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Microparticle Kit Standard Operating Procedures

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Kit Contains

PDMS Template (6, 10, 20, or 50 µm sized columns on PDMS mold) x 1
Biodegradable polymer (5 grams, your choice from non-block PolyVivo line)
Hydrogel Mixture (2 x 30 grams in packet)
Set of 10 Razor blades

You will need

100 ml bottle
Magnetic stir bar
Glass plate (or other flat surface)
Laboratory Oven set to ~60° C
Laboratory Refrigerator set to ~4° C
Centrifuge with capacity to hold 50 ml centrifuge tubes
Pipettes (~5 and 20 µL capacity)
Distilled water (or equivalent)
Dichloromethane (DCM)
Vacuum/Freeze dryer
Centrifuge tubes

Suggested Equipment

Microscope

Steps (See Figure 1. for schematic overview, steps lettered in red)

1. Combine the 30 gram packet of hydrogel mixture with enough water to make 100 ml in a sealed container to prevent evaporation. Stir at 60° C to dissolve. Maintain at 60° C to keep liquid.
2. Dissolve 1 to 2 grams of biodegradable polymer in 10 ml of dichloromethane along with drug substance to be loaded of your choice to generate a 10 to 20% w/v polymer solution respectively.
3. Onto flat glass plate pipette ~7 ml of hydrogel solution(**A**) (note solution is viscous, works best with pipette that has trimmed tip) place PDMS template with patterned side down (*Note – To tell which side of template is up look at oblique angle in light, patterned side will not reflect light as well as smooth side and will therefore appear dull. The patterned side is the one you want to use for hydrogel imprinting. Avoid pressing against patterned side as it can be damaged.)* on top of hydrogel solution(**B**)
4. Place plate with hydrogel and PDMS template in refrigerator for 5 minutes to cool and set the hydrogel
5. Afterwards remove from refrigerator and starting with one side gently peel the PDMS template off of the hydrogel scaffold.
6. Using razor blade cut away excess (non-patterned) portion of hydrogel. Cut the hydrogel up into strips roughly the width of the razor blade.(**C**)

7. Pipette 20 µL of DCM-polymer-drug solution along one edge of the cut portion(**D**).
8. Using the sharp side of the razor blade gently swipe the DCM solution along the length of the hydrogel template with gentle (**E**).
9. let set at room temperature for 5 minutes to evaporate off DCM
10. *(suggested)* view the microparticles under a microscope, preferably load a fluorescent dye in with the early batches (or dye conjugated AKPLGA-FL series) to confirm microparticle formation.
11. Cut hydrogel to easy to manage pieces (**F**) Place scaffold piece in 50 ml centrifuge tube, fill to top with hot (~60° C) distilled water to dissolve scaffold (**G**)
12. While tube is still hot, centrifuge to force microparticles to settle to bottom. Remove supernant solution. (Note centrifuge conditions will vary by centrifuge type and microparticle size, if no settling is initially observed centrifuge for longer time period or at higher speed)
13. Wash particles 2 more times with addition of distilled water and centrifuging.
14. Vacuum or freeze dry the microparticles.

Note: This kit is not intended for use to generate clinical products or for use on humans either directly or indirectly. This kit is intended for research purposes only.

