Preparation of elastic substrata for traction force microscopy under TIRF illumination

PROTOCOL FOR:
Molecular dynamics and forces of a motile cell simultaneously visualized by TIRF and force microscopies

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LEGEND

 '>' ATTENTION

 '*' HINT

 ' ' REST

REAGENTS

A pair of liquid silicones (Dow Corning Toray, Tokyo, Japan, CY52-276A and B)
Silane (Sigma-Aldrich Japan, Tokyo, Japan, 3-aminopropyl triethoxysilan)
Fluorescent red microspheres (Molecular Probes-Invitrogen Japan, Tokyo, Japan, F-8786)

PROCEDURE

COVER SLIP CLEANING
1. Immerse new 22 mm × 22 mm cover slips (Matsunami, Osaka, Japan, No. 1) in 61% nitric acid for 24 h
2. Wash with distilled water
3. Immerse in 2 M KOH for 24 h
4. Wash with distilled water
5. Wipe

 '*' The surface of cover slip becomes hydrophilic.
Without cover slip cleaning, many small air bubbles are sometimes produced at the boundary between cover slip and silicone.

SOLIDIFY LIQUID SILICONES
6. Mix a 300 mg of CY52-276A and a 250 mg of B
7. Spread the mixture on cover slips using a glass rod
8. Keep horizontally at room temperature (23°C) for 2 days
   * The mixing ratio of A and B can be changed to obtain optimal elasticity of substrata. Increasing the ratio of B, the elasticity become higher. During the long period (2 days) of fixation, the surface becomes flat and all of air bubbles diminish.

   CY52-276A and B should be mixed completely using a spatula. The thickness of the silicone layer should be 10–20 μm. If the thickness is larger, evanescent field should be created out of the field of view under microscopy.

BIND MICROSPHERES
9. Keep the cover slip with solidified substrata in a hermetically sealed case with 10–100 μl aliquot of a silane at 70°C for 1 h (Figure 1)
10. Assemble a round chamber (16 mm in diameter and 2 mm in depth) using the cover slip with the silicone substratum at the bottom of the chamber (Figure 2)
11. Add a 400 μl of microsphere solution to the solidified silicone
12. Keep horizontally for 1 min
13. Remove the solution
14. Add 400 μl of distilled water and remove it to eliminate unattached microspheres
15. Add 400 μl of distilled water to prevent drying of the surface

   Without procedure 9, microspheres cannot attach to the substrata. Probably, silane and the silicone surface bind each other by hydrophobic bonding, then silane and the microspheres bind each other through the binding between the amino group of the silane and the carboxyl group of the microspheres.

   Made-up chambers can be kept in dark-cold (4°C) condition for several days.
TABLES/FIGURES

Figure 1. Vapor Deposition of Silane

Figure 2. Assembly of Chenber

RECIPIES

Microsphere solution
Original solution containing microspheres is diluted 40,000× with distilled water.

Colophony wax
1:1 mixture of rosin (Wako Chemicals, Osaka, Japan) and beeswax (Wako Chemicals, Osaka, Japan).

TROUBLESHOOTING

NO FIXATION OF SILICONES
Cover slip is not clean. Follow the “COVER SLIP CLEANING” procedure carefully.

NONUNIFORM DISTRIBUTION OF MICROSPHERES
The microspheres cannot be recycled. Use new microspheres.

NO EVANESCENT FIEILD
Silicone layer is too thick. Evanescent field may exist far from the center of field of view under microscopy. Spread the silicone mixture more thinly.

NO MICROSPHERE MOVEMENT
Substratum is too hard. Reduce the ratio of CY52-276B in the mixture.

EQUIPMENT

Incubator (Panasonic, Osaka, Japan, FD-S35A1)