Obatala Sciences' Protocol 401
How Do I Create 2D Cultures with ObaGel®?

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Reagents, Materials, and Equipment

- Obatala Sciences' ObaGel® (Catalog #OS-301)
- Obatala Sciences' ObaVate™ (Catalog #OS-302)
- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product
- Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice
- Viability stains such as trypan blue or acridine orange/propidium iodide
- 70% ethanol
- Sterile paper towel or kimwipe
- 37°C, 5% CO_2 incubator
- BSL2 Biological Safety Cabinet
- Conical centrifuge tube
- Serological pipettes
- Pipette controller
- Micropipette and pipette tips
- Culture plate & micropipette for desired format, protocol is optimized for 150 mm plate or a T75 flask
- Wet ice for prolonged handling

Calculations

Reagent	150 mm plate	T75 flask	6-well plate
ObaGel®	5 mL	3 mL	1 mL/well
StromaQual™	10 mL	20 mL	3 mL
hSVF cells	1-10 x 10^4 cells/cm^2	1-10 x 10^4 cells/cm^2	1-10 x 10^4 cells/cm^2
hASC cells	1-10 x 10^3 cells/cm^2	1-10 x 10^3 cells/cm^2	1-10 x 10^3 cells/cm^2

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Protocol

Initial Handling of Your Obatala Sciences' Products

1. ObaGel® may arrive on wet ice or dry ice depending on the shipping conditions.

   Note: If you ordered your ObaGel® to arrive on a cold pack (4°C equivalent), it will arrive already thawed.

   a. Prior to use, thaw ObaGel® overnight at 4°C until completely thawed. Do not thaw at room temperature or attempt to warm products at higher temperatures.
   b. Aliquot necessary volumes for immediate use into separate containers to avoid repeated freeze/thaw cycles.
   c. After thawing, ObaGel® can be stored at 4°C for <48 hours prior to use. For longer term storage up to 3 months, store at -20°C. For storage >3 months, store at -80°C (shelf life 1 year).
2. After thawing, you may notice protein precipitant present in ObaGel®. This is normal and does not impact the function or quality of the product. In fact, it is beneficial to the formation of 3D constructs!

   Note: Do not attempt to spin or otherwise remove the precipitant from the product, as it will clump and aggregate. Unaltered, the precipitant will disperse when pipetting.

Seeding Cells on ObaGel® Coated Plates for 2-Dimensional Culture

1. Thaw ObaGel® overnight at 4°C until completely thawed and no ice crystals remain in solution (see handling instructions above). Keep refrigerated or on wet ice prior to use.

   Note: What could I do wrong at this step? The ObaGel® *product is temperature sensitive. If you were to immediately warm it up to 37°C, you will inactivate its gelling properties. Therefore, do not allow the thawed product's temperature to exceed refrigerator temperature (4°C) during the thawing process.*
2. Place the aliquot of ObaGel® on wet ice and transfer to a BSL2 biological safety cabinet.
3. Transfer the recommended volume of ObaGel® to the culture surface. Tilt to coat the entire surface of the plate or flask.
4. Transfer the coated surface to a cell culture incubator at 37°C for 30 minutes.
5. After 30 minutes, tilt the plate or flask and observe whether any excess volume collects at the periphery of the surface. Use a micropipette to remove any excess volume, pipetting slowly at the periphery to reduce shear stress on the coating surface.
6. Transfer the coated surface to a cell culture incubator at 37°C to dry overnight prior to seeding.
7. Following overnight incubation, observe the coated surface. The coating should appear homogenous in thickness with no bare patches of plastic visible when tilted.

8. Plate Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product at the recommended seeding density in the recommended volume of Obatala StromaQual™ Stromal Medium (Catalog #OS-001). Refer to *calculations* table.
9. Carefully transfer the plate or flask to a cell culture incubator at 37°C for 24 hr prior to additional manipulation.
10. After 24 hr, evaluate for cell adhesion under phase contrast microscopy. Proceed to feed and maintain cultures as appropriate for the cell type and conditions of interest.
    a. 2D ObaGel® culture is compatible with enzymatic harvest methods, including 0.05% trypsin solution. It is recommended that cultures are thoroughly washed with a serum-free buffer solution prior to the addition of enzymatic digest solution.

Recommended Protocols

Obatala Sciences' Protocol 101–How Do I Thaw Cryovials of Cells from Obatala Sciences?
Obatala Sciences' Protocol 102–How Do I Harvest Adherent Cells from Obatala Sciences?
Obatala Sciences' Protocol 103–How Do I Cryopreserve Culture-Expanded Cells from Obatala Sciences?

After you have established your 2-dimensional cultures, you can proceed to your next planned experimental endpoints, which might include but are by no means limited to proliferation, differentiation, or implantation into recipient mice or animal models *in vivo*.

We expect that you will have new ideas on how to use our product that extend beyond these boundaries and look forward to hearing about novel ways you can use ObaGel® in your discovery research. Please share your findings with us when they become available.

Remember, any laboratory that mentions Obatala Sciences' products by name in a publication is eligible for a 10% discount on their next order! We appreciate not only your business but your endorsement of our products!

