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Protocol for making 3D vitrogen gel

1. Warm up serum free media, vitrogen at 37°C.
2. For 10 ml of vitrogen,
    a. Add 3.3ml (1 volume) of vitrogen (at around 3.0 mg/ml concentration).
    b. Add 6.6ml (2volume) of serum free media.
    c. Add 0.2ml (0.06 volume) of 0.1 N NaOH.
    ⇒ pH~7.16 (if use serum media, pH will be around 7.4)
3. In order to make bound VEGF, add 1 µl of 1µg/µl VEGF into 10 ml of gel to make final concentration 100ng/ml.
4. Let vitrogen gel sit at 37°C for 1 hour to solidify in 12 well plates.
5. In order to apply the gel onto the cells to mimic the in vivo cell condition, cut 1 well of 4 well chamber slide's top part as "mold." (Or use a 17x100mm 14 cm loose cap falcon tube to cut.)
6. Sterilize scapula, "mold," in 70% ethanol for 10 min. UV for 15 min.
7. After gel gets solidified in 12 well plate, use the "mold" to cut the gel.
8. Use a scapula to lift the above cut gel and put the gel on top of the confluent cells, with medium been aspirated.
9. Incubate at different time points as desired.

Comments:

For BD plates:

- 6 well plates: 9.6 cm^2 as surface area, use 2 ml to cover the surface
- 12 well plates: 3.8 cm^2, use 800µl
- 24 well plates: 2 cm^2