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Review

In Vitro Sensitive Skin Models: Review of the Standard Methods and Introduction to a New Disruptive Technology

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Abstract: The skin is a protective organ, able to decode a wide range of tactile, thermal, or noxious stimuli. Some of the sensors belonging to the transient receptor potential (TRP) family, for example, TRPV1, can elicit capsaicin-induced heat pain or histamine-induced itching sensations. The sensory nerve fibers, whose soma is located in the trigeminal or the dorsal root ganglia, are able to carry signals from the skin's sensory receptors toward the brain via the spinal cord. In some cases, in response to environmental factors, nerve endings might be hyper activated, leading to a sensitive skin syndrome (SSS). SSS affects about 50% of the population and is correlated with small-fiber neuropathies resulting in neuropathic pain. Thus, for cosmetical and pharmaceutical industries developing SSS treatments, the selection of relevant and predictive in vitro models is essential. In this article, we reviewed the different in vitro models developed for the assessment of skin and neuron interactions. In a second part, we presented the advantages of microfluidic devices and organ-on-chip models, with a focus on the first model we developed in this context.

Keywords: neuron; skin; sensitive skin; organ-on-chip; microfluidic device; TRPV

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1. Introduction

Skin is a protective organ that decodes a wide range of tactile, thermal, and noxious stimuli. The perception of sensory stimuli involves various sensory receptors, such as mechanoreceptors (mechanical stimuli), thermoreceptors (warm or cold temperature), nociceptors (noxious stimuli) [1], and pruriceptors (itching sensations) [2]. All these receptors contain the axonal endings of sensory neurons, which can be divided into three types depending on their diameter and speed of impulse. The highest velocity is found in Aβ fibers, which are the biggest and are surrounded by several myelin sheaths. These nerve fibers are connected to hair follicles and mechanoreceptors (Merkel disks, Meissner, Pacinian, and Ruffini corpuscles) [3]. The small fibers are either surrounded by a thin myelin sheath that is lost in the dermis (Aδ fibers) or unmyelinated (C fibers), leading to slower nerve conduction, especially in C-fibers [1]. Their free nerve endings, located in the dermis (Aδ fibers) or the epidermis (C fibers), also largely innervate hair follicles [4]. They are both involved in temperature, pain [1], and itching sensations [5,6]. Some C-fibers also detect gentle mechanical stimuli, especially in skin on the face [7]. Both C and Aδ free nerve endings can detect a large panel of physical and chemical stimuli [1], being either specifically activated by mechanical, thermal, or chemical stimuli (unimodal fibers) or responding to several stimuli (polymodal fibers) [8]. Their sensitivity depends on the properties of their membrane sensors and associated proteins. The sensors mostly belong to the transient receptor potential (TRP) family and can be studied using various agonists and antagonists. For example, TRPV1 can elicit capsaicin-induced heat pain [9] or a histamine-induced itching sensation [10], while TRPV4 is known as a heat sensor [11]. The menthol-activated receptor TRPM8 is responsible for the detection of innocuous and

noxious cooling [8,12], and the wasabi-activated receptor TRPA1 is involved in a wide panel of stimuli, including chemo-nociception [12], thermal sensations [13,14], and itch [14].

All these sensory nerve fibers, for whom the cell body is located in the trigeminal or the dorsal root ganglia (DRG), can carry signals from skin sensory receptors toward the appropriate integration center of the brain via the spinal cord [8,15]. Besides this afferent sensory information transfer, some stimulated nociceptive C-fibers, called peptidergic C-fibers, can produce and release various neuropeptides, such as substance P and calcitonin-gene-related peptide (CGRP) at the skin level [16], thus provoking vasodilation and neurogenic inflammation [12]. To a lesser extent, this is also true for small myelinated Aδ fibers [1].

Sensitive skin syndrome (SSS) has been defined as "the occurrence of unpleasant sensations (stinging, burning, pain, pruritus, and tingling sensations) in response to various stimuli that normally should not provoke such sensations" [17]. A significant correlation between SSS and small-fiber neuropathies, especially those affecting unmyelinated C-fibers, has been established [18]. A decrease in the heat-pain threshold detection is currently considered to be responsible for the hyper-reactivity of nerve endings in response to environmental factors and the induction of neuropathic pain [11]. This detection threshold directly depends on the sensor's presence and properties. TRPV1 was found to be significantly increased in hypersensitive skin after nerve injury and repair [19]. TRPV1 also seems to play a key role in heat hyperalgesia following burn or inflammation [8], while TRPM8 and TRPA1 are involved in injury-evoked cold hypersensitivity [12]. TRPV1, TRPV4, and TRPA1 also contribute to mechanical hyperalgesia [8]. Studying the sensory perception mechanisms of sensory neurons is, therefore, essential for the better diagnosis, prevention, and treatment of sensitive skin. Nevertheless, some studies revealed that keratinocytes also express diverse sensory receptors, such as TRPV1, TRPV3, and TRPV4 [8,11,20]. Mainly expressed in keratinocytes, TRPV3 can stimulate CGRP secretion, which induces local vasodilation that plays a role in body thermoregulation [21]. The selective activation of the keratinocyte-located TRPV1 and TRPV4 sensors is sufficient to induce pain and itch, respectively [11]. It has been shown that the activation of keratinocyte TRPV1 elicits a calcium signal, leading to intraepidermal nerve-ending activation through synaptic-like contacts. Interestingly, the density of these synaptic-like contacts between keratinocytes and sensory neurons was found to be significantly higher in patients with sensitive skin compared to unsensitive controls [22]. Thus, sensitive skin not only depends on the sensory neurons' functionality but also on their physiological interactions with keratinocytes through those synaptic-like contacts.

As sensitive skin affects about 50% of the population [23], it is essential to find efficient active molecules and, thereby, develop relevant in vitro models for the cosmetical and pharmaceutical industries.

2. Review of In Vitro 2D/3D Models of Innervated Skin

2.1. Sensory Neuron Culture and Coculture

Skin nerve sensitivity relies primarily on sensory neurons. Thus, many teams have focused on 2D cultures of sensory neurons to better understand their function in correlation with skin sensitivity [24–26]. As the compositions and properties of primary neurons are much more similar to those of sensory neurons in tissue than those of immortalized cell lines [26], most in vitro models were developed using DRG sensory neurons. Such models have successfully demonstrated that histamine can induce itching by activating TRPV1 [10], which sheds light on the molecular mechanisms that explain how IL-31 secreted by T helper cells induces itching by activating a small population of TRPV1+/TRPA1+ sensory neurons [27] or how keratinocyte-secreted TSLP elicits itching through TRPA1 in atopic dermatitis [28].

