In Partnership With FUJIFILM Cellular Dynamics

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Value from Innovation

Measuring iPSC-derived Neuron Network Activity on CorePlate™ HD-MEA: Co-culture of iCell® Induced Excitatory Neurons with iCell® GABANeurons and iCell® Astrocytes 2.0

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1. Introduction

High-density microelectrode array (HD-MEA) technology provides a powerful platform for real-time, non-invasive monitoring of neuronal activity, making it invaluable in neurophysiological research and drug discovery. By capturing extracellular electrical signals from networks of neurons, MEAs deliver high-resolution data on neural communication, network connectivity, and functional responses to pharmacological treatments. This technology enables researchers to record complex signalling from large populations of neurons simultaneously, yielding deeper insights into network dynamics and cellular interactions.

Combining HD-MEA technology with human induced pluripotent stem cells (iPSC)-derived neural cells offers the ability to generate human-relevant, precise, and scalable studies of neuronal behaviour and response, paving the way for more predictive and relevant applications in neuroscience and therapeutic development.

This protocol describes culturing of high-quality human iPSC-derived neurons from FUJIFILM Cellular Dynamics on 3Brain's HD-MEA (CorePlate™). Briefly, human isogenic iPSC-derived glutamatergic (iCell® Induced Excitatory Neurons), inhibitory (iCell® GABANeurons), and glial cells (iCell® Astrocytes 2.0) are co-cultured under optimized proportions to support extended culture duration and development of neural network activity. Since MEA recordings are non-invasive and label-free, the development of synchronously bursting neural networks can be monitored over the course of the experiment.

This protocol generally follows FUJIFILM Cellular Dynamics application protocol: Measuring Neural Network Activity on MEA: Co-culture of iCell® Induced Excitatory Neurons with iCell® Astrocytes 2.0 (Scan QR Code Below)). It has been modified to incorporate iCell® GABANeurons and to accommodate the 3Brain CorePlate™.

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2. List of Materials

Table 1. Equipment List

Item	Vendor	Catalogue Number
CorePlate™	3Brain	CorePlate™ 24W 16/50 CorePlate™ 6W 38/60 CorePlate™ 1W 38/60
3Brain MEA platform	3Brain	HyperCAM Delta HyperCAM Alpha BioCAM DupleX
Pipette Kit, 0.2 µl – 1 mL	Multiple Vendors	

Table 2. Consumables List

Item	Vendor	Catalogue Number
iCell® Induced Excitatory Neurons Kit	FUJIFILM Cellular Dynamics, Inc.	R1245
iCell® Neural Supplement B, 2 ml (50X) †	FUJIFILM Cellular Dynamics, Inc.	M1029
iCell® Nervous System Supplement, 1 ml (100X) †	FUJIFILM Cellular Dynamics, Inc.	M1031
iCell® GABANeurons, 01279	FUJIFILM Cellular Dynamics, Inc.	R1011
iCell® Astrocytes 2.0, 01279	FUJIFILM Cellular Dynamics, Inc.	R1240
Borate Buffer (20X)	Thermo Fisher Scientific	28341
BrainPhys™ Neuronal Medium	STEMCELL Technologies	05790
Centrifuge Tubes, 1.5, 15, and 50 ml	Multiple Vendors	
DPBS, no calcium, no magnesium	Multiple Vendors	
Laminin Solution, from Mouse EHS Tumor	FUJIFILM Wako Pure Chemical Corp.	120-05751
N-2 Supplement (100X)	Thermo Fisher Scientific	17502-048

Item	Vendor	Catalogue Number
Gentamicin (50 mg/mL)	Thermo Fisher Scientific	15750060
50% Polyethyleneimine (PEI) Solution	Sigma-Aldrich	181978-100G
Sterile Disposable Reagent Reservoirs	Multiple Vendors	
0.22 µm Sterile Vacuum Filter Unit	Multiple Vendors	
Sterile Water	Multiple Vendors	

Table 3. Software List

Item	Vendor	Catalogue Number
BrainWave Software	3Brain	Latest Version

† Additional media supplements will be required to make enough complete BrainPhys™ medium to carry out the assay beyond two weeks.

