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Ethanol Dehydration for SEM Sample Preparation Protocol

Assay Description

The purpose of this assay is to prepare cell-seeded hydrogels or scaffolds for SEM imaging. Samples will be fixed and exchanged from a 100% aqueous solution to 100% ethanol solution through a series of graded ethanol washes. Each wash is conducted 3 times with a 5-minute soak before moving to the next exchange.

Materials Needed

- Cell-seeded hydrogels or scaffolds
- Fixative – 3.7% formaldehyde or Karnovsky's fixative
- Graded ethanol solutions (EtOH at 30%, 50%, 70%, 85%, 95% and 100%)
- 4% paraformaldehyde (ThermoFisher Cat # 047392-9M)
- Hexamethyldisilzane (Sigma-Aldrich, Cat # 379212)
- Ethanol
- 1× PBS
- DI water
- Transfer Pipette
- Serological pipettes
- Tweezer
- Spatula
- Cell culture plates
- Centrifuge tubes
- Orbital shaker

Procedure

1. Remove cell culture medium and rinse the hydrogel with 1X PBS twice to wash off the residual culture medium.

2. Fixation: add 4% formaldehyde solution to the hydrogel wells and incubate at room temperature for 1 hour.

3. Use tweezers or a spatula to transfer fixed scaffolds to a new dish or well-plate. *Soft hydrogels can remain in their wells.

4. Conduct ethanol serial dehydration referring to the wash/time table:

Solution	Time
DI water	5 min/soak, x2
30% EtOH	5 min/soak, x2
50% EtOH	5 min/soak, x3
70% EtOH	5 min/soak, x3
85% EtOH	5 min/soak, x3
95% EtOH	5 min/soak, x3
100% EtOH	5 min/soak, x3

5. After washing with 100% ethanol, move the samples to a ventilated fume hood.

6. Prepare the drying solution containing 1:1 hexamethyldisilzane (HMDS) and 100 v/v% ethanol and wash scaffolds with the solution twice.

7. Wash scaffolds with 100 v/v% HMDS once and store scaffolds in 100 v/v% HMDS in the well plate. Air dry samples for 6-12 hrs. in the ventilated fume hood.

8. Proceed to sputter coating and SEM imaging.

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Sample images

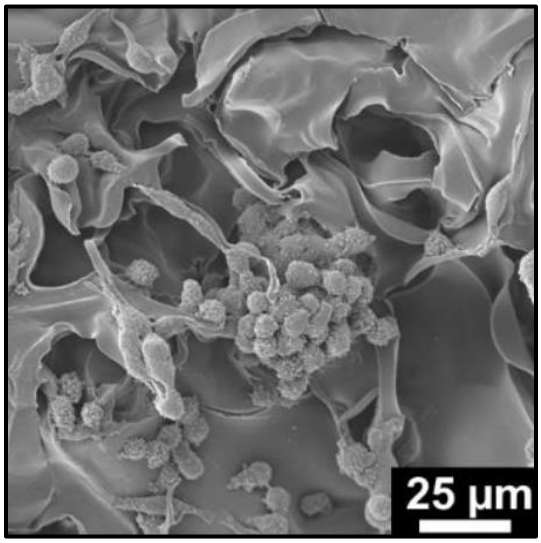

Fig.1. MDA-MB-231 breast cancer cells cultured in chitosan-based scaffold.

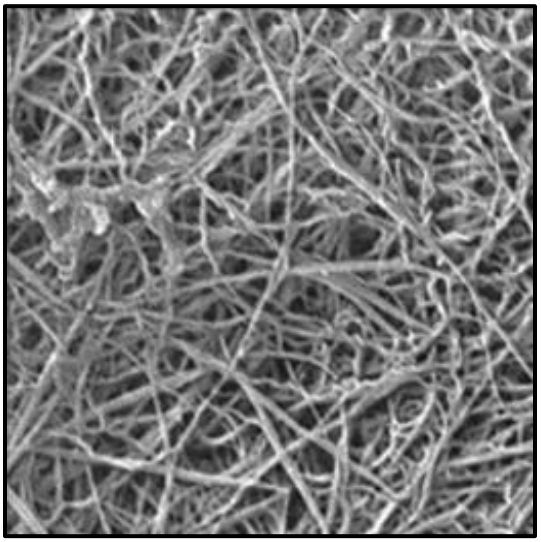

Fig.2. Collagen fibers in PureCol@ hydrogel.

