Influence of gap junctions upon Ca\(^{2+}\) propagation from stimulated keratinocytes to DRG neurons

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Epidermal cells, such as keratinocytes, are regarded as the first sensory cells to transmit nociception and mechanoreception to free nerve endings extended from the dorsal root ganglion (DRG). Previous studies suggested that this transmission occurs as Ca\(^{2+}\) propagation via ATP receptors. Conversely, the influence of gap junctions on this Ca\(^{2+}\) propagation is largely unknown. Thus, we examined the localization and the role of connexin 43 among keratinocytes and DRG neurons. We co-cultured keratinocytes and DRG neurons and investigated the effect of pharmacological blockade of gap junctions on Ca\(^{2+}\) propagation upon stimulation of a single keratinocyte. Immunocytochemical experiments showed that connexin 43 is localized between keratinocytes and between keratinocytes and DRG neurons. Octanol, a gap junction inhibitor, significantly suppressed the concentrical Ca\(^{2+}\) propagation. Therefore, we conclude that the Ca\(^{2+}\) propagation mechanism via gap junctions from stimulated keratinocytes to free nerve endings should be taken into account.

Key words: connexin 43, dorsal root ganglion, free nerve ending, octanol, skin

Keratinocytes can act as the first sensory cells to transmit tactile stimuli to free nerve endings extended from the dorsal root ganglion (DRG). Although previous studies suggested that this transmission occurs as Ca\(^{2+}\) propagation via ATP receptors, the present findings showed that connexin 43 is localized between keratinocytes and DRG neurons, and that the application of octanol suppresses the concentrical Ca\(^{2+}\) propagation, indicating that the transmission occurs as Ca\(^{2+}\) propagation via not only ATP receptors but also gap junctions. The effect of gap junctions between keratinocytes and DRG neurons should be considered on the transmission of nociception and mechanoreception.

Introduction

Nociception and mechanoreception in the skin activate keratinocytes, resulting in the excitement of free nerve endings elongated from dorsal root ganglion (DRG) neurons [1]. Ca\(^{2+}\) propagation via ATP receptors such as P2X and P2Y is believed to play a significant role in the first step of this information transmission [1-7].
If ATP is the only mediator for pain in the skin, ATP receptor inhibitors should alleviate pain; however, it is insufficient because ATP is not the only molecule engaged in Ca\(^{2+}\) propagation. The role of gap junctions in Ca\(^{2+}\) propagation must be investigated [8]. For example, inflammation is perceived as “pain” when transmitted through gap junctions between various cells including keratinocytes [9]. Gap junctions are also crucial for skin function as a barrier to protect against inflammation [10]. They are also involved in the Ca\(^{2+}\) wave caused by the wound [11]. Mechanical stimulation evokes Ca\(^{2+}\) waves in keratinocytes via gap junctions [12]. However, to the best of our knowledge, the effects of gap junctions on the transmission of information from keratinocytes to DRG neurons have not yet been thoroughly investigated. Transient receptor protein (TRP) channels, which are activated by mechanical stress, temperature and osmotic pressure, were found to be expressed in keratinocytes [13]. TRP channels have a calcium channel function, influencing Ca\(^{2+}\) propagation, even in keratinocytes. On the other hand, an optical recording was employed for studying cultured DRG neurite terminals, and TRP-mediated, capsaicin-induced Ca\(^{2+}\) and Na\(^{+}\) dynamics was analyzed in the nociceptive processes at an action potential time resolution [14]. These 2 independent results have also stirred us up to clarify how Ca\(^{2+}\) propagation occurs from keratinocytes to DRG neurons in detail.

In the present study, we examined the localization of gap junctions among keratinocytes and DRG neurons and investigated the effect of pharmacological blockade of gap junctions on Ca\(^{2+}\) propagation using co-cultured keratinocytes and DRG neurons.

