Video Article

Mechanical Stimulation of Stem Cells Using Cyclic Uniaxial Strain

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Abstract

The role of mechanical forces in the development and maintenance of biological tissues is well documented, including several mechanically regulated phenomena such as bone remodeling, muscular hypertrophy, and smooth muscle cell plasticity. However, the forces involved are often extremely complex and difficult to monitor and control in vivo. To better investigate the effects of mechanical forces on cells, we have developed an in vitro method for applying uniaxial cyclic tensile strain to adherent cells cultured on elastic membranes. This method utilizes a custom-designed bioreactor with a motorized cam-rotor system to apply the desired force. Here we present a step-by-step video protocol demonstrating how to assemble the various components of each "stretch chamber", including, in this case, a silicone membrane with micropatterned topography to orient the cells with the direction of the strain. We also describe procedures for sterilizing the chambers, seeding cells onto the membrane, latching the chamber into the bioreactor, and adjusting the mechanical parameters (i.e. magnitude and rate of strain). The procedures outlined in this particular protocol are specific for seeding human mesenchymal stem cells onto silicone membranes with 10 μm wide channels oriented parallel to the direction of strain. However, the methods and materials presented in this system are flexible enough to accommodate a number of variations on this theme: strain rate, magnitude, duration, cell type, membrane topography, membrane coating, etc. can all be tailored to the desired application or outcome. This is a robust method for investigating the effects of uniaxial tensile strain applied to cells in vitro.

Video Link

The video component of this article can be found at https://www.jove.com/video/242/

Protocol

Day 0 - Sterilization before day of experiment

1. Put materials into plastic tub and sterilize with 70% alcohol for 2 hours:
   - Chambers
   - Lids
   - Frames (all 3 pieces)
   - Screws
   - Rubber gaskets
   - Forceps
   - Scissors
   - Hex wrenches
2. Clean membranes with Aquet soap and distilled water.
3. Sonicate membranes in 70% alcohol for 10 minutes.
4. Place the clean membranes into plastic square dishes. If using patterned membranes, ensure that the patterned side is face up (spray alcohol onto one side of the membrane and watch for the liquid to run down the grooves).
5. Leave membranes covered in 70% alcohol for 2 hours.
6. Package gloves into aluminum foil for autoclaving tomorrow morning.
7. Make 2% (weight/volume) gelatin solution (2g/100mL) in distilled water for autoclaving tomorrow.
8. After the 2 hour sterilization in 70% alcohol, place all polymer materials and membranes (non-autoclavable materials) into hood for overnight UV:
   - Chambers
   - Lids
   - Frames (all 3 pieces)
   - Membranes
9. Pack remaining materials into autoclavable bag:
   - Screws

- Rubber gaskets
- Forceps
- Scissors
- Hex wrenches

Day 1 - Assembly of stretch chambers

1. Autoclave forceps, scissors, hex wrenches, screws, gaskets, gloves, and gelatin using small autoclave at 240°F for 20 minutes total
2. Remove membranes from UV and treat with O_2 plasma (patterned side up) for ~1 minute.
3. In TC hood, cover patterned area of plasma treated membranes with gelatin. Coat 30 for minutes under UV.
4. Wash each membrane with PBS 2X. After the 2nd wash, leave some PBS on the membranes to keep them slippery, for easier assembly into the chambers. (Should also use this PBS to lubricate the gaskets for easier assembly).
5. Assemble membranes into chambers (wear autoclaved gloves during assembly)
    1. Connect the two main pieces of the frame using a single screw.
    2. Flip the frame over and place a membrane on top so that the gelatin-coated side faces toward the frame (the patterned area should be in the center).
    3. Secure the membrane to the frame using a gasket at each side.
    4. Press the gaskets in, using gentle, even pressure, so as not to rip the membrane.
6. Attach assembled frame to T-bar.
7. Flip the frame and place into chamber upside down. UV back side of frame and membrane for 30 minutes
8. Flip the frame again, and screw into chambers (no change for control chamber, but for stretch chamber, need to use two screws to attach the end piece of the frame to the bottom of the chamber, and need to remove the single screw that is holding the two pieces of the frame together). UV front side for 30 min. Make sure the membranes are COMPLETELY dry before proceeding to the next step, or else the cell solution may slip off during seeding.
9. Seed cells. Area of 1 plate = Area of 3 membranes, so use 1/3 of a confluent plate per membrane. Use 1 mL of cell solution per membrane. Any more than 1.5mL will be difficult to keep solution on. Put cell solution only on patterned area, and use the pipette tip to spread the solution around. Cover chambers and let cells attach for 30 minutes RT in the hood.
10. Move chambers to incubator and let cells attach for 1 more hour. Be VERY careful moving the chambers to avoid letting cell solution slip off! The cells in that chamber will be ruined if the solution falls off at this point.
11. Return chamber to hood and add 20 mL media.
12. Place chambers in alcohol-cleaned tub that is covered with aluminum foil (also sprayed with alcohol). Move chambers to stretch incubator (10% CO_2).
13. Secure chambers into stretch machine. Let cells further attach overnight, and start stretch on the next day. (*Note*: when putting chambers into stretch machine, remember to lock the machine in the "zero" position before placing chambers, and then tighten the rubber band around the gears. Don't forget to unlock the gears before starting the machine).

Discussion

Banes et al. first reported the use of a system for mechanical stimulation of cells in vitro by using a flexible elastomeric substrate to deliver mechanical force to cells[1]. Since this time, many variations on this design have been conceived and utilized. Several mechanical stretch systems are commercially available under the name "Flexercell" (Flexcell International Corp.), while some labs use custom-built devices. In this video protocol we have described the setup and use of one such device in our lab.

The custom-built "stretch machine" depicted in this protocol has been used in various studies to investigate the effects of uniaxial cyclic strain on different cell types[2,3]. This machine is a versatile apparatus with several adjustable parameters that can be used for a variety of mechanical strain studies. The setup depicted herein represents a unique and robust method for delivering uniaxial cyclic strain to adherent cells in culture.

References

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