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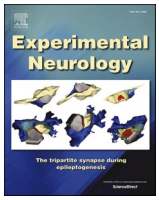

Review Article

Recent advances for using human induced-pluripotent stem cells as pain-in-a-dish models of neuropathic pain

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ABSTRACT

Neuropathic pain is amongst the most common non-communicable disorders and the poor effectiveness of current treatment is an unmet need. Although pain is a universal experience, there are significant inter-individual phenotypic differences. Developing models that can accurately recapitulate the clinical pain features is crucial to better understand underlying pathophysiological mechanisms and find innovative treatments. Current data from heterologous expression systems that investigate properties of specific molecules involved in pain signaling, and from animal models, show limited success with their translation into the development of novel treatments for pain. This is in part because they do not recapitulate the native environment in which a particular molecule functions, and due to species-specific differences in the properties of several key molecules that are involved in pain signaling. The limited availability of post-mortem tissue, in particular dorsal root ganglia (DRG), has hampered research using human cells in pre-clinical studies. Human induced-pluripotent stem cells (iPSCs) have emerged as an exciting alternative platform to study patient-specific diseases. Sensory neurons that are derived from iPSCs (iPSC-SNs) have provided new avenues towards elucidating peripheral pathophysiological mechanisms, the potential for development of personalized treatments, and as a cell-based system for high-throughput screening for discovering novel analgesics. Nevertheless, reprogramming and differentiation protocols to obtain nociceptors have mostly yielded immature homogenous cell populations that do not recapitulate the heterogeneity of native sensory neurons. To close the gap between native human tissue and iPSCs, alternative strategies have been developed. We will review here recent developments in differentiating iPSC-SNs and their use in pre-clinical translational studies. Direct conversion of stem cells into the cells of interest has provided a more cost- and time-saving method to improve reproducibility and diversity of sensory cell types. Furthermore, multi-cellular strategies that mimic *in vivo* microenvironments for cell maturation, by improving cell contact and communication (co-cultures), reproducing the organ complexity and architecture (three-dimensional organoid), and providing iPSCs with the full spatiotemporal context and nutrients needed for acquiring a mature phenotype (xenotransplantation), have led to functional sensory neuron-like systems. Finally, this review touches on novel prospective strategies, including fluorescent-tracking to select the differentiated neurons of relevance, and dynamic clamp, an electrophysiological method that allows direct manipulation of ionic conductances that are missing in iPSC-SNs.

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1. Introduction

Neuropathic pain represents a major clinical burden, affecting 7–8% of the global population(Bouhassira, 2019), with clinical presentation varying across a wide spectrum, even in related individuals(Mis et al., 2019). Available treatments are largely ineffective and can induce severe side effects(Sopacua et al., 2019). Therefore, there is urgency in better understanding patient-specific differences in pain pathophysiology and treatment responsiveness. However, current models have failed to accurately recapitulate the full phenotypic complexity of neuropathic pain, which further hinders development of novel and more effective analgesics.

The study of voltage-gated sodium channels (VGSC) that are expressed in dorsal root ganglion (DRG) neurons, in the context of painful neuropathies, has been a hot topic for several decades. VGSCs are key determinants of cellular excitability and action potential generation and propagation, which makes them essential for noxious stimuli transduction. Gain-of-function (GoF) mutations in *SCN9A* ($Na_V1.7$), *SCN10A* ($Na_V1.8$) and *SCN11A* ($Na_V1.9$) have been associated with a plethora of pain pathologies ranging from extreme painful disorders to the complete loss of pain sensation (Bennett et al., 2019; Dib-Hajj and Waxman, 2019; Huang et al., 2019; Huang et al., 2014; Huang et al., 2017). Although it is intuitively obvious why a GoF in sodium channels can lead to pain, these studies also showed that GoF mutations that cause a very large depolarization of the resting potential in DRG neurons lead to resting inhibition of VGSCs, which suppresses the excitability of these neurons. The development of transfecting methods of human channels into heterologous cell systems has been a major step forward in ion channel research(Dib-Hajj et al., 2009), allowing the identification of functional properties, downstream pathways, and characterization of VGSC mutations(Dib-Hajj et al., 2005; Geha et al., 2016; Yang et al., 2016). However, the human embryonic kidney 293 cell lines (HEK293) heterologous expression system, which is widely used in drug discovery, is limited to the sole expression of the channel of interest in a non-native cell background. While advantageous to study intrinsic channel properties, gating and pharmacological responses may not accurately reflect the behavior of the channel in its native cell background, reducing the model's validity. *Ex vivo* nucleofection of mutant channels into rodent DRG provides a more appropriate cell type for electrophysiological and pharmacological assessment; however, recordings of human DRG have highlighted important inter-species differences in these channel properties which alters neuronal firing(Han et al., 2015; Zhang et al., 2017). Additionally, expression of pain-related genes differs between humans and rodents, resulting in distinct pathophysiology and pharmacological response. Comparative transcriptome analyses have reported only 50–70% of DRG genes expressed in mice(Parisien et al., 2017; Ray et al., 2018; Shiers et al., 2020a; Tavares-Ferreira et al., 2022) and 80% in rats to overlap with those expressed in humans, with variations noted in pain-regulating proteins(Schwaid et al., 2018) which could impact the translation of animal data into successful human clinical trials. Thus, a human sensory neuronal model is critically needed. However, limited access to human DRG especially from pain patients has reduced their extensive use and highlights the need for a surrogate model that mimics human DRG neuronal properties(Middleton et al., 2021; Renthal et al., 2021).

The conversion of adult somatic cells into induced pluripotent stem cells (iPSCs) and their derivation into any desired cell type has revolutionized the study of patient-specific cells for mechanistic and preclinical studies(Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Specifically, minimally invasive collection of tissues from subjects has enabled studying molecular mechanisms and signaling pathways *in vitro* from both patients and healthy individuals, offsetting challenges of procuring post-mortem human neuronal tissues. Human iPSCs harbor and preserve the donor's full genetic background and transcriptional machinery throughout differentiation(Inoue et al., 2014). The differentiation of sensory neurons from iPSCs(Chambers et al., 2012) has led to studies of disease susceptibility(Chrysostomidou et al., 2021; Mis et al., 2019; Yuan et al., 2021). The markers that have been used to confirm a sensory neuron-like phenotype include RUNX1, BRN3A, ISL1, TRPV1, pRDM12. A recent paper posted on BioRxiv (Deng et al., 2022) reports a comprehensive transcriptomic analysis of iPSC-SNs, which confirms the sensory neuron-like nature of these cells, in addition to morphological evidence suggesting a pseudo-unipolar structure of these neurons. Additionally, being patient-specific, the cells already selectively express variants of interest driven by their endogenous promoters and retain intact endogenous signaling cascades. Their amenability to genome engineering has made them particularly appealing for studying rare mutations that can be introduced or corrected(Hockemeyer and Jaenisch, 2016; Lampert et al., 2020; McDermott et al., 2019a; Pettingill et al., 2019; Viventi and Dottori, 2018). Finally, differentiation of sensory neurons from iPSCs may provide insights into disease progression, bringing us a step closer to the bed side.

This review will cover the recent advances in using iPSCs in pain research, as well as the current limitations and prospective solutions to improve iPSC-based disease modeling.

2. Current state of iPSCs in pain research

The use of iPSC-nociceptors derived from chronic pain patients has been invaluable to the study of ion channels and human nociception, as well as to providing new avenues in personalized medicine and development of novel therapeutics. Demonstrative of their ability to recapitulate the clinical phenotype, iPSC-derived sensory neurons (iPSC-SNs) have been generated from patients with $Na_V1.7$-related inherited erythromelalgia (IEM), who exhibit severe pain episodes that are exacerbated by heat. Electrophysiological assessment showed aberrant ectopic activity and hyperexcitability in response to increasing temperatures, which correlated with different individual levels of pain severity(Cao et al., 2016; Meents et al., 2019; Mis et al., 2019; Yuan et al., 2021). Similar findings were reported in small fiber neuropathy (SFN)(Namer et al., 2019) and congenital insensitivity to pain (CIP) (McDermott et al., 2019a), where iPSC-SNs manifested increased spontaneous activity and reduced nociceptor excitability to depolarizing stimuli, respectively. Human iPSC-SNs from patients with hereditary sensory neuropathy type 1 (HSN1) revealed altered ganglioside biosynthesis as a major contributor to reduced axonal outgrowth and myelin stability(Clark et al., 2021). Finally, as stem cell-derived nociceptors are assumed to be naïve and uninjured(Chrysostomidou et al., 2021; Jones et al., 2018), they offer the possibility to study *de novo* injury-induced pain mechanisms compared to naïve counterparts. In a model of chemotherapy-induced peripheral neuropathy, healthy iPSC-SNs exposed to chemotherapeutics replicated the different responses observed in donors(Schinke et al., 2021).

The iPSC-SN model allowed the identification of two novel pain resilience genes. Utilizing whole-exome sequencing, patch-clamp and dynamic clamp recordings, *in vitro* analysis of iPSC-SNs from two families of IEM patients with $Na_V1.7$ GOF mutations, demonstrated neuronal firing behavior that paralleled the parent-child differences in pain phenotypes (less excitability of iPSC-SNs from the patient with less pain), but also pinpointed variants in potassium channels *KCNQ2* (Mis et al., 2019) and *KCNQ3* (Yuan et al., 2021), that explained at least some of the inter-individual differences in pain sensitivity within these families (Mis et al., 2019; Yuan et al., 2021).

Harvesting somatic cells from neuropathic pain patients with disease-causing mutations has also allowed the development of personalized treatment strategies. With recent advances in pharmacogenomics focusing on $Na_V1.7$-selective blockers, iPSC-SNs provide an ideal humanized *in vitro* platform to assay drug interventions and their application in specific patients (Cao et al., 2016; Capurro et al., 2020). Effective drug-induced VGSC blockade of iPSCs-SNs has been documented in both IEM and SFN patient-specific cells exposed to PF-05089771(Cao et al., 2016; Capurro et al., 2020) and lacosamide(Namer

et al., 2019), respectively. Follow-up clinical trials using PF-05089771 reported substantial reduction in several patients' pain scores(Cao et al., 2016; McDonnell et al., 2018) albeit without meeting the primary endpoint for the whole group. Treatment of one patient with lacosamide resulted in a reduction in C-fiber hyperexcitability(Namer et al., 2019) and amelioration of pain, demonstrating that patient-derived iPSCs can guide effective therapeutic design, bringing us a step closer to individualized treatments.

Moreover, the amenability to genome engineering of iPSC-SNs has facilitated the generation of isogenic lines, providing insights into genetic defects and new drug selectivity. Notably, CRISPR/Cas9-induced $Na_V1.7$ knock-out (KO) lines derived from healthy individuals paralleled the electrophysiological properties of CIP iPSC-SNs, while correcting one deleterious allele in CIP cells rescued the physiological phenotype(McDermott et al., 2019a). KO lines also offer the opportunity to assay the selectivity of clinically-relevant compounds(McDermott et al., 2019a). The $Na_V1.7$ blocker BIIB074, for example, while efficacious in trigeminal neuralgia patients(Zakrzewska et al., 2017), was confirmed to be non-selective to $Na_V1.7$ after reducing excitability in $Na_V1.7$ KO iPSC-SNs(Deuis et al., 2016), raising new prospects for using engineered iPSC-SNs in drug discovery(McDermott et al., 2019a).

Importantly, new treatments that have shown promising results in rodent models often do not successfully translate to the clinic (Mogil, 2009; Yezierski and Hansson, 2018); iPSC-SNs allow us to interrogate neurotoxicity and inter-species differences in a humanized system. The high scalability of iPSC-SNs enables high-throughput screening against large library of neurotoxic agents and chemotherapeutic drugs(Hoelting et al., 2016; Schinke et al., 2021), which has led to the identification of novel disease-modulating therapeutics and target-based mechanisms against relevant phenotypes(Elitt et al., 2018). Hence, drug-induced changes in iPSC excitability might become a useful tool to predict potential clinical outcomes in future trials(Cao et al., 2016; Namer et al., 2019).

