MIT Libraries | DSpace@MIT

MIT Open Access Articles

CNS disease models with human pluripotent stem cells in the CRISPR age

The MIT Faculty has made this article openly available. ***Please share*** how this access benefits you. Your story matters.

Citation: Muffat, Julien et al. "CNS Disease Models with Human Pluripotent Stem Cells in the CRISPR Age." Current Opinion in Cell Biology 43 (December 2016): 96–103 © 2016 Published by Elsevier Ltd

As Published: https://doi.org/10.1016/j.ceb.2016.10.001

Publisher: Elsevier

Persistent URL: http://hdl.handle.net/1721.1/117128

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Terms of use: Creative Commons Attribution-NonCommercial-NoDerivs License

Massachusetts Institute of Technology

CNS disease models with human pluripotent stem cells in the CRISPR age

Julien Muffat[1], Yun Li[1], Rudolf Jaenisch[#]
Whitehead Institute for Biomedical Research,
9Cambridge center, Cambridge, MA 02142

[1] Equal contribution
[#] Corresponding author: jaenisch@wi.mit.edu

In vitro differentiation of human pluripotent stem cells provides a systematic platform to investigate the physiological development and function of the human nervous system, as well as the etiology and consequence when these processes go awry. Recent development in three-dimensional (3D) organotypic culture systems allows modeling of the complex structure formation of the human CNS, and the intricate interactions between various resident neuronal and glial cell types. Combined with an ever-expanding genome editing and regulation toolkit such as CRISPR/Cas9, it is now a possibility to study human neurological disease in the relevant molecular, cellular and anatomical context. In this article, we review recent progress in 3D neural culture and the implications for disease modeling.

A need for human genetic models of CNS disorders

The derivation of human induced pluripotent stem (iPS) cells[1-4] allows limitless selection of donors carrying known genetic variants, including dominant single mutations, in order to study their effects using disease-relevant *in vitro* phenotypes[5,6]. In some cases, such effects may rise high above the system's variability, inherent to culture conditions and unknown genetic backgrounds. This may be true for fully penetrant mutations causing familial disorders such as early onset Alzheimer's disease (e.g. PS1 and APP mutations)[7,8], or Parkinson's disease (SYNCA, etc..)[5], or for rare monogenic diseases such as Adrenoleukodystrophy. Protein gain or loss-of-function can be monitored in relevant human cell types (such as neurons or astrocytes) with a previously unavailable precision. However, most neurological and psychiatric disorders, whether broadly defined as developmental or degenerative, are the product of complex environmental factors and combinations of genes, whose actions over the lifetime of the individual allow selective vulnerabilities to emerge in specific cell types of different tissues. Most instances of Alzheimer and Parkinson are so-called sporadic cases[9,10]. Genome wide association studies (GWAS) have begun to uncover the plethora of coding or noncoding sequences involved[11], and efforts are being made to tease out the individual variants' contributions to disease onset[9,12]. A common research goal is to make new variants fit within the decades old framework provided by the study of monogenic diseases, in model organisms and more traditional human cancer line cultures[13]. More interesting will be to see how these new associations can reshape our understanding of these diseases.

A fast evolving genetic engineering toolkit

To enable this paradigm shift, two complementary efforts must continue: 1) to use human donors as a cohort of spontaneously occurring mutants, while recognizing the great variability of

genetic backgrounds and life histories 2) to isolate the effects of individual genetic variations presenting statistical association with disease by keeping constant as many biological parameters as possible[14]. In an otherwise highly defined culture system, genome engineering is the natural way to perform such an isolation of hyperlocal genotype from background effects. Gene-targeting tools based on homologous recombination, which is efficient in model organisms, proved remarkably inefficient in human cells. The *status quo* changed when it was realized that combining traditional targeting methods (knock-out or knock-in) with ways of creating double-stranded DNA breaks at specific loci, could improve the targeting efficiency by orders of magnitude. In rapid succession, remarkably coinciding and accompanying the development of better culture systems for human pluripotent stem cells, various nucleases were used as mutagenic agents. While zinc-finger nucleases[15] provided an improvement over mega-nucleases[16] in our understanding of their pairing rules with specific DNA sequences and Tale-Effector nucleases (TALENs) made the system truly modular[17,18], allowing individual laboratories to design enzymes to cut specific sequences at will. This shortened the workflow for the generation of defined mutant cells from months to a few weeks. The true revolution allowing both widespread use of these technologies, and a more systematic trial-and-error approach, came, however, with the advent of the CRISPR/Cas9 system[19]. This bipartite system requires the transient co-expression of a constant moiety, the enzyme CAS9, alongside one or several sequences of RNA complementary of the sequence(s) of interest. The design of such guide RNAs (gRNAs) follows simple rules, and appropriate complementary targets are found at high density throughout the genome. Vectors for delivery of CAS9 and gRNAs were made readily available to the community, in a shining example of academic open-sourcing[20,21]. Today, individual gRNAs sequenced for a target of interest can be cloned into a delivery vector and tested for their cutting efficiency within a matter of days.