3. Workflow

The volumes, quantities, and cell numbers specified in the following workflow have been calculated to prepare enough material to culture on two 6-well CorePlate™.

3.1 Tips Before Starting

Refer to the User's Guides for iCell® Induced Excitatory Neurons, iCell® GABANeurons, and iCell® Astrocytes 2.0 for information on storage and handling of the cells and media supplements.

Refer to FUJIFILM Cellular Dynamics application protocol: Measuring Neural Network Activity on MEA: Co-culture of iCell® Induced Excitatory Neurons with iCell® Astrocytes 2.0 (Scan QR Code below) for additional handling and reagent preparation tips.

3.2 Sterilization and plate priming

Before starting, refer to the plate sterilization steps outlined on page 19 of the HyperCAM-Alpha User Guide.

To ensure optimal hydrophilicity, it is recommended to incubate the plates with 1.5 ml/well of complete medium overnight before proceeding with the coating procedure.

3.3 Preparing the PEI solution

To be done within a week prior to use.

1. Weigh a 15ml centrifuge tube. This will be required for step 3.
2. Prepare an intermediate solution (10% w/w) of PEI by pouring ~2 ml of the 50% PEI stock solution into the previous 15 ml centrifuge tube. PEI is a viscous material, therefore use a 25ml serological pipette and aspirate at lowest speed. Centrifuge at 400g for 5 minutes.
3. Determine the weight (in grams) of PEI solution by weighing out the PEI- containing 15ml centrifuge tube and subtract the initial weight. The stock solution is diluted with water to a known volume.

Note: An example calculation is 2 g of the 50% PEI stock solution is diluted with 8 ml of sterile water to a final volume of 10 ml to obtain an intermediate 10% PEI solution (w/w).

4. Mix the intermediate 10% PEI solution in the 15 ml centrifuge tube to dissolve. This intermediate 10% PEI solution should be aliquoted and stored at -20°C for future use, avoiding multiple freeze-thaw cycles.

Note: It may require extended vortexing or mixing overnight on a benchtop rocker at room temperature to dissolve completely.

The 10% PEI solution needs to be further diluted to 0.1% with 1X borate buffer before being used.

5. Prepare 1 ml of 1X borate buffer by diluting 50 µl 20X borate buffer with 950 µl sterile water.
6. Prepare a working 0.1% PEI solution by diluting 10 µl of the intermediate 10% PEI solution with 990 µl of 1X borate buffer. Sterile filter the working solution through a 0.22 µm pore filter.

Note: It is recommended to make the 0.1% PEI working solution fresh before each use. If needed, the 0.1% PEI working solution can be made some days in advance and stored at 4°C for up to one week.

3.4 Coating the 6-well CorePlate™ with PEI

To be done one day before thawing.

1. Aspirate entirely the medium from the wells.
2. Rinse the recording area with 80 µl sterile deionized water.
3. Aspirate water and let the recording area to completely dry before proceeding with step 4.
4. Dispense 80 µl/well of the 0.1% PEI working solution to the 6-well CorePlate™. Spread the matrix all over the chip, being sure that the entire culture area is covered (the drop must have a squared shape as in figure below, right). If not, use the pipette tip and ensure to shape the matrix drop to the shape of the chip.

