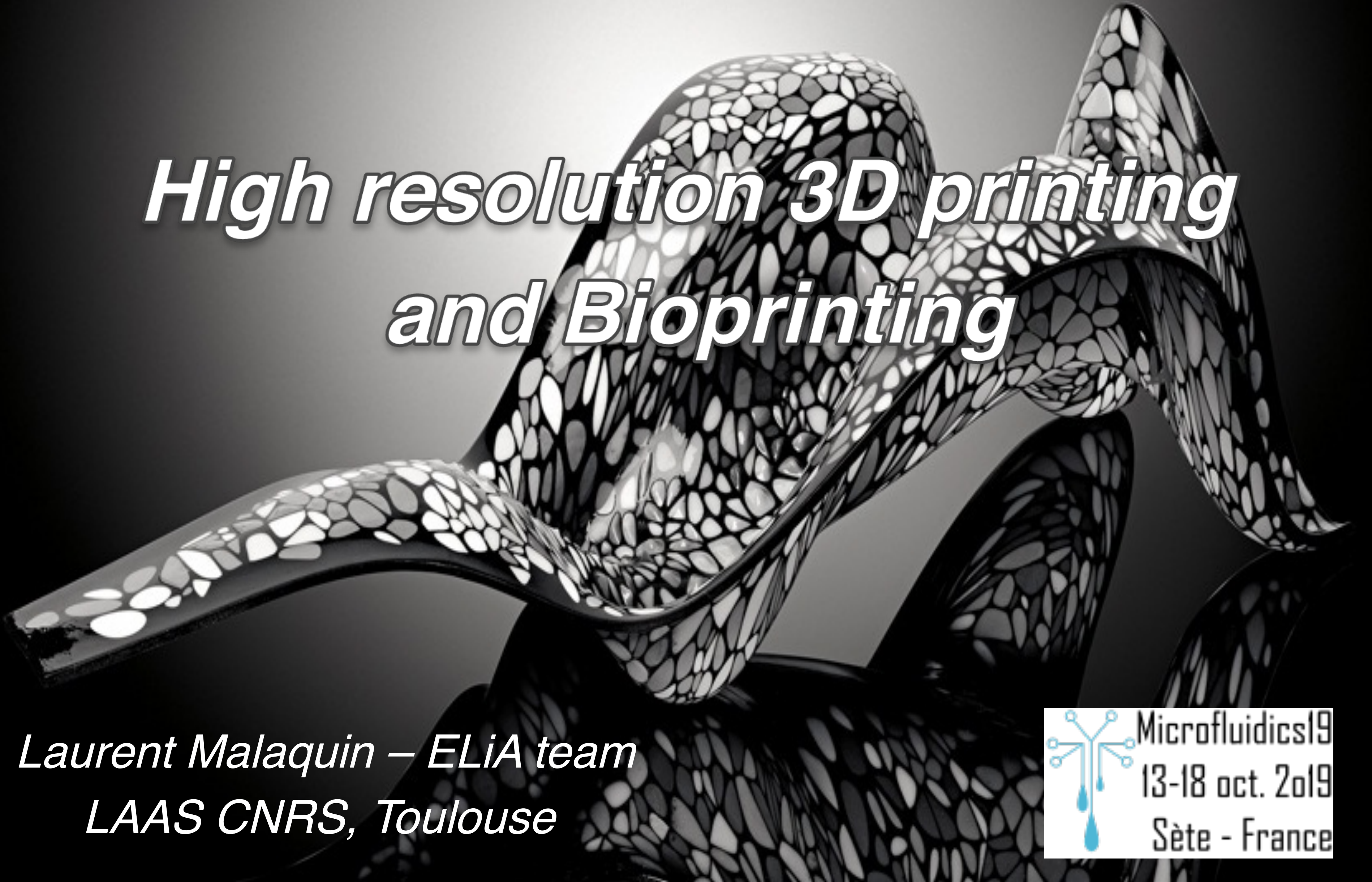
High resolution 3D printing and Bioprinting



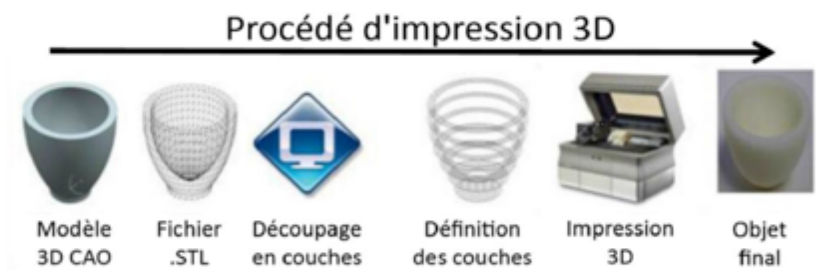
Laurent Malaquin – ELiA team
LAAS CNRS, Toulouse

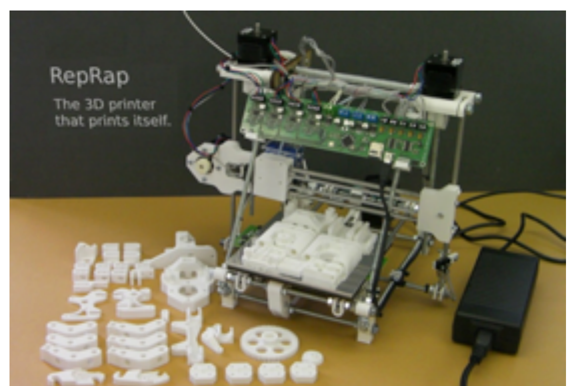
Microfluidics19
13-18 oct. 2019
Sète - France

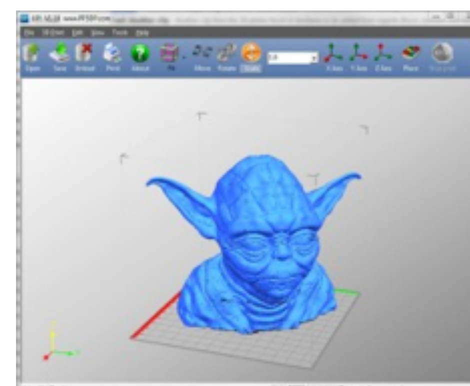
Fabrication additive

- 80 — First patent application for RP technology (Dr Kodama in Japan)
- 84 — First demonstration of stereolythography (Dr JC André)
- 89 — SLS patent was issued to Carl Deckard
- 90 — EOS sold its first “stereos” systems
- 92 — FDM patent was issued to Stratasys
- 96 — Sanders Prototype (Solidscape) and Z corporation set up
- 97 — Arcam was established
- 98 — Objet geometries launched

“**3D printing** (or **additive manufacturing**, **AM**) is any of various processes used to make a three-dimensional object. In 3D printing, additive processes are used, in which successive layers of material are laid down under computer control.[2] These objects can be of almost any shape or geometry, and are produced from a 3D model or other electronic data source. A 3D printer is a type of industrial robot.”





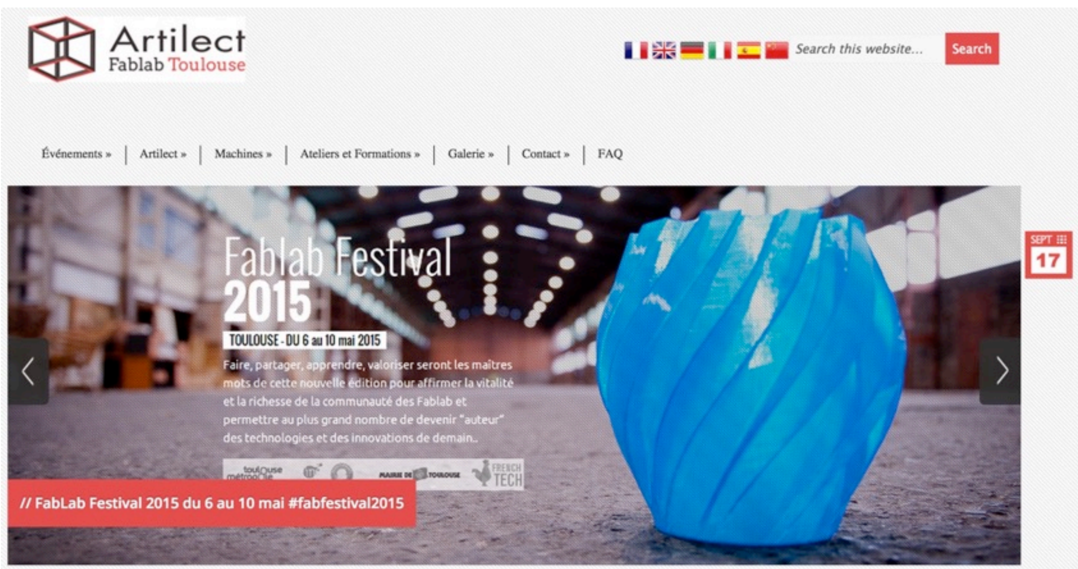


3D printing … where ?

Fab lab

Un ***fab lab*** (contraction de l'anglais ***fab**rication **lab**oratory*, « **laboratoire de fabrication** ») est un lieu ouvert au public où il est mis à sa disposition toutes sortes d'outils, notamment des machines-outils pilotées par ordinateur, pour la conception et la réalisation d'objets.

La caractéristique principale des *fab lab* est leur « ouverture ». Ils s'adressent aux entrepreneurs, aux designers, aux artistes, aux bricoleurs, aux étudiants ou aux hackers en tout genre, qui veulent passer plus rapidement de la phase de concept à la phase de prototypage, de la phase de prototypage à la phase de mise au point, de la phase de mise au point à celle de déploiement, etc. Ils regroupent différentes populations, tranches d'âge et métiers différents. Ils constituent aussi un espace de rencontre et de création collaborative qui permet, entre autres, de fabriquer des objets uniques: objets décoratifs, objets de remplacement, prothèses, orthèses, outils…



http://www.artilect.fr/

3D printing … where ?

http://www.sculpteo.com/fr/

<u>Many providers available !!</u>

MultiFAB Platform

L. Malaquin (LAAS CNRS) – P.Tailhades (CIRIMAT)
V. Raimbault, X. Dollat, H. Granier, L. Bary , A.M. Gue , S Assié Souleille, V. Conedera, F. Mesnilgrente, L. Boyer
R. Courson, J. Foncy ,

UNION EUROPÉENNE
La Région Occitanie Pyrénées - Méditerranée
INSTITUT CARNOT CIRIMAT
Feder / Région Occitanie

- ✓ Open platform (academic and industrial patners)
- ✓ Technological and process development & Dissemination

Technological challenges

- Multimaterial printing
- Multiprocesses
- High resolution printing and bioprinting (i.e. <50μm)

SLA (Dilase 3D , Dilase 3D HR, 2photon nanolithography)
Inkjet Printing (Ceradrop)
Dilase 3D Bio, Prototype LAMP, DWS, Wet spinning
Selective laser Melting/Sintering SLS, SLM

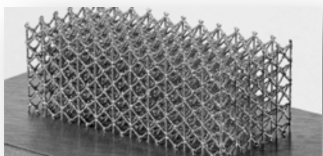
- Cell and Tissue Engineering
- MEMs
- Microfluidics
- Electronics, Optics, Aeronautics, ...

Web site
https://www.laas.fr/projects/MultiFAB

Contact
multifab@laas.fr

3D printing : what about resolution ?

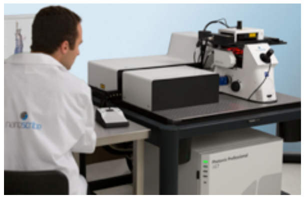
Selective Laser Sintering - Melting (SLS/SLM)



Fused deposition modeling (FDM) / InkJet printing

Stereolithography (SLA)

3D printing with two photons lithography



MACRO **MICRO** **NANO**

1m | 10 cm | 1cm | 1mm | 100µm | 10µm | 1µm | 100nm | 10nm | 1nm

<u>Additive manufacturing current limits :</u>

Resolution
Printing Volume /Writing speed

Multimaterials (SLA, 2 photons)

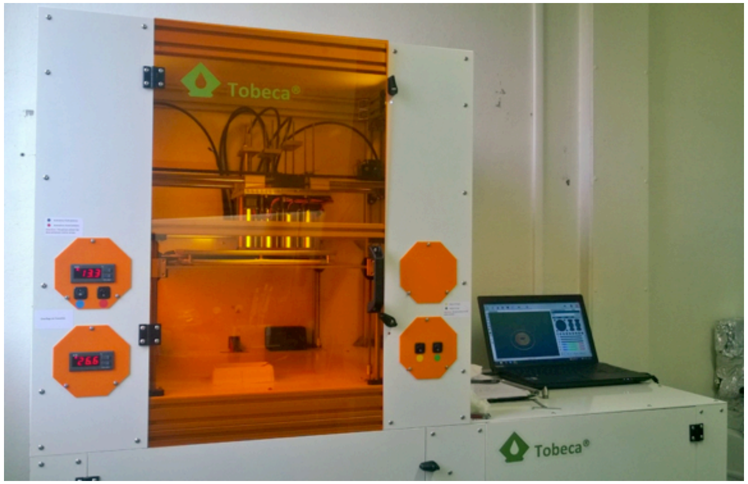
Applications :

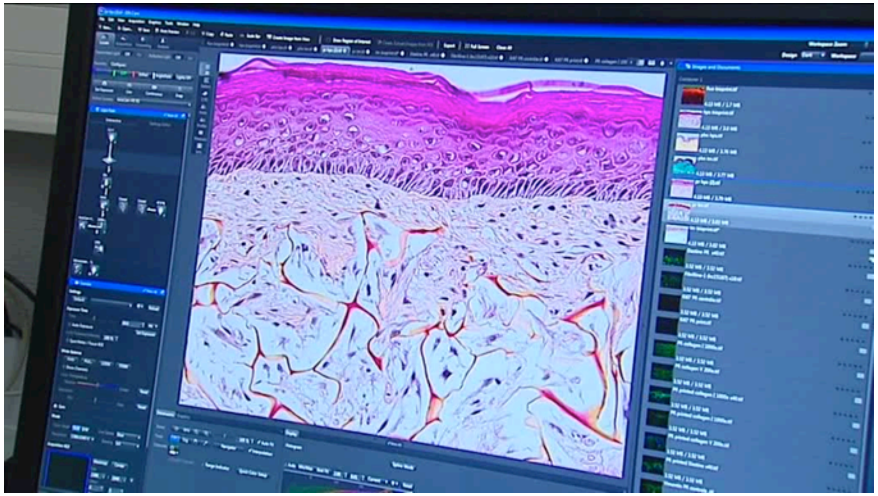
- Microfluidics
- MEMs devices
- Scaffolds for cell culture

3DFAB

3D FAB : 3D Fabric of Advanced Biology

3dfab
PRINTING FOR LIFE





http://fabric-advanced-biology.univ-lyon1.fr/

Reproducing Skin tissues

http://www.3dnatives.com/3dfab-francais-impression-3d-vivant-19022016/

Additive manufacturing market

Sized applications of 3D printing could have direct economic impact of $230 billion to $550 billion per year in 2025

Sized applications	Potential economic impact of sized applications in 2025 $ billion, annually	Estimated scope in 2025	Estimated potential reach in 2025	Potential productivity or value gains in 2025
Consumer use of 3D printing	100–300	▪ $4 trillion in sales of consumer products that might be 3D printed	▪ 5–10% of relevant products (e.g., toys) could be 3D printable, assuming easy consumer access	▪ 60–80% value increase per 3D-printed product – 35–60% cost savings to consumers – 10% added value from customization
Direct product manufacturing[1]	100–200	▪ $300 billion spending on complex, low-volume items such as implants and tools ▪ $470 billion spending on complex, low-volume parts in transportation	▪ 30–50% of products in relevant categories replaceable with 3D printing	▪ 40–55% cost savings to buyers of 3D-printed products
Tool and mold manufacturing	30–50	▪ $360 billion global market for injection-molded plastics	▪ 30–50% of injection-molded plastics produced with 3D-printed molds	▪ 30% production cost reduction using superior 3D-printed molds
Other potential applications (not sized)				
Sum of sized potential economic impacts	**230–550**			

1 Focuses on use of 3D printing to directly manufacture low-volume, high-value parts in the medical and transport manufacturing industries. Other potentially impactful applications might include manufacturing of low-volume, high-value replacement parts for other industries.

NOTE: Estimates of potential economic impact are for some applications only and are not comprehensive estimates of total potential impact. Estimates include consumer surplus and cannot be related to potential company revenue, market size, or GDP impact. We do not size possible surplus shifts among companies and industries, or between companies and consumers. These estimates are not risk- or probability-adjusted. Numbers may not sum due to rounding.

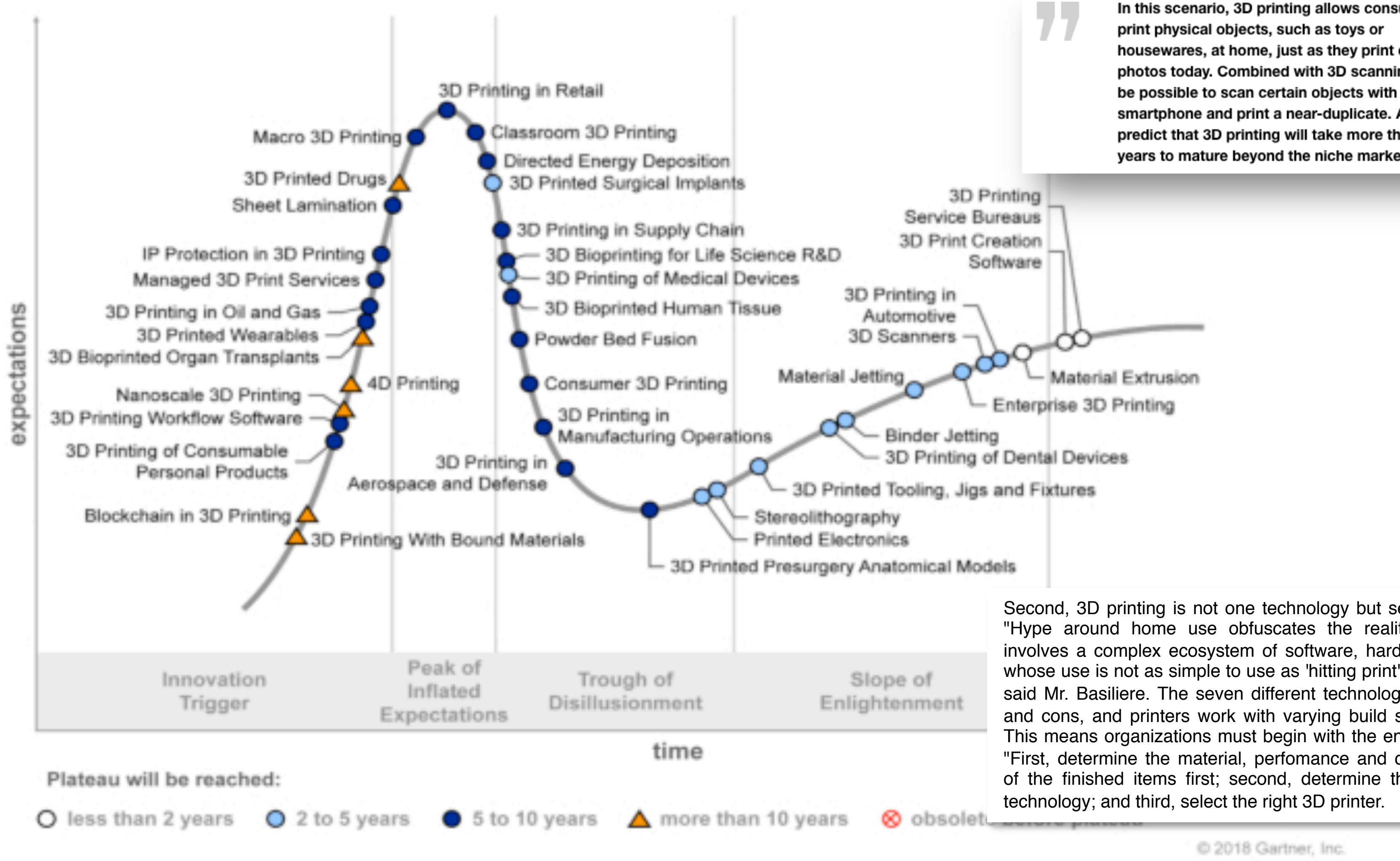
SOURCE: McKinsey Global Institute analysis

3D Complex parts
+
Rapid prototyping

↓

Small / medium Scale Production

3D printing on the Gartner cycle

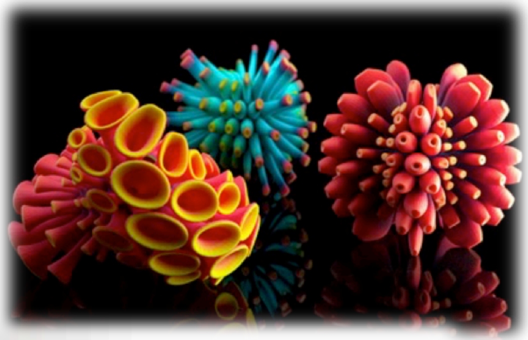


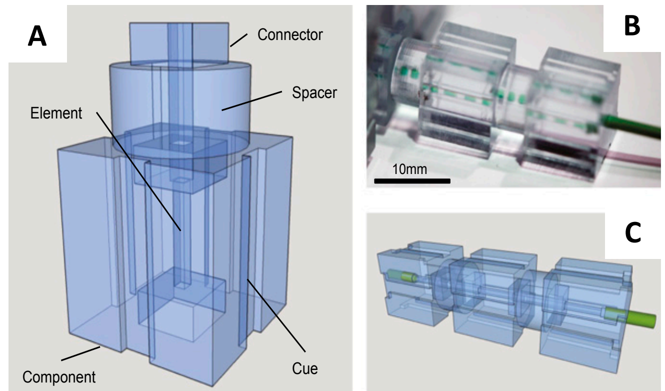
> In this scenario, 3D printing allows consumers to print physical objects, such as toys or housewares, at home, just as they print digital photos today. Combined with 3D scanning, it may be possible to scan certain objects with a smartphone and print a near-duplicate. Analysts predict that 3D printing will take more than five years to mature beyond the niche market.

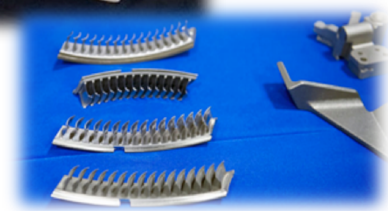
Second, 3D printing is not one technology but seven different ones. "Hype around home use obfuscates the reality that 3D printing involves a complex ecosystem of software, hardware and materials whose use is not as simple to use as 'hitting print' on a paper printer," said Mr. Basiliere. The seven different technologies each have pros and cons, and printers work with varying build sizes and materials. This means organizations must begin with the end products in mind: "First, determine the material, perfomance and quality requirements of the finished items first; second, determine the best 3D printing technology; and third, select the right 3D printer.

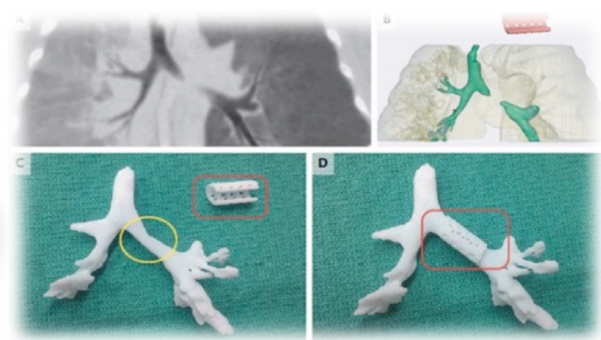
http://www.gartner.com/

What a 3D printer can do …









Motivation

Choosing the right *Material – Technology* combination

Material ⇄ **3D Printer Technology**

- Physical properties
- Chemical properties
- Availability
- Price
- …

- Volume production
- Resolution
- Throughput
- Dimensions
- Environment
- …

- **Price = f (Dimensions, resolution, repeatability, throughput …)**

Printing on (stacks of) paper ?

Why not ?

- Established technology
- Low cost
- High resolution (> 3000dpi)
- Low cost material & support

www.mcortechnologies.com/

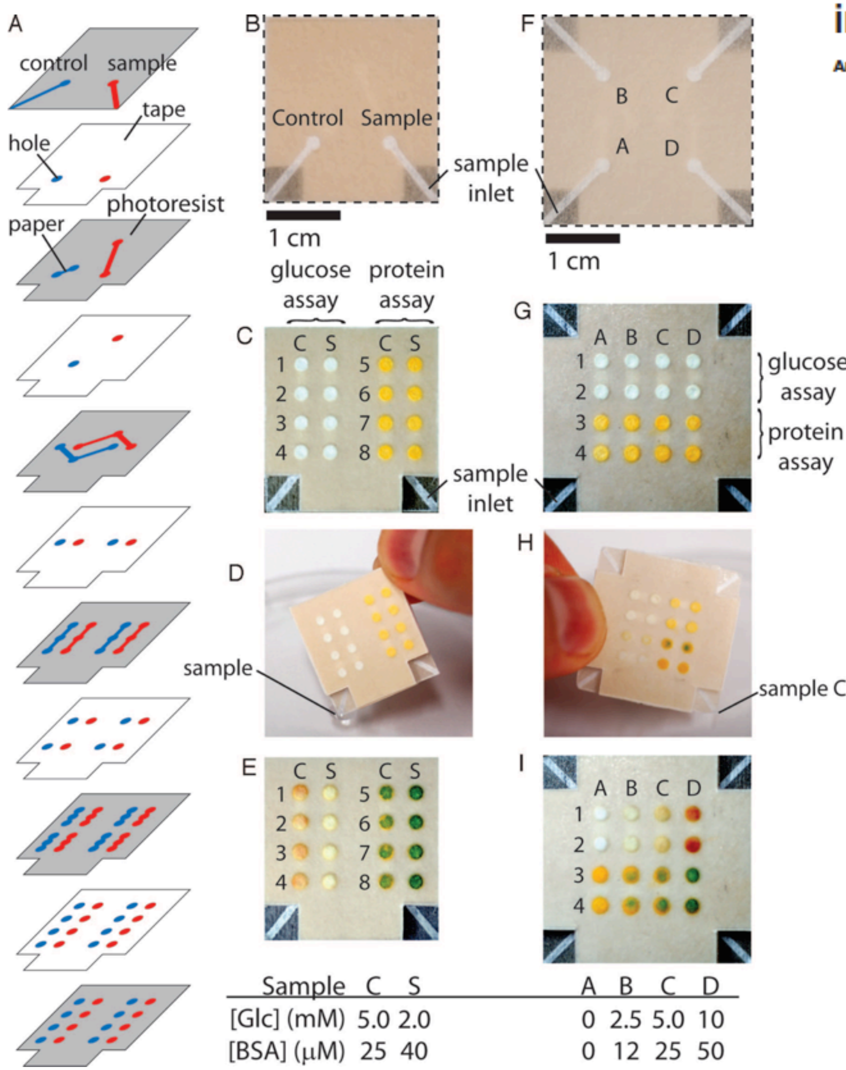
Printing on (stacks of) paper ?

start 1’40 / 2’05 / 3’10

http://mcortechnologies.com/3d-printers/iris/

mcor technologies
unfettered innovation™

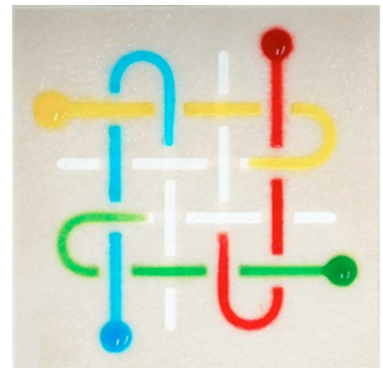
Printing stacks of paper … application to microfluidics ?

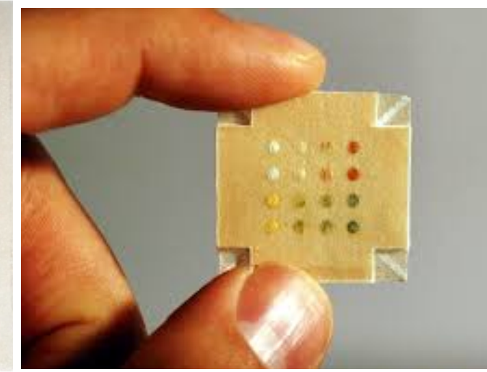


Three-dimensional microfluidic devices fabricated in layered paper and tape

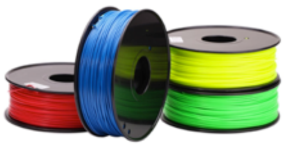
Andres W. Martinez, Scott T. Phillips, and George M. Whitesides[1]

PNAS, 2008, 19606



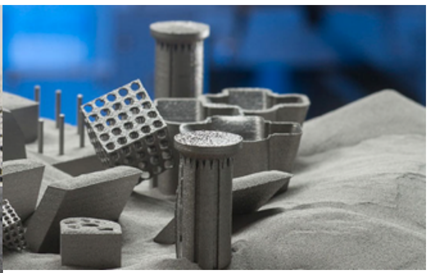


Choosing the right material ....



ABS, Nylon, COC … : FDM (Fused deposition Modeling technologies)

Polymer precursor **powder** (thermoplastic such as Nylon, Polyamide, Polystyrene, …)
Metal precursor **powder** (Ti, Ni, Cu, …)
SLS (Selective laser sintering)



Ni, Cu, Ti …

Photosensitive polymers, composites … :
SLA (Stereolithography)
Inkjet (Objet Stratasys)

Materials for cell culture
Cells
Hydrogels (natural , sythetic …)

3D printing – General workflow

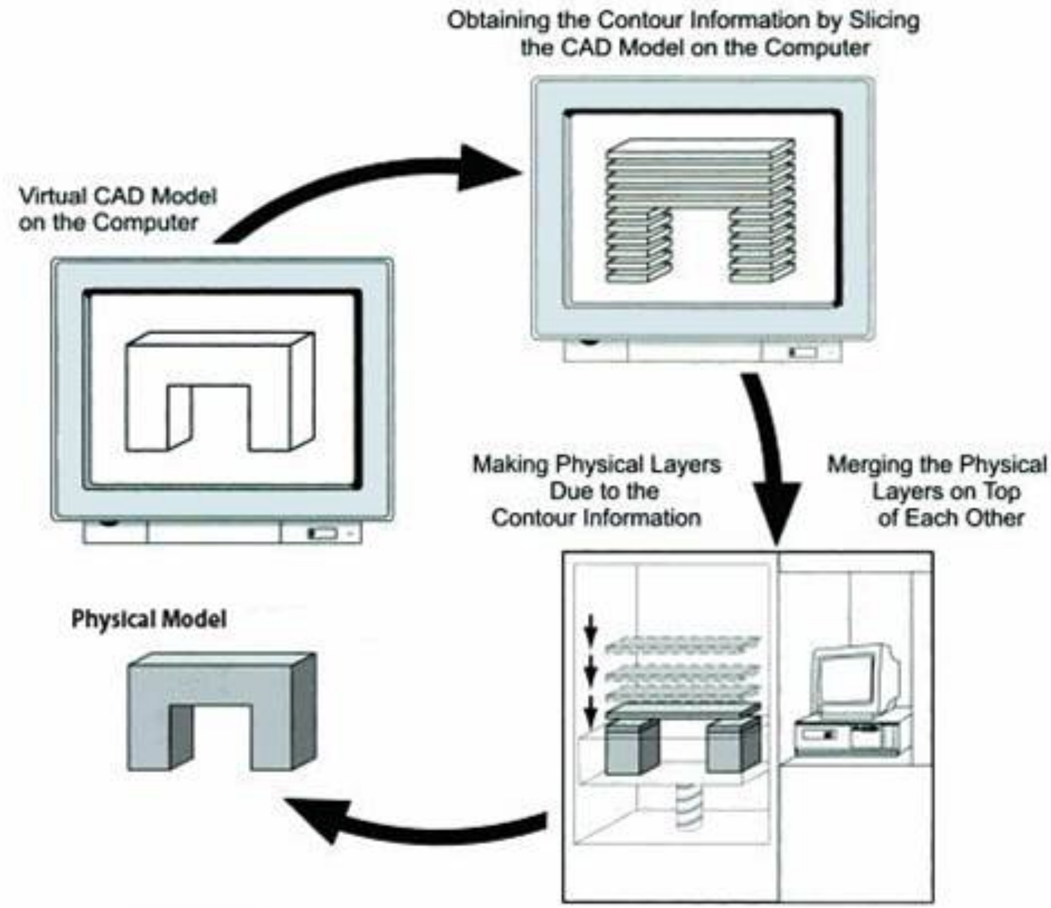
Points communs à tous les procédés

Les techniques de fabrication par couches sont mises en œuvre à partir d'une description numérique en strates de l'objet.

A partir d'un modèle 3D surfacique ou solide, il est généré un fichier STL (triangulation) sur lequel des sections parallèles sont calculées perpendiculairement à la direction de fabrication : c'est le processus de « tranchage »

Le procédé de fabrication se fait :

- **par solidification** d'une résine ou d'un matériau thermo fusible
- **par agglomération** de poudre
- **par collage** de matériaux en feuilles.

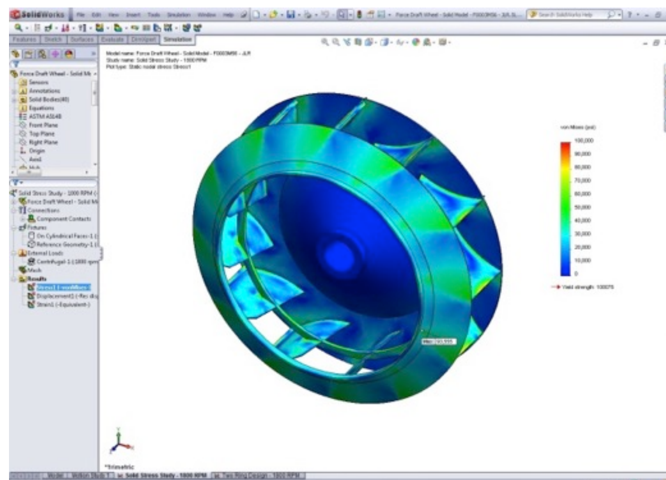


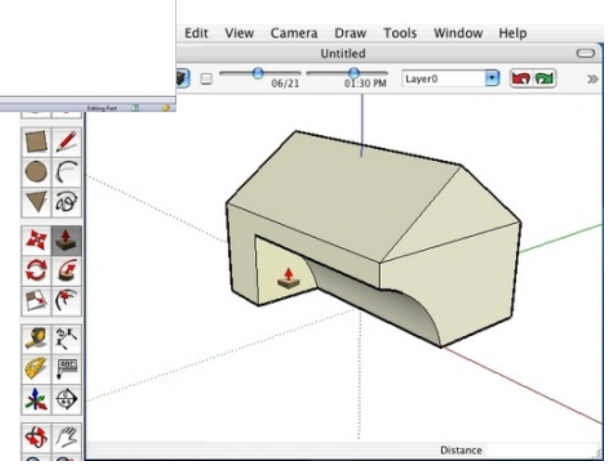
From a 3D Model to a 3D Object

Good 3D Model :

- Downloaded from a online resource
- 3D Laser Scanner
- **prepared using appropriate software: usually STL file format**

- OpenSCAD
- SketchUp
- Wings3D
- Scupltris
- Autodesk
- SolidWorks
- Inventor
- Catia
- Rhino
- AutoCad



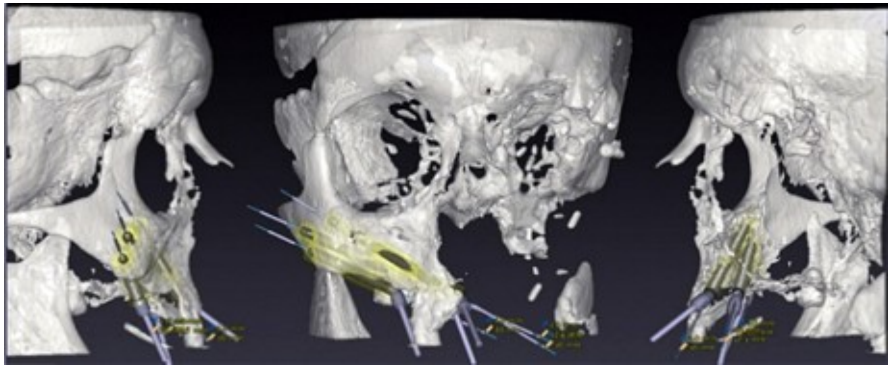


From a 3D Model to a 3D Object

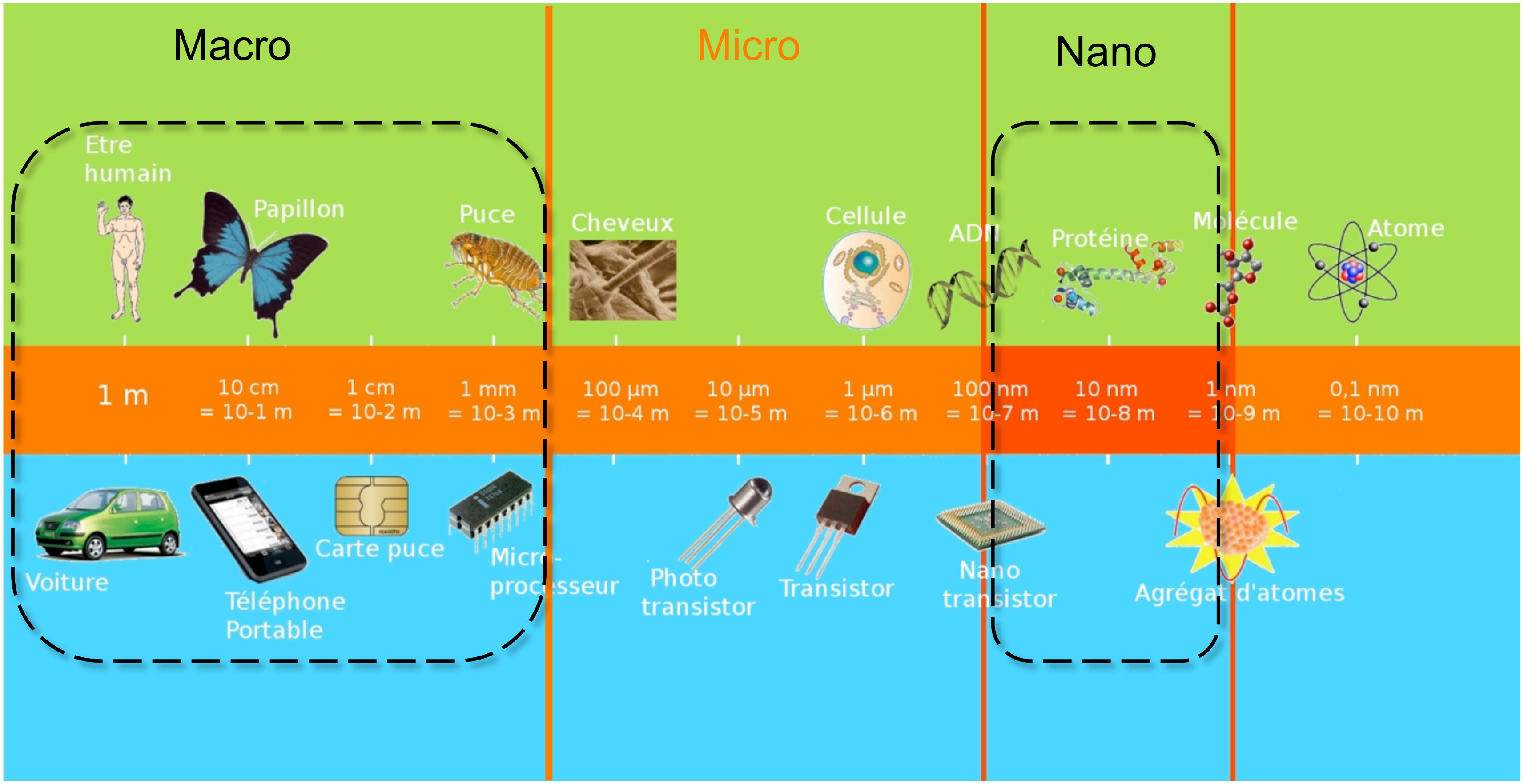
How doctors printed my new face

A man has had a new face printed for him after he lost the entire left side of his face in surgery to remove a tumour.

