Collagen Gel and Cyclical Stretcher Protocol

Materials Needed:
- Cell Media (DMEM containing 10% Fetal Bovine Serum)
- Serum Starved Media
- CellScale MechanoCulture B1 Devices
- 2 Forceps
- Surgical Scissors
- 150mm dishes with a 2mm layer of PDMS in the bottom (these are reusable)
- Cross-shaped PDMS negative mold (these are reusable)
- Green inserts made from a scotch-brite pad (these are reusable)
- Rotator
- Parafilm
- Pins taken from needle tips (sterile)
- Flat, plastic disk to help lift up the gels
- PBS
- Materials for Collagen
  - Collagen (Advanced BioMatrix, PureCol Collagen Solution (3 mg/ml), P/N 5005-B)
  - Ice bucket with ice
  - 0.2M HEPES, pH 9.0
  - 10x MEM
  - 1 50 ml centrifuge tube
- Pipettes 1-5-10-25 mL
- 1 50 mL centrifuge tube
- 400 mL waste beaker
- Titanium(IV) oxide powder (Sigma-Aldrich)

Method:

Making Collagen Gels
1. Serum starve the fibroblasts 18 hours before beginning.
2. Remove media from a T150 flask of fibroblasts, rinse with PBS, then add 3mL of trypsin and put in incubator for four minutes.
3. Remove flask and tap the side to dislodge any remaining stuck cells. Add 10mL of media and transfer everything to a 50mL conical tube and spin at 400g for 10 minutes (depending on the number of gels being made pool all the supernatant before spinning).
4. Reconstitute the cells in 6mL of media and count the cells on a hemocytometer.
5. Add enough media to cells to create a concentration of 1 million cells/mL.
6. Mix (1mL:1mL:8mL) ratio of [0.2M HEPES (pH 9.0)]:[10x MEM]:[3.1mg/mL soluble collagen] (all on ice). Once mixed keep mixture on ice until needed.
7. Mix collagen solution at a 4:1 ratio with the cells to create a final cell concentration of 200k cells/mL and collagen concentration of ~2mg/mL.
8. Wrap conical tube cap with parafilm and place the conical tube containing the gel mixture on the rotator that is in the incubator for 20 minutes.
9. Place PDMS cross-shaped form in a 150mm dish with a PDMS layer in the bottom.
10. Place green sponge inserts in the four corners of the mold. Wet them with sterile DI water first. Push two small pins through each sponge into the PDMS layer if making gels under biaxial restraint.
11. Add 4.6 or 9mL (depending on gel size) of gel per mold and make sure the green pads are saturated with gel (it helps if the green pads are slightly wet before gel addition). Make sure you mix the gel with a 10mL pipet before adding to the mold.
12. Transfer the gel to the incubator and let set for 4 hours.
13. Make 1 g/mL Titanium(IV) oxide solution with PBS
14. Paint dots on top of gel, image the dots to track strains.
15. Gently remove the mold from around the gel.
16. Add 30mL of collagen gel media (enough to submerge the gel), leaving the metal pins in the green pads for gels under biaxial constraint.
17. Place in incubator for 24 hours.

*Strain Tracking and Transferring to Stretcher Devices*
18. Take a picture of the gel and dots. Take the metal pins out of the gel and slide the plastic disk under the gel.
19. Add 75ml of media to the stretcher chamber.
20. Transfer the gel and disk to the stretcher and pin the green pads on the circle of pins within the stretcher. For uniaxial testing remove two opposing arms of the gel.
21. Take a picture of the dots and calculate strains (MATLAB) within the gel. $F_s$ should be around 1.00±.03. If the gel is under too much or not enough tension, readjust arms, take a new picture, and calculate the new strains.
22. Assemble the stretcher, place in the incubator and run for a predetermined amount of time.
23. Once the run is complete, cut the arms of the gel that are attached to the posts and transfer the gel to a dish containing formalin (no PBS rinse is needed and make sure the gel floats to ensure good fixing).
24. Keep in formalin for 30 minutes. Remove the formalin and rinse with PBS and let the gel sit for 10 minutes to get more formalin out.
24. Do a final change of PBS and store the gel in the refrigerator.

**Helpful tips:**
- The green pads can be reused by soaking them in .01M HCL for 30 minutes and then autoclaving them. If the pads are new, before using them for the first time rinse with 100% Ethanol to remove excess green dye.
- The PDMS can be reused by washing with soap and water, sterilize with 70% ethanol and dry in the hood. Before use rinse with sterile DI water.

*Last Modified January 30, 2018 by KC*