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3D SCAFFOLD DEGRADATION FOR CELL RECOVERY

GelMA Bioink, GelMA FIBRIN & GelMA C

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

This protocol provides instructions for the enzymatic digestion of 3D scaffolds printed using gelatin-based bioinks such as GelMA Bioink, GelMA C and GelMA FIBRIN. It includes preparation and treatment conditions for Cell Collect G (collagenase enzyme). The proposed protocol allows the recovery of cells from 3D scaffolds for their subsequent use in different downstream applications.

Materials needed

- Cell Collect G*
- 50 mL HBSS (1X) or PBS (1X) without calcium
- Light-protected 50 mL conical tube
- Sterile syringe filter, 0.20 µm
- Tip cap for syringe
- Cell-laden bioprinted constructs (GelMA Bioink, GelMA FIBRIN or GelMA C)

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

Protocol

This protocol has been optimized for the digestion of 3D constructs generated by photocrosslinking GelMA Bioink and GelMA C using Cell Collect G. For GelMA FIBRIN that received thrombin-crosslinking in addition to photocrosslinking, addition of Trypsin to the Cell Collect G solution can increase digestion efficiency. This protocol is intended to be used as a guide. It is always necessary to determine optimal experimental conditions for 3D construct degradation according to downstream applications and experimental setup. It is recommended to perform a preliminary acellular test to determine the best incubation time necessary to degrade the 3D constructs being studied and to get familiar with the protocol procedure.

1. Preparation of Cell Collect G solution

MATERIAL

Vial containing Cell Collect G lyophilizate

50 mL HBSS (1X) or PBS (1X)

Light-protected 50 mL conical tube

Light-protected tubes for aliquoting

50 mL syringe

Tip cap for syringe

Sterile syringe filter, 0.20 µm

DESCRIPTION

Cell Collect G is hazardous and should not be inhaled, ingested or come in contact with eyes or skin. Read SDS before use and work with proper PPE and in a flow hood.

- Remove the syringe plunger from the syringe, close the syringe using the tip cap and place the syringe vertically with the tip facing down. Use a rack for 50 mL conical tubes to keep the syringe in this position.
- Add 45 mL of HBSS (1X) or PBS (1X) into the syringe.
- Gently remove the rubber cap from the vial. The inside will be covered with powder and should carefully be placed facing up on the work bench.
- Add 1 mL of HBSS (1X) or PBS (1X) into the vial containing the Cell Collect G powder, release the volume onto the vial wall to bring down all the powder. Pipette up and down to reconstitute the solution. Transfer the solution into the 50 mL syringe containing previously added 45 mL of HBSS (1X) or PBS (1X), pipette up and down to rinse the pipette tip. Repeat this procedure four times to make sure all powder has been collected (using a total of 5 mL HBSS (1X) or PBS (1X)). After the final addition of buffer into the vial, the rubber lid can be returned and the vial gently inverted to retrieve the remaining powder, before final transfer to the 50 mL syringe.
- The total volume in the syringe should be 50 mL after adding the reconstituted Cell Collect G.
- Place the plunger back into the syringe. Flip the syringe, remove the tip cap and place the sterile syringe filter on the syringe. Keep vertically with tip facing up to avoid losing volume.
- Press the plunger to remove the air. There is a “pop” sound before the plunger starts moving.
- Filter the solution into a light-protected 50 mL conical tube by pressing the plunger to make the reconstituted Cell Collect G pass through the filter.

Note: Store reconstituted Cell Collect G not used immediately as aliquots in light-protected conical tubes at -20°C. After longer storage, up to one week, a decrease in enzyme activity can be expected and might require slightly longer degradation times. Thaw aliquot of Cell Collect G solution in fridge and avoid repeated freeze-thaw cycles.

2. Digestion of photocrosslinked 3D constructs

MATERIAL

Cell-laden 3D bioprinted constructs

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

Cell Collect G solution from step 1, frozen aliquot or freshly prepared.

DESCRIPTION

Photocrosslinking time affects the scaffold network density: denser networks require longer incubation times. Likewise, the size and shape of the constructs affects digestion enzyme accessibility: large dense structures require longer times than small and/or porous structures, e.g. grids. Values in Table 1 can be used as a reference.

- If frozen Cell Collect G solution is to be used, thaw aliquot in the fridge, make sure it is completely thawed before starting the digestion. If relevant, dilute Cell Collect G to 50% in PBS (Table 1). Keep in the fridge until used.
- 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 Cell Collect G to cover the construct(s).
- Incubate the samples at 37°C following the recommended incubation times in Table 1. Adjust incubation time if needed. For GelMA FIBRIN, after the initial Cell Collect G incubation, add 10X Trypsin (V_T=0.1$V_{CCG50\%}$) directly to the Cell Collect G solution digesting the constructs (e.g. 48-well with 270 µL Cell Collect G 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 enzymatic treatment incubation times using Cell Collect G (CCG) for GelMA Bioink, GelMA FIBRIN or GelMA C, photocrosslinked with 405 nm for 15 s at 4 cm distance with print-bed set to 22°C.

Bioink	Digestion solution	Construct volume/shape	Incubation time (minutes)
GelMA Bioink	CCG 100%	15-20 µL droplet	120
	CCG 100%	Discs Ø 8 mm h 0.5 mm cut to ≈2.5 mm^3	105
	CCG 100%	Discs Ø 8 mm h 0.5 mm cut to ≈1 mm^3	90
GelMA C	CCG 50%	Discs Ø 8 mm h 0.5 mm, cut to ≈1 mm^3	30
GelMA FIBRIN	CCG 100%	Discs Ø 8 mm h 0.5 mm, cut to ≈1 mm^3	75
	CCG 50% first, then mix with Trypsin (V_T=0.1$V_{CCG50\%}$)	Discs Ø 8 mm h 0.5 mm, cut to ≈1 mm^3	30 + 10

Note: There is an 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 the enzymatic treatment.

3. Cell isolation

MATERIAL

HBSS (1X) or PBS (1X)

Centrifuge tube(s)

DESCRIPTION

- Centrifuge the digested 3D construct at 200 *g* for 3-4 min.
- Remove supernatant.
- Add HBSS (1X) or PBS (1X) to resuspend the cell pellet.
- Centrifuge at 200 g for 3-4 min.
- Remove supernatant.

Cell pellet is ready to use for desired applications.