Materials and Methods

Reagents and Chemicals

Dispase (383-02281) and penicillin–streptomycin (168-23191) were purchased from Wako. IMMU-MOUNT (9990402) and TrypLE (12563011) were purchased from Thermo Fisher Scientific. Keratinocyte medium (CnTRP) was purchased from CELLnTEC. Collagenase (C7657), DAPI (D9543), normal goat serum (G9023) and octanol (95446-1ML-F) were obtained from Sigma-Aldrich. Anti-beta tubulin 3/tuj1 antibody (ab78078), anti-connexin 43/GJA1 antibody (ab235282), anti-cytokeratin 14 antibody (ab7800), goat anti-mouse IgG H&L (Alexa Fluor 555, ab150114) and PPADS (ab120009) were obtained from abcam. Goat anti-rabbit IgG H&L (Alexa Fluor 488, #A27034) was obtained from Invitrogen. Fura-2 AM (348-05831) was obtained from Dojindo. A BSS-Ca\(^{2+}\) buffer contained 130 mM NaCl, 5.4 mM KCl, 1 mM MgSO\(_4\), 2 mM CaCl\(_2\), 5.5 mM glucose, and 20 mM HEPES (pH 7.4).

Co-culture of Keratinocytes and DRG Cells

The animal study protocol was approved by the Institutional Review Board of Waseda University (protocol code 2019-A042, April 1, 2019; 2020-A005, April 1, 2020; and 2021-A004, April 1, 2021). The co-culture protocol of keratinocytes and DRG cells was modified from the previous study [15]. Wistar rats (5–7 days old, both males and females were used) were anesthetized with sevoflurane and decapitated, and then the skin was exfoliated. After washing with PBS, the skin was shaken in 10 mL of keratinocyte enzyme solution (4 mg/mL dispase in HBSS(−)) at 4°C for 12–18 h. The skin was spread and incubated with 500 μL of TrypLE, and the epithelium and dermis were peeled off. The epithelium was spread again and shaken in 500 μL of TrypLE at room temperature for 20 min. Two mL of a keratinocyte medium with penicillin-streptomycin solution was added to the epithelium, and the epithelium was rubbed on the bottom of the dish with forceps. The cell suspension was filtered with a 100 μL filter and centrifuged (180×g, room temperature, 5 min), and the supernatant was aspirated. The cell pellet was suspended with the keratinocyte medium (1.0 × 10\(^5\) cells) and cultured on a collagen-coated dish.

The DRGs were isolated as follows. The dorsal side of the rat was cut open to expose the spinal cord and the DRGs. The exposed DRGs were isolated and washed with HBSS(−). The DRGs were shaken with 1 mL of a DRG enzyme solution (0.75 mg/mL collagenase and 3 mg/mL dispase in HBSS(−)) at 37°C for 30 min. The DRG cells were washed using a DRG medium (MEM containing 5% FBS, 5% FCS, and penicillin–streptomycin solution) with centrifugation and suspension. DRG cells (5.0 × 10\(^4\)) were inoculated with keratinocytes and then co-cultured for 5–7 days in the keratinocyte medium.

Immunocytochemistry

The immunocytochemistry protocol was modified from the previous study [15]. After co-culturing keratinocytes and DRG cells for 5 days, the cells were fixed with 1 mL of 4% paraformaldehyde in PBS at room temperature for 10 min and penetrated with 1 mL of 0.1% Triton X-100 in PBS for 10 min. For a blocking procedure, 500 μL of 5% normal goat serum in PBS was added to the cells for 30 min. For the primary antibody reaction, anti-beta tubulin 3/tuj1 antibody (1 μg/mL), anti-cytokeratin 14 antibody (1 μg/mL), or anti-connexin 43/GJA1 antibody (15 μg/mL) in PBS was incubated with the cells at 4°C for 1 h. For the secondary antibody reaction and DAPI labeling, goat anti-rabbit IgG H&L or goat anti-mouse IgG H&L was diluted at 1:200 with 5% normal goat serum in PBS coupled with 5 μg/mL DAPI, followed by incubation with the cells at room temperature for 1 h. The cells were encapsulated with a drop of IMMU-MOUNT and...
Covered with a cover glass. Immunofluorescence was measured using a confocal laser scanning microscope (Olympus Fluoview FV1000).