3. Challenges

This exciting new cell-based "pain-in-a-dish" model faces its own set of challenges. First and foremost, the culture and maintenance of iPSCs is costly, highly technical and labor-intensive. Current protocols consist of multiple induction steps and necessitate weeks to months to reach

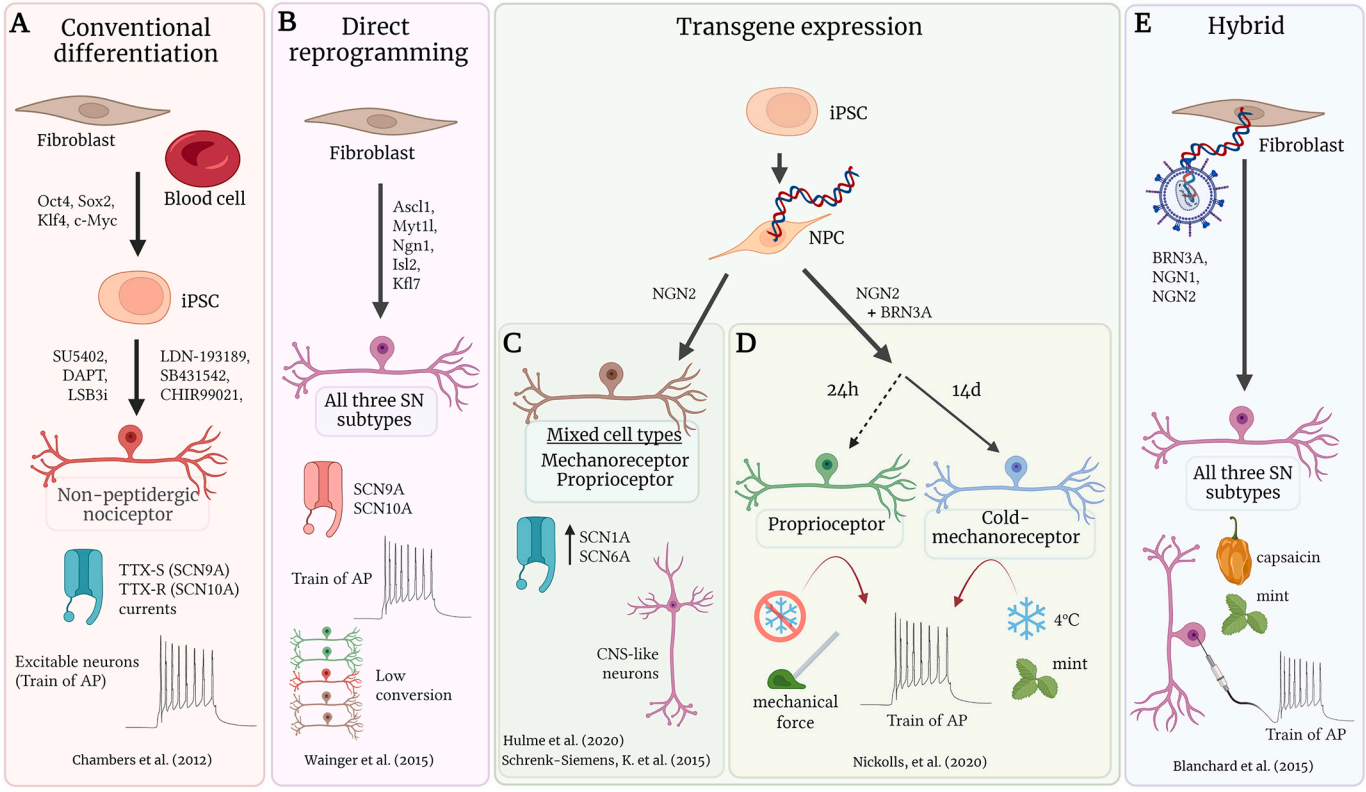

Fig. 1. Schematic representation of alternative protocols for deriving human somatic cells into sensory neurons (SN). (A) The conventional approach (Chambers et al., 2012) converts patient fibroblasts and blood cells into induced-pluripotent stem cells (iPSCs), which are then differentiated, using five small-molecule pathway inhibitors, into non-peptidergic nociceptors expressing sodium channels *SCN9A* ($Na_V1.7$, TTX-S), *SCN10A* ($Na_V1.8$, TTX-R) and *SCN11A* ($Na_V1.9$, TTX-R). Derived-nociceptors were reported to express functional $Na_V1.8$ channels and to produce TTX-S and TTX-R currents, as measured by current-clamp. However, the expression of $Na_V1.8$ channels has not been reproduced in subsequent studies using this protocol, and the TTX-R currents, when detected, have been argued to be produced by $Na_V1.5$ channels(Eberhardt et al., 2015). **(B)** Wainger et al. have implemented a direct reprogramming strategy, which bypasses the iPSC stage, *via* exposure to five alternative inhibitory molecules, and converts fibroblasts into diverse sensory neuronal subtypes that generate persistent sodium currents, typical of $Na_V1.8$ and $Na_V1.9$ sodium channels (Wainger et al., 2015). The limitation of this technique lays in its low conversion rate of nociceptors. On the other hand, **(C-D)** indirect (Hulme et al., 2020; Nickolls et al., 2020; Schrenk-Siemens et al., 2015) and **(E)** direct (or hybrid; (Blanchard et al., 2015)) transgene expression of lineage-specific transcription factors have led to factor- and time-dependent mixed neuronal population. **(C)** iPSC-derived neural precursor cells (NPC)-expressing *NGN2* alone led to sensory and CNS-like neurons, expressing higher mRNA levels of *SCN1A* and *SCN6A* than other sodium channels (Hulme et al., 2020; Schrenk-Siemens et al., 2015). **(D)** Additionally, length of reprogramming period affected the nature of the differentiated neurons, where short reprogramming (24 h) induced proprioceptors, based on their response to mechanical pressure but not cold stimuli (menthol, low temperature); and long reprogramming (14 days) induced mechanical- and cold-sensitive (menthol, low temperature) mechanoreceptors (Nickolls et al., 2020). **(E)** However, iPSC-NPCs co-expressing *NGN2* with *BRN3A* differentiated into proprioceptor and mechanoreceptor neurons (Blanchard et al., 2015). Direct virally-induced expression of *BRN3A*, *NGN1* and *NGN2* in fibroblasts yielded Trka+CGRP+ capsaicin-sensitive and Trpm8 + −mediated menthol-sensitive thermoreceptors, indicative of peptidergic C-fibers. *Created with BioRender.com.*

their nociceptive state, forming random, unorganized neuronal clusters that failed to recapitulate DRG cellular diversity and spatial architecture (Mazzara et al., 2020). The most commonly used method (Fig. 1A) (Chambers et al., 2012), adapted with minor modifications(Eberhardt et al., 2015; McDermott et al., 2019a; Mis et al., 2019; Pettingill et al., 2019; Schwartzentruber et al., 2018), exposes iPSCs to a cocktail of small inhibitory molecules that trigger signaling cascades responsible for cell fate determination. While very successful in producing neurons depicting a functional profile and markers of nociceptors, which is a promising start, this method has also been criticized for its inability to yield the diverse subtypes of mature sensory neurons that recapitulate most features described in native neurons.

3.1. Model validity

Although homogenous cultures allow us to perform phenotypic and functional analyses of specific cell cohorts, the generation of diverse iPSC-SN populations are important to effectively mimic DRG neuronal-neuronal and neuronal-glial interactions in the context of disease. Also, various cell types are involved in the etiology and mechanisms underlying chronic pain, which are lacking in the Chambers protocol; thus, we are unable to paint a complete picture of the pathology and the contribution of the complex cellular interactions that affect neuronal excitability(Wiegand and Banerjee, 2019).

Notably, differentiation of iPSC-SNs is assessed through their expression of canonical markers identified in rodents. Differential transcriptomic and proteomic make up of human and rodent DRG neurons impact the classification of iPSC-SNs based on rodent markers. For example, inter-species differences in the distribution and co-expression of sensory neuronal markers, including Nf-200, CGRP, P2x3r, Trpm8 and Piezo2(Chambers et al., 2012; Nickolls et al., 2020; Rostock et al., 2018; Saito-Diaz et al., 2021; Shiers et al., 2020b; Wainger et al., 2015) emphasize the need to define markers in specific subsets of nociceptors in human cells first to determine the identity of the iPSC-derived neuronal populations.

Differences between human DRGs and iPSC-SNs remain a challenge for their effective use as human cell surrogates. Current-clamp recordings showed similar distribution of rheobase (threshold current stimulus for firing action potentials) between DRG neurons and iPSC-SNs. However, there was substantial variation in the rheobase amongst donors, which may reflect differential expression levels of the underlying ionic conductances (Schwartzentruber et al., 2018). Also, the majority of Trpv1-expressing sensory neurons derived from the Chambers protocol are functionally unresponsive to capsaicin(Chrysostomidou et al., 2021; Eberhardt et al., 2015; Guimaraes et al., 2018), indicating that the presence of mRNA transcripts does not necessarily reflect the presence of functional channels at the cell surface to interact with the ligand. Furthermore, while substantial expression of the tetrodotoxin-sensitive (TTX-S) $Na_V1.7$ threshold channel, encoded by the *SCN9A* gene, is well-established in iPSC-SNs from many donors (Cao et al., 2016; McDermott et al., 2019b; Meents et al., 2019; Mis et al., 2019; Schwartzentruber et al., 2018; Yuan et al., 2021), functional expression of TTX-resistant (TTX-R) $Na_V1.8$ and $Na_V1.9$ channels in iPSC-SNs has long been debated. Previous studies have reported $Na_V1.8$ mRNA expression, and recorded TTX-R currents in iPSC-SNs(Meents et al., 2019). However, Eberhardt et al. have recently demonstrated that most of the TTX-R current is in fact produced by $Na_V1.5$ channels, which have previously been shown to be more highly expressed in rodent DRG neurons during the embryonic stages (Renganathan et al., 2002). These findings suggest an immature differentiation of these iPSC-SNs (Eberhardt et al., 2015), which is predicted to alter the cellular electro-responsiveness, compared to a mature DRG neurons(Middleton et al., 2021). These caveats underline the need for considering the presence of different cell types and maturity stages of the differentiated neurons in iPSC cultures when comparing them to human DRG neurons.

3.2. Experimental challenges

In addition, experimental challenges have arisen from currently used protocols, including poor replating efficiency and impracticality for multi-well systems(Hoelting et al., 2016; Stacey et al., 2018). Furthermore, iPSC-SNs are more uniform and smaller than human DRG neurons (McDermott et al., 2019a; Zhang et al., 2017), and thus, do not recapitulate the diversity of native sensory neurons. The long time in culture lead to longer neurites, causing space clamp artifacts in the recordings of ionic currents, which confound the interpretation of data regarding the functional properties of the channels that produce these currents (Hulme et al., 2020; Lampert et al., 2020; Meents et al., 2019). However, there are reports showing well-clamped sodium currents in iPSC-SNs(Alsaloum et al., 2021). Although space-clamp artifacts in the voltage-clamp recordings of iPSC-SNs can be a challenge, specific recording protocols which isolate somatic currents from those emanating in distal axons can mitigate this technical problem(Akin et al., 2021; Milescu et al., 2010).

3.3. Genetic and epigenetic challenges

3.3.1. Donor-based variability

Variability in genetic, epigenetic or environmental factors might account for reduced sample-to-sample reproducibility in iPSC-SN models, which could be mitigated by using samples from multiple donors (Popp et al., 2018; Volpato and Webber, 2020; Young et al., 2014). It is notable that iPSC-SNs from different healthy individuals have shown substantial differences in their electrogenic properties(Cao et al., 2016). Furthermore, the age of the donor has been shown to increase the likelihood of *de novo* mitochondrial DNA mutations, leading to metabolic loss effects(Kang et al., 2016). Nonetheless, these donor-specific differences in the properties of iPSC-SNs still provide an invaluable substrate for understanding inter-individual differences in nociceptive signaling, and will be essential for implementing a personalized medicine approach to treat pain.

3.3.1.1. Culture-induced variability. Heterogeneity has been reported between and within different iPSC lines(Viventi and Dottori, 2018; Young et al., 2014), arising from clonal selection and cell culture environment during reprogramming and differentiation(Schwartzentruber et al., 2018; Young et al., 2014). Small changes in cell media composition, environmental conditions and/or cell density have been associated with higher gene expression variability(Schwartzentruber et al., 2018) and can affect the neuronal content and *SCN9A* expression of iPSC-SNs (Young et al., 2014). Minor modifications of the coating (recombinant laminin-511 E8 fragments or matrigel instead of co-cultures with mouse embryonic fibroblasts), differentiation medium, and incubation time of the same Chambers protocol have yielded iPSC-SNs resembling pruriceptors(Umehara et al., 2020), proprioceptors (Dionisi et al., 2020) and C-low threshold mechanoreceptors(Chrysostomidou et al., 2021; Guimaraes et al., 2018). These technical issues necessitate careful head-to-head comparison between published studies, as well as the harmonization of these assays by the community, which might provide a future potential solution.

3.3.1.2. Differentiation-induced variability. Differentiation-induced variability has been shown to affect the neuronal composition of cultures(Schwartzentruber et al., 2018) and to contribute to stem cell genetic instability, whereby it increases the risk of *de novo* mutations (Young et al., 2014). Furthermore, cell reprograming has been suggested to induce epigenetic changes, which constitute another important bias in pain studies(Thanuthanakhun et al., 2021). Specifically, differentiation has been shown to reduce DNA methylation(Ankam et al., 2019; Efrat, 2020). Our current inability to distinguish between differentiation-induced epigenetic modifications and the donor epigenome suggests that caution is needed in interpreting results of subject-specific iPSC-

derived models.

