Protocol for Microcontact Printing Fibronectin - Sam Polio – Prototype

Once you have your wafer with the desired pattern (protocol elsewhere), the following procedure will be used to microcontact print protein, such as fibronectin or other, onto a glass coverslip with a polydimethylsiloxane (PDMS) stamp. This will be sufficient for producing micron sized and larger features. It is recommended that when performing this procedure that fluorescent protein or the protein be fluorescently labeled after the patterning be used to verify the pattern fidelity.

PDMS Preparation

* PDMS is thoroughly mixed at a 1:10 ratio (weight elastomer: weight base) for 2 minutes
* The PDMS is poured over the wafer which is contained within a petri dish
  + The desired amount should not create a stamp that is either extremely thick >2 cm nor thin (<5mm)
* The wafer is baked for 2hrs at 80oC under vacuum in a dessicator chamber to degas
* After baking, the wafer is allowed to cool
* The stamps are removed using a scalpel or suitable razor blade while ensuring one’s safety
* Place stamps with the pattern side facing up into a petri dish to avoid destroying the pattern. Cover it for cleanliness as well.

Microcontact Printing

* The stamps should be sonicated (or alternatively rinsed) in ethanol, then water to remove any impurities if the stamps are stable enough to withstand it or if stamps are being reused. Do not cross-contaminate stamps with different proteins.
* The stamps will be placed in the UV/Ozone treater for 5 minutes.
* 100 uL of protein solution in 1x PBS will be added per cm2 of stamp.
  + For FN, this will be at a concentration of 50ug/mL of solution
  + For larger areas, this amount should be sufficient enough to coat the stamp with a layer of fluid.
  + If the plasma treatment was done correctly, the solution should spread rapidly and should not need much aid in coating the surface of the stamp.
* The protein will incubate on the stamp for 1hr at room temperature.
  + The stamp should be placed in a petri dish or other container that will prevent the evaporation of the protein solution and protect against the deposition of dust as well as air currents.
* During this time, shortly (<15 mins) before the incubation is finished, a glass coverslip will be UV/Ozone treated for 5 minutes and placed into a covered petri dish.
  + The coverslip should be sonicated with ethanol, then water to help remove any debris. Alternatively, more harsh treatments such as 10M NaOH can be used without sonication.
* Stamp will then be dried using a stream of N­2
* The stamp will then be turned so that the pattern side with the protein is placed in contact with the glass coverslip. Gently, but firmly push on the stamp to ensure contact.
* Leave the stamp for 2 minutes before removal.
* After the stamp is removed, it can be rinsed with ethanol and water before storage in a petri dish for reuse.
* The glass coverslips will then be placed into a 6-well plate and incubated with 1% F108 pleuronics for 30 minutes.
* During this time, the 6 well plate will be placed under a UV light to help sterilize.
* After 30 minutes, the pleuronics will be rinsed from the coverslip with PBS and the experiment may proceed.