**Ca^{2+} Imaging**

The cells were incubated with 4 μM fura-2 AM solution in a BSS-Ca^{2+} buffer at 37°C for 20 min in an incubator (5% CO₂). The intracellular Ca^{2+} concentration was measured in a BSS-Ca^{2+} buffer using a CCD time-lapse microscope (Olympus IX81). The measurement wavelength of 510 nm for 340/380 nm excitation ratio for fura-2 allows accurate measurements of the intracellular Ca^{2+} concentration change. The sampling time for one image was 5 s. The total measuring time was 600 s. The data were analyzed with TI Workbench, which was provided at Waseda University. The gap junction blocker used was octanol, with a final concentration of 1000 μM. The P2 purinergic receptor blocker used was PPADS, with a final concentration of 400 μM. The glass micropipette tip was contacted to a single keratinocyte to stimulate it 90 s after adding the reagent (octanol or control). The cell number was assigned to each cell for the Ca^{2+} response analysis. On the other hand, the cells surrounding the stimulated keratinocyte were referred to as the cells in the 2nd zone, 3rd zone, and so on.

**Statistical Analysis**

Data are expressed as mean ± SEM, with statistical significance at \( P < 0.05 \). Data from multiple groups were analyzed using one-way analysis of variance followed by a post-hoc Tukey-Kramer test. The computer software used was a free software MEPHAS provided by Osaka University (http://www.gen-info.osaka-u.ac.jp/MEPHAS).

**Results**

**Co-culture of Keratinocytes and DRG Cells**

Immunocytochemistry was used to determine whether the co-culture of keratinocytes and DRG neurons was successful (Figure 1A). Keratinocytes—labeled using anti-cytokeratin 14 antibody—were cultured in a paving stone shape, and DRG neurons—labeled using anti-beta tubulin 3/tuj1 antibody—extended their thin neurites to keratinocytes. We decided to mechanically stimulate single keratinocytes distant from the cell body of DRG neurons in our experiments because keratinocytes in the epidermis and DRG neurons are distant from each other in the body (Figure 1B).

![Figure 1 Co-culture of keratinocytes and DRG neurons. (A) Triple immunostaining was performed for cytokeratin 14 (red), β-III tubulin (green), and DAPI (blue). (B) A single keratinocyte was stimulated using the tip of a glass micropipette. Scale bar = 100 μm.](image)

**Gap Junctions between Keratinocytes and between Keratinocytes and DRG Neurons**

Double staining of cytokeratin 14 and connexin 43 was performed to examine the protein expression of gap junctions in keratinocytes. Similarly, double staining of β-III tubulin and connexin 43 was performed to examine the protein expression of gap junctions in DRG neurons (Figure 2). Stained keratinocytes were observed like paving stones (Figure 2A-C). Co-staining of cytokeratin 14 and connexin 43 revealed that gap junctions exist around keratinocytes (Figure 2D-F). On the other hand, DRG neurons extended thin neurites (Figure 2G-I). β-III tubulin and connexin 43 were found to be co-localized around the cell body of neurons, and some double staining dots can be slightly seen around the neurite regions (Figure 2J-L). Especially, an arrow in Fig. 2K points to the staining spot of connexin 43 in a neurite.
Figure 2 Presence of gap junctions between keratinocytes and between keratinocytes and DRG neurons. (A-C) Single immunostaining was performed for cytokeratin 14 (red) to identify keratinocytes. (D-F) Triple immunostaining was performed for cytokeratin 14 (red), connexin 43 (green), and DAPI (blue). (G-I) Single immunostaining was performed for β-III tubulin (red) to identify DRG neurons. (J-L) Triple immunostaining was performed for β-III tubulin (red), connexin 43 (green), and DAPI (blue). Arrows indicate the connexin 43 stainings. Especially, the arrow in (K) points to the staining spot near a neurite. Scale bar = 20 μm.
Ca²⁺ Imaging after Stimulation of A Single Keratinocyte

Ca²⁺ imaging revealed that when a single keratinocyte was mechanically stimulated, Ca²⁺ spread concentrically, and DRG neurons located away from the stimulated keratinocyte also demonstrated an increase in Ca²⁺ (Figure 3A-C). Here we refer stimulated keratinocyte as the 1st keratinocyte, followed by 2nd, 3rd, and Nth keratinocyte in that order. Each of the 1st, 2nd, 3rd, and Nth cells is one cell thick. Administration of a gap junction inhibitor, octanol, to the co-cultured cells suppressed the diffusion of Ca²⁺ in keratinocytes and DRG neurons (Figure 3D-F).

