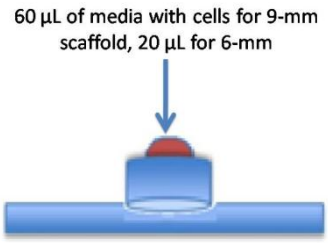
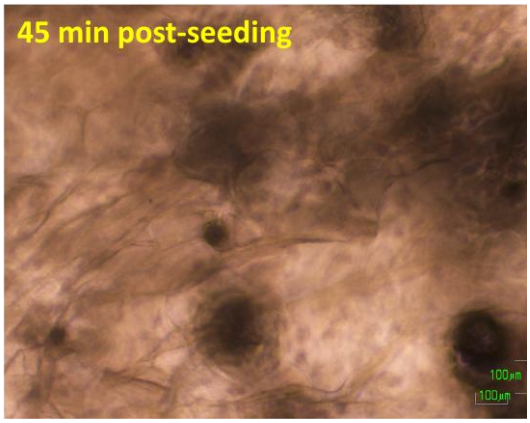
3D Cellusponge Cell Seeding Protocol_Multi-well Plate Format Sponge

1. If the diameter of your disk (scaffold) is 9-mm, obtaining cell suspension with the cell density of 0.3 million cells in 60 µL.(this is required number for Huh 7.5 cells and primary rat/human hepatocytes; for other cell types, the number can vary.) If the diameter of your disk is 6-mm, do it with cell density of 0.1 million in 20 µL.(In other words, the recommended cell density is $5*10^6$ per ml).

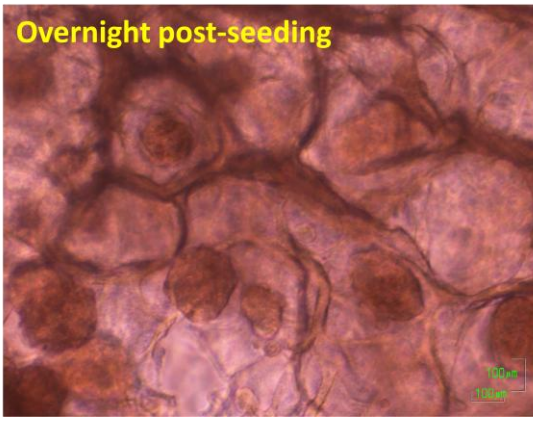


2. Adding 60 µL of the cell suspension obtained in point 1 on top of the sponge for 9-mm disk (20 µL for 6-mm disk).
3. Incubating 240 minutes for Huh 7.5 cells(cell lines applied) and 60 minutes for primary rat/human hepatocytes(primary cells applied), respectively. (By doing so, this will also remove the air bubble in the Cellusponge.)
4. <u>Phase contrast images of sponge upon cell seeding</u>:

   Huh7.5 cells in galactosylated cellulosic sponge(Cellusponge-Gal)



1) Cells already entered the sponge
2) Some bubbles still present, sponge will still not settle at the bottom of well plate
   (Still slowly get rid all air trapped in the pores)



1) Air bubbles trapped in the sponge pores have been removed
2) Compact spheroids already formed

5. After incubation, adding 500 µL of media to each well(for 24-well plate), 300µL for 48 well-plate and 100 µL for 96-well plate. Remember to slowly add the media along the edge of each well not directly onto the Cellusponge.
6. After overnight incubation, transferring the seeded sponge into new well plate, replenish the medium and continue the culture.