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CELLINK
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3D SCAFFOLD DEGRADATION FOR CELL RECOVERY

Photoinks GelMA 95%, PhotoGel®-INK 50% & 95%

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 with gelatin-based photoinks such as GelMA 95% DS (using LUMEN X), PhotoGel®-INK 50% DS & PhotoGel®-INK 95% DS (using BIONOVA X). 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 95% photoink, PhotoGel®-INK 50% or PhotoGel®-INK 95%)

*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 light-based printing following the Bioprinting Protocol GelMA 95% DS LUMEN X and Bioprinting Protocol PhotoGel-INKs BIONOVA X. 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 (that was 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.

Plastic spatula (sterile) to transfer samples

DESCRIPTION

Photocrosslinking time affects the scaffold network density: denser networks require longer incubation times. Likewise, the size and shape of the constructs affect digestion enzyme accessibility: large dense structures require longer time than small and/or porous structures, e.g. grids. Values in **Tables 1** & **2** 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. 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 1-2 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** For GelMA 95% and **Table 2** for PhotoGel®-INK 50% or 95%. Adjust incubation time if needed.
- Pipette the solution up and down directly onto the 3D construct pieces to complete the disintegration. Doing so intermittently can speed up degradation and shorten required degradation times.

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.

Table 1. Recommended enzymatic treatment incubation times using Cell Collect G solution for discs of 5 mm diameter and 1 mm height generated by DLP bioprinting following the LUMEN X protocol for photoink GelMA 95% using fixed build platform adhesion exposure time of 10 s and 70% intensity (~20 mW/cm^2).

Bioink	Layer height (µm)	Exposure time (s)	Construct shape	Incubation time (min)
GelMA 95% 7.5% (w/v)	100	10.0	solid disc	105
			cut to ~1-2 mm^3	45
	50	7.0	solid disc	105
			cut to ~1-2 mm^3	45
GelMA 95% 10% (w/v)	100	8.0	solid disc	210*
			cut to ~1-2 mm^3	100
	50	5.5	solid disc	240*
			cut to ~1-2 mm^3	120

*Long incubation times required for solid constructs can negatively affect cell viability and gene transcription. It is recommended to avoid them by cutting samples or including pores in the design to increase accessibility for the enzyme.

Table 2. Recommended enzymatic treatment incubation times using Cell Collect G solution for discs of 5 mm diameter and 1 mm height generated by light-based printing following the BIONOVA X protocol for PhotoGel®-INK 50% or 95%. Disc with pores refers to a structure with maximum distance of 250 µm between pores.

Bioink	Print mode	Intensity	Time or speed	Construct volume/shape	Incubation time (min)
PhotoGel®-INK 50%	1 layer	70%	25 s	solid disc	30
				cut to ≈1-2 mm^3	20
				disc with pores	20
	continuous	100%	0.008 mm/s	solid disc	40
				cut to ≈1-2 mm^3	20
PhotoGel®-INK 95%	1 layer	70%	15 s	solid disc	100
				cut to ≈1-2 mm^3	60
				disc with pores	60
	continuous	100%	0.015 mm/s	solid disc	100
				cut to ≈1-2 mm^3	60

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.