Figure 3  Ca²⁺ propagation from a single keratinocyte stimulated mechanically to surrounding keratinocytes and DRG neurons. (A-C) Ca²⁺ images were obtained using fura-2 labeling. F340 and F380 are the fluorescent images excited by 340 and 380 nm, respectively. The pseudocolor indicates the change in fluorescence, as F340 is set to become redder as calcium concentration increases, while F380 is set to become bluer. Cell # is assigned to keratinocytes and DRG neurons, and cell #1 indicates a single keratinocyte stimulated mechanically (as shown in black circles). The image of (B) was observed 60 s after stimulation. (D-F) Ca²⁺ propagation was suppressed in the presence of 1000 μM octanol. The image of (E) was observed 60 s after stimulation. Feeble Ca²⁺ responses were observed in DRG neurons. Scale bar = 50 μm.

Furthermore, the changes in the fluorescence intensity corresponding to Ca²⁺ concentration and the changes in the number of responded cells were examined (Figure 4). The maximum value of each Ca²⁺ response intensity was normalized by the maximum value of Ca²⁺ response in the 1st keratinocyte, which was stimulated mechanically. The Ca²⁺ response spread concentrically about keratinocytes in the 2nd, 3rd and 4th zones of keratinocytes with the attenuation of the Ca²⁺ response intensity (Figure 4A) and the decrease in the number of Ca²⁺ responsive cells (Figure 4B). Here, we have to note that the cells surrounding the stimulated keratinocyte were concentrically referred to as the cells in the 2nd zone, 3rd zone, and so on. The application of octanol suppressed the Ca²⁺ response intensity and the number of Ca²⁺-responsive cells in the 2nd, 3rd, and 4th zones of keratinocytes ($P < 0.05$, Figure 4A,B). Regarding DRG neurons, the Ca²⁺ responses were
observed in the 3rd and 4th zones of DRG neurons, but the number of Ca\(^{2+}\)-responsive cells was small regardless of the presence of octanol (Figure 4C,D). Because the blockade of gap junctions did not diminish the Ca\(^{2+}\) responses, we used a 400 \(\mu\)M solution of PPADS to inhibit P2 purinergic receptors. However, the Ca\(^{2+}\) responses were still observed in the 3rd and 4th zones of DRG neurons, further supporting the involvement of gap junctions in Ca\(^{2+}\) propagation.

**Figure 4** Changes in fluorescence intensity correspond to Ca\(^{2+}\) concentration and those in the number of responded cells. The cells surrounding the stimulated single keratinocyte are concentrically referred to as the cells in the 2nd zone, the 3rd zone, and so on. The number of rats used was at least 4. (A,B) Ca\(^{2+}\) responses in surrounding keratinocytes. The Ca\(^{2+}\) response intensity represented the maximum value of each cell that was normalized by the maximum value of Ca\(^{2+}\) response in the 1st keratinocyte, which was stimulated mechanically. The Ca\(^{2+}\) responses spread concentrically in the 2nd, 3rd, and 4th zones of keratinocytes. Octanol suppressed the Ca\(^{2+}\) response intensity and the number of Ca\(^{2+}\)-responsive cells in the 2nd, 3rd, and 4th zones of keratinocytes. (C,D) Ca\(^{2+}\) responses in surrounding DRG neurons. The Ca\(^{2+}\) responses were observed in the 3rd and 4th zones of neurons, but the number of Ca\(^{2+}\)-responsive cells was small regardless of the presence of octanol. \(^*P < 0.05\).
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Discussion

We attempted to establish an \emph{in vitro} model for free nerve endings (neurites of DRG neurons) and epidermal cells (keratinocytes). The presence of gap junctions between keratinocytes and keratinocytes and DRG neurons was confirmed with a high degree of certainty. We started using a lower concentration of octanol than in our present experiments to suppress the Ca$^{2+}$ propagation; however, the apparent effect of this blockade was obtained only at 1000 $\mu$M, as shown in Figures 3 and 4. Such a high concentration was consistent with other previous studies [16,17]. These findings suggest that gap junctions are used for signal transduction between keratinocytes and between keratinocytes and neurons.

