Collagen Gel Contraction Assay

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Method Article

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Abstract

Collagen contractility, a characteristic of activated fibroblasts, can be evaluated by Collagen Gel Contraction Assay.

Reagents

Materials 1. Dulbecco's Modified Eagle's Medium \(DMEM, Invitrogen); 2. PBS; 3. Fetal bovine serum \(FBS, Invitrogen); 4. 0.25%Trypsin; 5. Rat tail collagen type 1 \(R&D system); 6. Glacial acetic acid; 7. NaOH \(1 M); 8. 0.2-μm filter.

Procedure

Preparation of Collagen for Use in Collagen Gels 1) Prepare acetic acid solution \(0.1%): Mix 0.5ml 100% glacial acetic acid with 49.5ml deionized water, filter sterilize the solution with a 0.2-μm filter and cool to 4°C. 2) Make a 3 mg/mL collagen solution in 0.1% acetic acid: dilute the rat tail type 1 collagen\(from R&D system) in 0.1% acetic acid to make a concentration of 3mg/ml, this collagen solution should be stored at 4°C. **NaOH Titration of Collagen** \(Tips: Perform this step whenever using a new batch of collagen to optimize solidification.) 1) Prepare eight 1.6 mL Eppendorf tubes and add 0.4 mL of cell specific media or DMEM to each tube. 2) Add 0.2 mL of collagen solution \(3 mg/mL in 0.1% acetic acid) to each tube and mix well \(The final collagen concentration should be 1 mg/mL, and the addition of collagen solution will make the phenol red media turn into yellow.). 3) Immediately add 1 to 8 μL of 1 M NaOH to different tube and pipet mixture up and down with 1mL pipet. 4) Allow the mixture to solidify for 20 min at room temperature. 5) Compare rigidity and color of gels titrated with different volumes of NaOH to determine which volume of NaOH produces a well-solidified gel with neutral pH. \(The least amount of NaOH needed to turn the media to a light pink color and produce the most rigid collagen gels.) **Experiment Procedure** Populating collagen gels with cells requires careful attention to cell concentrations. For example, a final population of $1\sim2\times10^5$ cells/mL of fibroblasts will be good. 1) Detach cells from the culture vessel using warmed Trypsin solution and collect the cells by centrifugation. 2) Rinse the cells by PBS in order to remove the trypsin completely. 3) Suspend cells into complete culture media and count cells using counting chamber. 4) Adjust the cells concentration into 1.5×10^5 cells/mL. 5) Add 0.4mL cells suspension in a new 1.6 mL Eppendorf tubes. 6) Add 0.2 mL of collagen solution \(3 mg/mL in 0.1% acetic acid) to the cells suspension and mix well. 7) 1) Prepare eight 1.6 mL Eppendorf tubes and add 0.4 mL of cell specific media or DMEM to each tube. 8) 2) Add 0.2 mL of collagen solution \(3 mg/mL in 0.1% acetic acid) to each tube and mix well\(The final collagen concentration should be 1 mg/mL, and the addition of collagen solution will make the phenol red media turn into yellow.). 9) Quickly add the appropriate volume of 1 M NaOH in the cells/collagen mixture and mix the solution well with pipet. 10) Immediately transfer 500 μL of the mixture to a 24-well plate. 11) Allow gels to solidify at room temperature for 20 min. 12) Gently add 600uL culture media to each well. 13) Dissociate the gel from the well by gently running the tip of a 200-μL pipet tip along gel edges without shearing or tearing gels. 14) Gently swirl plate to make sure that gel is free from the plate. 15) Put the 24-well plate into incubator at

37°C, humidified 5% CO_2. 16) Observe the contraction of gels: record the diameter change of gels in several time-points\(Such as 0h, 3h, 6h, 12h, 24h) by using a digital camera at a fixed distance above the gels in order to obtain images at each time-point.