Supporting Information

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Modular Synthetic Tissues from 3D-Printed Building Blocks

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Supporting Information

Hierarchical assembly of modular synthetic tissues from 3D-printed building blocks

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Supplementary Note: Electrical properties of droplet interface bilayers

We have previously investigated the electrical properties of droplet interface bilayers[1]. The equivalent electrical circuit of a DIB where the lipid bilayer has been permeabilized with the membrane protein pore α-hemolysin (αHL) consists in a parallel RC circuit as shown below (left):

![Equivalent electrical circuit diagram](image)

The DIB will therefore have a resistive component $R_{DIB}$ and a capacitive component $C$. $R_{DIB}$ represents the resistance to the flow that the ions in solution meet as they translocate through a finite number of inserted membrane pores with nm-sized constrictions. $C$ represents the capacitance of the lipid bilayer. At steady state, there will be no current flow through the capacitor $C$, so the equivalent electrical circuit of the DIB can be reduced to the resistive component $R_{DIB}$ only, as shown in the diagram above (right).

We now consider the case of a linear chain of $n$ droplets interconnected by αHL-permeabilized DIBs. The equivalent electrical circuit at steady state will therefore consist in a $n – 1$ resistances $R_{DIB,i}$ connected in series:
The resulting resistance of the DIB chain $R_{\text{chain}}$ will therefore increase with the number of droplets $n$ as:

$$R_{\text{chain}} = \sum_{i=1}^{n-1} R_{\text{DIB},i}$$

At constant applied potential $V$, the current $I_n$ flowing through a droplet chain of length $n$ will decrease with $n$ following Ohm’s Law:

$$I_n = \frac{V}{R_{\text{chain}}} = \frac{V}{\sum_{i=1}^{n-1} R_{\text{DIB},i}}$$

as was previously demonstrated also experimentally\cite{2}. In the case of $n$ DIBs with equal resistance $R_{\text{DIB}}$, the current $I_n$ will therefore be:

$$I_n = \frac{V}{n R_{\text{DIB}}}$$

Similar considerations can be applied to networks of hundreds of 3D-printed droplets interconnected by lipid bilayers. However, in this case the equivalent electrical circuit at steady state will be described as a complex network of resistances as described in the simplified diagrams below (left):
In a real 3D-printed network we will have multiple layers of droplets contributing to the equivalent circuit. Additionally, local defects\(^3\) will interrupt the regularity of the lattice and consequently of the equivalent electrical circuit. The current flowing through the network at applied potential \(V\) will be:

\[
I_{\text{net}} = \frac{V}{f(R_{\text{DIB},i})}
\]

where \(f(R_{\text{DIB},i})\) is a complex unknown function of the resistances of all the DIBs in the network. Therefore, in this work we directly measured the effective resistance of the whole network:

\[
R_{\text{net}} = f(R_{\text{DIB},i})
\]

and used it to study the electrical properties of \(N\) assembled networks.

As we discussed in our Results section 2.2, we observed that the overall resistance \(R_N\) of \(N\) interconnected networks of resistance \(R_{\text{net}}\) has an additional component that can be described as electrical contact resistance \(R_C\):

\[
R_N = NR_{\text{net}} + (N - 1)R_C
\]

Using this model, we could accurately describe the drop in detected ionic current flowing through \(N\) interconnected networks, as shown in Figure 3d and discussed in Results section 2.2.
Supplementary Figures

**Figure S1.** Recordings of ionic currents flowing through an assembled synthetic tissue composed of 2 building blocks of $8 \times 9 \times 5$ droplets (teal trace, as seen in Fig. 2diii), and a synthetic tissue of $16 \times 9 \times 5$ droplets (black trace).
Figure S2. Cutting and re-assembling 3D-printed synthetic tissues. a, Diagram of the fracturing and re-assembly of a 3D-printed synthetic tissue containing a conductive droplet pathway (teal). b, Electrical recordings of a 3D-printed synthetic tissue with a conductive droplet pathway (containing αHL, green droplets) before (top) and after (middle) fracturing, and after re-assembly (bottom). The cutting was performed using a flat metal spatula. The re-assembled synthetic tissue regained its ability to transmit an electrical signal.
Figure S3. Electrical recordings of ionic currents flowing through synthetic tissues composed of $N = 1$ to 6 interconnected building blocks as in Fig. 3c. The average values of the steady state currents under an applied voltage of 100 mV were plotted in Fig. 3d.
**Figure S4.** a-b, Electrical recording demonstrating no flow of ionic current outside the conductive droplet pathway containing αHL (green droplets) in the assembled synthetic tissue in b and Fig. 3f-g.
Figure S5. a-b, Electrical recordings demonstrating no flow of ionic currents through droplets containing paramagnetic beads (orange, no αHL) as in b, confirming that the current detected in Fig. 4h flows through the aligned conductive droplet pathways (green droplets, with αHL) of the 3 building blocks.
Figure S6. Recordings of ionic currents flowing through the modular synthetic tissues shown in Fig. 5f, measured from each of the input building blocks as shown in b. Ionic currents can only be detected when Zn$^{2+}$ is not present in either input (inputs = {0, 0}, first and second traces from top). In the cases of mixed inputs (inputs = {0, 1}, third and fourth traces from top), no current can be detected flowing through the input building block that did not originally contain Zn$^{2+}$ (third trace from top), demonstrating that Zn$^{2+}$ diffuses from the input containing Zn$^{2+}$, resulting in the blockage of αHL-4H throughout the sensor module (yellow).
## Supplementary Tables

