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Date: 10-DEC-2024
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PROTOCOL FOR LIVE CELL IMAGING IN BIOINKS

CELL STAINING

Protocol aim

The aim of this protocol is to provide instructions for how to stain cells using CELLINK´s bioinks for experiments where tracking living cells over timer is needed, for example, for live cell imaging of 3D scaffolds. This protocol reduces preparation time of cells compared to the traditional workflow to stain cells with a fluorescent dye.

Materials needed

- Cells
- Live-cell imaging dye
- Bioink*
- Cell culture medium
- Sterile syringes with Luer lock connections
- Female/female Luer lock adaptor*
- Tip caps for syringes
- 3 mL cartridge(s)

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

Protocol

Live cell imaging of 3D scaffolds allows scientists to study biological processes overtime. Migration, interaction, and morphology of cells can be observed in real time. Altogether, this helps to gain a deeper understanding of the studied 3D tissue model. For live-cell imaging experiments, cells are stained or tagged with a fluorescent protein that helps with their visualization. If staining of cells is needed for 3D bioprinting experiments, the traditional workflow includes cell staining before mixing with the biomaterial. In our proposed workflow, the biomaterial is supplemented with the cell staining reagent, and cell staining starts once the cells are added to the mixture. This decreases the time needed to prepare the cells for the 3D bioprinting session, reducing the stress they are exposed to, and allows cells to be in longer contact with the dye increasing the time span to follow cells over time.

The following protocol was validated to stain 3 x 10^6 HEK 293 Linterna cells/ mL using 100 nM of MitoView 650 dye mixed with TeloCol 6 mg/mL. The cells were observed for 72 h after staining. The proposed workflow can be used to stain other types of cells with other live cell imaging dye reagents and other CELLINK biomaterials. Combining different bioinks, cells, and live-cell imaging dyes requires validation with appropriate controls to determine unspecific signal levels.

1. Mixing bioink with dye

MATERIAL

Live-cell imaging dye

Bioink

2 Sterile syringes with Luer lock connections

1 Luer lock adaptor

2 tip caps for syringes

DESCRIPTION

Preparation of live cell imaging dye

- Prepare the stock of live cell imaging reagent according to manufacturer´s instructions.
- Calculate the volume of reagent needed to obtain the desired concentration in the total volume of bioink.

  Note: It is recommended to try different dye concentrations to determine which gives better results under the studied experimental conditions.

Preparation of bioink and dye mixture

- Transfer the total volume of bioink needed for the experiment into a sterile syringe. Using a Luer lock adaptor, connect an empty syringe to the bioink containing syringe. Divide the total volume between the two syringes. For example, if the total volume of bioink is 1 mL, each syringe should contain approximately 500 µL of bioink.
- Detach one of the syringes and pipette the calculated volume of live-cell imaging dye into one of the syringes.
- Attach both syringes again and mix the bioink and the dye by moving the total volume from one syringe to the other. Make sure to synchronize the movement by pushing the plunger of one syringe and pulling the plunger of the other syringe at the same time, the movement should be fast but smooth to avoid bubble formation. Mixing 20 cycles (40 movements) is enough for most biomaterials, very viscous biomaterials might require extra mixing cycles.
- Divide the volume of the mixture between two syringes. Detach the syringes and place a tip cap on each of them. Determine if there are any bubbles present. In case of bubbles, it is necessary to further centrifuge the bioink to eliminate them before mixing with cells (see step 2); the speed and time of centrifugation depends on the biomaterial, ex. for TeloCol 6 mg/mL 200rpm for 30 s is enough.

  Note: It is important to keep in mind that CELLINK recommends a dilution of the bioinks 1:10 to keep the printability properties of the biomaterials. Therefore, the volume of the live cell imaging dye should be included in the volume of cell suspension to be mixed with the bioink.

2. Adding cells to bioink

MATERIAL

Cell pellet

Cell culture medium

Bioink mixture from step 1 in syringes

1 Luer lock adaptor

DESCRIPTION

Preparing cell suspension

- Resuspend the cell pellet containing the desired number of cells in the adequate volume of the cell culture medium. Take into account the final volume of bioink needed for the experiment. For example, if the total volume of bioink is 1 mL, resuspend cells in 100 µL of cell culture medium minus the volume of dye added to the bioink in step 1. This results in a dilution of 1:10 for the bioink.

Mixing cell suspension and bioink

- Use the bioink prepared in step 1. Remove the tip caps and place a Luer lock on one of the syringes.
- Add resuspended cells into one of the syringes.
- Attach both syringes using the Luer lock adaptor. Be careful to not introduce air. Mix by moving the total volume from one syringe to the other. Make sure to synchronize the movement by pushing the plunger of one syringe and pulling the plunger of the other syringe at the same time; the movement should be fast but smooth to avoid bubble formation. Mix using 20 cycles (40 movements).
- Transfer the total volume of the mixture into one of the syringes. Remove the empty syringe and keep the Luer lock adaptor on the syringe containing the bioink mixture.

3. Preparing for 3D bioprinting

MATERIAL

Bioink with cells in the syringe from step 2

Cartridge(s)

DESCRIPTION

Transferring the bioink containing cells into cartridges

- Use the mixture from step 2.
- Attach an empty cartridge to the syringe containing the bioink mixture using the Luer lock adaptor, make sure to do not introduce air at this step.
- Transfer the bioink mixture into the cartridge by pushing the syringe plunger. The cartridge capacity is 3 mL.
- The bioink is ready for the 3D bioprinting session, it contains the live-cell imaging dye and cells.
- Follow the recommended workflow for the bioprinting session according to the bioink used.