4. Alternative strategies to generate patient-specific sensory neurons

Human iPSCs are highly advantageous to study rare genetic diseases; however, the study of common regulatory variants remains limited. In order to achieve significance in 'recall-by-genotype' studies, Schwartzentruber et al. (2018) reported that over 40 samples from 20 to 80 different donors are required. However, it should be noted that the differentiation protocol that was implemented in this study yielded roughly 50% of the cells with fibroblast markers (Schwartzentruber et al., 2018). Whether the same number of samples will be needed if the cultures had more homogeneous sensory neuronal characteristics remains to be investigated.

Culture- and differentiation-induced variability may hamper the ability to distinguish between natural inter-individual variability and those resulting from the experimental conditions. Therefore, better reprogramming and differentiation methods are necessary for a more accurate disease modeling. The development of a cell-based system that overcomes the limitations described above and better replicates human endogenous cellular pathways driving differentiation can markedly advance the field (Fig. 1; Table 1).

4.1. Direct reprogramming

There are two main approaches to *in vitro* differentiation of sensory neurons. The first and most extensively used attempts to recapitulate the different stages of DRG embryonic development by enriching the culture media with growth factors and morphogens that replicate the neural tube environment and trigger signaling cascades responsible for cell determination (Fig. 1A) (Chambers et al., 2012). A second approach, which bypasses the progenitor stage and directly reprograms somatic cells into neurons, has yielded mixed populations in fewer steps than indirect reprogramming, providing a faster and more cost-effective differentiation strategy. For instance, direct neuronal differentiation of mouse fibroblasts into nociceptors was achieved by using only five key transcription factors: *ASCL1, MYTLl, NGN1, ISL2,* and *KFL7* (Fig. 1B) (Wainger et al., 2015). These transdifferentiated sensory neurons were functionally responsive to noxious agonists and showed different combinations of TTX-R slow and persistent sodium currents, consistent with $Na_V1.8$ and $Na_V1.9$ electrophysiological signatures. The sensory neurons generated from familial dysautonomia patients showed reduced neurite outgrowth compared to healthy controls, showcasing this study as a proof-of-concept that direct reprogramming can be used to model peripheral neuropathies. However, this approach remains limited by its low neuronal conversion efficacy and by its reduced capacity to maintain neurons in culture(Wainger et al., 2015) (Iyer et al., 2017).

Direct conversion of blood cells and hair follicles has shed light on the relevance of the cell type of origin to derive selective sensory neuronal subtypes, specifically capsaicin-sensitive peptidergic nociceptors, which current protocols have failed to produce consistently(Lee et al., 2015; Vojnits et al., 2019; Wilson et al., 2018). Importantly, fibroblasts are primed for neuronal potential, whereby their cell fate is restricted, while blood cells require *de novo* acquisition of neural crest development gene expression(Lee et al., 2015; Vojnits et al., 2019), hence reducing the risk of differentiation-induced variability(Schwartzentruber et al., 2018). Blood cell-converted nociceptors were functional and responsive to chemical irritants. Furthermore, the production from initial reprogramming to mature neurons was over three times faster than the conventional route and yielded high numbers of cells available for high-throughput screening of chemotherapeutic compounds (Vojnits et al., 2019). Taken together, these findings set the stage for using diverse somatic cells for direct conversion into specific sensory neuron lineages.

Several neural induction strategies have adopted protocols to trigger sensory neurogenesis, which occurs in two migratory waves. Large diameter neurons, including proprioceptors and mechanoreceptors, are first generated from neurogenin-2 $(Ngn2)^+$ cells, followed by small and medium diameter nociceptors during the second wave, stimulated by neurogenin-1 (Ngn1)(Marmigere and Ernfors, 2007). Peptidergic and non-peptidergic nociceptors emerge later following alternative expression of Trka and Ruxn1(Marmigere and Ernfors, 2007). Mimicking this phenomenon has been suggested to promote selective differentiation of nociceptor-like cells(Boisvert et al., 2015). Direct reprogramming of human fibroblasts through inducible lentiviral co-expression of Brn3a with Ngn1 or Ngn2 has achieved evenly mixed sensory neuron subtypes, belonging to the three primary DRG sensory neuronal groupings, namely mechanoreceptors, proprioceptors, and nociceptors (Fig. 1E) (Blanchard et al., 2015). The differentiated neurons showed electrophysiological activity, and were responsive to pruritic and noxious agonists and temperatures. Specifically, induced expression of *BRN3A/NGN1/NGN2* yielded $Trka^+CGRP^+$ capsaicin-sensitive and $Trpm8^+$-mediated menthol-sensitive thermoreceptors, indicative of peptidergic C-fibers (Fig. 1B, E) (Blanchard et al., 2015; Wainger et al., 2015).

The overall low conversion rates from direct induction of somatic cells to sensory neurons hinders adoption of this approach as an alternative to the iPSC model. A possible solution to circumvent this challenge is to expand fibroblasts in culture prior to induction, although this will add to the expense and labor and thus defeats its main purpose(Iyer et al., 2017). Previous studies have suggested that forced conversion of transcription factors greatly reduces differentiation efficiency(Nickolls et al., 2020; Schrenk-Siemens et al., 2015), which may be resolved by using other somatic cell types or by using additional differentiation steps, such as by converting iPSCs into neural precursor cells (NPCs) first (Lee et al., 2015; Vojnits et al., 2019; Wilson et al., 2018).

4.2. Transgene expression of lineage-specific transcription factors in iPSCs

Transducing iPSC-NPCs with *NGN2* has been shown to result in low *SCN9A, SCN10A* and *TRKA* gene expression and high levels of *SCN1A* and *SCN6A*, suggesting an upregulation of proprioceptor and mechanoreceptor differentiation (Fig. 1C) (Hulme et al., 2020). Furthermore, the $Na_V1.8$ immunoreactivity that was observed may be attributed to the presence of Aβ low threshold mechanoreceptors (Aβ-LTMR), some of which are known to express this channel *in vivo*(Hulme et al., 2020; Shields et al., 2012). However, the electrophysiology data in Hulme et al. is not definitive to make an unequivocal conclusion that functional $Na_V1.8$ channels are expressed in these neurons(Hulme et al., 2020). Selective Aβ-LTMR identity was reproduced in a separate study, where viral *NGN2*, combined with retinoic acid and neurotrophic factors, promoted the differentiation of neurons inducing rapidly adapting currents from mechanical stimuli (Fig. 1C). These findings underscore the capacity of *NGN2* to preferentially induce mechanoreceptors and suggest that overexpressing *NGN1* instead may drive cells to more nociceptor-like populations(Schrenk-Siemens et al., 2015). However, it has been argued that successful conversion requires addition of BRN3A, as neurons expressing Ngn2 alone have been suggested to more closely resemble CNS neurons(Nickolls et al., 2020). These divergent results may have resulted from the use of viral induction(Hulme et al., 2020; Schrenk-Siemens et al., 2015) over genetic engineering(Nickolls et al., 2020), and highlight the magnitude of protocol changes on the outcome of cellular differentiation.

Temporal regulation of the expression of *NGN2* and *BRN3A* also influence the identity of the cells that are differentiated by this approach. Stable transgene expression of *NGN2* and *BRN3A* generated uniform cultures of cold-mechanoreceptors sensitive to both 4 °C and menthol stimuli, while resistant to capsaicin and heat (Fig. 1D) (Nickolls et al., 2020). Interestingly, cell fate determination was time-dependent, whereby briefly induced (24 h) cells acquired a phenotype and gene expression profile consistent with LTMR, while those that underwent

Table 1
Novel induction strategies for obtaining sensory neuronal lineage derived from human cells. Summary of the publications that described alternative differentiation strategies, diverging from Chambers et al. (2012), to obtain mature, selective DRG neuronal subtypes from human somatic and induced pluripotent stem cells (iPSCs). The methodology used along with their advantages and limitations are listed accordingly. The table was adapted and expanded from Viventi and Dottori (2018).

Cell type of origin	Differentiation strategy/ Structure	Factors used	DRG subtypes	Characterization	Advantages	Disadvantages	Publication
Human fibroblast-derived iPSC	Small molecule pathway inhibitors 2D linear culture	SB431542, LDN-193189, SU5402, CHIR99021, DAPT, NGF, BDNF, GDNF	Non-peptidergic nociceptors	Immunostainings, Flow cytometry, Microarray Analysis, Electrophysiology (Current-clamp), Calcium Imaging	-Gold-standard (most used protocol) -High sensory neuron conversion efficiency	-Lack peptidergic nociceptors -Lack maturity -Lack neuronal diversity -<2% capsaicin-responsive cells	Chambers et al. (2012) ǂ
Direct reprogramming							
Mouse and human fibroblasts	Genetic manipulation of viral constructs 2D linear culture	ASCL1, MYT1L, NGN1, ISL2, KFL7, FGF, BDNF, CNTF, GDNF, NGF	Nociceptors	Immunohistochemistry, qPCR, Single cell RT-qPCR, Calcium imaging, CGRP ELISA, Electrophysiology (MEA, Current-clamp, Voltage-clamp), Morphology	-More cost- and time-effective -TTX-R neurons expressing $Na_V1.8$ and $Na_V1.9$ electrophysiological signatures -Bypass iPSC stage	-Limited neuronal conversion efficiency -Difficult culture maintenance —No progenitor stage, risk of heterogeneity	Wainger et al. (2015)
Mouse and human fibroblasts	Transient doxycycline-inducible lentiviral transduction 2D linear culture	Brn3A/NGN1 (BN1) Brn3A/NGN2 (BN2) NGF, BDNF, GDNF	MechanoreceptorsProprioceptors, Nociceptors	Immunohistochemistry, RT-qPCR, Electrophysiology (Current-clamp), Calcium Imaging	-Sensory neuronal diversity -Rapid and cost-effective -Functionally responsive to noxious stimuli -Peptidergic C-fibers -Capsaicin- and menthol-sensitive	-Low sensory neuronal (SN) conversion rates (5–10%)	Blanchard et al. (2015)
Human epidermal neural crest stem cells (hEPI-NCSC; hair follicles)	Small molecule pathway inhibitors 2D linear culture	SHH, CHIR99021, LDN193189, DAPT, NT-3, HGF	Peptidergic nociceptors	Indirect immunocytochemistry, RT-qPCR, Calcium imaging	-Fast, selective differentiation into capsaicin-responsive peptidergic neurons —No genetic manipulation —No cell purification	-Only 1/3 of cells obtained were capsaicin-responsive	Wilson et al. (2018)
Pre-conversion into neural precursor cells (NPC) from somatic cells							
Human CD34+ cord blood cells	Lentiviral transduction into NPC using OCT4 and SMAD+ GSK-3 Inhibition	*NPC:* SCF, Flt-3 L, IL3, TPO cytokines, SB431542, LDN-193189, Noggin, CHIR99021, bFGF, EGF *SN:* SU5402, DAPT, CHIR99021, BDNF, GDNF, NGF, NT3	(Peptidergic) nociceptors	Flow cytometry, Immunocytochemistry, RT-qPCR, Calcium imaging, Current-clamp, Comparative genomic hybridization array	- Reduced risk of differentiation-induced variability *(De novo* acquisition of neural crest genes) -Peptidergic sensory neurons -Bypass iPSC stage	-Slower and more steps than direct conversion	Lee et al. (2015)
CD34+ mononuclear cells	Lentiviral transduction 2D linear culture	*NPC:* OCT-4, SB431542, LDN-193189, CHIR99021, bFGF, EGF *SN:* DAPT, SU5402, CHIR99021, BDNF, GDNF, NGF, NT-3, ascorbic acid, forskolin	Nociceptors	Immunofluorescence, Flow cytometry, Calcium imaging	-Up to 3× faster (Less genetic drift) -Better for high-throughput compound screening (ability to expand cells prior)	-No major morphological or functional differences between CD34 + -SN and iPSC-SN	Vojnits et al. (2019)
Pre-conversion into NPC from iPSCs							
Skin fibroblast-derived iPSCs	Lentiviral transduction	lentivirus NGN2	Mechanoreceptors (LTMR)	Immunocytochemistry, *In situ* hybridization, RT-PCR,	-Selective differentiation into DRG subtype -Ability to differentiate hES	—No nociceptor	Schrenk-Siemens et al. (2015)

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Table 1 (*continued*)