Appendix A: 2D ObaGel® Culture Establishment Procedure Workflow

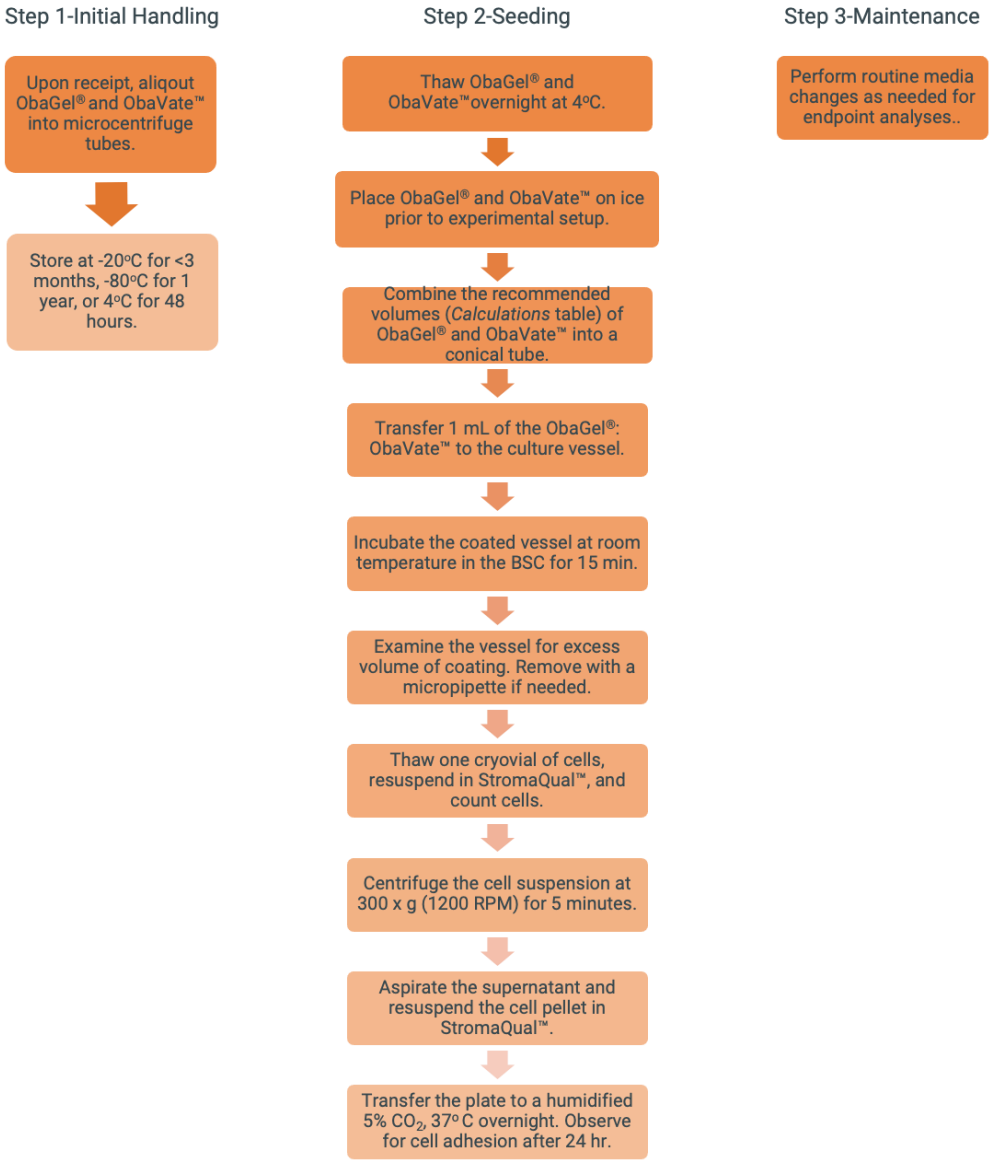

Figure 1. Workflow diagram outlining the steps required to create 2D cultures with ObaGel®. Steps for protocol 401 include: initial handling of ObaGel® and ObaVate™, coating of culture vessels with ObaGel®, seeding of coated culture vessels, and maintenance of the established cultures.

Appendix B: Troubleshooting

Problem	Reason	Solution
No plastic-adherent coating observed	ObaGel® pre-warmed at 37°C or equivalent prior to use	Do not heat ObaGel® prior to use, maintain on ice during use.
	Repeated freeze-thaws	Aliquot ObaGel® into smaller volumes and thaw as needed.
	Improper extended storage	Store at –20°C for up to one year from receipt date. Thaw and store at 4°C for no more than 48 hr prior to use.
Gelation observed during pipetting or handling	ObaGel® pre-warmed at 37°C or equivalent prior to use	Do not heat ObaGel® prior to use, maintain on ice during use.
	ObaGel® not maintained at 4°C or on ice prior to use	Ensure ObaGel® is kept at 4°C or on wet ice prior to use.
Poor cell detachment at harvest	Coated surface not thoroughly washed prior to addition of enzymatic digest solution	Wash 2-3 times with a serum-free buffer solution prior to harvest. You may choose to incubate the coated surface with the wash solution prior to removal.
Poor cell viability at harvest	Cells incubated too long with enzymatic digest solution	Cells may lift from the coated surface quicker than expected. Observe cell detachment frequently and minimize incubation time in digest solution.