All-inclusive 3D Osteogenesis-Angiogenesis Coupling Kit

3D-OAC

Cat. #3D-8748

Product Description

Bone formation and repair is a complex process involving the highly orchestrated interplay between different cell types, such as endothelial cells and osteoblasts. During skeletal development and postnatal bone repair, angiogenesis and osteogenesis must be tightly coupled for physiological bone function, as blood vessels bring a supply of oxygen, nutrients and osteogenic progenitor cells into the osteogenic environment [1]. In fact, the absence of a functional vasculature network can compromise physiological bone healing, leading to osteonecrosis, osteoporosis, and non-union fractures [2]. Thus, elucidation of the molecular crosstalk between angiogenesis and osteogenesis is critical in designing the therapeutic strategies for improving efficient vascularization and bone formation. To study such complex tissue and regulation processes, ScienCell's 3D Osteogenesis-Angiogenesis Coupling kit (3D-OAC) is designed to co-culture human primary osteoblasts and endothelial cells as 3D multicellular spheroids, mimicking the complex cellular interactions present in bone tissue.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
4600	1	Human Calvarial Osteoblasts (HCO)	5×10^5 cells	Liquid nitrogen
2000	1	Human Dermal Microvessel Endothelial Cells (HDMEC)	5×10^5 cells	Liquid nitrogen
3D-4621	1	3D-Osteo-Endo Spheroid Medium (3D-OESpM)	200 mL	2-8 °C
3D-4662	1	3D- Osteo-Endo Spheroid Supplement (3D-OESpS)	2 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
0343 (or) 0353 (or) 0383	2	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	2 plates	RT
2D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
4601	1	Osteoblast Medium – basal (ObM)	500 mL	2-8 °C
4652	1	Osteoblast Growth Supplement (ObGS)	5 mL	-20 °C
1001	1	Endothelial Cell Medium – basal (ECM)	500 mL	2-8 °C
1052	1	Endothelial Cell Growth Supplement (ECGS)	5 mL	-20 °C
0025	2	Fetal Bovine Serum (FBS)	25 mL	-20 °C
0503	2	Penicillin/streptomycin Solution (P/S)	5 mL	-20 °C

Additional Recommended Materials (Not Included)

Cat #	Product Name
0113	Trypsin Neutralization Solution (TNS)
0183	0.05% Trypsin/EDTA (T/E)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (PLL) (10 mg/mL)
8248	Bovine Plasma Fibronectin

Quality Control

3D-OAC is tested for the uniform formation of 3D osteoblast and endothelial cell co-culture spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

3D-OAC is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

4600, 2000, 3D-4662, 0010, 0583, 4652, 1052, 0025, and 0503 are shipping on dry ice. 3D-4621, 4601, 1001, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

[1] Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, and Maggio ND. (2017) "It Takes Two to Tango: Coupling of Angiogenesis and Osteogenesis for Bone Regeneration." *Front Bioeng Biotechnol* 5 (68): 1-7.

[2] Inglis S, Christensen D, Wilson DI, Kanczler JM, and Oreffo R. (2016) "Human Endothelial and Foetal Femur-derived Stem Cell Co-Cultures Modulate Osteogenesis and Angiogenesis." *Stem Cell Research & Therapy* 7 (13): 1 – 16.

Procedure:

A. Initiating cells in 2D culture

1. Please see the product sheet Cat. #4600 and #2000 for thawing and maintaining ScienCell's human primary osteoblasts (Cat. #4600) and human dermal microvascular endothelial cells (Cat. #2000) in 2D monolayer culture, respectively.

2. For expansion in 2D monolayer culture, use the included 2D culture kit components (Cat. #4601, 4652, 1001, 1052, 0025, and 0503).