With these tools in hands, it is now possible to target individual alleles with high efficiency in order to introduce or remove a particular genetic variant, while keeping the genetic background constant (**Figure 1A**). Human embryonic stem (ES) cells or iPS cells carrying such variants provide the essential starting material for any attempt at robust disease modeling in two or three dimensions. One can select a permissive genetic background, repairing the genome of a known disease-affected donor, or, alternatively, test the sufficiency of a particular genotype on phenotype presentation introducing the mutation in a random background. Given the flexibility of the system, actual epistatic analyses of various genetic contributions can now be performed. CRISPR is a fast-evolving technology, with modifications being introduced at a remarkable pace. For example, CAS9 was modified to be rendered inactive so as to simply block transcription where it preferentially binds following its gRNA[22]. Inactive CAS9 can be fused with trans-activator domains, to positively or negatively affect gene expression at control regions (promoter/enhancers)[23-25]. The enzyme can even be fused to epigenome-modifying enzymes[26] and affect genomic accessibility with pinpoint accuracy.

Recapitulating human neural development with 3D organoid cultures

In parallel to the development of better gene-modification techniques, the iPS boom fostered a diversification of methods (**Figure 1B and C)** to turn pluripotent stem cells into terminally differentiated cells of interest[27-34]. The neural lineage is a default differentiation path of human

pluripotent stem cells. Upon withdrawal of growth factors that maintain pluripotency, and without the requirement of external cues, cells with neural morphology and transcriptional signatures emerge spontaneously, which can be further differentiated into a mixture of neuronal and glial progenies. Using similar guiding principles and utilizing the self-organizing capacity of pluripotent stem cells, Yoshiki Sasai and colleagues demonstrated the generation of polarized neural tissues from dissociated mouse[35] and human[36] pluripotent stem cells, termed SFEBq (serum-free floating culture of embryoid body-like aggregates with quick reaggregation). This method was further developed by Juergen Knoblich and colleagues to include medium agitation via spinning or shaking (**Figure 1D**), and embedding into extracellular matrix for additional nutrient and structural support, allowing the generation of large neural tissues referred to as cerebral organoids[37]. In the absence of external patterning cues, neural cells of diverse anatomical and lineage identity can be generated within the organoids, including the predominant cortical forebrain lineage, as well as midbrain, hindbrain and hippocampal identity[37]. Addition of morphogens and growth factors can further promote the formation of organoids with refined regional identity[35,36,38-40]. Brain organoids can be maintained in suspension culture for prolonged period of time in neural maturation medium, facilitated by the addition of extracellular matrix and neurotrophic factors. Long-term culture of brain organoids allows the development of stratified cortex and the generation of post-mitotic neurons that form rudimentary cortical layers resembling early developing human brain. Furthermore, *in vitro* generated brain organoids display human-specific features such as the presence of an outer sub-ventricular zone and regulated spindle orientation at the ventricular surface[37,39]. These species-specific features are posited to underlie the expansion of human brain in size and complexity compared to lower organisms, and yet their investigation was precluded in model organisms such as rodents. Therefore, human brain organoids represent a novel method to investigate the molecular, cellular, and anatomical paradigms of human cortical development.

Disease modeling with patient-derived iPSCs

The most attractive application of human cerebral organoid culture system is to model the origin and pathogenesis of human neurological diseases, especially in aspects that involve organ structure formation and complex cell-cell interaction otherwise difficult to investigate using simplified 2D culture. Albeit still at an early stage, disease modeling utilizing patient-derived organoids has begun to shed light on the pathophysiology of early developmental disorder such as microcephaly[37], as well as adult-onset psychiatric disorder such as schizophrenia[38]. Starting from pluripotent stem cells, current methods of brain organoid generation mimic the early stages of neural development that parallel human fetal brain formation and therefore would be well adept at modeling neurodevelopmental disorders stemming from dysfunctional behaviors of neural precursors and immature neurons that can cause structural defects of the brain such as microcephaly, macrocephaly, lissencephaly, doublecortex syndrome, and polymicrogyria. Brain organoids containing mixed neuronal population, or pre-patterned towards specific subtype lineage can be powerful tools to investigate the establishment of proper cellular composite within a developing brain. Disturbance in the balance of excitatory and inhibitory neuron production has been reported in animal and human neuron models of autism spectrum disorders[41]. Indeed, overproduction of inhibitory neurons has been observed in brain organoids derived from patients with schizophrenia[38]. The ability to maintain brain organoids in culture for

many months allows the maturation of neurons and neuronal network, providing a more physiological platform to understanding developmental disorders with electrophysiological imbalance, such as Rett syndrome, Fragile X syndrome, ASDs and epileptic disorders. Furthermore, selective degeneration of vulnerable neuronal and glial subtypes can be modeled in brain organoids, from human pluripotent stem cells with genetic predisposition for neurodegenerative disorders such as AD, PD, HD and ALS.