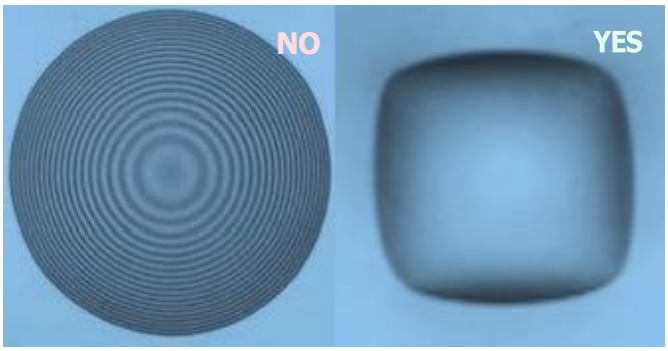

Figure 1. Examples of drop placement on the culture area (left: incorrect placement, right: correct placement)

5. Incubate at 37°C for 1 hour.
6. Aspirate the PEI solution from the plate. Do not allow the wells to dry out.
7. Immediately rinse the plate with 80 µl sterile water per well.
8. Rinse the plate with 80 µl sterile DPBS per well.
9. Allow the plate to air-dry under the hood with the lid removed.

3.5 Day of thawing

Prepare the culturing medium and let it reach room temperature:

Table 4. BrainPhys™ Medium

Component	Volume	Final Concentration
BrainPhys™ Neuronal Medium	48 ml	N/A
iCell® Neural Supplement B (50X)	1 ml	1X

Component	Volume	Final Concentration
iCell® Nervous System Supplement (100X)	0.5 ml	1X
N-2 Supplement (100X)	0.5 ml	1X
Laminin Solution (1mg/ml)	50 µl	1 µg/ml
Gentamicin Solution (50 mg/mL)	70 µl	70 µg/ml

a. Thaw iCell® Astrocytes 2.0 (Astro 2.0)

1. Add 1 ml of Complete BrainPhys™ Medium to a 50 ml sterile centrifuge tube.
2. Immerse the cryovial in a 37°C water bath for 2 minutes.
3. Gently transfer cells to the centrifuge tube using a 1 ml pipette while swirling the tube.
4. Rinse vial with 1 ml of Complete BrainPhys™ Medium and add it by slow, drop wise addition while swirling the tube.
5. Add additional 1 ml of Complete BrainPhys™ Medium drop wise.
6. Transfer to a 15 ml centrifuge tube. Gently invert the tube 2-3 times to allow cells to distribute evenly.
7. Remove 20 µl (add to a 0,5 ml tube) to perform a manual cell count.

b. Thaw iCell® GABAneurons (GABA)

1. Add 1 ml of Complete BrainPhys™ Medium to a 50 ml sterile centrifuge tube.
2. Immerse the cryovial in a 37°C water bath for 2 minutes.
3. Gently transfer cells to the centrifuge tube using a 1 ml pipette while swirling the tube.
4. Rinse vial with 1 ml of Complete BrainPhys™ Medium and add it by slow, drop wise addition while swirling the tube.
5. Add additional 5 ml of Complete BrainPhys™ Medium drop wise.
6. Transfer to a 15 ml centrifuge tube. Gently invert the tube 2-3 times to allow cells to distribute evenly.
7. Remove 20 µl (add to a 0,5 ml tube) to perform a manual cell count.

c. Thaw iCell® Induced Excitatory Neurons (IEN)

1. Add 1 ml of Complete BrainPhys™ Medium to a 50 ml sterile centrifuge tube.

2. Immerse the cryovial in a 37°C water bath for 2 minutes.
3. Gently transfer cells to the centrifuge tube using a 1 ml pipette while swirling the tube.
4. Rinse vial with 1 ml of Complete BrainPhys™ Medium and add it by slow, drop wise addition while swirling the tube.
5. Add additional 1 ml of Complete BrainPhys™ Medium drop wise.
6. Transfer to a 15 ml centrifuge tube. Gently invert the tube 2-3 times to allow cells to distribute evenly.
7. Remove 20 µl (add to a 0,5 ml tube) to perform a manual cell count.
8. Centrifuge cells at 400g x 5 minutes and proceed to the counting step.

d. Counting cells

1. Add 20 µl of Trypan Blue to each 0,5 ml tube.
2. Gently pipette and add 10 µl of solution to both sides of the Improved Neubauer Chamber on top of the grid that is visible by eye and cover with the coverslip.
3. Place the chamber under the microscope and count cells using a 4X or 10X objective.
4. Only cells inside the central 5x5 grid (A) are counted, following the method shown in B. In addition, count cells across the upper doubled line and right doubled line (edges) but not cells in the bottom doubled line and left doubled line (C).