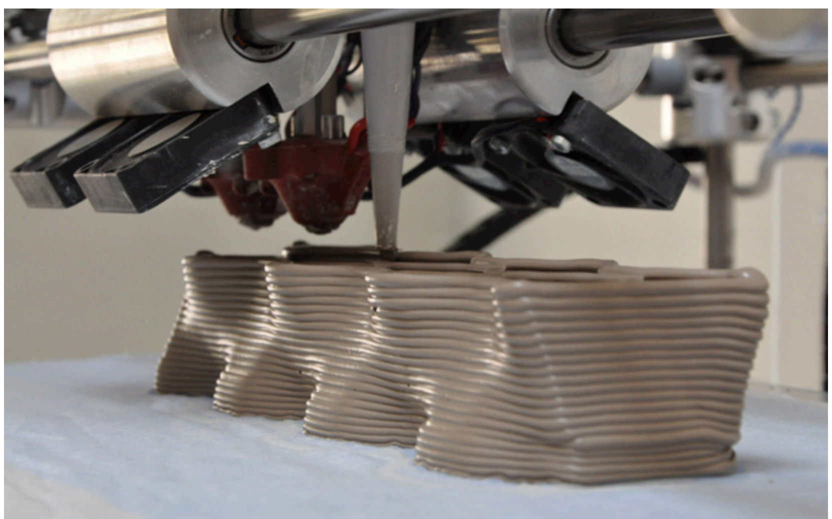
Dental surgeon Dr Dawood, London



What a 3D printer can do …



3D printing : printing volume and resolution



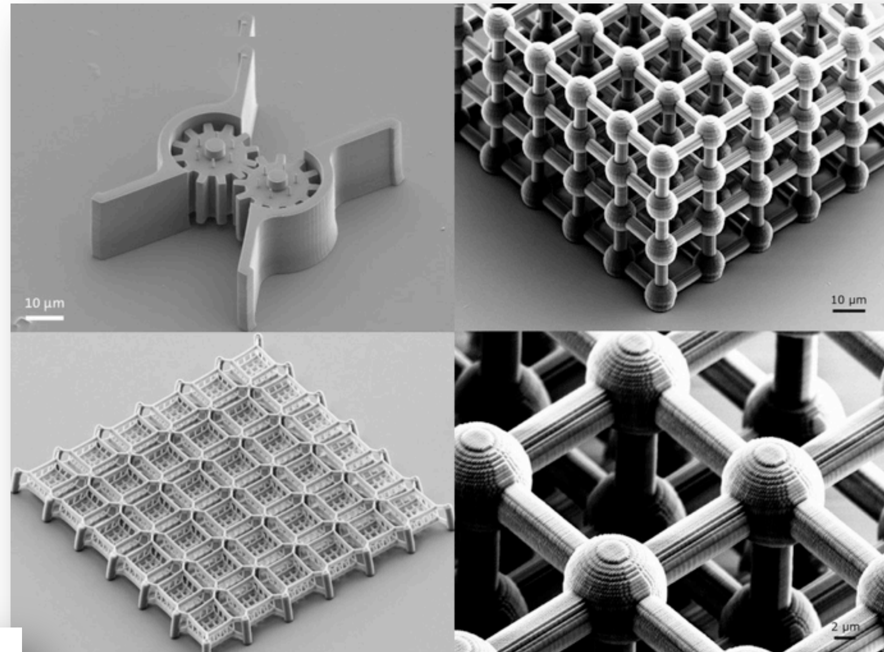
http://3dprinting.com/3dprinters/3d-concrete-printing-project-3dcp/

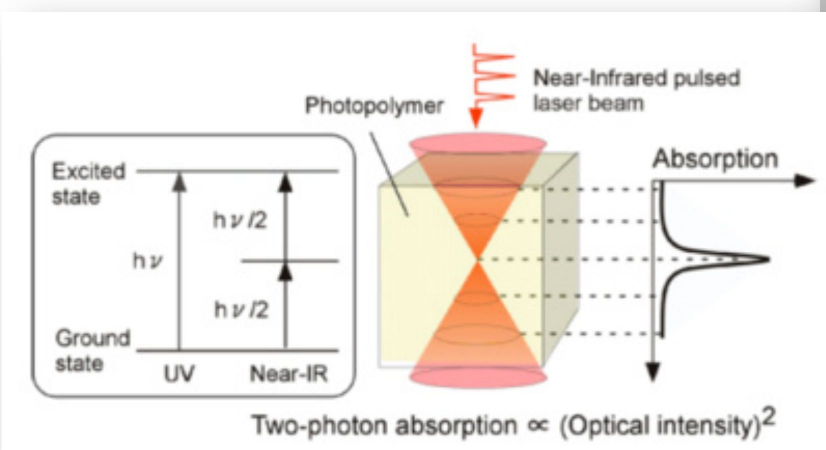
3D printing : printing volume and resolution

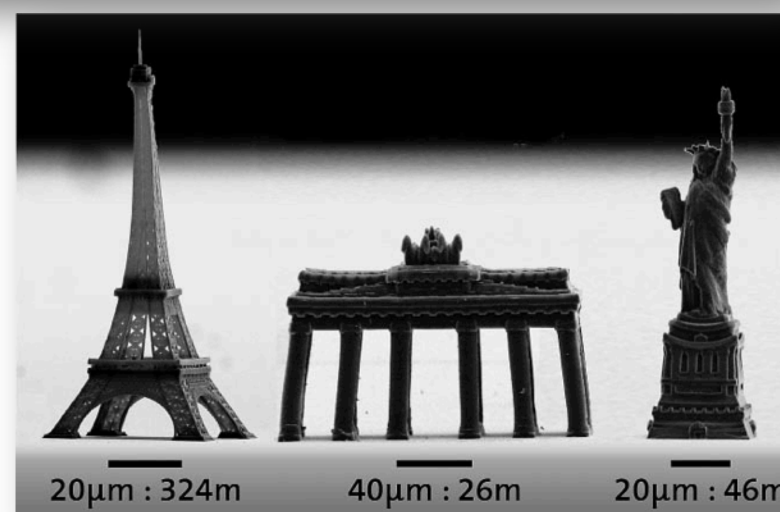
Nanoscribe system

Advantages :
- Resolution (<100nm ?)
- Flexibility
- No slicing

Disadvantages :
- Speed !
- Sample maximum size (mm³)







3D printing : printing volume and resolution

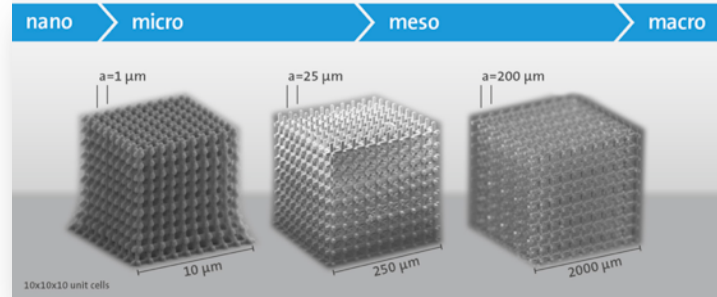
Article | OPEN

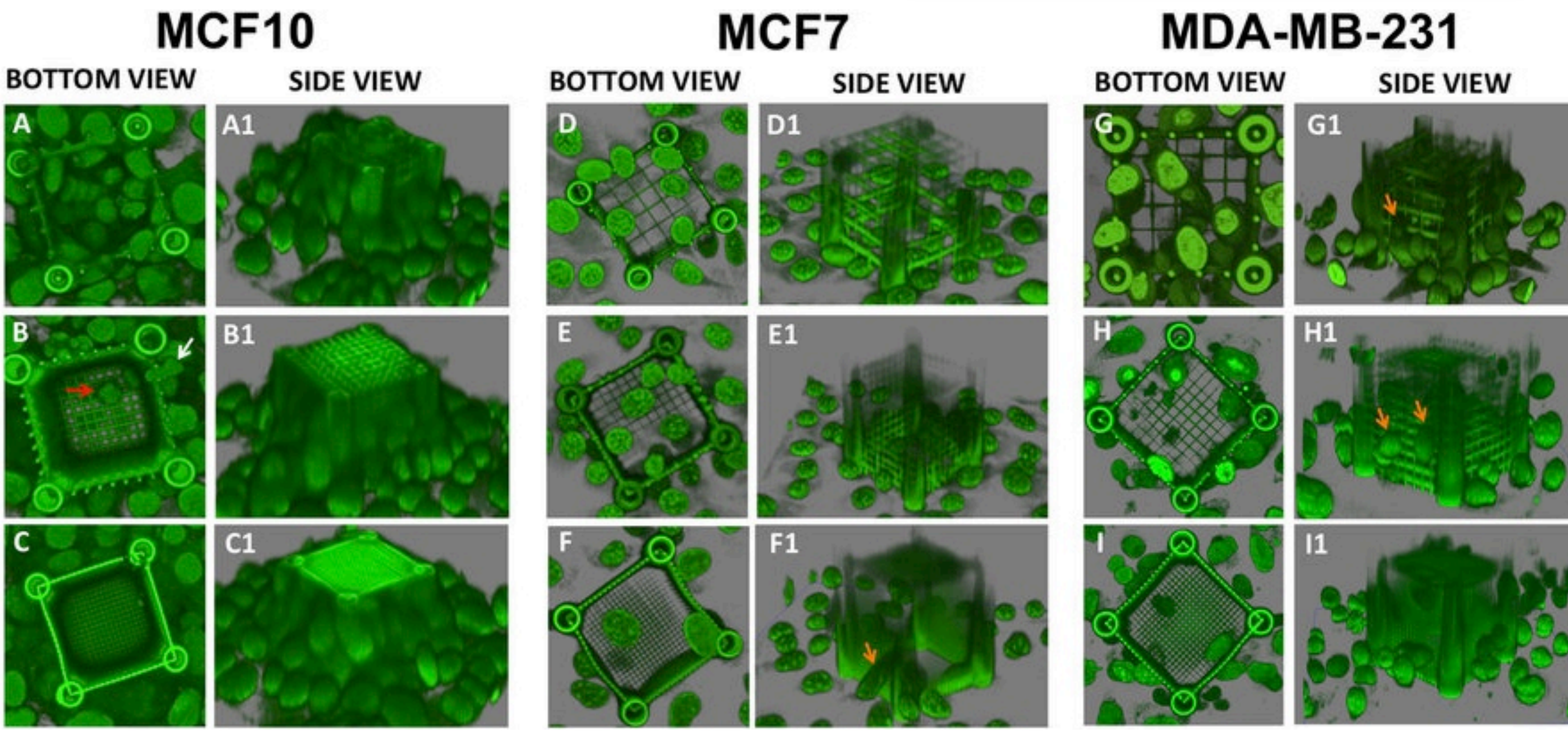
Three-dimensional cage-like microscaffolds for cell invasion studies

Barbara Spagnolo, Virgilio Brunetti, Godefroy Leménager, Elisa De Luca, Leonardo Sileo, Teresa Pellegrino, Pier Paolo Pompa, Massimo De Vittorio & Ferruccio Pisanello

Scientific Reports **5**, Article number: 10531 (2015)
doi:10.1038/srep10531

Received: 28 September 2014
Accepted: 23 April 2015
Published online: 27 May 2015





3D printing : printing volume and resolution

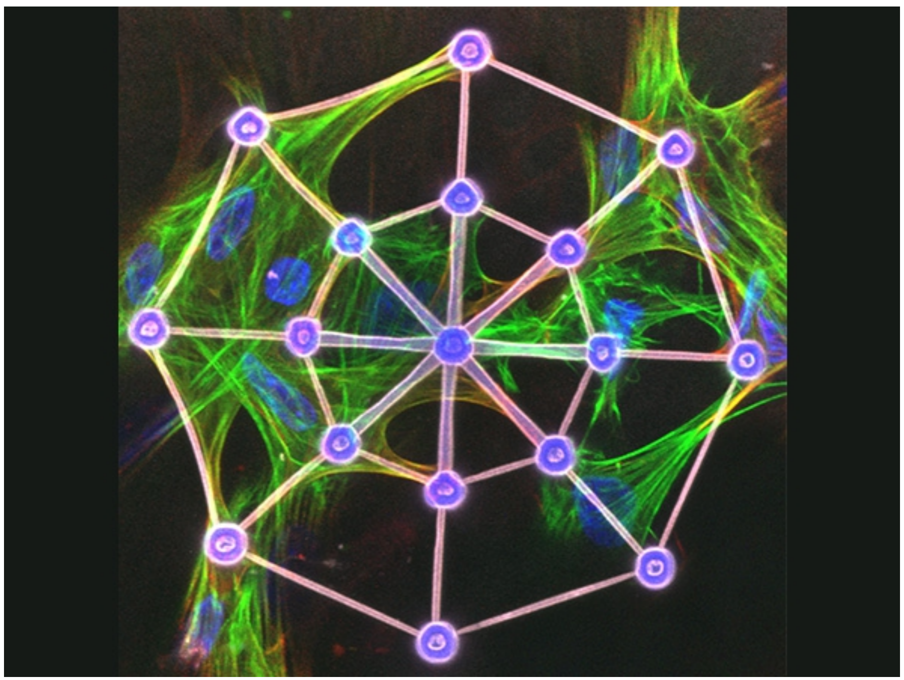
Microfluidics19
13-18 oct. 2019
Sète - France

ADVANCED MATERIALS
www.advmat.de

MaterialsViews
www.MaterialsViews.com

Elastic Fully Three-dimensional Microstructure Scaffolds for Cell Force Measurements

By *Franziska Klein, Thomas Striebel, Joachim Fischer, Zhongxiang Jiang, Clemens M. Franz, Georg von Freymann, Martin Wegener,* and *Martin Bastmeyer**



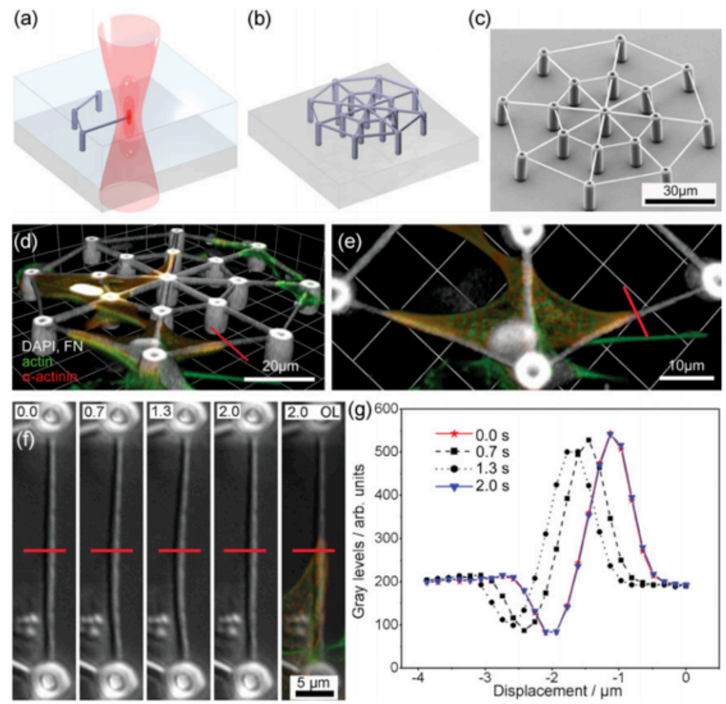
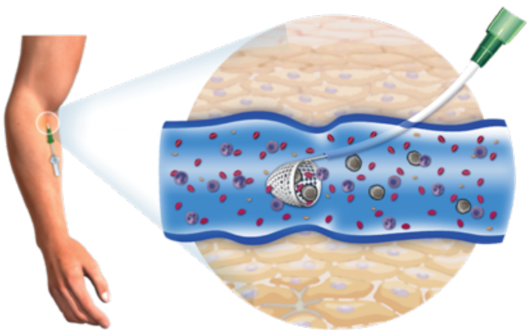
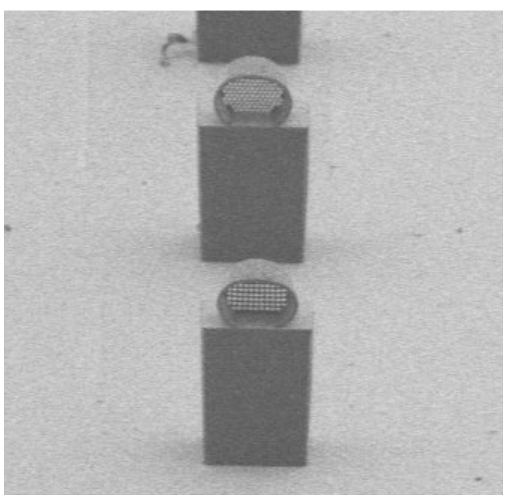


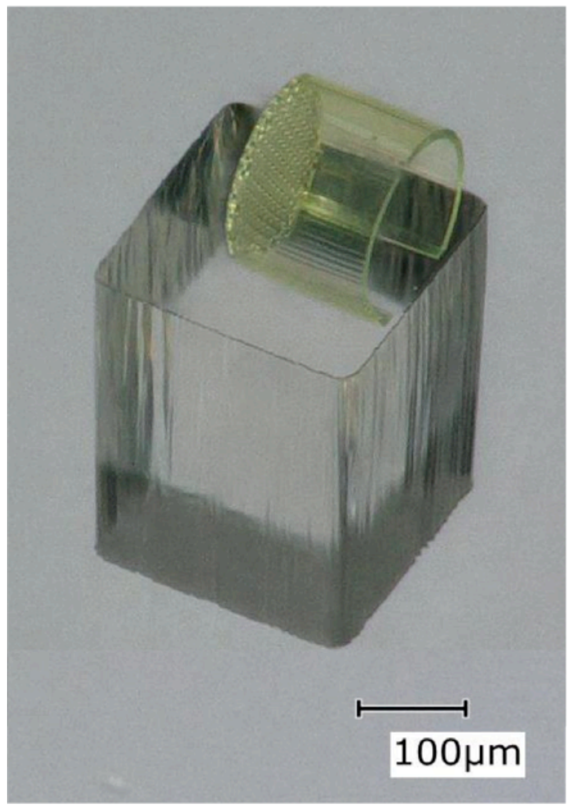
Figure 1. a,b) Scheme illustrating DLW, in which a photoresist is exposed to a laser focus (red region) via two-photon absorption. Scanning of the laser focus with respect to the resist leads to 3D structures. c) SEM image of a fully 3D scaffold. d,e) 3D reconstruction of a confocal image stack of chicken cardiomyocytes grown in an Ormocomp scaffold consisting of posts connected by beams with a diameter of 0.6 μm (oblique view (d); top view (e)). Labeling for f-actin and α-actinin illustrates the formation of regular myofibrils. f) Individual frames of a video demonstrate that the beam in contact with the cell is bent and stretched during a single contraction cycle of the cardiomyocyte (time in seconds). The overlay (OL) connects the video frames with (e). The red lines in (d–f) serve as guides to the eye. g) Gray levels along the red lines in (f) taken at the times in (f). After a maximum deflection of 0.8 μm the beam returns to its original position.

3D printing : printing volume and resolution

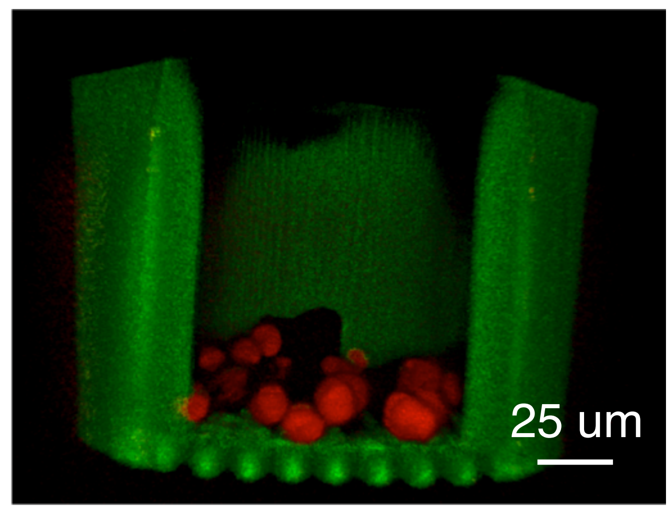
Circulating tumoral cell capture (A. Cerf , LAAS CNRS)







nanoscribe



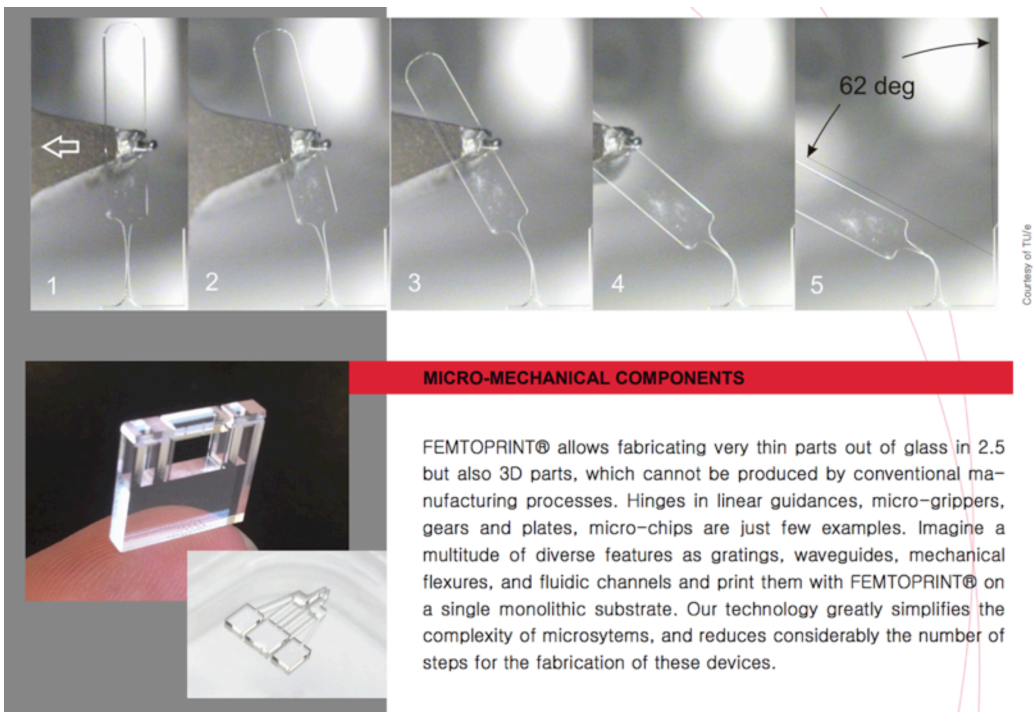
<u>Limits</u> : printed volume AND writing speed

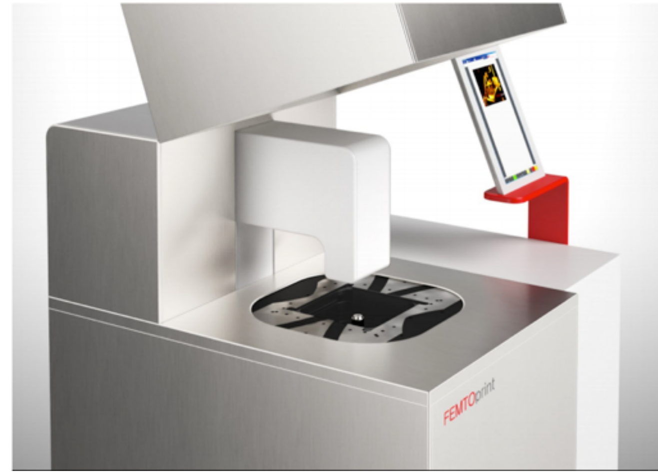
3D laser patterning

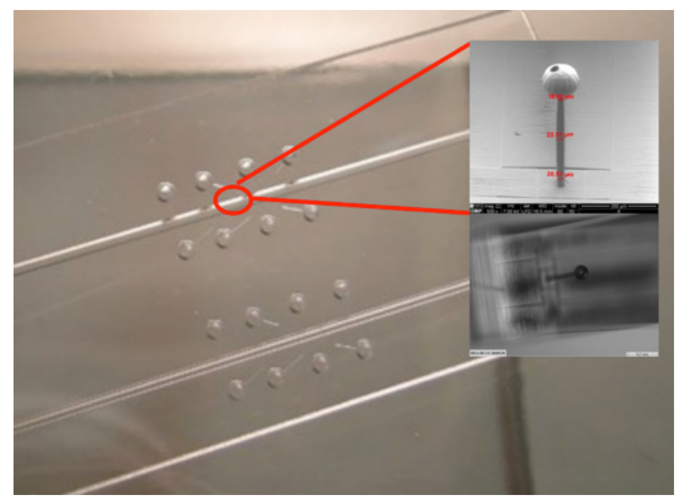
FEMTOPRINT®
Technology

FEMTOprint

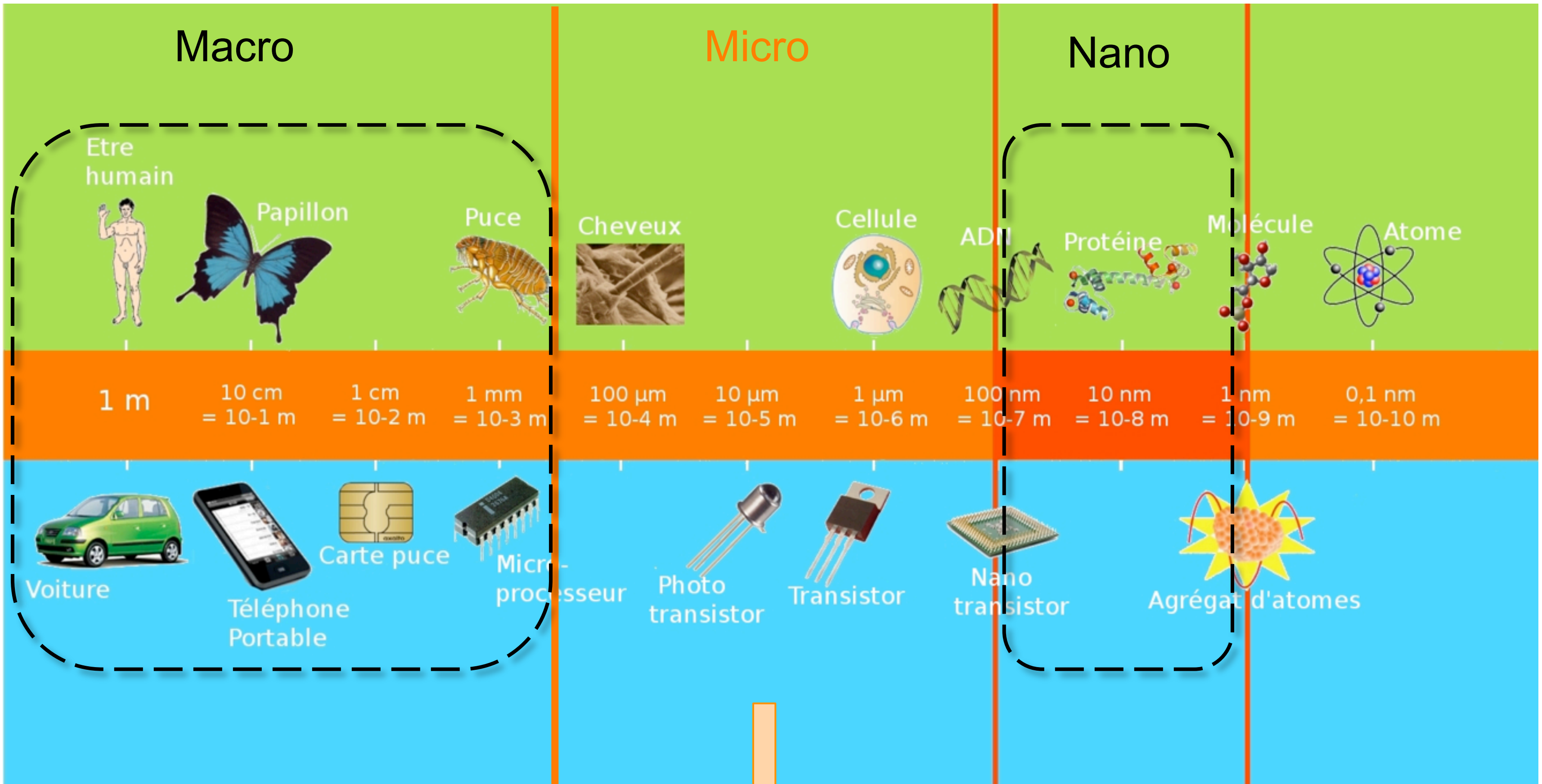
https://www.femtoprint.ch







What a 3D printer can do …



- Cell and Tissue Engineering
- MEMs
- Microfluidics

3D printing for microfluidics

Lab on a Chip

CRITICAL REVIEW

View Article Online
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CrossMark click for updates

Cite this: *Lab Chip*, 2016, 16, 1720

The upcoming 3D-printing revolution in microfluidics

Nirveek Bhattacharjee,[a] Arturo Urrios,[ab] Shawn Kang[a] and Albert Folch*[a]

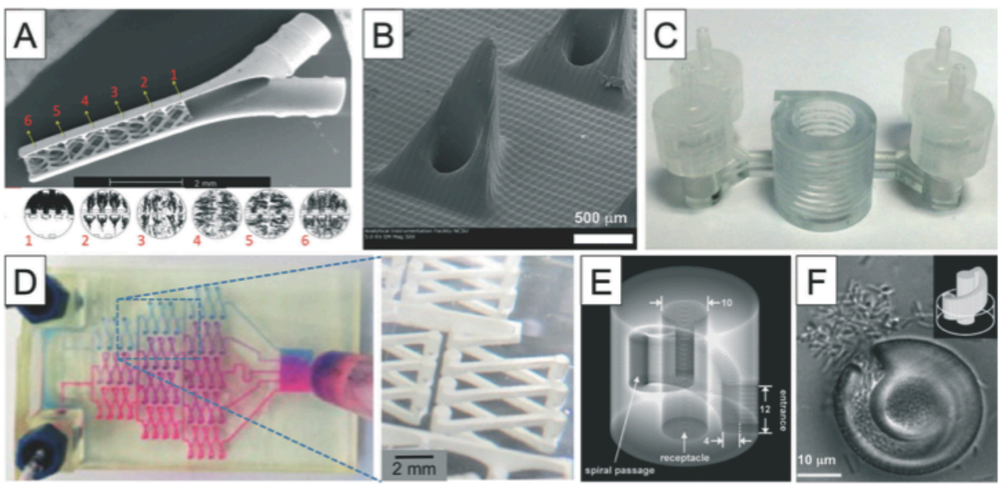


Fig. 3 Microfluidic devices printed with SL. (A) SEM micrograph of the first microfluidic device (micro-mixer) printed with SL. (below) numerical simulations of fluid mixing at the indicated cross-sections of the device. (B) SEM of hollow micro-needles fabricated in e-Shell-200 by DLP-SL. (C) Spiral microchannel with trapezoid cross-section (printed with Watershed) used for size-selective separation of bacterial cells. (D) A complex microfluidic mixer and gradient generator printed with a commercial desktop SL system. (E) A microfluidic "lobster trap" for bacteria fabricated in bovine serum albumin with multi-photon SL. (F) A colony of *E. coli* forming at the bottom of the "lobster trap". Panel (A) is reproduced from ref. 20 with permission of the Royal Society of Chemistry. Panel (B) is reproduced from ref. 22 with permission of the American Institute of Physics. Panel (C) is reproduced from ref. 24 under the Creative Commons Attribution-non Commercial-noderivs 4.0 International License. Panel (D) is reproduced from ref. 25 with permission of the American Chemical Society. Panels (E) and (F) are reproduced from ref. 37 with permission of John Wiley and Sons.

Advantages

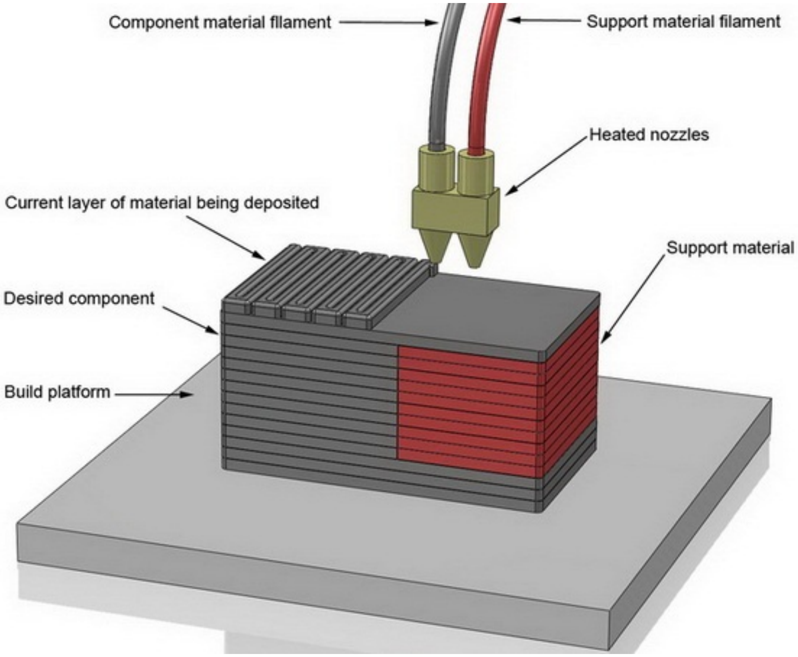
- Assembly free fabrication
- Low cost
- World to chip connections
- 3D design
- Rapid prototyping

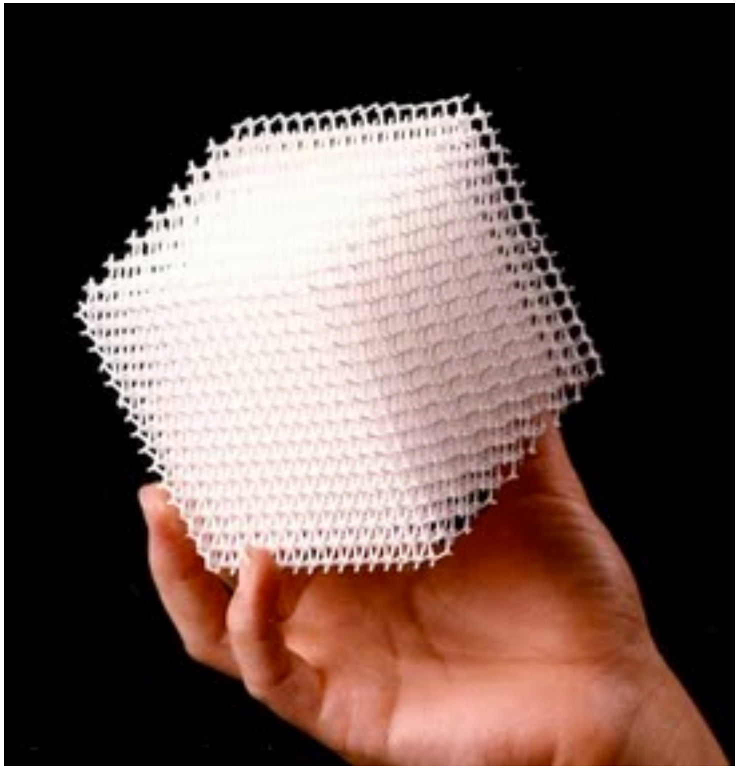
Limits

- Resolution ?
- Throughput ?
- Materials ?

