675 US Highway One
North Brunswick, NJ 08902
Tel: 732-729-6270
Fax: 732-745-7270
http://www.3DBiotek.com

Cell Seeding on Beta Tri-Calcium Phosphate (β-TCP) Disks

Materials and Equipment

Cell line of choice
TCP disk of specified diameter
Cell culture media
Pipettes

Procedure

1. Calculate the appropriate cell suspension concentration for cell seeding. For adhesion experiments with Osteoblast, we suggest 2,000-cells/cm^2. For proliferation experiments, we suggest 1,000-cells/cm^2 and time points at 1, 3, 5, and 7 days in culture.

Table I: Surface Area for Cell Seeding and Seeding Volumes on β-TCP Disks Formats

β-TCP Disk Size	Growth Area	Seeding Volume	Volume after 3 Hours	Total Volume
96-well	0.196 cm^2	30 ul	170 ul	200 ul
48-well	0.708 cm^2	100 ul	150 ul	250 ul

2. Once cells have been trypsinized, resuspend cells for specified concentration and seeding volumes (e.g. 30-ul for 96-well β-TCP, 100-ul for 48-well β-TCP).

3. Slowly pipette the correct seeding volume cell suspension onto the center, top surface of each β-TCP disk. **Note: Do not allow the cell suspension to contact the sides of the wells. This will result in reduced seeding efficiency.**

4. Gently place plates into incubator. Avoid agitating the plates!

5. After 3 h, remove the plates containing the β-TCP disk from the incubator. Add the appropriate volume (see chart above) of fresh medium into each well in a sterile biosafety hood. Make sure that the scaffolds are completely immersed and sitting at the bottom of the wells. Return the plate to the incubator.

6. To change media regularly, aspirate carefully without touching the disks. Add fresh medium into each well from the side. Continue with normal cell culture until analysis.

For further support, please visit us at http://www.3DBiotek.com

This file can be downloaded via this link: www.3dbiotek.com/Documents/CellSeedingProtocol_TCPDisk.pdf