To better mimic the physiological environment of sensory neurons, several teams created 2D and 3D coculture models, including sensory neurons and various cell types such as Schwann cells [29–31], fibroblasts [30–39], endothelial cells [31,35,36], and/or keratinocytes [29,31–38,40]. Indeed, some of these cells have been shown to modulate neurite growth [41], and one can expect a sensory neuron response to be closer to the in vivo response in a more physiological environment. However, besides this aspect, keratinocytes have been shown to be directly involved in cutaneous sensoriality. Indeed, they can both modulate and initiate sensory perception and sensory neuron response [21,42,43]. Thus, an appropriate model to study skin sensitivity and assess active molecule efficacy on sensitive skin needs to at least contain sensory neurons and keratinocytes (Table 1).

Table 1. In vitro models containing at least sensory neurons and keratinocytes in which the response to thermal or chemical stimulus has been tested. DGR: dorsal root ganglia; HDF: human dermal fibroblasts; HDMEC: human dermal microvascular endothelial cells; HEK: human primary epidermal keratinocytes; HUVEC: human umbilical vein endothelial cells; TRPV1: transient receptor potential vanilloid 1.

2D/3D Model	Origin of Neurons	Reference	Cell Types of the Model	Stimulus	Response
2D model	Animal	[29]	Tri-compartmented culture with (1) rat spinal cords cells; (2) rat DRG; (3) epidermal cells (keratinocytes, Merkel cells and melanocytes)	Hot medium in the epidermal/axonal compartment: - 22 °C (control) - 37 °C (heat stimulation) - 45 °C (painful stimulation)	Electrophysiological recording in the soma compartment by patch clamp showed spikes registered at 37 and 45 °C but not at 22 °C.
		[44]	Bi-compartmented culture with (1) rat DRG neurons; (2) rat primary epidermal keratinocytes	Systemic application of 100 nM capsaicin (TRPV1 agonist) in the epidermal/axonal compartment	Calcium imaging in the soma compartment showed a similar neuron activation by capsaicin either with or without keratinocytes.
3D model	Animal	[37]	Immortal human keratinocyte line of HaCaT cells, HDF, rat DRG neurons	Topical application of 5 μM capsaicin (TRPV1 agonist)	Calcium imaging on neurites showed calcium waves increase by capsaicin.
		[35]	HEK, HDF, HUVEC or HDMEC, mouse DRG neurons	Systemic application of 0.01 ng/mL capsaicin (TRPV1 agonist)	4-fold increase of substance P release in culture supernatant with capsaicin treatment.
		[40]	Human skin explant, rat DRG neurons	Topical application of 100 μM SLIGKV (PAR2 agonist), 10 μM capsaicin (TRPV1 agonist) or 3 μM polygodial (TRPA1 agonist)	- Electrophysiological recording by patch-clamp showed that all treatments stimulated reinnervating neurons. - SLIGKV treatment increased TSLP release and decreased VEGF expression. - Capsaicin treatment increased TSLP and CGRP release and decreased the expression of several genes in neurons, including CGRPα, MT5-MMP, and BDNF (involved in skin sensitivity). - Polygodial increased CGRP release and TNF, TSLP, and NGF expression in skin explants and decreased EGF and BDNF expression in neurons.
	Human	[39]	HEK, human iPSC-derived sensory neurons	Systemic application of 10 μM capsaicin (TRPV1 agonist)	Capsaicin treatment increased CGRP release in the culture supernatent. This effect was reduced by applying a soothing agent.

Unlike skin cells, which are easy to obtain from human skin biopsies, human primary neurons are difficult to collect for practical and ethical reasons, and they do not proliferate in vitro [45,46]. For this reason, most of the models were created by using human skin cells in association with rat [32,37,40], pig [33,34,38], or mouse DRG neurons [35,36], which are easier to obtain. Nevertheless, for regulatory reasons, pig and rodent DRG neurons cannot be used for cosmetic testing. Moreover, it has been shown that neurons from rodent DRG differ in their receptor types or expression levels, genes, and signaling

pathways compared to human DRG neurons [46], which makes them less relevant than human neurons for use in innervated human skin models.

The disruptive technology that creates induced pluripotent stem cells (iPSCs) [47] has allowed us to further obtain human sensory neurons derived from human iPSCs. Depending on the culture condition, human iPSCs can be differentiated into various neuron-like cells sharing characteristics with sensory neurons such as nociceptors, mechanoreceptors, and proprioceptors [48,49]. These human sensory neurons derived from iPSCs can produce substance P or CGRP under capsaicin activation and have been successfully included in innervated or reinnervated skin models [31,39]. They have been used, for example, to demonstrate the positive effect of an active ingredient on sensitive skin [39].

2.2. Two/Three-Dimensional Models and the Connection between Cell Types

More- and less-complex coculture models have been developed. The simplest ones are mixed 2D cocultures that enable easy cell–cell interactions [22]. To better mimic physiological conditions, sensory neurons have also been cultivated in coculture with 3D reconstructed skin [31,34–38,41,50], reconstructed human epidermis (RHE) [39], and full-skin explants [40,51].

To better mimic the anatomical distance and respective micro-environment between, on one side, neuronal cell bodies (somas) and, on the other side, axonal endings and skin cells, these cell types should be seeded and cultivated in distinct compartments. These compartments can be connected by microchannels [29,33] to study the behavior of a specific cell type. Thanks to the microchannels, Chateau et al. established a functional synaptic-like connection between two compartments containing neurons and skin cells [29]. With this model, they demonstrated the possibility of activating the soma by only applying heat-stimulation in the axon–keratinocyte compartment.