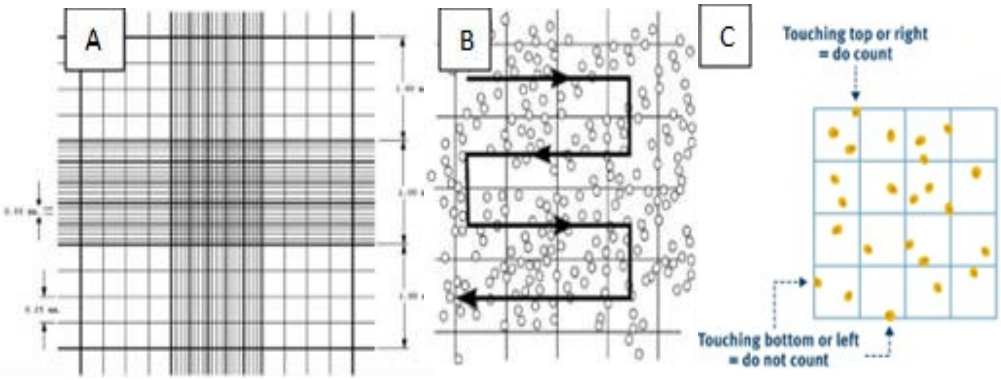

Figure 2. Schematic methodology for counting cells using a Neubauer Chamber

5. Wipe chamber and coverslip with 70% ethanol before passing to the next cell type.

4. Knowing the right number of cells in solution

First, calculate the average of cells counted on both sides of the Chamber. Since the volume of the square is:

0.1 cm x 0.1 cm = **0.01 cm2 of area counted.**

Since the depth of the chamber is 0.01cm:

0.01cm2 x 0,01 cm = 0.0001 cm2 = 0.0001ml = **0.1 µl**

To have the number of cells/ml:

Counted cells x 10000 **(1ml=0.1µl x10000)**

Since cell were diluted 1:1 with Trypan Blue, we multiply the result x2.

Finally, to have the exact number of cells in the tubes, multiply x final solution volume.

Ex.

iCell® Astro 2.0=25x10k=250k x2=500k x4(**ml**)=2mln

iCell® GABA=36x10k=360k x2=720k x8(**ml**)=5.76mln

iCell® IEN=21x10k= 210k x2= 420k x4(**ml**)=1.68mln

Collect cells from centrifuge and remove supernatant by manually pouring the liquid. The remaining volume should be ≈ 100 µl. Gently resuspend cells by adding 900 µl of medium to have the calculated number in a final volume of 1ml.

4.1 Calculate the right number of cells to seed.

The suggested proportion of cells to seed is per well is:

58k iCell® Induced Excitatory Neurons (IEN)

19k iCell® GABANeurons (GABA)

13k iCell® Astrocytes 2.0 (Astro 2.0)

The following is an example for the appropriate number of cells to seed in 3 wells:

The seeding volume is fixed to 80 µl, for this reason prepare each condition in a 1,5 ml tube that will reach the final volume of 560 µl (7+1 wells).

Calculate the volume of each cell type to be added in the 1,5 ml tube:

Example. iCell® IEN

58k x 7 (wells) = 406k 1680000 (total n° cells) : 1000 (µl final volume) = 406000 (required cells) : x=242 µl

Following the example, we will have:

iCell® IEN 406k = 242 µl | iCell® GABA 133k = 23 µl | iCell® Astro 2.0 91k = 45,5 µl | Medium = 249,5 µl (to reach 560 µl final volume).

