

# Scaffold Fabrication Tutorial: Salt-Leaching & Gas Foamed Scaffolds

## SALT-LEACHED SCAFFOLDS (48-WELL PLATE):

**Polymer Solution:** Make a 30% (by mass) solution of PCL [poly( $\epsilon$ -caprolactone), MW 80 kDa] (3 g of PCL + 7 g chloroform).

**Molds:** Construct molds out of Teflon sheet (4 mm thick) by drilling 12 mm dia. holes. Use a second sheet of 4 mm-thick Teflon sheet as the base of the mold. Clip base to the holed-sheet with binder clips.

**Making Scaffolds:** Put 2 g of 30% PCL solution into a 20 mL glass vial. Add 8 g of sieved NaCl (250  $\mu$ m to 425  $\mu$ m; sieves #40 and #60). Mix with a spatula into a paste. Cut the tip off of a 5 mL syringe, put your finger over the end of the syringe and scoop the polymer/salt paste into the syringe (back fill the syringe). When syringe is full, insert plunger and press the paste against your finger to compact it. Use the syringe to deposit the paste into the Teflon molds (full syringe can fill 4 or 5 molds). Use the cutoff end of the syringe to scrape off the paste flush with the top of the mold. Air dry scaffolds for 2 h. Chloroform is volatile and nearly all of it will evaporate (there is no residual chloroform odor on the scaffolds after 2 h). Wet scaffolds in ethanol and put in excess deionized water to leach NaCl for 5 d. Air dry for 3 d and store in desiccator.

**Cell Culture:** 12-mm dia. scaffolds fit snugly in a 48-well plate for cell culture (can apply a small dab of vacuum grease to bottom of scaffolds to keep them from floating in medium if necessary). Sterilize with ethylene oxide. Anprolene Products sells table top ethylene oxide sterilizers for \$4100 ([www.anpro.com](http://www.anpro.com)). De-gas after sterilization by putting scaffolds under house vacuum for 2 d. To wet scaffolds for cell culture, add cell medium to scaffolds and apply house vacuum for 2 min (use a desiccator or vacuum oven) (should see bubbles). Break and reapply vacuum two more times to fully wet the scaffolds with medium. Remove medium and seed with cells.

**GAS-FOAMED SCAFFOLDS (48-WELL PLATE):** Everything is the same as for salt-leached scaffolds except the porogen and the leaching. For the porogen, use sieved ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) (250  $\mu$ m to 425  $\mu$ m). For the leaching, first immerse in warm water (40°C) for 2 h (until bubbling stops), then immerse in room temperature water for 5 d.

**96-WELL PLATE SCAFFOLDS (SALT-LEACHED):** Make a 10% (mass/volume) solution of PCL (80 kDa) (1 g of PCL + 10 mL dioxane). Use a polypropylene 96-well plate as the mold. Put 0.13 g of sieved NaCl (250  $\mu$ m to 425  $\mu$ m) in each well of the 96-well plate. Use a flat ended implement to level NaCl in each well. Dispense 30  $\mu$ L of PCL solution into each well with glass syringe. Spin plate in a swinging bucket centrifuge at low speed (1.5K rpm for 1 min) to settle PCL solution to bottom of wells. Immerse plate in liquid nitrogen in a small tray for 5 min to freeze scaffolds. Remove from liquid nitrogen and immediately place in freezedryer overnight. Next day, immerse plate in deionized water to leach NaCl for 5 d and air dry 3 d (store in desiccators).

