Mixing Cells Protocol

CELLINK Series

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions for how to mix cells with any bioink within the CELLINK® Series of bioinks, both small volumes below 2 mL and large volumes up to 10 mL. The CELLINK Series of bioinks includes CELLINK Bioink, CELLINK BONE, CELLINK FIBRIN, CELLINK LAMININK, CELLINK RGD and CELLINK SKIN.

Materials needed

- Cells in suspension
- Culture medium
- CELLINK Series 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 store at *www.cellink.com/store/*.

Protocol

This protocol is adjusted for mixing either 1 mL or 3 mL of bioink with cell suspensions to a final cell concentration of 10 million cells/mL bioink. For other quantities and cell concentrations, the same protocol can be used with adjusted calculations.

Step	Title	Material	Description
1	Prepare cell suspension	- Cells in suspension - Culture medium	If preparing for quantities < 2 mL of CELLINK Series bioink. - Resuspend 11 million cells in 100 µL cell culture medium if mixing with 1 mL bioink. - Move on to Step 2a. If preparing for quantities > 2 mL of CELLINK Series bioink. - Resuspend 33 million cells in 300 µL cell culture medium if mixing with 3 mL bioink. - Move on to Step 2b.
2a	Mixing small volumes of CELLINK Series bioink with cells	- 1 mL CELLINK bioink variation - Cells in suspension - 3 mL syringes - Female/female Luer lock adapters	At this point, mix ten parts of a bioink with one part of a cell suspension, taking care not to introduce air bubbles to the mixture. - Transfer the 100 µL cell suspension to a 3 mL syringe using a female/female Luer lock adaptor. - Transfer 1 mL of bioink to a 3 mL syringe 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 between the syringes. - Transfer the cell containing bioink back to the 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 CELLINK Series bioink of choice before attaching the syringe with the cell suspension.
2b	Mixing larger volumes CELLINK Series	- 3 mL CELLINK Series bioink variation - Cells in suspension - CELLLMIXER	At this point, mix ten parts of a bioink with one part of a cell suspension, taking care not to introduce air bubbles to the mixture.

	bioink with cells		- Transfer the 300 µL cell suspension to the 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). - Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing unit's 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*

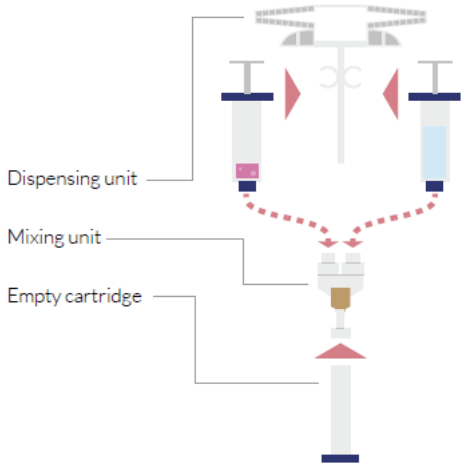

Figure 1. Illustration of how to assemble the CELLMIXER components.