We found that $\beta$-III tubulin and connexin 43 were co-localized mainly around the cell bodies of DRG neurons, whereas the double staining dots were less observed around the neurites (Figure 2). Previous studies showed the gap junctions between the cell bodies of DRG neurons and those of satellite glial cells and between the neurites of DRG neurons [18]. Many previous studies only focused on the gap junctions between the cell bodies of DRG neurons and those of satellite glial cells, but not keratinocytes [18]. Furthermore, gap junctions are generally abundant in the cell bodies and less in the neurites [19]. More importantly, we would like to insist that the immuno-positive spots of connexin 43 were observed in the neurites (Figure 2), showing that the neurites of DRG neurons can communicate with keratinocytes that are localized far from DRG neuron cell bodies via gap junctions. We do not deny the influence of ATP by any means in Ca$^{2+}$ propagation from keratinocytes and DRG neurons, but our results strongly suggest that there is an influence of gap junction on this propagation at the same time. We need to obtain more evidence about gap junctions between neurites of DRG neurons and keratinocytes.

Skin cells, as the first sensory receptors that respond to external stimuli, are of great interest. As described in the Introduction, receptors activated by mechanical stress, temperature, and osmotic pressure have been reported to be expressed in keratinocytes, suggesting that skin cells are involved in sensory reception [20]. For example, in the TRP channel family that acts on sensory reception of temperature and physical and chemical stimuli, the expression of TRPV1, TRPV3, and TRPV4 has been reported [13,21,22]. TRPV1 is activated by heat (>43°C), acidic pH (<6.6), and capsaicin [23,24]; TRPV3 is activated by heat (>35°C) and mechanical stimulation [25,26]; and TRPV4 is activated by osmotic pressure [27].

Recent studies have revealed that DRG neurons contain oxytocin and its related receptors including TRP channels. Oxytocin has been reported to play some roles in the DRGs [28–32], because oxytocin acts on DRG neurons and suppresses the firing of action potentials [33,34]. The expression of oxytocin was also confirmed in DRG neurons [35]. A recent study suggested that oxytocin-expressing DRG neurons with small cell bodies and large cell bodies probably correspond to C-fiber neurons and A$\beta$-fiber neurons, respectively. Furthermore, the oxytocin-expressing neurons contained not only TRPV1 but also piezo-type mechanosensitive ion channel component 2 (Piezo2), suggesting that oxytocin may be released by mechanical stimulation regardless of nociception. Thus, mechanoreception and nociception themselves may induce the autocrine/paracrine function of oxytocin in the DRG, contributing to alleviation of pain [36].

We would like to perform these experiments using human cells in the future. Human keratinocytes are commercially available from several companies, for example, Thermo Fisher Scientific and Zen-Bio. The present question is how we can obtain human free nerve endings. One possible answer is the use of human dental pulp stem cells [37,38]. If an \emph{in vitro} model for a human free nerve ending model in the skin is established, the development of analgesics will advance considerably.

Conclusion

Keratinocytes can act as the first sensory cells to transmit tactile stimuli to free nerve endings extended from the DRG. To our best knowledge, it is known that mechanical stimulation evokes Ca$^{2+}$ waves in keratinocytes [12], but the Ca$^{2+}$ propagation from keratinocytes to DRG neurons via gap junctions has not yet been clarified. The present findings clearly showed that this transmission occurs as Ca$^{2+}$ propagation via ATP receptors and gap junctions. Thus, we insist that the effect of gap junctions between keratinocytes and DRG neurons on the transmission of nociception and mechanoreception should be considered.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, K.O. and E.I.; methodology, E.I.; investigation, C.S., K.T., and K.I.; writing—original draft preparation, C.S. and E.I.; writing—review and editing, K.T. and K.O.; project administration, E.I.; funding acquisition,
K.O. and E.I. All authors have read and agreed to the published version of the manuscript.

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References

[1] Shindo, Y., Fujita, K., Tanaka, M., Fujio, H., Hotta, K., Oka, K. Mechanical stimulus-evoked signal transduction between keratinocytes and sensory neurons via extracellular ATP. Biochem. Biophys. Res. Commun. 582, 131–136 (2021). https://doi.org/10.1016/j.bbrc.2021.10.046

[2] Pillai, S., Bikle, D. D. Adenosine triphosphate stimulates phosphoinositide metabolism, mobilizes intracellular calcium, and inhibits terminal differentiation of human epidermal keratinocytes. J. Clin. Invest. 90, 42–51 (1992). https://doi.org/10.1073/JCI115854