Fused filament fabrication (FFF), *fused deposition modeling (FDM)*

Developed by Scott Crump (Stratasys) in the late 1980s





- heated thermoplastic material is extruded according to computer-controlled paths and then solidifies

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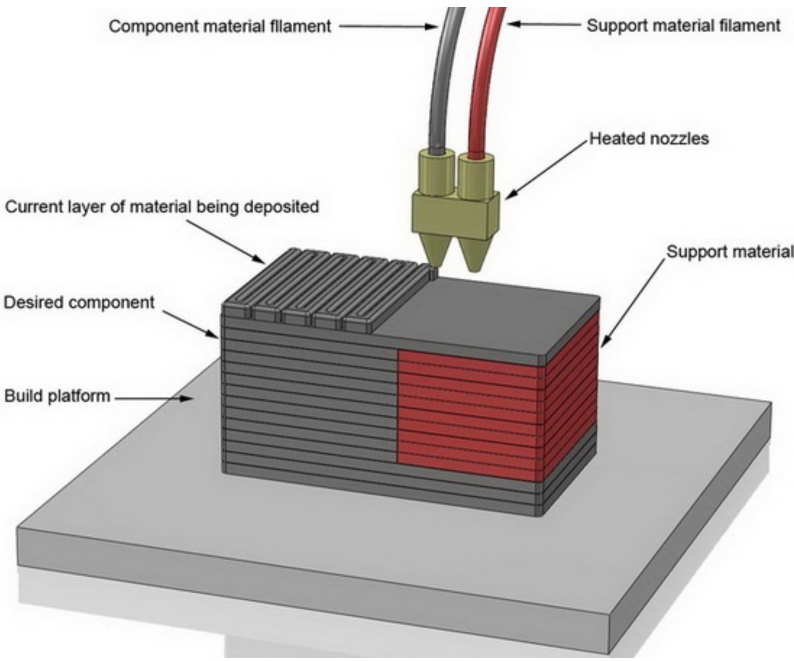


start 45s

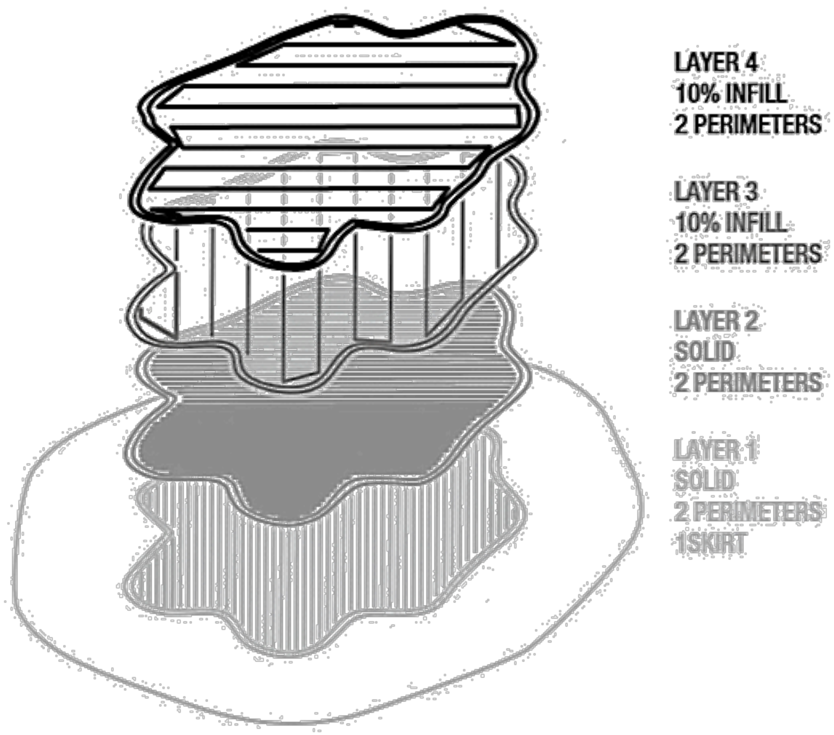
- heated thermoplastic material is extruded according to computer-controlled paths and then solidifies (PLA, ABS, PET, PVA, Nylon …)

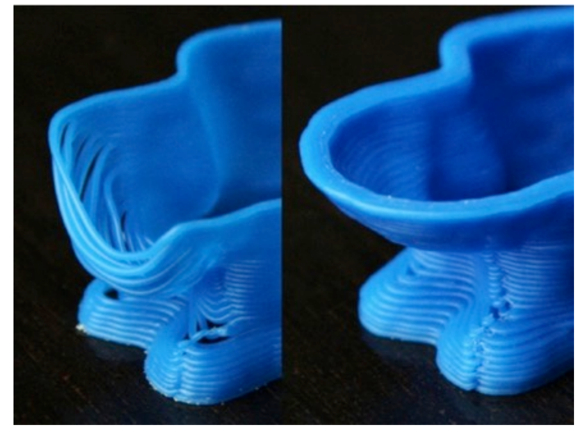
Fused filament fabrication (FFF), *fused deposition modeling (FDM)*

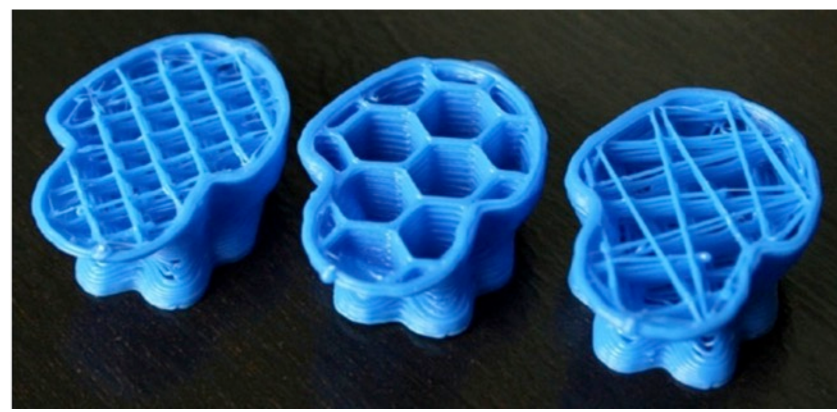
Slicing and Toolpath

- slice the model into discrete layers and generate the toolpath (depending on the printer technology)

Example of a toolpath







Fused filament fabrication (FFF), *fused deposition modeling (FDM)*

e.g. Stratasys's uPrint

- **Dimensions**: 8''x8''x6''
- **Resolution :** 100-200um
- **Materials** : ABS (+ PET) / Matériau sacrificiel
- **Medium Throughput (3-4 parts /day)**

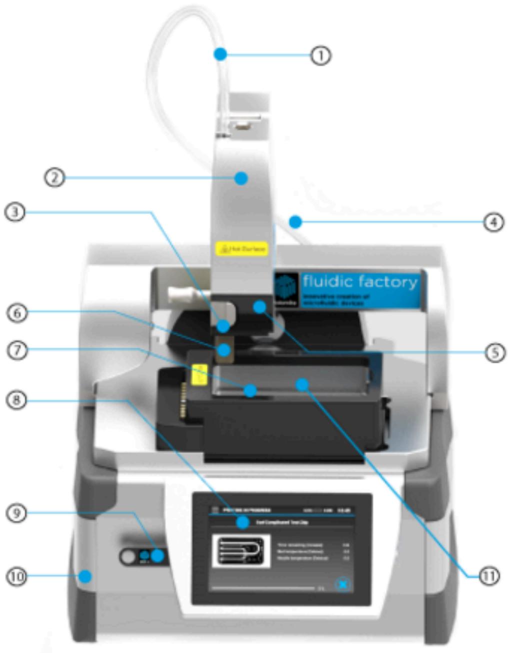
Fused filament fabrication (FFF) for microfluidics

dolomite

Features:

① **COC (cyclic olefin copolymer)** the Fluidic Factory prints COC devices
② **Motor feedback** ensures correct polymer feed rate
③ **Inductive heating coil** ensures high speed heating and accurate control, and enables disposable print nozzle to be easily replaced
④ **Polymer reel** contains 60 m of polymer with a disposable nozzle that is changed for every reel to ensure that high quality of print is maintained over time. The reel can be changed in seconds. Auto-alert when COC is running low

⑤ **Future enabled print head:** A user-changeable print head and print bed plus easy to upgrade software enables future developments such as printing different polymers, ultra high definition printing, micromilling, fluid dispensing and bio-printing

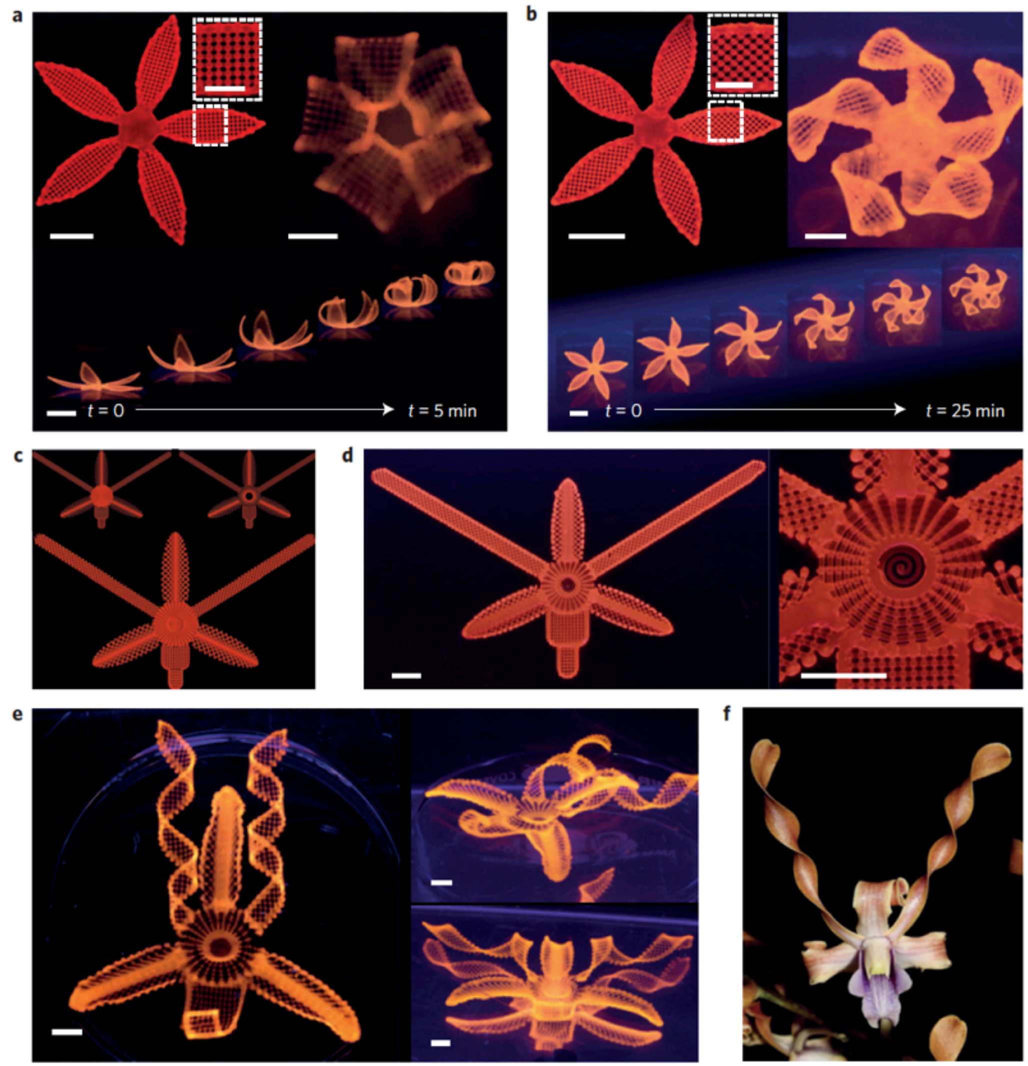


General COC device specifications

Material:	COC (cyclic olefin copolymer), grade 8007S-04
Maximum size:	85 mm (l) x 50 mm (w) x 25 mm (h)
Maximum pressure:	10-20 bar, subject to design geometry
Temperature range:	Up to 77°C
Chemical compatibility:	COC is one of the most resistant plastics to a wide range of polar solvents and molecules
Method of printing:	Features are created by adding layers with an obround cross sectional area ("cylinder" with flattened, parallel top and bottom and semicylinder sides). As adjacent layers are printed, the polymer flows into the areas above and below the semicylindrical layers to create one seamless layer
Printing resolution (dimensions of layer):	Fine printing mode: 320 μm (w) x 150 μm (h). Increased operating pressure and greater fluidic sealing Fast printing mode: 400 μm (w) x 200 μm (h). Quicker prototyping, useful for larger print items
Print time (size):	20mins (small 15 x 15 x 2 mm), 1hr (medium 40 x 15 x 4 mm), 24hr (large 85 x 50 x 25 mm)

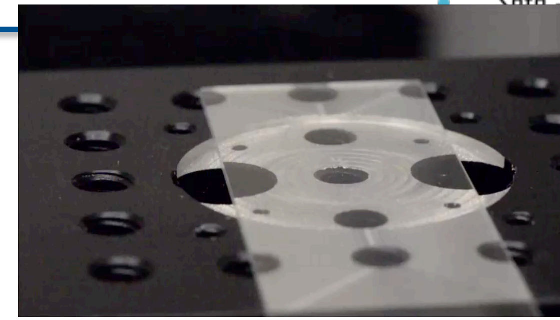
4D Printing

- Biomimetic 4D Printing



A. Gladman, Nature Materials, 15, 2016 (Scale Bar : 5mm)

Fused filament fabrication (FFF) for microfluidics



Lab on a Chip

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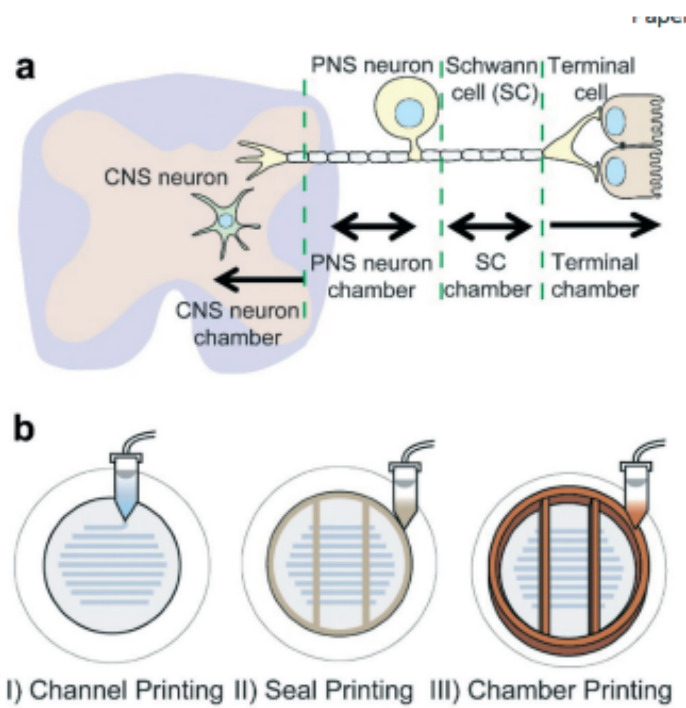
Cite this: *Lab Chip*, 2016, **16**, 1393

3D printed nervous system on a chip†

Blake N. Johnson,[ab] Karen Z. Lancaster,[c] Ian B. Hogue,[c] Fanben Meng,[bd] Yong Lin Kong,[b] Lynn W. Enquist[c] and Michael C. McAlpine*[bd]



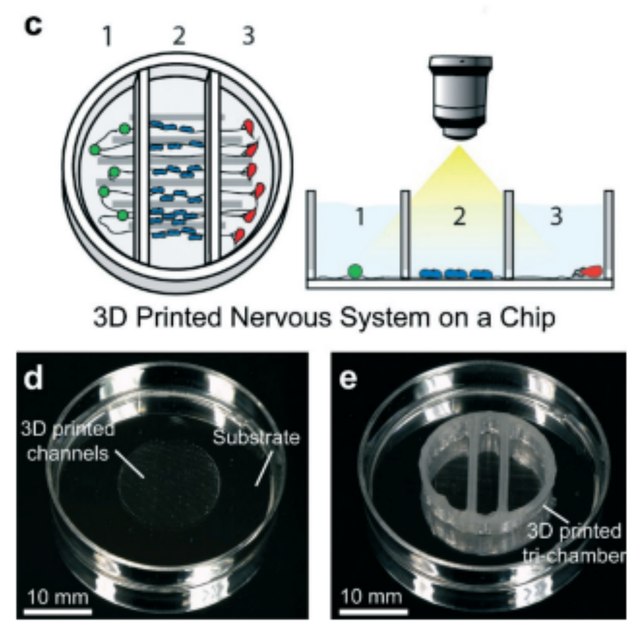


Fig. 1 a) Schematic of the nervous system, modelled as four primary components: CNS neurons, PNS neurons, axon-associated Schwann cells, and epithelial cells. b) 3D printing of the model nervous system on a chip, consisting of (I) parallel microchannels, (II) a sealant layer, and (III) a top tri-chamber. c) Schematic of a representative 3DNSC for peripheral nervous system applications, showing (1) PNS neurons in chamber 1, (2) Schwann cells in chamber 2, and (3) terminal cell junctions in chamber 3. The Schwann cells and the terminal cells interact with the neurons and each other solely *via* the axonal network. d) Circular pattern of 3D printed silicone microchannels for axonal guidance in the centre of a plastic 35 mm dish. e) A 3DNSC showing perpendicular assembly of microchannel and tri-chamber components.

Printing of Silicone (RTV), Polycaprolactone, Cells

Fused filament fabrication (FFF) for microfluidics

SMARTFIL® by smart materials 3D

COMPARATIVE CHART SMARTFIL FILAMENTS

	PRINTING TEMPERATURE	BED TEMPERATURE	PRINT DIFFICULTY	STRENGTH	FLEXIBILITY	DURABILITY	RESISTANCE TO TEMPERATURE	SOLUBLE	FOOD SAFE
PLA	200 / 220 / 240	0 / 60	●○○	●●○	●○○	●●○	60	✗	✓
PLA 3D850	200 / 210 / 220	0 / 60	●○○	●●○	●○○	●●○	75	✗	✓
PLA 3D870	200 / 210 / 220	0 / 60	●○○	●●●	●○○	●●○	75	✗	✗
EASY PRINT	190 / 200 / 210	0 / 60	●○○	●○○	●○○	●●○	55	✗	✓
WOOD	200 / 220 / 240	0 / 60	●○○	●○○	●○○	●○○	60	✗	✗
BOUN	210 / 220 / 230	0 / 60	●●○	●○○	●●○	●○○	65	ACETONE	✗
ABS	230 / 240 / 250	80 / 100	●●○	●●○	●●○	●●●	100	ACETONE	✓
ABS HIGH IMPACT	230 / 240 / 250	80 / 100	●●○	●○○	●●○	●●●	100	ACETONE	✗
ABS FIREPROOF	210 / 220 / 230	80 / 100	●●○	●●○	●●○	●●●	95	ACETONE	✗
ABS MEDICAL	230 / 240 / 250	80 / 100	●●○	●●●	●●○	●●●	100	ACETONE	✓
FLEX	215 / 225 / 235	0 / 100	●●●	●○○	●●●	●●○	105	✗	✗
HIPS	225 / 235 / 245	80 / 100	●●○	●●○	●●○	●●●	100	LIMONENE	✓
PETG	215 / 235 / 255	60 / 90	●●○	●●○	●●○	●●●	85	✗	✓
PP	210 / 220 / 230	60 / 100	●●●	●●●	●●○	●●●	60	✗	✓
NYLSTRONG	245 / 255 / 265	95 / 110	●●●	●●●	●○○	●●●	210	✗	✗
GLACE	205 / 220 / 235	70	●○○	●●○	●●○	●●●	75	ALCOHOL	✗
CLEAN	190 / 220 / 250	—	—	—	—	—	—	—	—
SUPPORT	210 / 240 / 270	70 / 100	●●○	●○●	●●○	●○○	—	LIMONENE	✗

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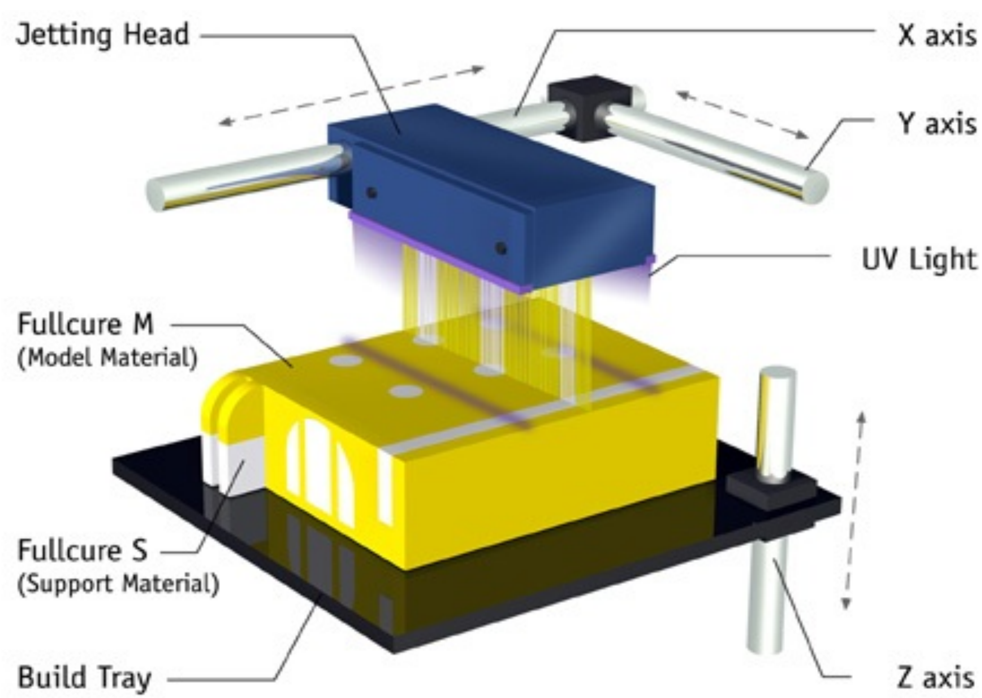
https://www.smartmaterials3d.com/blog/en/filamentos3d/tabla-smartfil/

					Range	Required?	Difficulty
ABS	Acrylonitrile Butadiene Styrene	High Strength	Moving Parts	High	210°C - 250°C	50°C - 100°C	Moderate
PLA	Polylactic Acid	User Friendly	Consumer Products	Fair to Good	180°C - 230°C	No	Easy
PET	PolyEthylene Terephthalate	High Strength	Moving Parts	High	220°C - 250°C	No	Moderate
HIPS	High Impact Polystyrene	Dual extrusion w/ ABS	Support Structure	High	210°C - 250°C	50°C - 100°C	Moderate
PVA	Polyvinyl alcohol	Dual extrusion w/ PLA	Support Structure	Good	180°C - 230°C	No	Easy
Nylon	Polyamide	High Strength	Moving Parts	High	220°C - 260°C	50°C - 100°C	Moderate
Wood	PLA + Wood	Wood Finish	Home Decor	Fair to Good	195°C - 220°C	No	Moderate
Sandstone	Co-Polyester + Sandstone	Sandstone Finish	Architectural	Low	165°C - 210°C	No	Moderate
Metal	Metal + PLA	Metal Finish	Jewelry	High	195°C - 220°C	No	High
Magnetic	Iron + PLA	Magnetic	Moving parts	High	195°C - 220°C	No	High
Conductive	Carbon + PLA	Conductive	Electronics	Low	215°C - 230°C	No	Easy
Temp Changing	PLA	Changes Color	Novelty	Fair to Good	215°C	No	Easy
Carbon Fiber	Carbon Fiber + PLA	High Strength	Moving Parts	High	195°C - 220°C	No	Moderate
Flexible / TPE	Thermoplastic Elastomer	Elastic	Wearables	Good	225°C - 235°C	No	High
Glow-In-The-Dark	PLA	Luminous	Novelty	Fair to Good	215°C	No	Easy

https://www.elveflow.com/microfluidic-tutorials/soft-lithography-reviews-and-tutorials/how-to-choose-your-soft-lithography-instruments/microfluidic-3d-printer/

PolyJet 3D Printer … multimaterial printing

Stratasys Objet Series

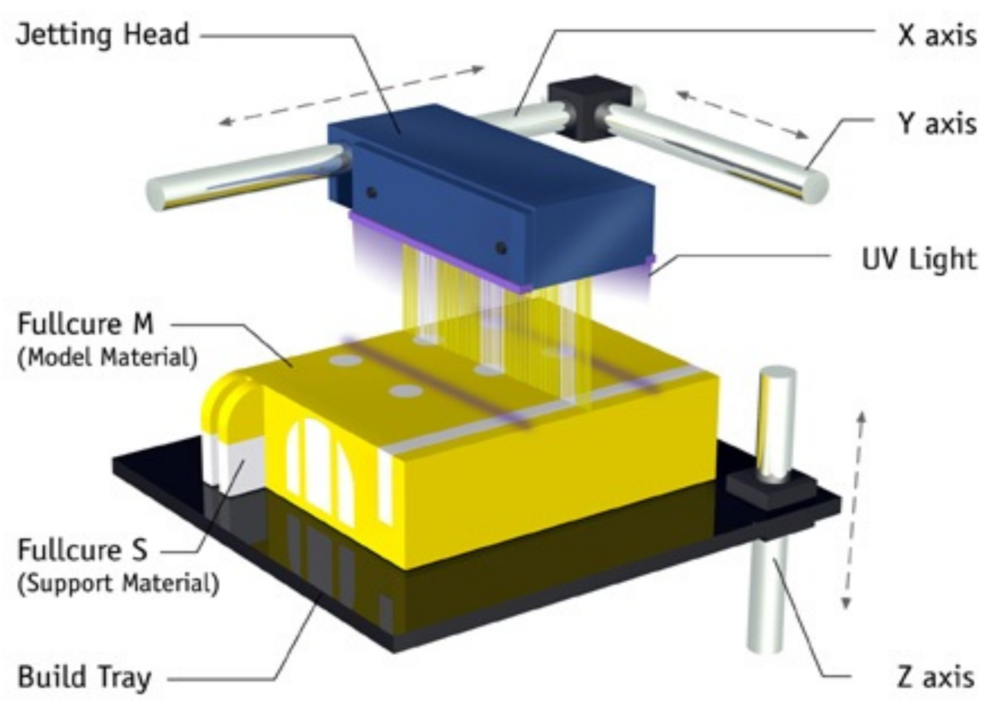


start 1min

- layers of liquid photopolymer layered onto a build tray and cure them with UV light

PolyJet 3D Printer … multimaterial printing

Stratasys Objet Series



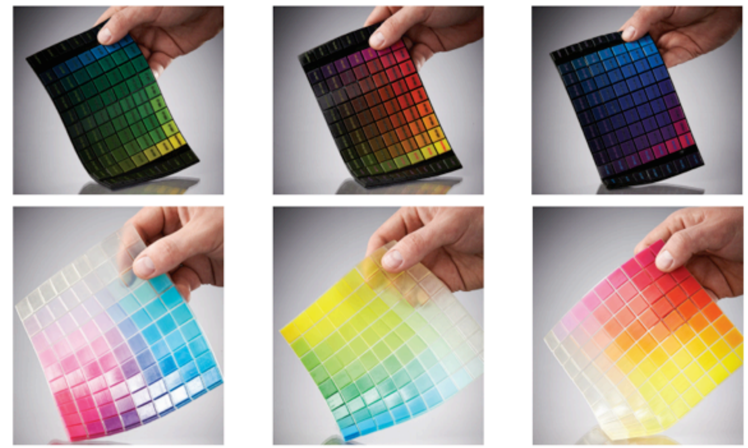
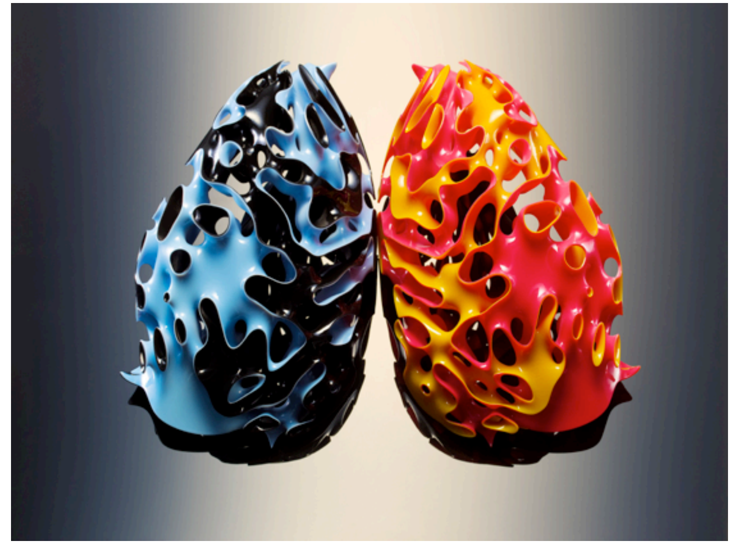


Figure 9: These six flexible color palettes provide a range of color options and Shore A values. Three 72-color palettes feature combinations of TangoPlus with VeroCyan, VeroMagenta and VeroYellow, while three 68-color palettes combine those same vivid colors with TangoBlackPlus.

- layers of liquid photopolymer layered onto a build tray and cure them with UV light

PolyJet 3D Printer

Stratasys Objet Series



- **Max dimensions**: 25x25x20 cm
- **Resolution :** r 50um
- **Materials :** ABS like, elastomer like, multicolor …
- **Application**: Products prototypes, art, architecture …

PolyJet 3D Printer … multimaterial printing

Lab on a Chip

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Cite this: Lab Chip, 2016, 16, 668

3D printed microfluidic circuitry *via* multijet-based additive manufacturing†

R. D. Sochol,‡*[abcd] E. Sweet,[ab] C. C. Glick,[be] S. Venkatesh,[ab] A. Avetisyan,[f] K. F. Ekman,[ab] A. Raulinaitis,[ab] A. Tsai,[bg] A. Wienkers,[ab] K. Korner,[ab] K. Hanson,[bg] A. Long,[bg] B. J. Hightower,[abh] G. Slatton,[ab] D. C. Burnett,[bi] T. L. Massey,[bi] K. Iwai,[ab] L. P. Lee,[bg] K. S. J. Pister[bi] and L. Lin§*[ab]

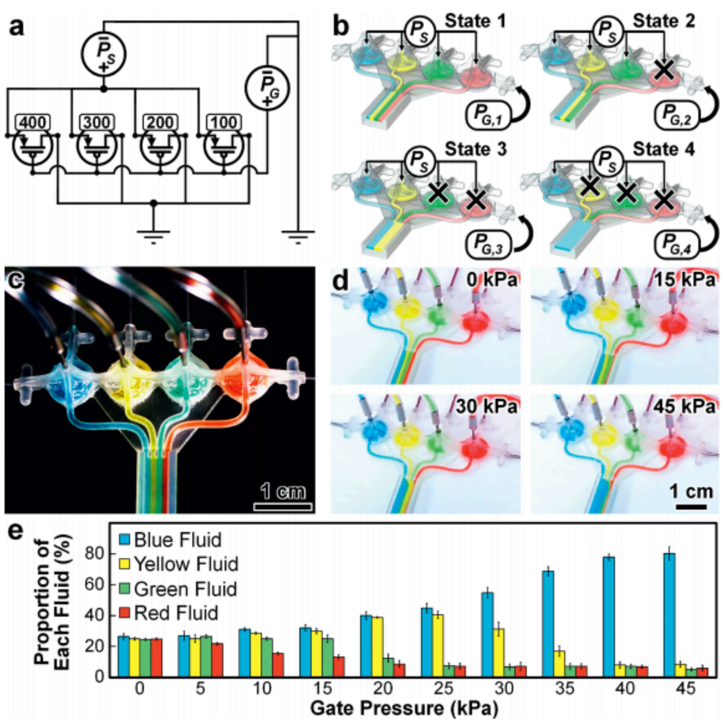

Fig. 6 3D P_G-actuated multi-flow controller. (a) Circuit diagram. Numeric values denote the relaxed state distance between the top surface of the piston element and the source outlet for each fluidic transistor (D_{PS}; ESI† Fig. S8d). Units are in μm. (b) Conceptual illustrations of the four primary flow states ($P_{G,1} < P_{G,2} < P_{G,3} < P_{G,4}$; P_S = constant). Ports without arrows are sealed during device operation. (c) Fabrication results. (d) Experimental results for four distinct fluidic streams under constant P_S = 1 kPa and varying P_G (see also ESI† Movie S6). (e) Quantified fluidic stream proportions for constant P_S = 1 kPa and varying P_G. All error bars/bands denote standard deviation.

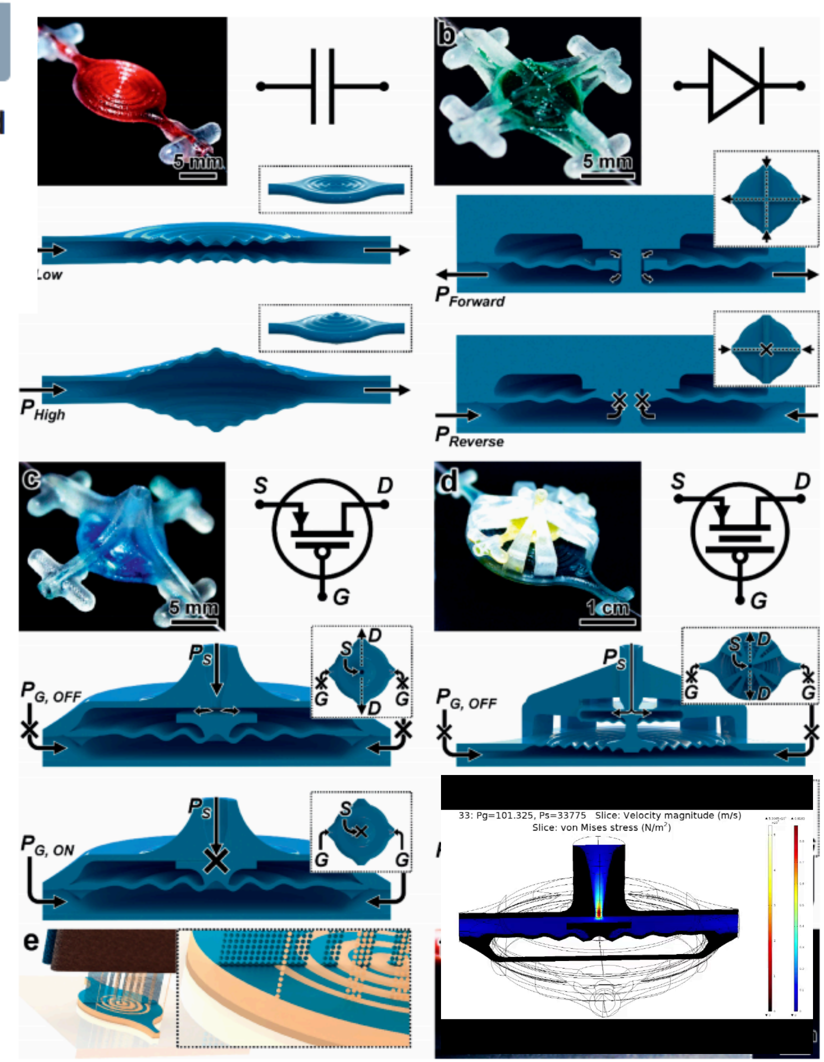

Fig. 1 3D printed fluidic circuit components *via* multijet modelling (MJM). (a–d) Fabrication results, analogous electronic circuit symbols, and conceptual operating principles for 3D printed: (a) fluidic capacitors, (b) fluidic diodes, (c) fluidic transistors, and (d) enhanced-gain fluidic transistors. The fluidic components operate based on pressure (*P*) inputs. The 3D fluidic transistors (c, d) are analogous to p-channel MOSFET transistors, with gate (G) regulation of source (S) to drain (D) fluid flow (Q_{SD}). (e) Conceptual illustration of the MJM process for simultaneous inkjet deposition of photoplastic (blue) and sacrificial support (beige) materials. (f) A 3D printed DNA-inspired architecture comprised of eight fluidic channels (750 μm in diameter) filled with discrete solutions of dye-coloured fluid.

