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Ossiform ApS

PROTOCOL

How to 3D culture human mesenchymal stem cells — Seeding hMSCs on P3D Scaffolds

Application

This protocol provides you with guidelines for seeding and 3D culturing of human mesenchymal stem cells (hMSCs) on P3D Scaffolds (Ø12 mm) in a 24-well plate. Please refer to the table with suggested seeding densities in our technical data sheet if you are using another size of P3D Scaffold – download data sheet here.

Here, you will find guidelines on how to create 3D multicellular *in vitro* tissue constructs based on hMSCs and subsequently differentiation of hMSCs into osteoblasts to establish a 3D bone cell culture. For more protocols, please visit our Resources Platform.

Materials

- P3D Scaffolds (supplied sterile)
- Pipette
- Cell culture incubator
- Cell culture flask
- Cryopreserved hMSCs
- Hemocytometer
- Growth medium (for instance Human Mesenchymal Stem Cell Growth Medium (MSCGM™))
- Trypsin/EDTA
- PBS
- Low-attachment 24 well plates
- Maintenance medium (MEM medium with 1% Penicillin/Streptomycin (P/S) and 10% FBS)
- Osteogenic medium (MEM with 1% P/S, 10%FBS, 10mM β-glycerol phosphate, 10nM dexamethasone, 10nM calcitriol and 250nM ascorbic acid)

Flowchart

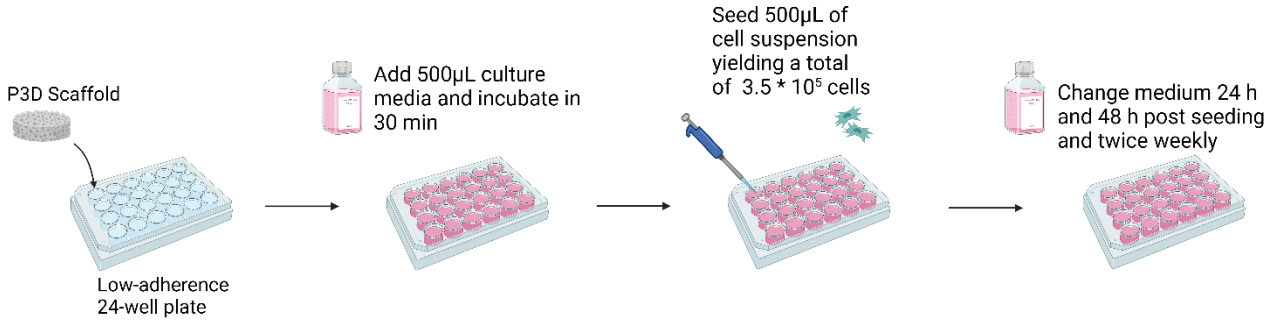

FIGURE 1 *OVERVIEW OF THE WORKFLOW FOR HANDLING P3D SCAFFOLDS TO CREATE A 3D TISSUE MODEL. PREPARE THE CELL SUSPENSION AND CALCULATE THE CELL DENSITY, THEN, BEFORE SEEDING THE CELLS ONTO THE 24-WELL PLATE, ADD ONE STERILE P3D SCAFFOLD TO EACH WELL.*

Notes before starting and general advice on material handling

- All handling of the P3D Scaffolds should be performed using gloves, according to the standard aseptic methods.
- The scaffolds are supplied sterile by dry heat sterilization and remain sterile until opened.

Procedure

Preparation of the scaffolds

1. Place one P3D scaffold in each well of a low-adherence 24-well plate.
2. **Optional:** Wash the P3D Scaffolds in 500 uL PBS x3 for one minute to remove any particles.

Note: The material and structure of the P3D Scaffold are not compromised during sterilization or disinfection, but loose particles may occur.

Preparing the cell suspension

The procedure uses cryopreserved hMSCs which needs to be thawed properly for preserving the cell culture health.

1. Follow your preferred method for cell thawing (1 vial of cells per culture flask).
2. Incubate the flasks at 37 °C and change culture media two to three times per week. This allows the cells to enter the growth phase.

Conduct the passaging of hMSCs to proliferate the cells until confluency of 90%.

1. Wash the flasks with 1xPBS. The solution should cover the surface. Discard the PBS.
2. Add trypsin/EDTA to the flask.
3. Tilt the flask gently to distribute trypsin/EDTA evenly. Incubate for five minutes.

Ensure that cells are no longer attached to the flask before proceeding to the next step.

1. Transfer the cell suspension into a centrifuge tube, and centrifuge at 500g/1200rpm for five minutes.
2. Discard the supernatant – do not disturb the pellet.
3. Resuspend the pellet in 1-5 mL media.
4. Count the cells and calculate number of cells/mL to determine cell density.
5. Make final cell suspension by adding the appropriate amount of media to the centrifuge flask. Seeding cell density in 24-well plates should be $3.5 * 10^5$ cells/Ø12 Scaffold.

Plating cells

1. If not done already, add one sterilized or disinfected P3D Scaffolds to each well of a 24-well plate.
2. Add 500µL culture media to each well and let the scaffold soak. The scaffold must be completely submerged in medium.
3. Incubate the plate in a humidified incubator for at least 30 minutes, at 37ºC and 5% CO_2.
4. Add 500uL of the final cell suspension generated above to each well by placing the cells on top of the scaffold. Be careful not to touch the scaffold (see Fig. 1).
5. Replace the medium and evaluate cell status after 24 and 48 hours with maintenance medium.
6. To differentiate the hMSCs to osteoblasts, replace the maintenance medium with osteogenic medium after 48 hours.
7. Change the medium twice weekly.

8. The organoids are now ready for analysis and further experiments.

   Optional: If needed, quantify osteogenesis by following the guidelines in this protocol.

   Optional: If needed, you may retrieve the seeded cells from the scaffold as described in this protocol.

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Patent status:

The product is protected by one or more US, European, and/or foreign patents.

Safety Data Sheet:

Download from our website here.

Technical Data Sheet:

Download from our website here.