Effect of Retinoic Acid on HaCaT and NIH-3T3 cells in an *in vitro* 3D Collagen Cell Culture Skin Model

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APPENDIX: Seeding Cells in Collagen Gels (final 1 mg/mL)

<u>To prepare 1.5 mL of 2 mg/mL collagen (all components should be sterile):</u>
1 mL collagen (4 mg/mL) (KEEP COLD) - * variable based on a lot so adjust as necessary
+ 0.15 mL 10x PBS (COLD)
+ 0.20 mL medium (COLD)
+ 10-100 µL 1 M NaOH in 10 mL increments (COLD), look for indication of pH (should go from yellow to orange/red; avoid turning pink!
+ medium to 1.5 mL (COLD)

<u>Embedding cells within the collagen solution:</u>
Mix collagen gel 1:1 with cells in cell medium (COLD)
Prepare cells by trypsinization, spinning, resuspending in fresh medium, and adjusting to desired 2X concentration. Aliquot as needed into plates or molds.
Gels should solidify within 30 minutes at 37 ℃.

If you want to grow cells on top of the gels, use 2 mg/mL collagen so that the collagen is stiffer. Choose a volume that completely covers the bottom of the well, allow it to gel, and then seed cells directly on top. If you seed fibroblasts in the gel and want keratinocytes on the surface, allow the fibroblasts to grow for 3-7 days (7 days are safer and easier for a lab class – it may be worth testing first) to allow the fibroblast to contract the gel before seeding keratinocytes. Previously, we tried stiffening the collagen by adding 1.25% low-melt agarose to a final concentration of 0.25%. The issue was the collagen must stay cold and the agarose warm, so it gels quickly. The cells did not respond well to the collagen-agarose matrix *vs.* a gel composed of collagen only.