Considering the key role of small sensory fibers and TRPV1, sensitive skin is usually mimicked by activating TRPV1 in an innervated skin model. TRPV1 can be directly activated by temperatures above 43 °C [9], pH below 6 [52], endogenous molecules such as anandamide [53], and exogenous agonists such as capsaicin (the main pungent component in hot chili peppers) or its analog, resiniferatoxin [9,54,55]. Many other molecules, including histamine, indirectly activate TRPV1 [10,56]. As TRPV1 is a non-selective calcium channel, this results in calcium influx, leading to neuron depolarization, which is responsible for both the afferent signal genesis and the local secretion of neuropeptides [57]. To validate innervated skin functionality and/or mimic sensitive skin, several teams have, therefore, evaluated the reactivity of their model to a temperature increase from 22 to 37 or 45 °C [29], capsaicin added in the medium [31,35,39] or topically applied [37,40], and/or histamine added in the medium [39] (Table 1). Active ingredients have been considered to have positive effects when they could reduce the temperature-, histamine-, or capsaicin-induced activation of innervated skin [39].

Based on the review of the existing models (Table 1), it seems that the ideal model to mimic sensitive skin and activate the TRPV1 pathway would be a compartmentalized model in which neurons and skin cells are independently cultured but interconnected by microchannels, with both cell types being derived from humans. In addition, for the drug screening or toxicity assessment, this model should also be standardized to ensure reproducibility and large-scale production.

3. Organ-on-Chip Models and Their Use in Dermatology

3.1. Introduction to Microfluidic Devices and Organ-on-Chip Models

The organ-on-chip (OoC) model consists of an in vitro microfluidic platform with micrometer-sized chambers that allow the combination of different cell types in a 3D structure to recreate the functionality of an organ close to that of a human. The dynamic culture of cells inside and their interaction allow the characterization of the organ's properties, its interaction with other tissues, and its behavior in certain physiological and

pathological conditions. These models aim to replace animal testing and be more predictive, while, with miniaturization, they could reduce the costs of experimental setups by reducing the use of tissue or specific cells, especially non-proliferative cells (e.g., iPSC-derived neurons), medium, growth factors, and drugs. Another advantage of microfluidic devices is the control of the interaction between cells or tissues with the possibility to use microchannels, which enables the interconnection between independent compartments—for example, to isolate the soma of a neuron to its axonal endings [58]. The fluidic isolation created by the microchannels is of great interest to limit diffusion from one compartment to another, which allows the use of different optimal culture media for each cell type, the selective application of stimuli (e.g., actives) in a compartment, or the sampling of the supernatant in a specific compartment. Microfluidic devices usually consist of microchannels to guide neurites' growth and membranes to create cellular barriers or air–liquid interfaces. Many applications and new perspectives arise from microfluidic device technologies, such as functional studies, mechanistic studies, drug screening, toxicology, and pharmacokinetics.

In the past decade, there has been an increasing number of reports published about the development of microfluidic devices for testing various organs, such as the liver, kidney, brain, intestine, and heart [59]. The development of an OoC involving skin is more recent and essentially consists of using microfluidic technology, channels, and porous membranes to reconstruct the different layers of the skin [60,61]. These first models may include endothelial cells to study drug toxicity, inflammation [62], skin permeability, or the immune response to the presence of bacteria on the skin [63]. Few studies have focused on the coculture of skin with other organs, such as the liver [64–68], intestine [66], kidney [66], and hair [69]. The main objectives were to observe cell viability or to study the crosstalk between the skin, liver, or kidneys for drug toxicity.

In recent years, the interaction between cutaneous nerves, the neuroendocrine axis, and the immune system has been established. It has been found that neurocutaneous interactions influence various physiological and pathophysiological functions, including cell growth, immunity, inflammation, pruritus, and wound healing [70,71]. Thus, compartmentalized microfluidic devices represent a relevant model to assess the interaction of these cells' networks; however, to our knowledge, only one article reported the development of a real microfluidic device to assess the interaction between skin and neurons. Tsantoulas et al. cocultured keratinocytes and neurons in a microfluidic model and assessed whether their coculture interacted with nerve activation induced by capsaicin [44]. The results were promising; however, the main limitation was the use of animal cells, which limited the transferability of the results to humans.

3.2. Development of an Innervated Skin-on-a-Chip

3.2.1. Interest and Objectives

Psoriasis, atopic dermatitis, eczema, wound healing, skin irritation, herpes infection, hyperhidrosis, neuropathy, and skin sensitivity syndrome are conditions involving both skin cells, i.e., keratinocytes and neurons. Preclinical research is limited by in vitro models, which are not always relevant either because they often use rodent neurons cells or because the cocultures of skin cells and neurons do not fully recapitulate the anatomical structure, i.e., a neuronal cell body compartmentalized from the skin. The association between compartmentalized microfluidic device technology and human-derived sensory neurons may address these limitations. The interest in such a model is double: 1. to evaluate the impact on neurons of a stimulus applied on the skin, and 2. to evaluate the impact on skin of a stimulus applied on neurons. Stimuli are various and might be physical (UV, mechanical forces, electrical stimulation of neurons), chemical (toxins or drugs), or molecular (capsaicin, oxytocin, substance P, histamine, bradykinin, etc.). In the field of dermatology and cosmetics, the applications are varied and include, for example, drug efficacy screening, pharmacokinetic studies, skin penetration and drug diffusion, drug–drug

interaction, and toxicological studies (sensitization, irritation, phototoxicity). This model is even more relevant in cosmetics that avoid animal testing by using human cells in vitro.

3.2.2. Design of an Innervated Skin-on-Chip

OoC models developed by Netri™ are versatile technologies that allow the 3D coculture of different cell types in isolated compartments interconnected by microchannels, which allow the unidirectional or bidirectional growth of neurites. Thus, the chip's architecture is tailored according to the objective of the trial. These devices are made with polydimethylsiloxane (PDMS), a biocompatible silicon material that has several advantages: 1. Its transparency allows the use of standard analytical methods, such as optical, immune-fluorescence, or confocal microscopies. 2. It is permeable to gas, and thus, no pump or mechanical stirrer is needed, and the culture is compatible with standard incubators. 3. Its suppleness allows biopsy and standard histology. These associated features contribute to the long-term culture and survival, up to several months.

