Ref No: PPR-TP60505
Approval date: 01-SEPT-2025
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PRINTING PROTOCOL

PCL

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

The aim of this protocol is to provide instructions for printing biocompatible and biodegradable 3D structures with CELLINK's polycaprolactone (PCL). PCL can be extruded through both heating and mixing with a solvent. This protocol has been optimized for heat extrusion at 200°C using the Thermoplastic printhead and the BIO X but can also be extruded using the aluminum cartridge and tips with the INKREDIBLE+.

Materials needed

- CELLINK PCL*
- Thermoplastic printhead*
- BIO X*
- Petri dish*
- Thermoplastic high-precision nozzle*

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

Protocol

1. Filling cartridge with PCL

MATERIAL

PCL

Thermoplastic printhead

DESCRIPTION

- Load the cartridge as recommended in the *Thermoplastic Printhead User Manual*.

Note: To ensure the most efficient heating of PCL, fill maximum a quarter of the cartridge with PCL (*ca.* 80 pellets).

Note: If mixing with a solvent, ensure that the solvent is compatible with the Thermoplastic printhead (BIO X) or the stainless-steel cartridge, plunger, and the tips (INKREDIBLE+).

2. Pre-heating

MATERIAL

BIO X

DESCRIPTION

- Attach the Thermoplastic printhead to the BIO X and start pre-heating at 200°C. Heat for 90 min until the PCL is fully melted and homogenized.

3. Printing

MATERIAL

BIO X

Petri dish

DESCRIPTION

Printing with the 400 µm thermoplastic nozzle:

- Set layer height at 80% of the nozzle diameter. Start with a printing temperature of 200°C and the pressures shown in Figure 1.

Note: Due to the need to fuse filaments of successive layers together, it is recommended to use a layer height that is smaller than the nozzle diameter. This is to allow the filaments to fuse and to account for shrinkage of the layers during the cooling process.

Note: If PCL is extruding inconsistently, the tip may be dirty. Wipe the tip with steel wool to remove the excess polymer. Re-start from step 1, then flush the nozzle with new PCL from the printer overview page or the utilities menu. If necessary, run a calibration script to reoptimize the printing characteristics (pressure and speed).

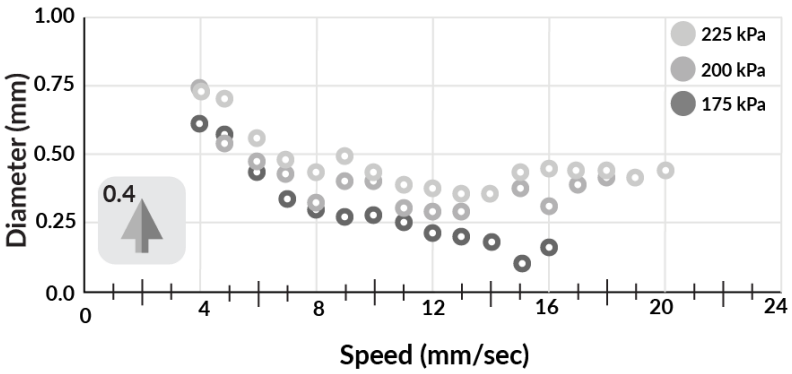

Figure 1. *PCL filament thickness dependency on printing speed and pressure when extruded at 200°C.*

Printing with the 150 µm thermoplastic high-precision nozzle:

- Set the first layer height at 100% of the nozzle diameter, the rest of the layers can be set at 66% of the nozzle diameter. Start with a printing temperature of 200°C and the pressure of 200 kPa to 500 kPa using external compressor (see Table 1).

Note: Due to the need to fuse filaments of successive layers together, it is recommended to use a layer height that is smaller than the nozzle diameter (except for the first layer). This is to allow the filaments to fuse and to account for shrinkage of the layers during the cooling process.

Note: For the initial extrusion, temporarily set the external compressor to a high pressure of 700 kPa. Activate the extrusion and continue only until the material flows from the printhead as a stable, continuous filament. Immediately after that, you MUST stop extrusion and reduce the pressure to the operational printing range (200-500 kPa) before starting your print. For a visual guide, please refer to the accompanying video.

Note: If you observe that the extruded filament is discontinuous during a print, key parameters such as speed, pressure, and layer height can be adjusted in real-time. Access the *Utilities* menu while the print is active and modify these settings in small increments to fine-tune the extrusion until a stable, continuous line is achieved (Figure 2). The primary goal is to extrude a continuous filament whose measured width precisely matches the nozzle's internal diameter. The ink may change its properties when heated over time, resulting in a decreased viscosity and lower printing pressures, ex. going from 500 to 200 kPa during printing. If the PCL is turning brown, the temperature has been too high for too long. Replace the PCL in the cartridge then.

Note: If PCL is extruding inconsistently, the tip may be dirty. Wipe the tip with steel wool to remove the excess polymer. Re-start from step 1, then flush the nozzle with new PCL from the printer overview page or the utilities menu. If necessary, run the Pressure/Speed calibration Gcode scripts to reoptimize the printing characteristics, (speed and pressure).

Note: Before starting, use the provided clamps to firmly secure the Petri dish to the print bed. This prevents shifting during printing, which can cause layer misalignment and print failure. For a detailed guide on proper clamp placement, please refer to the "Printing Setup" video.

