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WEBINAR Q&A REPORT

Functional Characterization of iPSC-Derived Neurons and Glial Cells Using MEA and Calcium Flux Technologies

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How do you distinguish calcium flux signals between neurons and astrocytes in co-culture?

In the FDSS assay, astrocytes do show changes in calcium levels, but they don't produce them spontaneously. The oscillations we're seeing in the trace are produced by a synchronous electrical activity, which is generally generated by neurons. So what we're seeing in this assay is coming from neurons, not astrocytes.

Have you ever done MEA in neuron monoculture? If so, how does it compare to that in co-culture?

Yes, we have run those assays in monocultures. In general, our protocol uses a co-culture system because astrocytes support neuronal health and maturation — the cultures look healthier overall, which matters especially since we culture for extended periods. Monocultures generally aren't as active as co-cultures. You can actually see this in our oligodendrocyte data: wherever a condition is marked "olig minus," that's a neuronal monoculture, and there are differences versus the co-culture condition — slight in healthy controls, more pronounced in the Alzheimer's disease lines. In general, we advise co-culturing with astrocytes to support the health and maturation of the culture.

Is the co culture CW13049/CW50107 derived from patients?

Yes — the excitatory neurons are derived from Alzheimer's disease patient lines. The oligodendrocytes used in that co-culture were derived from healthy donors.

What is the maximum duration that cells can be cultured and matured on the MEA?

We generally see the most robust, stable network activity starting around week five to six. After that it tends to plateau, and sometimes activity even declines, simply because the culture is aging. We have cultured well beyond that point, but culture quality does decrease the longer you go. Our general recommendation is to work somewhere in the week five to week eight window, with eight weeks probably the latest we'd recommend.

How long do you run the MEA readings/recordings?

We have a lot of flexibility here. Our general protocol uses 10-minute recordings, but we can go shorter or longer depending on what a client needs — we have experience measuring for as long as half an hour. We can also provide optical (photo) recordings alongside the electrical data.

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Have you ever measured organoids in your MEA system? If so, how do you attach them to the array?

We have not done this yet, but it's an interesting direction to explore. If this is something you're interested in, we'd be glad to discuss how we could optimize our protocols to accommodate 3D cultures.

Have you done action potential recordings from single cells?

No, we have not — we don't have much experience with single-cell recordings. Our Maxwell MaxTwo instrument has the capability to do higher-level analysis to help resolve signals coming from single cells, but in general our assays are looking at population-level activity across the culture.

What patient cell types are used to develop the neurons and glia?

We work with a variety of patient cell types. Depending on the need, we have lines for Parkinson's disease, Alzheimer's disease, and ALS — essentially any neurodegenerative disease you can think of, we can likely either procure the iPSCs for or already have in stock.

Have you done the FDSS calcium flux assay on a disease line? If so, which disease, and how does it compare to healthy controls?

We have not yet done this for our own internal purposes, but if it's something a client is interested in, it's totally doable — we have a stock of patient lines available for that kind of experiment.

For the MEA co-culture work with Alzheimer's disease neurons and healthy OPCs (oligodendrocyte precursor cells), did you also run AD neurons together with AD-derived OPCs?

No, we have not done that combination yet, but it's certainly something we could do. I'm not sure unhealthy OPCs would provide any advantage for the culture — probably not — but it really depends on the specific question you're trying to answer. It's totally doable; we can differentiate oligodendrocytes from our Alzheimer's disease iPSC lines as well.

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Are there any specific challenges you encounter culturing co-cultures versus monocultures, in the context of the assays you're developing?

In the context of the assay itself, no — it's a very straightforward, easy-to-follow protocol. The culture duration is fairly long, which makes it a little tedious, and the longer a culture runs the more opportunities there are for something to go wrong. That said, so far we've been fortunate — our cultures have been very easy to maintain.

What are the differences between FDSS (calcium flux) and MEA?

It largely depends on the scale you're working at. FDSS is higher throughput — we can run 96-well or 384-well formats. MEA is more limited: going beyond a 24- or 48-well format means sacrificing electrode density, and therefore data quality, so we currently stay in those smaller formats. If MEA throughput technology develops further, we'd be happy to adopt it. There's also a difference in what's being measured: FDSS is an indirect readout of neuronal electrical activity, while MEA measures it directly. MEA has another advantage — we can record the same culture as many times as we want, weekly or even every few days. FDSS, unfortunately, is an endpoint assay: the cells are exposed to a non-sterile instrument environment, so they can't be used again afterward. Which one to use really depends on your needs and what kind of information you're trying to get — either can work well.

How do you acquire cell lines from healthy living patients?

We have partnerships with cell banks — recently we acquired a number of iPSC lines through CIRM, if I recall correctly. We also maintain a large internal bank of frozen stock: once we have a line, we can expand it and create additional frozen stock. In general, the initial reprogramming step — taking a blood sample, applying Yamanaka factors, and driving the cells to a pluripotent state — is not something we do in-house; that's done by our line-sourcing partners. We receive the iPSCs and then apply our own differentiation protocols to generate the cell types we work with.

Do you plan to detect synaptic function?

We'd want a bit more detail on exactly what's meant by "synaptic function" to answer precisely, but broadly, yes — our analysis software is comprehensive and gives us multiple metrics to work with, so this is something we can look into.

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For the oligodendrocyte-neuron rescue effect seen in the Alzheimer's disease lines, what's the therapeutic hypothesis — is it remyelination, neuroprotection, or something else?

We're not certain yet exactly what the mechanism of action is. There are published studies suggesting that oligodendrocyte support programs are altered in Alzheimer's disease, and that this may contribute to reduced neuronal electrical activity. Our working hypothesis is that pairing Alzheimer's disease neurons with healthy oligodendrocytes can rescue that phenotype, and that's broadly consistent with what we're seeing in our data — but we can't yet speak to the exact mechanism of action.

After differentiation and functional analysis, can the neurons and astrocytes be harvested (e.g., by FACS sorting) and remain viable?

We believe they can. We routinely harvest cells, freeze them down, and thaw them in-house, and their viability doesn't decrease as a result — they're able to survive multiple freeze/thaw cycles.

Do you have plans to develop quad-cultures (excitatory and inhibitory neurons plus astrocytes)? And do you have plans to use cells from patients with psychiatric disease?

That's a very interesting question. So far we haven't gone beyond triple cultures, but quad cultures are definitely something we could explore — in fact, our excitatory neuron population already includes some inhibitory neurons, so we're already seeing some of that effect in our current co-cultures. True quad cultures are an exciting future direction. On psychiatric disease lines: yes, definitely — if there's interest from clients in exploring that direction, it's something we can pursue further.

Have you tried the same healthy oligodendrocyte line paired with neurons from the same donor versus a different donor — do they behave the same on MEA?

Not yet tested directly, since we currently have only one oligodendrocyte line (CW50065) and no second donor to compare it against; what we do have on MaxTwo is CW50065 paired with donor-matched healthy neurons and with two AD lines (CW13049, CW50107), where healthy oligodendrocytes partially rescue network activity — most clearly for CW50107 at DIV43 — though every mismatched pairing here is also disease-mismatched, so it can't isolate a pure donor effect (our astrocyte co-culture data has the same confound). The clean test would be one healthy neuron line paired with oligodendrocytes from multiple healthy donors tracked DIV18–43, which requires differentiating oligodendrocytes from additional donors; there's no near-term timeline yet, but it's a conversation worth having.