

## Creating Cell-Laden OkaGel Protocol and Tips

### Protocol:

1. Calculate the number of cells needed which will vary depending on application and cell type.
2. Centrifuge desired number of cells and resuspend them in a small volume of cell culture media to create a high-density cell suspension. NOTE: Volume of cell suspension may affect final concentration of hydrogel and should be factored into all calculations.
  - Pre-warm biomaterial to 37°C to limit temperature stress on cells.
  - Pre-warm OkaGel to 35°C using an incubator, water bath or pneumatic printhead.
3. Prepare high-density cell suspension.
  - Centrifuge desired number of cells and resuspend them in a small volume of cell culture media. NOTE: The final concentration of the hydrogel may vary based on the volume of cell suspension and biomaterial used.
4. Carefully mix GelMA OkaGel with cell suspension. It is recommended to mix ten parts OkaGel with one part cell suspension.
  - Using a female/female luer-lock adaptor, transfer the cell suspension to a 3ml foil wrapped syringe.
  - Using a female/female luer-lock adaptor, transfer the desired amount of OkaGel to a 3ml foil wrapped syringe.
  - Connect the OkaGel syringe with the cell suspension syringe and carefully push the plungers back and forth to mix the GelMA OkaGel with the cell suspension.

### Notes:

- Mixing volumes > 2ml of GelMA OkaGel with desired cells will require larger syringes.
- Tip: It's preferred not to vortex or shake OkaGel in order to avoid bubble formation, especially with the viscous higher concentrations. If bubbles occur, centrifugation at around 1500 rpm for 3 mins can help get rid of the bubbles.