



Collagen I Coating of 3D PETG Scaffold

1. Prepare 3D PETG Scaffold by treating with 70% ethanol followed by two PBS washes as described in the previous protocol. Leave scaffolds in the second PBS wash until ready to apply the collagen solution.
2. Dilute rat tail collagen I to a concentration of 0.8 mg/mL using cell culture grade water. Handle the reagents on ice, using pre-chilled pipette tips to perform the dilution and subsequent application onto the 3D PETG Scaffolds.
3. Aspirate the second PBS wash from the respective Scaffold disc and carefully pipette 500 μ L of the diluted collagen solution onto each disc. Please note, it is important to place scaffolds in the correct orientation prior to this step (film base in contact with well floor). Replace plate lids and leave to stand for 1 hour at room temperature.
4. Aspirate to remove any residual coating agent from the bottom of the wells.
5. Prepare cells for seeding in the appropriate culture media and seed directly on the wet collagen coated 3D PETG Scaffolds. Place seeded scaffolds back into the incubator for 3h to allow cell attachment. Following 3h incubation, flood wells with 1.4ml complete media respectively.