Microglia, the missing link

One of the great limitations in CNS disease modeling has been the absence of several key cell types such as microglia that, unlike the resident neural cell types that originate from the neural tube, derive from primitive macrophages at early stages of yolk-sac hematopoiesis[42-45], or endothelial cells that vascularize the early nervous system from the outside in. Microglia are particularly relevant to CNS disorders, as the only resident innate immune cell[46]. It is now recognized that microglia play a role in normal physiology during the early stages of CNS development, for example as phagocytes of apoptotic bodies from supernumerary progenitors[47], or later as gardeners of the extensive arborization of neurons, pruning away synaptic contacts following signals under active investigation. As a result, the potential for microglial dysfunction to trigger, accelerate, or modulate the resolution of disease is becoming the focus of intense scrutiny. For example, recent GWAS of AD, PD or schizophrenia have highlighted several genes which are specifically expressed in microglia[48,49]. Thus, any study of disease biology without evaluating the role of microglia misses a key biological interaction and may have limited value. In the developing and mature CNS, microglia are the first responders to pathogenic insults, whether it be invasion by pathogens such as viruses or bacteria, or overt injury from trauma or stroke. Microglia activated by certain triggers can turn directly cytotoxic to neurons or oligodendrocytes. They can also alter the permeability of the blood-brain-barrier through the release of proteases[50], and secrete cytokines and chemokines to recruit and activate cells of the peripheral immune system (innate and adaptive) in a process known as neuro-inflammation[46]. Once neuro-inflammation sets in, its unfolding can have more deleterious consequences than the original insult. This is particularly true in cases of bacterial or viral encephalitis[51], or during stroke recovery, where conservative treatments attempt at dampening the reactivity of these cells. Importantly, while the ontogenic nature of microglia singles them out, their identity is shaped by residence in the CNS microenvironment. Primary microglia are notoriously difficult to study in culture owing to their incredible plasticity and their reactivity to isolation from their tissue of residence, highlighting a need for tissue-like cultures of these cells. Given microglia's early embryonic origin, and their inherent plasticity, pluripotent stem cells combined with defined minimal media culture conditions represent an excellent source of microglia-like cells for *in vitro* studies. We found (Nature Medicine, Accepted) that it is possible to efficiently generate primitive yolk-sac macrophages from pluripotent stem cells, and mature them in fully defined conditions into microglia-like cells. We also showed that exposure to neural conditioned medium can further refine their signature (as shown in **Figure 1B**). Finally, we showed that these cells can be physically re-embedded into tissue-like three-dimensional neural cultures (as in **Figure 1C**). As they mature in these conditions, microglia-like cells adopt morphological characteristics of so-called quiescent ramified microglia, and display the purine-sensing motility observed *in vivo.*

Environmental insults: the example of neurotropic viruses

The etiology of monogenic developmental disorders such as Rett syndrome or microcephaly may be studied using cerebral organoids as described above. The tools developed for such research are being established in a timely manner to tackle our mechanistic understanding of abnormal CNS development triggered by environmental insults. Several viruses have known neurotropic teratogenic consequences, and the recent outbreak of Zika virus (ZKV) is further highlighting the need for better humanized models. Until recently ZKV had not been linked to microcephaly. In just a few months, statistical analysis of case reports acknowledged the damning correlation between maternal infection and fetal malformations[52-55]. Concurrently, *in vitro* models highlighted the remarkable cytotoxicity of ZKV for human neural stem cells[56], and the ability of the virus to limit the growth of brain organoids[39,54,57]. However, much remains to be done to understand how maternal viremia may ultimately lead to fetal brain malformations, or what happens to an infected nervous system in absence of overt microcephaly. Pluripotent stem cell cultures present a unique opportunity to tease apart ZKV tropism for different cell types. These models may allow the study of viral dissemination from cell type to cell type. Enhanced brain organoids displaying cortical gyrification can be used to study the appearance of lissencephaly as well as microcephaly. Inclusion of microglia may model the early interactions of these immune cells with the virus, and their consequences on the surrounding developing CNS. Such studies will identify cellular viral carriers (Trojan horses), innate defenses, as well as long term reservoirs. In doing so, they will provide platforms to screen for therapeutic intervention at each instance of virus/cell interaction along the way.