Cell type of origin	Differentiation strategy/ Structure	Factors used	DRG subtypes	Characterization	Advantages	Disadvantages	Publication
and embryonic stem cells (hES)	Neuro-ectodermal spheres	FGF, EGF, NGF, NT-3, BDNF, GDNF, RA		Deep-sequencing analysis, Electrophysiology (Current-clamp), Calcium Imaging	or hiPSC interchangeably with the same protocol		
hiPSCs	Lentiviral transduction Caudal neural progenitors (CNP) into neurospheres	lentivirus NGN2 CHIR99021, SB431524, FGF2, BMP2, BDNF, GDNF, NT-3 ß-NGF	Pan-sensory neurons (mostly mechanoreceptors, proprioceptors)	Immunocyochemistry, RT-qPCR, Electrophysiology (Current- and Voltage-clamp), Pharmacology	-Heterogenous sensory neuron populations and electrophysiological profiles -High conversion rates (80–90%) -C-fiber electrophysiological properties (30% neurons) -$Na_V1.7$ and $Na_V1.8$ currents -Functional expression of K_V, Ca_V and ASIC channels	-Low levels of *SCN10A*, but high *SCN1A, SCN6A* -Left-shifted I_{Na} steady-state inactivation -Low proportions of nociceptors -Low TRPV1 signal	Hulme et al. (2020)
hiPSCs	Transgene expression Neural crest spheroids	NGN2-BRN3A plasmid bFGF, EGF, SB431542, BDNF, GDNF, β-NGF, NT-3, RA	Cold-mechanoreceptor, proprioceptors	Immunocytochemistry, RNA *in situ* hybridization, RT-PCR, Bulk RNA sequencing, Single-nuclei RNAseq, Calcium imaging, Electrophysiology (Current- and Voltage-clamp)	-Time-dependent induction into divergent, yet specific subtypes -High sensory neuron conversion -Detects cold and mechanical stimuli	-No nociceptor —No $Na_V1.8$ or $Na_V1.9$ expression -Devoid of most pain-related genes	Nickolls et al. (2020)
hiPSCs	Pathway inhibitors Neurospheres ('nocispheres') CompacT SelecT platform (Robotic cell culture)	A83–01, CHIR98014, CEPT, DBZ, PD173074, PD0332991, BDNF, GDNF, NGF, NT-3	Nociceptors	Immunocychemistry, electron microscopy, Bulk RNAseq, Electrophysiology (MEA, manual and automated patch-clamp), Calcium imaging, RNAscope, FAAH inhibition assay	-Morphology of peptidergic and non-peptidergic nociceptors -Pseudo-unipolar anatomy -Express relevant pain genes (including $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ transcripts) -Robotic cell culture: Fast production of large quantities of sensory neurons (over 150 million nociceptor per workflow, scalable up 30-fold) -Functionality of Ca_V, TRP, K_V and Na_V channels	-Transcriptomcally still distinct from hDRGs -Limited $Na_V1.7$ functional availability compared to native DRGs	Deng, et al. (2022)
Multicellular strategies:							
hiPSCs	Linear 2D co-culture iPSC-SN + Rat bone marrow stromal cells-derived Schwann cells (SC) or Rat SCs	LDN-193189, A83–01, CHIR99021, RO4929097, SU5402, RA, NT-3, NGF, BDNF, GDNF	Myelinated sensory neurons	Immunofluorescence, Flow cytometry, Western Blot, RT-PCR, Electrophysiology (Current-clamp)	-Co-culture reciprocally influence cell fate of BMSC and iPSC-SN *via* contact-dependent cues	-Not fully human -Long process -Immature myelin	Cai et al. (2017)
Human fibroblasts-iPSCs	Linear 2D co-culture iPSC-SN + Rat SCs	SB431542, LDN-193189, CHIR99021, SU5402, DAPT, NGF, GDNF, BDNF, NT-3	Myelinated sensory neurons	Immunocytochemistry, Western blot, Transmission electron microscopy	- Functional VGSCs and $K_V1.2$ similar to human DRGs -Nodes of Ranvier formation -Mature myelin sheath -Reproducible and stable myelination levels across cultures	-Non-human SC -Few nociceptive neurons	Clark et al. (2017) [‡]

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Table 1 (*continued*)

Cell type of origin	Differentiation strategy/ Structure	Factors used	DRG subtypes	Characterization	Advantages	Disadvantages	Publication
					-Longer culture longevity (>9 months)		
Human fibroblast- and PBMC- iPSC	3D collagen sponge structure and co-culture Fibroblast and/or endothelial cells + hiPSC-SN + hiPSC-SCs (tissue-3D skin model)	SB431542, LDN193189, CHIR99021, DAPT, BDNF, GDNF, NGF, hydrocortisone, insulin, hEGF, cholera toxin	Sensory neurons, Schwann cells	ELISA, Immunofluorescence	-Fully human model -Almost 100% successful conversion -Over 2× prolonged neuronal culture compared to 2D -Higher maturity -Longer neurites extending to epidermal layers (nerve network) -Responsive to capsaicin	-Absence of non-peptidergic neurons - TRP receptors-induced SP and CGRP release was more limited in 3D cultures than in 2D iPSC-SN	Muller et al. (2018)
Human fibroblast-derived iPSCs	3D structure and co-culture iPSC-DRG organoids (DRGO) + human intrafusal muscle fibers	SB431542, LDN193189, CHIR99021, SU5402, DAPT, BDNF, GDNF, NGF, NT-3	Sensory neurons (mechanoreceptors, proprioceptors, and nociceptors), Glial cells (S100+ Satellite cells, SCs)	Repeat-primed PCR, Bulk and Single-cell RNA seq, CHIP-qPCR, meDIP-qPCR, Immunocytochemistry, Electron microscopy, Western Blot, Electrophysiology (Current- and Voltage-clamp), Single-cell RT-PCR, ROS FACS	-Reconstitute muscle spindles of proprioceptors -Better cell survival, expression of mature neuronal genes, and active neurotransmitter trafficking -Higher maturity and diversity - Express higher *SCN10A* mRNA levels -Similar transcription profile, morphological structure, and cellular diversity to native hDRGs -Long-term culture (>80 days)	-Nociceptors are the least represented cell type -Incomplete electro-physiological maturity	Mazzara et al. (2020)[i]
hiPSCs	3D sensorimotor organoid Free-floating sphere culture + Adherent plating	GSK-3 Inhibitor IX, FGF2, Forskolin	Motor neurons Sensory neurons, Skeletal muscle, Astrocytes, Microglia, and Endothelial cells (vasculature)	Immunocytochemistry, FACS, Single-cell RNA seq, RT-RT-qPCR, Electron microscopy, Calcium imaging, Electrophysiology	-Functional neuromuscular junctions -Complex structure with diverse cell types -Capsaicin- and TTX -responsive -Large portion of TTX-R cells + similar activation kinetics to $Na_V1.8$ channels -Reduced within-line variance in isogenic lines by 5-10×	-Slow and varying maturation levels within organoid -Only 30% $Brn3A^+$ sensory neurons -Structural complexity: difficulty elucidating specific mechanisms	Pereira et al. (2021)
DRG-targeted differentiation							
Mouse and human fibroblasts	Direct reprogramming 3D organoid	Doxycycline–inducible lentiviruses Ascl1, Brn3a/3b, and Isl1 bFGF, IGF-1, BDNF, GDNF, NGF, NT-3, forskolin	Sensory neurons (Nociceptors, proprioceptors, mechanoreceptors), Retinal ganglion cells	RT-qPCR, immunohistochemistry, immunocytochemistry, Time-lapse recording, Electrophysiology, (Current- and Voltage-clamp), RNAseq, scRNA-seq, Calcium imaging	-Physiological characteristics of mature neurons -Extensive cell diversity -Peptidergic nociceptors -Key Na_V (incl. *SCN11A*), Ca_V and K_V channel gene expression -Expression of pain and touch-regulating genes -Responsive cells	-No *SCN10A* expression -Difficulty distinguishing between retinal and sensory neurons for functional assessment	Xiao et al. (2020)

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Table 1 (*continued*)

Cell type of origin	Differentiation strategy/ Structure	Factors used	DRG subtypes	Characterization	Advantages	Disadvantages	Publication
					to KCl, capsaicin (56.8%), and menthol (70.4%) -Equivalent proportion (30%) of $TrkA^+$, $TrkB^+$ and $TrkC^+$ neurons -Majority $TrkA^+$ nociceptors -Integrate, form connections with endogenous neurons, epidermis innervation		
	Ex vivo injection organoid into rat DRG explant						
hESCs, hiPSCs		Sensory neurospheres (NSP)	FGF2, BMP2 NGF, NT-3, BDNF	Sensory neurons (Nociceptors, mechanoreceptors, and proprioceptors), Glial cells (S100+, GFAP+)	RT-qPCR, Immunostaining	- Markers of myelinated and unmyelinated, peptidergic and non-peptidergic nociceptors	
In vivo injection NSP into rat DRG	Viventi et al. (2021)				-Long-term survival (8 weeks) NSP *in vivo* - *In vivo* differentiation into diverse DRG neuron subtype populations	-Nociceptors are the least represented -Technical challenges injecting and removing DRG for functional testing	

† Induction protocols based on Chambers et al. (2012).

long exposure (14 days) corresponded to cold-mechanoreceptors, suggesting that extending *NGN2/BRN2A* transgene activation overrides the LTMR transcription and result in Piezo2+/Trpm8+ neurons(Nickolls et al., 2020). While the obtained cells lacked a nociceptive identity and expression of many genes involved in pain and itch, the aforementioned studies provide important insights on alternative strategies to yield sensory neuron specific subtypes and emphasize on the importance of timing and duration of developmental signals to manipulate cell fate determination.

4.3. Microniche and multi-cellular strategies

Nociception requires neurons to act in concert with various cell types for physiological functions. Hence, the incomplete differentiation of iPSCs into fully mature sensory neurons might result from the lack of ligands in the media to activate appropriate signaling cascades and/or the absence of cell-cell interaction with normal constituents of the native DRG tissue (Iyer et al., 2017) necessary to replicate the *in vivo* extracellular environment (Fig. 2; Table 1).

4.3.1. Linear co-cultures

Because of their ability to recapitulate cell-neuron communication, co-cultures are emerging as a powerful *in vitro* tool(Abdo et al., 2019; Moehring et al., 2018; Talagas et al., 2020a; Talagas et al., 2020b). Schwann cells (SCs) have been a particularly attractive cell type for sensory neuron co-cultures as they are required for both maintenance and development of peripheral nerves, and myelinated neurons are crucial for pain signaling(Wei et al., 2019). Xenogeneic co-cultures of human iPSC-SNs with rat SCs have enabled the production of myelinated fibers with unlimited capacity for remyelination. Electrophysiological characterization of myelinated iPSC-SNs demonstrated characteristic properties to those of functional neurons(Cai et al., 2017). In a separate study, co-culture of SCs with iPSC-SNs yielded VGSCs at the node of Ranvier and shaker-type potassium channels at the juxtaparanode, which were molecularly comparable to human DRG neurons (Fig. 2A) (Clark et al., 2017). Both protocols provided net improvements over existing methods; however, a clear limitation is the use of non-human SCs. Recently, a protocol for directly inducing human SC precursors has been published, which might serve as a missing puzzle piece for generating fully human-derived myelinated peripheral neurons. Nonetheless, myelin generation in these cultures remained limited, with as few as twelve myelin internodes per well reported (Kim et al., 2020b). Other co-culture systems exist, including astrocytes, microglia, muscle and skin cells to improve neuronal differentiation and functions(Canfield et al., 2019; di Domenico et al., 2019; Haenseler et al., 2017; Schutte et al., 2021). Incorporating alternative cell types, including non-nociceptive and even neuronal cells, may reconstitute the crosstalk between sensory neurons and neighboring cells *in vitro*.

4.3.2. 3D-organoids

Human iPSCs can be grown three-dimensionally (3D) to create complex tissue structures that include multiple sensory neuronal populations and supporting glial cells, mimicking *in vivo* conditions and consequently, improving neuronal differentiation (Mazzara et al., 2020). In contrast to 2D-adherent monolayer strategies, such as in the Chambers protocol (Fig. 1A) (Chambers et al., 2012), 3D suspension cultures have yielded more diverse cell types that better reflect endogenous neurogenesis (Chiaradia and Lancaster, 2020). For example, 3D organoids derived from human fibroblasts, virally transduced with *ASCL1*, *BRN3B* and *ISL11*, yielded self-organized ganglia, closely resembling native DRGs. The 3D neuronal structure displayed molecular features such as relative expression of *SCN9A, SCN11A, CGRP* and *NF200* transcripts, supporting sensory neuron subtype diversity (Fig. 2B) (Xiao et al., 2020). Although in another study, nociceptors were the least represented cell type from the iPSC-derived DRG organoids (Mazzara et al., 2020). Additionally, 3D structures are reported to better overcome

