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CELL RECOVERY PROTOCOL

Cell Collect A

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

This protocol provides instructions for the use of Cell Collect A, an enzyme-based proprietary formulation, that allows degradation of 3D scaffolds created using ionically crosslinked alginate-containing bioinks. The proposed method allows the recovery of cells from 3D scaffolds – these cells can subsequently be used in different downstream applications.

Materials needed

- Cell Collect A*
- PBS (1X)
- Cell-laden bioprinted scaffolds
- Cell strainer (40-70 µm nylon)
- Centrifuge tubes that fit the selected cell strainer and have enough volume capacity

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

Protocol

This protocol has been optimized for the use of Cell Collect A reagent to degrade small volume droplets of CELLINK Bioink and ionically crosslinked GelXA Bioink. This protocol is intended to be used as a guide. It is always necessary to determine optimal experimental conditions for 3D scaffold degradation according to downstream applications, size and shape of the scaffolds. It is recommended to perform a preliminary acellular test to determine the best incubation time necessary to degrade the 3D constructs being studied. For scaffolds stabilized using dual crosslinking of the bioink, or in cases when the cultured cells secrete significant amounts of ECM, it is necessary to combine Cell Collect A with other enzymes to obtain a complete degradation.

1. Selecting the degradation conditions

MATERIAL

Cell-laden 3D constructs

DESCRIPTION

To select the incubation time for harvesting cells from 3D constructs, it is necessary to consider the size and shape of the bioprinted constructs, and incubation temperature. The incubation temperature should be decided according to the intended downstream applications: if slowing down cellular pathways is necessary, 4°C incubation should be considered. Table 1 shows incubation conditions for degradation of 15 µL bioink droplets.

Table 1. Recommended Cell Collect A conditions for complete degradation of bioink droplets.

Cell Collect A	Bioink droplet volume (µL)	Minimum time to digest at 4°C (min)	Minimum time to digest at 37°C (min)
10 x bioink volume*	10-15	180	60

*A volume ratio of 10:1 for Cell Collect A:Bioink is recommended. For example, to digest 15 µL droplets, it is recommended to use a minimum of 150 µL of Cell Collect A reagent. The final volume depends on experimental set-up since the reagent must cover the scaffold completely.

The conditions may require modifications if one of the following applies:

- High infill-density constructs without macroscopic pores will have slower enzyme diffusion and require longer incubation times.
- Larger 3D scaffolds would require longer incubation times. Alternatively, constructs should be cut into small pieces before starting the digestion.

2. Degrading the cell-laden 3D constructs

MATERIAL

Cell-laden bioprinted constructs

PBS (1X)

DESCRIPTION

- Remove the cell culture medium from the 3D construct(s) and wash with PBS.
- Add 10 times as much volume of Cell Collect A as the volume of bioink in the construct. Make sure that the solution covers the construct.
- Place the vessel containing the 3D constructs at the desired incubation temperature, refer to Table 1 for recommended incubation times at respective temperatures.
- After incubation time is finished, complete the disintegration by pipetting the Cell Collect A volume directly onto the 3D construct, repeat 3 to 5 times.

Note: Check digestion status every 20-30 min in the preliminary test by pipetting the volume of Cell Collect A onto the 3D constructs.

3. Isolating the cells

MATERIAL

Cell strainer

Centrifuge tube(s) that fit the selected cell strainer and have enough volume capacity

PBS (1X)

DESCRIPTION

- Place a cell strainer over a centrifuge tube and wet the bottom of the strainer with sterile PBS to facilitate flow-through.
- In the same tube, use the cell strainer to filter the digested 3D construct.

Note: After filtering the digested 3D construct, some residues of nanocellulose from the bioink might be present.

Note: Filtering decreases the final cell number, therefore, pooling replicates of the 3D constructs might be necessary depending on the desired downstream application.

4. Collecting cells

MATERIAL

Cell suspension from step 3

Centrifuge

DESCRIPTION

- Centrifuge the collected cell suspension according to recommended conditions for the specific cell type used.
- Remove supernatant.
- Cell pellet is ready to use for desired applications.
  Note: It might be necessary to wash the cell pellet to remove more impurities, this will increase cell loss.