PolyJet 3D Printer … multimaterial printing

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3D printed microfluidic circuitry *via* multijet-based additive manufacturing†

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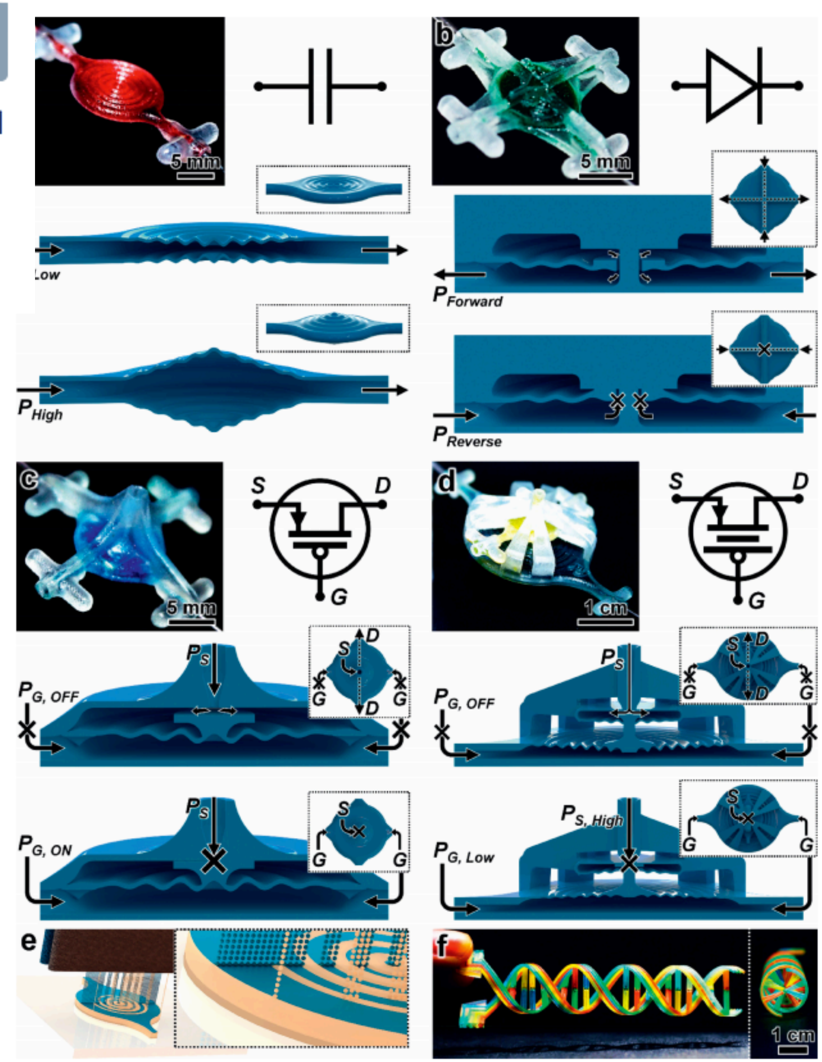

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PolyJet 3D Printer … multimaterial printing

SCIENTIFIC REPORTS

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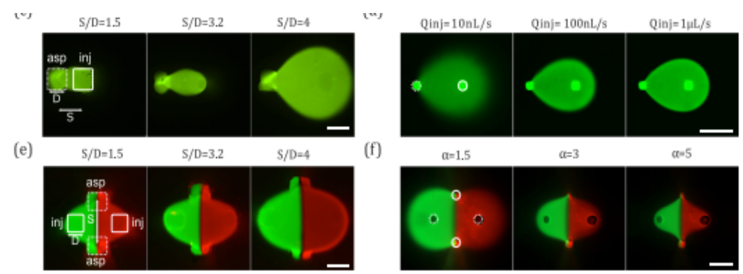
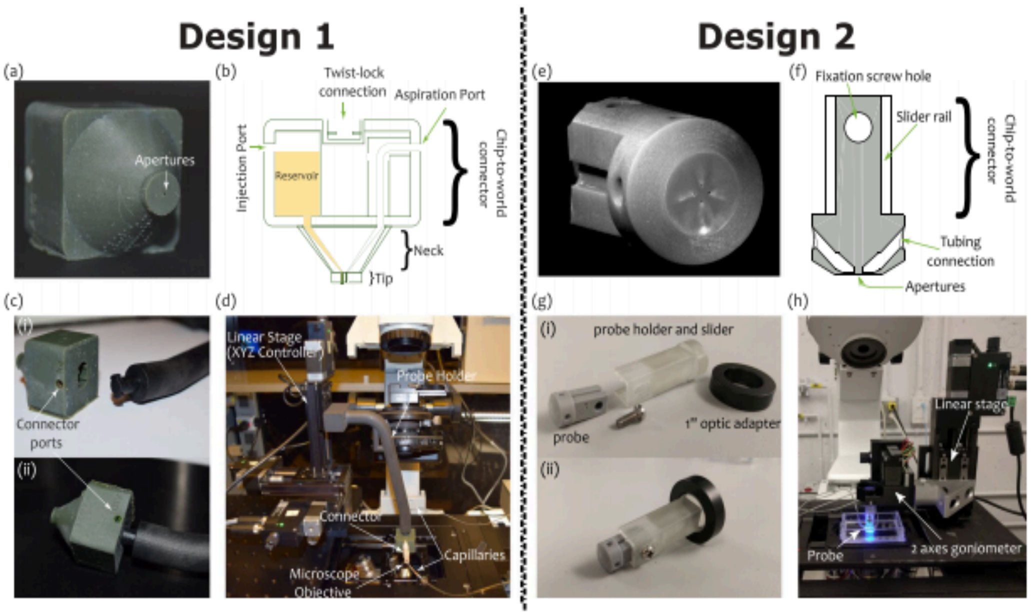
3D Printed Microfluidic Probes

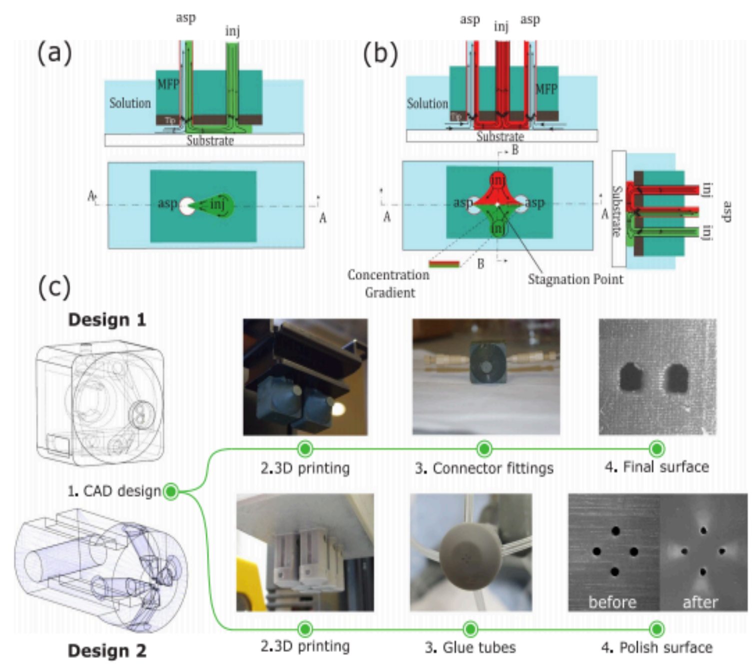
Ayoola Brimmo[1,2], Pierre-Alexandre Goyette[3], Roaa Alnemari[1], Thomas Gervais[3,4,5] & Mohammad A. Qasaimeh[1,2]

Received: 16 April 2018
Accepted: 9 July 2018
Published online: 20 July 2018

In this work, we fabricate microfluidic probes (MFPs) in a single step by stereolithographic 3D printing and benchmark their performance with standard MFPs fabricated via glass or silicon micromachining. Two research teams join forces to introduce two independent designs and fabrication protocols, using different equipment. Both strategies adopted are inexpensive and simple (they only require a stereolithography printer) and are highly customizable. Flow characterization is performed by reproducing previously published microfluidic dipolar and microfluidic quadrupolar reagent delivery profiles which are compared to the expected results from numerical simulations and scaling laws. Results show that, for most MFP applications, printer resolution artifacts have negligible impact on probe operation, reagent pattern formation, and cell staining results. Thus, any research group with a moderate resolution ($\leq$100 μm) stereolithography printer will be able to fabricate the MFPs and use them for processing cells, or generating microfluidic concentration gradients. MFP fabrication involved glass and/or silicon micromachining, or polymer micromolding, in every previously published article on the topic. We therefore believe that 3D printed MFPs is poised to democratize this technology. We contribute to initiate this trend by making our CAD files available for the readers to test our "print & probe" approach using their own stereolithographic 3D printers.







PolyJet 3D Printer (Objet 350)

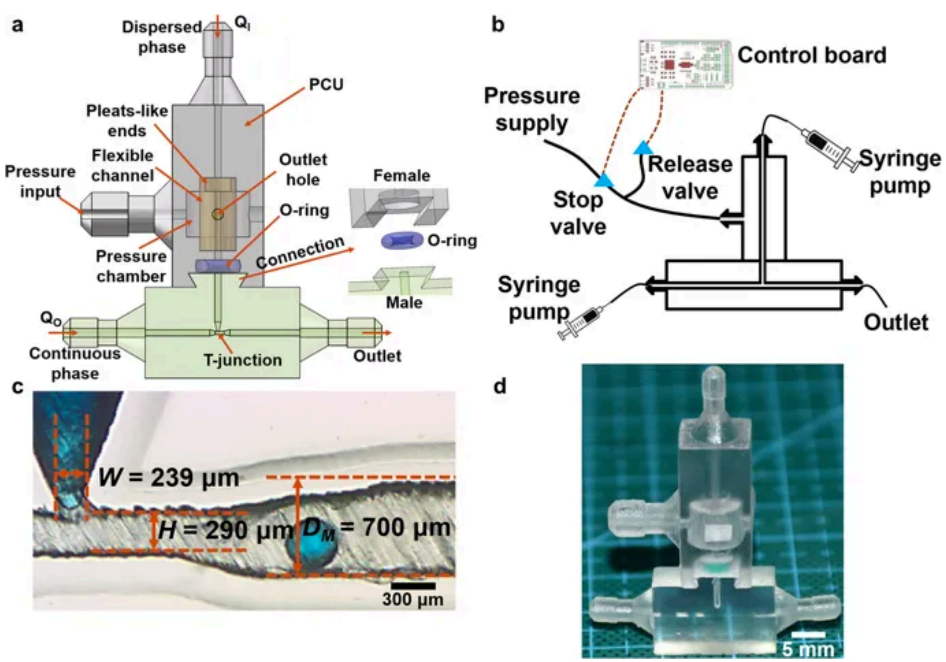
SCIENTIFIC REPORTS

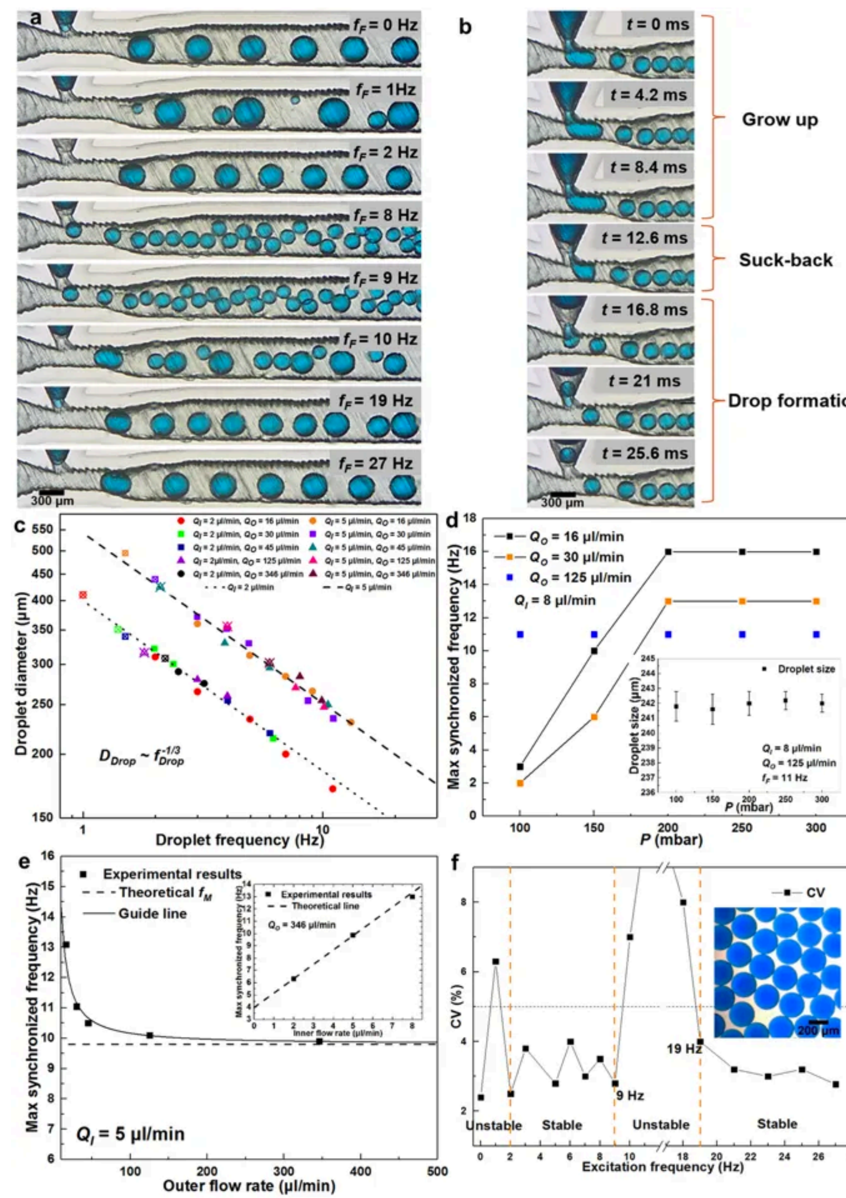
Article | Open Access | Published: 19 March 2018

A Modular Microfluidic Device *via* Multimaterial 3D Printing for Emulsion Generation

Qinglei Ji, Jia Ming Zhang, Ying Liu, Xiying Li, Pengyu Lv, Dongping Jin & Huiling Duan

Scientific Reports 8, Article number: 4791 (2018) | Download Citation





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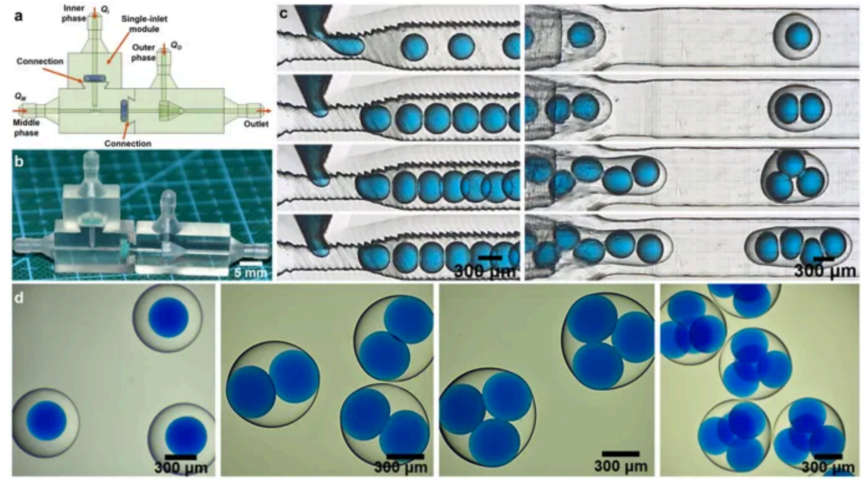
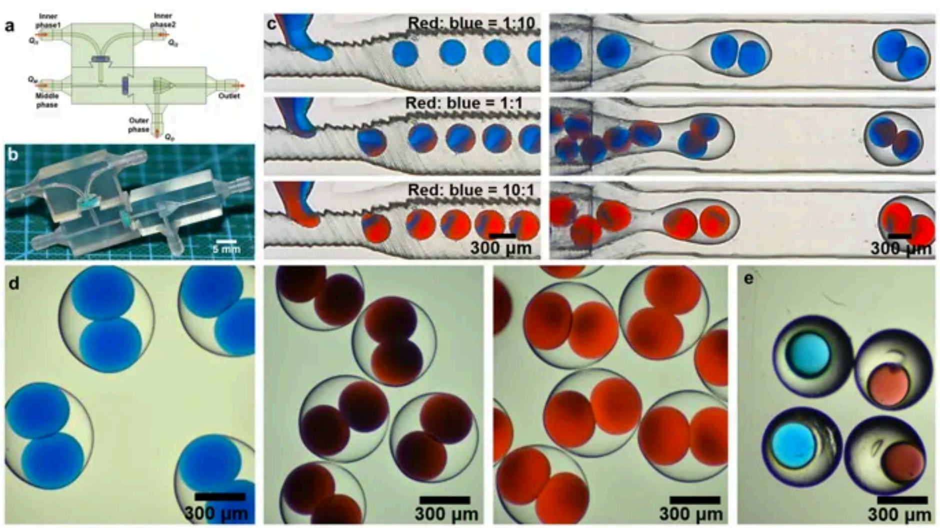
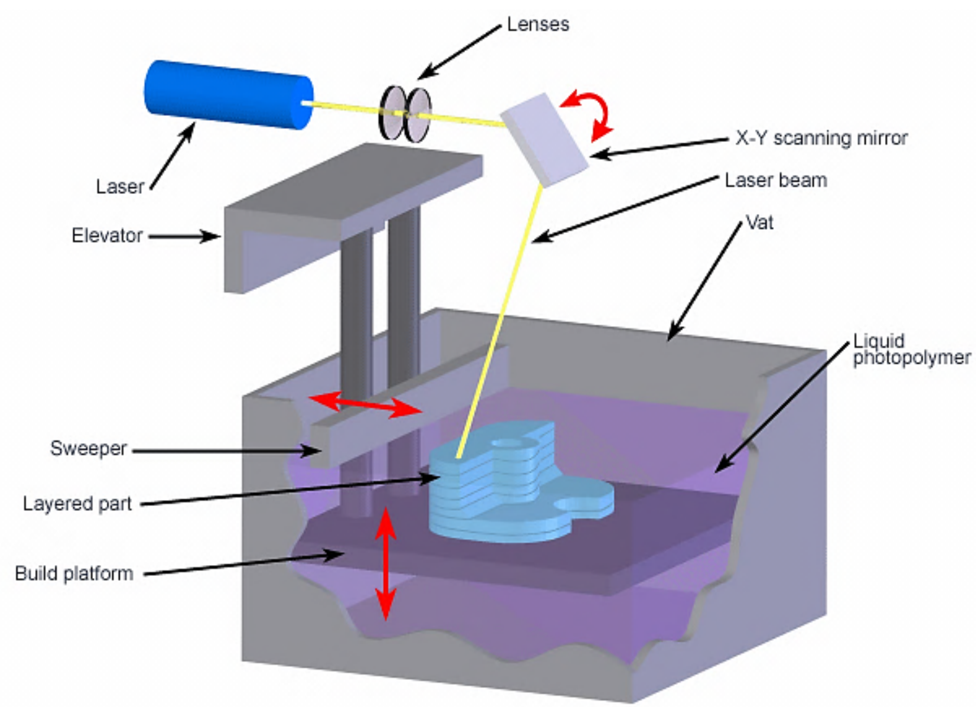
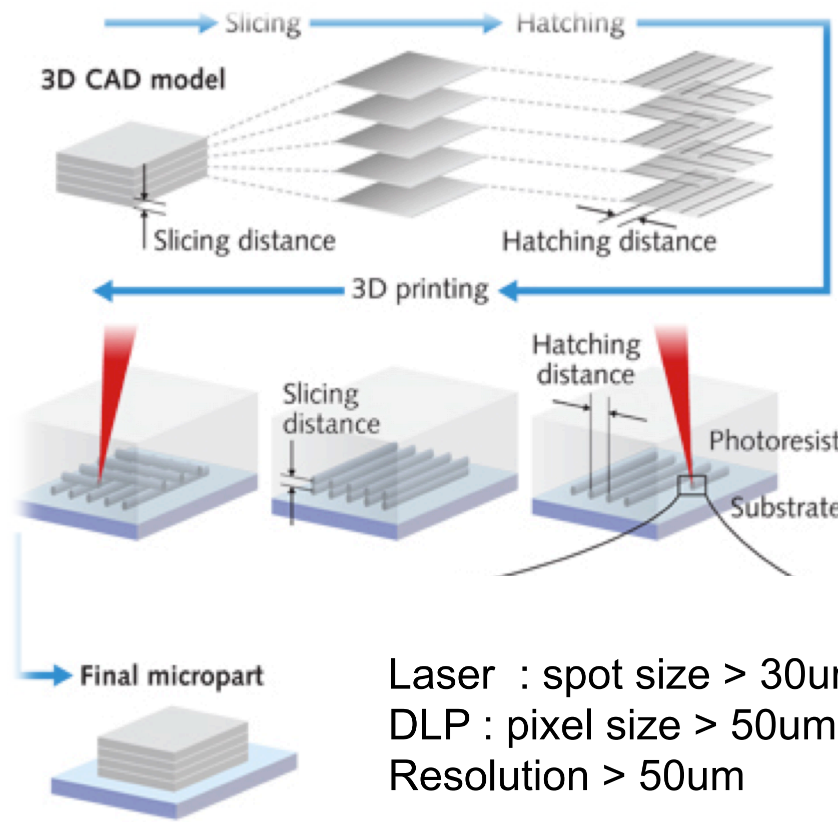




Silicone oils (Beijing Hagibis Technology Ltd.) of different viscosities were used as the oil phase (O), whereas water-glycerin (Sinopharm Chemical Reagent Ltd.) mixtures with different viscosities were used as the aqueous phase (W). A 3D-printed resin (**Clear FLGPCL04**) was used as the solidification phase for microsphere fabrication. RSN-749 resin (CosBond Ltd.), as a surfactant, was used in the oil phase with 0.25% (v/v), and Tween-20 (Beijing Huabo Ltd.), as a surfactant, was used in the aqueous phase with 0.25% (w/v). 1. Soluble food dyes with different colors (PT. Gunacipta Multirasa Co.) were used in the aqueous phases for a better observation.

Stereolithography (SLA)



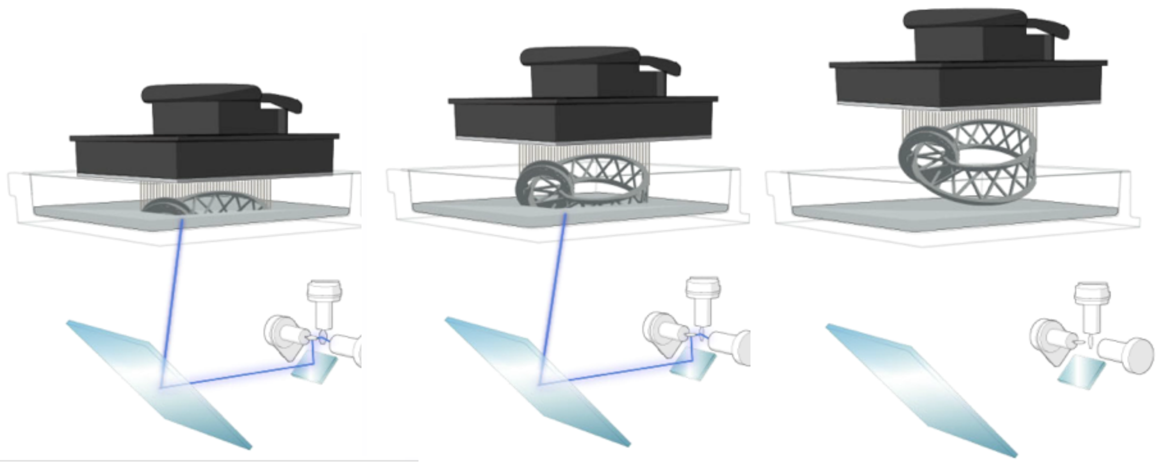


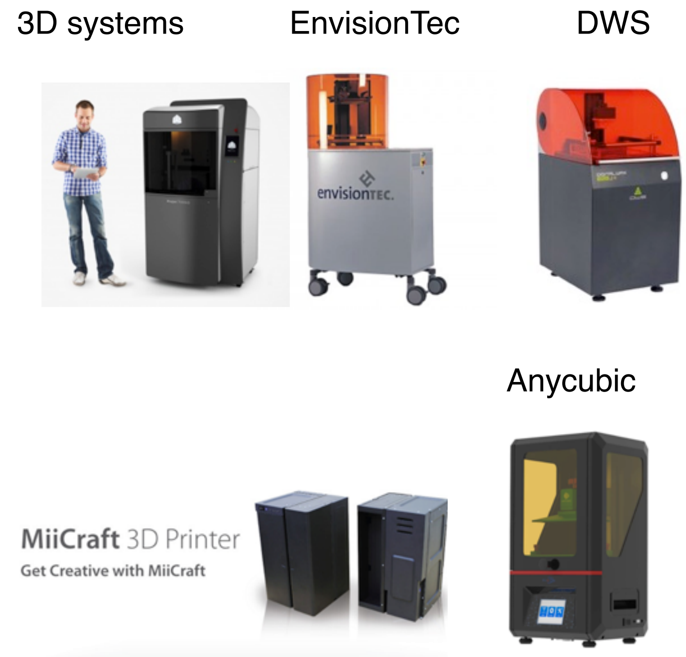
Laser : spot size > 30um
DLP : pixel size > 50um
Resolution > 50um

- parts are built from a liquid photopolymer
- each layer is created by a UV laser that cures one cross section at a time.

Stereolithography (SLA)

What currently means “Stereolithography” :

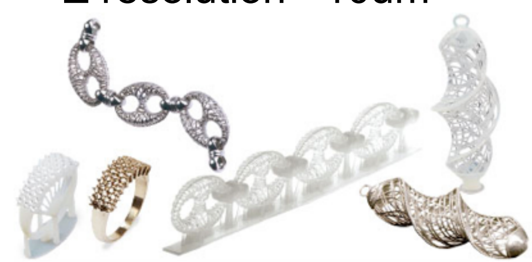




Laser : spot size > 30um
DLP : pixel size > 50um
X,Y Resolution > 50um
Z resolution >10um

Application domains :

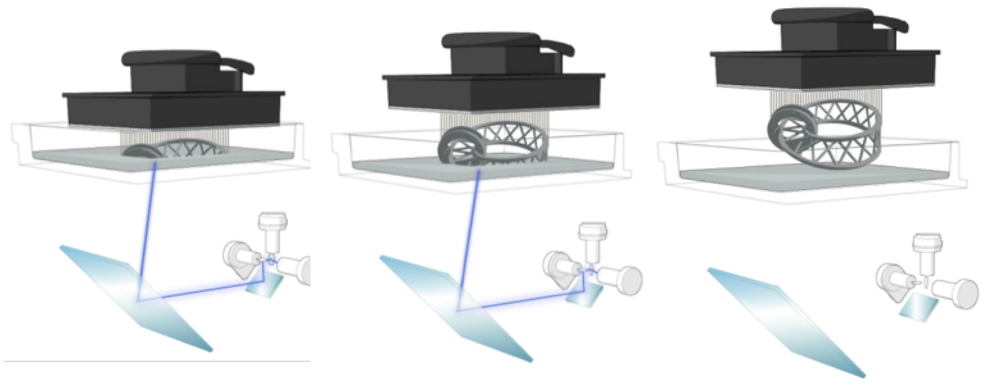
- Dental health
- Jewellery
- Micromechanics



Stereolithography (SLA)

start 1’45`

In the mid-1980s, Chuck Hull



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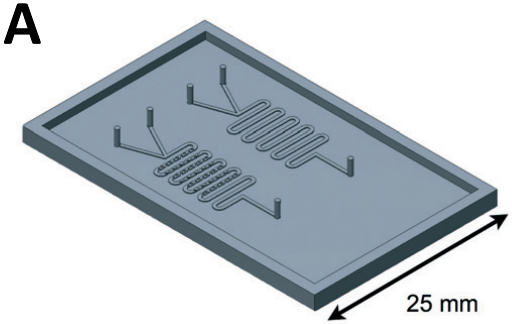
Z resolution >10um

- parts are built from a liquid photopolymer
- each layer is created by a UV laser that cures one cross section at a time.

Stereolithography for microfluidics

- Master fabrication for molding

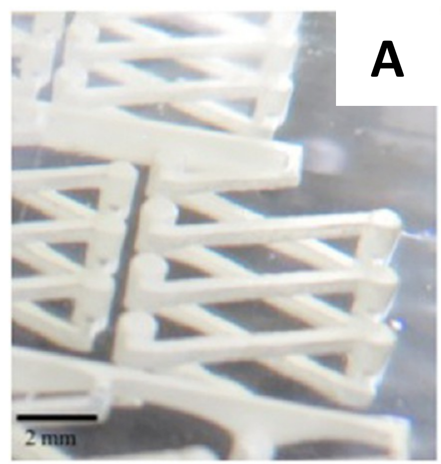
A

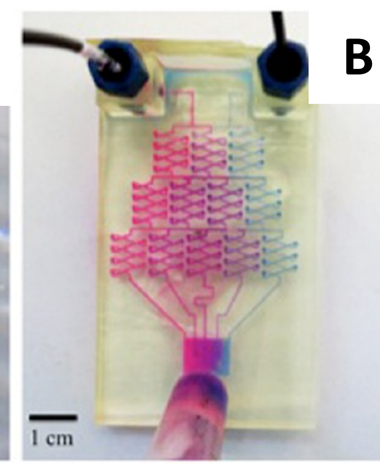


B

G. Comina, A. Suska and D. Filippini. *PDMS lab-on-a-chip fabrication using 3D printed templates.* Lab Chip 2014, 14, 424-430.

- Direct fabrication of devices

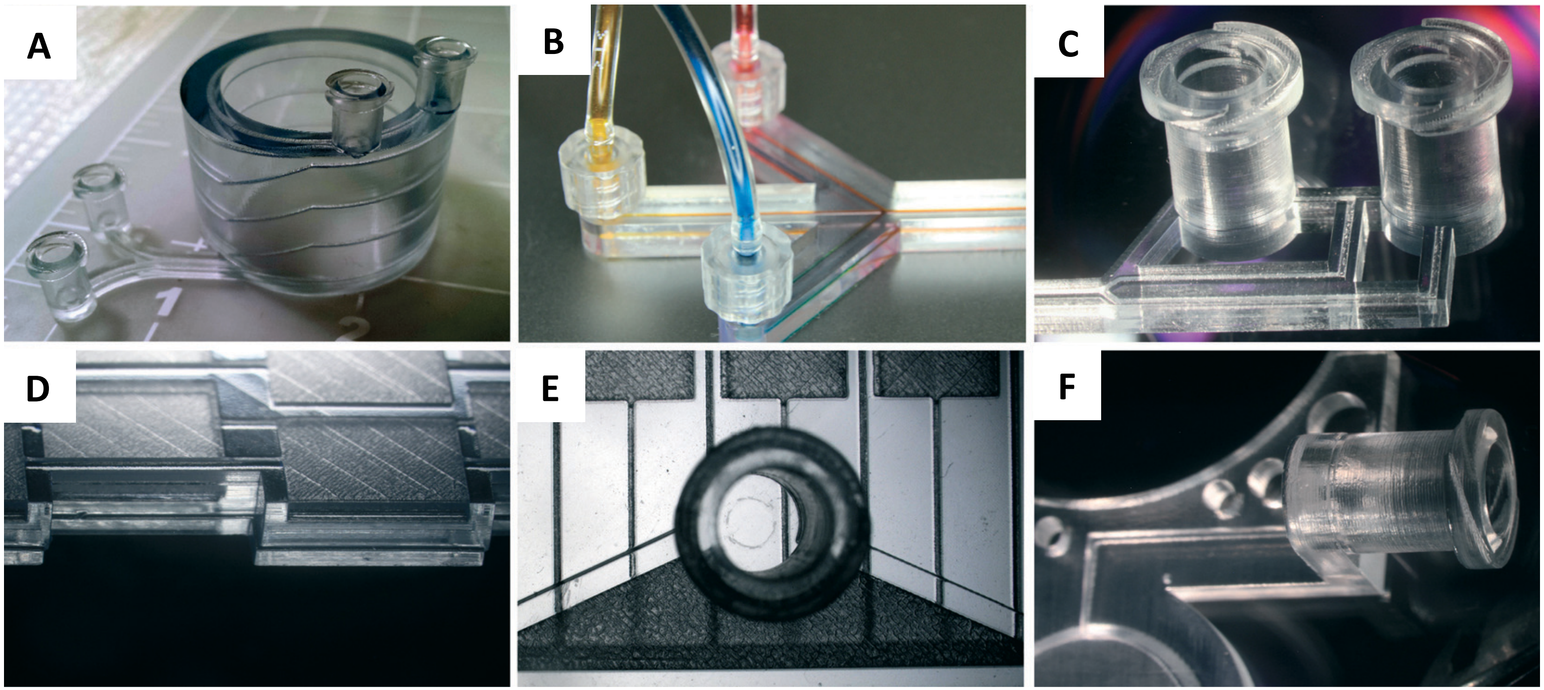




A. I. Shallan, P. Smejkal, M. Corban, R. M. Guijt and M. C. Breadmore. *Cost-effective three-dimensional printing of visibly transparnet microchips within minutes.* Anal. Chem. 2014,86, 3124-3130.

Stereolithography for microfluidics

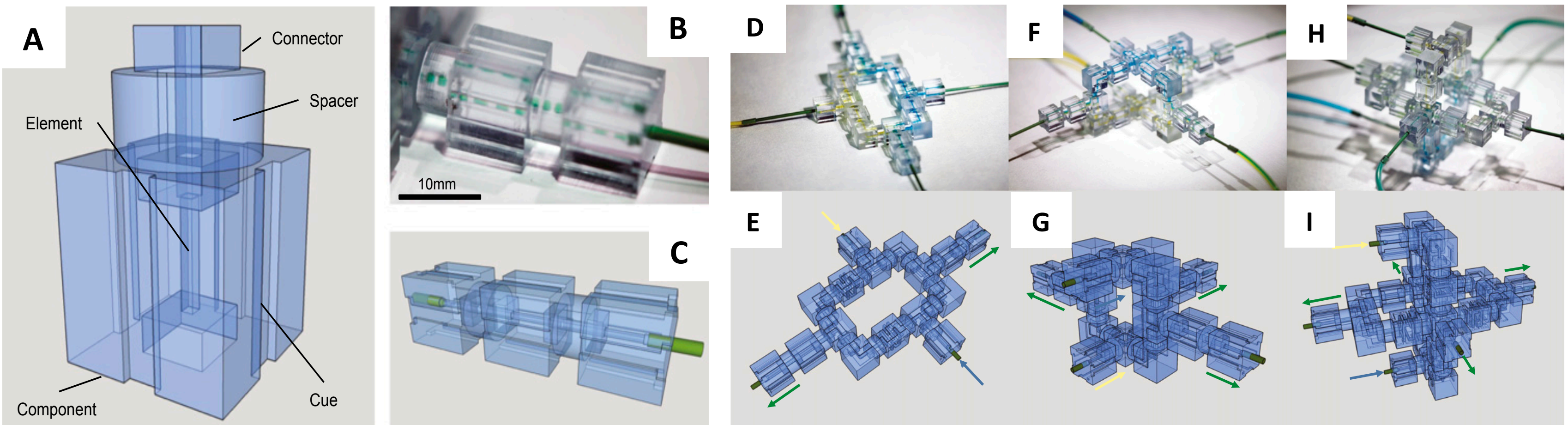
- **Solving the world to chip problem**



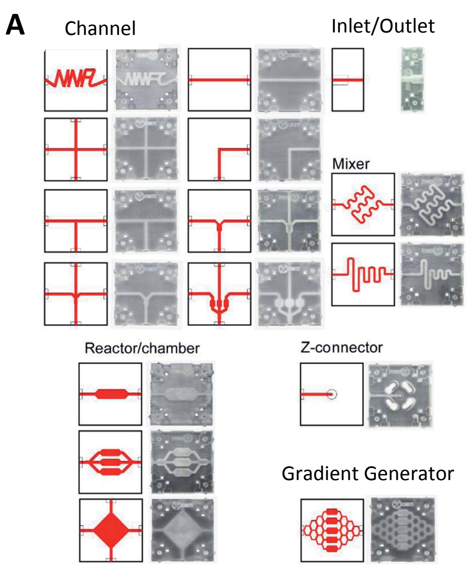
O. H. Paydar, C.N. Parede, Y. Hwang, J. Paz, N.B. Shah and R.N. Candler. *Characterization of 3D-printed microfluidic chip interconnects with integrated O-rings.* Sensors and Actuators A : Physical 2014, 205, 199-203.

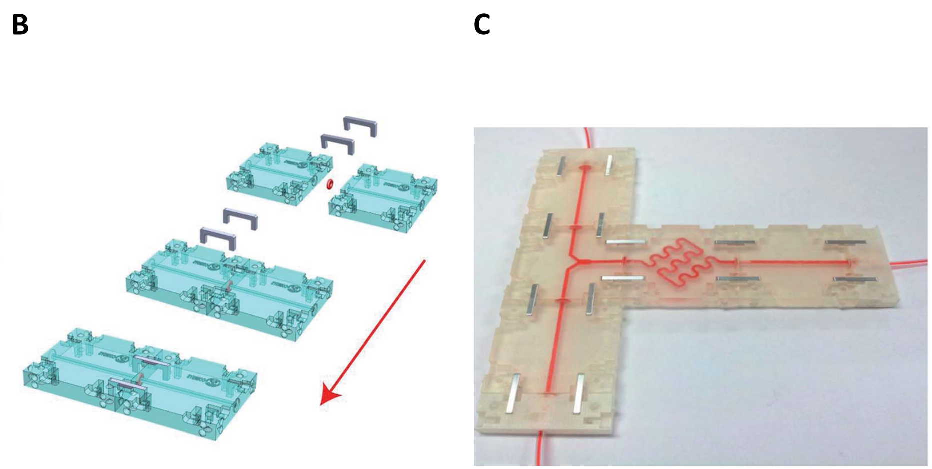
Stereolithography for microfluidics

- « LEGO » like microfluidics



K. C. Bhargava, B. Thompson and N. Malmstadt. *Discrete elements for 3D microfluidics*. PNAS 2014





K. G. Lee, K. J. Park, S. Seok, S. Shin, D. H. Kim, J. Y. Park, Y. S. Heo, S. J. Lee and T. J. Lee. *3D printed modules for integrated microfluidic devices.* RSC Advances 2014, 4, 32876-32880.