Long-term 3D cultures: beyond developing mini-brains

Traditional 2D cell culture guarantees that most of the membrane of a given cell is in contact with either the artificial plastic substrate, or the acellular medium. The brain's extracellular matrix is a combination of amorphous interstitial components and compacted perineural nets, composed of proteoglycans and link proteins not found in other tissues[58,59]. Classical bioengineering approaches using synthetic hydrogels and various biomaterials as scaffolds do not replicate the *in vivo* cell-to-cell, or cell-to-ECM contacts either. In contrast, self-organizing organoids[37] do allow cells to establish normal contacts with each other, as they lay out their own extracellular matrices. Cells are then free to differentiate, sort out or migrate in the structures they form, following some of the signals they would experience *in vivo*. In the case of CNS development, where signals necessary for the establishment of the dorso-ventral and rostro-caudal axes are relatively well understood, one can bioengineer methods to impose such polarities onto organoids as they form[60,61]. Given the ability of pluripotent stem cells or neural progenitors to aggregate, grow and differentiate as three-dimensional objects, one can also envisage the purposeful re-aggregation of defined numbers of the main cell types of the nervous system, neurons, astrocytes, oligodendrocytes and microglia in static cultures, more amenable to longitudinal study than free-floating organoids. Individual cell types can be pre-differentiated separately, validated and sorted, before re-aggregation in thick three-dimensional stacks, in absence of additional scaffolds (**Figure 1C**). This would allow the study of contact, autocrine and paracrine non-cell-autonomous interactions. Such cultures can be used for drug screening or to compare isogenic lines and follow phenotypic output of engineered genotype variants.

Cells of the nervous system experience disease alterations in a complex system that is composed of many post-mitotic cells with limited lifelong turnover, with genetic and environmental factors exerting cumulative damage. Such damage may be difficult to model in two dimensional cultures, in no small part because the current culture systems include improperly designed rodent-centric media that do not match the normal human milieu, and require feeding schedules which are far from replicating the constant homeostasis found *in vivo*. As a simple example, it is not uncommon to see the pH of a routine neuronal culture drop from 7.3 upon feeding, to 6.0 before the next feeding. In the clinic, such a change would be associated with irreversible cellular damage, and negative vital prognostic. Energetic substrates' and excreted metabolites' concentrations vary widely with feeding schedules. Large insoluble byproducts of cellular metabolism, such as extracellular peptidic aggregates, are on the other hand diluted and washed off in the extracellular volume of medium bathing the cells. Canonical cell culture is performed at non-physiological atmospheric oxygen tensions (21%), when brain oxygen tensions are reported to be below 5%. As a result, large amounts of disease-modifying antioxidants (e.g. SOD, Catalase, Vitamin E) are part of most media recipes[62]. Teasing out the contributions of proteotoxic, metabolic or oxidative stresses to disease in those conditions is less than ideal. For this reason, new media, formulated to better mimic the human extracellular milieu and to allow synchronized maturation of multiple different cell types, must be developed[63].

The major liability of dense tissue-like cellular structures comes from poor access to nutrients and oxygen, and poor excretion of the more soluble toxic metabolic byproducts (e.g. purine catabolites, excess lactate). In order to avoid these one must limit the greatest distance between a cell and the medium. For example, 500μm thick 3D cultures in Boyden chambers, exposed to medium from both apical and basal side, can remain viable for months (J.M., personal communication). Such cultures are well-suited to the study of neurodegenerative disorders, and the relative vulnerabilities of different cell types. Remarkable success was already achieved using mouse-derived ECM (albeit non-neural) gels as scaffold for 3D cultures of neurons and glia derived from immortalized human cell lines engineered to produce large amounts of beta-amyloid[64]. These efforts showed the recapitulation of the two hallmarks of AD, extracellular amyloid plaque formation, and intra-neuronal accumulation of tau tangles. In such models, it is likely that the hyper-production of amyloid coupled with the lower clearance of the secreted peptides in the ECM gel, leads to nucleation, plaque growth, and consequent taupathy. A more physiological model would rely on a single familial AD mutation, growing cells in a xeno-free 3D culture without exogenously added ECMs (organoid or transwell stack). Organotypic cellular density and interstitial makeup would allow the study of plaque and tangle formation in a context further mimicking the patient's own cortical tissue. To manage long-term viability of thick cultures, iPS-derived neurons and glia can be grown in perfusable bioreactors, aggregating cells on 3D-printed artificial vasculature[65,66], allowing medium to be continuously flowed past and through the tissue (**Figure 1E**). Such bioreactors can have an open-circuit architecture, buffering metabolite variations due to feeding regimens, mimicking physiological homeostasis.

