4Dcell
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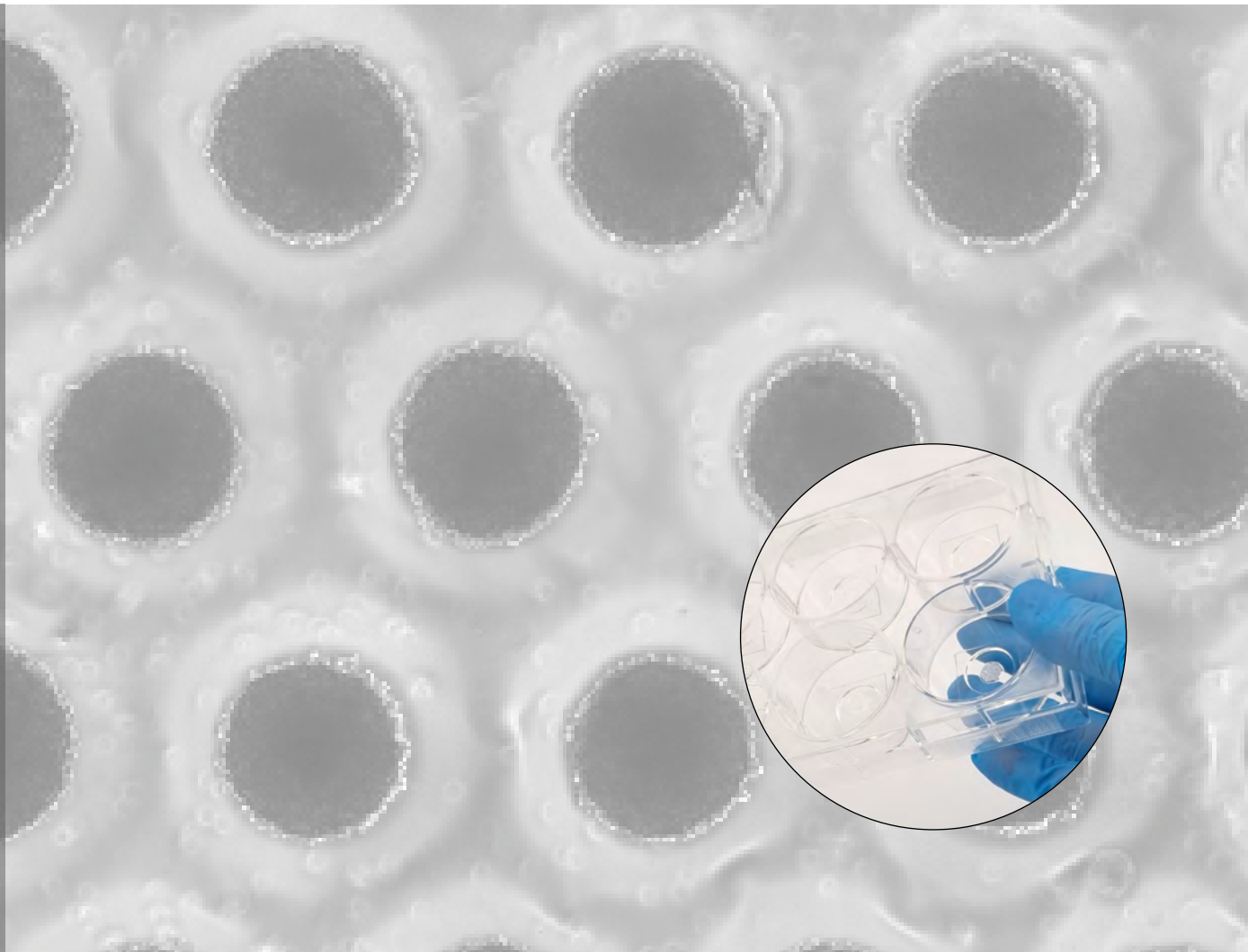
4Dcell Anchored Spheroid 24-well plate

User protocol

USER PROTOCOL



4Dcell spheroids User Guide

How to use 4Dcell (anchored) spheroid plates: 24 wells

Materials included

Glass-bottom plates with microstructured gels facilitating spheroid formation.

Materials not included

10 - 1000 µL micropipette
Distilled water
PBS

Optional

> Coating solution
e.g. fibronectin, collagen, laminin. The fibronectin solution provided by 4Dcell has a concentration of 50 µg/ml. It can be adjusted according to the cell type.

