BioScaffolder
Tools at a Glance

3D-Printing & Nanolitre Pipetting in one Process

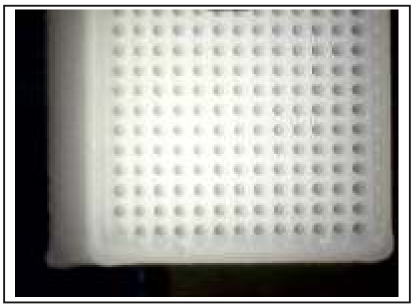
3D-Printing of BioInks & Polymer Melts

Polycaprolactone (PCL), polylactic acid (PLA) and similar polymers are bio-degradable, with melting points between 60 and 200 °C. They degrade in a physiological environment and are therefore attractive for a potential use as implantable bio-matrices. The BioScaffolders' cartridge-based printing tools accept a wide variety of granules / powders of such materials and blends.

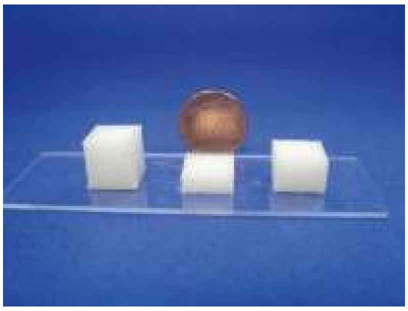
3D bioprinting for tissue engineering requires porous objects co-printed from very different materials. Both a stiff polymer matrix providing the stable 3D scaffold (free 3D forms are possible) and a bioink of lower viscosity containing living cells, unable to shape 3D parts by itself, are required.

The GeSiM BioScaffolder platform enables different approaches for co-printing:

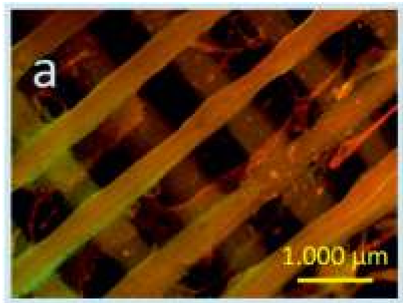
– Printing from up to three cartridges (optional: with different nozzles at different temperatures)
– Coaxial printing of two different materials with core/shell dispensers
– Application of e.g. cell suspensions or protein samples by nanolitre pipetting



Cubic PCL scaffold structure:
Edge length: 10 mm
Layer height: 50 µm
Number of layers: 200
Nozzle diameter: 250 µm



Cubes of PCL at a molecular weight of 14000 g/mole. Cubes have X/Y dimensions of 10 mm by 10 mm. The heights are 10 mm, 3 mm and 7 mm (left to right).



Fluorescence microscope image of a bioscaffold co-printed from PCL-PEG and ADA-GEL (alginate dialdehyde gelatin hydrogel) loaded with a stromal cell line (St2) [1]

