Preparation of Alginate-encapsulated Spheroids and Organoids

Protocol

Some cell lines have poor or non-natural aggregation, making the initial formation of spheroids and organoids a challenge. To overcome this spheroids and organoids can be forced to aggregate by a scaffold material. Alginate crosslinked with Ca^{2+} has been popularized for in vitro cell culture and tissue engineering applications due to their biocompatibility, low toxicity, relatively low cost, and mild gelation properties. They have a relatively inert aqueous environment, high gel porosity which can increase diffusion rate and are biodegradable. This protocol describes how to encapsulate cells in alginate to initiate spheroid or organoid formation.

Reagents and Materials

- PVC blocks 5x5x0.5 cm clean, sterile, and single wrapped
- Parafilm® M foil or similar 8x8 cm
- 70 % Ethanol Solution
- Pure sterile water
- Petri dish or similar for bath containers and drying space
- 2.5 % sodium alginate in PBS, sterilized (e.g. In autoclave)
- Cross-linker solution (50 mM $CaCl_2$ and 150 mM NaCl) filtered through 0.2 µm syringe filter
- Automatic pipette (recommended)
- Cell culture media with supplements
- Wide bore pipet tips
- Petri dish (10 cm)

Additional information

During embedding sheets preparation, the extensive washing steps are necessary for two reasons: 1) Ensure a clean surface; 2) Ensure that the ethanol solution is completely removed to avoid biased results due to the effect of ethanol on the cells.
For preparation of the ClinoReactor® see **003_Protocol_Preparation_of_ClinoReactor**. Sub-cultivation of cells in 2D is described here **002_Protocol_Sub_Cultivating_Cells_in_2D**.

Protocol

Preparation of embedding sheets

1. PVC blocks are washed with detergent, rinsed with deionised water, and sterilized. The blocks can be prepared in advance and stored in sterile bags.
2. Wash the Parafilm® M sheets in 1) pure water; 2) 70 % Ethanol solution; 3) pure water; 4) pure water. Allow the 70 % Ethanol solution to drip off before proceeding.
3. After the final wash in pure water allow the sheets to dry on a sterile surface.
4. Prepare the desired number of embedding sheets. Note: each sheet can contain approximately 100 µL droplets.
5. Unpack a sterile PVC block under sterile conditions.
6. Carefully wrap dry Parafilm® M around it so that the Parafilm® M foil creates a flat even surface on the PVC block. Avoid stretching the Parafilm®M on the surface that will contain the droplets.
7. Place the casting blocks covered with Parafilm® M into a 10 mL Petri dish. Press it firmly so the block sticks to the Petri dish bottom.

8. Fill the Petri dish with sterile water to reach half of the height of the casting block. Note: This creates a moist environment to slow the polymerisation process of the gel.

Encapsulation of cells in sodium alginate

1. Cultivate your cells in 2D to 80% confluency.
2. Prepare the 2.5 % sodium alginate in PBS and cross-linker solution.
3. Prepare a single cell suspension and evaluate the cell count.
4. Pellet the cells by centrifugation of the cell suspension 140xg for 5 min at 37°C and discard the supernatant.
5. Step 6 and 7 should be carried out within 5-6 min as the gel starts to polymerize, therefore make sure everything is prepared before proceeding.
6. Resuspend the cells in 37°C sodium alginate suspension to a concentration of 2000 cells/µL.
7. Quickly place 1 µL droplets of the cell/alginate suspension onto the previously prepared embedding sheets on the PVC blocks. Note: The alginate will polymerize so speed is of the essence, but do not compromise the uniformity of the droplets. We recommend to dispense the droplets in a 10x10 droplet matrix for convenience.
8. Add 0.5 µL cross-linker solution to each of the droplets, cover them with the Petri dish lid and let it polymerize for 3 min at room temperature. Repeat if the droplets are not solidified.
9. Wash the alginate spheroids off the parafilm with prewarmed cell culture media into a petri dish.
10. Use a wide bore tip to transfer the alginate spheroids to an equilibrated ClinoReactor® 5 % CO_2, 37°C. The rotation speed is adjusted correctly when the spheroids are in stationary orbit (approximately 16 RPM).