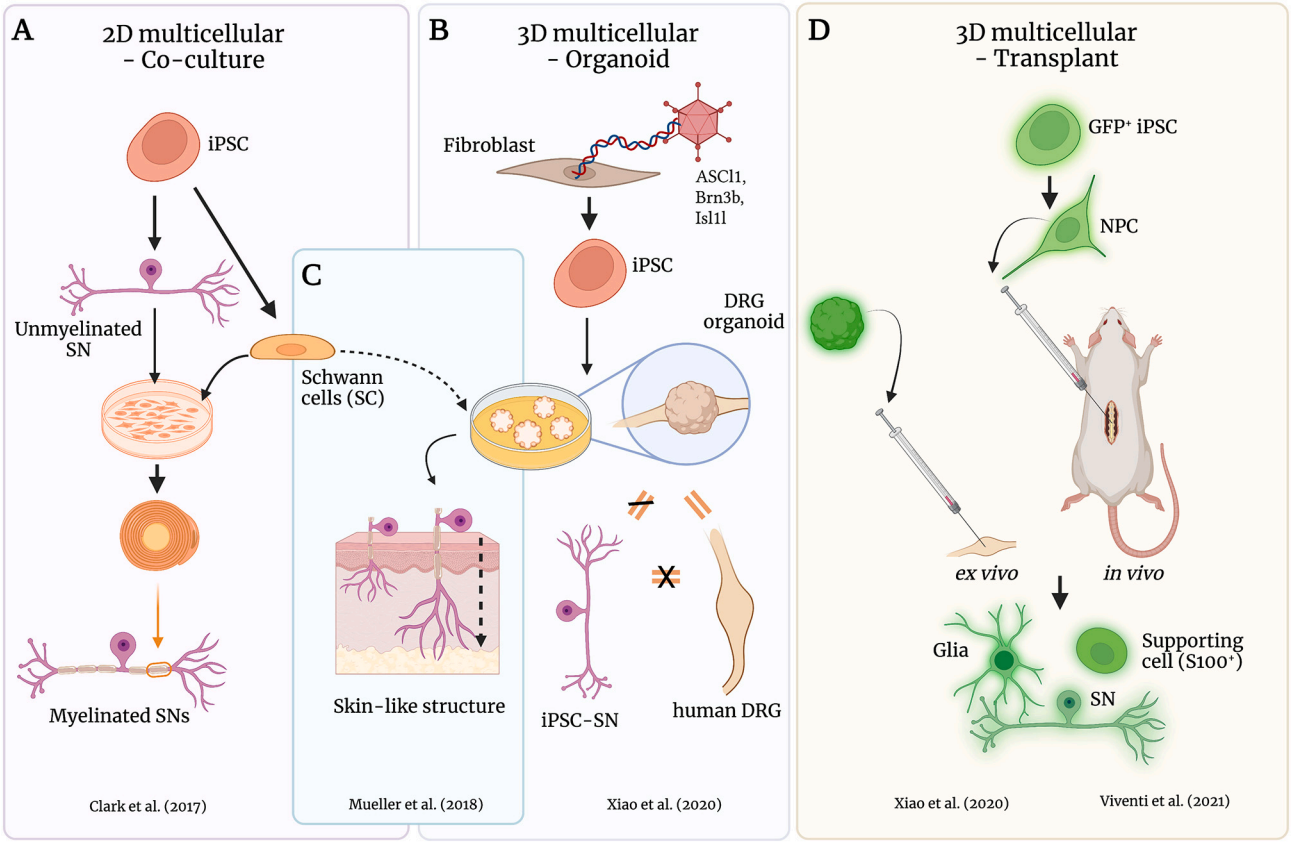

Fig. 2. Multi-cellular strategies for inducing iPSC-derived sensory neurons. Multicellular approaches aim to obtain sensory neurons by replicating the *in vivo* extracellular environment; **(A)** 2-D co-cultures provide additional cell types, such as Schwann cells (SCs), in culture, to recapitulate cell-to-cell communication in the dish. For example, induced-pluripotent stem cell (iPSC) cultures with SCs yielded myelinated sensory neurons (SN) (Clark et al., 2017). **(B)** 3D-organoids mimic the organized cellular structure of native dorsal rot ganglia (DRG) tissues. iPSC-derived DRG organoids displayed molecular features more closely resembling those of human DRGs than to iPSC-SNs derived using conventional protocols (Xiao et al., 2020). **(C)** Combined co-culture and organoid strategies enabled the formation of fully human skin-like structures, which allowed neurite elongation into epidermal-like layers, an important feature for pain disorders involving nerve degeneration (Muller et al., 2018). **(D)** *In vivo* (Viventi et al., 2021) and *ex vivo* (Xiao et al., 2020) transplantation of GFP-tagged iPSC-derived organoids and neural precursor cells (NPCs) into rodent DRGs provides an environmental microniche to the cells, including cell-cell communication and nutrients to differentiate into mature sensory neurons. The authors reported iPSCs to yield diverse DRG-specific cell types, including sensory neurons, glial and supporting cells. *Created with BioRender.com*.

the lack of maturity of sensory neurons that is observed in cells cultured in the 2D configuration(Lee et al., 2009; Wiegand and Banerjee, 2019), and to express higher *SCN10A* mRNA levels compared to 2D iPSC-SNs (Mazzara et al., 2020). However, without electrophysiological recordings, the relative expression of VGSC transcripts cannot guarantee channel functionality, and has yet to be evaluated (Mazzara et al., 2020). On the other hand, growing iPSC-SN in suspension allows them to be cultured for twice as long, compared to 2D strategies(Muller et al., 2018). Furthermore, non-adherent 3D cultures better replicate native spatial organization, cellular composition expression patterns, dynamic cell-matrix interactions and organ functions (Mazzara et al., 2020), facilitating the assessment of drug-tissue penetration (Kapalczynska et al., 2018; Mofazzal Jahromi et al., 2019). In a sensorimotor model of amyotrophic lateral sclerosis (ALS), organoid cultures adopted a DRG-like structure and produced physiologically functional neuromuscular junctions, along with diverse neuronal, glial, endothelial and skeletal muscle cellular cohorts (Pereira et al., 2021).

However, 3D models are more laborious and costly, they have limited high-throughput capacity and hinder microscopy and dynamic readouts (Wiegand and Banerjee, 2019). Additionally, limited control over their formation increases the risk of phenotypic variability, which can however be reduced by changing scaffolds, somewhat improving uniformity (Candiello et al., 2018; Wiegand and Banerjee, 2019). For instance, 3D cultured iPSC-SNs in fibroblast-populated collagen sponge release significantly more substance P after capsaicin stimulation than 2D models(Muller et al., 2018). Adopting a 3D architecture has also been shown to reduce extensive cell death, commonly observed in 2D cultures, due to a deficiency in glial supporting cells and cell-to-cell contact.

4.3.3. *Organoids and co-culture*

Combining 3D-organoid and co-culture strategies may further bridge the gap to obtaining mature sensory neurons. In a first-of-a-kind fully human tissue-engineered skin model, human keratinocytes and fibroblasts were co-cultured with iPSC-derived sensory neurons and SCs (or murine SCs) in a 3D collagen sponge model (Fig. 2C). This protocol allowed neuronal cells to reach higher levels of maturity and to extend neurites long enough to reach epidermal-like layers, promising valuable insights for studying skin-associated pain pathologies(Muller et al., 2018). Additionally, iPSC-DRG organoids cultured with human intrafusal muscle fibers enabled sensory neurons to contact their required targets and reconstitute the muscle spindle of proprioceptors, which further improved cell survival, expression of mature neuronal genes, and active neurotransmitter trafficking machinery(Mazzara et al., 2020).

4.3.4. *Targeted iPSC differentiation in rodent DRGs*

The potential of the microniche influence on iPSCs' fate can be further examined by providing the endogenous spatiotemporal context, critical nutrients and cellular interactions lacking in *ex vivo* cultures. Introducing iPSCs to the native DRG environment *in vivo* provides

signaling cues necessary for sensory neurons to express their full proteome range(Martinez-Cerdeno et al., 2017), by analogy to an *in vivo* differentiated iPSC-derived cardiomyocyte model, where xenotransplanted cells acquired additional protein markers, at similar levels to native cardiac cells(Yu et al., 2013).

Targeted DRG injection has provided promising therapeutic avenues in degenerative diseases such as Friedreich's Ataxia (FRDA)(Viventi et al., 2021) and has been highly informative about *in vivo* cellular behavior. Viventi et al. and Xiao et al. are amongst the first to attempt DRG injection of human GFP-labelled iPSC-SNs in rodents (Fig. 2B,D) (Viventi et al., 2021; Xiao et al., 2020). Injected iPSC-DRG organoids into rat DRG explants *ex vivo* showed high survival rate and maintained nociceptive, proprioceptive and mechanosensitive identity (Fig. 2D). Furthermore, transplanted cells spontaneously aggregated with endogenous neurons and formed interconnected structures, demonstrating their strong mutual affinity(Xiao et al., 2020). Propelling these findings further, *in vivo* transplant of FRDA iPSC-progenitor neurospheres into athymic "nude" rodent DRGs demonstrated effective survival and differentiation for up to 8 weeks post-graft (Fig. 2D) (Viventi et al., 2021). Within this timeframe, the number of cells almost doubled, indicative of their expansion potential. Likewise, injected cells showed markers of all three sensory neuronal subtypes, confirming their ability to differentiate *in vivo* into heterogeneous populations, with neuronal projections that extended into the spinal cord and along the neuronal tracts. Transplanted cells also differentiated into S100+ supporting cells and surrounded the host DRG neurons in a similar manner to endogenous cells, indicative of their functionality *in vivo*.

Altogether, this strategy highlights the importance of the microenvironment in influencing cell behaviors and in promoting diversity, encouraging the use of transplanted iPSCs for neuronal differentiation and maturation(Viventi et al., 2021).

However, the anatomical location and small structure of DRG make this technique challenging. Also, the elevated tumorigenic potential of xenotransplanted iPSCs(Fu et al., 2012) along other transplantation-related concerns, including cell survival, migration and further differentiation, has restricted their use for disease modeling. However, pre-differentiated neurons *in vitro* within modified fibrin gels controlling the delivery of growth factors and small molecules have been able to improve neurite growth, cell survival and differentiation post-transplant (Iyer et al., 2017). Strategies that eliminate injected iPSC tumorigenicity are also under development, such as bee venom, which selectively target undifferentiated, still pluripotent cells(Kim et al., 2020a). Finally, differences between human and rodent DRG micro-environments may confound the composition and protein expression levels of *in vivo* derived iPSC-SNs and may not reflect the phenotype of sensory neurons differentiated in native human tissues.

5. Outlook and future prospects

5.1. Modeling iPSC-SN missing TTX-R current using dynamic clamp

While several strategies have allowed for the differentiation of heterogeneous sensory neuron populations, none thus far have reliably produced functional expression of $Na_V1.8$ and $Na_V1.9$ channels. This is important because these channels harbor mutations in patients with painful disorders(Bennett et al., 2019; Dib-Hajj and Waxman, 2019; Eijkenboom et al., 2019) and the lack of their robust expression hamper mechanistic and pharmacological studies in patient-specific iPSC-SNs. Alsaloum et al. (2022) have recently used dynamic clamp, an electrophysiological approach which injects or removes modeled currents in a patched neuron, in order to investigate the effect of this specific conductance on neuronal excitability (Prinz et al., 2004). These modeled currents are developed based on empirical data obtained by recording this conductance. Alsaloum et al. injected modeled $Na_V1.8$ and $Na_V1.9$ currents based on the native properties of these channels in human DRGs, and studied the effects of these channels on iPSC-SN excitability (Alsaloum et al., 2021). The contribution of each channel to the cell's current density, action potential overshoot and repetitive firing was measured from human DRG recordings and from injecting varying current levels into iPSC-SNs with dynamic clamp. Building on these findings, they have been able to precisely 'inject' the newly-established amount of current representative of the two missing channels to study, for the first time, the effect of a $Na_V1.8$ mutation in iPSC-SNs. Taken together, this method allows the study of patient-specific iPSC-SNs with the full complement of sodium channel currents, hence more closely mimicking human physiological action potentials.

5.2. Grouping iPSC-SN select subtypes

Sensory neuron cultures with diverse cell types provide the opportunity to test new analgesic compounds, improving the translation of knowledge based on *in vitro* cultures. A drawback, however, is in the ability to identify and isolate cell groups of interest for functional assessment. Immunopanning, an antibody-based purification technique, has the potential of segregating selective subtypes from a large pool of mixed neuron types, by pre-coating dishes with cell-surface antibodies to which cells of interest bind, for example using Trka expression to isolate nociceptors(Saito-Diaz et al., 2021). The generation of sensory neuronal reporter cell lines may further enable the identification of specific subtypes and explore their electrogenic profile(Wainger et al., 2015).

5.3. Improving organoid development

The future of iPSC-SNs points to multicellular and *in/ex vivo* cultures as they allow complex cellular connections and better mimic the organ's microniche. Nonetheless, these models still lack cell survival and scalability. Chimera formation of iPSC-neural stem cells (NSC) instead of direct transplant has provided insights into their *in vivo* behavior in their native environment(Choi et al., 2017). Chimeric embryos are formed by aggregating iPSCs with morulas before transferring them to a pseudo-pregnant mother; after which, the embryos' brain is cultured into neurospheres. *In vivo* differentiated iPSC-NSCs expressed markers more closely resembling the endogenous gene profile of the fetal brain than *in vitro* differentiated cells. The iPSC-NSCs also actively contributed to chimeric embryo brain tissue development(Choi et al., 2017). Adopting a similar approach to obtain iPSC-DRG might produce cells that are more representative of native neurons. Furthermore, providing a scaffold for iPSCs to differentiate and mature has been suggested to better control the disorganized, sporadic cell growth by creating spatial resolution mimicking the endogenous architecture. Examples include using 3D bioprinting(Hirano et al., 2021) and microfluidic chambers, which involve culturing cells in small amounts of fluids, further easing drug discovery(Mofazzal Jahromi et al., 2019). In a proof-of-concept study, iPSC-derived brain organoids formed in a microfluidic 'organ-on-a-chip' demonstrated key features of human brain development, including neuronal differentiation and cortical organization (Wang et al., 2018). An analogous DRG organoid -on-a-chip would substantially advance the field.