Tools for Bulk Dispensing and 3D Printing

Tool	Printing by...	Function / Specs	Reservoir / Volume	Porosity / Interior Patterns for 3D	Viscosity Range	Use with...
High-power syringe extruder	Displacement force by motor-driven syringe plunger	Stainless steel cartridge/nozzle; manual filling/cleaning, dosage pressure > 100 bar, T < 250 °C (482 °F), two-zone heater	10 ml	Solid lines, grids, curves; width depends on nozzle size	PCL80 (12600 Pa·s) at 70 °C was succesfully printed	Thermoplastic materials such as PCL, PLA, PLGA (blends), etc. with appropriate melting point
Pneumatic cartridge dispenser w/o heater	Compressed air, CO_2, nitrogen or other inert gases	Disposable cartridge, disposable nozzle (e.g Nordson EFD); manual filling, dispensing pressure 500 … 600 kPa	30 ml	Solid lines, grids, curves, meanders; line width 100 … 400 µm (depends on nozzle and material)	Approx. 550 Pa·s (Na-alginate 16% at RT)	Hydrogels, alginate, hydroxyapatite, ceramic pastes, calcium phosphate cement etc.
Pneumatic cartridge dispenser with shell heater	Compmressed air, CO_2, nitrogen or other inert gases	Stainless steel cartridge/nozzle; manual filling, dispense pressure 500 … 600 kPa, temperature up to 100 °C or 190 °C	10 ml	Solid lines, grids, curves, meanders; line width 100 … 400 µm (depends on nozzle and material)	Approx. 550 Pa·s (16% Na-alginate at RT), approx. 1350 Pa·s (PCL50 at 100 °C)	Thermoplastic materials such as PCL, PCL-PEG blends etc. with appropriate melting point, bioinks with cells at 37 °C
Pneumatic cartridge dispenser with Peltier cooler	Compressed air, CO_2, nitrogen or other inert gases	Disposable cartridge, disposable nozzle (e.g. Nordson EFD); manual filling, dispense pressure 500 … 600 kPa, $\Delta T \approx -20$ K	10 ml	Solid lines, grids, curves, meanders; line width 100 … 400 µm (depends on nozzle and material)	Similar to pneumatic cartridge dispenser w/o heater	Hydrogels
Pneumatic core/shelll dispenser with replacable pair of nozzles	Compressed air, CO_2, nitrogen or other inert gases	Two separate disposable cartridges, disposable nozzles (E.g. Nordson EFD); manual filling, dispense pressure 500 … 600 kPa	2 x 10 ml	Coaxially printed two-component strands („macaroni style" or hollow fibres), lines, grids, curves	Smaller than for pneumatic dispenser	Pair of two materials (core/shell), e.g. hydrogels, alginate, hydroxyapatite, ceramic pastes etc.
Melt electrospinning writing	Electrostatic force and pressurized air, nitrogen or other inert gases	Stainless steel cartridge/nozzle; manual filling, dispense pressure 500 … 600 kPa T up to 100 °C or 190 °C, voltage ± 10 … 30 kV	10 ml	Solid lines, grids, meanders; line width 10 … 20 µm for regular patterns	> 10000 Pa·s	Thermoplastic materials, e.g. PCL, PCL-PEG blends, with appropriate melting point
FDM extruder	Mechanical force (displacement/extrusion) and melting	Wire drawn from a coil and melted	Filament coils	Solid lines, grids, meanders; line width 70 … 400 µm	Not applicable	Commercially available filaments with appropriate melting point

1 bar = 14.5 psi

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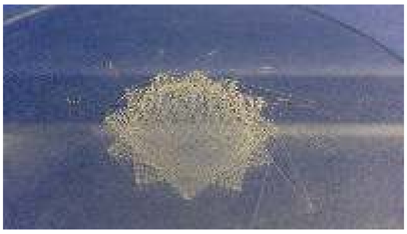
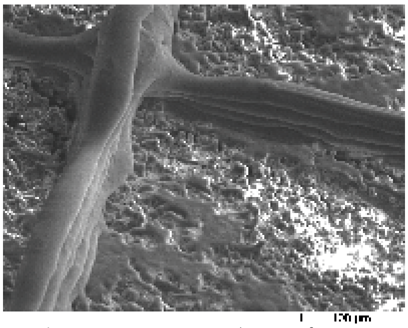
Melt Electrospinning Writing

The Melt Electrospinning Writing Module (MES) uses electrical charge to draw very thin fibres, typically in the micrometre range, from a liquid or polymer melt. Depending on the experimental set-up, arbitrary and regular patterns can be generated.

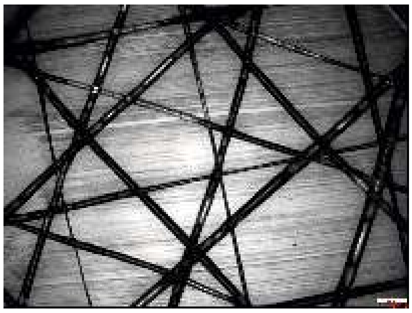


The MES module for the BS3.x contains a high-voltage generator and a special substrate support. Special dispense nozzles and metal cartridges are required.



