and complex segmentation challenges. They suggest that these limitations may be overcome in future studies by using software guided image acquisition to locate IENFs and more sophisticated deep learning algorithms guided by artificial intelligence.

"We have investigated the neuro-cutaneous unit in human skin and provide evidence for nerve fiber ensheathment by keratinocytes and Cx43 contact sites between keratinocytes and IENFs," concludes senior author Nurcan Üçeyler, a professor at the Department of Neurology, University Hospital of Würzburg.

"These findings are crucial for better understanding the role of neuronal and non-neuronal cells in the development and maintenance of neuropathy, and could have major implications for future treatment approaches," says Üçeyler.

More information: Christoph Erbacher et al, Interaction of human keratinocytes and nerve fiber terminals at the neuro-cutaneous unit, *eLife* (2024). DOI: 10.7554/eLife.77761

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