5.4. Tracking iPSCs in vivo

Imaging of human iPSCs can provide new avenues to monitor cell distribution, differentiation, proliferation and survival. Notably, genetic knock-in of fluorescent and epitope tag proteins, where the protein of interest transduces a fluorescent marker, can give us insights on *in vivo* cellular behaviors and patterns of expression (Luo et al., 2014). This strategy might further help evaluate the feasibility of cell engraftment therapy in humans and their sustainability in regenerative medicine. Also, future studies might consider using Ca^{2+} reporter lines to assess iPSC-SN activity in DRG *in vivo* (Viventi et al., 2021).

5.5. Direct donor cell comparison

To better assess inter-individual variability in characteristics of cell-based sensory neuron models, post-mortem DRGs could be compared to the same donor's iPSC-SNs to delineate the true proteomic expression range and refine our assignment of canonical markers. Over time a biobank of well characterized iPSC-derived libraries would be invaluable to allow the standardization of *in vitro* data and improve our understanding of global pathophysiological mechanisms and therapy options *via* iPSC-data combined with patient data. It should be noted that large scale comparisons will benefit from the development of validated automated high throughput platforms for the differentiation of iPSC-SNs. A recent posting in BioRXiv (Deng et al., 2022) of a study of this nature suggests that such an approach might be feasible in the foreseeable future despite the fact that major challenges remain before achieving the rich mixture of heterogenous sensory neurons that is characteristic of native DRG neurons.

6. Conclusions

Although current advances in developing iPSC-SN systems for mechanistic and pharmacological studies related to pain research and drug development represent major progress when compared to widely used heterologous expression systems and rodent DRG neurons, there are limitations to this approach that must be considered when we interpret the data that is produced by this cell-based system. The limited knowledge of sensory neurogenesis and maturation *in vivo* hinders our ability to more effectively model human DRG neurons using iPSCs. Furthermore, neural induction and differentiation depend on intrinsic cell qualities and the extracellular environment, including cell-to-cell communication and spatial cues, which are still poorly understood (Iyer et al., 2017). Genetic and epigenetic modifications of the cells that are used to generate donor-specific iPSCs are likely to impact the nature of the differentiated cells. Additionally, better cell type classification of the differentiated cultures will depend on using a more complete list of markers, including those of the cells of origin, so that attribution of a sensory modality to a specific population will be more appropriate than current practices. The first step towards advancing disease modeling using iPSCs is to identify the critical key components of the human DRG microniche influencing cell fate, in order to find adequate enrichment strategies and improve establishing the full complement of sensory neuronal subtypes (Nickolls et al., 2020). Another important issue is inter-laboratory variability and poor reproducibility even when using the same protocols, which might be caused by minor modifications that do not get reported or other environmental factors and batch-to-batch variability of reagents. More efforts are still needed to circumvent the limitations of this system, but rapid technological advances raise new possibilities for iPSC-based disease modeling, and will undeniably facilitate the discovery of new aspects of sensory biology including the neurobiology of pain.

Despite current limitations, iPSC models are helping us to unravel, molecule by molecule, key players in human pain and inter-individual phenotypic differences, bringing us a step closer towards innovative therapeutic strategies powerful enough to substitute current trial-and-error attempts by personalized medicine.

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Data availability

This is a review of literature

References

Abdo, H., Calvo-Enrique, L., Lopez, J.M., Song, J., Zhang, M.D., Usoskin, D., El Manira, A., Adameyko, I., Hjerling-Leffler, J., Ernfors, P., 2019. Specialized cutaneous Schwann cells initiate pain sensation. Science 365, 695–699.

Akin, E.J., Alsaloum, M., Higerd, G.P., Liu, S., Zhao, P., Dib-Hajj, F.B., Waxman, S.G., Dib-Hajj, S.D., 2021. Paclitaxel increases axonal localization and vesicular trafficking of Nav1.7. Brain 144, 1727–1737.

Alsaloum, M., Labau, J.I.R., Liu, S., Estacion, M., Zhao, P., Dib-Hajj, F., Waxman, S.G., 2021. Contributions Na. Sci Rep 11, 24283.

Alsaloum, M., Labau, J.I.R., Liu, S., Effraim, P., Waxman, S.G., 2022. Stem cell-derived sensory neurons modelling inherited erythromelalgia: normalization of excitability. Brain. (awac031) https://doi.org/10.1093/brain/awac031.

Ankam, S., Rovini, A., Baheti, S., Hrstka, R., Wu, Y., Schmidt, K., Wang, H., Madigan, N., Koenig, L.S., Stelzig, K., Resch, Z., Klein, C.J., Sun, Z., Staff, N.P., 2019. DNA methylation patterns in human iPSC-derived sensory neuronal differentiation. Epigenetics 14, 927–937.

Bennett, D.L., Clark, A.J., Huang, J., Waxman, S.G., Dib-Hajj, S.D., 2019. The role of voltage-gated sodium channels in pain signaling. Physiol. Rev. 99, 1079–1151.

Blanchard, J.W., Eade, K.T., Szucs, A., Lo Sardo, V., Tsunemoto, R.K., Williams, D., Sanna, P.P., Baldwin, K.K., 2015. Selective conversion of fibroblasts into peripheral sensory neurons. Nat. Neurosci. 18, 25–35.

Boisvert, E.M., Engle, S.J., Hallowell, S.E., Liu, P., Wang, Z.W., Li, X.J., 2015. The specification and maturation of nociceptive neurons from human embryonic stem cells. Sci. Rep. 5, 16821.

Bouhassira, D., 2019. Neuropathic pain: definition, assessment and epidemiology. Rev. Neurol. (Paris) 175, 16–25.

Cai, S., Han, L., Ao, Q., Chan, Y.S., Shum, D.K., 2017. Human induced pluripotent cell-derived sensory neurons for fate commitment of bone marrow-derived Schwann cells: implications for Remyelination therapy. Stem Cells Transl. Med. 6, 369–381.

Candiello, J., Grandhi, T.S.P., Goh, S.K., Vaidya, V., Lemmon-Kishi, M., Eliato, K.R., Ros, R., Kumta, P.N., Rege, K., Banerjee, I., 2018. 3D heterogeneous islet organoid generation from human embryonic stem cells using a novel engineered hydrogel platform. Biomaterials 177, 27–39.

Canfield, S.G., Stebbins, M.J., Faubion, M.G., Gastfriend, B.D., Palecek, S.P., Shusta, E.V., 2019. An isogenic neurovascular unit model comprised of human induced pluripotent stem cell-derived brain microvascular endothelial cells, pericytes, astrocytes, and neurons. Fluids Barriers CNS 16, 25.

Cao, L., McDonnell, A., Nitzsche, A., Alexandrou, A., Saintot, P.P., Loucif, A.J., Brown, A. R., Young, G., Mis, M., Randall, A., Waxman, S.G., Stanley, P., Kirby, S., Tarabar, S., Gutteridge, A., Butt, R., McKernan, R.M., Whiting, P., Ali, Z., Bilsland, J., Stevens, E. B., 2016. Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. Sci. Transl. Med. 8, 335ra356.

Capurro, A., Thornton, J., Cessac, B., Armstrong, L., Sernagor, E., 2020. Nav1. 7 gating in human iPSC derived sensory neurons: an experimental and computational study. bioRxiv.

Chambers, S.M., Qi, Y., Mica, Y., Lee, G., Zhang, X.J., Niu, L., Bilsland, J., Cao, L., Stevens, E., Whiting, P., Shi, S.H., Studer, L., 2012. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat. Biotechnol. 30, 715–720.

Chiaradia, I., Lancaster, M.A., 2020. Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. Nat. Neurosci. 23, 1496–1508.

Choi, H.W., Hong, Y.J., Kim, J.S., Song, H., Cho, S.G., Bae, H., Kim, C., Byun, S.J., Do, J. T., 2017. In vivo differentiation of induced pluripotent stem cells into neural stem cells by chimera formation. PLoS One 12, e0170735.

Chrysostomidou, L., Cooper, A.H., Weir, G.A., 2021. Cellular models of pain: new technologies and their potential to progress preclinical research. Neurobiol. Pain 100063.

Clark, A.J., Kaller, M.S., Galino, J., Willison, H.J., Rinaldi, S., Bennett, D.L.H., 2017. Co-cultures with stem cell-derived human sensory neurons reveal regulators of peripheral myelination. Brain 140, 898–913.

Clark, A.J., Kugathasan, U., Baskozos, G., Priestman, D.A., Fugger, N., Lone, M.A., Othman, A., Chu, K.H., Blesneac, I., Wilson, E.R., Laura, M., Kalmar, B., Greensmith, L., Hornemann, T., Platt, F.M., Reilly, M.M., Bennett, D.L., 2021. An iPSC model of hereditary sensory neuropathy-1 reveals L-serine-responsive deficits in neuronal ganglioside composition and axoglial interactions. Cell Rep Med 2, 100345.

Deng, T., Tristan, C.A., Weber, C., Chu, P.-H., Ryu, S., Jovanovic, V.M., Ormanoglu, P., Twumasi, P., Shim, J., Jayakar, S., Zhang, H.-X.B., Jo, S., Voss, T.C., Simeonov, A., Bean, B.P., Woolf, C.J., View ORCID Profile Singeç, I, 2022. Scalable Generation of Pseudo-Unipolar Sensory Neurons from Human Pluripotent Stem Cells.

Deuis, J.R., Wingerd, J.S., Winter, Z., Durek, T., Dekan, Z., Sousa, S.R., Zimmermann, K., Hoffmann, T., Weidner, C., Nassar, M.A., Alewood, P.F., Lewis, R.J., Vetter, I., 2016. Analgesic effects of GpTx-1, PF-04856264 and CNV1014802 in a mouse model of NaV1.7-mediated pain. Toxins (Basel) 8.

di Domenico, A., Carola, G., Calatayud, C., Pons-Espinal, M., Munoz, J.P., Richaud-Patin, Y., Fernandez-Carasa, I., Gut, M., Faella, A., Parameswaran, J., Soriano, J., Ferrer, I., Tolosa, E., Zorzano, A., Cuervo, A.M., Raya, A., Consiglio, A., 2019. Patient-specific iPSC-derived astrocytes contribute to non-cell-autonomous neurodegeneration in Parkinson's disease. Stem Cell Rep. 12, 213–229.

