Protocol for Attachment of Cells in Suspension to Glass Coverslips.

Marek Malecki.
University of Wisconsin, Integrated Microscopy Resource

A) Pretreatment of Glass Coverslips.

1) Dry clean glass coverslips in an oven 200 to 500° C for 24 hours.

2) Immerse the glass coverslips into vials (Nalgene, Naperville, IL) filled with 2% 3-aminopropyltriethoxysilane (APTES; Aldrich, Milwaukee, WI)[1] in acetone. Decant excess of liquid and leave the vials in 45° C for 24 hours.

3) Rinse off with plenty of water.

The further steps will differ for fixed or living cells. If you plan to attach cells, which were fixed in suspension by adding glutaraldehyde (GA) to the final concentration 0.25 – 1% in cell culture medium or buffer, then follow the steps in B) below[2]. If you plan to attach living cells, which were spun down and resuspended in phosphate buffered saline (PBS: 138 mM NaCl, 27 mM KCl, 10 mM phosphate buffer pH 7.4) or HEPES buffered saline (HBS: 138 mM NaCl, 27 mM KCl, 10 mM HEPES buffer pH 7.4) while alive, follow the steps in C) below[3]. In both cases the essential requirement for the successful attachment is that the unreacted reagents (APTES and GA) are completely rinsed off.

B) Attachment of Glutaraldehyde Fixed Cells.

1) Spin down the cells, which were fixed in suspension with glutaraldehyde, at 800 *g* for 10 minutes at room temperature.

2) Resuspend them in water or low salt buffer.

3) Place the APTES treated glass coverslips on the bottom of a flat-bottom centrifuge tube and spin the cells down onto the APTES treated glass coverslips.

4) Remove the coverslips with the attached cells from the tube and immerse them in 1% sodium borohydride (Sigma, St. Louis, MO) in Pb on ice for 10 minutes, This step is also important for restoration of antigenicity and quenching fluorescence.

5) Further processing of the cells attached to the APTES/GA treated glass coverslips can be pursued according to standard protocols established for processing of adherent cells for fluorescence microscopy, SEM, AFM, or TEM[4].

C) Attachment of Living Cells.

1) Place the glass coverslips treated as in 1) to 4) into 1% glutaraldehyde in water for 1 hour at room temperature (APTES/GA treated glass).

2) Rinse with plenty of water.

3) Place the APTES/GA treated glass coverslips on the bottom of a flat-bottom centrifuge tube, add the living cells in PBS or HBS, and spin the cells down onto the APES/GA treated glass coverssiips at 800 *g* for 10 minutes.

4) Remove the glass cover-slips with attached living cells and continue their processing according to protocols suitable for adherent cells.

Footnotes

1) Old APTES will not work. After receiving the fresh bottle, make small aliquots in small vials filled to the top (again, we prefer small cell freezing vials from Nalgene). The higher concentrations of APTES will result in fluffy coats on the glass chips. The smooth cells may adhere better, but the background in the pictures will not be as clean as with the 2% APTES protocol described above (Weettall 1969, Robinson *et al.* 1971, Malecki & Ris 1992).

Incorporation of glutaraldehyde may take place during chemical fixation or freeze-substitution of high pressure or jet frozen cells (Malecki 1991).

3) Attachment of unfixed cells results in distortion of their morphology, therefore this version is more suitable for studies on isolated cell organelles (*e.g.*, Nermut & Eason 1989) or viruses (*e.g.*, Buechi & Bachi 1979).

4) Other groups reported applications of this or similar protocols involving attachment of isolated cell organelles, proteins, nucleic acids for various types of microscopy (*e.g.*, attachment of chromosomes, proteins, or DNA for AFM or SEM as reviewed by Lyubchenko *et al.* 1996). Good luck! ■

References

Buechi & Bachi 1979 J Cell Biol. 33: 338.
Lyubchenko *et al.* 1996 SM Sup.10: 97.
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Weettall 1969 Science 166: 616.

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