BioScaffolder Tools at a Glance

3D-Printing & Nanoliter Pipetting in one Process

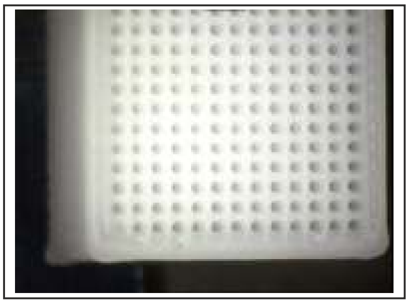
3D-Printing of BioInks & Polymer Melts

Polycaprolactone (PCL), Polylactic acid (PLA) and derivatives are biodegradable polymers with melting points between 60°C and 200°C. They degrade in a physiological environment (such as in the human body) and are therefore attractive for potential use as implantable biomatrices. The BioScaffolder's cartridge based print tools accept a wide variety of granules/ powders of such materials and blends.

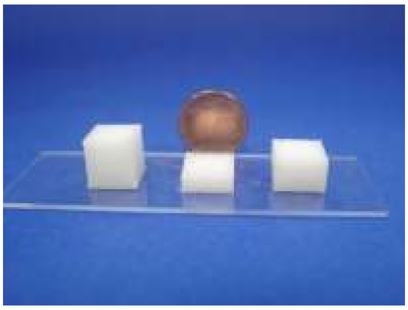
3D bioprinting for tissue engineering requires porous objects co-printed from very different materials. Both a stiff polymer matrix providing the 3D scaffold (Free 3D forms are possible) and a much lower viscosity bioink containing living cells, unable to shape 3D parts by itself, are required.

The GeSiM BS3.x platform enables three different approaches for co-printing:

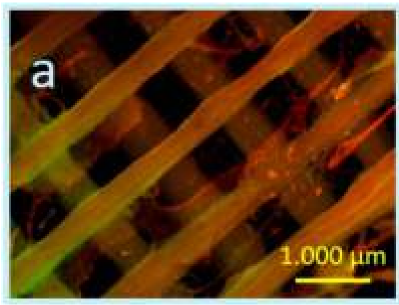
i) Printing from two or three cartridges with different nozzles (at different temperatures)
ii) Innovative coaxial printing of two different materials with Core/Shell dispensers
iii) Application of cell suspensions or protein samples by Nanoliter pipetting



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 14,000. The cubes have X, Y dimensions of 10 mm by 10 mm. The heights of these examples 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 gelatine hydrogel) loaded with a stromal cell line (St2) [1]

Tools for Bulk Dispensing and 3D Printing

Tool	Material Application by...	Function/ Specs	Reservoir/ Volume	Porosity/ Interior Patterns for 3D Objects	Viscosity Range	For Use with...
High-Power Syringe Extruder (from Q4 2017)	Displacement force by motor driven syringe plunger	Stainless steel cartridge/ nozzle; manual filling/ cleaning, dosage pressure > 100 bar, T < 250°C (482°F), Two zones heater	10 Milliliter	Solid lines, grids, curves, width depends on nozzle size	PCL80 (12.600 Pa*s) at 70°C was succesfully printed	Thermoplastic materials like PCL, PLA, PLGA (blends), etc. with appropriated melting point
Pneumatic cartridge dispenser w/o heater	Pressurized air, Carbondioxide, Nitrogen or other inert gas	Disposable cartridge, disposable nozzle (E.g Nordson EFD); manual filling, dispense pressure 0.5 … 6 bar	30 Milliliter	Solid lines, grids, curves, meanders, line width 100 … 400 microns (depends on nozzle and material)	Approx. 550 Pa*s (Na-Alginat 16% at RT), ca. 1.350 Pa*s (PCL50 at 100°C)	Hydrogels, Alginate, Hydroxyapatite, Ceramic pastes, Calcium phosphat cement, etc.
Pneumatic cartridge dispenser with shell heater	Pressurized Air, Carbondioxide, Nitrogen or other inert gas	Stainless steel cartridge/ nozzle; manual filling, dispense pressure 0.5 … 6 bar, T < 120°C (248°F) (< 180°C (356°F) from Q4 2017)	10 Milliliter	Solid lines, grids, curves, meanders, line width 100 … 400 microns (depends on nozzle and material)	Approx. 550 Pa*s (Na-Alginat 16% at RT), ca. 1.350 Pa*s (PCL50 at 100°C)	Thermoplastic materials like PCL, PCL-PEG blends, etc. with appropriated melting point, bioinks with cells at 37°C
Pneumatic cartridge dispenser with Peltier chiller (from Q4 2017)	Pressurized Air, Carbondioxide, Nitrogen or other inert gas	Disposable cartridge, disposable nozzle (E.g Nordson EFD); manual filling, dispense pressure 0.5 … 6 bar, ΔT ≈ -20K	10 Milliliter	Solid lines, grids, curves, meanders, line width 100 … 400 microns (depends on nozzle and material)	Similar to Pneumatic cartridge dispenser w/o heater	Hydrogels
Pneumatic core/shelll dispenser with replacable pair of nozzles	Pressurized Air, Carbondioxide, Nitrogen or other inert gas	Two separate disposable cartridges, disposable nozzles (E.g Nordson EFD); manual filling, dispense pressure 0.5 … 6 bar	2 x 10 Milliliter	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 forces plus pressurized air, Nitrogen or other inert gas	Stainless steel cartridge/ nozzle; manual filling, dispense pressure 0.5 … 6 bar, T < 120°C (248°F) (< 180°C (356°F) from Q4 2017)., V= +/-10…30 kV	10 Milliliter	Solid lines, grids, meanders, line width 10 … 20 microns for regular patterns	> 10.000 kPa*s	Thermoplastic materials like PCL, PCL-PEG blends, etc. with appropriated melting point
FDM Extruder (From August 2017)	Mechanical force by displacement	Wire from an unwinding coil	Filament coils	Solid lines, grids, meanders, line width 70 … 400 microns	Doesn't matter	Commercially available filaments with appropriated melting point

