Mixing Cells Protocol

GelMA Series

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for how to mix cells with any ink within the GelMA Bioink series, both small volumes below 2 ml and large volumes up to 10 ml. The GelMA Bioink Series includes GelMA, GelMA A, GelMA C, GelMA HA, GelMA HIGH C and Bio Conductink. GelMA can be purchased with the STARTINK-Kit.

Material needed

- Cells in suspension
- Cell culture medium
- GelMA bioink variation*
- Pipette and pipette tips
- 3 ml syringes with luer lock connections
- Female/female luer lock adaptor*
  or
- CELLMIXER*

* The product can be purchased in the CELLINK shop at www.cellink.com/store/.

KEEP THE INK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

This protocol is adjusted for mixing either 1 ml or 3 ml of bioink with cells to a final cell concentration of 10 million cells/ml bioink. For other quantities and cell concentrations recalculations need to be made but the same protocol can be followed.

Step	Title	Material	Description
1	Preheat bioink	- GelMA bioink variation	- Heat the bioink in the amber cartridge to 35°C. The heating of the bioink can be performed in a pneumatic printhead, water bath or incubator.

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2	Prepare cell suspension	- Cells - Culture medium	If preparing for quantities < 2 ml of GelMA bioink. - Resuspend 11 million cells in 100 µl cell culture medium if mixing with 1 ml bioink. - Move on to Step 3a. If preparing for quantities > 2 ml of GelMA bioink. - Resuspend 33 million cells in 300 µl cell culture medium if mixing with 3 ml bioink. - Move on to Step 3b.
3a	Mixing small volumes GelMA bioink with cells	- 1 ml GelMA bioink variation - Cell suspension - 3 ml syringes - Female/female luer adapters	At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture. - Transfer the 100 µl cell suspension to a 3 ml syringe wrapped in aluminium foil, using a female/female luer lock adaptor. - Transfer 1 ml of bioink to a 3 ml syringe wrapped in aluminium foil, using a female/female luer lock adaptor. - Attach the bioink syringe to the syringe with cell suspension. - Carefully mix the bioink with the cell suspension by gently pushing the bioink back and forth. - Transfer the cell containing bioink back to the amber cartridge and cap it. - *Video link for a detailed illustration on how to perform the mixing process:* https://www.youtube.com/watch?v=NmdOTNLrV-Q Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the luer lock adaptor with GelMA bioink before attaching the syringe with the cell suspension.
3b	Mixing larger volumes GelMA bioink with cells	- 3 ml GelMA bioink variation - Cell suspension - CELLLMIXER	At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture. - Transfer the 300 µl cell suspension to a 1 ml cell syringe (PART 1) using a female/female luer lock adaptor. - Transfer 3 ml of bioink to the 12 ml syringe (PART 2) using a female/female luer lock adaptor. - Clip both syringes to the Dispensing unit (PART 3).

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			- Connect the two syringes to the Mixing unit (PART 4) then connect the Empty cartridge (PART 5) to the Mixing units other side. - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. - *Video link for a detailed illustration on how to perform the mixing process using the CELLMIXER: https://www.youtube.com/watch?v=CmSYL1-oltI* Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the luer lock adaptor with GelMA bioink before attaching the syringe with the cell suspension. Note: PART 2 is prefilled with bioink if using the STARTINK-Kit.

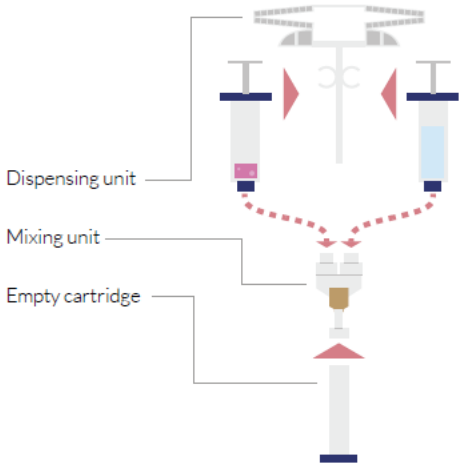

Figure 1. Illustration of how to assemble the CELLMIXER components.

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