Cell Seeding on Hydroxyapatite (HA) Disc

Materials and Equipment

Cell line of choice
HA disc 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 1: Surface Area for Cell Seeding and Seeding Volume on various disc formats

HA Disc Size	Diameter (mm)	Growth Area (cm^2)	Seeding Volume (ul)	Volume after 3 hours (ul)	Total Volume (ul)
96-well	5.0	0.196	30	170	200
48-well	9.5	0.708	100	150	250

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

3. Slowly pipette the correct seeding volume cell suspension onto the center, top surface of each disc. 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 hours, remove the plate containing the disc from the incubator. Add the appropriate volume (see Table 1 above) of fresh medium into each well in a sterile biosafety hood. Make sure that the Discs 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 disc. 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 or send email to info@3dbiotek.com