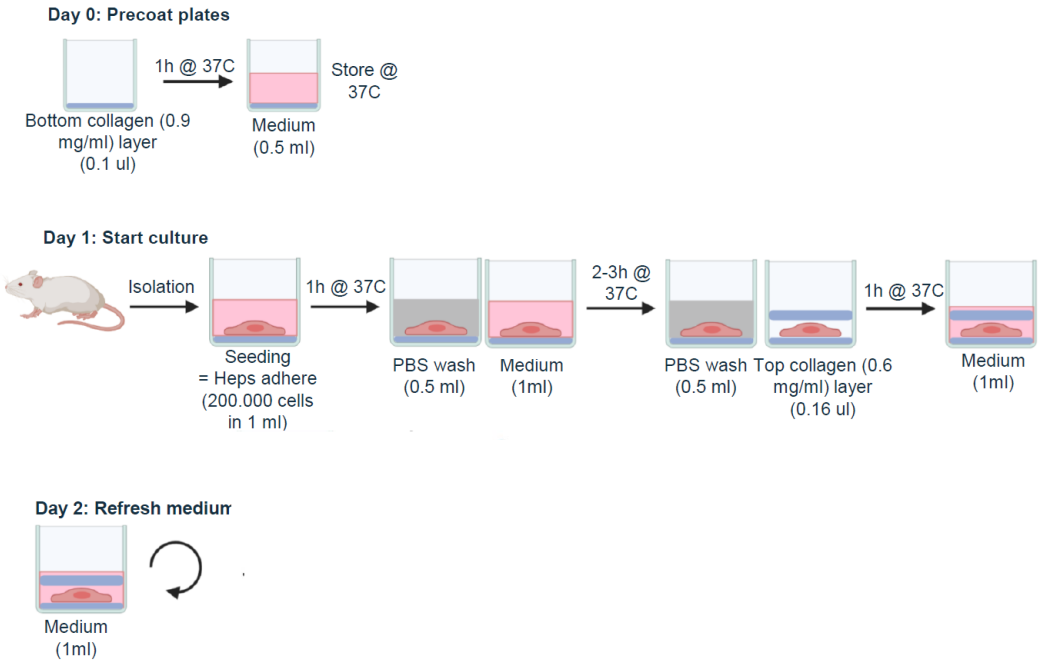
Plating of isolated primary hepatocytes in collagen sandwich culture – 24 well format

Reference: Zeigerer et al.; Nature 485, 465–470 (2012). https://doi.org/10.1038/nature11133

Overview



Reagents

Williams Medium E	Pan Biotech P04-29150
Spezial processed FBS (Sera Plus)	Pan Biotech P40-37500
Collagen	Roche 11179179001
Pen/Strep	Gibco 15140-122
Dexamethasone	Sigma D4902-500MG
BSA	Serva 11926 -500g
Acetic Acid	1.00063.2511 2.5L
10x DMEM (without phenol red)	

Method

After isolation, the cells should be kept on ice. They can be stored on ice for up to 20 hours, but the viability drops significantly. If the hepatocytes are to be shipped or stored, it is better to fill the falcon tube completely (50 ml) with resuspension buffer, to minimize air in the tube. For later use, spin the cells down again with 50x g for 5 min and resuspend the cells in 10 ml buffer again.

Determine cell number and vitality of the prep by using Trypan blue exclusion procedures:

Dilute 100 µl of the cell suspension (make sure to invert the tube gently before taking an aliquot to dissolve all cells; and use a cut-off tip) into 400 µl of resuspension buffer. Take 50 µl and mix this in 50 µl of Trypan blue solution. Apply to the Neubauer counting chamber and count all four squares.

To determine vitality, count dead cells and live cells separately. Calculate the concentration by using the following equation: mean number of live cells/square x 10 000 x dilution factor = number of cells/ml.

Usually the prep of one BL6 male mouse (8 to 12 weeks old) results in total of 30 to 100 mio cells. Cells with a vitality of <75% should not be used.

Dissolve the Roche rat tail collagen in 10 ml of sterile 0.2 % (v/v) acetic acid and incubate at 4°C for at least 6 hours (better overnight): concentration is now 1 mg/ml. Don't store the solution longer than 2 weeks!

For sandwich culture, the plates have to be coated before the isolation.

Prepare the bottom collagen layer. For one well of a 24-well plate you need to prepare 100 µl collagen solution (0.9 mg/ml).

Prepare a collagen solution with a concentration of 0.9 mg/ml in 10x DMEM. Adjust pH to 7.4 with 1 M NaOH using pH-stripes. Keep the solution always on ice in a 50 ml tube. Pipet 100 µl per well and evenly spread the collagen solution. Let the collagen polymerize at 37°C for 1 hour in the incubator. After that add 0.5 ml medium (William's E Medium, with 50 ml FCS, 5 ml PenStrep, 31.25 µl Dexamethasone stock solution at 1.6 mM – final 100 nM) to equilibrate the collagen. Store in the incubator until plating the cells.

For plating use app. 80000 cells / cm^2 i.e. 200 000 cells in 1 ml medium per well of a 24 well plate. Calculate how many cells you need and dilute them in pre-warmed medium. Use a cut-off culture tip to take the appropriate volume from the cells – make sure to resuspend them again before plating by gently inverting the 50 ml falcon tube (keep the isolated hepatocytes on ice the whole time). Mix the cells with the medium also gently by inverting the tube.

Remove the medium rom the prepared plates. Distribute 1 ml cell solution per well - regularly invert the tube. This way you ensure the same number of cells in each well. The primary cells settle down fast!

Incubate the cells for 1 hour for attachment (37°C, 5% CO_2, humidified atmosphere).

Remove the medium and wash the cells with PBS once to remove dead and unattached cells. Aspirate PBS completely and add 1 ml medium. Incubate the cells for another 2 hours.

Prepare the top collagen solution with a concentration of 0.6 mg/ml in 10x DMEM. Dilute the collagen solution in the falcon tube 2:3 again in 0.2% acetic acid in order to achieve a lower concentration in the top layer compared to the bottom. Adjust the pH again. Keep the solution always on ice.

Wash the cells 1x with PBS. Aspirate PBS and add 160 µl collagen solution per well. Put collagen-coated cells into the incubator (37°C, 5% CO_2, humidified atmosphere) for 1 hour to form the sandwich. Afterwards add 1 ml medium and keep in the incubator.

Check the morphology of the cells in the next morning. Replace with fresh medium. Bile canaliculi are fully formed from day 3 of the culture. Cells are healthy up to 6-7 days. Do not use wells were > 50% of the cells are depolarized.