UV Crosslinking Protocol and Tips

Optimizing the UV light setup:

To ensure successful UV crosslinking, the setup of the UV light system is very important. Position the 365nm UV light source above the product to be crosslinked, whether it is bioprinted or cell culture plates. Lower intensity UV lamps require the product to be physically closer than higher intensity UV lamps. Lower intensity UV lamps require longer exposure time than higher intensity UV lamps.

Recommended conditions for crosslinking a thin (~150um) layer of OkaGel:

- Place a high intensity light source 14 cm above the printed structure and expose for 5-45 seconds according to the final properties required for the construct.
- UV light intensity of 10mw/cm^2 exposed for 25-45s [1]
- UV light intensity of 13mw/cm^2 exposed for 15-35s [1]

Protocol:

1. If you are using OkaGel Liquid, proceed directly to step 1. If you are using OkaGel Solid, begin by creating your preferred w/v concentration of OkaGel in sterile reverse osmosis (RO) water. If you are unsure, we recommend starting with a 7.5% w/v concentration (75mg/mL).
    - Dissolve the Irgacure photoinitiator in sterile PBS to form a 0.5% w/v solution by heating for 1-2 hours at 60°C.
    - Dissolve OkaGel Solid in sterile RO water.
    - Leave the OkaGel solution at 37°C until dissolved.
2. Dispense warm OkaGel (37 °C), either from a bioprinter or manually into your vessel (petri dish or multi-well plate).
3. Turn on the UV lamp for your desired amount of time, then turn off.
4. Let the structure sit for 2 minutes for crosslinking to occur.
5. To verify that crosslinking has occurred, heat the sample to 37°C if cells are present, or 50°C if no cells present. If the sample remains stiff, it has successfully crosslinked, if it liquifies, continue UV exposure.

Notes

- If you are using a UV light source of lower intensity, 5.5-8.5 mW/cm2 for example, increase the exposure time to 1 or 2 minutes to allow for complete crosslinking to occur.
- Results from a viability assay at day 1 showed that bioprinted cell-laden 10% GelMA photopolymerized for 60 s were associated with lower viability than gels photopolymerized for 15 and 30 s [2].
- UV intensity and time of exposure lead to lots of room for variation in physical characteristics and your required needs.
- The various protein concentrations in different OkaGels impact pore size. When using a higher

concentration of OkaGel, using a shorter exposure time will result in larger pore size when cured.
- When using OkaGel with a low concentration (5%), use a longer exposure time to fully cure.
- The petri dish lid reduces the UV intensity by around a third.
- The UV flashlight lamp emits light at an intensity of 16 -18 mW/cm2, and reduces to about 14 mW/cm2 after adding the lid, at a distance of 14 cm/5.5 inches.
- The Thermofisher lamp emits UV intensity of around 8-10 mW/cm2 and around 6-8 mW/cm2 with the petri dish lid on.
- The lamp can be placed directly in contact with the top or bottom of the culture plates. Its intensity decreases with increasing distance.
- For small samples at the micro-level, a high intensity UV source is better. This is because the generated free radicals from the photoinitiation process are very reactive with oxygen in the air. So, the higher the exposed surface area with lower volume/surface ratio, the higher the free radical generation intensity is required for crosslinking. As a result, higher UV intensity for a shorter time (to provide the same energy level) is a better choice for small sample sizes as it allows for crosslinking and maintains cell viability.
- Otherwise, small samples would require a relatively longer time to crosslink which might affect cell viability.

Tip: It's preferred not to vortex or shake liquid OkaGel in order to avoid bubble formation, especially with the viscous higher concentrations. If bubbles occur, warm OkaGel in a 37°C water bath and centrifuge at around 1500 rpm for 3 mins.

References

[1] Wadnap, S., Krishnamoorthy, S., Zhang, Z., & Xu, C. (2019). Biofabrication of 3D cell-encapsulated tubular constructs using dynamic optical projection stereolithography. Journal of Materials Science: Materials in Medicine. https://doi.org/10.1007/s10856-019-6239-5

[2] Liu, W., Zhong, Z., Hu, N., Bertassoni, L. E., Cardoso, J. C., Manoharan, V., Cristino, A. L., Bhise, N. S., Araujo, W. A., Zorlutuna, P., Vrana, N. E., Ghaemmaghami, A. M., Dokmeci, M. R., & Khademhosseini, A. (2014). Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication, 6, 24105–24116. https://doi.org/10.1088/1758-5082/6/2/024105