OkaSciences

Mixing Cells with OkaGel Protocol

Overview

Cell-laden hydrogels are the centre of many biotechnological applications. OkaGel can support many different cell types, including progenitor and stem cells. OkaGel embedded with cells can be used for bioprinting biological structures, fast-tracking cell culture and studying cell behaviour.

Protocol

Note: This protocol is adapted for use with OkaGel liquid. If you plan to use OkaGel Solid, please refer to [this] protocol with instructions on how to prepare OkaGel Solid for use.

1. Determine the final volume of OkaGel required to conduct your experiment.

    Note: It is recommended to increase the total volume of cell-laden OkaGel by about 20% to account for losses during preparation. The minimum recommended volume of OkaGel preparation is 1mL to allow for successful pipetting.

2. If using OkaGel liquid premixed with photoinitiator, proceed to step 6.

3. If using OkaGel solid: Add 4⁄5 of your final required volume of a 1.25x OkaGel solution to an appropriate capacity centrifuge tube. For example, if you require 10mL of 10% OkaGel, add 8mL of 12.5% OkaGel. This is to account for the dilution caused after addition of a photoinitiator solution later in the protocol.

    If using OkaGel solid, prepare a fresh photoinitiator solution in PBS with a concentration of 2.5mg/ml. Refer to [this] protocol outlining photoinitiator prep.

4. Add one-fifth the total OkaGel volume of photoinitiator solution to the four-fifths OkaGel to a photoinitiator solution. Place on a gentle rocker for 1 hour to ensure complete mixing.

5. Determine your cell suspension concentration. Detach your cells of choice from their culture vessel using your standard protocol. Wash the cell pellet with PBS to remove trace amounts detachment tool (eg trypsin, Versene) and resuspend in enough fresh cell media to determine the cell suspension concentration via cell counter or hemocytometer.

6. Determine the required number of cells (n_{cells}) based on the desired final cell concentration (c_{cells}) and the total volume of OkaGel solution prepared (V_{GelMA}) using the equation $n_{cells} = c_{cells} \times V_{GelMA}$. Take the volume ($V_{cells}$) containing n_{cells} from the cell suspension prepared corresponding to $V_{cells} = n_{cells}/c_{suspension}$, and transfer to a fresh 50-ml tube. Pellet the cells by centrifugation at 100–300*g* for 5 min at RT and discard the supernatant.

7. Resuspend the pellet in OkaGel by pipetting the final volume of liquid OkaGel into the tube and pipette up and down ~10 times to agitate the pelleted cells and ensure complete mixing.