Stereolithography for microfluidics

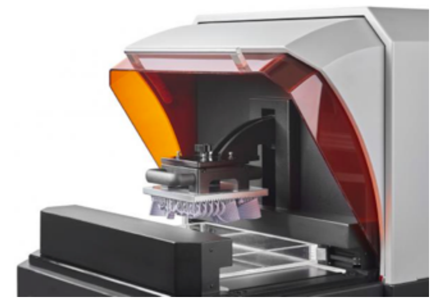
- **SLA system : DWS 29J+**

<u>Technical specifications :</u>

- **Resolution X,Y : 40 um**
- Resolution Z : 10-100um
- Laser Wavelength : 405 nm
- Samples size (15 x 15 x 10 cm - X,Y,Z)
- **Laser speed : 6m/s**

<u>Materials :</u>

- Commercial UV photosensitive resists
- Commercial SLA resists (PU, acrylate + microparticles …)
- Home made materials (Hydrogels …)



Stereolithography for microfluidics

Static micromixers based on large-scale industrial mixer geometry

Arnaud Bertsch,*[a] Stephan Heimgartner,[a] Peter Cousseau[b] and Philippe Renaud[a]

[a] *Swiss Federal Institute of Technology, EPFL, DMT-IMS, 1015 Lausanne, Switzerland*
[b] *Debiotech SA, Avenue de Sévelin 28, 1004 Lausanne, Switzerland*

Received 30th April 2001, Accepted 17th July 2001
First published as an Advance Article on the web 9th August 2001

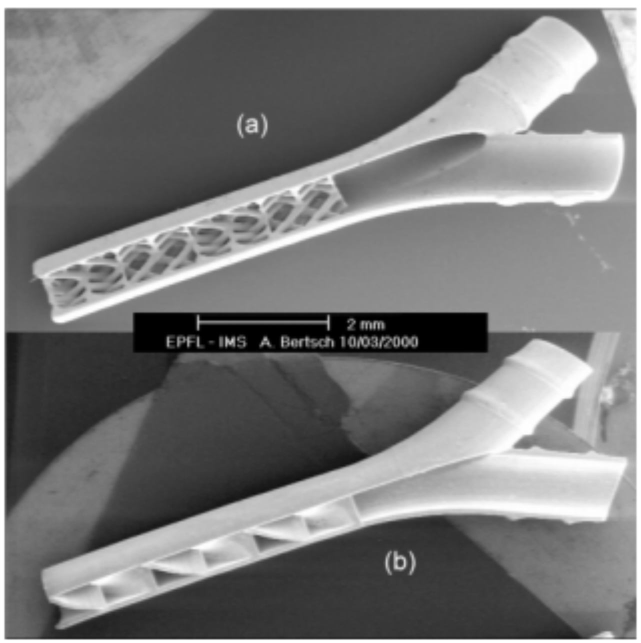
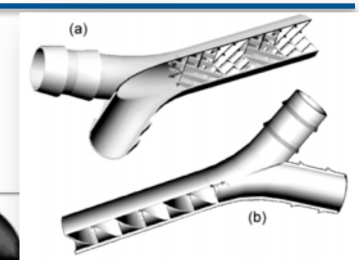


Fig. 2 Cut-out view of the micromixer structures built by micro-stereolithography. (a) Micromixer made of intersecting channels. (b) Micromixer made of helical elements.



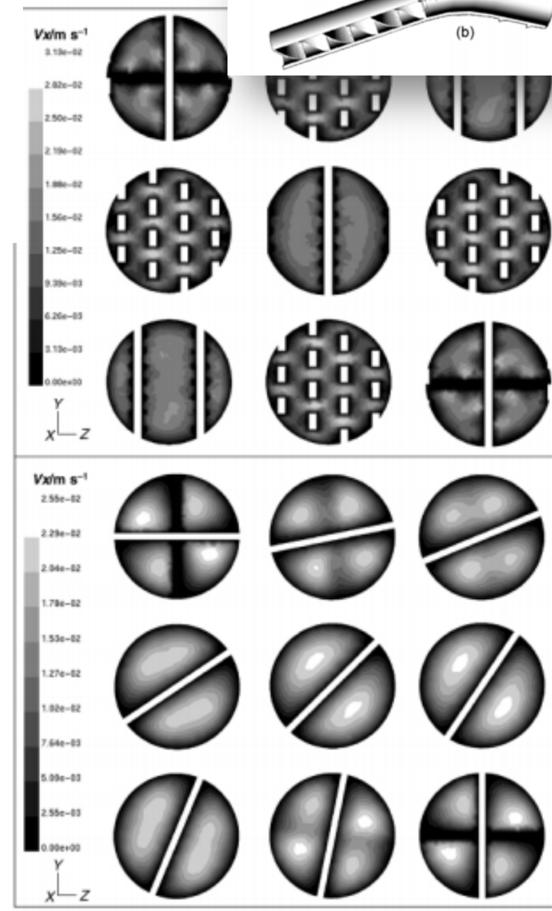
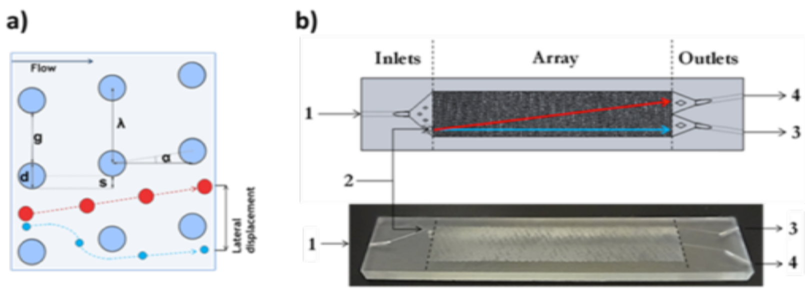


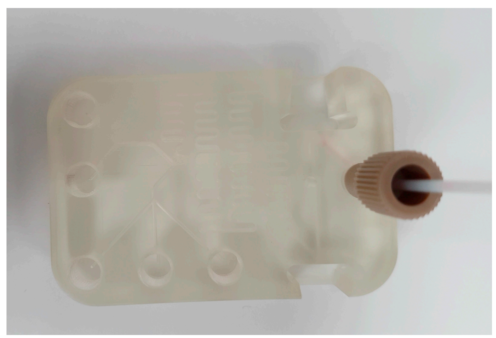
Fig. 5 Cross sectional profiles of velocity computed for flow with $Re = 12$ at regularly spaced locations of one element of each type of micromixer (from top left to bottom right). The top part corresponds to the 3rd element of the micromixer made of intersecting channels. The bottom part corresponds to the 4th element of the micromixer made of helical elements.

Stereolithography for microfluidics

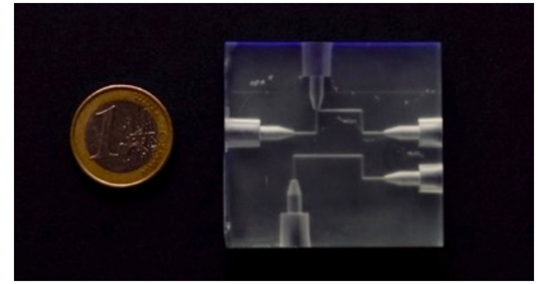
→ European project HOLIFAB : Pilot Line (Sculpteo, Fluigent)

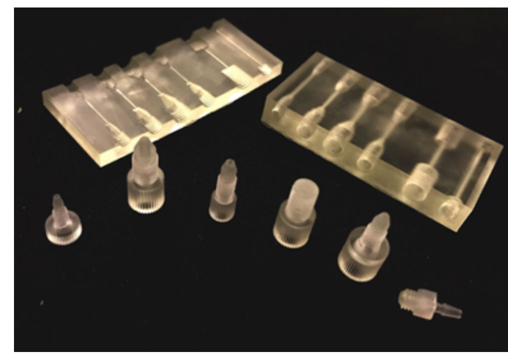


Juskova, P.; Ollitrault, A.; Serra, M.; Viovy, J.-L.; Malaquin, L. Analytical, Chimica Acta, 2017.

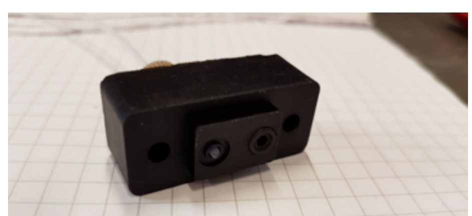


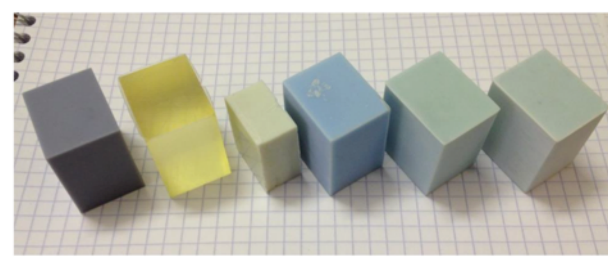
DS3000 (DWS) –
Transparent Biocompatible





GM08b (black, rubber like)



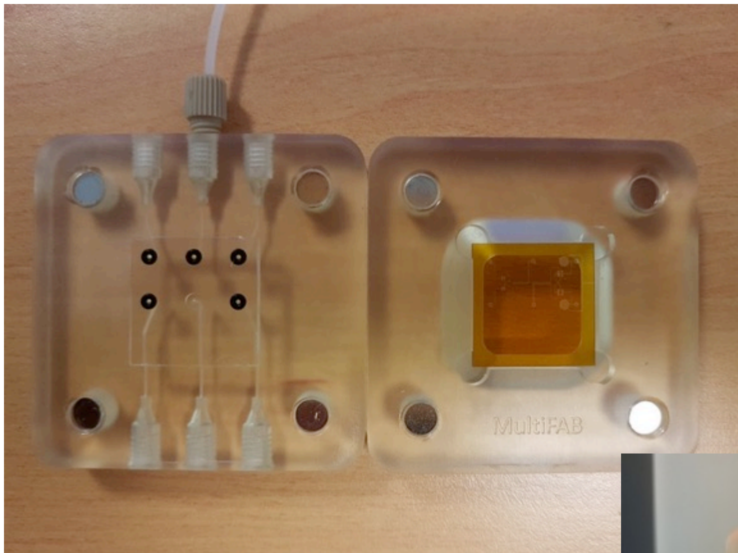


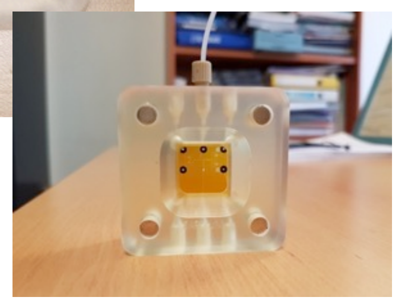
dl-260 ds-2000 ds-2000+ HA rd-096-b dm-220 dm-220 2h @ 120°C

Stereolithography for microfluidics

Coupling silicon technologies & SLA

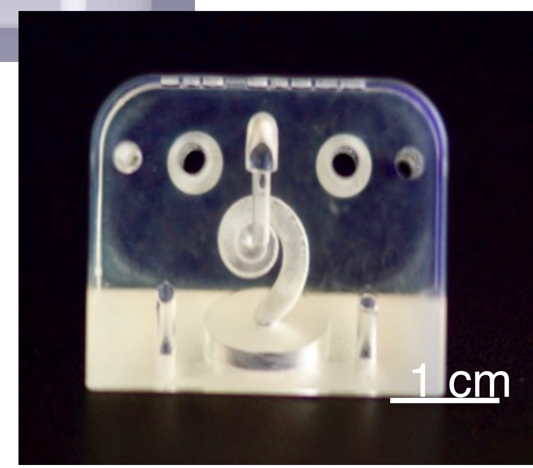
DS3000 material, DWS 29J+, R. Courson, V. Raimbault, J. Foncy





Magnetic clamping system
Integrated channels (300μm)
Integrated o-rings for glass/dryfilm device connection
Integrated Upchurch ports

Cochlea models for electrode implantation and fabrication (OTICON)

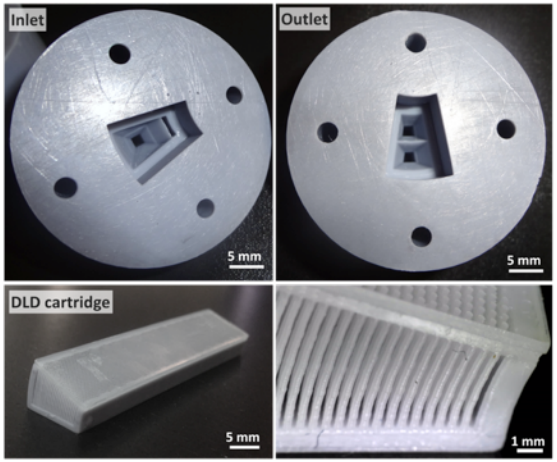


Objectives :

- **Integration of sensors / electrodes**
- **Integration of optical sensors / waveguides for detection**

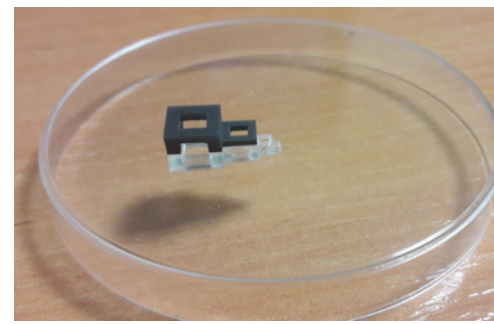
Stereolithography for microfluidics

- Composite materials for improved mechanical properties



→ Alumina/Silica doped photoresist
- Lower X,Y resolution
- Higher Z resolution
  (compared to DS3000)

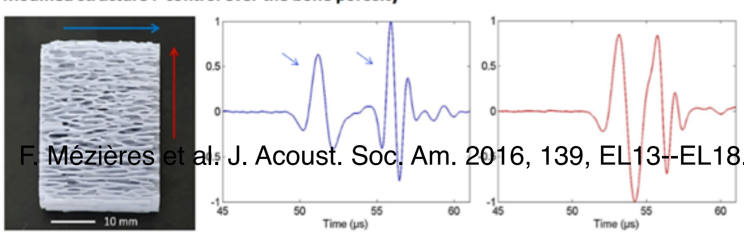
- High mechanical resistance
  / Low deformation



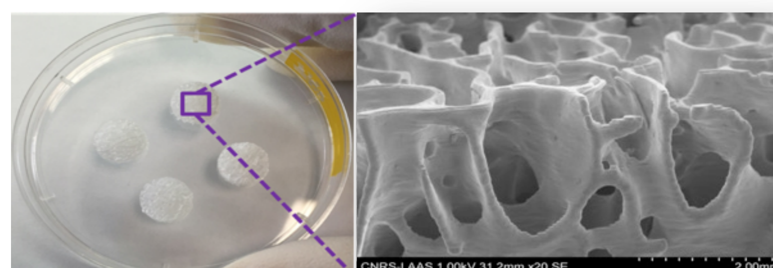
- Hybridization : Magnetic Transparent materials

- Composite materials for improved acoustic/biological properties : Trabecular bone s

Modified structure : control ever the bone porosity



F. Mézières et al. J. Acoust. Soc. Am. 2016, 139, EL13--EL18.



Photosensitive PDMS ?

COMMUNICATION
Microfluidics
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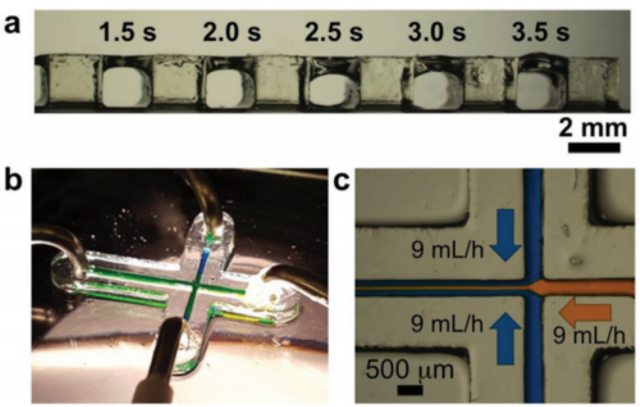
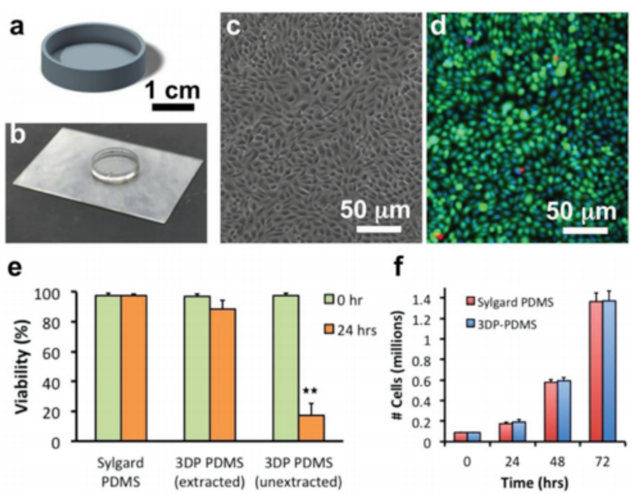
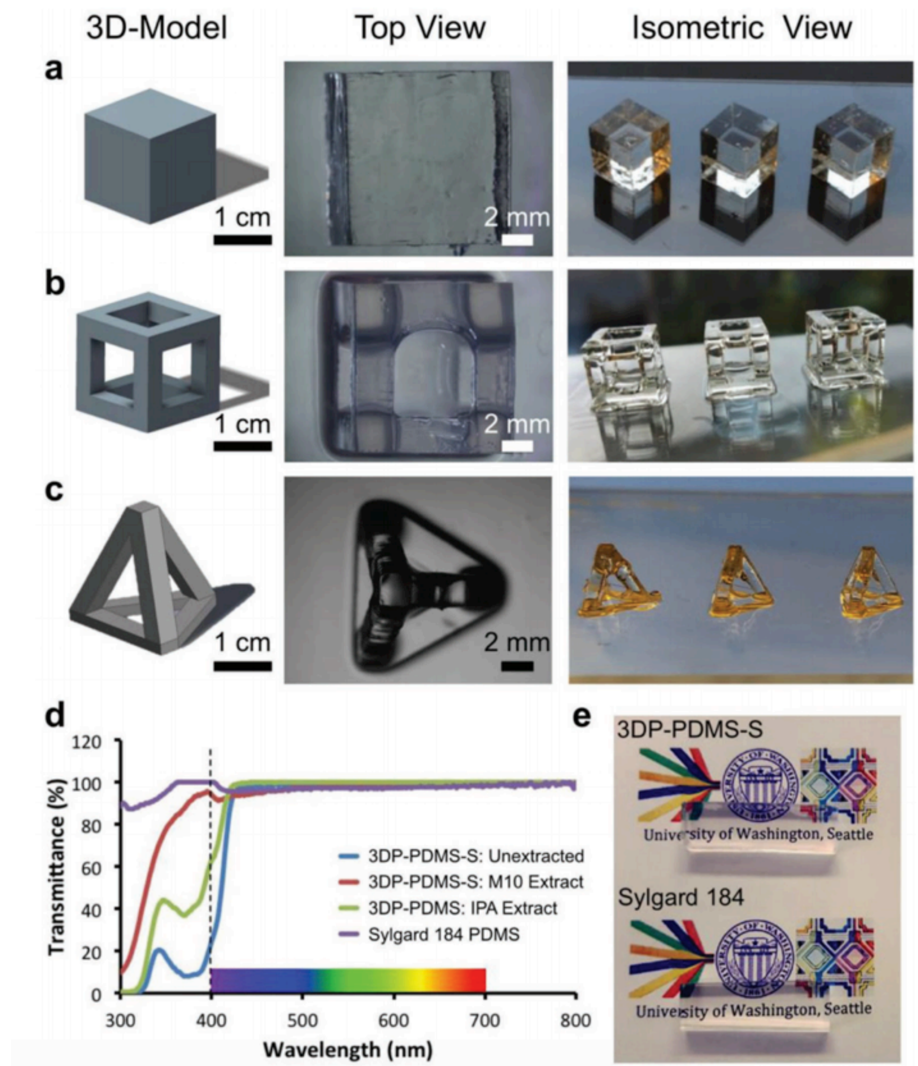
Desktop-Stereolithography 3D-Printing of a Poly(dimethylsiloxane)-Based Material with Sylgard-184 Properties

Nirveek Bhattacharjee, Cesar Parra-Cabrera, Yong Tae Kim, Alexandra P. Kuo, and Albert Folch**

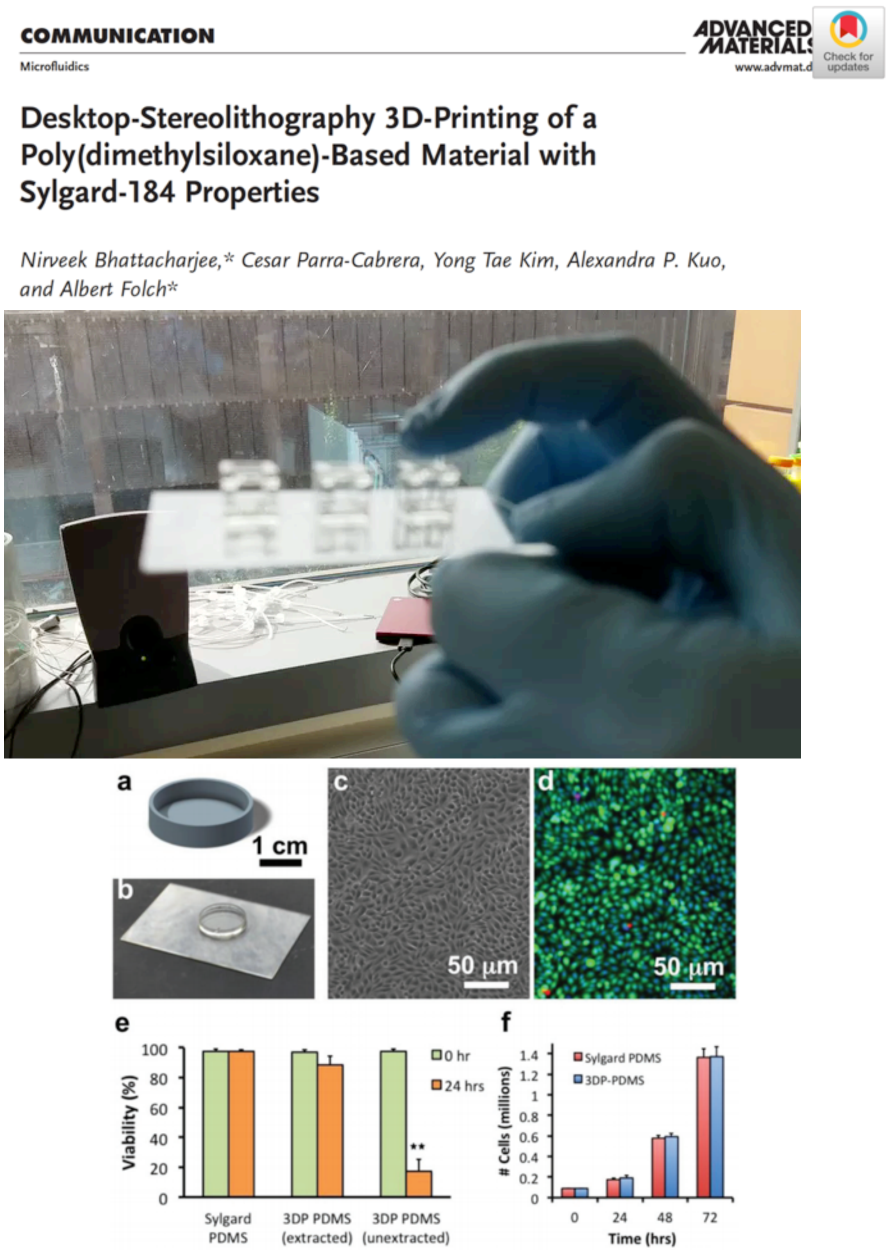


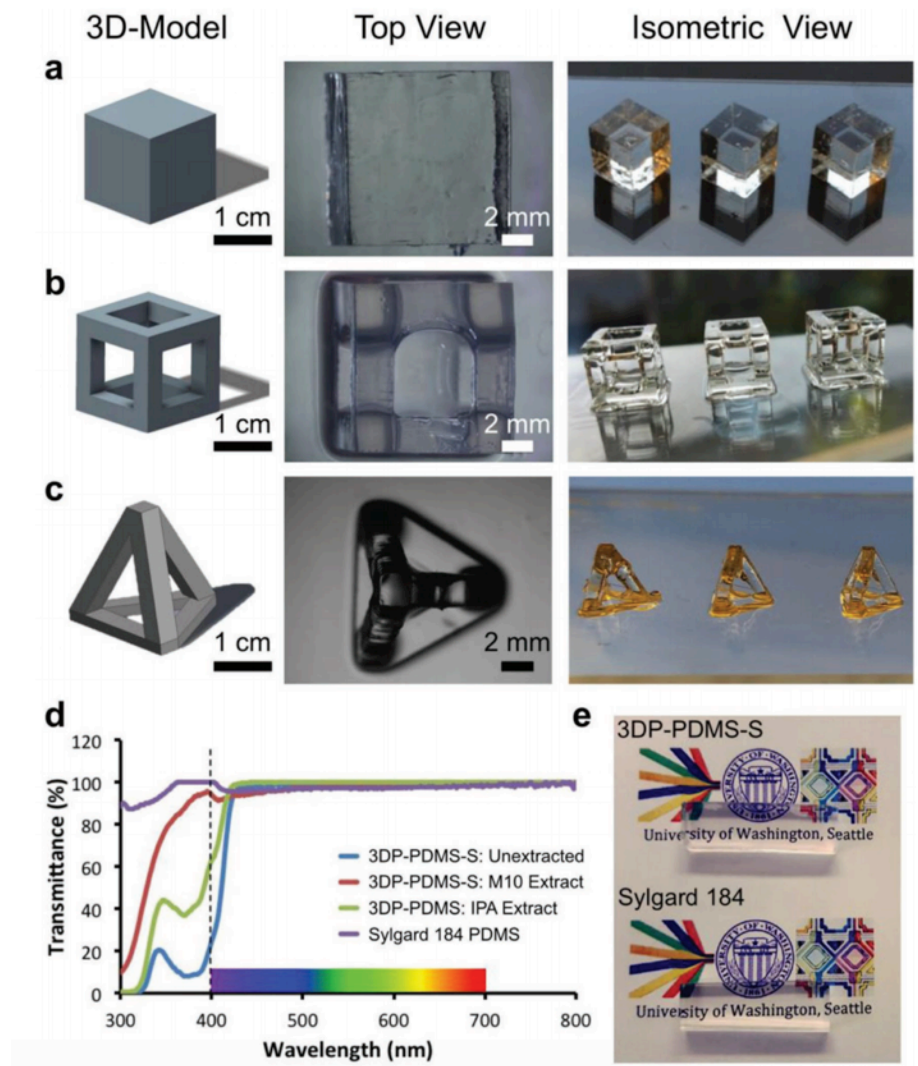




Long exposure time + baking
Resolution : 50 μm ?

Photosensitive PDMS ?





Long exposure time + baking

Resolution : 50 μm ?

X,Y *vs* Z resolution …

ROYAL SOCIETY OF CHEMISTRY

Lab on a Chip

PAPER

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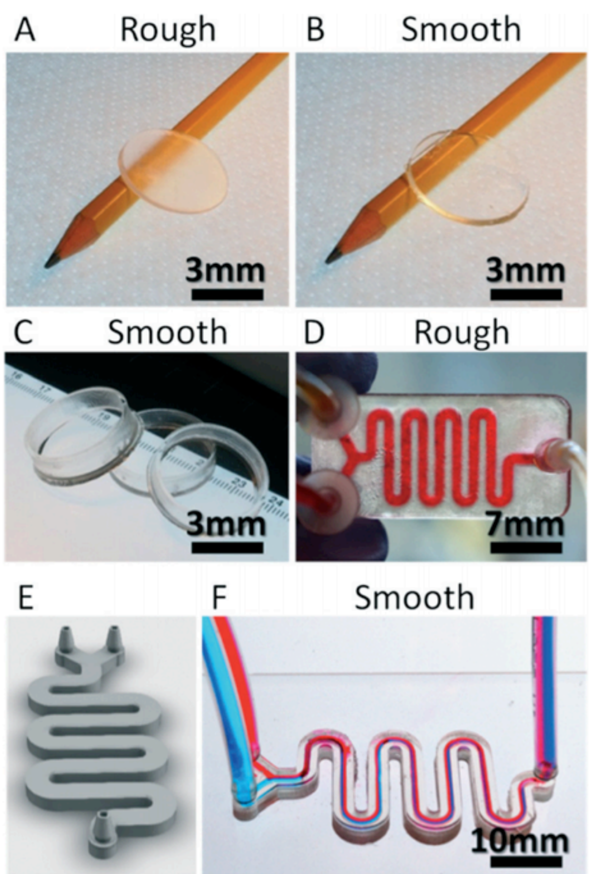
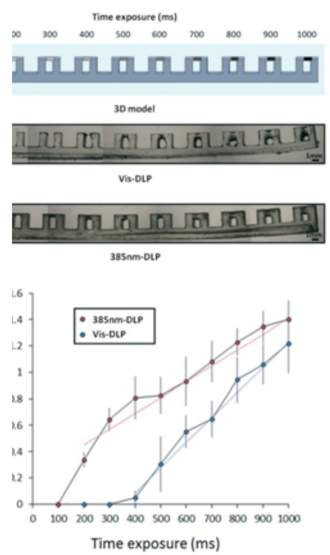
Cite this: *Lab Chip*, 2016, 16, 2287

3D-printing of transparent bio-microfluidic devices in PEG-DA†

Arturo Urrios,‡[ag] Cesar Parra-Cabrera,‡[g] Nirveek Bhattacharjee,‡[g] Alan M. Gonzalez-Suarez,‡[bg] Luis G. Rigat-Brugarolas,[cg] Umashree Nallapatti,[g] Josep Samitier,[cde] Cole A. DeForest,[f] Francesc Posas,[a] José L. Garcia-Cordero[b] and Albert Folch*[g]





Issue 8, 2018

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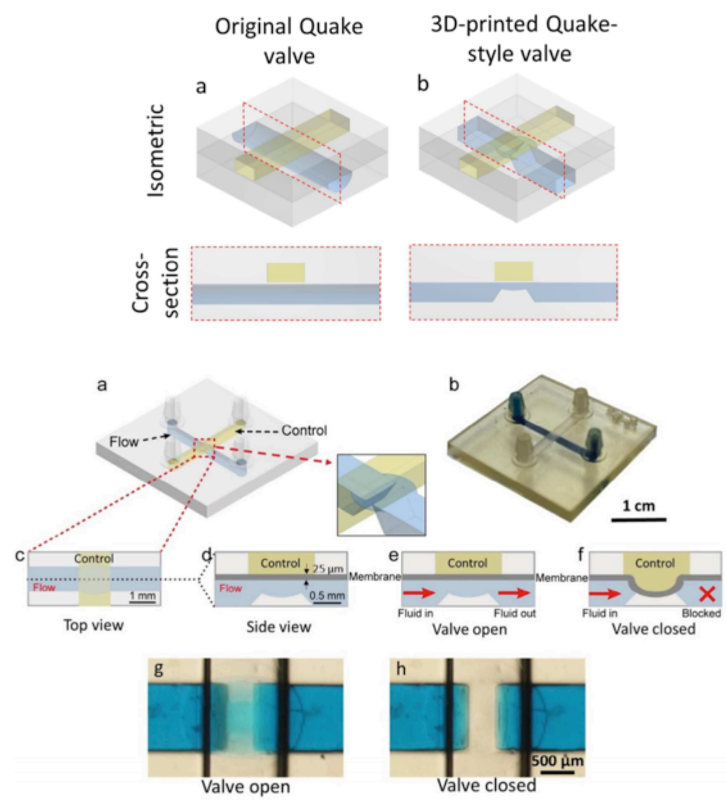
From the journal:
Lab on a Chip

3D-printed Quake-style microvalves and micropumps†

Check for updates

Yuan-Sheng Lee, *a Nirveek Bhattacharjee b and Albert Folch b

Author affiliations



When limit is mechanics ...

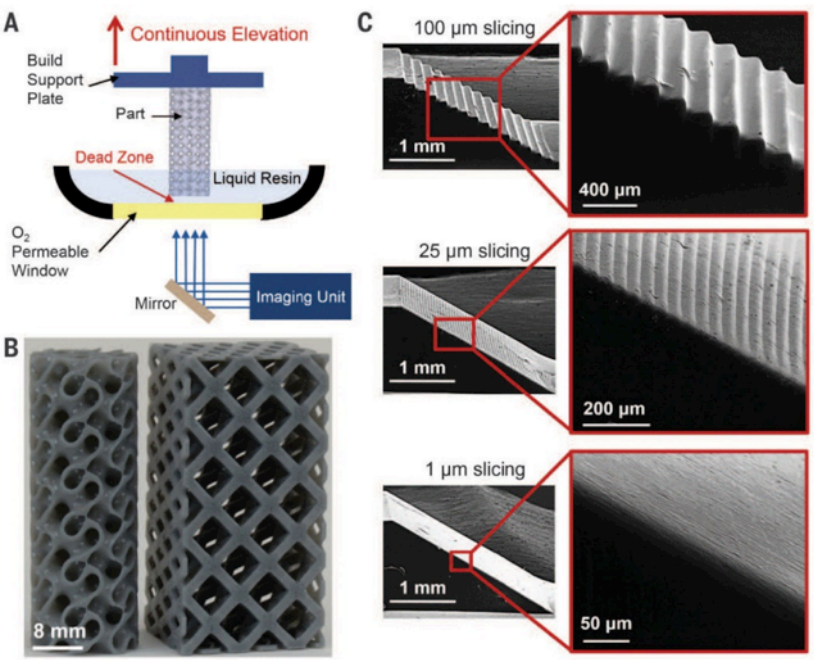
Sciencexpress

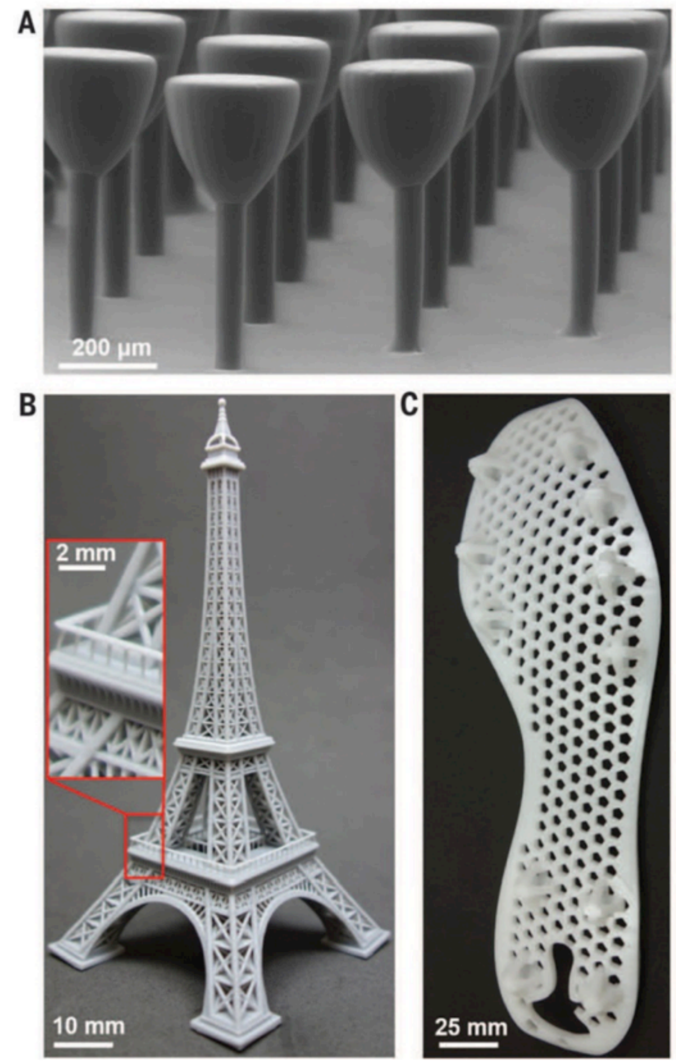
Continuous liquid interface production of 3D objects

John R. Tumbleston,[1] David Shirvanyants,[1] Nikita Ermoshkin,[1] Rima Janusziewicz,[2] Ashley R. Johnson,[3] David Kelly,[1] Kai Chen,[1] Robert Pinschmidt,[1] Jason P. Rolland,[1] Alexander Ermoshkin,[1*] Edward T. Samulski,[1,2*] Joseph M. DeSimone[1,2,4*]

[1]Carbon3D Inc., Redwood City, CA 94063, USA. [2]Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, USA. [3]Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University. [4]Department of Chemical andBiomolecular Engineering,. North Carolina State University, Raleigh, NC 27695, USA.

*Corresponding author. E-mail: alex@carbon3d.com (A.E.);et@unc.edu (E.T.S.); desimone@email.unc.edu (J.M.D.)