B. Establishing 3D spheroid culture

Step I: Prepare the complete 3D culture medium

3. Thaw 3D-Osteo-Endo spheroid supplement (3D-OESpS; Cat. #3D-4662), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Gently mix 3D-OESpS, FBS and P/S solution into the 3D-Osteo-Endo spheroid medium (3D-OESpM; Cat. #3D-4621) by swirling the medium bottle around.

   a. 3D-OESpM medium is **viscous** and optimized for homogenous spheroid formation.
   b. Warm the complete 3D-OESpM medium only **to room temperature** before use.
   c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Harvest cells for 3D culture

Table A: An Example of Suggested Cell Numbers and Culture Volumes Per Sample

1	2	3	4
Plate formats	**HCO cell number per well**	**HDMEC cell number per well**	**Volume per well**
24-well	2.3×10^5 cells	1.3×10^5 cells	~ 1000 µL
48-well	9.4×10^4 cells	5.0×10^4 cells	~ 500 µL
96-well	4.0×10^4 cells	2.1×10^4 cells	~ 200 µL

4. Please see **Table A** for the suggested cell densities for different plate formats. A confluent T-75 and T-175 flasks should yield about 5×10^6 and 1×10^7 cells, respectively.

5. When desired amount of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.

6. Rinse the cells with DPBS.

7. Add 5 mL of DPBS and 5 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

8. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).

9. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

10. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

Step III: Resuspend and seed cells in 3D culture medium

11. Count cells using hemacytometer. Please see **Table A** for the suggested cell numbers for both osteoblasts (Cat. #4600) and endothelial cells (Cat. #2000) for different plate formats.

12. Aliquot the suggested cell numbers for both osteoblasts and endothelial cells into a fresh 50 ml conical tube.

    Note: It is recommended to make a minimum of 5 mL cell suspension in 3D medium for easier pipetting due to the viscosity of 3D medium.

13. Centrifuge the 50 ml conical tube at 1000 rpm for 5 minutes.

14. Aspirate the supernatant while leaving behind the 100-200 µl supernatant above the pellet in the tube.

15. Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.

16. Next, add the appropriate volume of the 3D-OESpM medium (see **Table A; column 4**) to obtain the suggested density of cell suspension.

17. Slowly pipette up and down for ~ 10 times and make sure you have uniform cell suspension in 3D medium before proceeding to next step.

    Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid too much bubble formation.

18. Add the suggested volume of cell mixture (see **Table A; column 4**) to each well in the provided ultra-low binding plate by using a p1000 pipette. Do not use the serological pipette to minimize pipette errors while adding small volumes to the wells.

    Note: Add cell suspension to the center of the well instead of adding to the side.

19. Incubate the cells at 37°C in a 5% CO_2 humidity incubator.

20. Change the 3D culture medium every four days by only changing 60-70% of the top layer of the medium using a pipette by hand (Do not use a vacuum aspirator).

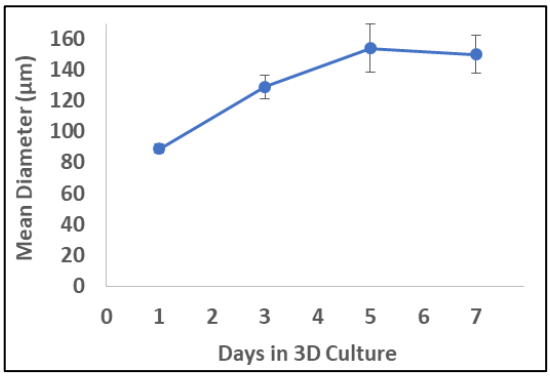
    Note: Spheroids are situated at the bottom of the well due to the viscosity of the 3D culture medium. Thus, centrifugation of the plates is not necessary, and spheroid loss will not occur by changing 60-70% of the top layer of the medium by pipetting.

21. Monitor the growth and formation of spheroid every day under the microscope. Mature osteoblast-endothelial cell co-culture spheroids develop at ~ 5 days post seeding (Figure 1).

    Note: You may see the disorganized spheroid morphology around days 2-4 post seeding. However, the compact and mature spheroids will develop around days 5-6 post seeding as the cell-cell junction proteins express and accumulate over time.

Figure 1 – Growth of osteoblast and endothelial cell co-culture spheroids over 7 days. (A) Spheroids were generated using primary osteoblasts and endothelial cells according to the provided protocol. (B) Brightfield images of spheroids were taken at 100X magnification at days 1, 3, and 7.

A.



B.

