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PERSPECTIVE

In preprints: shaping the developing human brain

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Tissues undergo complex shape changes during development that are crucial for their later architecture and function. Understanding how these changes in shape, the morphodynamics, occur is important not only to understand developmental processes, but also to reveal how dysregulation of these processes leads to developmental defects. The precise spatiotemporal events and morphological dynamics of human brain development have remained relatively poorly understood, partly due to limited access to early human neural tissues. In the last decade, cerebral organoids have provided a novel approach to address this open question, enabling early human neural development to be observed and manipulated *in vitro* (Pasca et al., 2015; Camp et al., 2015; Lancaster et al., 2013).

A recent preprint by Jain and colleagues (Jain et al., 2023 preprint) used a combination of single-cell RNA-sequencing (scRNA-seq) with a novel imaging and cell-labelling approach to track the changes in patterning and morphology of early organoids over several weeks. In order to visualise the morphodynamics in intact organoids, Jain and colleagues used an elegant labelling system, combining induced pluripotent stem cells (iPSCs) lines, each carrying a GFP- or RFP-tagged protein to label one of five cellular structures: the plasma membrane (CAAX), actin cytoskeleton (actin), microtubules (tubulin), nucleus (histone) and nuclear envelope (lamin). These cell lines were then combined together with a non-labelled iPSC line to generate mosaic and sparsely labelled organoids, which enabled single cells to be tracked. The morphology of the organoids was then analysed using measurements of overall size, as well as lumen number and volume.

This long-term imaging of whole organoids provided a window into the cellular- and tissue-level changes in shape that occur in early neural development. Combining this with the scRNA-seq data, the authors started to pick apart the genetic changes linked to the morphodynamics observed. One key family of genes that was upregulated at times of morphological change were extracellular matrix (ECM) and ECM-associated genes. ECM has long been reported to play a role in development and morphogenesis, due to its known functions in cell migration, proliferation and organisation of the developing tissues (Barros et al., 2011; Long and Huttner, 2019; Hynes, 2009).

Jain and colleagues then explored the role of exogenously supplied ECM, using Matrigel, in regulating this morphogenesis and patterning. Organoids were generated using an unguided protocol, with or without Matrigel (or 0.6% agarose) added at the onset of live imaging at day 4 of culture. The authors showed that, without Matrigel, organoids developed different morphologies, with slower growth of both organoid and lumen volume and disrupted cell polarity. Leveraging the multi-mosaic labelling of cellular structures, the authors next analysed the morphology at the cellular level. Using partition-based graph abstraction (PAGA) trajectory analysis, they observed that membrane elongation and compression of nuclei occur during a key morphological change: the transition from neuroectodermal to neuroepithelial cells.

This change in cellular morphology was also accompanied by the perpendicular alignment of the cells to the organoid surface, which was increased in the presence of Matrigel. Matrigel also increased the homogeneity of cell morphologies, with cells displaying a higher degree of alignment and a more elongated morphology at earlier time points. This morphological change at the cellular level was associated with a change in cell identity, with Matrigel-exposed organoids containing significantly more telencephalic progenitors and fewer neural crest cells at day 13 than those grown in the no-Matrigel or agarose conditions.

In the absence of Matrigel, scRNA-seq showed an increase in Wnt and Hippo pathway components, suggesting that Matrigel might be altering both the rostral-caudal and dorso-ventral patterning of the organoids. When the authors activated the YAP1 pathway in the presence of Matrigel, the organoids displayed altered overall and lumen morphologies and expressed genes associated with the caudalisation of the developing neuroepithelium. They suggest that Matrigel is, therefore, promoting lumen expansion and patterning of mainly rostral prosencephalic regions, which instead pattern into more caudal regions in the absence of Matrigel.

Overall, Jain and colleagues have shown a significant role of Matrigel in the morphodynamics of organoid development across the scales, from gene expression to cell shape and alignment to organoid architecture. How Matrigel drives morphodynamics is an interesting question and one that others are also trying to address. Two recent papers (Chiaradia et al., 2023; Martins-Costa et al., 2023) also investigated the effect of Matrigel on guided telencephalic organoids and guided or unguided cerebral organoid development. Both papers report some similar effects of Matrigel on organoid size and structure as that reported by Jain and colleagues. Chiaradia and colleagues confirm that Matrigel improved the neuroepithelial-like structure, although they also reported that this can sometimes be observed in no Matrigel organoids too. Martins-Costa and colleagues go one step further, reporting that from day 20 onwards telencephalic organoids grown in the absence of Matrigel eventually arranged in the correct apico-basal axis without displaying any morphological differences.

There are several possibilities for this contrast in results, one of which is currently hotly debated in the field; the heterogeneity of organoids (Velasco et al., 2019; Lancaster and Knoblich, 2014; Di Lullo and Kriegstein, 2017). There are many variations of protocols used to generate organoids, which can also yield intra-batch variability and variability between different cell lines. For example, the addition of WNT inhibitors by Chiaradia and colleagues and Martins-Costa and colleagues (Chiaradia et al., 2023; Martins-Costa et al., 2023) and the timing of the addition of Matrigel, could result in differences in organoid development when compared with the

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unguided organoid protocol used by Jain and colleagues. Navigating the effect of this when interpreting results is not easy, but there are some consistent changes that have been observed. For example, Chiaradia and colleagues report that organoids with an initial proper morphology will then display the correct cellular identity regardless of culture conditions, but that Matrigel may help to set up this initial morphology. This report is consistent with the findings of Jain and colleagues that Matrigel helped to pattern the structure of early organoids, and with the findings of Martins-Costa and colleagues that Matrigel can accelerate the formation of correct tissue polarity.