Thanks to the microfluidic technology and compartmentalization, we developed a microfluidic model including human sensory neurons and keratinocytes (Figure 1). The device consisted of two isolated channels linked by microchannels in which only axons can pass through over dendrites. Such a configuration allows the creation of a selective barrier while also connecting the skin compartment with keratinocytes and the spinal cord compartment with the neuronal cell body. Human-iPSC-derived sensory neurons were seeded in one channel, and human primary keratinocytes were seeded in the other. Cell seeding density and timing between the two cell types were optimized to ensure proper cell maturation and proliferation. Thanks to compartmentalization and fluidic isolation, cells were cultivated with their respective culture medium, allowing optimal growth. This model can be used to mimic sensitive skin, for example, by adding capsaicin to axonal endings and assessing its effect on either the skin compartment or the neuronal cell body.

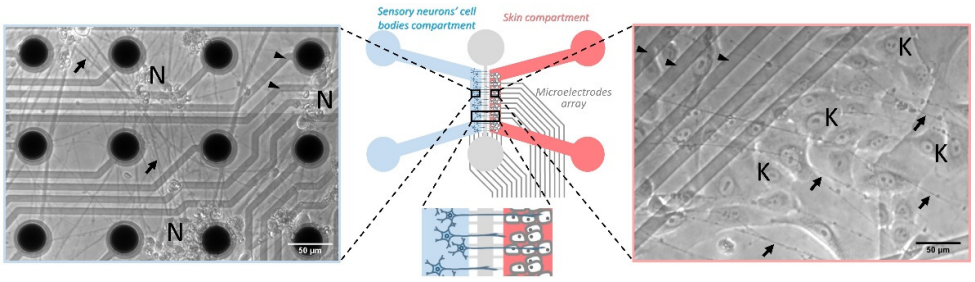

Figure 1. Development of an innervated skin-on-chip. In the center, the micro-fluidic device architecture, with on the bottom, a close-up view of three compartments. On the left side, a representative transmission light pictures of the compartmentalized culture of human iPSC-derived sensory neurons (N). On the right side, a representative transmission light pictures of the compartmentalized culture of human primary keratinocytes (K) with neurites (arrows) which grow from one channel to the opposite one. The culture was done on a micro-electrode array (arrow heads) allowing electrophysiological recording of the neuronal activity.

3.2.3. From Standard to Innovative Specific Readouts

The versatility of the OoC developed by Netri™ allows the use of a large variety of readouts. Cells and tissues might be visualized by optical, immunofluorescence, or confocal microscopy, and their viability is assessed using standard methods (MTT, live/dead assay, etc.). The real-time follow up of cell migration is also possible. Moreover, collecting the cells or the supernatant allows the dosage of molecules, mediators, or biomarkers to be determined by standard methods such as ELISA, PCR, etc. PDMS suppleness allows slicing and standard histology on every compartment, either skin cell or neuron. In addition to these standard methods, neural activity might also be specifically evaluated.

Calcium imaging is a common method and enables the visualization of the activity of hundreds of individual neurons simultaneously using fluorescent activity sensors. Changes in fluorescence indicate fluctuations in intracellular calcium, which is an indirect indicator of neural activity. This technique may induce long-term cytotoxicity and disruption of cell behavior. In addition, the field of view of the sample is limited. On the other hand, a micro-electrode array (MEA) has more advantages because, being not cytotoxic, it allows a repeated and continuous electrophysiological recording of functional activity. The MEA is an array of microscopic electrodes distributed over a small surface area that provides a macroscopic view of neural networks by recording the electrical activity of brain slices, dissociated tissues, organoids, spheroids, or the 2D neuronal cell culture. An MEA records the field potential electrical activity from the extracellular space of a population of neurons, whereas patch-clamp electrophysiology records the action potential of electrical activity from the intracellular space of a single neuron. MEA technology associated with compartmentalized microfluidic devices allows the monitoring of the electrical shift of neuron activity induced by skin cells.

4. Future Perspectives

Current limitations in traditional in vitro skin models are the correlation between skin cell culture or reconstructed skin and the clinical conditions. Our future development involves introducing an air–liquid interface to recreate conditions close to in vivo to enable the differentiation of the stratum corneum and the topical application of products or microbiota on the skin. To study specific pathological conditions, such as psoriasis or eczema, a patient's skin biopsy might be directly inserted into the chip and colonized by neurites of human sensory neurons, as Lebonvallet et al. have previously demonstrated [40].

Intense noxious stimuli and tissue inflammation produce a pain hypersensitivity resulting from peripheral and central sensitization. This hypersensitivity of the receptor might be explained by the fact that the activation threshold of TRPV1 might be lowered by a pro-inflammatory environment [72]. Thus, to better mimic sensitive skin and TRPV1 hyperexcitability, pro-inflammatory cytokines might be added into the OoC.

As recently popularized, the skin, gut, and microbiota are closely linked, and their interactions directly influence both normal physiology and disease processes, such as atopic dermatitis [28,33,34] or psoriasis. Therefore, a multi-organ-on-chip combining these entities seems relevant to better understand their interaction and develop new treatments.

To go one step further in the predictability of these models, the future could involve a personalized and innervated skin-on-chip. From the subject's skin biopsy, it would be possible to derive neurons that can be associated together with the subject's skin cells into a unique and personalized chip. This personalization is especially relevant in rare diseases or in fragile populations such as the pediatric population, pregnant women, the elderly, or immunocompromised patients.

5. Conclusions

The prevalence of sensitive skin varies according to gender, ethnicity, and environmental factors (e.g., climate, exposures to products and chemicals, and cultural influences), but it is generally agreed that this condition affects a substantial portion of the population. Recently, the importance of skin and nerve interaction was highlighted by the 2021 Nobel Prize in physiology. Indeed, David Julius and Ardem Patapoutian shared the Nobel Prize for the identification of receptors that enable cells to detect temperature and touch. Dr. Julius worked on TRPV1 and capsaicin, the latter of which is currently considered to be the reference marker of sensitive skin.

Several in vitro models have been developed to assess the interaction between the skin and neurons, essentially to mimic sensitive skin. Most of them consisted of the coculture of both cell types in the same system. However, to better reproduce the physiological distance between neuron cell bodies (soma) and skin cells, these cell types should be

cultivated in distinct compartments. Thus, compartmentalized microfluidic devices such as the OoC represent a relevant alternative.