Seed cells onto the chip of the **6-well CorePlate™** distributing evenly all around the surface area, starting from the perimeter.

Place the plates into the incubator.

4.2 Adding Medium

After 90 minutes, gently add 350 µl of medium in a circular fashion around the cells-containing drop until the two liquid phases comes in contact each other. Use a 1ml pipette tip. Place the multi-well plates into the incubator.

After 2-3 hours, gently and slowly add 1 ml of medium to each well. Place the multi-well plates into the incubator.

The medium is then 50% replaced every 2-3 days or the day prior to the recording session.

5. Results

Electrical activity in the cells can be recorded within 2-3 weeks (usually starting from day 18), but timing may vary depending on culturing conditions and number of cells on the chip. Stable network activity is observed between 4-6 weeks, with cells retaining activity up to day 69, however it's always suggested to find the optimal experimental window by performing a pilot experiment.

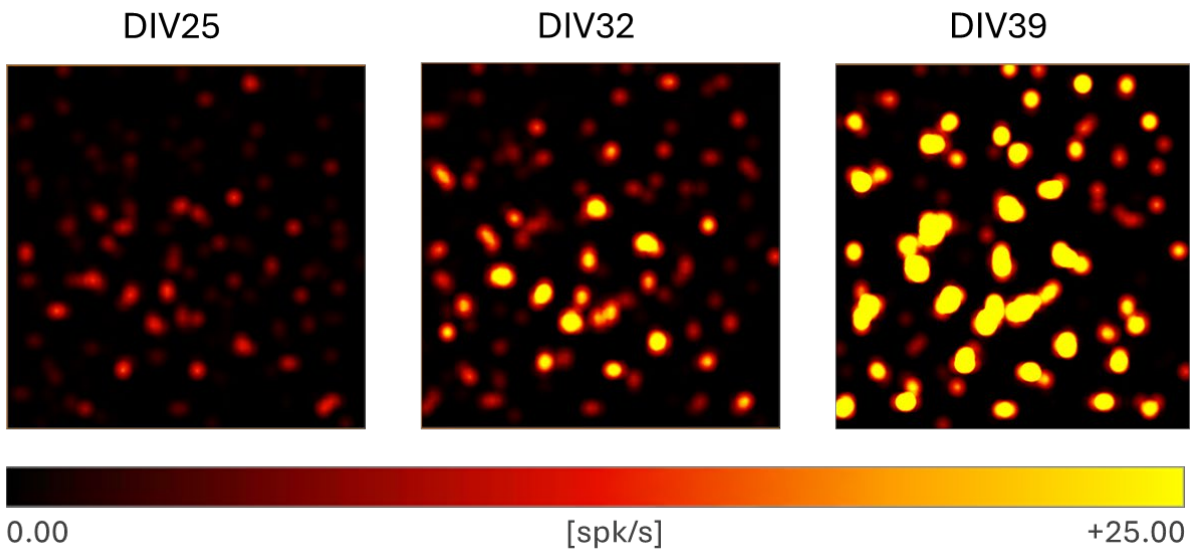

Figure 3. Representative Spike Map showing the spatial mean firing rate of the culture on days 25, 32 and 39 post-seeding. As the culture matures, functional activity measured in spikes per second (spikes/s) intensifies.