However, the exact role of the ECM compared with the other components of Matrigel is still an open question. Martins-Costa and colleagues report that the addition of purified mouse laminin 1 or collagen IV was not sufficient to replicate the effects of Matrigel, which is perhaps unsurprising given the complex mixture of components it contains. Jain and colleagues show that encapsulation of organoids in 0.6% agarose promotes neuroepithelium and lumen formation and affects organoid patterning by promoting more telencephalic fates. This organoid encapsulation can induce a similar phenotype to organoids grown with Matrigel, indicating that capture of molecules secreted by the organoid or the extracellular mechanical environment might also be one of the roles of the ECM. Matrigel is often used as a source of ECM, but is known to contain signalling factors that could also affect morphodynamics, many of which may interact with the ECM components. Untangling the role of ECM is further complicated by our incomplete understanding of what ECM is present in the fetal brain, hindering our ability to replicate it in organoids. Once this information is known, developing an ECM composition with a more spatial and temporal approach could help recreate a more *in vivo*-like situation and enable the role of ECM to be explored further. Synthetic, tuneable ECM may be a way to achieve this and could also be used to generate basement-membrane-like microenvironments in which to grow organoids.

How the ECM shapes the developing neuroepithelium and how it is directly or indirectly linked to some of the major signalling pathways remains to be defined, but Jain and colleagues add exciting new data to the growing evidence that it contributes to the morphodynamics of brain development, in both organoids and tissue-based models (Jain et al., 2023 preprint; Chiaradia et al., 2023; Martins-Costa et al., 2023; Long et al., 2018; Long and Huttner, 2019).

Competing interests
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References

Barros, C. S., Franco, S. J. and Müller, U. (2011). Extracellular matrix: functions in the nervous system. *Cold Spring Harbor Perspect. Biol.* **3**, a005108. doi:10.1101/cshperspect.a005108

Camp, J. G., Badsha, F., Florio, M., Kanton, S., Gerber, T., Wilsch-Bräuninger, M., Lewitus, E., Sykes, A., Hevers, W., Lancaster, M. et al. (2015). Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. *Proc. Natl. Acad. Sci. USA* **112**, 15672-15677. doi:10.1073/pnas.1520760112

Chiaradia, I., Imaz-Rosshandler, I., Nilges, B. S., Boulanger, J., Pellegrini, L., Das, R., Kashikar, N. D. and Lancaster, M. A. (2023). Tissue morphology influences the temporal program of human brain organoid development. *Cell Stem Cell* **30**, 1351-1367.e10. doi:10.1016/j.stem.2023.09.003

Di Lullo, E. and Kriegstein, A. R. (2017). The use of brain organoids to investigate neural development and disease. *Nat. Rev. Neurosci.* **18**, 573-584. doi:10.1038/nrn.2017.107

Hynes, R. O. (2009). The extracellular matrix: not just pretty fibrils. *Science* **326**, 1216-1219. doi:10.1126/science.1176009

Jain, A., Gut, G., Sanchis-Calleja, F., Okamoto, R., Streib, S., He, Z., Zenk, F., Santel, M., Seimiya, M., Holtackers, R. et al. (2023). Morphodynamics of human early brain organoid development. *bioRxiv*. doi:10.1101/2023.08.21.553827

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

Lancaster, M. A., Renner, M., Martin, C. A., Wenzel, D., Bicknell, L. S., Hurles, M. E., Homfray, T., Penninger, J. M., Jackson, A. P. and Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373-379. doi:10.1038/nature12517

Long, K. R. and Huttner, W. B. (2019). How the extracellular matrix shapes neural development. *Open Biol.* **9**, 180216. doi:10.1098/rsob.180216

Long, K. R., Newland, B., Florio, M., Kalebic, N., Langen, B., Kolterer, A., Wimberger, P. and Huttner, W. B. (2018). Extracellular matrix components HAPLN1, lumican, and collagen I cause hyaluronic acid-dependent folding of the developing human neocortex. *Neuron* **99**, 702-719.e6. doi:10.1016/j.neuron.2018.07.013

Martins-Costa, C., Pham, V. A., Sidhaye, J., Novatchkova, M., Wiegers, A., Peer, A., Möseneder, P., Corsini, N. S. and Knoblich, J. A. (2023). Morphogenesis and development of human telencephalic organoids in the absence and presence of exogenous extracellular matrix. *EMBO J.* **42**, e113213. doi:10.15252/embj.2022113213

Pasca, A. M., Sloan, S. A., Clarke, L. E., Tian, Y., Makinson, C. D., Huber, N., Kim, C. H., Park, J. Y., O'Rourke, N. A., Nguyen, K. D., et al. (2015). Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* **12**, 671-678. doi:10.1038/nmeth.3415

Velasco, S., Kedaigle, A. J., Simmons, S. K., Nash, A., Rocha, M., Quadrato, G., Paulsen, B., Nguyen, L., Adiconis, X., Regev, A., et al. (2019). Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* **570**, 523-527. doi:10.1038/s41586-019-1289-x