Obatala Sciences Protocol 307
How Do I Use Obatala Sciences' ObaGel® ECM to Create 3D Cultures?

Written by: Obatala Sciences Scientific Team
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Reagents, Materials, and Equipment

- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101), Human Stromal Vascular Fraction Cells (Catalog # OS-107-01), or equivalent cryopreserved primary cell product
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical tubes
- Multi-well plate, or equivalent plasticware suitable for cell culture
- Obatala Sciences' ObaGel®-ECM^{A} (Catalog#OS-314)
- Obatala Sciences' ObaGel®-ECM^{B} (Catalog #OS-314)
- Cell culture medium of choice
- Viability stains such as trypan blue or acridine orange/propidium iodide
- P-1000 pipette and tips
- 37°C, 5% CO_2 incubator
- Serological pipet
- Wet ice for prolonged handling

Calculations

Plate Size	Surface area per well (cm^2)	Hydrogel Volume (mL)	Media Volume - Total (mL)	Media Volume – Feedings (mL)
24-well	1.9	0.25	0.5	0.25
96-well	0.3	0.05	0.1	0.05

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General Requirements

1. All personnel should be trained and certified by the Principal Investigator regarding Universal Precautions and Handling of Bloodborne Pathogens.
2. All procedures should be conducted by investigators always using appropriate personal protective equipment. Any waste materials should be decontaminated (bleached) and disposed of using appropriate biohazard waste containers.

Protocol

Preparation of Cells for Seeding

Note: Perform all tasks in Level 2 Biosafety Cabinet using aseptic technique.

1. Determine the number of cells needed for seeding plate.
    a. Recommended density for seeding ASC is 200,000 cells per mL of hydrogel.
2. Thaw cryopreserved cells and resuspend in the appropriate cell culture media.
3. Count cells using a live/dead stain and prepare cell pellets for resuspension.
   For example: Prepare 3 wells with 50,000 cells/well (250 µL constructs/well)
        i. Plate size: 24-well
        ii. # wells to be seeded: 3
        iii. Volume of ObaGel®-ECM^{A}: ObaGel®-ECM^{B} needed for seeding: 750 µL total (250 µL per well)
        iv. Volume of gel to prepare: 1 mL
        v. Number of cells needed: 200,000

Seeding of Cells in ObaGel® ECM

Note: Final Concentration of ObaGel®-ECM^{A}:ObaGel®-ECM^{B} *should be 1:1 (v/v).*

Note: Prepare more of the hydrogel solution than expected to use due to loss of material in pipette.

1. In BSC, place ObaGel®-ECM^{A} and ObaGel®-ECM^{B} on wet ice or frozen steel beads while handling.
2. Transfer appropriate volume ObaGel®-ECM^{B} to conical tube using a P-1000 pipette or a serological pipette.
    a. Pipette slowly and allow for excess gel to gather in pipette tip after dispensing to prevent loss of material.
    b. Following example provided in *Preparation of Cells for Seeding*
        i. Total volume of gel needed: 1 mL
        ii. Volume of ObaGel®-ECM^{A}: 500 µL
        iii. Volume of ObaGel®-ECM^{B}: 500 µL
3. Aspirate media from cell pellet and resuspend cells in ObaGel®-ECM^{A} at 2X the desired seeding density using a P-1000 pipette.
    a. Following example provided in *Preparation of Cells for Seeding*, resuspend 200,000 cells in 500 µL of ObaGel®-ECM^{A}
    b. Pipette gently and slowly to avoid lysing cells. It is <u>not</u> recommended to use a smaller pipette for resuspension due to risk of lysing cells.

4. Add ObaGel®-ECM^{A} to pre-measured amount of ObaGel®-ECM^{B} in conical tube using a P-1000 pipette and mix solutions slowly and thoroughly to avoid creating bubbles in the mixture. You may also use a serological pipette to mix the solution.
    a. Following example provided in *Preparation of Cells for Seeding*, mix 500 µL ObaGel®-ECM^{A} + cells with 500 µL of ObaGel®-ECM^{B}
5. Seed appropriate volume of hydrogel + cells mixture into cell culture plate.
    a. See recommended seeding volumes in *Calculations*.
6. Slowly rotate the plate to ensure the well surface is evenly coated.
7. Transfer plate to cell culture incubator at 37°C and allow crosslinking for 30 minutes to 1 hour.
8. After crosslinking occurs, add appropriate cell culture media. Always add media to the side of well and never directly on top of constructs.
9. For maintenance after cell seeding, change media every 2-3 days by removing half of the well volume and replacing it with an equal volume of media.
    a. See *Calculations* for recommended media volume–feedings.
    b. Always add media to side wall of the well, and never directly on top of the gel.

Appendix A: ObaGel®-ECM 3D Culture Establishment Procedure Flowchart

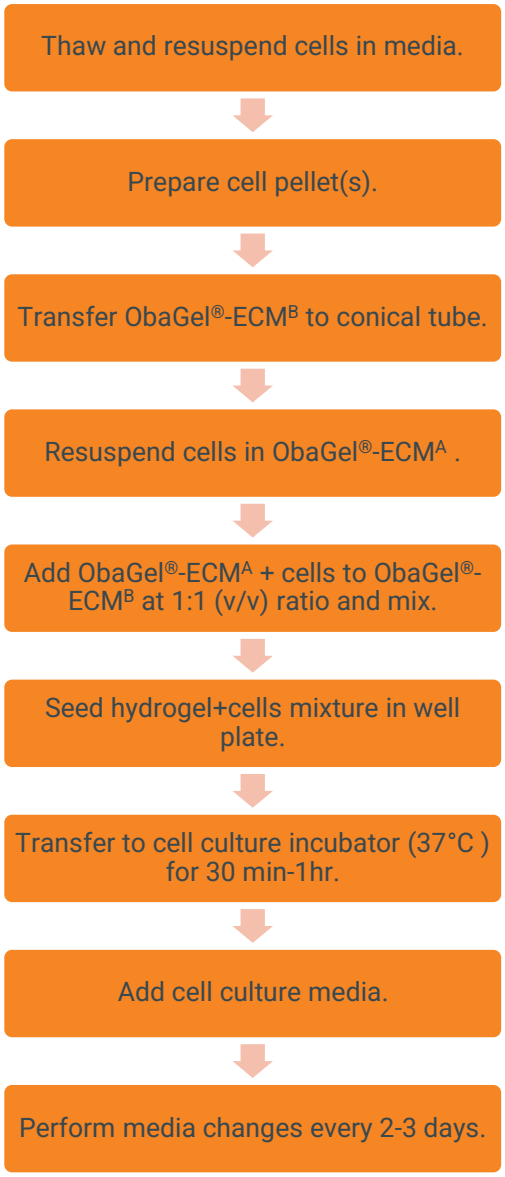

Figure 1. Workflow diagram outlining the steps required to create 3D cultures with ObaGel®-ECM. Steps for protocol 307 include: initial handling of ObaGel®-ECM, thawing and resuspension of cells, seeding of the ObaGel®-ECM cultures, and maintenance of the established ObaGel®-ECM cultures.