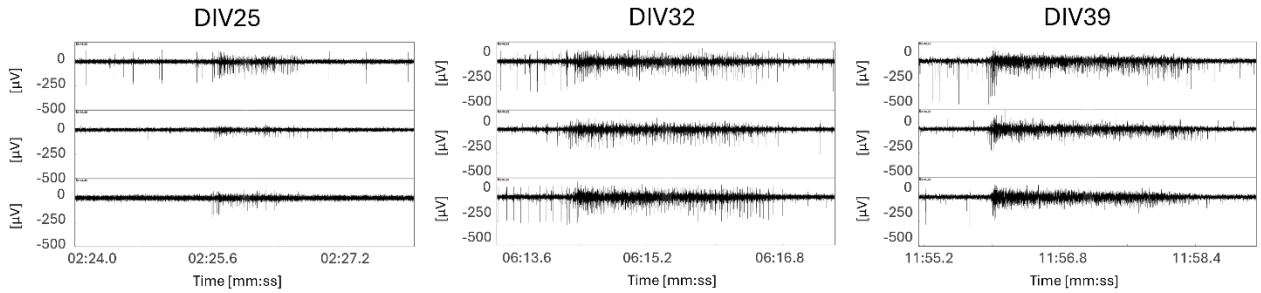

Figure 4. Representative images of the same three channels observed at various developmental time points. It is evident that the duration of the bursts increases with a denser clustering of spikes

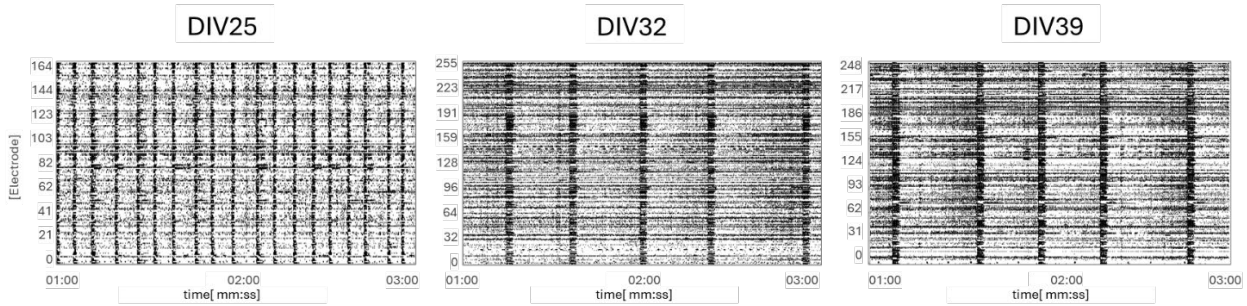

Figure 5. Raster Plots showing the functional activity of the culture at days 25,32 and 39 post seeding. The graphs display units with a mean firing rate of ≥0.1 spikes per second (spk/s).

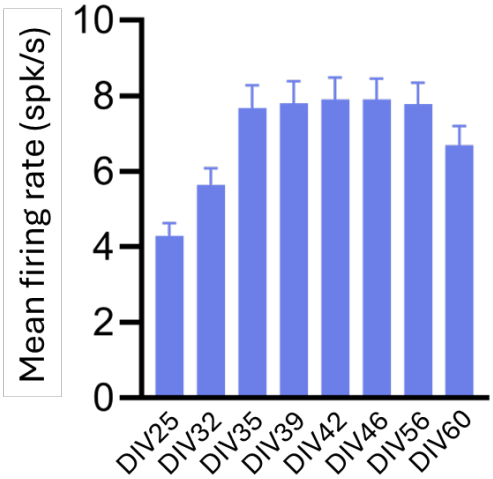

Figure 6. Mean Firing Rate measured from day 25 to day 60. It can be observed that the network achieves stability by day 35 on-chip, which is maintained until approximately day 60, after which it begins to decline.

