Seeding, Culture and Harvesting of Cells from 3D PETG Scaffold

Notes Before Starting

• Prior to use, scaffolds must be rendered hydrophilic with a 70 % ethanol wash for 15 minutes.

• Add enough ethanol to completely cover the scaffold, remove, and wash 2x with appropriate culture media (2ml)

• To avoid drying, leave the scaffold in the final medium (2ml) wash until required.

• We recommend leaving scaffolds in this final medium for 4h to enhance subsequent cell attachment.

Seeding Cells on Scaffold

• Remove all cell culture media from the well prior to seeding, via pipette.

• Slowly pipette your desired cell density via a 100µl cell media solution onto the top of the scaffold, taking care to ensure the solution permeates the interconnected pores as much as possible. We recommend using several cell densities initially to optimise your culture protocol (recommended range: 75,000 - 200,000 cells per scaffold).

• Place seeded scaffolds back into the incubator for 3h to allow cell attachment.

• Fill the remaining well with 1.4ml of complete medium.

• Change culture medium every 2 days until the end of culture. Do not culture beyond 21 days.

Harvesting Cells on Scaffold

- Cells can be recovered from the scaffold via a 1X trypsin wash for 10 minutes. Ensure that the entire scaffold is submerged in 1X trypsin solution, before adding a further 2ml of media per scaffold.

- Pipette up and down to disturb cells inside the scaffold, and to encourage optimal mixing of the trypsin and media solutions.

- Remove the remaining solution from the scaffold well and centrifuge at 1000 rpm for 5 minutes to form a cell pellet.