In the next few years, it can be envisioned that animal studies might be at least in part replaced by OoC technology, leading to more reliable results close to clinical studies. Progress in iPSC manipulation during these last years is a key element that may contribute to the development of all-human in vitro models. In dermatology and especially in cosmetology, the combination of compartmentalized microfluidic technology and iPSC-derived human sensory neurons to create a standardized all-human in vitro model of innervated skin makes sense. In addition, nociception, itch, or inflammation might be recreated and controlled in these models by adding specific modulators such as capsaicin, menthol, cytokines, and drugs. Furthermore, besides the standard readouts, microfluidic devices can be coupled to MEA technology to allow the recording of the electrical signals' of the soma after axonal molecular stimulation.

Innervated skin-on-a-chip represents several advantages at the different stages of drug or cosmetic development. The model is expected to reduce R&D costs by avoiding animal costs through reducing the sample size and duration of the trial. All in all, these predictive preclinical models aim to better discriminate new drug candidates and, thus, to reduce the risk of clinical study failures (phases I and II).

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References

1. Laverdet, B.; Danigo, A.; Girard, D.; Magy, L.; Demiot, C.; Desmoulière, A. Skin innervation: Important roles during normal and pathological cutaneous repair. *Histol. Histopathol.* **2015**, *30*, 875–892. https://doi.org/10.14670/HH-11-610.
2. Misery, L.; Brenaut, E.; Le Garrec, R.; Abasq, C.; Genestet, S.; Marcorelles, P.; Zagnoli, F. Neuropathic pruritus. *Nat. Rev. Neurol.* **2014**, *10*, 408–416. https://doi.org/10.1038/nrneurol.2014.99.
3. Siemionow, M.; Gharb, B.B.; Rampazzo, A. The Face as a Sensory Organ. *Plast. Reconstr. Surg.* **2011**, *127*, 652–662. https://doi.org/10.1097/prs.0b013e3181fed6fd.
4. Abraira, V.E.; Ginty, D.D. The Sensory Neurons of Touch. *Neuron* **2013**, *79*, 618–639. https://doi.org/10.1016/j.neuron.2013.07.051.
5. Schmelz, M.; Schmidt, R.; Bickel, A.; Handwerker, H.O.; Torebjörk, H.E. Specific C-Receptors for Itch in Human Skin. *J. Neurosci.* **1997**, *17*, 8003–8008. https://doi.org/10.1523/jneurosci.17-20-08003.1997.
6. Potenzieri, C.; Undem, B.J. Basic mechanisms of itch. *Clin. Exp. Allergy* **2012**, *42*, 8–19. https://doi.org/10.1111/j.1365-2222.2011.03791.x.
7. Wessberg, J.; Olausson, H.; Fernström, K.W.; Vallbo, A.B. Receptive Field Properties of Unmyelinated Tactile Afferents in the Human Skin. *J. Neurophysiol.* **2003**, *89*, 1567–1575. https://doi.org/10.1152/jn.00256.2002.
8. Dubin, A.E.; Patapoutian, A. Nociceptors: The sensors of the pain pathway. *J. Clin. Investig.* **2010**, *120*, 3760–3772. https://doi.org/10.1172/jci42843.
9. Caterina, M.J.; Schumacher, M.A.; Tominaga, M.; Rosen, T.A.; Levine, J.D.; Julius, D. The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* **1997**, *389*, 816–824. https://doi.org/10.1038/39807.
10. Shim, W.-S.; Tak, M.-H.; Lee, M.-H.; Kim, M.; Koo, J.-Y.; Lee, C.-H.; Oh, U. TRPV1 Mediates Histamine-Induced Itching via the Activation of Phospholipase A2 and 12-Lipoxygenase. *J. Neurosci.* **2007**, *27*, 2331–2337. https://doi.org/10.1523/jneurosci.4643-06.2007.
11. Misery, L. Sensitive Skins May Be Neuropathic Disorders: Lessons from Studies on Skin and Other Organs. *Cosmetics* **2021**, *8*, 14. https://doi.org/10.3390/cosmetics8010014.