Dib-Hajj, S.D., Waxman, S.G., 2019. Sodium channels in human pain disorders: genetics and pharmacogenomics. Annu. Rev. Neurosci. 42, 87–106.
Dib-Hajj, S.D., Rush, A.M., Cummins, T.R., Hisama, F.M., Novella, S., Tyrrell, L., Marshall, L., Waxman, S.G., 2005. Gain-of-function mutation in Nav1.7 in familial erythromelalgia induces bursting of sensory neurons. Brain 128, 1847–1854.
Dib-Hajj, S.D., Choi, J.S., Macala, L.J., Tyrrell, L., Black, J.A., Cummins, T.R., Waxman, S.G., 2009. Transfection of rat or mouse neurons by biolistics or electroporation. Nat. Protoc. 4, 1118–1126.
Dionisi, C., Rai, M., Chazalon, M., Schiffmann, S.N., Pandolfo, M., 2020. Primary proprioceptive neurons from human induced pluripotent stem cells: a cell model for afferent ataxias. Sci. Rep. 10, 7752.
Eberhardt, E., Havlicek, S., Schmidt, D., Link, A.S., Neacsu, C., Kohl, Z., Hampl, M., Kist, A.M., Klinger, A., Nau, C., Schuttler, J., Alzheimer, C., Winkler, J., Namer, B., Winner, B., Lampert, A., 2015. Pattern of functional TTX-resistant sodium channels reveals a developmental stage of human iPSC- and ESC-derived nociceptors. Stem Cell Rep. 5, 305–313.
Efrat, S., 2020. Epigenetic memory: lessons from iPS cells derived from human beta cells. Front Endocrinol (Lausanne) 11, 614234.
Eijkenboom, I., Sopacua, M., Otten, A.B.C., Gerrits, M.M., Hoeijmakers, J.G.J., Waxman, S.G., Lombardi, R., Lauria, G., Merkies, I.S.J., Smeets, H.J.M., Faber, C.G., Vanoevelen, J.M., Group, P.S, 2019. Expression of pathogenic SCN9A mutations in the zebrafish: a model to study small-fiber neuropathy. Exp. Neurol. 311, 257–264.
Elitt, M.S., Barbar, L., Tesar, P.J., 2018. Drug screening for human genetic diseases using iPSC models. Hum. Mol. Genet. 27, R89–R98.
Fu, W., Wang, S.J., Zhou, G.D., Liu, W., Cao, Y., Zhang, W.J., 2012. Residual undifferentiated cells during differentiation of induced pluripotent stem cells in vitro and in vivo. Stem Cells Dev. 21, 521–529.
Geha, P., Yang, Y., Estacion, M., Schulman, B.R., Tokuno, H., Apkarian, A.V., Dib-Hajj, S. D., Waxman, S.G., 2016. Pharmacotherapy for pain in a family with inherited Erythromelalgia guided by genomic analysis and functional profiling. JAMA Neurol 73, 659–667.
Guimaraes, M.Z.P., De Vecchi, R., Vitoria, G., Sochacki, J.K., Paulsen, B.S., Lima, I., Rodrigues da Silva, F., da Costa, R.F.M., Castro, N.G., Breton, L., Rehen, S.K., 2018. Generation of iPSC-derived human peripheral sensory neurons releasing substance P elicited by TRPV1 agonists. Front. Mol. Neurosci. 11, 277.
Haenseler, W., Sansom, S.N., Buchrieser, J., Newey, S.E., Moore, C.S., Nicholls, F.J., Chintawar, S., Schnell, C., Antel, J.P., Allen, N.D., Cader, M.Z., Wade-Martins, R., James, W.S., Cowley, S.A., 2017. A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. Stem Cell Rep. 8, 1727–1742.
Han, C., Estacion, M., Huang, J., Vasylyev, D., Zhao, P., Dib-Hajj, S.D., Waxman, S.G., 2015. Human Na(v)1.8: enhanced persistent and ramp currents contribute to distinct firing properties of human DRG neurons. J. Neurophysiol. 113, 3172–3185.
Hirano, M., Huang, Y., Vela Jarquin, D., De la Garza Hernandez, R.L., Jodat, Y.A., Luna Ceron, E., Garcia-Rivera, L.E., Shin, S.R., 2021. 3D bioprinted human iPSC-derived somatosensory constructs with functional and highly purified sensory neuron networks. Biofabrication 13.
Hockemeyer, D., Jaenisch, R., 2016. Induced pluripotent stem cells meet genome editing. Cell Stem Cell 18, 573–586.
Hoelting, L., Klima, S., Karreman, C., Grinberg, M., Meisig, J., Henry, M., Rotshteyn, T., Rahnenfuhrer, J., Bluthgen, N., Sachinidis, A., Waldmann, T., Leist, M., 2016. Stem cell-derived immature human dorsal root ganglia neurons to identify peripheral neurotoxicants. Stem Cells Transl. Med. 5, 476–487.
Huang, J., Han, C., Estacion, M., Vasylyev, D., Hoeijmakers, J.G., Gerrits, M.M., Tyrrell, L., Lauria, G., Faber, C.G., Dib-Hajj, S.D., Merkies, I.S., Waxman, S.G., Group, P.S, 2014. Gain-of-function mutations in sodium channel Na(v)1.9 in painful neuropathy. Brain 137, 1627–1642.
Huang, J., Vanoye, C.G., Cutts, A., Goldberg, Y.P., Dib-Hajj, S.D., Cohen, C.J., Waxman, S.G., George, A.L., 2017. Sodium channel NaV1.9 mutations associated with insensitivity to pain dampen neuronal excitability. J. Clin. Invest. 127, 2805–2814.
Huang, J., Estacion, M., Zhao, P., Dib-Hajj, F.B., Schulman, B., Abicht, A., Kurth, I., Brockmann, K., Waxman, S.G., Dib-Hajj, S.D., 2019. A novel gain-of-function Nav1.9 mutation in a child with episodic pain. Front. Neurosci. 13, 918.
Hulme, A.J., McArthur, J.R., Maksour, S., Miellet, S., Ooi, L., Adams, D.J., Finol-Urdaneta, R.K., Dottori, M., 2020. Molecular and functional characterization of neurogenin-2 induced human sensory neurons. Front. Cell. Neurosci. 14, 600895.
Inoue, H., Nagata, N., Kurokawa, H., Yamanaka, S., 2014. iPS cells: a game changer for future medicine. EMBO J. 33, 409–417.
Iyer, N.R., Wilems, T.S., Sakiyama-Elbert, S.E., 2017. Stem cells for spinal cord injury: strategies to inform differentiation and transplantation. Biotechnol. Bioeng. 114, 245–259.
Jones, I., Yelhekar, T.D., Wiberg, R., Kingham, P.J., Johansson, S., Wiberg, M., Carlsson, L., 2018. Development and validation of an in vitro model system to study peripheral sensory neuron development and injury. Sci. Rep. 8, 15961.
Kang, E., Wang, X., Tippner-Hedges, R., Ma, H., Folmes, C.D., Gutierrez, N.M., Lee, Y., Van Dyken, C., Ahmed, R., Li, Y., Koski, A., Hayama, T., Luo, S., Harding, C.O., Amato, P., Jensen, J., Battaglia, D., Lee, D., Wu, D., Terzic, A., Wolf, D.P., Huang, T., Mitalipov, S., 2016. Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs. Cell Stem Cell 18, 625–636.
Kapalczynska, M., Kolenda, T., Przybyla, W., Zajaczkowska, M., Teresiak, A., Filas, V., Ibbs, M., Blizniak, R., Luczewski, L., Lamperska, K., 2018. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. Arch. Med. Sci. 14, 910–919.
Kim, A., Lee, S.Y., Kim, B.Y., Chung, S.K., 2020a. Elimination of teratogenic human induced pluripotent stem cells by bee venom via calcium-Calpain pathway. Int. J. Mol. Sci. 21.
Kim, H.S., Kim, J.Y., Song, C.L., Jeong, J.E., Cho, Y.S., 2020b. Directly induced human Schwann cell precursors as a valuable source of Schwann cells. Stem Cell Res Ther 11, 257.
Lampert, A., Bennett, D.L., McDermott, L.A., Neureiter, A., Eberhardt, E., Winner, B., Zenke, M., 2020. Human sensory neurons derived from pluripotent stem cells for disease modelling and personalized medicine. Neurobiol Pain 8, 100055.
Lee, G., Papapetrou, E.P., Kim, H., Chambers, S.M., Tomishima, M.J., Fasano, C.A., Ganat, Y.M., Menon, J., Shimizu, F., Viale, A., Tabar, V., Sadelain, M., Studer, L., 2009. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461, 402–406.
Lee, J.H., Mitchell, R.R., McNicol, J.D., Shapovalova, Z., Laronde, S., Tanasijevic, B., Milsom, C., Casado, F., Fiebig-Comyn, A., Collins, T.J., Singh, K.K., Bhatia, M., 2015. Single transcription factor conversion of human blood fate to NPCs with CNS and PNS developmental capacity. Cell Rep. 11, 1367–1376.
Luo, Y., Liu, C., Cerbini, T., San, H., Lin, Y., Chen, G., Rao, M.S., Zou, J., 2014. Stable enhanced green fluorescent protein expression after differentiation and transplantation of reporter human induced pluripotent stem cells generated by AAVS1 transcription activator-like effector nucleases. Stem Cells Transl. Med. 3, 821–835.
Marmigere, F., Ernfors, P., 2007. Specification and connectivity of neuronal subtypes in the sensory lineage. Nat. Rev. Neurosci. 8, 114–127.
Martinez-Cerdeno, V., Barrilleaux, B.L., McDonough, A., Ariza, J., Yuen, B.T.K., Somanath, P., Le, C.T., Steward, C., Horton-Sparks, K., Knoepfler, P.S., 2017. Behavior of xeno-transplanted undifferentiated human induced pluripotent stem cells is impacted by microenvironment without evidence of tumors. Stem Cells Dev. 26, 1409–1423.
Mazzara, P.G., Muggeo, S., Luoni, M., Massimino, L., Zaghi, M., Valverde, P.T., Brusco, S., Marzi, M.J., Palma, C., Colasante, G., Iannielli, A., Paulis, M., Cordiglieri, C., Giannelli, S.G., Podini, P., Gellera, C., Taroni, F., Nicassio, F., Rasponi, M., Broccoli, V., 2020. Frataxin gene editing rescues Friedreich's ataxia pathology in dorsal root ganglia organoid-derived sensory neurons. Nat. Commun. 11, 4178.
McDermott, L.A., Weir, G.A., Themistocleous, A.C., Segerdahl, A.R., Blesneac, I., Baskozos, G., Clark, A.J., Millar, V., Peck, L.J., Ebner, D., Tracey, I., Serra, J., Bennett, D.L., 2019a. Defining the functional role of NaV1.7 in human nociception. Neuron 101, 905–919.
McDermott, L.A., Weir, G.A., Themistocleous, A.C., Segerdahl, A.R., Blesneac, I., Baskozos, G., Clark, A.J., Millar, V., Peck, L.J., Ebner, D., Tracey, I., Serra, J., Bennett, D.L., 2019b. Defining the functional role of NaV1.7 in human nociception. Neuron 101, 905–919.
McDonnell, A., Collins, S., Ali, Z., Iavarone, L., Surujbally, R., Kirby, S., Butt, R.P., 2018. Efficacy of the Nav1.7 blocker PF-05089771 in a randomised, placebo-controlled, double-blind clinical study in subjects with painful diabetic peripheral neuropathy. Pain 159, 1465–1476.
Meents, J.E., Bressan, E., Sontag, S., Foerster, A., Hautvast, P., Rosseler, C., Hampl, M., Schuler, H., Goetzke, R., Le, T.K.C., Kleggetveit, I.P., Le Cann, K., Kerth, C., Rush, A. M., Rogers, M., Kohl, Z., Schmelz, M., Wagner, W., Jorum, E., Namer, B., Winner, B., Zenke, M., Lampert, A., 2019. The role of Nav1.7 in human nociceptors: insights from human induced pluripotent stem cell-derived sensory neurons of erythromelalgia patients. Pain 160, 1327–1341.
Middleton, S.J., Barry, A.M., Comini, M., Li, Y., Ray, P.R., Shiers, S., Themistocleous, A. C., Uhelski, M.L., Yang, X., Dougherty, P.M., Price, T.J., Bennett, D.L., 2021. Studying human nociceptors: from fundamentals to clinic. Brain 144, 1312–1335.
Milescu, L.S., Bean, B.P., Smith, J.C., 2010. Isolation of somatic Na+ currents by selective inactivation of axonal channels with a voltage prepulse. J. Neurosci. 30, 7740–7748.
Mis, M.A., Yang, Y., Tanaka, B.S., Gomis-Perez, C., Liu, S., Dib-Hajj, F., Adi, T., Garcia-Milian, R., Schulman, B.R., Dib-Hajj, S.D., Waxman, S.G., 2019. Resilience to pain: a peripheral component identified using induced pluripotent stem cells and dynamic clamp. J. Neurosci. 39, 382–392.
Moehring, F., Cowie, A.M., Menzel, A.D., Weyer, A.D., Grzybowski, M., Arzua, T., Geurts, A.M., Palygin, O., Stucky, C.L., 2018. Keratinocytes mediate innocuous and noxious touch via ATP-P2X4 signaling. Elife 7.
Mofazzal Jahromi, M.A., Abdoli, A., Rahmanian, M., Bardania, H., Bayandori, M., Moosavi Basri, S.M., Kalbasi, A., Aref, A.R., Karimi, M., Hamblin, M.R., 2019. Microfluidic brain-on-a-chip: perspectives for mimicking neural system disorders. Mol. Neurobiol. 56, 8489–8512.
Mogil, J.S., 2009. Animal models of pain: progress and challenges. Nat. Rev. Neurosci. 10, 283–294.
Muller, Q., Beaudet, M.J., De Serres-Berard, T., Bellenfant, S., Flacher, V., Berthod, F., 2018. Development of an innervated tissue-engineered skin with human sensory neurons and Schwann cells differentiated from iPS cells. Acta Biomater. 82, 93–101.
Namer, B., Schmidt, D., Eberhardt, E., Maroni, M., Dorfmeister, E., Kleggetveit, I.P., Kaluza, L., Meents, J., Gerlach, A., Lin, Z., Winterpacht, A., Dragicevic, E., Kohl, Z., Schuttler, J., Kurth, I., Warncke, T., Jorum, E., Winner, B., Lampert, A., 2019. Pain relief in a neuropathy patient by lacosamide: proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. EBioMedicine 39, 401–408.
Nickolls, A.R., Lee, M.M., Espinoza, D.F., Szczot, M., Lam, R.M., Wang, Q., Beers, J., Zou, J., Nguyen, M.Q., Solinski, H.J., AlJanahi, A.A., Johnson, K.R., Ward, M.E., Chesler, A.T., Bonnemann, C.G., 2020. Transcriptional programming of human mechanosensory neuron subtypes from pluripotent stem cells. Cell Rep. 30 (932–946), e937.
Parisien, M., Khoury, S., Chabot-Dore, A.J., Sotocinal, S.G., Slade, G.D., Smith, S.B., Fillingim, R.B., Ohrbach, R., Greenspan, J.D., Maixner, W., Mogil, J.S., Belfer, I., Diatchenko, L., 2017. Effect of human genetic variability on gene expression in dorsal root ganglia and association with pain phenotypes. Cell Rep. 19, 1940–1952.