Conclusion

We expect the combination of genome engineering, novel perfusable 3D culture techniques, improved culture media, differentiation and inclusion of all relevant cell types, and morphogen-imposed tissue polarity, to contribute to the establishment of greatly enriched human CNS disease models. Given that such concepts can be applied to other pluripotent stem cell-derived tissues, one can envisage studying the interactions of different organs through a common engineered vascular system, placing each organ behind relevant biological barriers. Efforts to miniaturize individual tissue models in microfluidic devices are on their way. As a timely example, we expect that in the near future, viral dissemination of Zika from the skin bite to the maternal bloodstream, through the placenta to the fetal brain, can be followed in a single dish so that the effects of drugs and genes on viral propagation and cytotoxicity may be assessed with ease. From 3D brain-in-a-dish, to 3D brain-on-a-chip, novel culture models combined with *ad hoc* genetic engineering are revolutionizing disease-modeling efforts. Robust differentiation and culture systems should make these technologies as user-friendly and open-source as genomic engineering has already become.

Acknowledgements

We thank Richard Ransohoff for helpful discussions on microglial identity and differentiation, We also thank Linda Griffith and Pierre Sphabmixay for their help with perfused bioreactors. Work in the R.J. laboratory was supported by grants from the Simons Foundation, NIH grants HD 045022, R37-CA084198, the ELA foundation, the Emerald foundation and the Simons Center for the Social Brain at MIT. J.M. received support from the European Leukodystrophy Association, and a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation. Y.L. was supported by a Simons Postdoctoral Fellowship, an International Rett Syndrome Foundation Postdoctoral Fellowship, and a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation. RJ is an advisor to Stemgent and a cofounder of Fate Therapeutics and Fulcrum Therapeutics.

Reference and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1 Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872, doi:10.1016/j.cell.2007.11.019 (2007).

2 Wernig, M. *et al.* In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448**, 318-324, doi:10.1038/nature05944 (2007).

3 Yu, J. *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917-1920, doi:10.1126/science.1151526 (2007).

4 Scudellari, M. How iPS cells changed the world. *Nature* **534**, 310-312, doi:10.1038/534310a (2016).

5 Soldner, F. *et al.* Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* **136**, 964-977, doi:10.1016/j.cell.2009.02.013 (2009).

6 Li, Y. *et al.* Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. *Cell stem cell* **13**, 446-458, doi:10.1016/j.stem.2013.09.001 (2013).

7 Paquet, D. *et al.* Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. *Nature* **533**, 125-129, doi:10.1038/nature17664 (2016).
8 Kondo, T. *et al.* Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. *Cell stem cell* **12**, 487-496, doi:10.1016/j.stem.2013.01.009 (2013).
9 Soldner, F. & Jaenisch, R. Dissecting risk haplotypes in sporadic Alzheimer's disease. *Cell stem cell* **16**, 341-342, doi:10.1016/j.stem.2015.03.010 (2015).
10 Nalls, M. A. *et al.* Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet* **46**, 989-993, doi:10.1038/ng.3043 (2014).
11 Bertram, L. & Tanzi, R. E. The genetics of Alzheimer's disease. *Prog Mol Biol Transl Sci* **107**, 79-100, doi:10.1016/B978-0-12-385883-2.00008-4 (2012).
12 Young, J. E. *et al.* Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell stem cell* **16**, 373-385, doi:10.1016/j.stem.2015.02.004 (2015).
13 Soldner, F. *et al.* Parkinson-associated risk variant in distal enhancer of alpha-synuclein modulates target gene expression. *Nature* **533**, 95-99, doi:10.1038/nature17939 (2016).
14 Soldner, F. & Jaenisch, R. Medicine. iPSC disease modeling. *Science* **338**, 1155-1156, doi:10.1126/science.1227682 (2012).
15 Soldner, F. *et al.* Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. *Cell* **146**, 318-331, doi:10.1016/j.cell.2011.06.019 (2011).
16 Cohen-Tannoudji, M. *et al.* I-SceI-induced gene replacement at a natural locus in embryonic stem cells. *Mol Cell Biol* **18**, 1444-1448 (1998).
17 Christian, M. *et al.* Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics* **186**, 757-761, doi:10.1534/genetics.110.120717 (2010).
18 Hockemeyer, D. *et al.* Genetic engineering of human pluripotent cells using TALE nucleases. *Nat Biotechnol* **29**, 731-734, doi:10.1038/nbt.1927 (2011).
19 Jinek, M. *et al.* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816-821, doi:10.1126/science.1225829 (2012).
20 Shalem, O. *et al.* Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84-87, doi:10.1126/science.1247005 (2014).
21 Ran, F. A. *et al.* Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* **8**, 2281-2308, doi:10.1038/nprot.2013.143 (2013).
22 Gilbert, L. A. *et al.* CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* **154**, 442-451, doi:10.1016/j.cell.2013.06.044 (2013).
23 Liu, H. *et al.* CRISPR-ERA: a comprehensive design tool for CRISPR-mediated gene editing, repression and activation. *Bioinformatics* **31**, 3676-3678, doi:10.1093/bioinformatics/btv423 (2015).
24 Gilbert, L. A. *et al.* Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell* **159**, 647-661, doi:10.1016/j.cell.2014.09.029 (2014).
25 Cheng, A. W. *et al.* Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. *Cell Res* **23**, 1163-1171, doi:10.1038/cr.2013.122 (2013).
26 Choudhury, S. R., Cui, Y., Lubecka, K., Stefanska, B. & Irudayaraj, J. CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter. *Oncotarget*, doi:10.18632/oncotarget.10234 (2016).
27 Du, Z. W. *et al.* Generation and expansion of highly pure motor neuron progenitors from human pluripotent stem cells. *Nat Commun* **6**, 6626, doi:10.1038/ncomms7626 (2015).
28 Takazawa, T. *et al.* Maturation of spinal motor neurons derived from human embryonic stem cells. *PLoS One* **7**, e40154, doi:10.1371/journal.pone.0040154 (2012).