1 bar = 14.5 psi

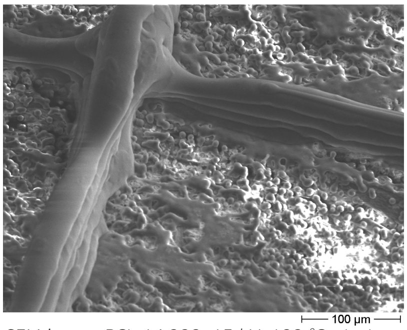
BioScaffolder

Tools at a Glance

Melt Electrospinning Writing

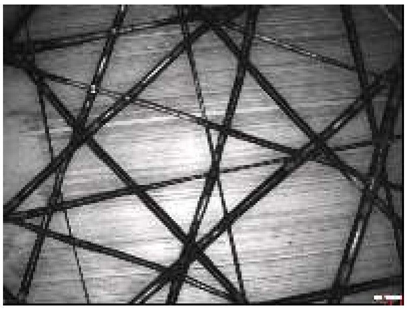
The Melt Electrospinning Writing Module (MES) uses an electrical charge to draw very fine (typically in the micrometer range) fibres from a liquid or polymer melt. Depending on the experimental setup, arbitrary and regular patterns can be achieved.

The MES module for BS3.1 comprises a high voltage generator. Further particular substrate supports and dispense nozzles will be required for the regular deposition of thin fibres.



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

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.

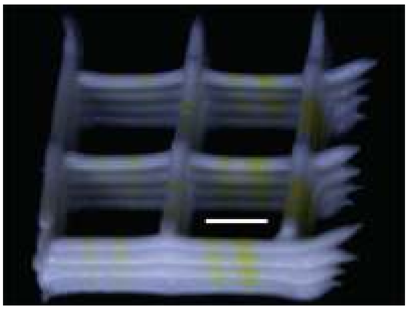


Bottom layer with 20 micron strands, top layer with 100 micron strands.

Nanoliter Pipetting

The pipetting module enables partial functionalization of 3D-printed structures by the application of Nanoliter amounts of cell suspensions or protein samples.
Alternatively, micro-scaffolds from curable liquid samples become feasible.

Right: Fluorescence dots (green) at pre-programmed XY positions in a scaffold printed from CPC (calcium-phoshate-cement) [2]



Tools for Liquid & Powder Pipetting

Tool	Material Application by...	Function/ Specs	Reservoir/ Volume	Interior Patterns for 3D Objects	Viscosity Range	For Use with...
Piezoelectric pipet (Optionally heatable)/ Twin-tip piezoelectric pipet (from Q4 2017)	Ultrasonic wave, Twin-tip pipet allows kinetic mixing	Pipettor(s) with wash system, Drop-on-demand dispensing, single drop volume 100 … 400 Picolitre, Up to 1 Microlitre, Automatic fill-up, T < 100°C (212°F)	96 well micro plate, max. 120 Microliters/ well	Single spots from 80 microns, arrays, lines	Up to 10 mPa*s	All liquid samples, e.g protein solutions, cell suspensions, solved polymers (Two component systems)
Solenoid valve pipet (from Q3 2017)	Solenoid valve plus pressurized air	Pipettor with wash system, Drop-on-demand dispensing, single drop volume 50 Nanolitre, Range up to several Microlitres, Automatic fill-up	96 well micro plate, max. 120 Microliters/ well	Single spots, arrays, lines	Up to 40 mPa*s	All liquid samples, e.g. protein solutions, cell suspensions, solved polymers
Passive pipet tips (Metal/ Teflon coated)	Diluter syringe displacement	Pipettor with wash system, Microliter range, Automatic fill-up	96 well micro plate, max. 120 Microliters/ well	Bulk dispensing of Microlliter volume on printed patterns	Liquids (depends on tip size)	All liquid samples, e.g protein solutions, cell suspensions, solved polymers
Piezoelectric dispense valves (OEM components)	Piezoelectric valve pluse pressurized air	Cartridge dispenser, Drop-on-demand dispensing, drop volume in the Nanolitre range (Optional heatable)	3 Milliliter	Single spots, arrays, lines	Approx. 50 … 200.000 mPa*s	High-viscous liquids, e.g. glue, solved polymers
Powder pipet	Vacuum, pressurized Air	Aspiration/ Dispensing of powder aliquots in the Microgram range	Min. 1 Milliliter	Spots	Solid materials	Granular materials & powder

Optical (OEM-) Components

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

References:

[1] Tobias Zehnder, Tim Freund, Merve Demir, Rainer Detsch and 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-Nuremberg, 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., 2016
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