Table 1. *Bioprinting parameters for PCL with a 150 µm nozzle using Model Print function and G-code on BIO X.*

Printing parameters	
Surface	*Petri dish*
Print bed temperature	*30°C*
Printhead temperature	*200°C*
Speed	*7- 12 mm/s*
Pressure	*200 kPa-500 kPa (external compressor)*
Z-offset	*0.150 mm*

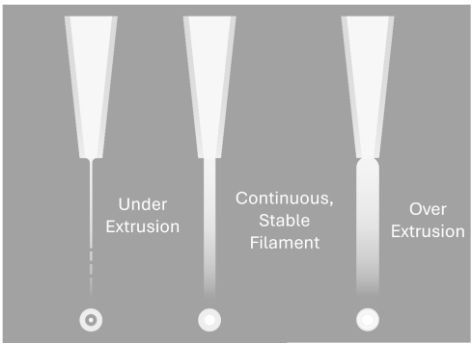

Figure 21. *Nozzle fidelity and characterization. Left – under-extrusion, middle – optimal extrusion, right – over-extrusion.*

4. Solidification

DESCRIPTION

- Let PCL cool down and solidify after printing. Do not touch the PCL construct until it is completely cooled as this can deform the structure.

Note: The temperature-controlled print bed on the BIO X can be used to accelerate or slow down this cooling process. Sintering of PCL filaments is possible. If a PCL/solvent mixture is printed, make sure that the resulting construct is placed in a well-ventilated area for drying to ensure the solvent is removed prior to cell seeding.

Note: If the structure contracts inwards during the printing process, there may be a need for additional cooling between successive layers. Modify the G-code to pause the print for 1 min between layers to allow solidification of already deposited material.

5. Procedure completion and cleanup

Follow these steps after the print is complete to ensure the equipment is properly shut down, cleaned, and materials are stored correctly. The procedure is ordered to perform hot tasks first, followed by a full system cooldown and final cleaning.

DESCRIPTION

Immediate post-print procedure

- Immediately after finishing the print, purge a small amount of PCL through the nozzle while it is still hot to clear it of any residual material and prevent clogging.

Printer shutdown

- After purging the nozzle, use the BIO X software interface to turn off the heating for all components. Set the target temperatures for the thermoplastic printhead and print bed to "OFF" or room temperature.
- Allow the printhead and print bed to cool down completely to a safe handling temperature before proceeding. This may take several minutes.
- Once cooled, carefully remove the PCL cartridge from the printhead.
- Power down the BIO X bioprinter using the main power switch.

Printhead and workspace cleaning

- After the printhead has cooled, carefully wipe any external polymer residue from the nozzle tip using a lint-free cloth. For stubborn residue, the nozzle can be removed and cleaned more thoroughly according to the manufacturer's guidelines and *Thermoplastic Printhead User Manual.*
- Wipe down the print bed and surrounding surfaces with 70% ethanol to maintain a clean environment.

Material storage

- Store the PCL pellets in their original container inside a sealed bag in the fridge at 2-10°C, as recommended by the manufacturer.
- Ensure the container and the bag are tightly sealed to protect the polymer from moisture, which can cause degradation over time.

6. Troubleshooting guide

DESCRIPTION

To ensure the highest quality and consistency for your PCL constructs, please follow these best practices. This guide also provides solutions for common issues that may arise during printing.

Observed problem	Potential cause(s)	Recommended solution(s)
PCL FILAMENT QUALITY		
Filament is discontinuous, inconsistent, too thin, or breaks (under-extrusion).	1. **Parameter mismatch:** The extrusion pressure is too low for the set printing speed. The print height is too far from the surface. 2. **Dirty/clogged nozzle:** Polymer residue on the nozzle tip is obstructing the flow.	1. **Real-time adjustment:** While the print is active, access the Utilities menu. Increase the pressure in small increments (e.g., 5-10 kPa) or adjust print height until a stable, continuous filament is achieved. 2. **Clean nozzle:** Pause the print. Carefully wipe the nozzle tip with steel wool to remove any built-up polymer. If the problem persists, flush the nozzle with fresh PCL from the Utilities menu.
Filament is significantly wider than the nozzle diameter (over-extrusion).	**Parameter mismatch:** The extrusion pressure is too high for the set printing speed. The print height is too close to the surface.	**Real-time adjustment:** While the print is active, access the Utilities menu. Decrease the pressure in small increments or adjust print height until the extruded filament width matches the nozzle diameter.
PCL MATERIAL CONDITION		
PCL extrudes with a brown or yellowish discoloration.	**Temperature too high:** The PCL is being heated above its degradation point, causing it to scorch.	**Lower the temperature:** Lower the printhead temperature in 5°C increments. If the PCL in the cartridge is already coloured, replace the cartridge with fresh PCL to avoid printing degraded material.
Extruded PCL filament is brittle and snaps easily, lacking its usual flexibility.	**Thermal degradation of the polymer:** This is typically caused by: 1. **Overheating:** The printhead temperature is set too high. 2. **Prolonged heating:** The PCL has been kept molten for an excessive amount of time (e.g., several hours/days).	1. **Immediate action (purge and replace):** Discard the degraded PCL. If the cartridge has been heated for a long time, replace it with a fresh cartridge. 2. **Preventative action (parameter adjustment):** • **Verify and reduce temperature:** Confirm the printhead temperature is set correctly (e.g., 200°C). Lower it in 5°C increments if needed. • **Minimize idle heating time:** Plan your printing session to avoid leaving the PCL molten in the printhead for extended periods.
PRINTED STRUCTURE FIDELITY		
The printed structure warps or contracts inwards as it builds.	**Insufficient cooling:** The heat from newly deposited layers is causing lower, still-warm layers to deform.	**Add pause between layers:** Modify the G-code to introduce a cooling pause. At the end of the G-code for each layer, add the command **G4 S60**. This will pause all activity for 60 seconds, allowing the layer to cool and solidify before the next one is printed.