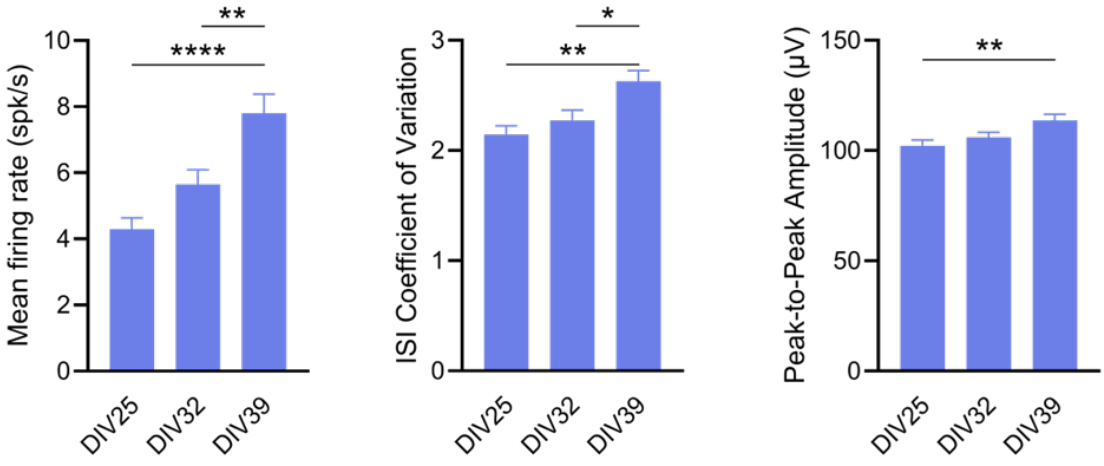

*Figure 7. Mean Firing Rate (a), Inter-Spike Interval coefficient of variation (b), and Peak-to-Peak Amplitude (c) were measured at days 25, 32 and 39 post-seeding. Statistical significance is indicated as follows: a)** p value =0.0029, **** p value < 0.0001; b) ** p value = 0.0138, **p value = 0.0022; c) * p value < 0.0091, determined by one-way ANOVA*

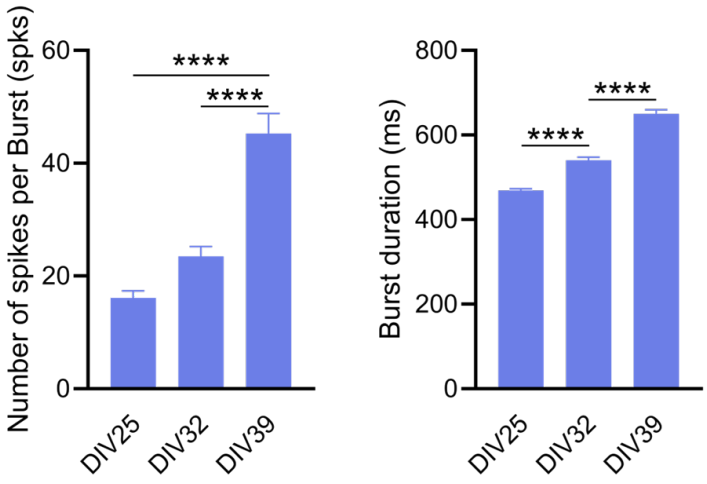

*Figure 8. Number of Spikes per Burst (a) and Burst Duration (b) were measured at Days 25, 32 and 39 post-seeding. Statistical significance is indicated as ****p<0.0001, determined by one-way ANOVA.*