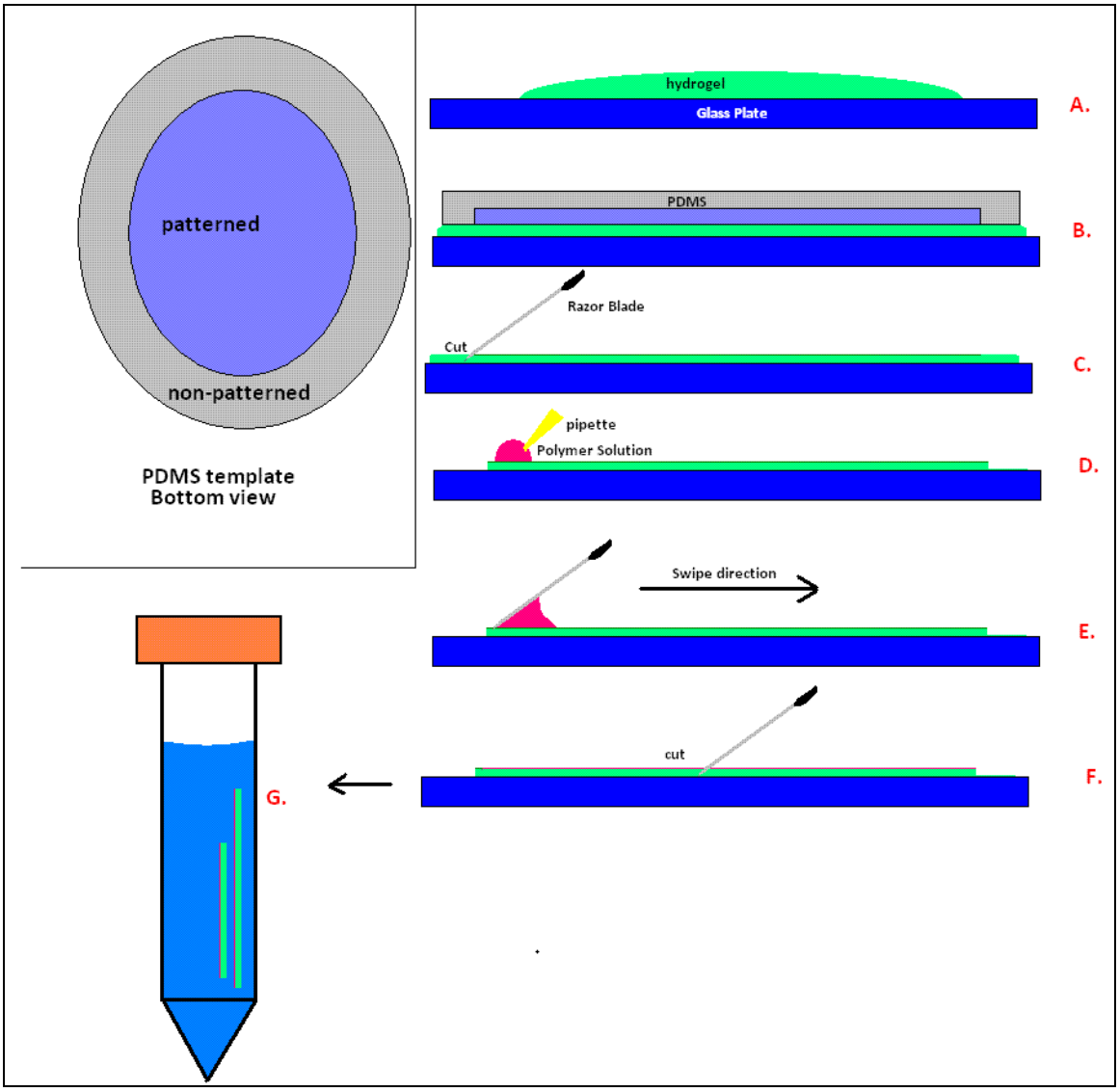

Figure 1. Directions schematic overview.

How It Works (see Figure 2 for schematic)

Briefly, the PDMS provides a positive mold of columns (1) onto which a hydrogel solution is poured (2) and removed to form the negative mold with wells (3). This mold is filled with organic solvent containing polymer and drug substance (4) and dissolved in water (5). Afterwards the mold is washed away and the particles collected (6). Note this requires that whatever organic solvent is used be very hydrophobic (i.e. acetone, DMSO, etc. do not work as well) or else a film may form connecting the wells.

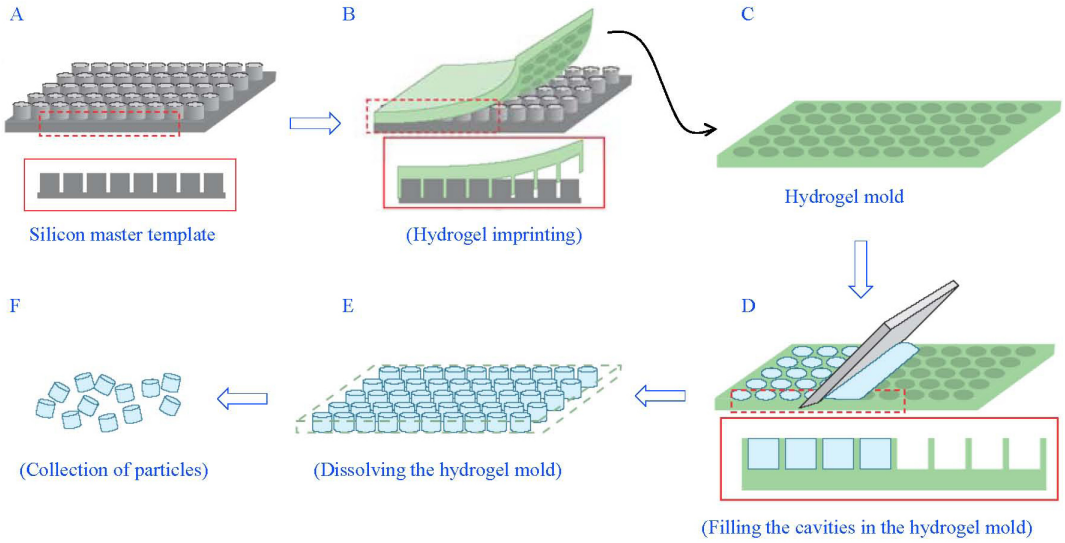

Figure 2. Mold method of microparticle fabrication explanation.