References

1. Smit T, et al. Characterization of an Alginate Encapsulated LS180 Spheroid Model for Anti-colorectal Cancer Compound Screening. ACS Medicinal Chemistry Letters (2020). doi:10.1021/acsmedchemlett.0c00076

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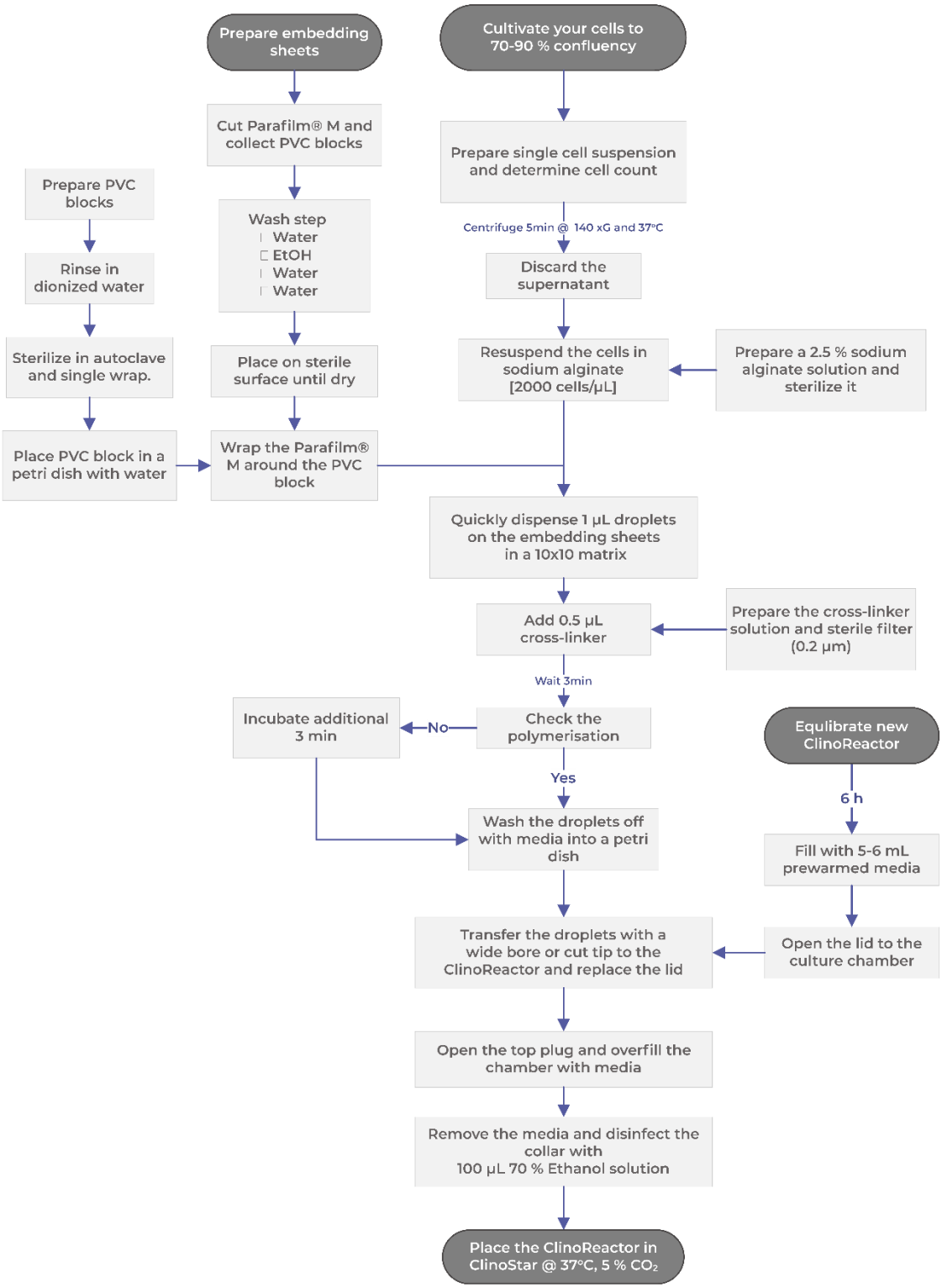
Prepare embedding sheets
Cut Parafilm® M and collect PVC blocks
Wash step
Water
EtOH
Water
Water
Place on sterile surface until dry
Wrap the Parafilm® M around the PVC block
Prepare PVC blocks
Rinse in dionized water
Sterilize in autoclave and single wrap.
Place PVC block in a petri dish with water
Cultivate your cells to 70-90 % confluency
Prepare single cell suspension and determine cell count
Centrifuge 5min @ 140 xG and 37°C
Discard the supernatant
Resuspend the cells in sodium alginate [2000 cells/µL]
Prepare a 2.5 % sodium alginate solution and sterilize it
Quickly dispense 1 µL droplets on the embedding sheets in a 10x10 matrix
Add 0.5 µL cross-linker
Prepare the cross-linker solution and sterile filter (0.2 µm)
Wait 3min
Check the polymerisation
No
Incubate additional 3 min
Yes
Wash the droplets off with media into a petri dish
Equlibrate new ClinoReactor
6 h
Fill with 5-6 mL prewarmed media
Open the lid to the culture chamber
Transfer the droplets with a wide bore or cut tip to the ClinoReactor and replace the lid
Open the top plug and overfill the chamber with media
Remove the media and disinfect the collar with 100 µL 70 % Ethanol solution
Place the ClinoReactor in ClinoStar @ 37°C, 5 % CO2