Photocrosslinking of OkaGel Microspheres

GelMA microspheres are a useful method for delivering specialized cells to various locations, both *in vitro* and *in vivo* while maintaining high cell viability. Here, a flow-focused microfluidic device is used to create cell-laden GelMA microspheres. The cell-laden GelMA acts as the aqueous phase and an oil solution is prepared to emulsify the GelMA into droplets.

Protocol:

1. Prepare Okagel solution to include cells and photoinitiator of choice.
    a. See “Embedding cells in Okagel” protocol
    b. Recommended concentration contains a 7.5 wt% GelMA (between 4.0 wt% and under 8.0 wt%) and 1.0 wt% photoinitiator
2. Form an oil solution of perfluorinated oil (3M HFE 7500). This becomes the oil phase.
    a. Add a biocompatible triblock perfluorinated copolymer surfactant to stabilize the droplets (for example 1.0 WT% of, Krytox-PEG, RAN Biotech).
3. Flow GelMA into a capillary microfluidic flow-focusing device. This will create the microspheres.
    a. Oil phase flow rate should be kept constant at 10mL/h.
    b. GelMA flow rate should vary from 100mL/h - 2000mL/h as to control the size of microsphere., This will result in droplet diameters varying between 90 and 230 um.
4. Subject the resultant droplets to UV light (~365nm) for 20 seconds.
5. Wash the microspheres in 20% perfluorooctane in HFE oil to remove surfactant.
6. Wash the microspheres in 1:1 volume distilled water.
7. Repeat Steps 7 and 8 once more to ensure complete removal of residual oil and immerse the crosslinked microspheres in water for 24 hours.

Notes:

- 20 seconds of UV exposure time was selected to be long enough to allow full conversion but short enough to ensure high cell vitality.
- The size of microspheres was selected so that it is larger than 60 um to ensure a sufficient number of encapsulated cells to promote cell contact and proliferation and smaller than 200 um to allow ready oxygen exchange across hydrogels for cell viability.
- The resultant GelMA microspheres can be easily injected through a syringe head. GelMA microsphere stiffness can be measured with the nanoindentation technique AFM.

References

Zhao, X., Liu, S., Yildirimer, L., Zhao, H., Ding, R., Wang, H., Cui, W., & Weitz, D. (2016). Injectable stem cell-laden photocrosslinkable microspheres fabricated using microfluidics for rapid generation of osteogenic tissue constructs. *Advanced Functional Materials*, *26*(17), 2809–2819. https://doi.org/10.1002/adfm.201504943