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Microparticle Kit Standard Operating Procedures Last Revised 12/15/15

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

You will need
1000 mL bottle
Magnetic stir
bar
Hot plate
Shaking incubator set to 37°C
Distilled water (or equivalent)
Ethanol
Glass plate (or other flat surface)
Oven set to 60°C
Centrifuge with capacity to hold 50 ml centrifuge tubes
Pipettes (~5 mL and 20 µL capacity)
Dichloromethane (DCM)
Vacuum/Freeze dryer
Centrifuge tubes

Suggested Equipment
Microscope

Steps (See Figure 1. for schematic overview)

a. Combine the 20 gram packet of hydrogel mixture with 300 mL of distilled water and 200 mL of Ethanol in the 1000 mL bottle. Stir and heat until boiling to dissolve. Remove from heat and add 250 mL of Ethanol. Stir to dissolve. Cool to room temperature.
b. 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.
c. Place PDMS template with patterned side up on a flat glass plate. (*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.)*
d. With a 5 mL pipette, transfer 4.5 mL of the hydrogel solution onto the patterned area of the PDMS template. With the pipette tip, carefully spread the hydrogel solution over the entire patterned area of the PDMS. Remove any air bubbles that are generated when pouring the hydrogel solution.

e. Place plate with hydrogel and PDMS template in oven for approximately 2 hours to dry. The hydrogel is dry when not tacky to the touch and has a frosty appearance.
f. Remove from refrigerator and starting with one side gently peel the hydrogel film off the PDMS template. ENSURE THAT YOU MONITOR THE ORIENTATION OF THE HYDROGEL FILM SO AS NOT TO LOSE TRACK OF THE SIDE OF THE FILM CONTAINING THE PATTERN.
g. Tape the hydrogel film down onto a flat glass plate, ensuring that the patterned side is up.
h. Pipette 100 µL of DCM-polymer-drug solution along one edge of the hydrogel film.
i. Using the sharp side of the razor blade, gently swipe the DCM solution along the length of the hydrogel template.
j. Let set at room temperature for 5 minutes to evaporate off DCM.
k. *(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.
l. Cut hydrogel into easy to manage pieces. Place piece in 50 ml centrifuge tube, fill to top with warm (~37° C) distilled water to dissolve hydrogel. Place tube in shaking incubator to facilitate dissolution.
m. Centrifuge tube to force microparticles to settle to bottom. Remove supernatant 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)
n. Wash particles 2 more times with addition of distilled water and centrifuging.
o. 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.

How It Works (see Figure 1 for schematic)

Briefly, the PDMS provides a positive mold of columns (A) onto which a hydrogel solution is poured (B) and removed to form the negative mold with wells (C). This mold is filled with organic solvent containing polymer and drug substance (D) and dissolved in water (E). Afterwards the mold is washed away and the particles collected (F). 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.

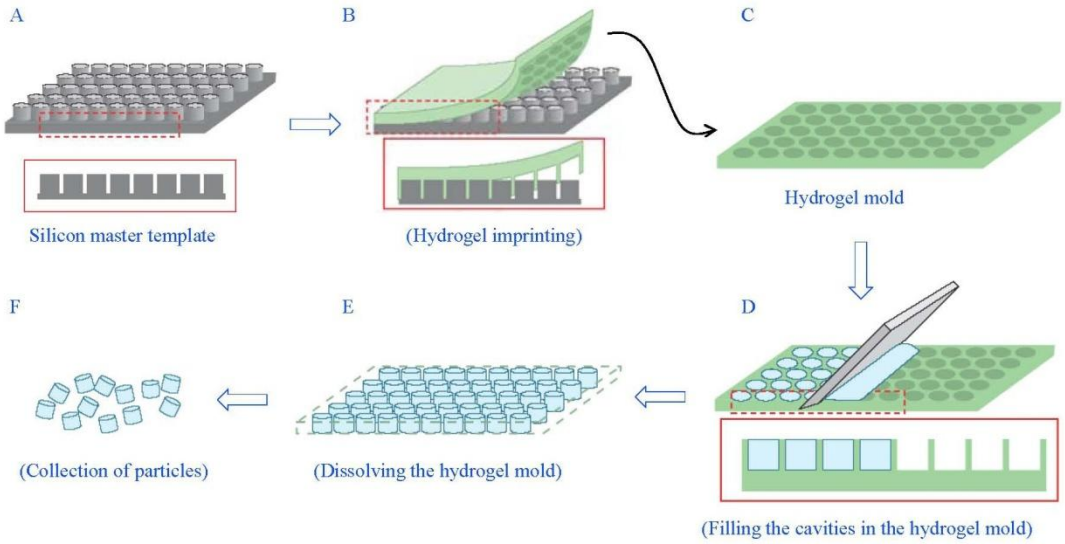

Figure 1. Mold method of microparticle fabrication explanation.