Shipping & storage

The plates are shipped in sealed sterile bags. The gels inside of the wells are submerged in an aqueous solution encapsulated with a protective film. The plates can be stored at room temperature for up to one month from the date of production.

Before starting

To avoid contamination, only manipulate the plates using aseptic techniques in a laminar flow hood.

The hydrogel structures are bound to the surface of a glass coverslip in the middle of each seeding well. To preserve the integrity of the structures, avoid touching the gels with any hard or sharp objects and always keep them submerged.

This assay was optimized for mesenchymal stem cells, with every spheroid containing 3000 cells. The protocol may need adjustments to satisfy your specific needs. The number of cells per seeding well can be changed according to the desired spheroid size or for your specific cell typeby adjusting the seeding concentration.

Please refer to the schematic below and the appendix of this document for definitions and nomenclature.

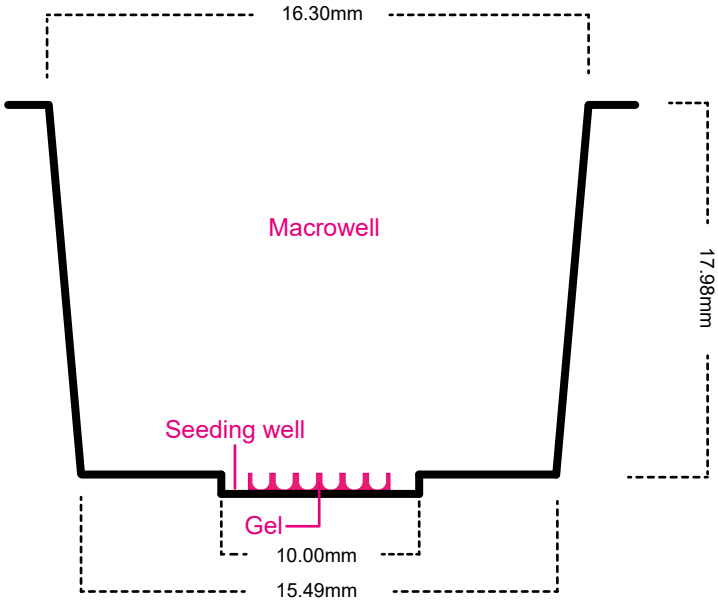


Fig. 1: Schematic of the cross-section from a well (not to scale).

Protocol

a. Using an aseptic technique in a laminar flow hood, open the sealed bag and expose the seeding well by gently removing the sealing film by pulling on the tab with a set of clean tweezers.

b. Aspirate the storage liquid, making sure not to touch the gel structure located in the middle of the seeding well. Preferentially aspirate out of the macro well to avoid damaging the gel.

If coating with an adhesion protein is necessary, proceed to step b.1. Otherwise, proceed directly to step c.

b.1. *Optional: Pipette 100 µL of your adhesive protein onto each gel*[1].

b.2. *Optional: Incubate for 30 minutes to 1 hour at room temperature.*

b.3. *Optional: Discard the adhesive protein solution from the wells.*

b.4. *Optional: Rinse each gel with 2 mL of distilled water in the macro well before proceeding to the cell seeding step.*

c. Rinse each gel by adding 2mL of PBS to the macro well.

d. Remove the PBS, taking care not to touch the gel to avoid damaging it[2].

e. Add 100 µL of culture medium to the seeding wells and incubate for 15 minutes under cell culture conditions.

f. While the gels are incubating, prepare the cell solution to the desired concentration[3]. A total volume of 2400µL of cell suspension is necessary to seed the entire 24-well plate.

g. Move the plate back into the laminar flow hood and gently pipet 100 µL of cell suspension straight on top of the gel, without touching the gel[4]. Make sure to deposit the cell suspsention close to the gel and drop-by-drop to avoid bubbles getting trapped.

h. Once every well is seeded, transfer the plate into an incubator and incubate for 1 hour under cell culture conditions[5].

i. After the cells have sedimented into the seeding wells, gentrly add 2 mL of pre-warmed medium to the macro well[6].

j. To change the medium, first remove the medium from the macro well and add 2mL of pre-warmed medium. It is not recommended to pipette directly from the seeding well as this may damage the gel. However, a small amount of medium will remain in the seeding well if the medium is only removed from the macro well. To ensure that the medium is sufficiently replenished, the medium can be changed twice.