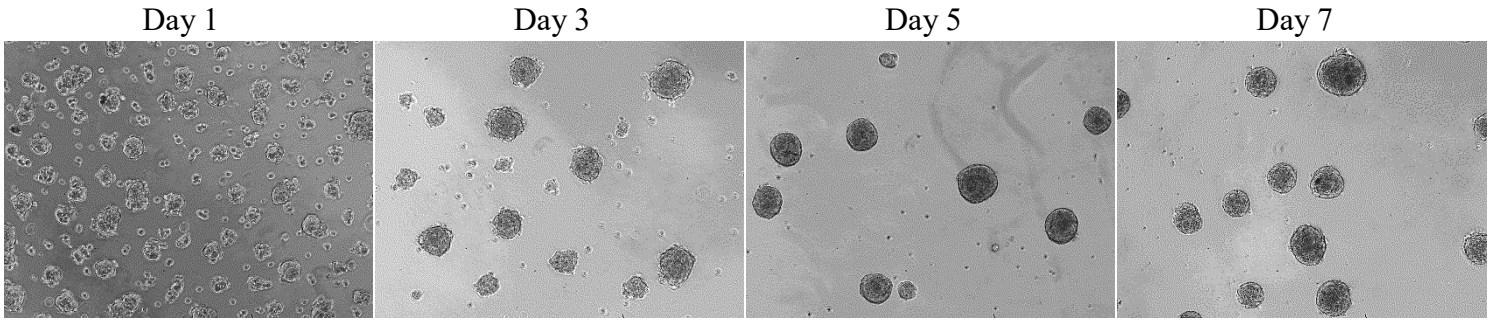
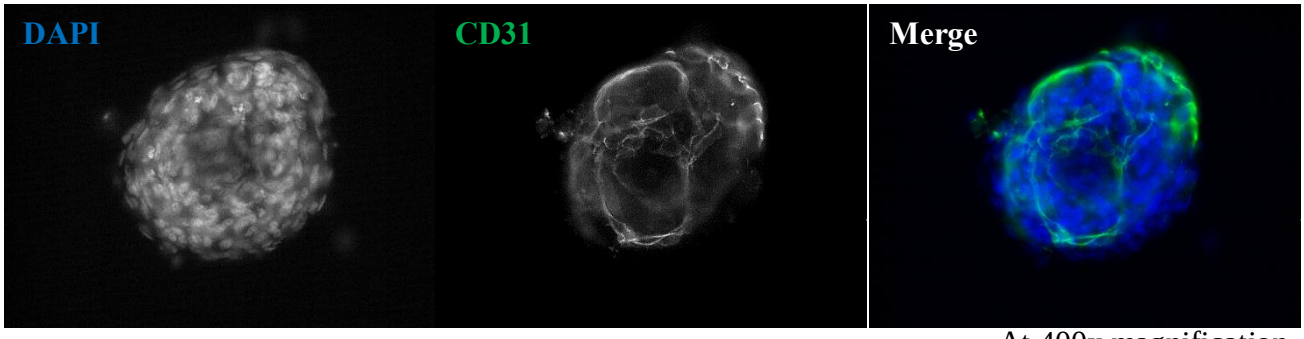


Figure 2 – Day 7; Immunofluorescence staining of osteoblast and endothelial cell co-culture spheroids. IF analysis showed that within 3D spheroids, the CD31+ endothelial cells are directly in contact with the CD31- osteoblasts, mimicking the complex cellular interactions in bone tissue.



At 400x magnification

Troubleshooting Guide

Problem	Possible Cause	Potential Solution
Cells do not form spheroids.	Cells are not healthy.	- Check cell viability (should be >90%) and cell proliferation using trypan blue. - Reduce extensive sub-culturing in 2D culture.
Spheroid formation is not homogenous.	1. Cells are not resuspended well.	- First, obtain single cell suspension in the residual supernatant by gently pipetting up and down for approximately 10-15 times (see step 14). - Next, obtain uniform cell suspension in 3D culture medium by pipetting up and down for approximately 10 – 15 times. Additionally, you can rotate the tube around to help mixing cells in 3D medium (see step 16).
	2. Shelves in the cell culture incubator are not level.	- Level your shelves of the CO_2 incubators.