Pereira, J.D., DuBreuil, D.M., Devlin, A.C., Held, A., Sapir, Y., Berezovski, E., Hawrot, J., Dorfman, K., Chander, V., Wainger, B.J., 2021. Human sensorimotor organoids derived from healthy and amyotrophic lateral sclerosis stem cells form neuromuscular junctions. Nat. Commun. 12, 4744.
Pettingill, P., Weir, G.A., Wei, T., Wu, Y., Flower, G., Lalic, T., Handel, A., Duggal, G., Chintawar, S., Cheung, J., Arunasalam, K., Couper, E., Haupt, L.M., Griffiths, L.R., Bassett, A., Cowley, S.A., Cader, M.Z., 2019. A causal role for TRESK loss of function in migraine mechanisms. Brain 142, 3852–3867.
Popp, B., Krumbiegel, M., Grosch, J., Sommer, A., Uebe, S., Kohl, Z., Plotz, S., Farrell, M., Trautmann, U., Kraus, C., Ekici, A.B., Asadollahi, R., Regensburger, M., Gunther, K., Rauch, A., Edenhofer, F., Winkler, J., Winner, B., Reis, A., 2018. Need for high-resolution genetic analysis in iPSC: results and lessons from the ForIPS consortium. Sci. Rep. 8, 17201.
Prinz, A.A., Abbott, L.F., Marder, E., 2004. The dynamic clamp comes of age. Trends Neurosci. 27, 218–224.
Ray, P., Torck, A., Quigley, L., Wangzhou, A., Neiman, M., Rao, C., Lam, T., Kim, J.Y., Kim, T.H., Zhang, M.Q., Dussor, G., Price, T.J., 2018. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. Pain 159, 1325–1345.
Renganathan, M., Dib-Hajj, S., Waxman, S.G., 2002. Na(v)1.5 underlies the 'third TTX-R sodium current' in rat small DRG neurons. Brain Res. Mol. Brain Res. 106, 70–82.
Renthal, W., Chamessian, A., Curatolo, M., Davidson, S., Burton, M., Dib-Hajj, S., Dougherty, P.M., Ebert, A.D., Gereau, R.W., Ghetti, A., Gold, M.S., Hoben, G., Menichella, D.M., Mercier, P., Ray, W.Z., Salvemini, D., Seal, R.P., Waxman, S., Woolf, C.J., Stucky, C.L., Price, T.J., 2021. Human cells and networks of pain: transforming pain target identification and therapeutic development. Neuron 109, 1426–1429.
Rostock, C., Schrenk-Siemens, K., Pohle, J., Siemens, J., 2018. Human vs. mouse nociceptors - similarities and differences. Neuroscience 387, 13–27.
Saito-Diaz, K., Street, J.R., Ulrichs, H., Zeltner, N., 2021. Derivation of peripheral nociceptive, Mechanoreceptive, and proprioceptive sensory neurons from the same culture of human pluripotent stem cells. Stem Cell Rep. 16, 446–457.
Schinke, C., Fernandez Vallone, V., Ivanov, A., Peng, Y., Kortvelyessy, P., Nolte, L., Huehnchen, P., Beule, D., Stachelscheid, H., Boehmerle, W., Endres, M., 2021. Modeling chemotherapy induced neurotoxicity with human induced pluripotent stem cell (iPSC) -derived sensory neurons. Neurobiol. Dis. 155, 105391.
Schrenk-Siemens, K., Wende, H., Prato, V., Song, K., Rostock, C., Loewer, A., Utikal, J., Lewin, G.R., Lechner, S.G., Siemens, J., 2015. PIEZO2 is required for mechanotransduction in human stem cell-derived touch receptors. Nat. Neurosci. 18, 10–16.
Schutte, S.C., Kadakia, F., Davidson, S., 2021. Skin-nerve co-culture Systems for Disease Modeling and Drug Discovery. Tissue Eng Part C Methods 27, 89–99.
Schwaid, A.G., Krasowka-Zoladek, A., Chi, A., Cornella-Taracido, I., 2018. Comparison of the rat and human dorsal root ganglion proteome. Sci. Rep. 8, 13469.
Schwartzentruber, J., Foskolou, S., Kilpinen, H., Rodrigues, J., Alasoo, K., Knights, A.J., Patel, M., Goncalves, A., Ferreira, R., Benn, C.L., Wilbrey, A., Bictash, M., Impey, E., Cao, L., Lainez, S., Loucif, A.J., Whiting, P.J., Consortium, H., Gutteridge, A., Gaffney, D.J., 2018. Molecular and functional variation in iPSC-derived sensory neurons. Nat. Genet. 50, 54–61.
Shields, S.D., Ahn, H.-S., Yang, Y., Han, C., Seal, R.P., Wood, J.N., Waxman, S.G., Dib-Hajj, S.D., 2012. Nav1. 8 expression is not restricted to nociceptors in mouse peripheral nervous system. PAIN® 153, 2017–2030.
Shiers, S., Klein, R.M., Price, T.J., 2020a. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. Pain 161, 2410–2424.
Shiers, S., Ray, P.R., Wangzhou, A., Sankaranarayanan, I., Tatsui, C.E., Rhines, L.D., Li, Y., Uhelski, M.L., Dougherty, P.M., Price, T.J., 2020b. ACE2 and SCARF expression in human dorsal root ganglion nociceptors: implications for SARS-CoV-2 virus neurological effects. Pain 161, 2494–2501.
Sopacua, M., Hoeijmakers, J.G.J., Merkies, I.S.J., Lauria, G., Waxman, S.G., Faber, C.G., 2019. Small-fiber neuropathy: expanding the clinical pain universe. J. Peripher. Nerv. Syst. 24, 19–33.
Stacey, P., Wassermann, A.M., Kammonen, L., Impey, E., Wilbrey, A., Cawkill, D., 2018. Plate-based phenotypic screening for pain using human iPSC-derived sensory neurons. SLAS Discov 23, 585–596.
Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676.
Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.
Talagas, M., Lebonvallet, N., Berthod, F., Misery, L., 2020a. Lifting the veil on the keratinocyte contribution to cutaneous nociception. Protein Cell 11, 239–250.
Talagas, M., Lebonvallet, N., Leschiera, R., Sinquin, G., Elies, P., Haftek, M., Pennec, J.P., Ressnikoff, D., La Padula, V., Le Garrec, R., L'herondelle, K., Mignen, O., Le Pottier, L., Kerfant, N., Reux, A., Marcorelles, P., Misery, L., 2020b. Keratinocytes communicate with sensory neurons via synaptic-like contacts. Ann. Neurol. 88, 1205–1219.
Tavares-Ferreira, D., Shiers, S., Ray, P.R., Wangzhou, A., Jeevakumar, V., Sankaranarayanan, I., Cervantes, A.M., Reese, J.C., Chamessian, A., Copits, B.A., Dougherty, P.M., Gereau, R.W., Burton, M.D., Dussor, G., Price, T.J., 2022. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci. Transl. Med. 14, eabj8186.
Thanuthanakhun, N., Kino-Oka, M., Borwornpinyo, S., Ito, Y., Kim, M.H., 2021. The impact of culture dimensionality on behavioral epigenetic memory contributing to pluripotent state of iPS cells. J. Cell. Physiol. 236, 4985–4996.
Umehara, Y., Toyama, S., Tominaga, M., Matsuda, H., Takahashi, N., Kamata, Y., Niyonsaba, F., Ogawa, H., Takamori, K., 2020. Robust induction of neural crest cells to derive peripheral sensory neurons from human induced pluripotent stem cells. Sci. Rep. 10, 4360.
Viventi, S., Dottori, M., 2018. Modelling the dorsal root ganglia using human pluripotent stem cells: a platform to study peripheral neuropathies. Int. J. Biochem. Cell Biol. 100, 61–68.
Viventi, S., Frausin, S., Howden, S.E., Lim, S.Y., Finol-Urdaneta, R.K., McArthur, J.R., Abu-Bonsrah, K.D., Ng, W., Ivanusic, J., Thompson, L., Dottori, M., 2021. In vivo survival and differentiation of Friedreich ataxia iPSC-derived sensory neurons transplanted in the adult dorsal root ganglia. Stem Cells Transl. Med. 10, 1157–1169.
Vojnits, K., Mahammad, S., Collins, T.J., Bhatia, M., 2019. Chemotherapy-induced neuropathy and drug discovery platform using human sensory neurons converted directly from adult peripheral blood. Stem Cells Transl. Med. 8, 1180–1191.
Volpato, V., Webber, C., 2020. Addressing variability in iPSC-derived models of human disease: guidelines to promote reproducibility. Dis. Model. Mech. 13.
Wainger, B.J., Buttermore, E.D., Oliveira, J.T., Mellin, C., Lee, S., Saber, W.A., Wang, A. J., Ichida, J.K., Chiu, I.M., Barrett, L., Huebner, E.A., Bilgin, C., Tsujimoto, N., Brenneis, C., Kapur, K., Rubin, L.L., Eggan, K., Woolf, C.J., 2015. Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. Nat. Neurosci. 18, 17–24.
Wang, Y., Wang, L., Guo, Y., Zhu, Y., Qin, J., 2018. Engineering stem cell-derived 3D brain organoids in a perfusable organ-on-a-chip system. RSC Adv. 8, 1677–1685.
Wei, Z., Fei, Y., Su, W., Chen, G., 2019. Emerging role of Schwann cells in neuropathic pain: receptors, glial mediators and myelination. Front. Cell. Neurosci. 13, 116.
Wiegand, C., Banerjee, I., 2019. Recent advances in the applications of iPSC technology. Curr. Opin. Biotechnol. 60, 250–258.
Wilson, R., Ahmmed, A.A., Poll, A., Sakaue, M., Laude, A., Sieber-Blum, M., 2018. Human peptidergic nociceptive sensory neurons generated from human epidermal neural crest stem cells (hEPI-NCSC). PLoS One 13, e0199996.
Xiao, D., Deng, Q., Guo, Y., Huang, X., Zou, M., Zhong, J., Rao, P., Xu, Z., Liu, Y., Hu, Y., Shen, Y., Jin, K., Xiang, M., 2020. Generation of self-organized sensory ganglion organoids and retinal ganglion cells from fibroblasts. Sci. Adv. 6, eaaz5858.
Yang, Y., Huang, J., Mis, M.A., Estacion, M., Macala, L., Shah, P., Schulman, B.R., Horton, D.B., Dib-Hajj, S.D., Waxman, S.G., 2016. Nav1.7-A1632G Mutation from a Family with Inherited Erythromelalgia: Enhanced Firing of Dorsal Root Ganglia Neurons Evoked by Thermal Stimuli. J. Neurosci. 36, 7511–7522.
Yezierski, R.P., Hansson, P., 2018. Inflammatory and neuropathic pain from bench to bedside: what went wrong? J. Pain 19, 571–588.
Young, G.T., Gutteridge, A., Fox, H., Wilbrey, A.L., Cao, L., Cho, L.T., Brown, A.R., Benn, C.L., Kammonen, L.R., Friedman, J.H., Bictash, M., Whiting, P., Bilsland, J.G., Stevens, E.B., 2014. Characterizing human stem cell-derived sensory neurons at the single-cell level reveals their ion channel expression and utility in pain research. Mol. Ther. 22, 1530–1543.
Yu, T., Miyagawa, S., Miki, K., Saito, A., Fukushima, S., Higuchi, T., Kawamura, M., Kawamura, T., Ito, E., Kawaguchi, N., Sawa, Y., Matsuura, N., 2013. In vivo differentiation of induced pluripotent stem cell-derived cardiomyocytes. Circ. J. 77, 1297–1306.
Yuan, J.H., Estacion, M., Mis, M.A., Tanaka, B.S., Schulman, B.R., Chen, L., Liu, S., Dib-Hajj, F.B., Dib-Hajj, S.D., Waxman, S.G., 2021. KCNQ variants and pain modulation: a missense variant in Kv7.3 contributes to pain resilience. Brain Commun 3, fcab212.
Zakrzewska, J.M., Palmer, J., Morisset, V., Giblin, G.M., Obermann, M., Ettlin, D.A., Cruccu, G., Bendtsen, L., Estacion, M., Derjean, D., Waxman, S.G., Layton, G., Gunn, K., Tate, S., study, i, 2017. Safety and efficacy of a Nav1.7 selective sodium channel blocker in patients with trigeminal neuralgia: a double-blind, placebo-controlled, randomised withdrawal phase 2a trial. Lancet Neurol. 16, 291–300.
Zhang, X., Priest, B.T., Belfer, I., Gold, M.S., 2017. Voltage-gated Na(+) currents in human dorsal root ganglion neurons. Elife 6.