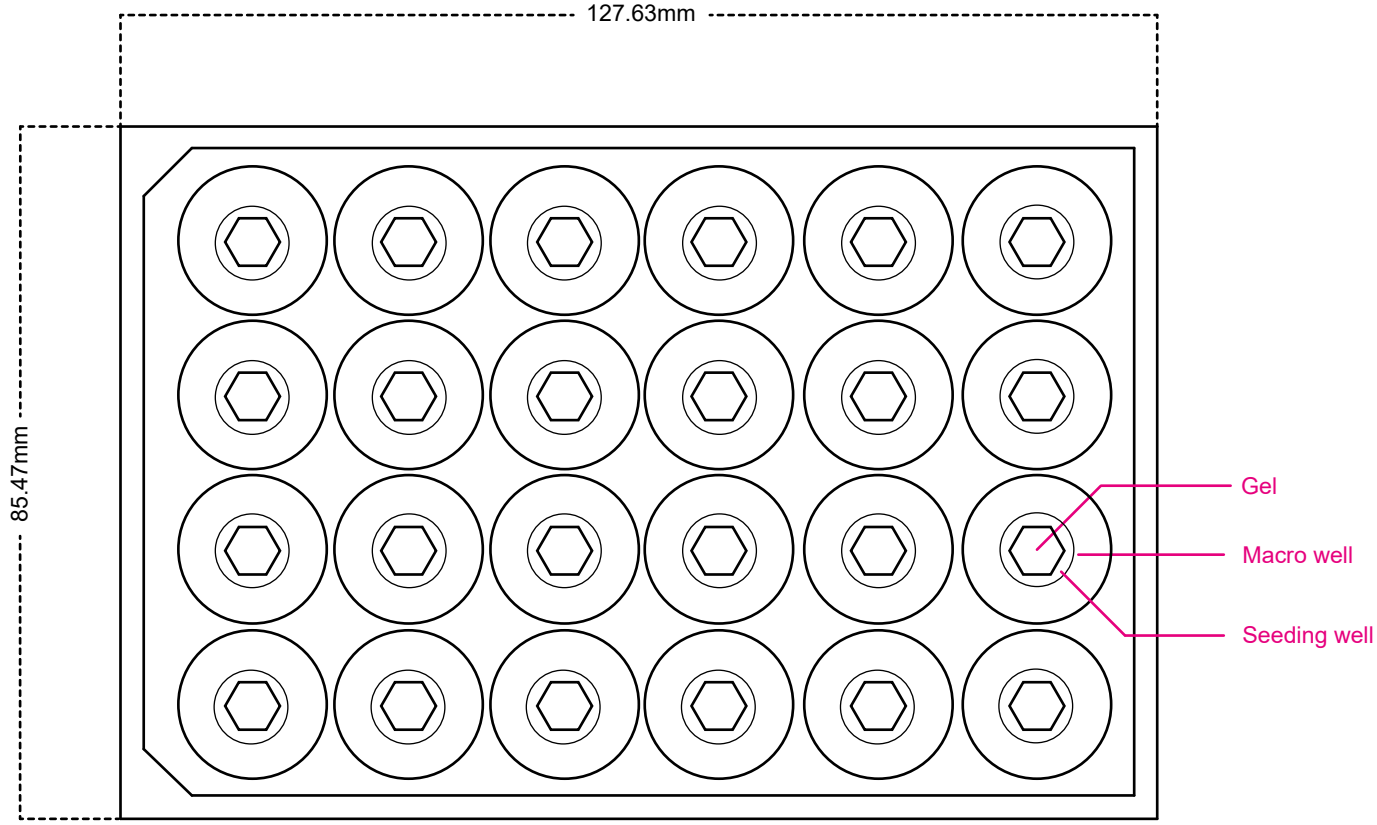
k*. Optional: When working with a target molecule, add the molecule dissolved in a solution at the step you want to study instead of the medium.*

Notes

(1) The adhesive protein provided by 4DCell is 50µg/mL fibronectin. The concentration and the choice of the protein can be adjusted according to the cell type.
(2) To avoid going over the gel and distorting it, the plate can be tilted to transfer the solution in the seeding well to the macro well.
(3) The concentration used for 3000 MSCs per spheroid is 2.73×10^6 cells/mL. The concentration, and thus the number of cells per spheroid, can be changed according to the desired spheroid size or the cell type.
(4) The cell suspension should be pipetted all around the seeding well to obtain homogeneous seeding.
(5) This period of sedimentation has been optimized for MSCs. It may vary according to the cell type.
(6) The cells should have sedimented before adding more medium. The cells should be at the bottom of the seeding wells, which can be observed as round aggregates of cells separated from each other.

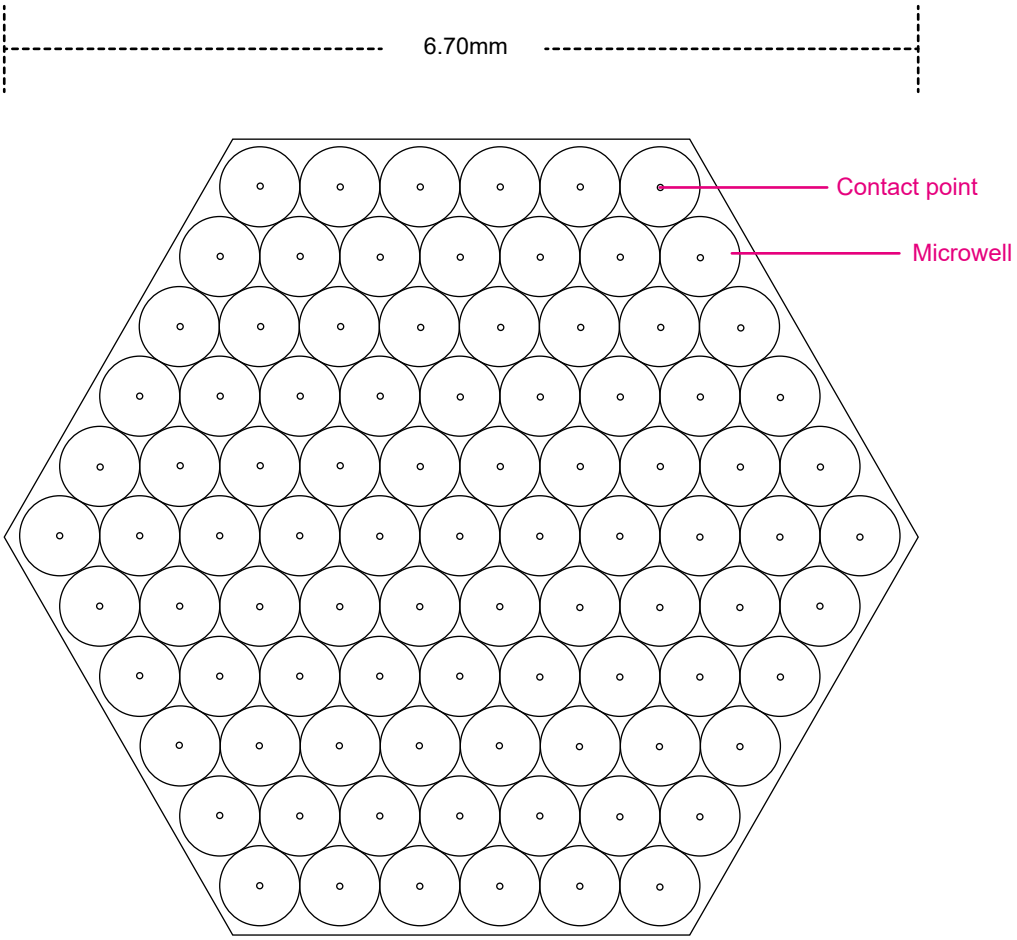
Appendix

24-well plate

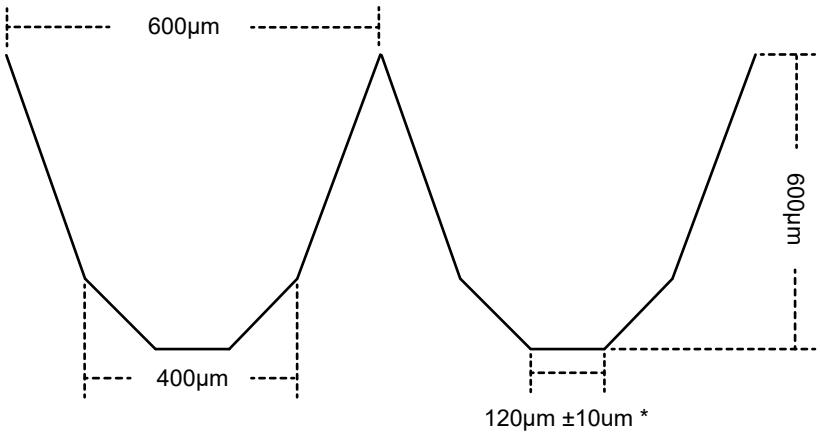


Appendix

Microstructured gel



Cross-sec on microwell



* Customizable