

Mixing cells Protocol

Collagen 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 biomaterial within the Collagen series, both small volumes below 2 mL and large volumes up to 10 mL. The Collagen Series includes Coll 1, ColMA and Collagen PREMIUM.

Material needed

- Cells in suspension
- Culture medium
- Collagen biomaterial variation*
- Pipette and pipette tips
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
- OR
- CELLMIXER*

1

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

KEEP THE BIOMATERIALS COOLED TO 4°C TO AVOID THERMAL GELATION BEFORE CASTING. KEEP COLMA SOLUTION PROTECTED FROM LIGHT IF TRANSFERRED FROM THE UV PROTECTED BOTTLE. 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 5 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	Cool collagen biomaterials	- Collagen biomaterial variation	- Keep collagen biomaterials and all solutions cooled to 4°C throughout the whole experiment by placing the solutions and tools on ice.

Boston, USA
75 Kneeland Street
Boston, MA 02111

Gothenburg, Sweden
Arvid Wallgrens Backe 20,
Gothenburg, 41346

Blacksburg, USA
2000 Kraft Dr, Suite 2125
Blacksburg, VA 24060

Kyoto, Japan
46-29 Yoshida-Shimo Adachi-
cho, Sakyo-ku, Kyoto

2	Prepare cell suspension	<ul style="list-style-type: none"> - Cells in suspension - Culture medium 	<p>If preparing for quantities < 2 mL of collagen biomaterial.</p> <ul style="list-style-type: none"> - Resuspend 5.5 million cells in 100 µL cell culture medium if mixing with 1 mL collagen biomaterial. - Move on to Step 3a. <p>If preparing for quantities > 2 mL of collagen biomaterial.</p> <ul style="list-style-type: none"> - Resuspend 16.5 million cells in 300 µL cell culture medium if mixing with 3 mL bioink. - Move on to Step 3b.
3a	Mixing small volumes of collagen biomaterials with cells	<ul style="list-style-type: none"> - 1 mL collagen biomaterial variation - Cells in suspension - 3 mL syringes - Female/female Luer adaptors 	<p>At this point, mix ten parts of pre-cooled collagen with one part of cell suspension without introducing air bubbles to the mixture.</p> <ul style="list-style-type: none"> - Transfer the 100 µL cell suspension to a 3 mL syringe using a female/female Luer lock adaptor. - Transfer 1 mL of pre-cooled collagen to a 3 mL syringe using a female/female Luer lock adaptor. - Attach the collagen syringe to the syringe with cell suspension. - Carefully mix the collagen with the cell suspension by gently pushing the bioink back and forth between the syringes. - Transfer the cell containing biomaterial back to the cartridge and cap it. - <i>Video link for a detailed illustration on how to perform the mixing process:</i> https://www.youtube.com/watch?v=NmdOTNLrV-Q <p>Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the Luer lock adaptor with collagen biomaterial before attaching the syringe with the cell suspension.</p>
3b	Mixing larger volumes collagen with cells	<ul style="list-style-type: none"> - 3 mL collagen biomaterial variation - Cells in suspension - CELLLMIXER 	<p>At this point, mix ten parts of collagen with one part of cell suspension without introducing air bubbles to the mixture.</p> <ul style="list-style-type: none"> - 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 collagen to the 12 mL syringe (PART 2) using a female/female Luer lock adaptor.

			<ul style="list-style-type: none"> - 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 other side of Mixing unit. - Apply gentle pressure onto the Dispensing unit to mix the content of both syringes into the empty cartridge. - <i>Video link for a detailed illustration on how to perform the mixing process using the CELLMIXER: https://www.youtube.com/watch?v=CmSYL1-oltI</i>
--	--	--	---

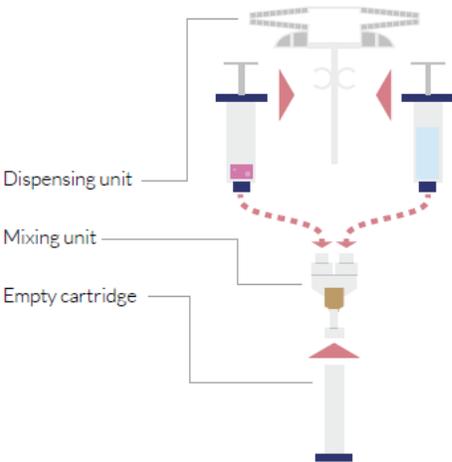


Figure 1. Illustration of how to assemble the CELLMIXER components.