12. Julius, D. TRP Channels and Pain. *Annu. Rev. Cell Dev. Biol.* **2013**, *29*, 355–384. https://doi.org/10.1146/annurev-cellbio-101011-155833.
13. Laursen, W.J.; Anderson, E.O.; Hoffstaetter, L.J.; Bagriantsev, S.N.; Gracheva, E.O. Species-specific temperature sensitivity of TRPA1. *Temperature* **2015**, *2*, 214–226. https://doi.org/10.1080/23328940.2014.1000702.
14. Maglie, R.; de Araujo, D.S.M.; Antiga, E.; Geppetti, P.; Nassini, R.; de Logu, F. The Role of TRPA1 in Skin Physiology and Pathology. *Int. J. Mol. Sci.* **2021**, *22*, 3065. https://doi.org/10.3390/ijms22063065.
15. Rice, F.; Albrecht, P. Cutaneous Mechanisms of Tactile Perception: Morphological and Chemical Organization of the Innervation to the Skin. *Senses A Compr. Ref.* **2008**, *6*, 1–31. https://doi.org/10.1016/b978-012370880-9.00340-6.
16. Le Bars, D.; Adam, F. Nocicepteurs et médiateurs dans la douleur aiguë inflammatoire. In *Ann Fr Anesth Reanim*; Elsevier Masson: Amsterdam, The Netherlands, 2002; Volume 21, pp. 315–335. https://doi.org/10.1016/s0750-7658(02)00592-0.
17. Misery, L.; Ständer, S.; Szepietowski, J.; Reich, A.; Wallengren, J.C.; Evers, A.W.M.; Takamori, K.; Brenaut, E.; Gall-Ianotto, C.; Fluhr, J.; et al. Definition of Sensitive Skin: An Expert Position Paper from the Special Interest Group on Sensitive Skin of the International Forum for the Study of Itch. *Acta Derm. Venereol.* **2017**, *97*, 4–6. https://doi.org/10.2340/00015555-2397.
18. Buhé, V.; Vié, K.; Guéré, C.; Natalizio, A.; Lhéritier, C.; Gall-Ianotto, C.; Huet, F.; Talagas, M.; Lebonvallet, N.; Marcorelles, P.; et al. Pathophysiological Study of Sensitive Skin. *Acta Derm. Venereol.* **2016**, *96*, 314–318. https://doi.org/10.2340/00015555-2235.
19. Facer, P.; A Casula, M.A.; Smith, G.D.; Benham, C.D.; Chessell, I.P.; Bountra, C.; Sinisi, M.; Birch, R.; Anand, P. Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. *BMC Neurol.* **2007**, *7*, 11. https://doi.org/10.1186/1471-2377-7-11.
20. Inoue, K.; Koizumib, S.; Fuziwaraa, S.; Dendaa, S.; Inoueb, K.; Dendaa, M. Functional Vanilloid Receptors in Cultured Normal Human Epidermal Keratinocytes. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 124–129. https://doi.org/10.1006/bbrc.2002.6393.
21. Fromy, B.; Josset-Lamaugarny, A.; Aimond, G.; Pagnon-Minot, A.; Marics, I.; Tattersall, G.J.; Moqrich, A.; Sigaudo-Roussel, D. Disruption of TRPV3 Impairs Heat-Evoked Vasodilation and Thermoregulation: A Critical Role of CGRP. *J. Investig. Dermatol.* **2018**, *138*, 688–696. https://doi.org/10.1016/j.jid.2017.10.006.
22. Talagas, M.; Lebonvallet, N.; Leschiera, R.; Sinquin, G.; Elies, P.; Haftek, M.; Pennec, J.-P.; Ressnikoff, D.; La Padula, V.; Le Garrec, R.; et al. Keratinocytes Communicate with Sensory Neurons via Synaptic-like Contacts. *Ann. Neurol.* **2020**, *88*, 1205–1219. https://doi.org/10.1002/ana.25912.
23. Misery, L. Irritated Skin Is Not Sensitive Skin. *JID Innov.* **2021**, *1*, 100031. https://doi.org/10.1016/j.xjidi.2021.100031.
24. Hall, A.K.; Ai, X.; Hickman, G.E.; MacPhedran, S.E.; Nduaguba, C.O.; Robertson, C.P. The Generation of Neuronal Heterogeneity in a Rat Sensory Ganglion. *J. Neurosci.* **1997**, *17*, 2775–2784. https://doi.org/10.1523/JNEUROSCI.17-08-02775.1997.
25. Hall, A.K. Rodent Sensory Neuron Culture and Analysis. *Curr. Protoc. Neurosci.* **2006**, *36*, 3–19. https://doi.org/10.1002/0471142301.ns0319s36.
26. Lin, Y.-T.; Chen, J.-C. Dorsal Root Ganglia Isolation and Primary Culture to Study Neurotransmitter Release. *J. Vis. Exp. JoVE* **2018**, *140*, e57569. https://doi.org/10.3791/57569.
27. Cevikbas, F.; Wang, X.; Akiyama, T.; Kempkes, C.; Savinko, T.; Antal, A.; Kukova, G.; Buhl, T.; Ikoma, A.; Buddenkotte, J.; et al. A sensory neuron–expressed IL-31 receptor mediates T helper cell–dependent itch: Involvement of TRPV1 and TRPA1. *J. Allergy Clin. Immunol.* **2014**, *133*, 448–460. https://doi.org/10.1016/j.jaci.2013.10.048.
28. Wilson, S.R.; Thé, L.; Batia, L.M.; Beattie, K.; Katibah, G.E.; McClain, S.P.; Pellegrino, M.; Estandian, D.M.; Bautista, D.M. The Epithelial Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch. *Cell* **2013**, *155*, 285–295. https://doi.org/10.1016/j.cell.2013.08.057.
29. Château, Y.; Dorange, G.; Clément, J.-F.; Pennec, J.-P.; Gobin, E.; Griscom, L.; Baudrimont, M.; Rougier, N.; Chesné, C.; Misery, L. In Vitro Reconstruction of Neuro-Epidermal Connections. *J. Investig. Dermatol.* **2007**, *127*, 979–981. https://doi.org/10.1038/sj.jid.5700646.
30. Guéniche, A.; Bastien, P.; Ovigne, J.M.; Kermici, M.; Courchay, G.; Chevalier, V.; Breton, L.; Castiel-Higounenc, I. Bifidobacterium longum lysate, a new ingredient for reactive skin. *Exp. Dermatol.* **2009**, *19*, e1–e8. https://doi.org/10.1111/j.1600-0625.2009.00932.x.
31. Muller, Q.; Beaudet, M.-J.; de Serres-Bérard, T.; Bellenfant, S.; Flacher, V.; Berthod, F. Development of an innervated tissue-engineered skin with human sensory neurons and Schwann cells differentiated from iPS cells. *Acta Biomater.* **2018**, *82*, 93–101. https://doi.org/10.1016/j.actbio.2018.10.011.
32. Khammo, N.; Ogilvie, J.; Clothier, R.H. Development of an innervated model of human skin. *Altern. Lab. Anim. ATLA* **2007**, *35*, 487–491. https://doi.org/10.1177/026119290703500511.
33. Roggenkamp, D.; Falkner, S.; Stäb, F.; Petersen, M.; Schmelz, M.; Neufang, G. Atopic Keratinocytes Induce Increased Neurite Outgrowth in a Coculture Model of Porcine Dorsal Root Ganglia Neurons and Human Skin Cells. *J. Investig. Dermatol.* **2012**, *132*, 1892–1900. https://doi.org/10.1038/jid.2012.44.
34. Roggenkamp, D.; Köpnick, S.; Stäb, F.; Wenck, H.; Schmelz, M.; Neufang, G. Epidermal Nerve Fibers Modulate Keratinocyte Growth via Neuropeptide Signaling in an Innervated Skin Model. *J. Investig. Dermatol.* **2013**, *133*, 1620–1628. https://doi.org/10.1038/jid.2012.464.
35. Blais, M.; Mottier, L.; Germain, M.-A.; Bellenfant, S.; Cadau, S.; Berthod, F. Sensory Neurons Accelerate Skin Reepithelialization via Substance P in an Innervated Tissue-Engineered Wound Healing Model. *Tissue Eng. Part A* **2014**, *20*, 2180–2188. https://doi.org/10.1089/ten.tea.2013.0535.

