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Date: 05-JAN-2023
Author: JH. Version: 2

Mixing cells and bioink Protocol

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 CELLINKs portfolio such as CELLINK bioink, GelXA, GelMA and Coll 1 solution. This protocol describes mixing small volumes using two syringe or larger volumes using the CELLINK CELLMIXER.

Materials needed

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

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

FOR BIOINKS WITH PHOTOINITIATIOR: 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.

FOR COLLAGEN BASED BIOINKS: KEEP THE BIOMATERIALS COOLED TO 4°C TO AVOID THERMAL GELATION BEFORE DEPOSITION.

Protocol

This protocol is adjusted for mixing either 1 mL or 3 mL of bioink with a cell suspension with desired number of cells. The bioink to cell suspension ratio is 10+1. For other quantities and dilutions, the same protocol can be used with adjusted calculations. The desired number of cells in the bioink is cell type and application dependent.

1. Preparing the bioink

MATERIAL

Bioink variation

DESCRIPTION

- Prepare the bioink according to the bioink specific *Bioprinting Protocol*. The preparation can include heating or cooling of the bioink before mixing with cells.

2. Preparing cell suspension

MATERIAL

Cells in suspension

Culture medium

DESCRIPTION

If preparing 1 mL of bioink

- Resuspend the desired number of cells in 100 µL cell culture medium if mixing with 1 mL bioink.

Note: If the desired final cell concentration is 1 million cells/mL of bioink, dissolve 1.1 million cells in 100 µL cell culture medium.

- *Move on to Step 3a.*

If preparing 3 mL of bioink

- Resuspend the desired number of cells in 300 µL cell culture medium if mixing with 3 mL bioink.
- Note: If the desired final cell concentration is 1 million cells/mL of bioink, dissolve 3.3 million cells in 300 µL cell culture medium.
- *Move on to Step 3b.*

3a. Mixing 1 mL of bioink with cells

MATERIAL

1 mL bioink

Cells in 100 µL suspension

3 mL syringes

Female/female Luer lock adapters

DESCRIPTION

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.
- Carefully pre-fill the Luer lock adaptor with the bioink and attach the bioink syringe to the syringe with cell suspension. This is to avoid introducing air when connecting the syringes.
- Carefully mix the bioink with the cell suspension by gently pushing the bioink back and forth between the syringes until a homogeneous mixture is achieved.

Note: If a bubble is detected, push the bubble to the Luer lock adaptor, disconnect the syringe and carefully push out the air by filling the Luer lock with bioink instead. Connect the syringe and continue the mixing.

- Transfer the cell containing bioink to a cartridge.
- *Video link for a detailed illustration on how to perform the mixing process:* https://www.youtube.com/watch?v=NmdOTNLrV-Q

3b. Mixing 3 mL of bioink with cells

MATERIAL

3 mL bioink

Cells in 300 µL suspension

CELLMIXER

DESCRIPTION

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. See Figure 1 for how to connect the different parts of the CELLMIXER.

- 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

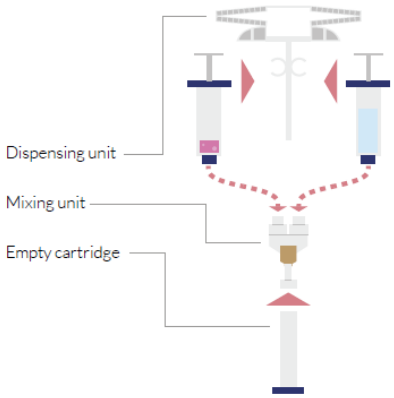

Figure 1. *Illustration of how to assemble the CELLMIXER components.*