SEM image, PCL 14,000, 15 kV, 100 °C, strut width is between 20 and 40 µm

The spun scaffold consists of stacked layers, each rotated at 30 degrees. Printing was done with PCL 14,000 at 100 °C and 10 kV.



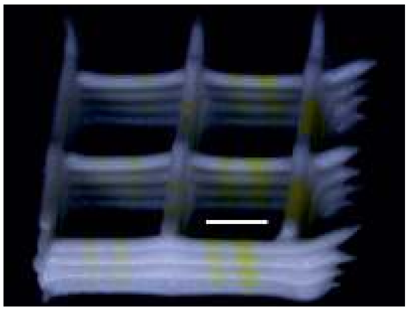
Bottom layer with 20 µm strands, top layer with 100 µm strands

Nanolitre Pipetting

The pipetting module enables the partial functionalization of 3D-printed structures by applying nanolitre amounts of cell suspensions or protein solutions.

Alternatively, micro-scaffolds from curable liquid samples are feasible.

On the right: Fluorescein-labelled dots (green) printed at defined XY positions on a scaffold that was printed from oil-based CPC (calcium-phoshate cement) paste [2]



Tools for Liquid and Powder Pipetting

Tool	Printing by…	Function / Specs	Reservoir / Volume	Interior Patterns for 3D Objects	Viscosity Range	Use with…
Piezoelectric pipette (optionally heatable) or Twin-Tip	Ultrasonic wave; Twin-Tip pipette allows kinetic mixing	Pipettor(s) with wash system, drop-on-demand dispensing, single drop volume 100 … 400 picolitres, automatic fill-up, T < 100 °C	96 well microtitre plate, max. 120 µl/well	Single spots of at least 80 µm, arrays, lines	Up to 10 mPa·s	All liquid samples, e.g. protein solutions, cell suspensions, solved polymers (two-component systems)
Solenoid valve pipette	Solenoid valve and pressurized air	Pipettor with wash system, drop-on-demand dispensing, single pulse 60 nanolitres, range up to microlitres, automatic fill-up	96 well microtitre plate, max. 120 µl/well	Single spots, arrays, lines	Up to 40 mPa·s	All liquid samples, e.g. protein solutions, cell suspensions, dissolved polymers
Passive pipette tips (Metal/Teflon-coated)	Diluter syringe displacement	Pipettor with wash system, µl range, automatic fill-up	96 well microtitre plate, max. 120 µl/well	Bulk dispensing of ml volumes on printed patterns	Liquids (depends on tip size)	All liquid samples, e.g. protein solutions, cell suspensions, dissolved polymers
Piezoelectric dispense valves (OEM components)	Piezoelectric valve and pressurized air	Cartridge dispenser, drop-on-demand dispensing, drop volume in the nanolitre range (also heatable)	3 ml	Single spots, arrays, lines	Approx. 50 … 200.000 mPa·s	Highly viscous liquids, e.g. glue, dissolved polymers
Powder pipette	Vacuum, compressed air	Aspiration / dispensing of powder aliquots in the µg range	min. 1 ml	Spots	Solid materials	Granular materials and powder

Optical (OEM) Components

OmniCure S1500: 200 W mercury UV lamp with selectable filters covering wavelengths from 250 nm to 500 nm. Typical irradiation is in the range of 6 … 28 W/cm².	Camera with a wide range of lenses at different magnifications

References:

[1] Tobias Zehnder, Tim Freund, Merve Demir, Rainer Detsch, Aldo R. Boccaccini: Fabrication of cell-loaded two-phase 3D constructs for tissue engineering, Materials 2016, 9, 887 (Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nürnberg, Germany)

[2] Stefan Giron, Anja Lode, Michael Gelinsky: In situ functionalization of scaffolds during extrusion-based 3D plotting using a piezoelectric nanoliter pipette, J. 3D Print. Med., 2017, 1, 25 (Centre for Translational Bone, Joint & Soft Tissue Research, University Hospital Carl Gustav Carus & Faculty of Medicine, TU Dresden, Germany)