36. Cadau, S.; Leoty-Okombi, S.; Pain, S.; Bechetoille, N.; André-Frei, V.; Berthod, F. In vitro glycation of an endothelialized and innervated tissue-engineered skin to screen anti-AGE molecules. *Biomaterials* **2015**, *51*, 216–225. https://doi.org/10.1016/j.biomaterials.2015.01.066.
37. Martorina, F.; Casale, C.; Urciuolo, F.; Netti, P.A.; Imparato, G. In vitro activation of the neuro-transduction mechanism in sensitive organotypic human skin model. *Biomaterials* **2017**, *113*, 217–229. https://doi.org/10.1016/j.biomaterials.2016.10.051.
38. Reichert, O.; Fleming, T.; Neufang, G.; Schmelz, M.; Genth, H.; Kaever, V.; Wenck, H.; Stäb, F.; Terstegen, L.; Kolbe, L.; et al. Impaired glyoxalase activity is associated with reduced expression of neurotrophic factors and pro-inflammatory processes in diabetic skin cells. *Exp. Dermatol.* **2017**, *26*, 44–50. https://doi.org/10.1111/exd.13118.
39. Bize, C.; Lavigne, E.G.; Monneuse, J.-M.; Chrétien, A.; Anchartechahar, V.; Hubaux, R.; Skorski, G.; Salmon, M.; Steinschneider, R. Evaluation of Soothing Activity of Aphloia Theiformis Extract Using an Innovative Model of Human Innerved Reconstructed Epidermis. The IFSCC Congress 2020 Yokohama, Virtual Event, 21–30 October 2020; pp. 1500–1508.
40. Lebonvallet, N.; Fluhr, J.W.; Le Gall-Ianotto, C.; Leschiera, R.; Talagas, M.; Reux, A.; Bataille, A.; Brun, C.; Oddos, T.; Pennec, J.; et al. A re-innervated in vitro skin model of non-histaminergic itch and skin neurogenic inflammation: PAR2-, TRPV1- and TRPA1-agonist induced functionality. *Ski. Health Dis.* **2021**, *1*, e66. https://doi.org/10.1002/ski2.66.
41. Gingras, M.; Bergeron, J.; Déry, J.; Durham, H.D.; Berthod, F. In vitro development of a tissue-engineered model of peripheral nerve regeneration to study neurite growth. *FASEB J.* **2003**, *17*, 2124–2126. https://doi.org/10.1096/fj.02-1180fje.
42. Baumbauer, K.M.; DeBerry, J.J.; Adelman, P.C.; Miller, R.H.; Hachisuka, J.; Lee, K.H.; Ross, S.E.; Koerber, H.R.; Davis, B.M.; Albers, K.M. Keratinocytes can modulate and directly initiate nociceptive responses. *eLife* **2015**, *4*, e09674. https://doi.org/10.7554/elife.09674.
43. Pang, Z.; Sakamoto, T.; Tiwari, V.; Kim, Y.-S.; Yang, F.; Dong, X.; Güler, A.D.; Guan, Y.; Caterina, M.J. Selective keratinocyte stimulation is sufficient to evoke nociception in mice. *Pain* **2015**, *156*, 656–665. https://doi.org/10.1097/j.pain.0000000000000092.
44. Tsantoulas, C.; Farmer, C.; Machado, P.; Baba, K.; McMahon, S.B.; Raouf, R. Probing Functional Properties of Nociceptive Axons Using a Microfluidic Culture System. *PLoS ONE* **2013**, *8*, e80722. https://doi.org/10.1371/journal.pone.0080722.
45. Valtcheva, M.V.; Copits, B.A.; Davidson, S.; Sheahan, T.D.; Pullen, M.Y.; McCall, J.G.; Dikranian, K.; Gereau, R.W. Surgical extraction of human dorsal root ganglia from organ donors and preparation of primary sensory neuron cultures. *Nat. Protoc.* **2016**, *11*, 1877–1888. https://doi.org/10.1038/nprot.2016.111.
46. Schutte, S.C.; Kadakia, F.; Davidson, S. Skin-Nerve Co-Culture Systems for Disease Modeling and Drug Discovery. *Tissue Eng. Part C: Methods* **2021**, *27*, 89–99. https://doi.org/10.1089/ten.tec.2020.0296.
47. Takahashi, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **2006**, *126*, 663–676. https://doi.org/10.1016/j.cell.2006.07.024.
48. Marmigere, F.; Ernfors, P. Specification and connectivity of neuronal subtypes in the sensory lineage. *Nat. Rev. Neurosci.* **2007**, *8*, 114–127. https://doi.org/10.1038/nrn2057.
49. Chambers, S.M.; Qi, Y.; Mica, Y.; Lee, G.; Zhang, X.-J.; Niu, L.; Bilsland, J.; Cao, L.; Stevens, E.; Whiting, P.; et al. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat. Biotechnol.* **2012**, *30*, 715–720. https://doi.org/10.1038/nbt.2249.
50. Lebonvallet, N.; Boulais, N.; Le Gall, C.; Pereira, U.; Gauché, D.; Gobin, E.; Pers, J.-O.; Jeanmaire, C.; Danoux, L.; Pauly, G.; et al. Effects of the re-innervation of organotypic skin explants on the epidermis. *Exp. Dermatol.* **2012**, *21*, 156–158. https://doi.org/10.1111/j.1600-0625.2011.01421.x.
51. Chéret, J.; Lebonvallet, N.; Buhé, V.; Carre, J.L.; Misery, L.; Le Gall-Ianotto, C. Influence of sensory neuropeptides on human cutaneous wound healing process. *J. Dermatol. Sci.* **2014**, *74*, 193–203. https://doi.org/10.1016/j.jdermsci.2014.02.001.
52. Tominaga, M.; Caterina, M.J.; Malmberg, A.B.; Rosen, T.A.; Gilbert, H.; Skinner, K.; Raumann, B.E.; I Basbaum, A.I.; Julius, D. The Cloned Capsaicin Receptor Integrates Multiple Pain-Producing Stimuli. *Neuron* **1998**, *21*, 531–543. https://doi.org/10.1016/s0896-6273(00)80564-4.
53. Zygmunt, P.M.; Petersson, J.; Andersson, D.A.; Chuang, -H.; Sørgård, M.; Di Marzo, V.; Julius, D.; Högestätt, E.D. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **1999**, *400*, 452–457. https://doi.org/10.1038/22761.
54. Thresh, L. Isolation of Capsaicin. *Pharm. J.* **1846**, *6*, 941–947.
55. Guimarães, M.Z.P.; de Vecchi, R.; Vitória, G.; Sochacki, J.K.; Paulsen, B.S.; Lima, I.; Rodrigues da Silva, F.; da Costa, R.F.M.; Castro, N.G.; Breton, L.; et al. Generation of IPSC-Derived Human Peripheral Sensory Neurons Releasing Substance P Elicited by TRPV1 Agonists. *Front. Mol. Neurosci.* **2018**, *11*, 277.
56. Sulk, M.; Steinhoff, M. Chapter 17—Role of TRP Channels in Skin Diseases. In *TRP Channels as Therapeutic Targets*; Szallasi, A., Ed.; Academic Press: Boston, MA, USA, 2015; pp. 293–323. ISBN 978-0-12-420024-1.
57. Armstrong, S.A.; Herr, M.J. *Physiology, Nociception*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
58. Taylor, A.M.; Blurton-Jones, M.; Rhee, S.W.; Cribbs, D.H.; Cotman, C.W.; Jeon, N.L. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat. Methods* **2005**, *2*, 599–605. https://doi.org/10.1038/nmeth777.
59. Li, J.; Chen, J.; Bai, H.; Wang, H.; Hao, S.; Ding, Y.; Peng, B.; Zhang, J.; Li, L.; Huang, W. An Overview of Organs-on-Chips Based on Deep Learning. *Research* **2022**, *2022*, 1–20. https://doi.org/10.34133/2022/9869518.
60. Ponmozhi, J.; Dhinakaran, S.; Varga-Medveczky, Z.; Fónagy, K.; Bors, L.; Iván, K.; Erdő, F. Development of Skin-On-A-Chip Platforms for Different Utilizations: Factors to Be Considered. *Micromachines* **2021**, *12*, 294. https://doi.org/10.3390/mi12030294.

