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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, to enhance cell isolation or reduce viscosity of alginate-based bioinks. The proposed method allows for digesting of bioinks either fully or partially and harvesting cells that can subsequently be used for cell viability analyses, RNA isolation, protein extraction, western blots, and qPCR among other downstream applications. Under sterile conditions, recovered cells can be re-plated for culture or used for analysis.

Materials needed

- Cell Collect A*
- PBS (1X)
- Cell-laden bioprinted constructs
- Cell shaker
- 1 M NaOH (optional)
- Cell culture media (optional)
- Cell strainer (40-70 µm nylon)
- Centrifuge tubes

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

Protocol

1. Diluting Cell Collect A (optional)

MATERIAL

Cell Collect A

PBS (1X)

DESCRIPTION

- Dilute Cell Collect A in PBS to achieve desired concentration. See Table 1 for recommended concentrations.

Table 1. Recommended Cell Collect A concentrations for partial or complete digestion of bioink droplets.

Digestion	Cell Collect A dilution	Bioink droplet volume (µL)	Minimum time to digest at 4°C (min)
Partial**	Diluted more than twice	10-100	15–30
Complete	Non-diluted	10-25	15–30
		25-100***	30–60

**Partial bioink digestion may be desired for improved proliferation, porosity or delivery of small molecules. For this, diluted concentrations of Cell Collect A can be added to constructs. Adjust dilution according to application.
***For bioprinted constructs larger than 100 µL longer incubation times may be required.

2. Dissociation

MATERIAL

Cell-laden bioprinted constructs

Cell shaker

NaOH (optional)

PBS (1X) (optional)

Cell culture media (optional)

DESCRIPTION

- Remove the cell culture medium from construct(s).
- Add 10 times as much volume of Cell Collect A as the volume of bioink construct to desired well(s). Make sure that the solution covers the construct.
- Place the entire well plate on a cell shaker at 4°C for ~30 min, or until fully dissolved, see Table 1 recommended incubation times.

Note: Gentle disintegration with a large orifice pipette tip is required to fully break down constructs.

- For partial degradation, the degradation process can be inhibited after the desired degradation time by adding 1 M NaOH in a 1:10 volume ratio to initially added Cell Collect A, waiting for 15 min, washing once and then carefully replacing it with fresh media.

Note: To slow cell signalling pathways, it's recommended to use Cell Collect A at 4°C. If desired, 3D constructs in Cell Collect A can be incubated at room temperature or 37°C.

3. Cell isolation

MATERIAL

Cell strainer

Centrifuge tube(s)

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 dissolved ECM-cell suspension.

4. Centrifuge

DESCRIPTION

- Centrifuge the collected cell suspension at 200 *g* for 3-4 min.
- Remove supernatant.
- Cell pellet is ready to use for desired applications.