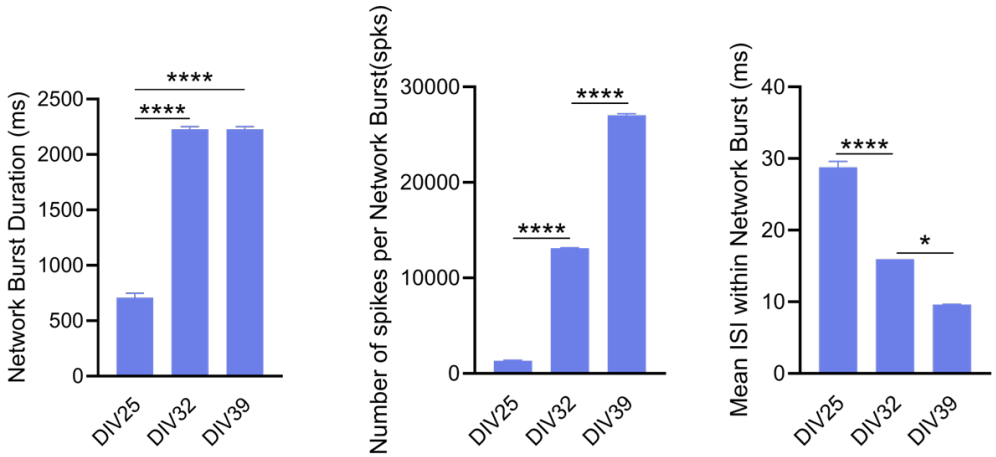

*Figure 9. Network Burst Duration (a), Number of Spikes per Network Burst (b), and Mean Inter-Spike Interval within Network Bursts (c) were measured on Days 25, 32, and 39 post-seeding. While network burst duration stabilizes by Day 32, the figure demonstrates an increasing spike density within network bursts, as indicated by the number of spikes per burst (b) and the inter-spike interval in milliseconds (ms) within each burst (c). **** p value < 0.0001; c) * p value = 0.0138, determined by one-way ANOVA.*

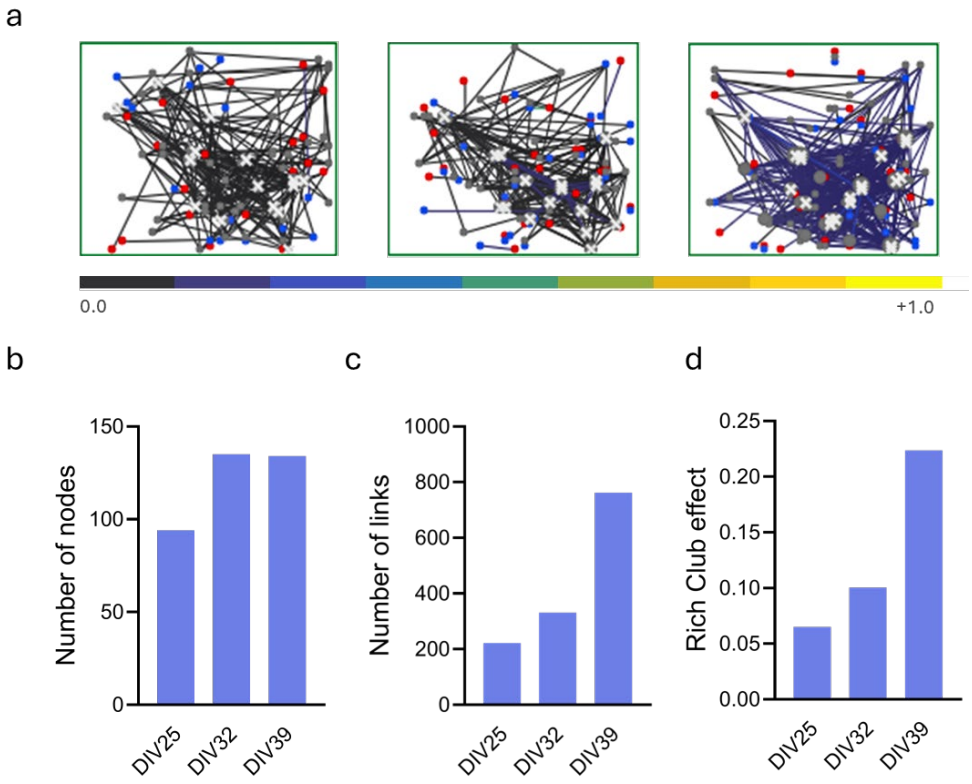

Figure 10. Connectivity graph (a) at days 25, 32, and 39 post-seeding, with the number of nodes (b), number of links (c), and rich-club effect measured at the same time points. Although the number of nodes – defined as units with at least one incoming or outgoing link – reaches a plateau by day 32, the network's complexity continues to increase, as indicated by a rise in the number of links and the strengthening of the rich-club effect.