29 Hu, B. Y. & Zhang, S. C. Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc* **4**, 1295-1304, doi:10.1038/nprot.2009.127 (2009).

30 Douvaras, P. *et al.* Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. *Stem Cell Reports* **3**, 250-259, doi:10.1016/j.stemcr.2014.06.012 (2014).

31 Hu, B. Y., Du, Z. W., Li, X. J., Ayala, M. & Zhang, S. C. Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects. *Development* **136**, 1443-1452, doi:10.1242/dev.029447 (2009).

32 Krencik, R., Weick, J. P., Liu, Y., Zhang, Z. J. & Zhang, S. C. Specification of transplantable astroglial subtypes from human pluripotent stem cells. *Nat Biotechnol* **29**, 528-534, doi:10.1038/nbt.1877 (2011).

33 Zimmer, B. *et al.* Derivation of Diverse Hormone-Releasing Pituitary Cells from Human Pluripotent Stem Cells. *Stem Cell Reports* **6**, 858-872, doi:10.1016/j.stemcr.2016.05.005 (2016).

34 Chambers, S. M. *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* **27**, 275-280, doi:10.1038/nbt.1529 (2009).

35 Eiraku, M. *et al.* Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* **3**, 519-532, doi:10.1016/j.stem.2008.09.002 (2008).

36 Kadoshima, T. *et al.* Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci U S A* **110**, 20284-20289, doi:10.1073/pnas.1315710110 (2013).

37 Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373-379, doi:10.1038/nature12517 (2013).

38 Mariani, J. *et al.* FOXG1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders. *Cell* **162**, 375-390, doi:10.1016/j.cell.2015.06.034 (2015).

39 Qian, X. *et al.* Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. *Cell* **165**, 1238-1254, doi:10.1016/j.cell.2016.04.032 (2016).

40 Pasca, A. M. *et al.* Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat Methods* **12**, 671-678, doi:10.1038/nmeth.3415 (2015).

41 Nelson, S. B. & Valakh, V. Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. *Neuron* **87**, 684-698, doi:10.1016/j.neuron.2015.07.033 (2015).

42 Goldmann, T. *et al.* Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol* **17**, 797-805, doi:10.1038/ni.3423 (2016).

43 Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841-845, doi:10.1126/science.1194637 (2010).

44 Tay, T. L., Hagemeyer, N. & Prinz, M. The force awakens: insights into the origin and formation of microglia. *Curr Opin Neurobiol* **39**, 30-37, doi:10.1016/j.conb.2016.04.003 (2016).

45 Ransohoff, R. M. Neuroinflammation: Surprises from the sanitary engineers. *Nature* **532**, 185-186, doi:10.1038/nature17881 (2016).

46 Ransohoff, R. M. & El Khoury, J. Microglia in Health and Disease. *Cold Spring Harb Perspect Biol* **8**, a020560, doi:10.1101/cshperspect.a020560 (2016).

47 Fourgeaud, L. *et al.* TAM receptors regulate multiple features of microglial physiology. *Nature* **532**, 240-244, doi:10.1038/nature17630 (2016).

48 Korvatska, O. *et al.* R47H Variant of TREM2 Associated With Alzheimer Disease in a Large Late-Onset Family: Clinical, Genetic, and Neuropathological Study. *JAMA Neurol* **72**, 920-927, doi:10.1001/jamaneurol.2015.0979 (2015).

49 Chan, G. *et al.* CD33 modulates TREM2: convergence of Alzheimer loci. *Nat Neurosci* **18**, 1556-1558, doi:10.1038/nn.4126 (2015).

50 Rempe, R. G., Hartz, A. M. & Bauer, B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. *J Cereb Blood Flow Metab*, doi:10.1177/0271678X16655551 (2016).