|                     | Average I (pA) | SD  | R (MΩ) |
|---------------------|---------------|-----|--------|
| **Block 1 (green)** | 9673.7        | 99.1| 5.17   |
| **Block 2 (red)**   | 11471.7       | 225.0| 4.36  |
| **Assembled Blocks**| 4614.1        | 45.5| 10.84  |
| **Double-sized Block** | 7030.4      | 84.3| 7.11   |

**Table S1.** Average recorded current, standard deviation and effective resistance of building blocks and assembled synthetic tissues shown in Fig. 2. *SD* is the standard deviation of the current in the voltage protocol interval with $V = +50$ mV.

| Number of Building Blocks | Average I (pA) | SD  | R (MΩ) |
|---------------------------|---------------|-----|--------|
| 1                         | 6244.8        | 105.7| 16.01  |
| 2                         | 2891.3        | 105.3| 34.59  |
| 3                         | 2035.6        | 94.1 | 49.12  |
| 4                         | 1551.7        | 71.7 | 64.45  |
| 5                         | 1506.1        | 64.2 | 66.40  |
| 6                         | 1363.8        | 50.5 | 73.32  |

**Table S2.** Average recorded current, standard deviation and effective resistance of N = {1, 2, … , 6} assembled building blocks as shown in in Fig. 3a-d. *SD* is the standard deviation of the current in the voltage protocol interval with $V = +100$ mV.

| Figure reference          | Average I (pA) | SD  |
|---------------------------|---------------|-----|
| Fig. 3g top               | 7809.5        | 91.0|
| Fig. 3g bottom            | 3209.8        | 114.4|
| Fig. S4a                  | 11.2          | 106.8|

**Table S3.** Average recorded current and standard deviation of assembled synthetic tissues shown in Fig. 3g and Fig. S4a. *SD* is the standard deviation of the current in the voltage protocol interval with $V = +100$ mV.
Table S4. Average recorded current and standard deviation of assembled synthetic tissues shown in Fig. 4h and Fig. S5a. SD is the standard deviation of the current in the voltage protocol interval with $V = +10$ mV.

| Figure reference          | Average I (pA) | SD  |
|---------------------------|---------------|-----|
| Fig. 4h interrupted path  | 0.9           | 15.8|
| Fig. 4h interconnected path | 692.5        | 18.5|
| Fig. S5a                  | 0.5           | 21.6|

Table S5. Average recorded current and standard deviation of assembled synthetic tissues shown in Fig. 6c and Fig. S6. SD is the standard deviation of the current in the voltage protocol interval with $V = +10$ mV.

| Figure reference          | Average I (pA) | SD  |
|---------------------------|---------------|-----|
| Fig. 6c top               | 1693.4        | 163.4|
| Fig. 6c bottom            | 2.6           | 78.7 |
| Fig. 6f trace 1           | 7697.5        | 210.8|
| Fig. 6f trace 2           | 29.1          | 158.6|
| Fig. 6f trace 3           | 383.3         | 180.6|
| Fig. 6f trace 4           | 4.7           | 175.9|
| Fig. S6 trace 1           | 8270.2        | 250.6|
| Fig. S6 trace 2           | 7960.4        | 288.5|
| Fig. S6 trace 3           | 50.3          | 182.6|
| Fig. S6 trace 4           | 4.1           | 110.6|
| Fig. S6 trace 5           | 5.1           | 186.8|
| Fig. S6 trace 6           | 4.6           | 170.8|

References
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