61. Risueño, I.; Valencia, L.; Jorcano, J.L.; Velasco, D. Skin-on-a-chip models: General overview and future perspectives. *APL Bioeng.* **2021**, *5*, 030901. https://doi.org/10.1063/5.0046376.
62. Wufuer, M.; Lee, G.; Hur, W.; Jeon, B.; Kim, B.J.; Choi, T.H.; Lee, S. Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. *Sci. Rep.* **2016**, *6*, 37471. https://doi.org/10.1038/srep37471.
63. Kim, J.J.; Ellett, F.; Thomas, C.N.; Jalali, F.; Anderson, R.R.; Irimia, D.; Raff, A.B. A microscale, full-thickness, human skin on a chip assay simulating neutrophil responses to skin infection and antibiotic treatments. *Lab Chip* **2019**, *19*, 3094–3103. https://doi.org/10.1039/c9lc00399a.
64. Wagner, I.; Materne, E.-M.; Brincker, S.; Süßbier, U.; Frädrich, C.; Busek, M.; Sonntag, F.; Sakharov, D.A.; Trushkin, E.V.; Tonevitsky, A.G.; et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. *Lab Chip* **2013**, *13*, 3538–3547. https://doi.org/10.1039/c3lc50234a.
65. Maschmeyer, I.; Hasenberg, T.; Jaenicke, A.; Lindner, M.; Lorenz, A.K.; Zech, J.; Garbe, L.-A.; Sonntag, F.; Hayden, P.; Ayehunie, S.; et al. Chip-based human liver–intestine and liver–skin co-cultures—A first step toward systemic repeated dose substance testing in vitro. *Eur. J. Pharm. Biopharm.* **2015**, *95*, 77–87. https://doi.org/10.1016/j.ejpb.2015.03.002.
66. Maschmeyer, I.; Lorenz, A.K.; Schimek, K.; Hasenberg, T.; Ramme, A.P.; Hübner, J.; Lindner, M.; Drewell, C.; Bauer, S.; Thomas, A.; et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip* **2015**, *15*, 2688–2699. https://doi.org/10.1039/c5lc00392j.
67. Kühnl, J.; Tao, T.P.; Brandmair, K.; Gerlach, S.; Rings, T.; Müller-Vieira, U.; Przibilla, J.; Genies, C.; Jaques-Jamin, C.; Schepky, A.; et al. Characterization of application scenario-dependent pharmacokinetics and pharmacodynamic properties of permethrin and hyperforin in a dynamic skin and liver multi-organ-chip model. *Toxicology* **2021**, *448*, 152637. https://doi.org/10.1016/j.tox.2020.152637.
68. Tavares, R.S.N.; Tao, T.P.; Maschmeyer, I.; Maria-Engler, S.S.; Schäfer-Korting, M.; Winter, A.; Zoschke, C.; Lauster, R.; Marx, U.; Gaspar, L.R. Toxicity of topically applied drugs beyond skin irritation: Static skin model vs. Two organs-on-a-chip. *Int. J. Pharm.* **2020**, *589*, 119788. https://doi.org/10.1016/j.ijpharm.2020.119788.
69. Ataç, B.; Wagner, I.; Horland, R.; Lauster, R.; Marx, U.; Tonevitsky, A.G.; Azar, R.P.; Lindner, G. Skin and hair on-a-chip: In vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. *Lab Chip* **2013**, *13*, 3555–3561. https://doi.org/10.1039/c3lc50227a.
70. Misery, L. Skin, immunity and the nervous system. *Br. J. Dermatol.* **1997**, *137*, 843–850. https://doi.org/10.1046/j.1365-2133.1997.19762090.x.
71. Roosterman, D.; Goerge, T.; Schneider, S.W.; Bunnett, N.W.; Steinhoff, M. Neuronal Control of Skin Function: The Skin as a Neuroimmunoendocrine Organ. *Physiol. Rev.* **2006**, *86*, 1309–1379. https://doi.org/10.1152/physrev.00026.2005.
72. Ji, R.-R.; Samad, T.A.; Jin, S.-X.; Schmoll, R.; Woolf, C.J. p38 MAPK Activation by NGF in Primary Sensory Neurons after Inflammation Increases TRPV1 Levels and Maintains Heat Hyperalgesia. *Neuron* **2002**, *36*, 57–68. https://doi.org/10.1016/s0896-6273(02)00908-x.