When limit is mechanics …

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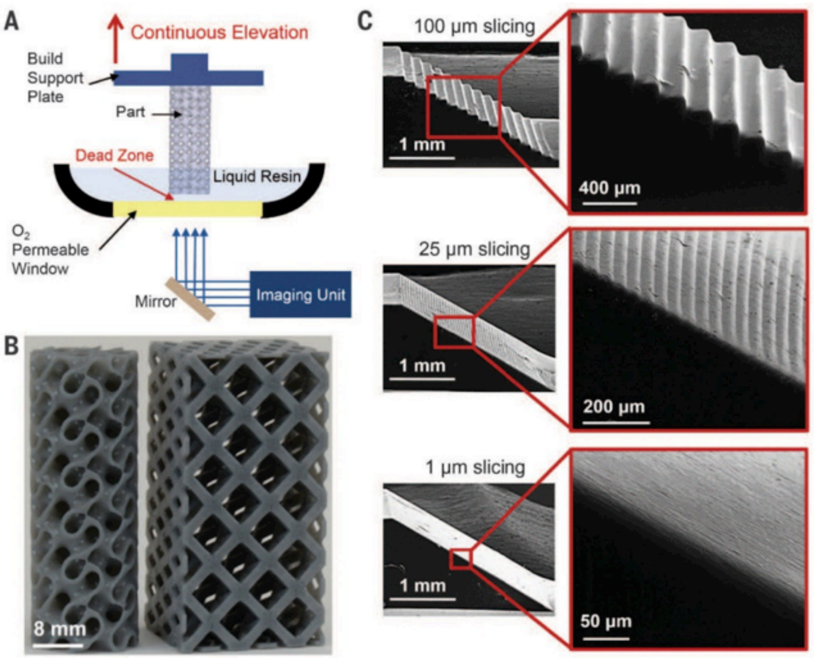
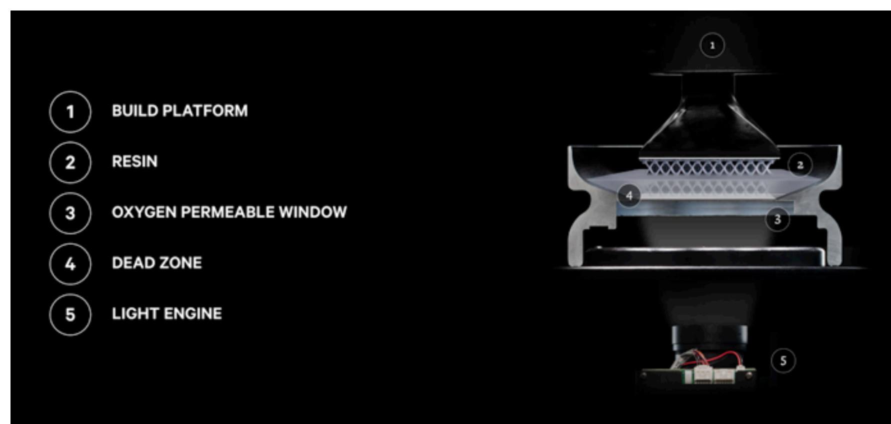
[1]Carbon3D Inc., Redwood City, CA 94063, USA. [2]Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, USA. [3]Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University. [4]Department of Chemical andBiomolecular Engineering,. North Carolina State University, Raleigh, NC 27695, USA.

***Corresponding author. E-mail: alex@carbon3d.com (A.E.);et@unc.edu (E.T.S.); desimone@email.unc.edu (J.M.D.)**



https://www.carbon3d.com/



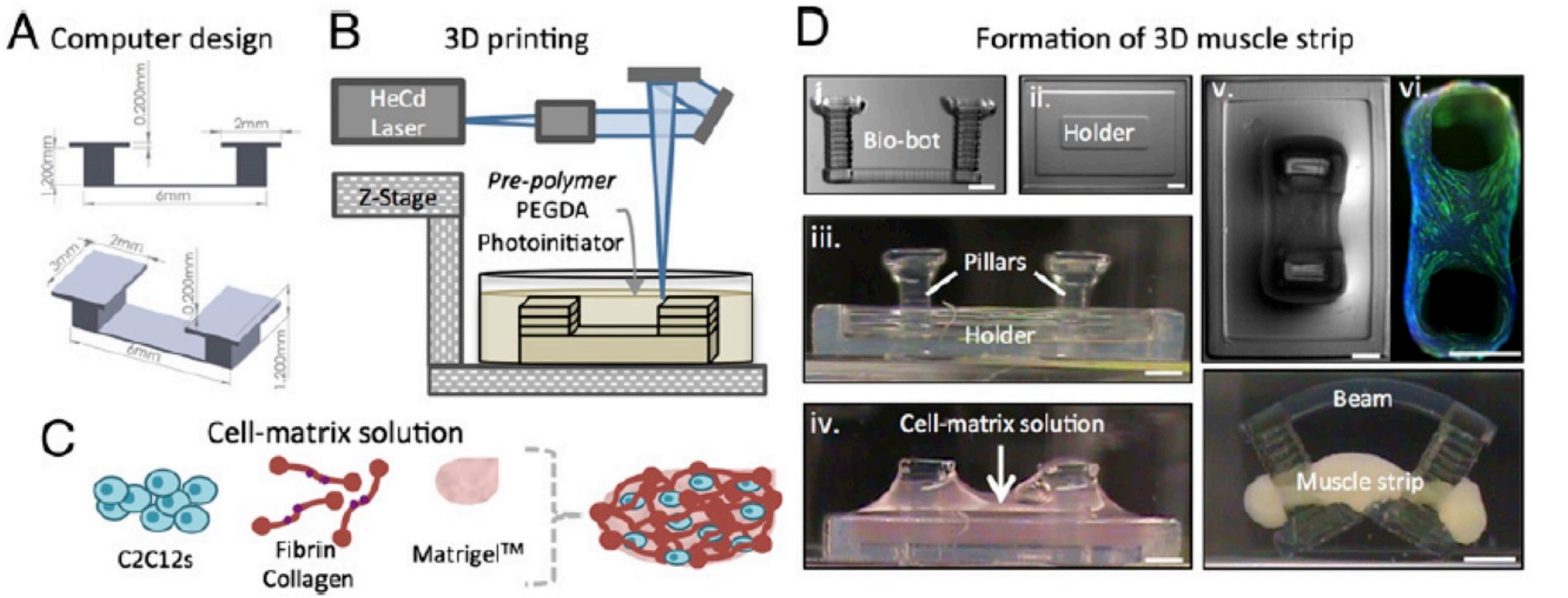
Biological machines …

Three-dimensionally printed biological machines powered by skeletal muscle

Caroline Cvetkovic[a,b,1], Ritu Raman[b,c,1], Vincent Chan[a,b,d], Brian J. Williams[b,c], Madeline Tolish[e], Piyush Bajaj[a,b,2], Mahmut Selman Sakar[d,3], H. Harry Asada[d], M. Taher A. Saif[b,c], and Rashid Bashir[a,b,4]

[a]Department of Bioengineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [b]Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [c]Department of Mechanical Science and Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [d]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; and [e]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37325

Edited by Stephen R. Quake, Stanford University, Stanford, CA, and approved May 30, 2014 (received for review January 26, 2014)



Cvetkovic, C., Raman, R., Chan, V., Williams, B. J., Tolish, M., Bajaj, P., ... & Bashir, R. (2014). Three-dimensionally printed biological machines powered by skeletal muscle. Proceedings of the National Academy of Sciences, 111(28), 10125-10130.

Biological machines …

PNAS

Three-dimensionally printed biological machines powered by skeletal muscle

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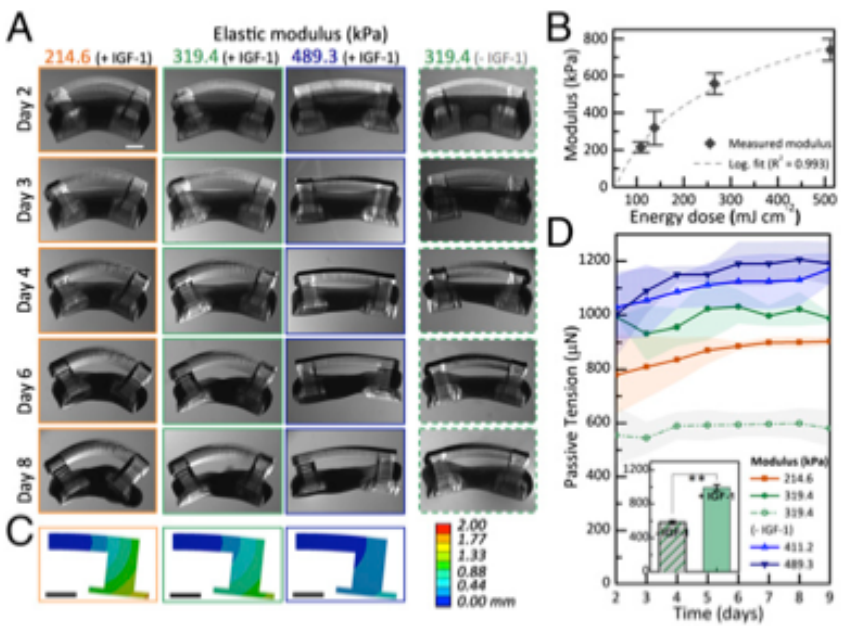
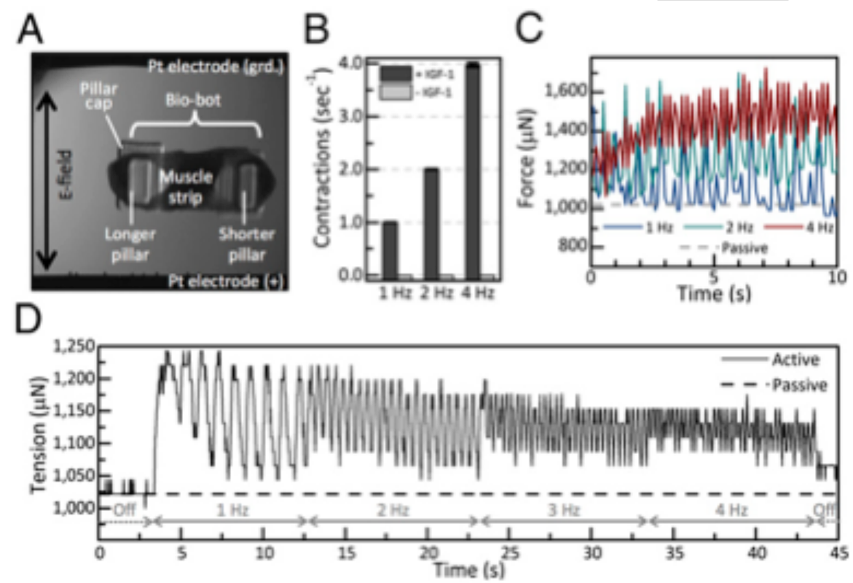
[a]Department of Bioengineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [b]Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [c]Department of Mechanical Science and Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [d]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; and [e]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37325

Edited by Stephen R. Quake, Stanford University, Stanford, CA, and approved May 30, 2014 (received for review January 26, 2014)

Movie





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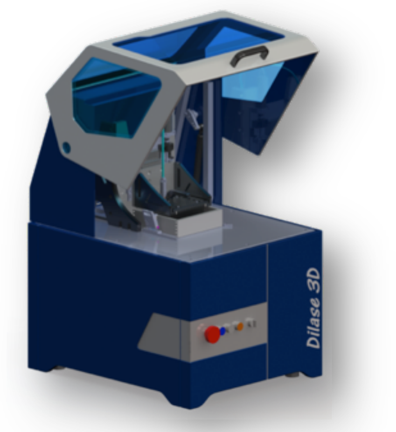
Stereolithography for microfluidics

- **Objective : combining High Resolution Laser Lithography to SLA concepts**

DILASE 3D (LAAS / KLOE partnership, LEAF Equipex)

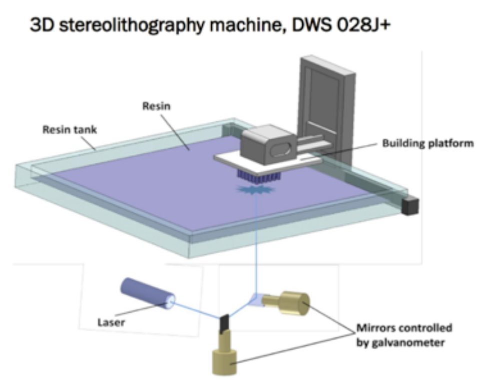


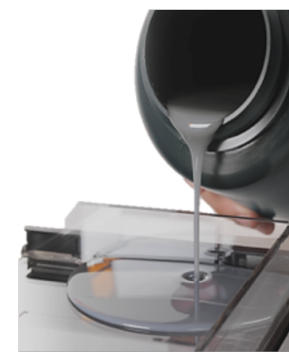
<u>Technical specifications :</u>

- Targeted Resolution X,Y : 5um
- Targeted Resolution Z : 5-100um
- Laser Wavelength : 405 nm (50 mW)
- Samples size (10 x 10 x 5 cm - X,Y,Z)

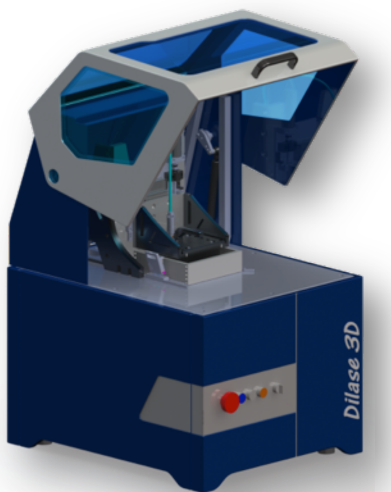
<u>Materials :</u>

- KLOE's materials
- Commercial UV photosensitive resists
- Commercial SLA resists
  (PU, acrylate + microparticles …)
- Home made materials (Hydrogels …)

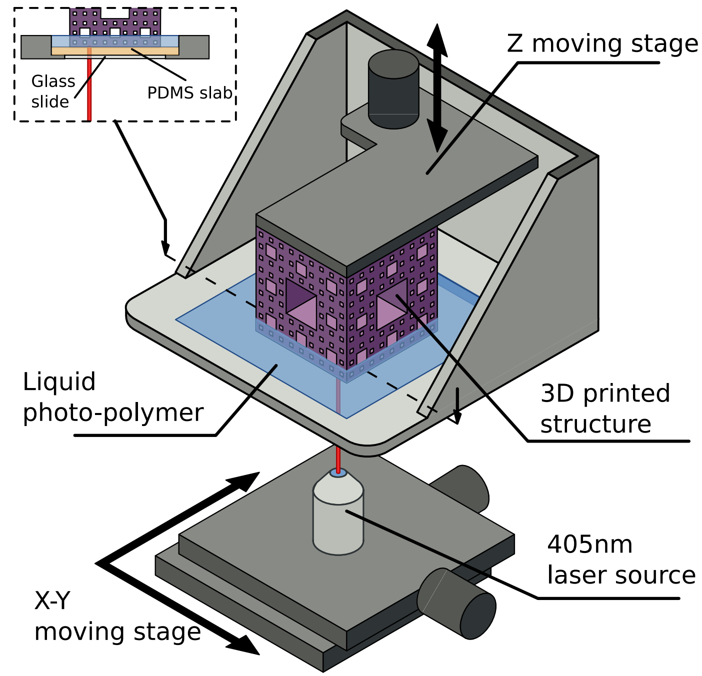




High resolution Stereolithography



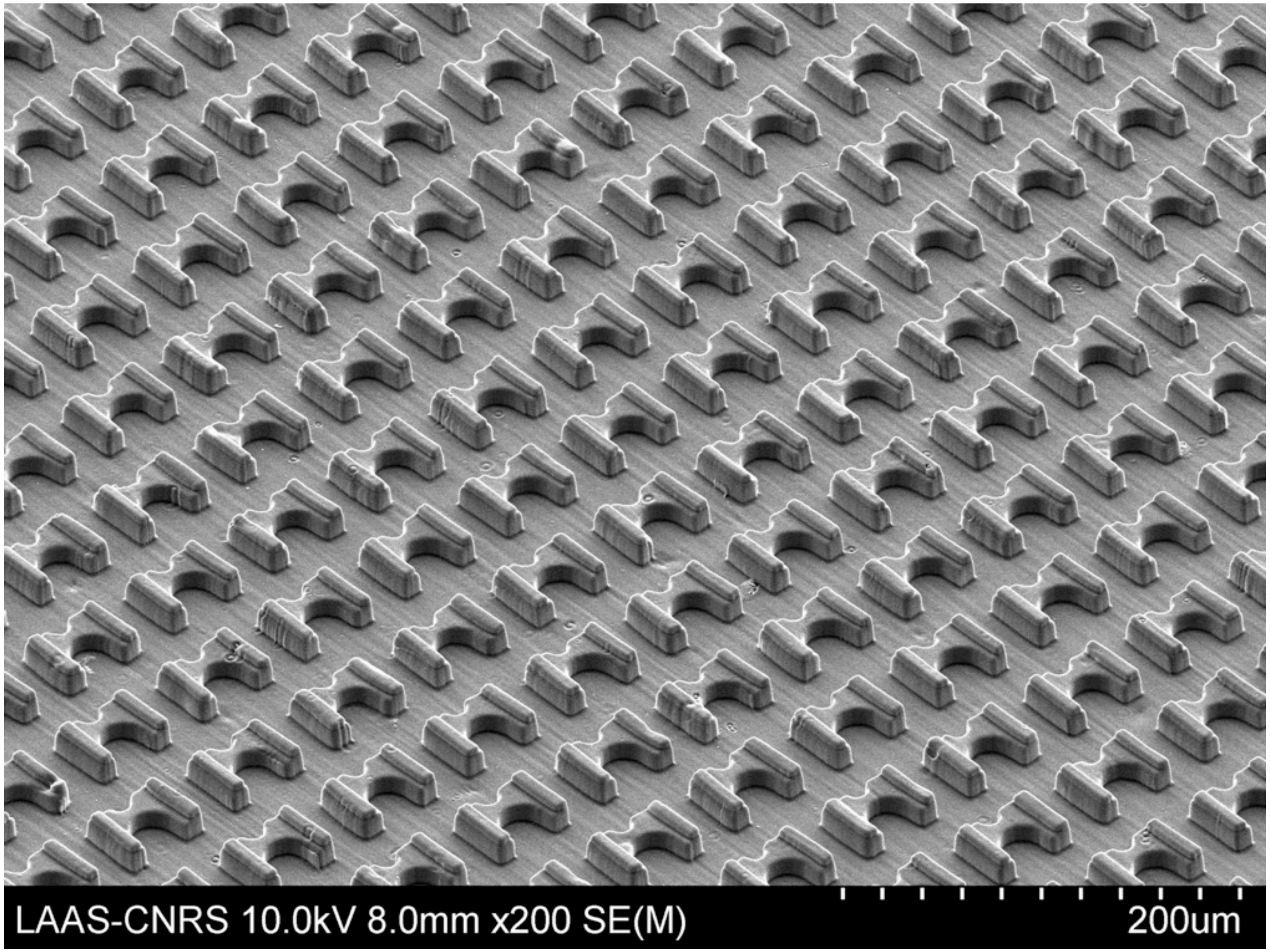
DILASE 3D (KLOE SA / LAAS CNRS)

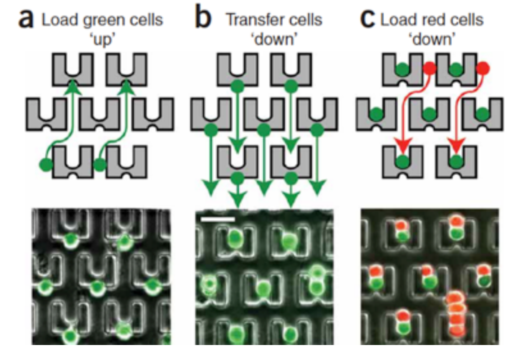


<u>Technical specifications :</u>

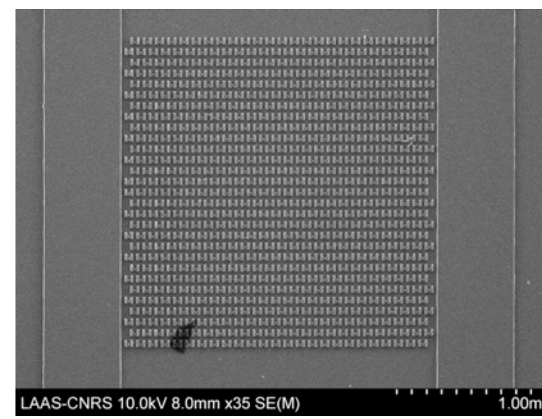
- Targeted Resolution X,Y : 5um
- Targeted Resolution Z : 5-100um
- Laser Wavelength : 405 nm (50 mW)
- Samples size (10 x 10 x 5 cm - X,Y,Z)

High resolution Stereolithography





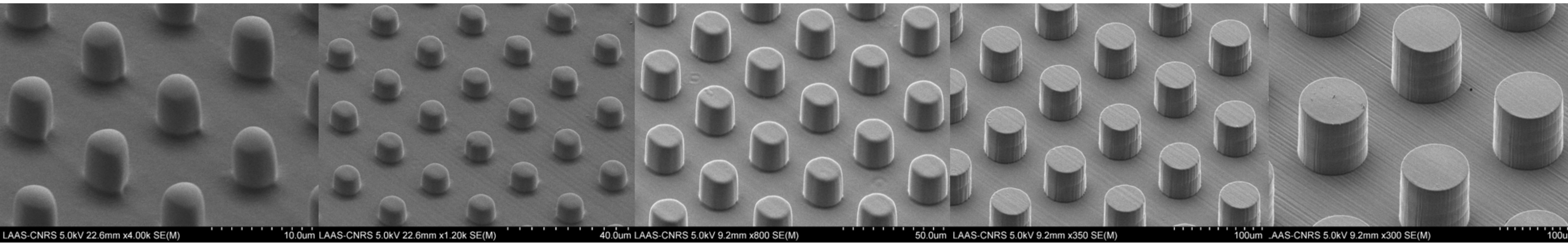
Voldman et al , Nature Methods, 2009

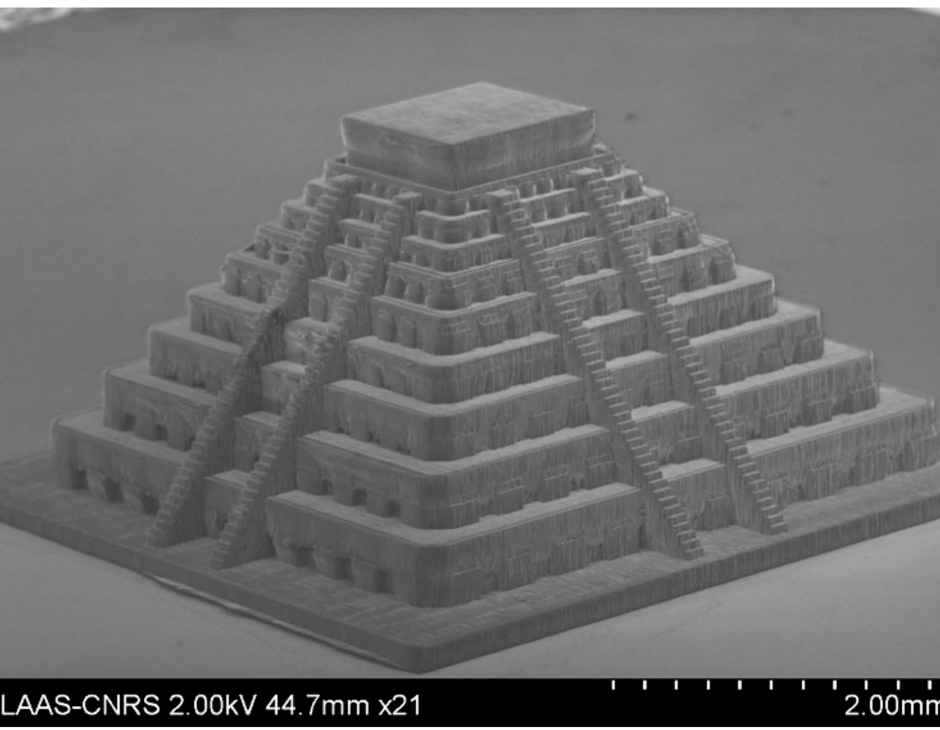


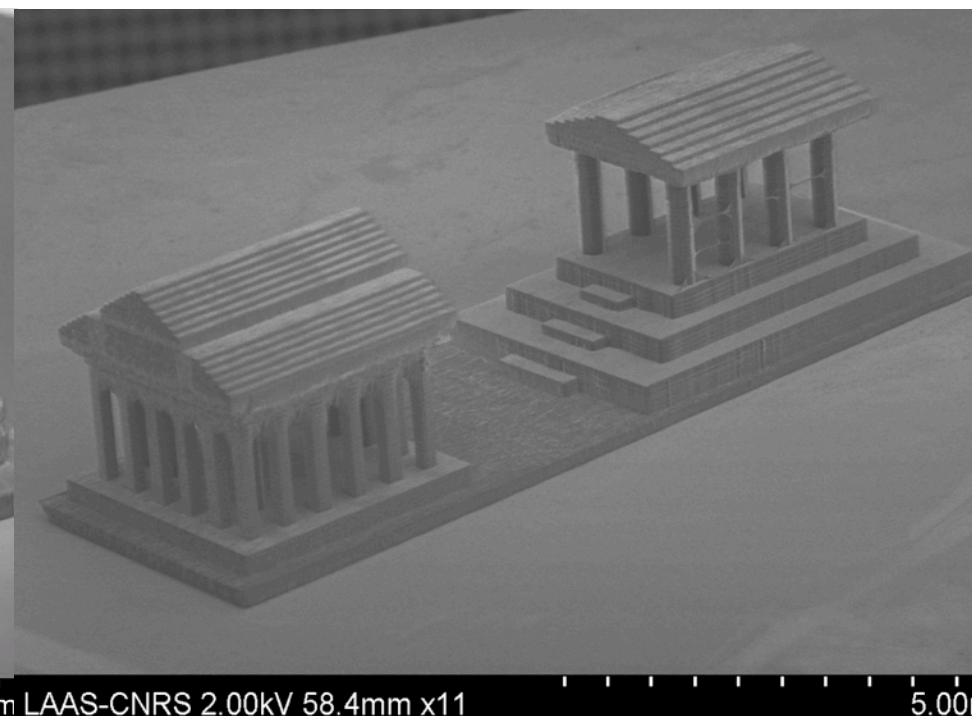
A. Accardo et al , Additive Manufacturing, 2018ccc

Microfluidics19
13-18 oct. 2019
Sète - France

High resolution Stereolithography

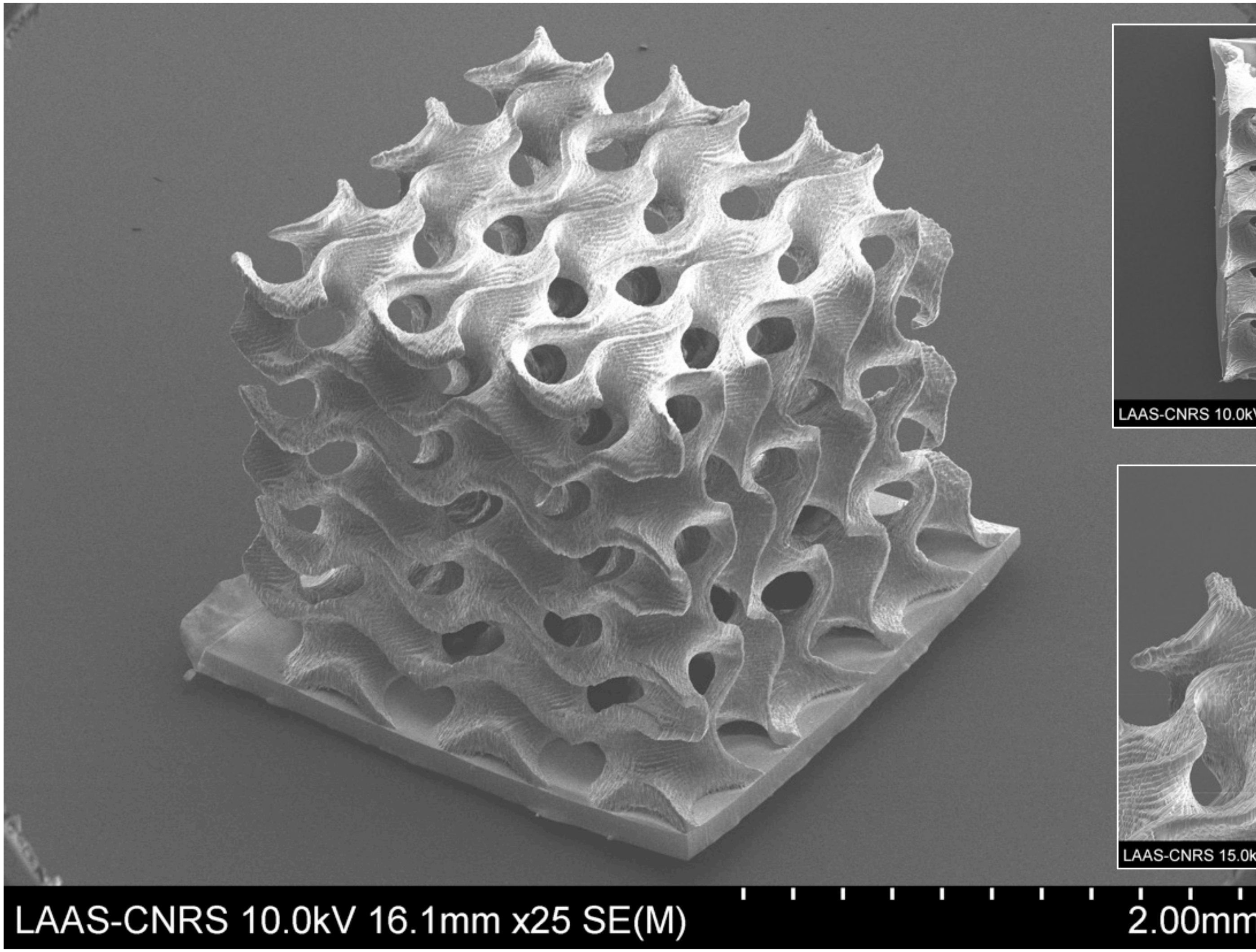


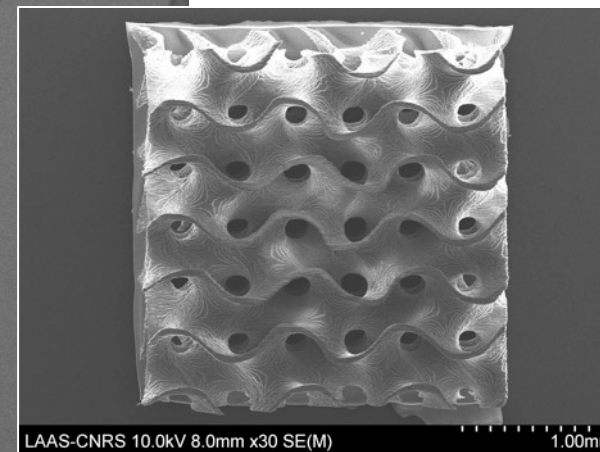


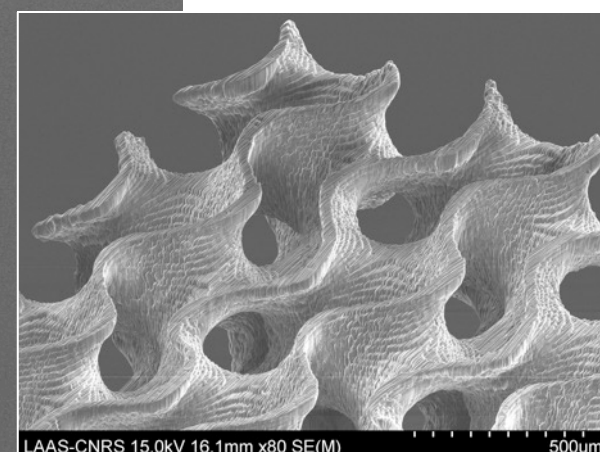


A. Accardo et al , Additive Manufacturing, 2018ccc

High resolution Stereolithography



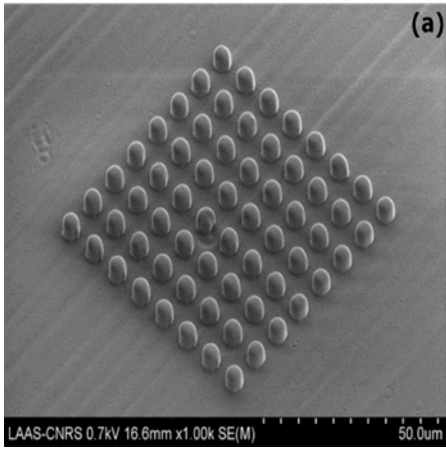




A. Accardo et al , Additive Manufacturing, 2018ccc

Improving the resolution of stereolithography

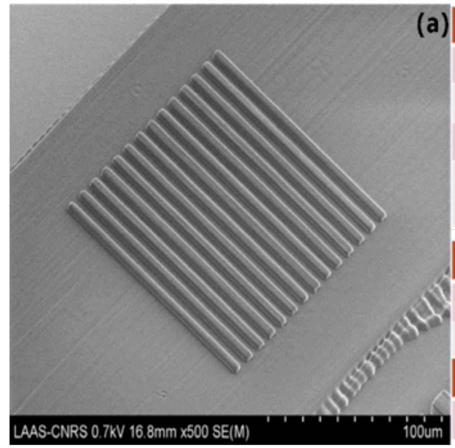
Ormocomp resists (Microresist Technology)



Dilase 3D (b)	
Modulation	24 %
Velocity	4 $^{mm}/_{s}$
#Layers	1
Layer thickness	5 µm

Laser power density & Dose (c)	
Laser power density	26.66 $^{kW}/_{cm^2}$
Dose, Laser beam width 3 µm	20 $^{J}/_{cm^2}$

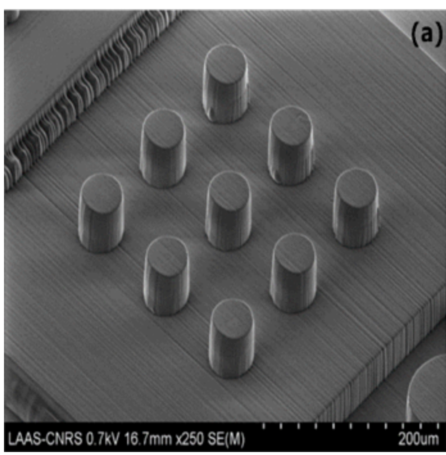
Confocal microscopy (d)	
Avg. width (µm) ± standard deviation (µm)	4.77 ± 0.29
Avg. height (µm) ± standard deviation (µm)	5.36 ± 0.05
SEM	
Avg. width (µm) ± standard deviation (µm)	5.13 ± 0.13



Dilase 3D (b)	
Modulation	19 %
Velocity	2 $^{mm}/_{s}$
#Layers	1
Layer thickness	5 µm

Laser power density & Dose (c)	
Laser power density	21.11 $^{kW}/_{cm^2}$
Dose, Laser beam width 3 µm	31.66 $^{J}/_{cm^2}$

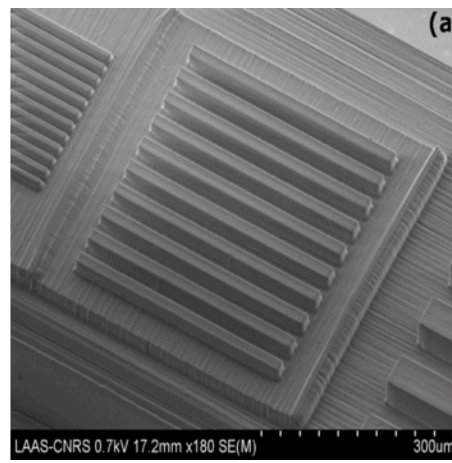
Confocal microscopy (d)	
Avg. width (µm) ± standard deviation (µm)	5.11 ± 0.14
Avg. height (µm) ± standard deviation (µm)	3.06 ± 0.11
SEM	
Avg. width (µm) ± standard deviation (µm)	5.52 ± 0.29



Dilase 3D (b)	
Modulation	35 %
Velocity	12 $^{mm}/_{s}$
#Layers	2
Layer thickness	25 µm

Laser power density & Dose (c)	
Laser power density	38.88 $^{kW}/_{cm^2}$
Dose, Laser beam width 3 µm	9.72 $^{J}/_{cm^2}$

Confocal microscopy (d)	
Avg. width (µm) ± standard deviation (µm)	49.36 ± 0.16
Avg. height (µm) ± standard deviation (µm)	48.25 ± 0.5
SEM	
Avg. width (µm) ± standard deviation (µm)	45.65 ± 0.53

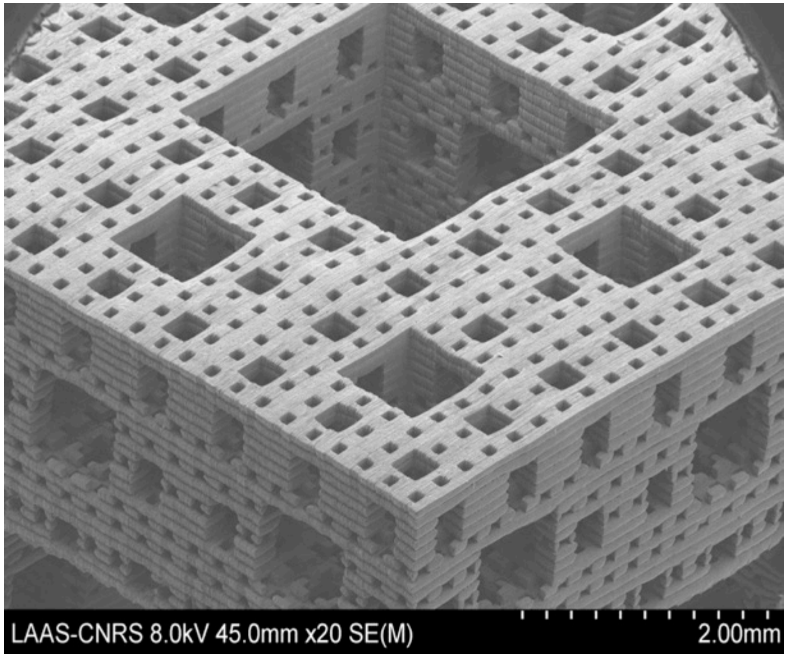


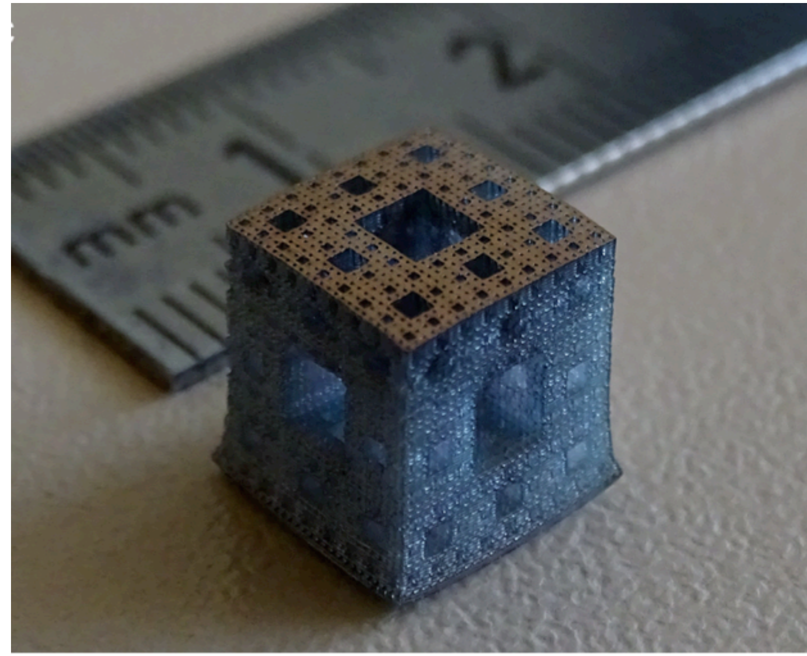
Dilase 3D (b)	
Modulation	80 %
Velocity	12 $^{mm}/_{s}$
#Layers	1
Layer thickness	20 µm
# of laser passes	2