[3] Boarder, M. R., Hourani, S. M. The regulation of vascular function by P2 receptors: Multiple sites and multiple receptors. Trends. Pharmacol. Sci. 19, 99–107 (1998). https://doi.org/10.1016/s0165-6147(98)01170-5

[4] Dixon, C. J., Bowler, W. B., Littlewood-Evans, A., Dillon, J. P., Bilbe, G., Sharpe, G. R., et al. Regulation of epidermal homeostasis through P2Y2 receptors. Br. J. Pharmacol. 127, 1680–1686 (1999). https://doi.org/10.1038/sj.bjp.0702653

[5] Koizumi, S., Fujishita, K., Inoue, K., Shigemoto-Mogami, Y., Tsuda, M., Inoue, K. Ca2+ waves in keratinocytes are transmitted to sensory neurons: the involvement of extracellular ATP and P2Y2 receptor activation. Biochem. J. 380, 329–338 (2004). https://doi.org/10.1042/BJ20031089

[6] Suzuki, T. Recent progress in sensory mechanism. Bull. Tokyo Dent. Coll. 48, 1–7 (2007). https://doi.org/10.2209/tdcpublication.48.1

[7] Moehring, F., Cowie, A. M., Menzel, A. D., Weyer, A. D., Grzybowski, M., Arzua, T., et al. Keratinocytes mediate innocuous and noxious touch via ATP-P2X4 signaling. eLife 7, e31684 (2018). https://doi.org/10.7554/eLife.31684

[8] Yamane, Y., Shiga, H., Asou, H., Ito, E. GAP junctional channel inhibition alters actin organization and calcium propagation in rat cultured astrocytes. Neuroscience 112, 593–603 (2002). https://doi.org/10.1016/s0306-4522(02)00095-7

[9] Hansson, E., Sköldebrand, E. Coupled cell networks are target cells of inflammation, which can spread between different body organs and develop into systemic chronic inflammation. J. Inflamm. 12, 44 (2015). https://doi.org/10.1186/s12950-015-0091-2

[10] Lee, S. E., Lee, S. H. Skin barrier and calcium. Ann. Dermatol. 30, 265–275 (2018). https://doi.org/10.5021/ad.2018.30.3.265

[11] Hudson, L., Begg, M., Wright, B., Cheek, T., Jahoda, C. A. B., Reynolds, N. J. Dominant effect of gap junction communication in wound-induced calcium-wave, NFAT activation and wound closure in keratinocytes. J. Cell. Physiol. 236, 8171–8183 (2021). https://doi.org/10.1002/jcp.30488

[12] Tsutsumi, M., Inoue, K., Denda, S., Ikeyama, K., Goto, M., Denda, M. Mechanical-stimulation-evoked calcium waves in proliferating and differentiated human keratinocytes. Cell Tissue Res. 338, 99–106 (2009). https://doi.org/10.1007/s00441-009-0848-0

[13] Denda, M., Fuziwaara, S., Inoue, K., Denda, S., Akamatsu, H., Tomitaka, A., et al. Immunoreactivity of VR1 on epidermal keratinocyte of human skin. Biochem. Biophys. Res. Commun. 285, 1250–1252 (2001). https://doi.org/10.1006/ijbc.2001.5299

[14] Goldstein, R. H., Katz, B., Lev, S., Binshok, A. M. Ultrafast optical recording reveals distinct capsaicin-induced ion dynamics along single nociceptive neurite terminals in vitro. J. Biomed. Opt. 22, 76010 (2017). https://doi.org/10.1117/1.JBO.22.7.076010

[15] Ulmann, L., Rodeau, J. L., Danoux, L., Contet-Audonneau, J. L., Pauly, G., Schlichter, R. Trophic effects of keratinocytes on the axonal development of sensory neurons in a coculture model. Eur. J. Neurosci. 26, 113–125 (2007). https://doi.org/10.1111/j.1460-9568.2007.05649.x

[16] Rose, C. R., Ransom, B. R. Gap junctions equalize intracellular Na+ concentration in astrocytes. Glia 20, 299–307
[38] Arimura, Y., Shindo, Y., Yamanaka, R., Mochizuki, M., Hotta, K., Nakahara, T., et al. Peripheral-neuron-like properties of differentiated human dental pulp stem cells (hDPSCs). PLoS One 16, e0251356 (2021). https://doi.org/10.1371/journal.pone.0251356