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Immunocytochemistry of cells in nanofiber scaffolds*

1. Wash cells or spheroids cultured in the nanofiber scaffolds using preheated PBS (with 1 mM $CaCl_2$ and 1 mM $MgCl_2$).

2. Fix cells using a 4 % paraformaldehyde in PBS (pH 7.4) for 10 min at room temperature. 0.1 % glutaraldehyde may be added to enhance cell fixation.
   Note: Avoid using organic solvents that may deteriorate the nanofiber scaffold, e.g. methanol or acetone.

3. Rinse cultures 3 x 5 min with PBS.

4. Cells may be permeabilized and non-specific binding blocked by incubating cultures for 1 h at room temperature using 0.3 % (v/v) Triton X-100 and 5 % (w/v) BSA (or other suitable blocking agent) in 100 mM PBS.
   Note: Increase blocking agent concentration if trouble with the nanofibers adsobing the antibodies occur.

5. Rinse cultures 3 x with PBS.

6. ICC can now be performed using standard protocols.
   Note: For high-magnification microscopy (>10X) see the protocol "Mounting nanofiber scaffolds for microscopy" for suggestions on how to mount your samples.

*Suggested procedure, please adjust according to your experimental needs.

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