**APTES-DSC Coverslips**

**Materials**

Glass coverslips (15mm -- 24 well plate or 18mm -- 12 well plate, Fisher)

(3-aminopropyl)triethoxysilane (APTES, Sigma)

Toluene

95% Ethanol

Water (nanopure or equivalent)

N,N-disuccinimidyl carbonate (DSC, Sigma)

Diisopropylethylamine (DIEA, Sigma)

Acetone

MA-PEG24 (VWR)

Devcon 5 Minute Epoxy (Fisher or RS Hughes)

**Day 1 (20min, longer for more samples)**

1. UV-ozone or plasma treat glass coverslips for 10 minutes. Stand coverslips up in a well plate so both sides are exposed to air. i.e., two 18mm coverslips can be stood to make a V in a well of a 12 well plate. If you use 15mm coverslips, use a 24 well plate. If you use 5mm coverslips, use custom 3D printed holder.
	1. This is important because you will not be able to keep track of directionality! Do not use any coverslips that have fallen over.
2. Add 75ul of APTES to 6 places in the well plate, but in places that your coverslips are not. Do not put silane directly onto coverslips, this is a vapor deposition reaction.
	1. 12 well plate: I use the 6 spaces in between wells (spaced throughout the plate)
	2. 24 well plate: I keep 6 wells empty evenly spaced throughout the plate



1. Put lid on plate, seal around edges with autoclave tape to contain silane, being careful not to knock over any coverslips. Wrap plate in aluminum foil to contain silane again, label with autoclave tape.
2. Put in oven at 85-90C overnight (12-18h minimum).
	1. Too hot: At 90C or above, the well plate will warp and coverslips will get stuck and break when you try to take them out! If this is an issue, decrease temperature slightly. Also be aware of where the heating element is in the oven – ours is on the bottom, so I put my plates with coverslips on the top rack to decrease heating issue.

**Day 2 (3.5-4 hours)**

1. Take coverslips out of oven and rinse each sequentially in toluene, 95% ethanol, water with tweezers by dipping.
	1. I make 50ml tubes of each solvent and use tweezers to dip one coverslip in toluene, ethanol, and then water, then place to dry on foil.
		1. Drying: take a piece of foil and fold ridges into it so coverslips can be stood up to dry more quickly and evenly
2. After all coverslips have been rinsed this way, place foil containing coverslips back into the oven at 85-90C to dry (1h minimum)
3. Place coverslips into a solution of 10 g/L DSC, 5 v/v% DIEA in acetone and shake at room temperature for 2h. 30ml is sufficient for 24 18mm coverslips in a clean, dry beaker.
	1. I tape the beaker onto shaker at ~115rpm, just fast enough that coverslips move around in beaker. Larger beakers allow for better movement of coverslips.
	2. Alternative: use a stir bar and stir plate to move solution and coverslips, but be careful that it’s not so fast that it breaks them.
4. Rinse coverslips individually 3x in acetone and dry on folded foil tray in air. When dry, ~10 min, fold up foil and store in dessicator for 1 day, or use immediately.

**Day 3 (or continuation of day 2; 6+ hours)**

1. Make protein solution in sterile PBS so that desired surface concentration of proteins is in 95uL (18mm) or 70uL (15mm). This can be done on the bench if UV-sterilization is available, or in the tissue culture hood. Sterile PBS is key to keeping these clean!

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| Protein | Concentration | Volume needed of protein per 1 slide |
| Collagen | 3 ug/ul | 2.54 ug/ 3 ug/ul = 0.846 ul |
| Laminin | 1.19 ug/ul | 2.54 ug/ 1.19 ug/ul = 2.13 ul |
| Fibronectin | 1 ug/ul | 2.54 ug/ 1 ug/ul = 2.54 ul |

1. Use a container large enough to hold all coverslips and line the bottom with paper towels. Add some water to soak the paper towels and keep coverslips hydrated.
2. Add parafilm into the container, remove the paper, and add 95ul (or 70ul) droplets of protein solution spaced out on the parafilm. They should stay as droplets because of hydrophobicity, but do not space them too close because then your coverslips might touch.
3. Carefully use tweezers to place a coverslip on each protein solution droplet. Use the tweezers to carefully move the coverslip if the whole surface is not wetted or there is an air bubble.
4. Incubate for 3h! **Now the coverslips have directionality! Protein side is down.**
5. Move coverslips into a well plate, flipping the orientation (protein side up), and rinse wells 4x in sterile PBS. Again, better in the tissue culture hood but ok on the bench with sterile PBS.
	1. 18mm coverslips can be rinsed in a 12 well plate and removed
	2. 15mm coverslips cannot be removed once placed into 24 well plate, so rinse in 12 well plate.
6. Replace the parafilm and add new droplets of the blocking solution (10ug/cm2 PEG24 in PBS).
7. Incubate for 2h!
8. Move the coverslips back into the well plate, rinse 4x in sterile PBS.
9. Epoxy the coverslips to the well plate if you will be doing time lapse microscopy.
	1. Mix a little bit of each of the 2 components of the epoxy on some foil with a pipet tip
	2. 18mm: use tweezers to lift coverslip, add a small dab of epoxy to bottom, and place coverslip back in well. Push down with tweezers to stick it to the bottom. It should cure in 5 min, even in PBS.
	3. 15mm: add epoxy BEFORE putting coverslip into 24 well plate. Wait 5min before rinsing because this will agitate epoxy all over the coverslip.
		1. Application of epoxy can either be done with a pipet tip or 1ul using a positive displacement pipet. Do not add too much because it is difficult to image through.
10. Either use immediately, or seal plate with parafilm and store at 4C for 1 day. UV-sterilization for 30-60min minimum is preferred, but not necessary, for short-term experiments.
	1. If contamination problems, do all in the hood, UV sterilize, and make sure PBS is filtered/autoclaved.