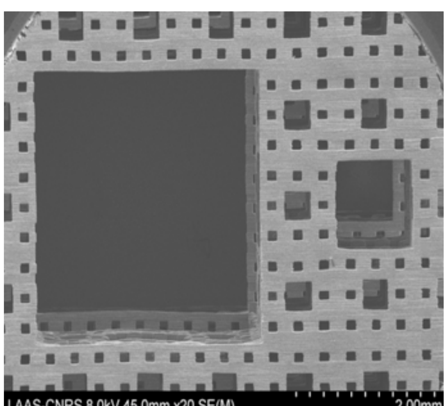
Laser power density & Dose (c)	
Laser power density	88.8 $^{kW}/_{cm^2}$
Dose, Laser beam width 3 µm	22.2 $^{J}/_{cm^2}$

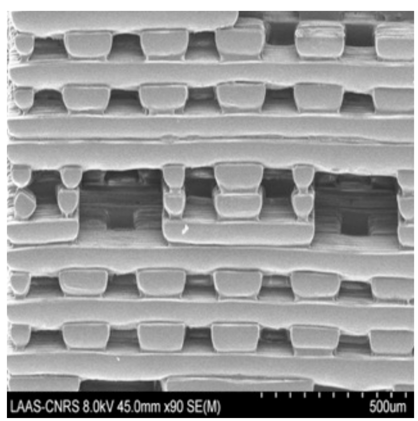
Confocal microscopy (d)	
Avg. width (µm) ± standard deviation (µm)	21.42 ± 1.28
Avg. height (µm) ± standard deviation (µm)	17.1 ± 0.15
SEM	
Avg. width (µm) ± standard deviation (µm)	21.35 ± 0.70

Printing complex architectures









<u>Menger sponge design</u>

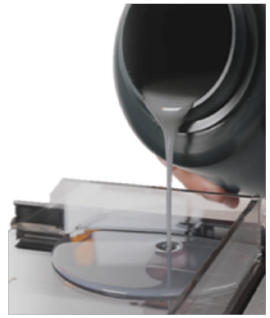
Volume : $1x1x1cm^3$

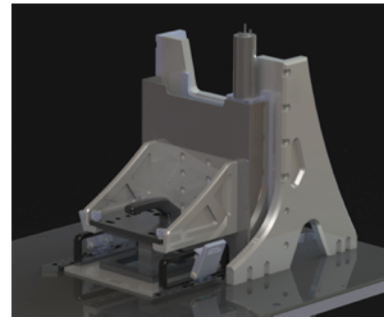
Smaller channels : 100μm

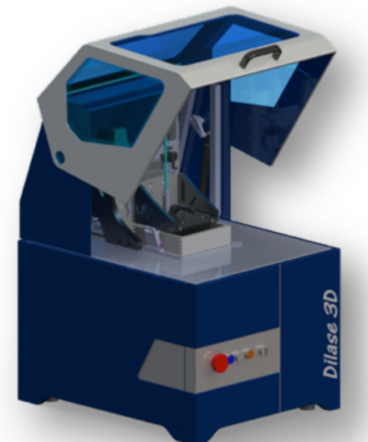
A. Accardo et al , Additive Manufacturing, 2018ccc

Stereolithography for microfluidics

Multiresolution printing



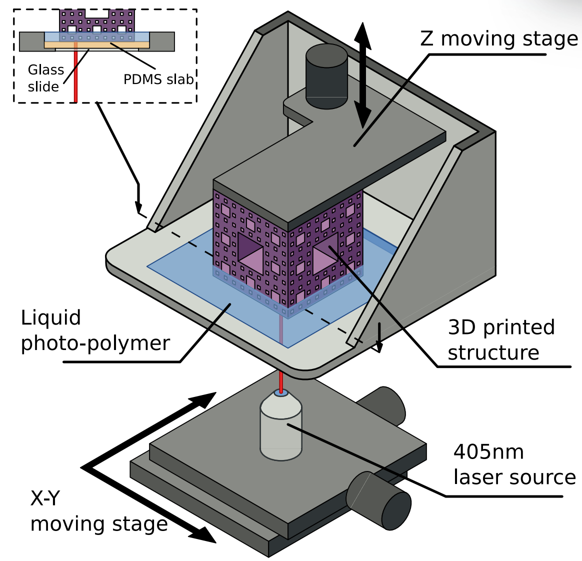




KLOÉ

Technical specifications :

- ***DILASE MR targeted resolution X,Y : 5 - 20 µm***
- ***DILASE Bio targeted resolution : 10 - 65 µm***
- ***Automatic switch***
- ***Alignment in progress***
- Targeted Resolution Z : 5 - 100 µm
- Laser Wavelength : 405 nm (50 mW)
- Samples size (10 x 10 x 5 cm - X,Y,Z)
- Targeted Laser speed : 100 mm/s
- Targeted Roughness : < 2µm



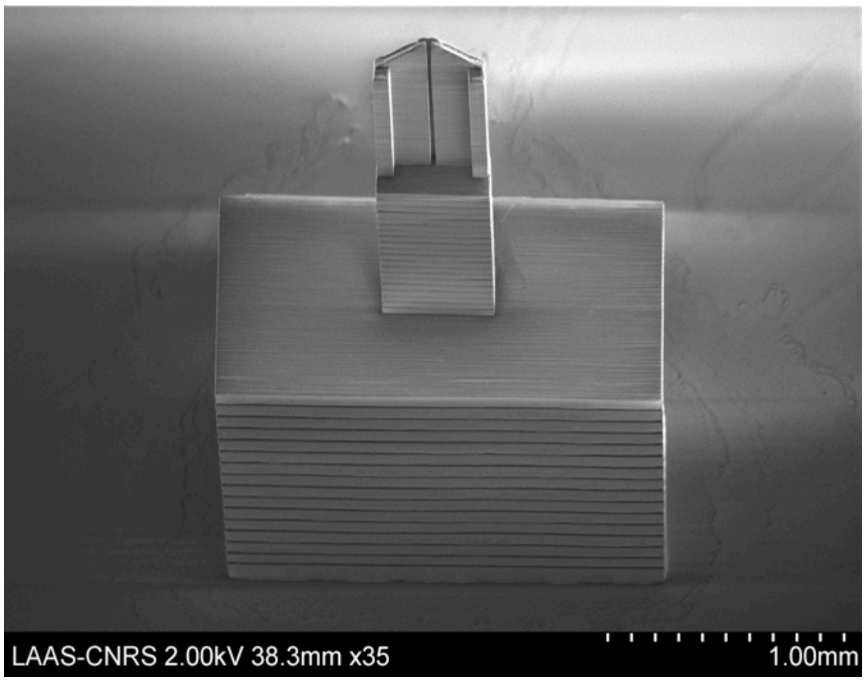
Writing time of a simple square of 6 mm^3

- *Dilase V1 :* ***60 min***
- *Dilase V2 :* ***<1 min***

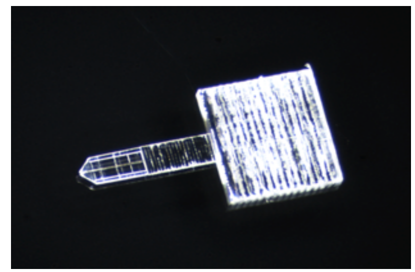
MEMs fabrication

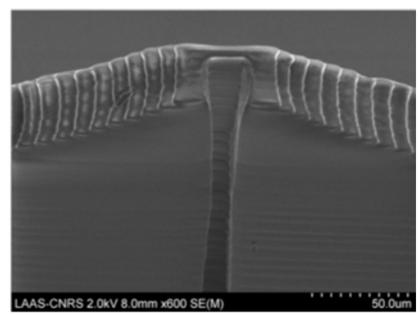
Bioplume : MEMS-based picoliter droplet dispenser

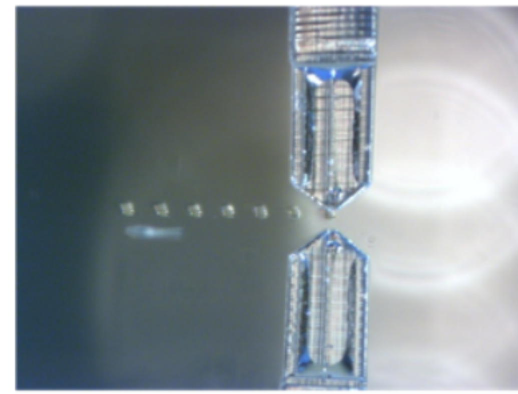
A.Maziz, R. Courson, T. Leichlé, Ali Maziz

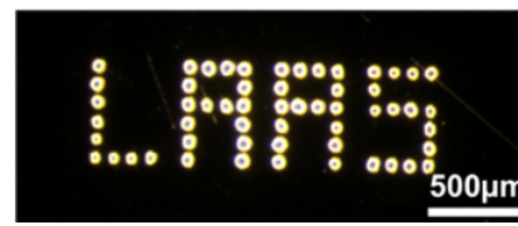


A. Maziz et al. submitted to Analytical Bioanalytical Chemistry









→ Future Integration of piezo sensors /
→ Improving mechanical properties

Towards 4D printing

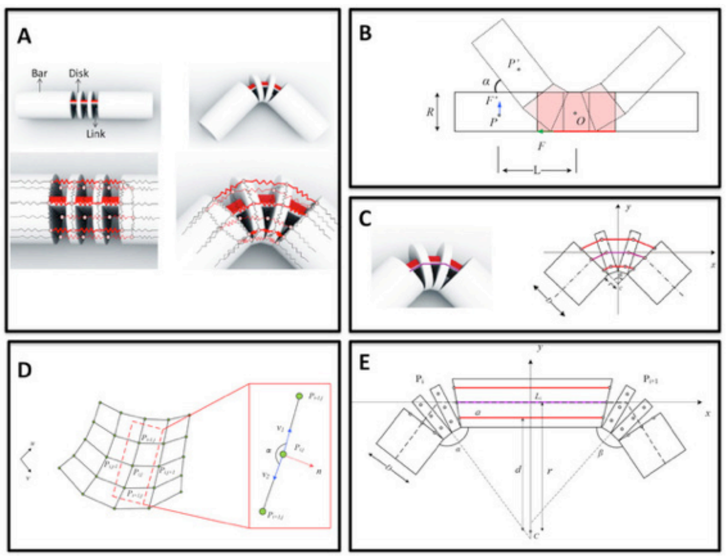
Active Printed Materials for Complex Self-Evolving Deformations

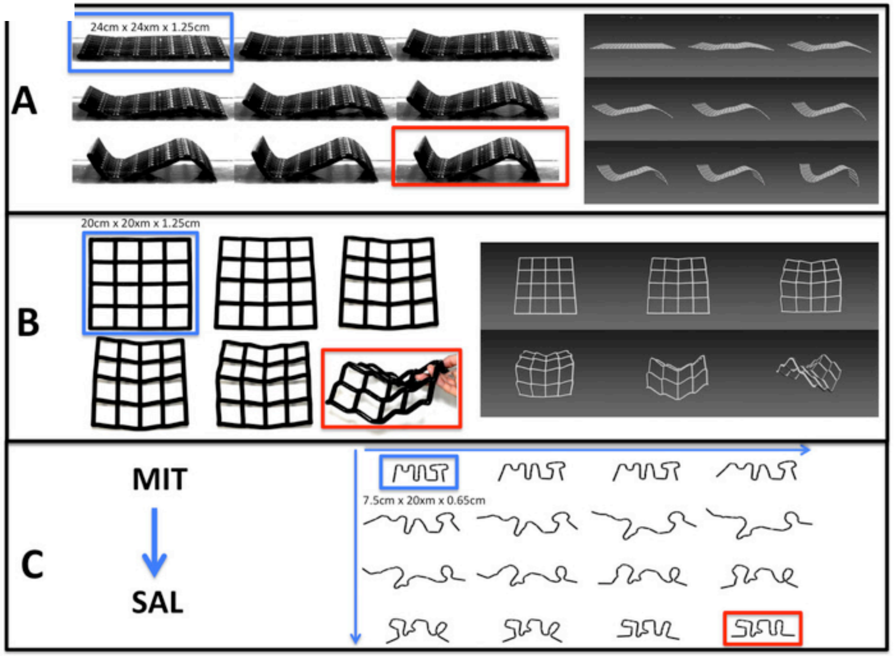
Dan Raviv, Wei Zhao, Carrie McKnelly, Athina Papadopoulou, Achuta Kadambi, Boxin Shi, Shai Hirsch, Daniel Dikovsky, Michael Zyracki, Carlos Olguin, Ramesh Raskar & Skylar Tibbits

Affiliations | Contributions | Corresponding author

Scientific Reports **4**, Article number: 7422 | doi:10.1038/srep07422

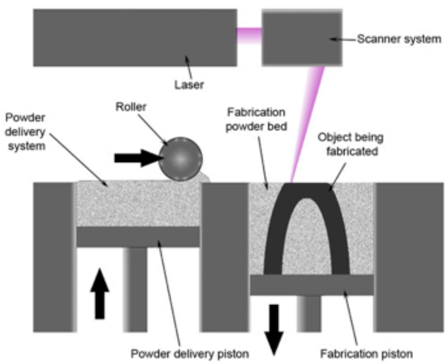
4D printing movie

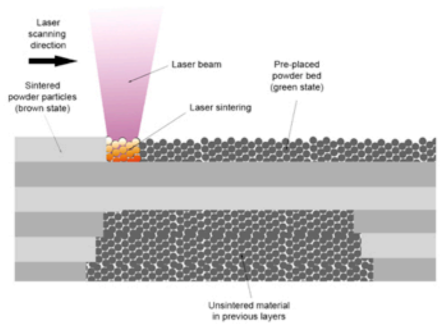


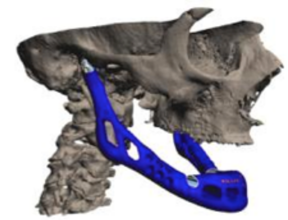


Selective Laser Sintering

Selective Laser Sintering (SLS) uses a laser as the power source to sinter powdered material







start 15s or 40s

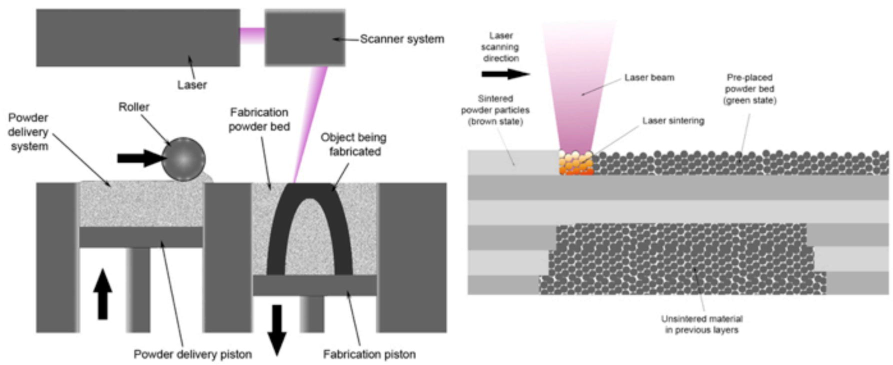
Suitable for …

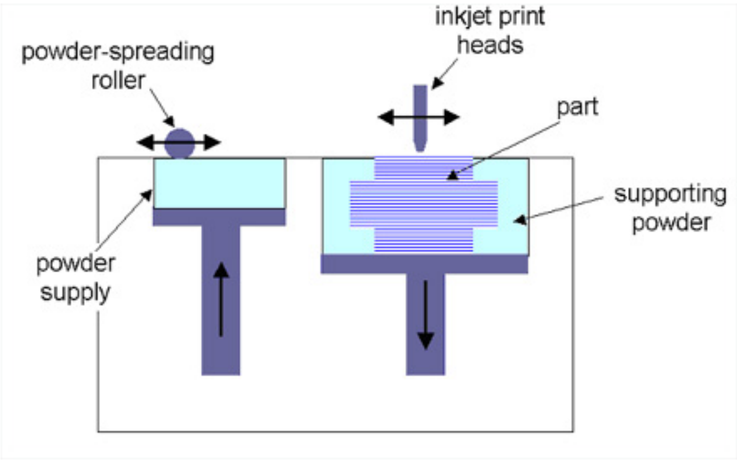
- plastic
- metal
- ceramic
- glass powders
- …

Price ?

Resolution (>100um)

SLS / Ink Jet Printing on polymer powder

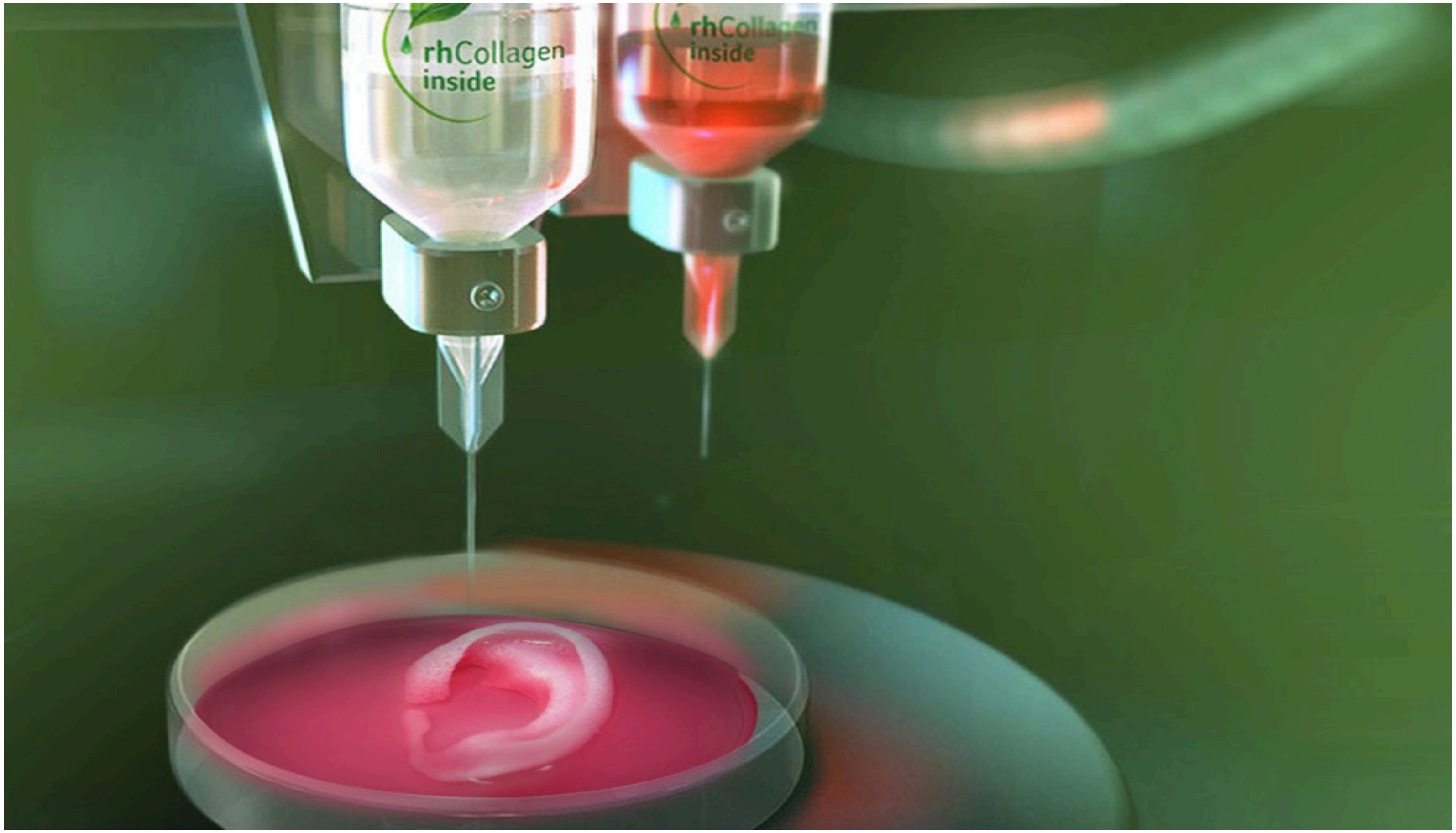




- **Dimensions**: up to 1m
- **Resolution:** around200 um (function of size distribution)
- **Materials:** Metals, ceramics, polymer, composites …
- **Application**: Engines, implants, prosthesis,
- **Post procession & cleaning**

Price > 100kEuros - Controlled environment !

Bioprinting



3D Bioprinting …what does it mean ?

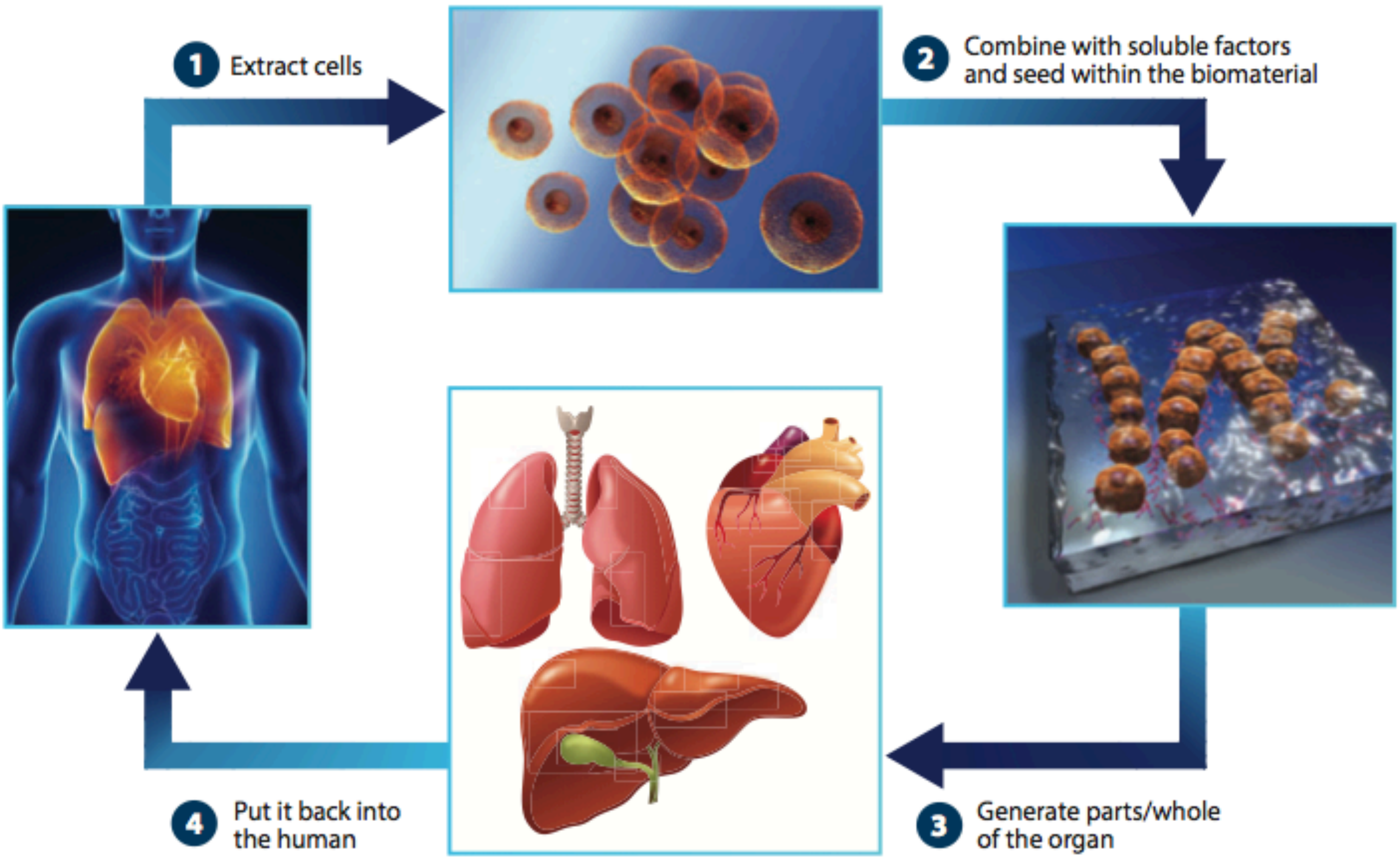
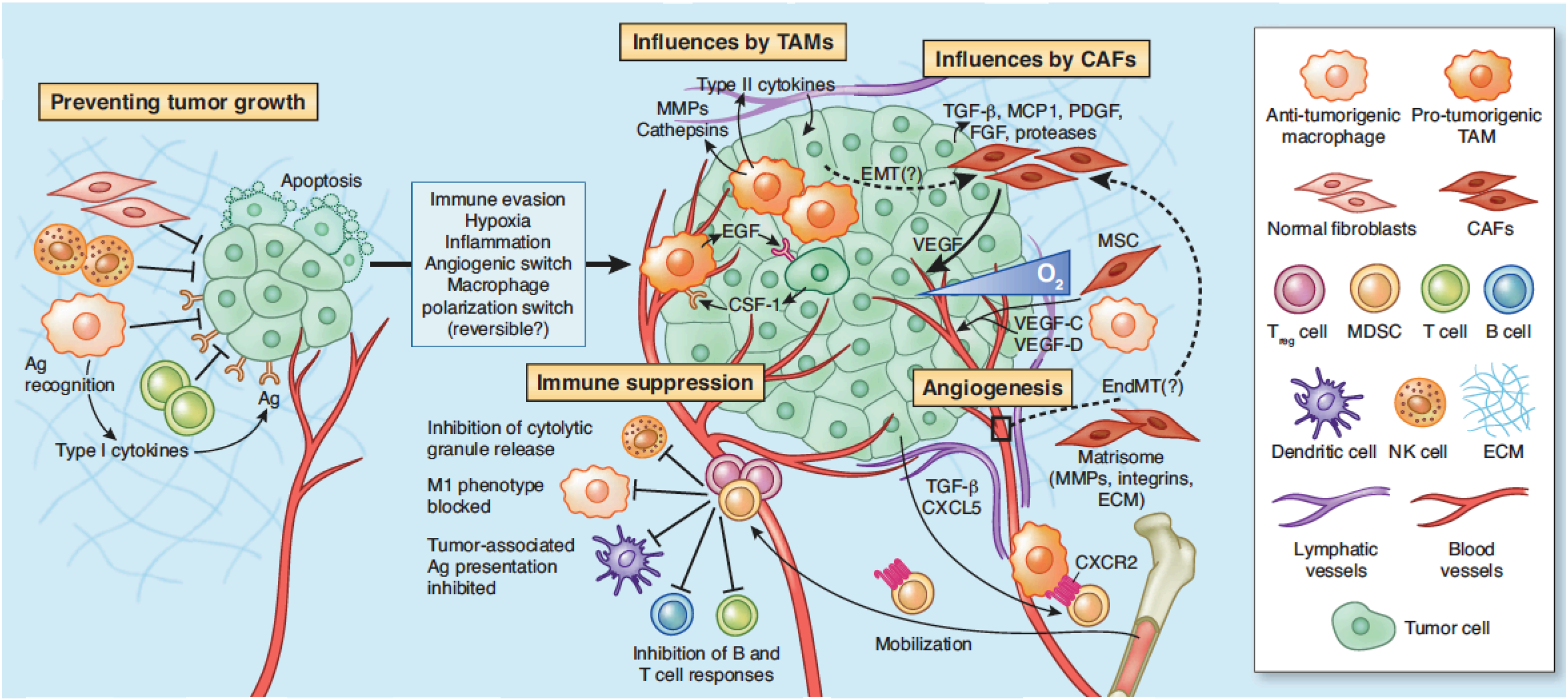


Figure 2

Overview of the tissue engineering–based approach using three-dimensional (3D) biofabrication for de novo organogenesis.

3D Bioprinting …what does it mean ?



NATURE MEDICINE VOLUME 19 | NUMBER 11 | NOVEMBER 2013 1423

→ Organogenesis (too ambitious so far ?)
→ Creating tissue models
→ Building cell microenvironments

→ What is the complexity required for a functional tissue ?

What is needed :

- *3D topography*
- *Porosity*
- *Stiffness*
- *Cell heterogeneity*
- *Environment control*
- *Vascular network*

3D printing for cell and tissue engineering …

… a Sci Fi movie ?

Too much anthusiasm ?

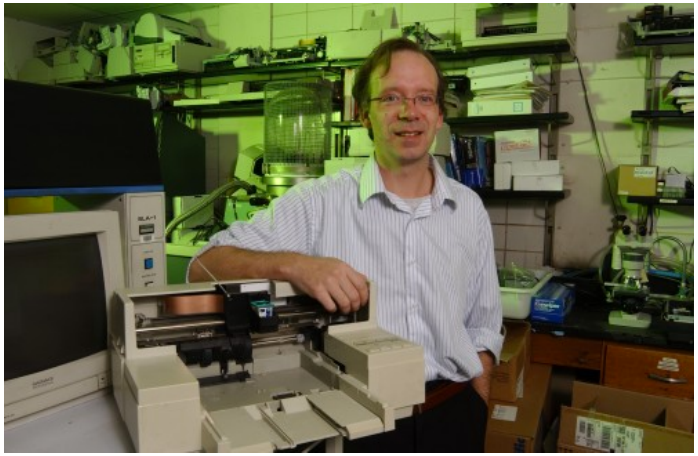
Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature biotechnology

Bioprinting … how to ?

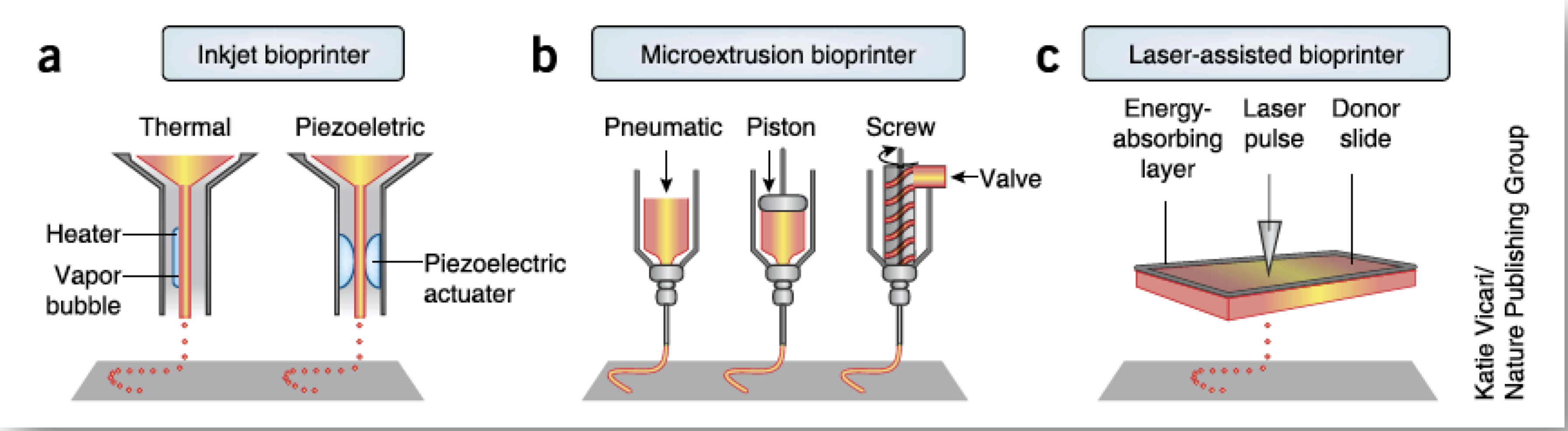
- First demonstration in 2004

Thomas Boland (Univ. South Carolina)

→ Hydrogel & cells in a HP Inkjet printer



- Most current approaches



Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature biotechnology

Bioprinting tissues … how to ?

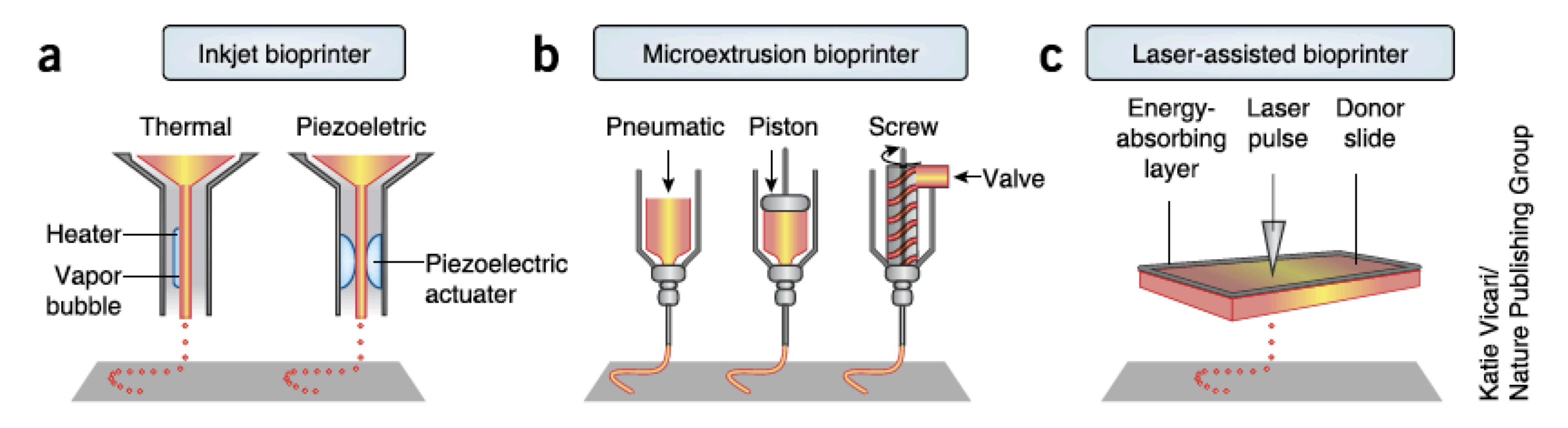


Table 1 Comparison of bioprinter types

	Bioprinter type			
	Inkjet	Microextrusion	Laser assisted	Refs.
Material viscosities	3.5–12 mPa/s	30 mPa/s to >6 × 10^7 mPa/s	1–300 mPa/s	48,63,78,107
Gelation methods	Chemical, photo-crosslinking	Chemical, photo-crosslinking, sheer thinning, temperature	Chemical, photo-crosslinking	64,85,106,110
Preparation time	Low	Low to medium	Medium to high	38,64,94,107
Print speed	Fast (1–10,000 droplets per second)	Slow (10–50 µm/s)	Medium-fast (200–1,600 mm/s)	49,58,76,90
Resolution or droplet size	<1 pl to >300 pl droplets, 50 µm wide	5 µm to millimeters wide	Microscale resolution	49,68,69,76
Cell viability	>85%	40–80%	>95%	42,54,80,104
Cell densities	Low, <10^6 cells/ml	High, cell spheroids	Medium, 10^8 cells/ml	42,49,88,89
Printer cost	Low	Medium	High	77

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature biotechnology

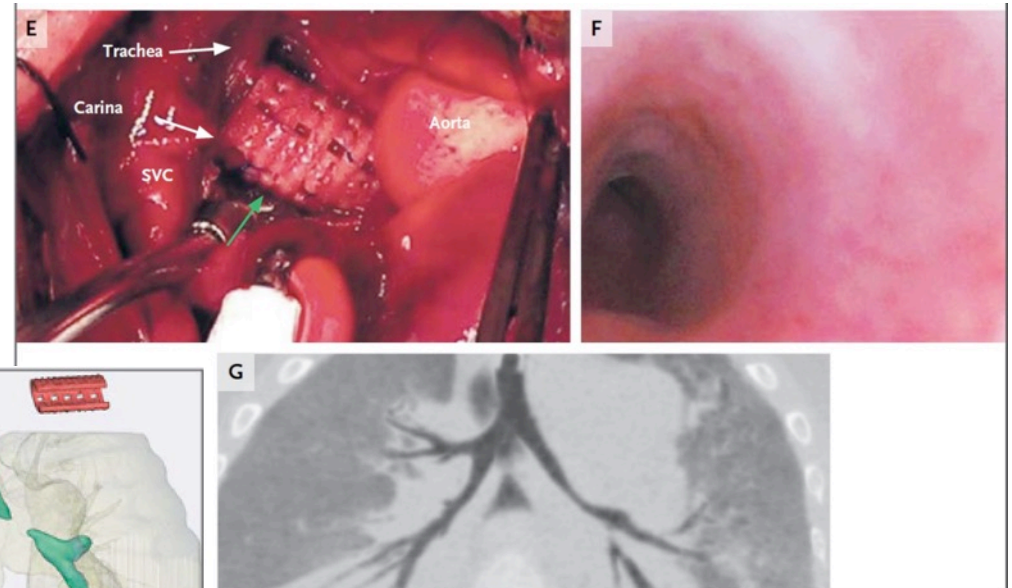
Implantable devices

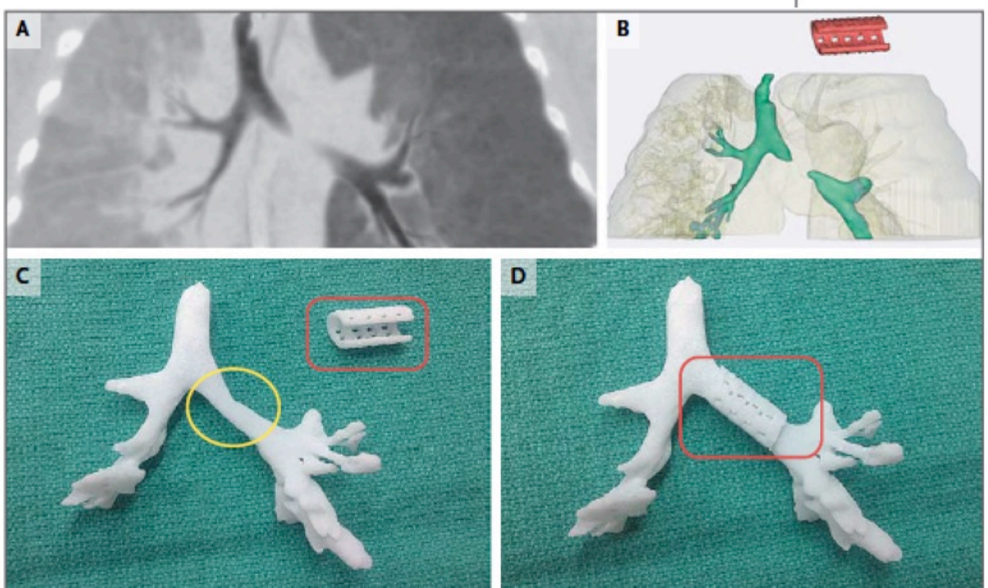
Bioresorbable Airway Splint Created with a Three-Dimensional Printer

TO THE EDITOR: Tracheobronchomalacia in newborns, which manifests with dynamic airway collapse and respiratory insufficiency, is difficult to treat.[1,2] In an infant with tracheobronchomalacia, we implanted a customized, bioresorbable tracheal splint, created with a computer-aided design based on a computed tomographic image of the patient's airway and fabricated with the use of laser-based three-dimensional printing, to treat this life-threatening condition.

custom-fabricated resorbable airway splint. Our bellowed topology design, similar to the hose of a vacuum cleaner, provides resistance against collapse while simultaneously allowing flexion, extension, and expansion with growth. The splint was manufactured from polycaprolactone with the use of a three-dimensional printer (Fig. 1A through 1D).

