Printing Protocol

PCL

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

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 180° 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

- PCL*
- Thermoplastic Printhead*
- BIO X*
- Well plate or Petri dish*

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

Protocol

Step	Title	Material	Description
1	Filling cartridge with PCL	- PCL - Thermoplastic Printhead	- Load the cartridge as recommended in the *THERMOPLASTIC Printhead Manual*. Note: To ensure the most efficient heating of PCL, fill maximum half of the cartridge with PCL. 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	- BIO X	- Attach the Thermoplastic Printhead to the BIO X and start pre-heating at 180°C. Heat for 15 min until the PCL is melted.
3	Printing	- BIO X	- Set layer height at 80% of the nozzle diameter. Start with a printing temperature of 180°C and the pressures showed 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. Please wipe the tip with steel wool to remove the excess polymer. If the PCL is turning brown, the temperature is too high. Lower the temperature or replace the PCL in the cartridge. 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.
4	Solidification		- Let PCL cool down and solidify after printing. Do not touch the PCL construct until it is completely cooled: 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.

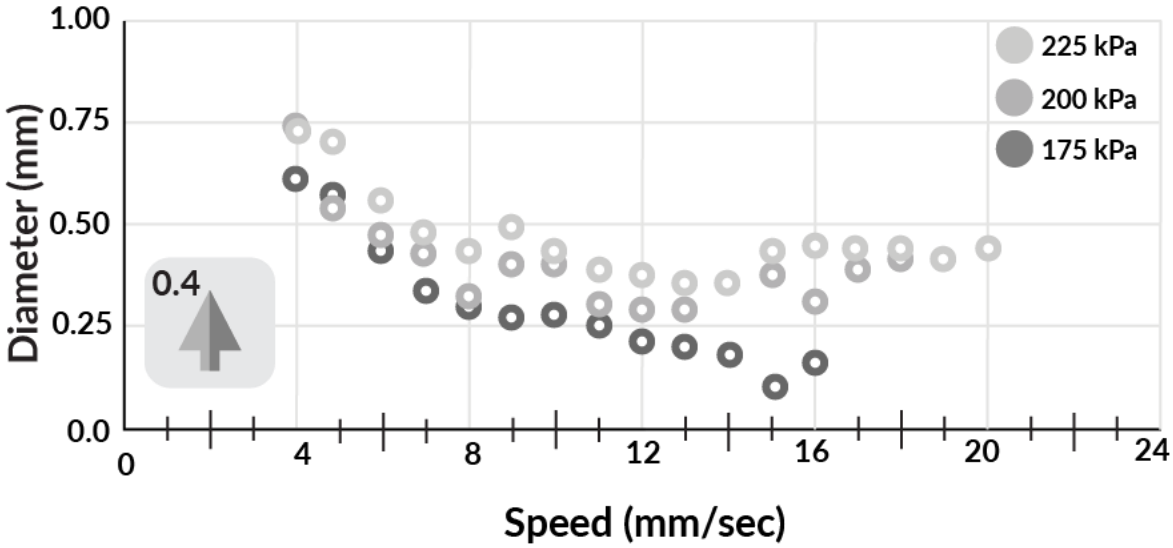

Figure 1. PCL filament thickness dependency on printing speed and pressure when extruded at 180°C.