## REFERENCES

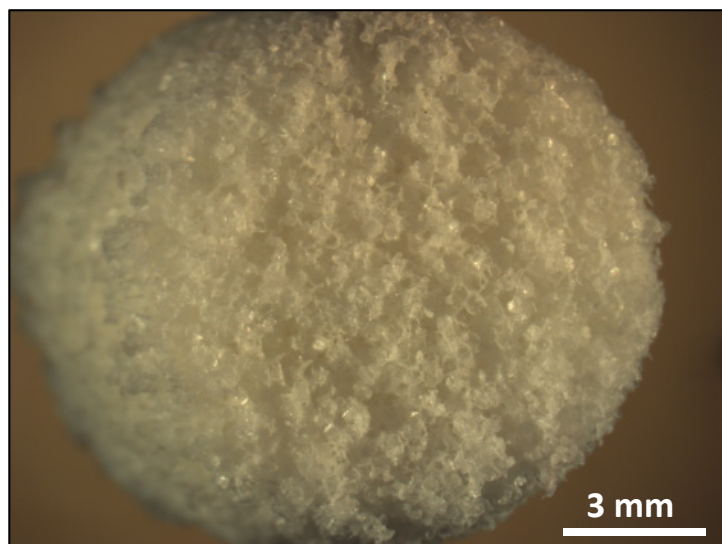
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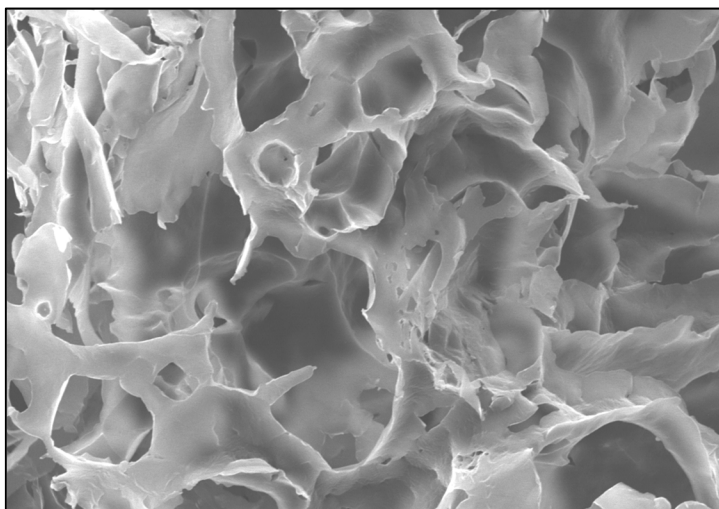
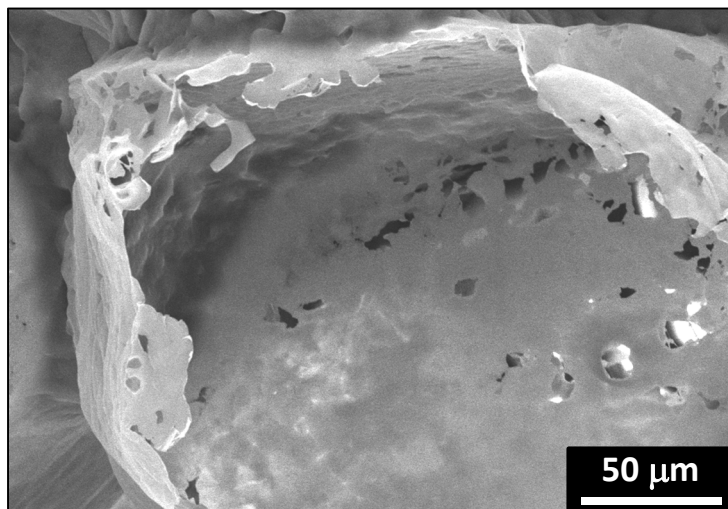
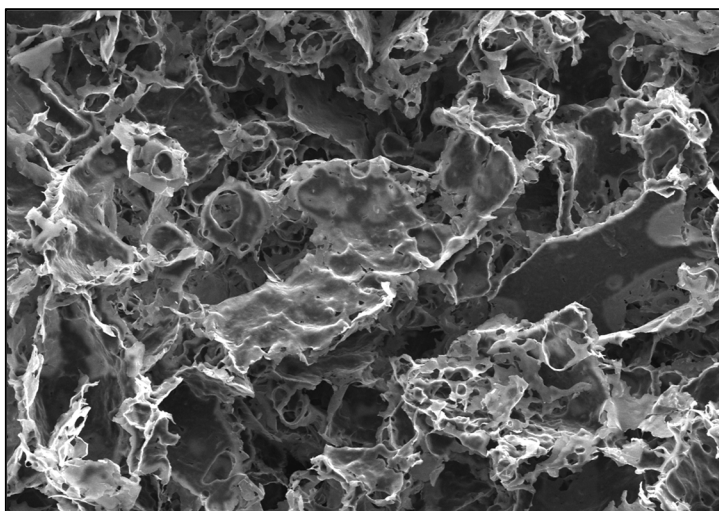
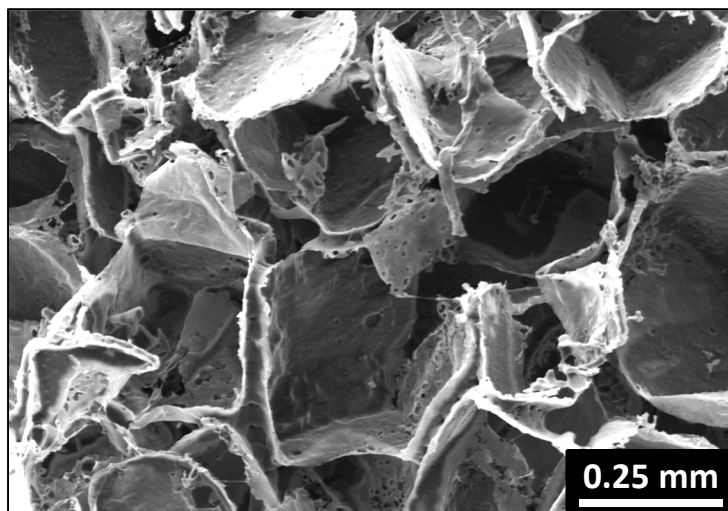
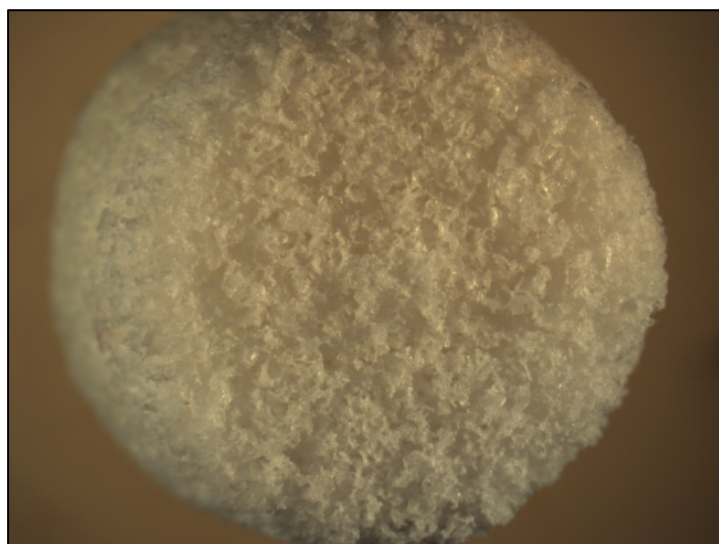
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# 12 mm Diameter Scaffolds for 48-Well Plates Made from 30% PCL Solution

## Salt-Leached



## Gas-Foamed



# Salt-Leached Scaffolds Fabricated in Polypropylene 96-well Plates Using 10% PCL Solution

