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3D SCAFFOLD DEGRADATION FOR CELL RECOVERY

CELLINK series bioinks

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 enzymatic digestion of 3D scaffolds printed using CELLINK series bioinks such as CELLINK Bioink, CELLINK RGD, CELLINK LAMININKs, CELLINK BONE and CELLINK SKIN/FIBRIN. It includes preparation and treatment conditions for Alginate Lyase, an enzyme that degrades ionically crosslinked alginate-containing bioinks. The proposed method allows the recovery of cells from 3D scaffolds for their subsequent use in different downstream applications.

Materials needed

- Alginate Lyase powder (≥10 000 U/g)*
- PBS (1X) solution
- Sterile filter setup
- Cell-laden bioprinted 3D constructs
- Cell strainer (40-70 µm nylon)
- Centrifuge tubes that fit the selected cell strainer and have enough volume capacity

*The recommended product version can be purchased at https://www.sigmaaldrich.com (catalog number A1603-100MG).

Protocol

This protocol has been optimized for the use of Alginate Lyase reagent to degrade bioprinted constructs created with CELLINK Bioink series. 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 constructs stabilized using dual crosslinking of the bioink (ex. CELLINK SKIN/FIBRIN), or in cases when the cultured cells secrete significant amounts of ECM, it is necessary to combine Alginate Lyase with a protease such as trypsin to obtain complete degradation

1. Preparation of Alginate Lyase enzyme solution

MATERIAL

Alginate Lyase powder (≥10 000 U/g)

PBS (1X) solution

Sterile filter setup (50 mL syringe + filter for ≤50 mL, funnel + filter + bottle for volumes >50 mL)

DESCRIPTION

Alginate Lyase is hazardous and should not be inhaled. Read SDS before use and work with proper PPE and in a flow hood.

- Per 50 mL of PBS solution add 5 mg of Alginate Lyase powder.
- Place on shaker until dissolved.
- Bring the sterile filter setup into the hood and sterile filter the enzyme solution.
- Store the solution at 4-8°C for further use (for up to 4 months).

Note: Always sterile filter the solution immediately after preparation to avoid growth of microbes.

2. Digestion of 3D constructs

MATERIAL

Cell-laden 3D constructs

Alginate Lyase solution from step 1, refrigerated aliquot or freshly prepared

Trypsin 10X (only relevant for CELLINK SKIN/FIBRIN)

HBSS (1X) or PBS (1X) without calcium

DESCRIPTION

The size and shape of constructs affect digestion enzyme accessibility: large dense structures require longer time than small and/or porous structures, e.g. grids. For this reason, it is recommended to cut constructs into 1 mm^3 pieces before digestion. However, there is inherent damage to the cells caused by cutting the construct. It is important to have this in mind while optimizing the experimental conditions to find a balance between reduced cell viability due to cutting and the incubation time for enzymatic treatment. 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. Values in Table 1 can be used as a reference.

- Remove cell culture medium from construct(s). Wash construct(s) twice using either HBSS or PBS.
- Using a scalpel, cut the 3D constructs into small pieces, aim for 1 mm^3 size. It is recommended to transfer the constructs to a glass surface to perform the cutting step to avoid plastic debris. The smaller the pieces the faster the digestion would occur.
- Add enough volume of Alginate Lyase solution to cover the construct(s).
- Incubate the samples following the recommended incubation times in Table 1. Adjust incubation time if needed. For CELLINK SKIN/FIBRIN bioinks, after the initial Alginate Lyase (AL) incubation, add 10X

Trypsin (V_T=0.1V_{AL}) directly to the Alginate Lyase solution digesting the constructs (e.g. 48-well with 270 µL pure Alginate Lyase for 30 min, then add 30 µL 10X Trypsin for final 300 µL volume and additional 10 min incubation)

- Pipette the solution up and down directly onto the 3D construct pieces to complete the disintegration.

Table 1. Recommended Alginate Lyase (and Trypsin) conditions for complete degradation of bioink droplets.

Bioink	Construct volume/shape	Digestion solution	Incubation time (minutes)
CELLINK Bioink series	10-15 µL droplet	Alginate Lyase	60 (37°C) 180 (4°C)
CELLINK Bioink series	Discs Ø 8 mm h 0.5 mm, cut to ≈1 mm^3	Alginate Lyase	30 (37°C)
CELLINK SKIN/FIBRIN	Discs Ø 8 mm h 0.5 mm, cut to ≈1 mm^3	Pure Alginate Lyase first, then mix with Trypsin (V_T=0.1V_{AL})	30 + 10 (37°C)

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

3. Cell isolation

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.