51 Kleinschmidt-DeMasters, B. K. & Beckham, J. D. West Nile Virus Encephalitis 16 Years Later. *Brain Pathol* **25**, 625-633, doi:10.1111/bpa.12280 (2015).

52 Rasmussen, S. A., Jamieson, D. J., Honein, M. A. & Petersen, L. R. Zika Virus and Birth Defects--Reviewing the Evidence for Causality. *N Engl J Med* **374**, 1981-1987, doi:10.1056/NEJMsr1604338 (2016).

53 Petersen, L. R., Jamieson, D. J., Powers, A. M. & Honein, M. A. Zika Virus. *N Engl J Med* **374**, 1552-1563, doi:10.1056/NEJMra1602113 (2016).

54 Cugola, F. R. *et al.* The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* **534**, 267-271, doi:10.1038/nature18296 (2016).

55 Check Hayden, E. Proving Zika link to birth defects poses huge challenge. *Nature* **530**, 142-143, doi:10.1038/530142a (2016).

56 Tang, H. *et al.* Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. *Cell Stem Cell* **18**, 587-590, doi:10.1016/j.stem.2016.02.016 (2016).

57 Garcez, P. P. *et al.* Zika virus impairs growth in human neurospheres and brain organoids. *Science* **352**, 816-818, doi:10.1126/science.aaf6116 (2016).

58 De Luca, C. & Papa, M. Looking Inside the Matrix: Perineuronal Nets in Plasticity, Maladaptive Plasticity and Neurological Disorders. *Neurochem Res* **41**, 1507-1515, doi:10.1007/s11064-016-1876-2 (2016).

59 Carulli, D., Kwok, J. C. & Pizzorusso, T. Perineuronal Nets and CNS Plasticity and Repair. *Neural Plast* **2016**, 4327082, doi:10.1155/2016/4327082 (2016).

60 Lancaster, M. A. & Knoblich, J. A. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**, 1247125, doi:10.1126/science.1247125 (2014).

61 Lancaster, M. A. & Knoblich, J. A. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc* **9**, 2329-2340, doi:10.1038/nprot.2014.158 (2014).

62 Chen, Y. *et al.* NS21: re-defined and modified supplement B27 for neuronal cultures. *J Neurosci Methods* **171**, 239-247, doi:10.1016/j.jneumeth.2008.03.013 (2008).

63 Bardy, C. *et al.* Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proc Natl Acad Sci U S A* **112**, E2725-2734, doi:10.1073/pnas.1504393112 (2015).

64 Choi, S. H. *et al.* A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* **515**, 274-278, doi:10.1038/nature13800 (2014).

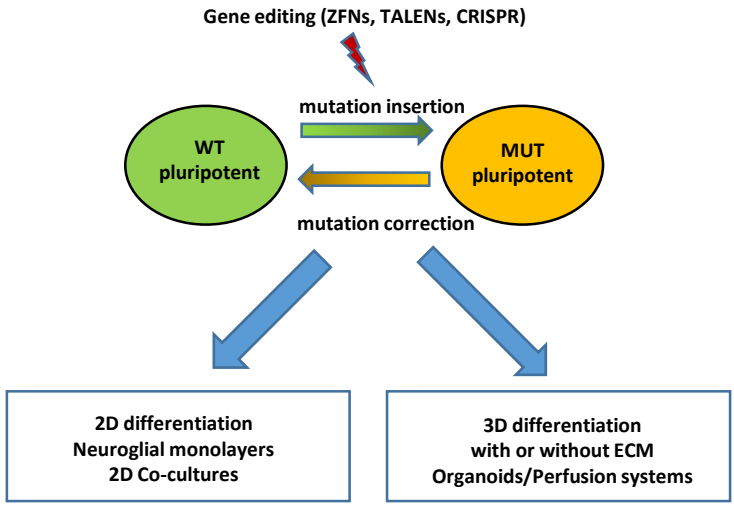
65 Griffith, L. G., Wells, A. & Stolz, D. B. Engineering liver. *Hepatology* **60**, 1426-1434, doi:10.1002/hep.27150 (2014).

66 Neiman, J. A. *et al.* Photopatterning of hydrogel scaffolds coupled to filter materials using stereolithography for perfused 3D culture of hepatocytes. *Biotechnol Bioeng* **112**, 777-787, doi:10.1002/bit.25494 (2015).