The institutional review board of the University of Michigan consulted with the Food and





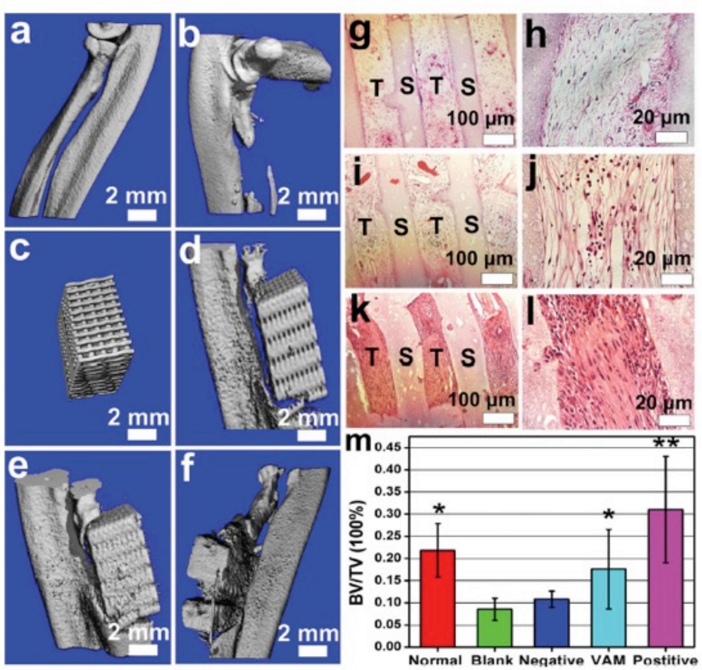
Zopf, D. A., Hollister, S. J., Nelson, M. E., Ohye, R. G., & Green, G. E. (2013). Bioresorbable airway splint created with a three-dimensional printer. New England Journal of Medicine, 368(21), 2043-2045.

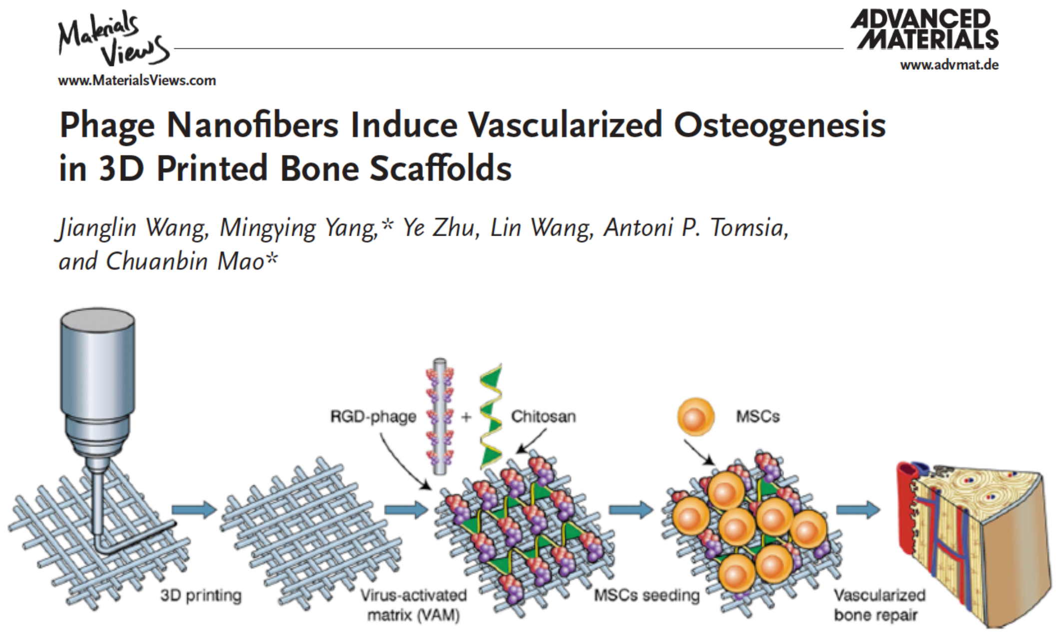
An implanted polycaprolactone scaffold for pulmonary-artery hypoplasia

Bioprinting tissues

Two steps approach
1) Scaffold, 2) Cell seeding

- Controlling the rigidity of the scaffold
- Controlling the chemistry of the surface
- Cell density/heterogeneity during seeding
- Degradability




Materials Views
www.MaterialsViews.com
ADVANCED MATERIALS
www.advmat.de

Phage Nanofibers Induce Vascularized Osteogenesis in 3D Printed Bone Scaffolds

Jianglin Wang, Mingying Yang, Ye Zhu, Lin Wang, Antoni P. Tomsia, and Chuanbin Mao**

- calcium phosphate (BCP) with a composition of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) at a mass ratio of 60/40, was produced using a 3D printing technique in Pluronic F127
- RGD-phage (peptide fiber) could induce the differentiation of mesenchymal stem cells (MSCs) into bone forming cells (osteoblasts) and

Wang, J., Yang, M., Zhu, Y., Wang, L., Tomsia, A. P., & Mao, C. (2014). Phage Nanofibers Induce Vascularized Osteogenesis in 3D Printed Bone Scaffolds. Advanced Materials.

Bioprinting tissues

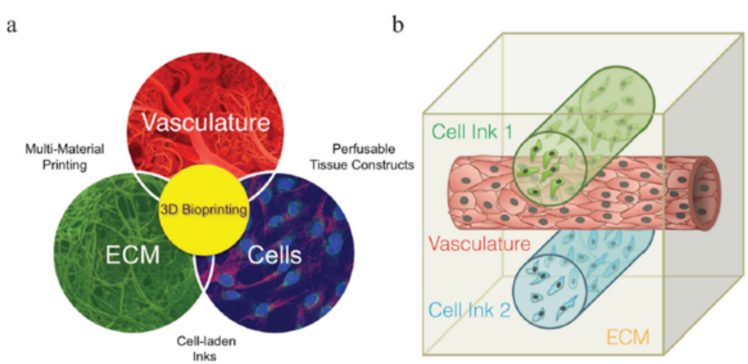
One step approach :
Cell w/o ECM printing

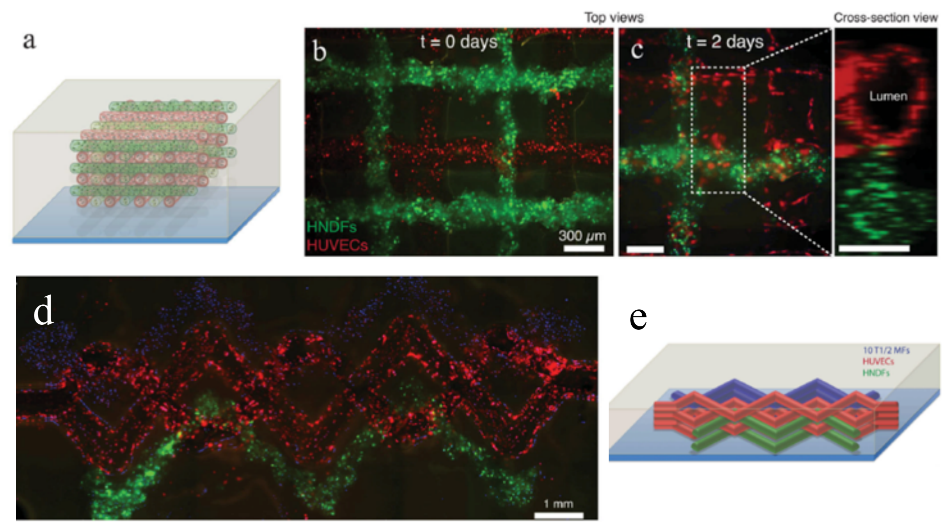
- Controlling cell viability
- Controlling cell density, heterogeneity
- Stability of the structures …
- Vascularization …
- Possibility of self evolving → 4D printing ?

ADVANCED MATERIALS
www.advmat.de
Materials Views
www.MaterialsViews.com

3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs

*David B. Kolesky, Ryan L. Truby, A. Sydney Gladman, Travis A. Busbee, Kimberly A. Homan, and Jennifer A. Lewis**





Schematic view (a) and fl uorescence images of an engineered tissue construct cultured for 0 and 2 days, respectively, in which red and green fi laments correspond to channels lined with RFP HUVECs and GFP HNDF-laden GelMA ink respectively. The cross-sectional view ishows that endothelial cells line the lumens within the embedded 3D microvascular network

Kolesky, D. B. et al. (2014). 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. Advanced Materials, 26(19), 3124-3130.

Bioprinting tissues … what does it mean ?

- **Materials** (Topography, stiffness, cell viability, soluble factors, angiogenesis, …)

Box 1 Ideal material properties for bioprinting

The selection of appropriate materials for use in bioprinting and their performance in a particular application depend on several features. These are listed below.

- Printability
  Properties that facilitate handling and deposition by the bioprinter may include viscosity, gelation methods and rheological properties.
- Biocompatibility
  Materials should not induce undesirable local or systemic responses from the host and should contribute actively and controllably to the biological and functional components of the construct.
- Degradation kinetics and byproducts
  Degradation rates should be matched to the ability of the cells to produce their own ECM; degradation byproducts should be nontoxic; materials should demonstrate suitable swelling or contractile characteristics.
- Structural and mechanical properties
  Materials should be chosen based on the required mechanical properties of the construct, ranging from rigid thermoplastic polymer fibers for strength to soft hydrogels for cell compatibility.
- Material biomimicry
  Engineering of desired structural, functional and dynamic material properties should be based on knowledge of tissue-specific endogenous material compositions.

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature biotechnology

Selecting the right material to print

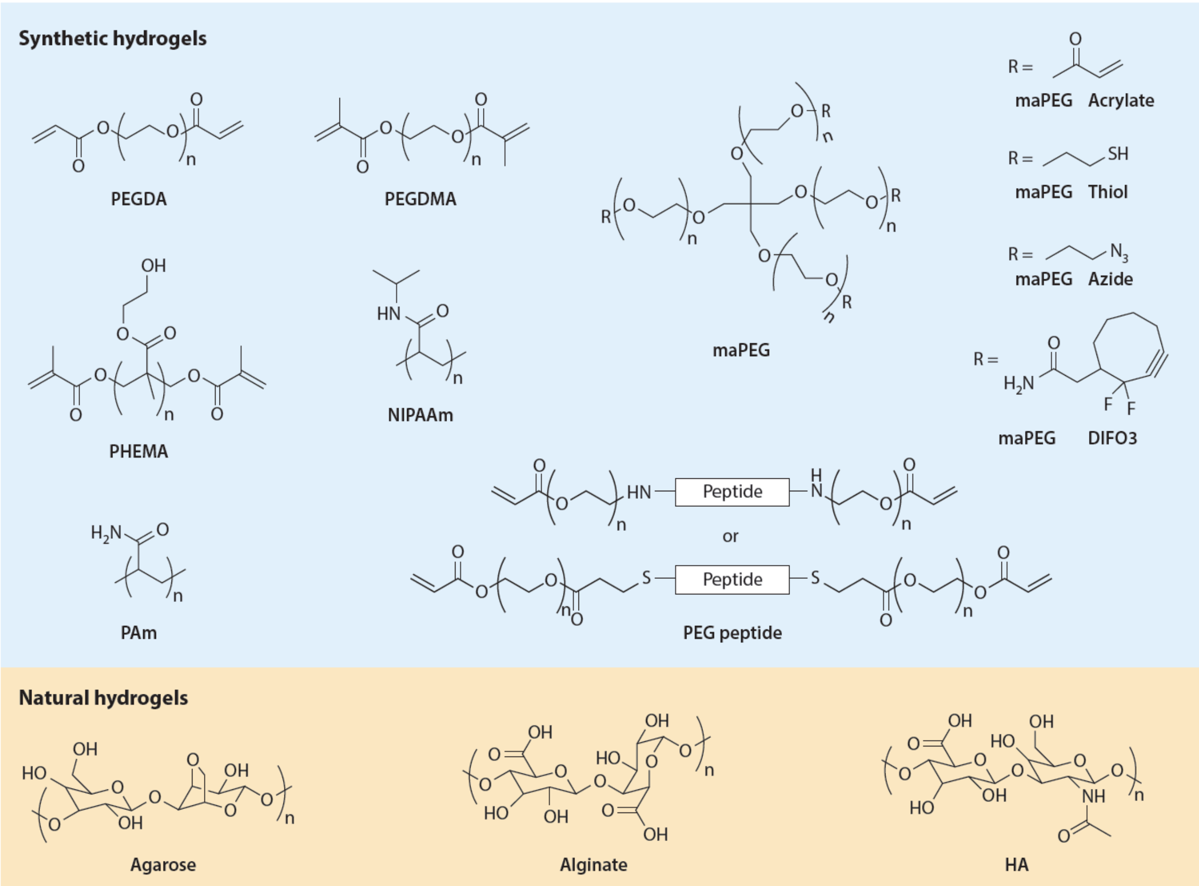


Figure 3

Chemical structures of common polymers for biofabrication. Abbreviations: DIFO3, difluorinated cyclooctyne; HA, hyaluronic acid; maPEG, multiarm PEG; NIPAAm, *N*-isopropyl acrylamide; PAm, poly(acrylamide); PEG, poly(ethylene glycol); PEGDA, PEG-diacrylate; PEGDMA, PEG-dimethacrylate; PHEMA, poly(2-hydroxy ethyl methacrylate).

Parameters :
- Porosity
- Stiffness
- Compatibility
- Degradability (kinetics, byproducts)
- Mechanical stability
- Biomimicry (Proteins, soluble factors …)

Polycaprolactine, Pluronic F 127, composite materials …

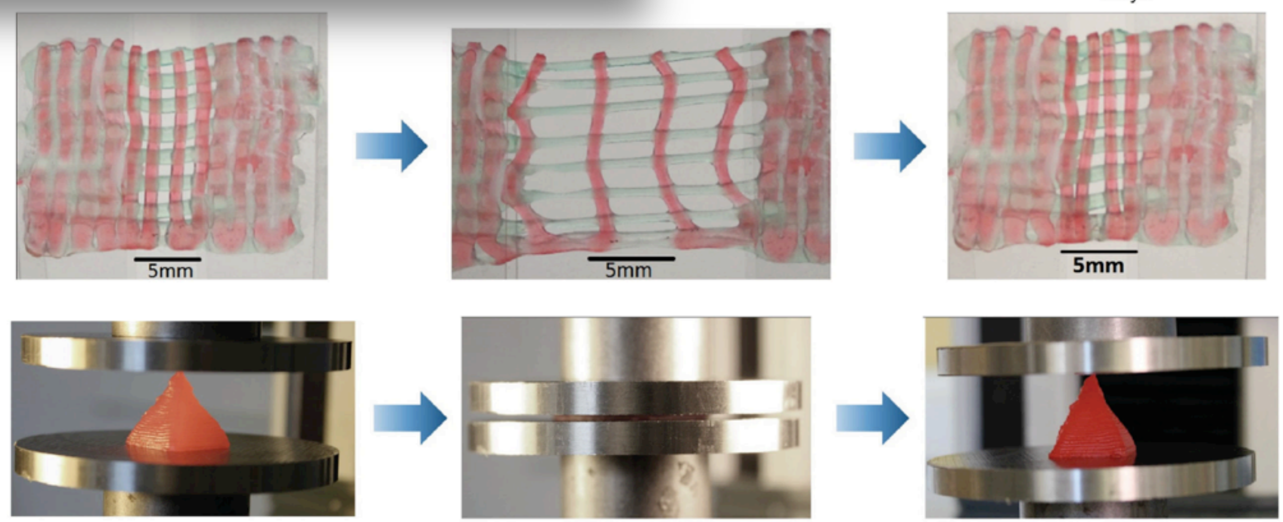
Bajaj, P.; Schweller, R. M.; Khademhosseini, A.; West, J. L.; Bashir, R. 3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine. Annu. Rev. Biomed. Eng. 2014, 16, 247–276.

Selecting the right material to print

Materials Views
www.MaterialsViews.com
ADVANCED MATERIALS
www.advmat.de

3D Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures

*Sungmin Hong, Dalton Sycks, Hon Fai Chan, Shaoting Lin, Gabriel P. Lopez, Farshid Guilak, Kam W. Leong, and Xuanhe Zhao**



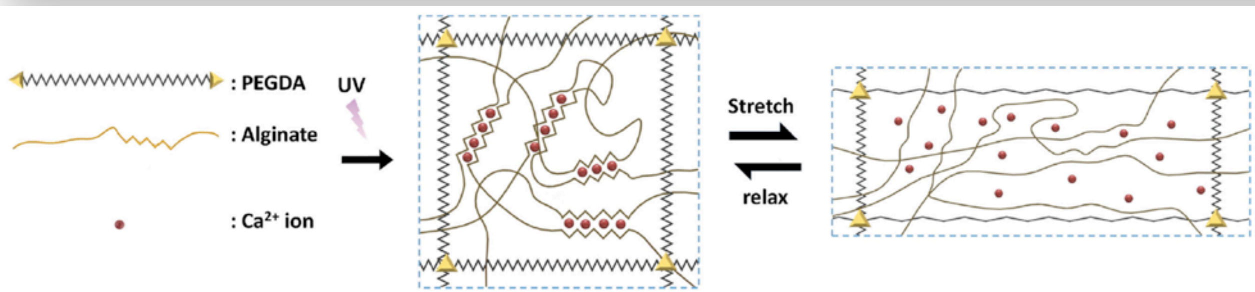


Figure 1. Schematic diagrams of the biocompatible and tough hydrogel. PEG and alginate polymers are covalently and ionically crosslinked through UV exposure and Ca^{2+}, respectively. As the hydrogel is deformed, the alginate chains are detached from the reversible ionic crosslinks and mechanical energy is dissipated. Once the hydrogel is relaxed from deformation, it regains its original configuration since the covalently crosslinked PEG network maintains the elasticity of the hydrogel. Over time, some of the ionic crosslinks in the alginate network can reform in the deformed and relaxed hydrogel.

Selecting the right material to print

ARTICLE

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Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink

Falguni Pati[1,*], Jinah Jang[2,3,*], Dong-Heon Ha[1], Sung Won Kim[4,5], Jong-Won Rhie[6], Jin-Hyung Shim[7], Deok-Ho Kim[3,8,9] & Dong-Woo Cho[1]

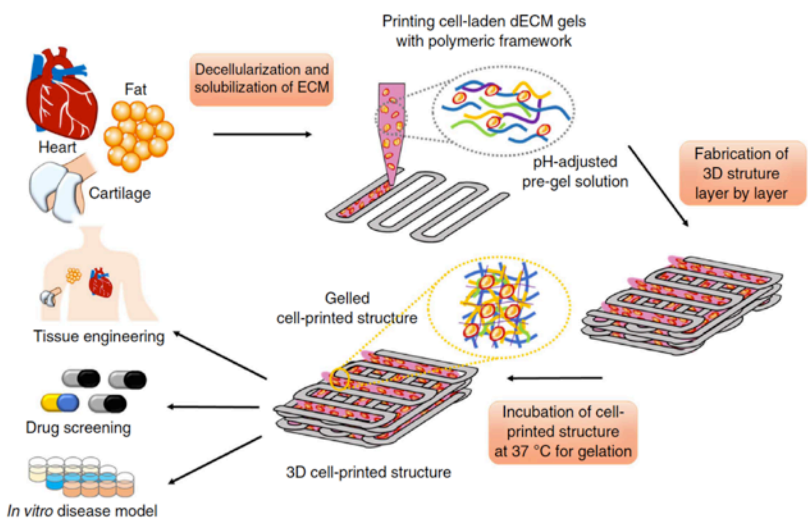
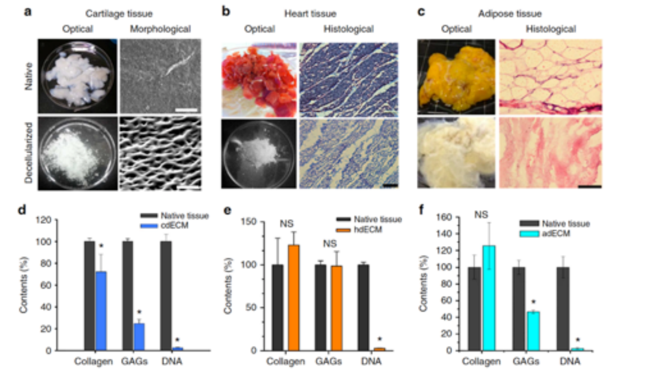


Figure 1 | Schematic elucidating the tissue printing process using dECM bioink. Respective tissues were decellularized after harvesting with a combination of physical, chemical and enzymatic processes, solubilized in acidic condition, and adjusted to physiological pH. Tissue printing was performed with the dECM bioink encapsulating living stem cells via a layer-by-layer approach followed by gelation at 37 °C. The 3D cell-printed structure has applications in various border areas including tissue engineering, *in vitro* drug screening and tissue/cancer model.



re 2 | Decellularization of the native tissues and their biochemical analysis. Optical and microscopic images of native and decellularized (**a**) cartilage e (scale bar, 50 μm), (**b**) heart tissue (scale bar, 100 μm), and (**c**) adipose tissue (scale bar, 100 μm). ECM components (Collagen and GAGs) and contents of native and decellularized (**d**) cartilage (cdECM), (**e**) heart (hdECM) and (**f**) adipose (adECM) tissue. All experiments were performed in cate. Error bars represent s.d. (*$P<0.05$; NS, no significance).

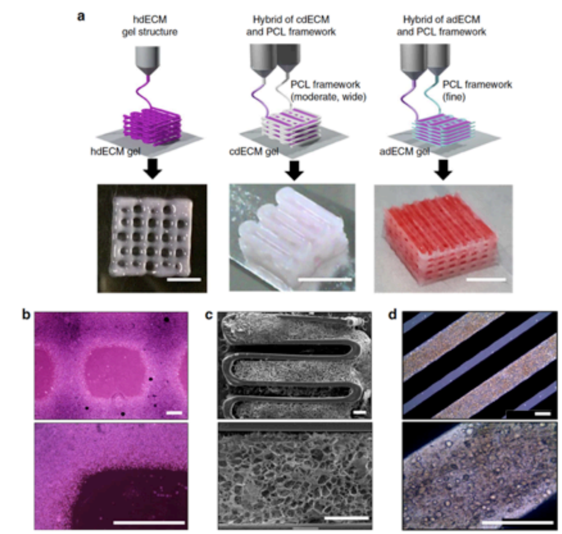
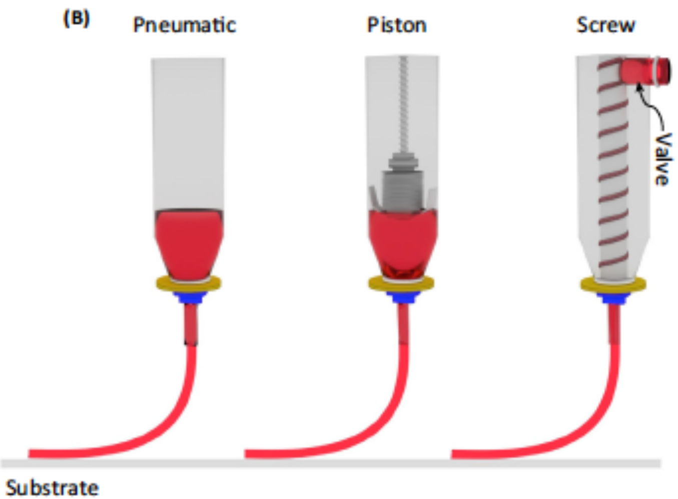


Figure 4 | Printing process of particular tissue constructs with dECM bioink. (**a**) Heart tissue construct was printed with only heart dECM (hdECM). Cartilage and adipose tissues were printed with cartilage dECM (cdECM) and adipose dECM (adECM), respectively, and in combination with PCL framework (scale bar, 5 mm). (**b**) Representative microscopic images of hdECM construct (scale bar, 400 μm), (**c**) s.e.m. images of hybrid structure of cdECM with PCL framework (scale bar, 400 μm) and (**d**) microscopic images of cell-printed structure of adECM with PCL framework (scale bar, 400 μm).

Extrusion Bioprinting … for multimaterial scaffolds



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- Continuous extrusion of material bead
- Temperature controlled material
- Nozzle with variable diameters
- Moving printing heads

Performances :
Common Resolution : 100 – 200 μm
Speed : 10 – 50μm/s

Materials :

- Large range of viscosities (30mPa.s / 6.10^7mPa.s)
- UV / Thermically cross linked (35°C-40°C)
- Shear thinning materials

Potential issues :

- Cell viability (survival rate (40-86%)
- Viability depends on pressure
- Viability depends on nozzle diameter (shear stress)
- Viability <Inkjet, LIFT

Vascular structures … an essential step towards organs

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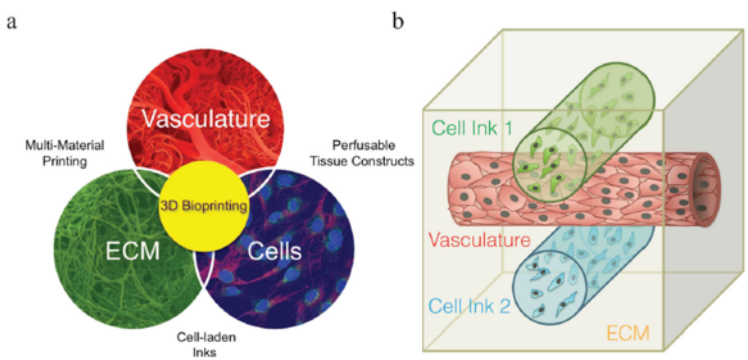
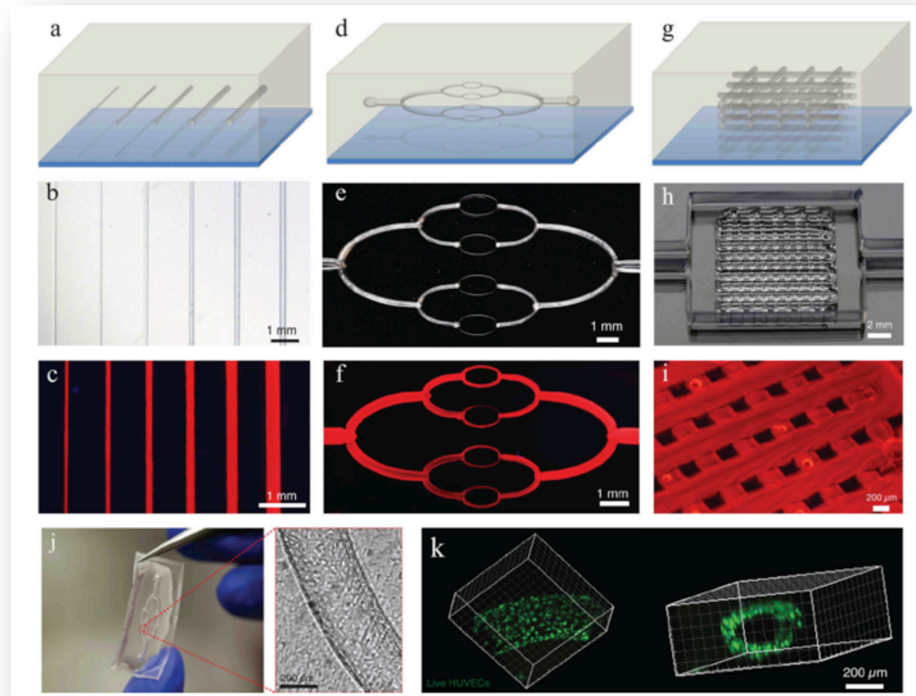
3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs

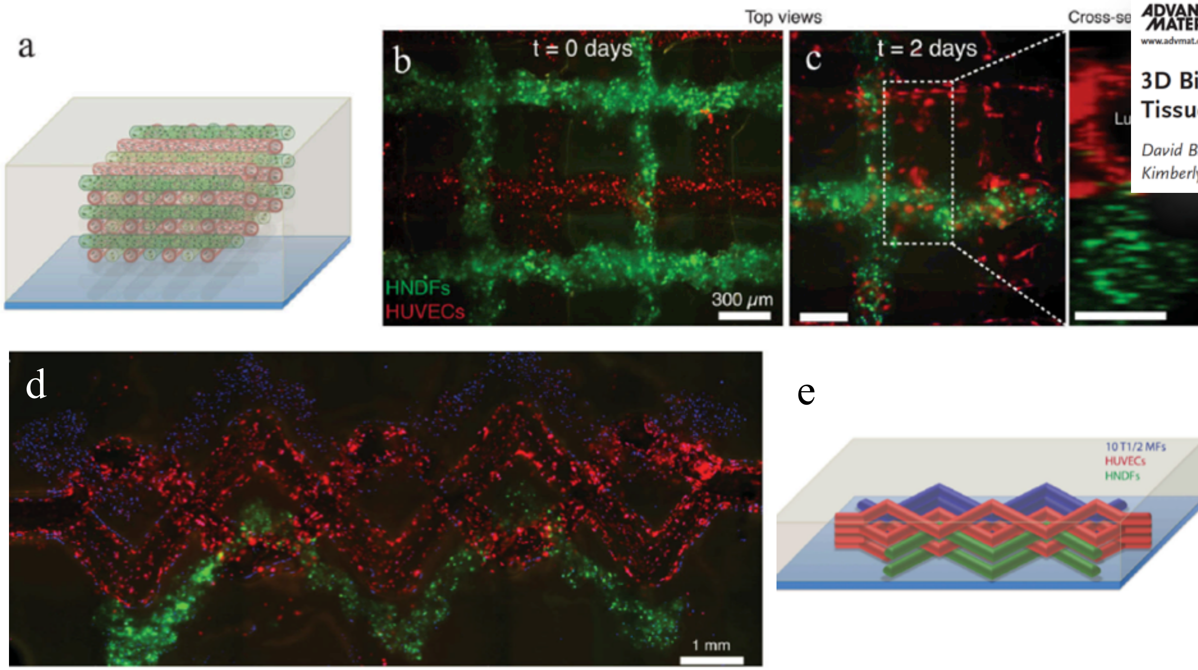
*David B. Kolesky, Ryan L. Truby, A. Sydney Gladman, Travis A. Busbee, Kimberly A. Homan, and Jennifer A. Lewis**





Kolesky, D. B., Truby, R. L., Gladman, A., Busbee, T. A., Homan, K. A., & Lewis, J. A. (2014). 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. Advanced Materials, 26(19), 3124-3130.

Vascular structures … an essential step towards organs



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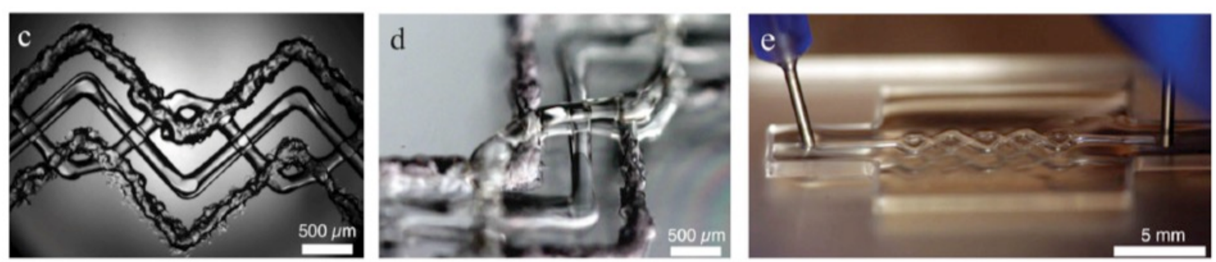
3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs

*David B. Kolesky, Ryan L. Truby, A. Sydney Gladman, Travis A. Busbee, Kimberly A. Homan, and Jennifer A. Lewis**

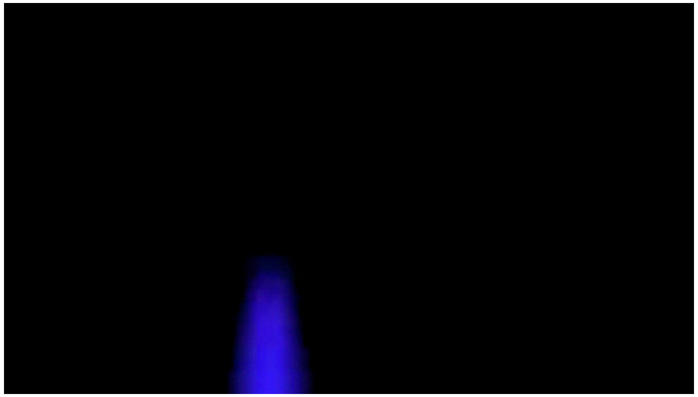
- Cell seeding (HUVECS, Fibroblasts …)
- Functionnal network
- ECM

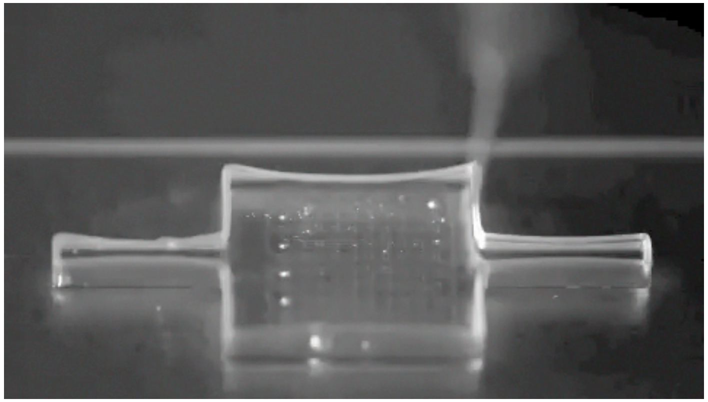
In summary, we report a new approach for creating vascularized, heterogeneous tissue constructs on demand based on 3D bioprinting. This highly scalable platform allows one to produce engineered tissue constructs in which vasculature and multiple cell types are programmably placed within extracellular matrices. These 3D microengineered environments open new avenues for drug screening and fundamental studies of wound healing, angiogenesis, and stem cell niches. With further refinement, our technique may lead to the rapid manufacturing of functional 3D tissues and, ultimately, perhaps organs.

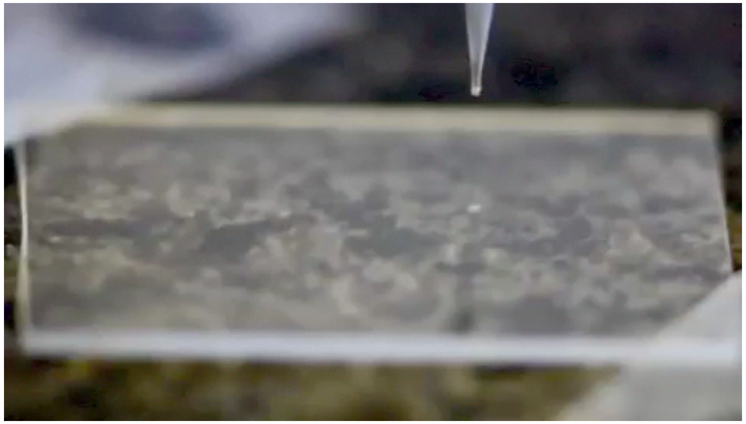


Kolesky, D. B., Truby, R. L., Gladman, A., Busbee, T. A., Homan, K. A., & Lewis, J. A. (2014). 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. Advanced Materials, 26(19), 3124-3130.

Vascular structures … an essential step towards organs







- