C-Well™ Technical Guide

Versatile, high-throughput and size-controllable cell spheroid culture protocols for using **INCYTO C-Well™**

Contents

Preface

How to Obtain Services and Support

For more information or technical assistance, call, write, fax, or e-mail.

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We welcome your comments and suggestions for improving our products.
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Introduction

General Information

Purpose of the Guide:

The C-Well™ Technical Guide contains information about the C-Well™ cell spheroid culture platform. Complete protocols for spheroid formation and reagents preparation are provided in this guide.

For additional information, contact Technical Assistance (see page 3).

Storage and Shelf life:

The C-Well™ have a shelf life of 36 months when stored at room temperature.

Do not reuse, otherwise you will encounter the following problems: contamination, failure of the cell spheroid formation and protein-rich surface.

Do not store the C-Well™ in UV radiation.

Intended Use:

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Description of C-Well™

C-Well™:

The C-Well™ is a revolutionary cell spheroid culture platform generating various types of size-controlled cell spheroids in a high-throughput way. The most widely used technique for culturing cell spheroids is a hanging drop method. The gravity environment in each hanging drop aggregates the cells, which then form single spheroids. However, the hanging drop method has several limitations. The major limitations of the hanging drop method are: ‘labor intensiveness’, ‘low throughput’, ‘relatively short period of culture’, ‘no accessibility of additional fresh growth medium’ and ‘non-uniform size of the cell spheroids’.

The C-Well™ provides following advantages over the hanging drop method and any other commercialized spheroid culture platforms: ‘extended culture period of 10-30 days due to easy access of medium change’, ‘extremely shortened culture period of 2-3 days for some application by controlling cell seeding density’, ‘co-culture ready system without direct cell-cell contact using, e.g., Transwell inserts’, ‘generating size-controlled cell spheroids’, ‘easy to change the growth medium in the device due to shear stress-free structure’, ‘applicability to various cell types’ and ‘unlimited throughput (361 cell spheroids can be obtained from one device)’.

Methods

Preparing Reagents and Materials for Cell Spheroid Culture using C-Well™

- ✓ Growth medium of target cell
- ✓ Trypsin/EDTA or ACCUTASE™
- ✓ Trypan Blue
- ✓ Phosphate buffered saline (PBS) 1X
- ✓ 70 % Ethanol
- ✓ 100 % Ethanol
- ✓ Deionized distilled water (DDW), optional
- ✓ Bovine albumin serum (BSA) 4 % solution, optional

Preparing Equipment for Cell Spheroid Culture using C-Well™

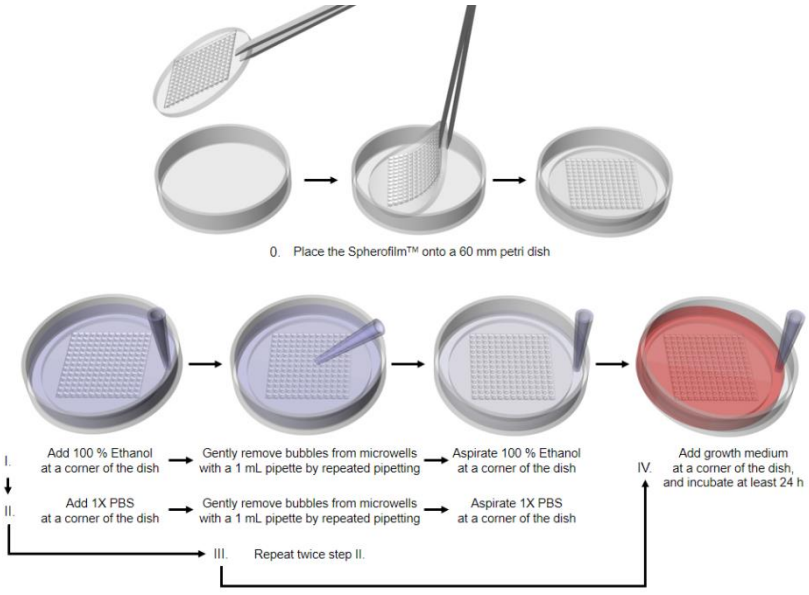
- ✓ C-Well™
- ✓ Conical tubes (15 mL or 50 mL)
- ✓ T75 flasks or cell culture dishes
- ✓ Cell strainers (100 μm pore)
- ✓ Petri dishes (60 mm or 100 mm, no treated)
- ✓ Hemocytometers (e.g. DHC-N01, INCYTO)
- ✓ Pipettes and tips (1 mL and 200 μL)
- ✓ Serological pipette aid and tips (10 mL)
- ✓ Clean bench
- ✓ CO_2 Incubator
- ✓ Phase-contrast microscopy
- ✓ Aspirator
- ✓ Centrifuge
- ✓ Alcohol lamp

A. Pretreatment of C-Well™ prior to Cell Seeding

1. Warm up growth medium, 1X PBS (or DDW) and 100 % Ethanol to room temperature.

   Recommended approximate volumes of growth medium is 14 mL/1 device, 1X PBS is 24 mL/1 device and 100 % Ethanol is 8 mL/1 device.

2. Remove the packaging of the C-Well™ in a clean bench.
3. Using a sterile tweezer, plate the C-Well™ onto a 60 mm petri dish from the one outside edge toward the other for preventing bubbles between the C-Well™ and the petri dish.
4. Add 8 mL of 100 % Ethanol to the prepared dish.
5. Gently remove bubbles from the microwells with a 1 mL pipette by repeated pipetting.
6. Aspirate 100 % Ethanol at a corner of the dish. Do not aspirate the solution in each microwells.
7. Add 8 mL of 1X PBS to the dish, and remove bubbles again.
8. Aspirate 1X PBS at a corner of the dish. Do not aspirate the solution in each microwells.
9. Repeat above 7, 8 steps two times.
10. Add 7 mL of growth medium to the dish, and remove bubble thoroughly.
11. Place the dish in an incubator for at least 24 hours.
12. Gently remove bubbles from the microwells with a 1 mL pipette by repeated pipetting.
13. Aspirate the growth medium at a corner of the dish, and add 7 mL of fresh growth medium prior to cell seeding.



B-1. Preparing of Single Cell Suspension of Target Cells (for adherent cells)

1. Warm up growth medium and Trypsin/EDTA solution at an appropriate concentration to 37 °C.
2. Add 10 mL of 1X PBS, and gently wash the cell culture flask (T75) or the dish (100 mm).
3. Aspirate the 1X PBS solution.
4. Add 2 mL of the Trypsin/EDTA solution, and place the flask in a 37 °C CO_2 incubator for 1~2 minutes (depends on cell types).
5. Gently tap the flask, and observe the cells. Most of the cells should be detached
6. Neutralize the Trypsin/EDTA treated cell suspension with 4 mL of the growth medium or appropriate neutralizing solutions.
7. Detach the cells thoroughly with repeated pipetting.
8. Count the cell number with a hemocytometer (e.g. INCYTO C-Chip, DHC-N01)
9. Centrifuge the cell suspension at an appropriate centrifugal force.
10. Remove the supernatant by aspiration.
11. Resuspend the cell pellet in the growth medium. The final seeding density and the final volume of the cell suspension should be 0.1~0.5 million cells/mL and 7 mL, respectively. These values vary by the cell types.

B-2. Preparing of Single Cell Suspension of Target Cells (for suspended cells)

1. Warm up growth medium and Trypsin/EDTA solution (optional) at an appropriate concentration to 37 °C.
2. Homogenize and disaggregate the cell suspension with repeated pipetting.
3. Count the cell number with a hemocytometer (e.g. INCYTO C-Chip, DHC-N01)
4. Centrifuge the cell suspension at an appropriate centrifugal force.
5. Remove the supernatant by aspiration.
6. (Optional) Add 1~2 mL of the Trypsin/EDTA solution, and place the tube in a 37 °C CO_2 incubator for 1~2 minutes (vary by the cell types).
7. (Optional) Neutralize the Trypsin/EDTA treated cell suspension with 2~4 mL of the growth medium or appropriate neutralizing solutions (vary by the cell types).
8. (Optional) Centrifuge the cell suspension at an appropriate centrifugal force.
9. (Optional) Remove the supernatant by aspiration.
10. Resuspend the cell pellet in the growth medium. The final seeding density and the final volume of the cell suspension should be 0.1~0.5 million cells/mL and 7 mL, respectively. These values vary by the cell types.

C. Formation of Cell Spheroids using C-Well™

1. Warm up growth medium to 37 °C.
2. Gently remove bubbles from the microwells with a 1 mL pipette by repeated pipetting.
3. Aspirate the growth medium at a corner of the dish, and add 7 mL of the cell suspension having the cell seeding density of 0.1~0.5 million cells/mL
4. After 10~15 minutes (vary by the cell types), gently aspirate the cell suspension to remove the supernatant cells.
5. Add 7 mL the growth medium at a corner of the dish.
6. Aspirate the suspension, and add 7 mL of the growth medium at a corner of the dish. Repeat this step two times. It's very important to be sure that there is no suspended cells in the end of this step. If not, these suspended cells might impede the formation of the cell spheroids.
7. Incubate the dish in a 37 °C CO_2 incubator for 24 hours.
8. Change the growth medium in the dish every 24 hours.

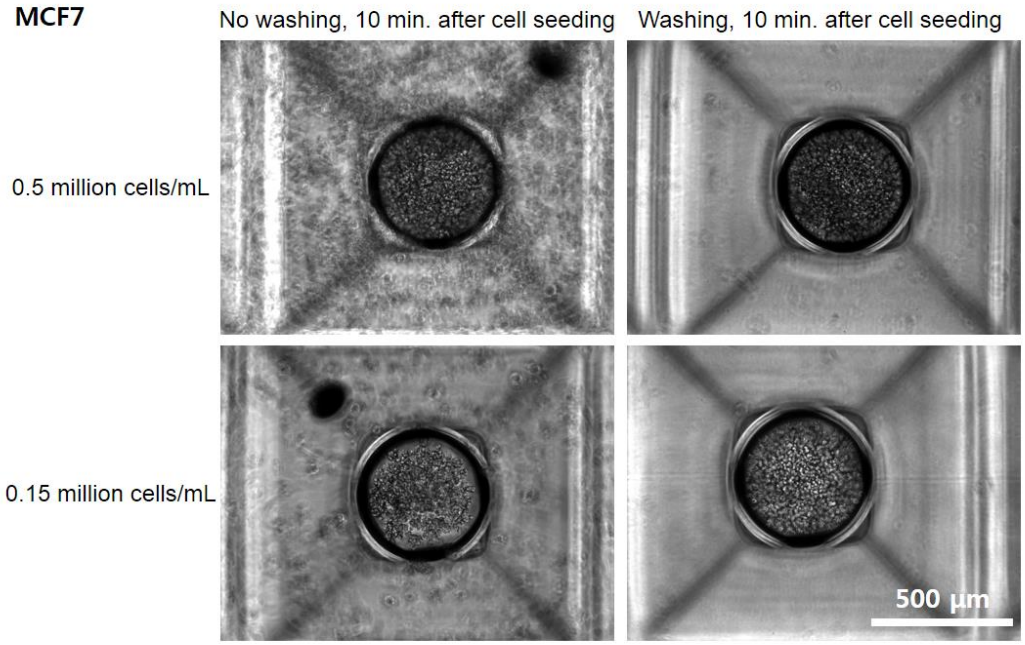


Figure 1 The culture conditions of the MCF7 spheroids. The cell seeding density and the washing timing vary by the cell types. Appropriate seeding density and washing timing should be determined by pre-experiments.

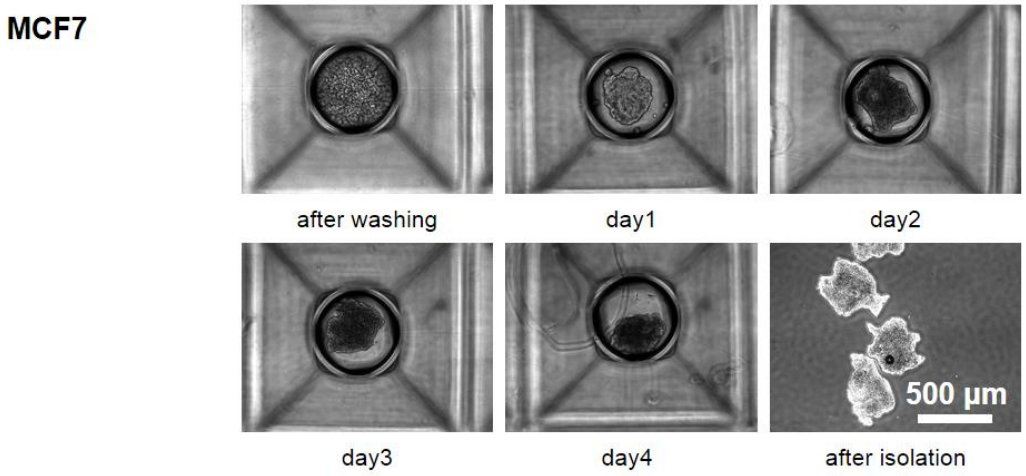


Figure 2 The formation of the MCF7 spheroids.

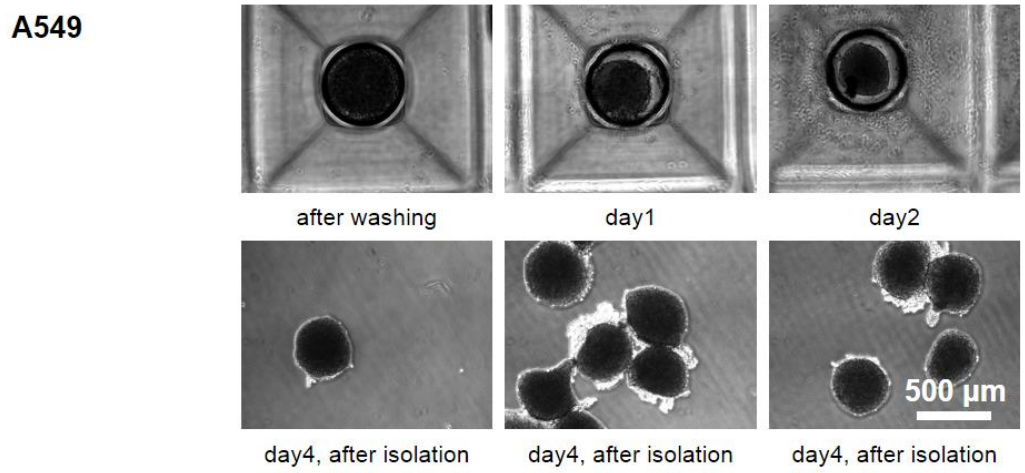


Figure 3 The formation of the A549 spheroids.

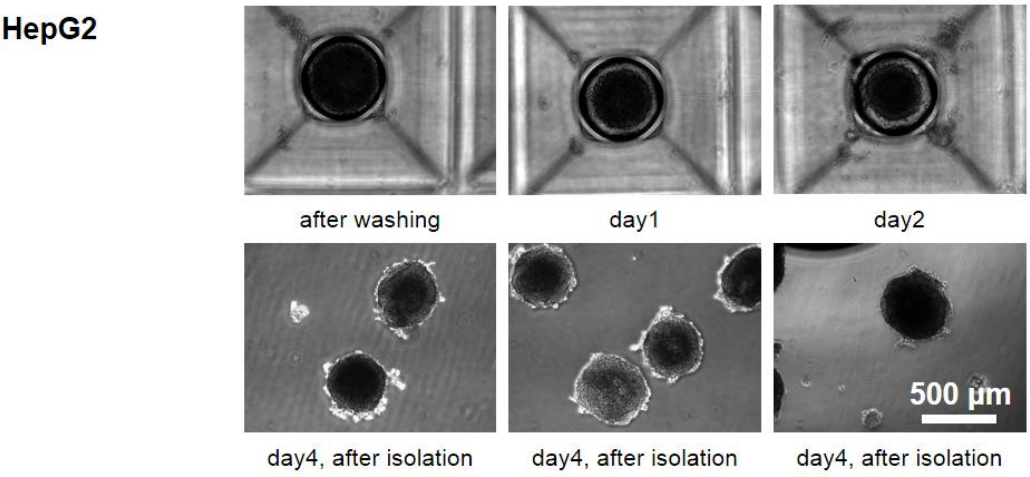


Figure 4 The formation of the HepG2 spheroids.

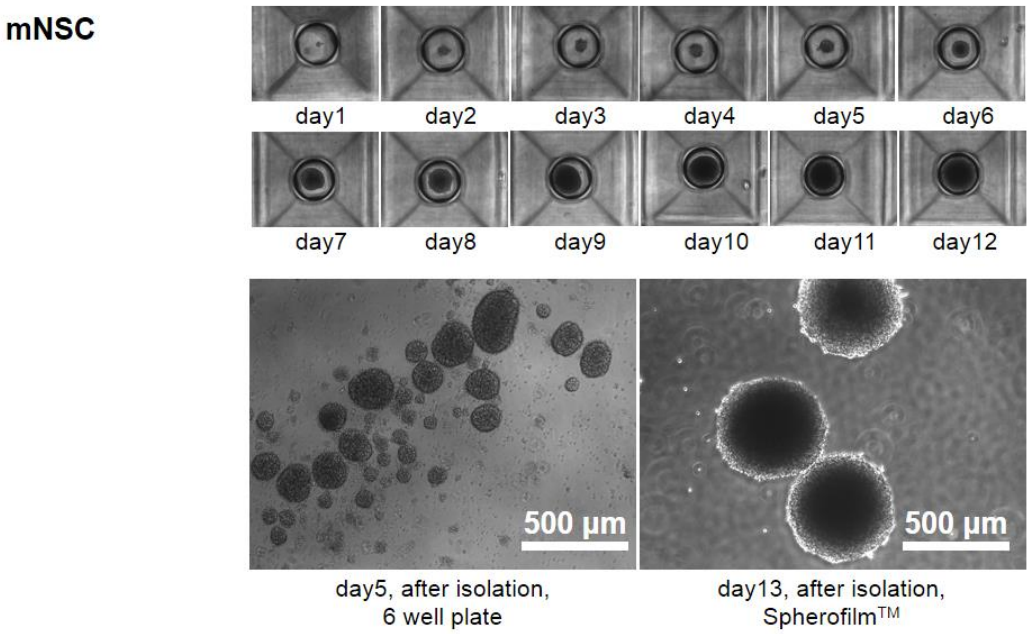


Figure 5 The formation of the mouse neural stem cells (mNSC) spheroids. The C-Well™ can generate size-controlled, uniform-shaped cell spheroids when compared to the other conventional spheroid culture methods.

D. Isolation of Cell Spheroids from C-Well™

1. Warm up growth medium to 37 °C.
2. Directly pipette (with 1 mL tip) the growth medium onto the C-Well™.
3. Repeat above step 2 until most of the cell spheroids is collected.
4. Pipette gently the cell spheroids solution.
5. Add the solution to the one surface (top) of the cell strainer.
6. Flip the strainer, and add the growth medium to the other surface (bottom) of the strainer.
7. Plate the cell spheroids in a no treated petri dish.