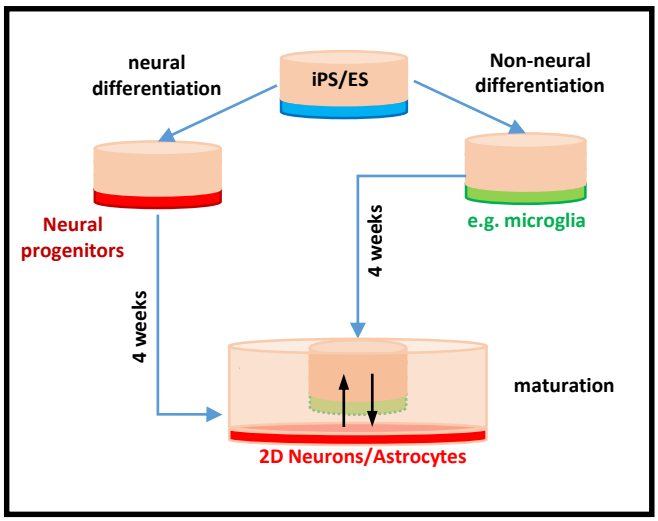
FIGURE 1: A: gene editing technique applied to pluripotent stem cells allow the generation of isogenic pairs for phenotypic comparisons without genetic background interference. **B:** canonical 2D cell cultures of differentiated cells can be mixed and matched, to study interactions of cells deriving from different lineages (e.g. nerve cells and immune cells). **C:** cultures differentiated separately can be reaggregated into 3D cultures, with or without ECM scaffolds. **D:** free-floating organoids can be generated from the pluripotent state, allowing the study of

developmental aspects of tissue morphogenesis. **E:** static 3D culture viability may be improved by active perfusion systems, forcing medium through an artificial 3D-printed vascular system.

A

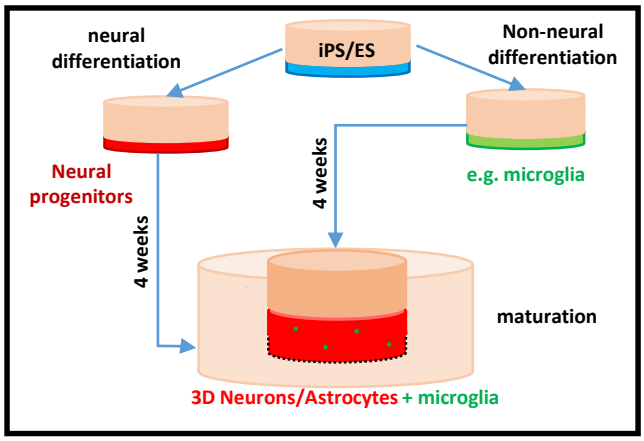


B



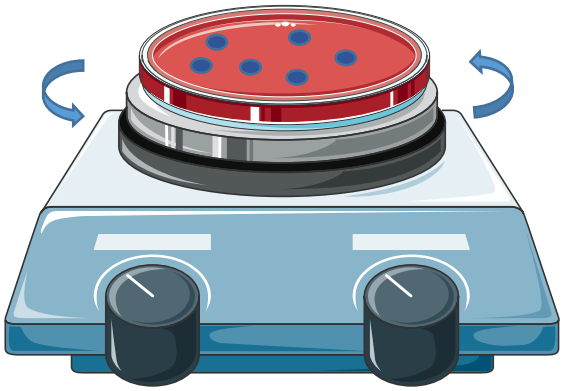
Reciprocal exposure to secreted factors in 2D

C



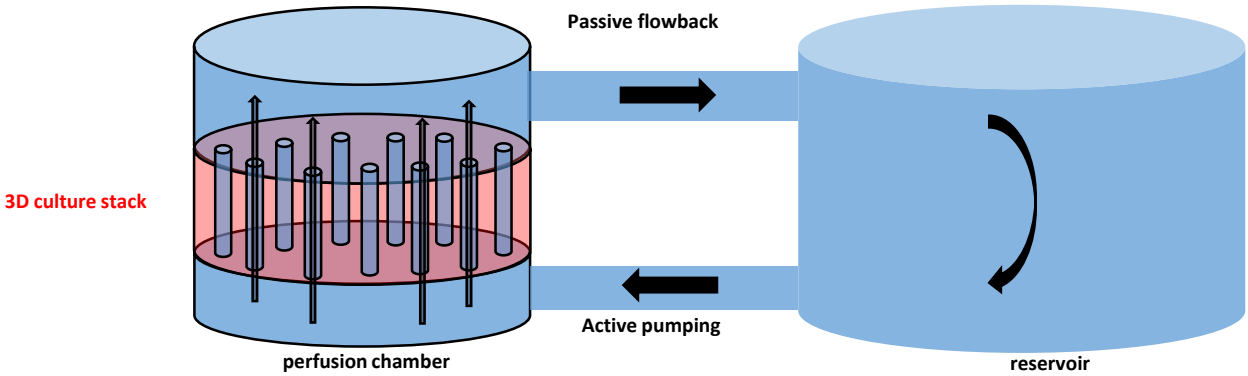
Physical re-aggregation in 3D

D



Rotating 3D organoids in suspension

E



Active perfusion system for thick 3D cultures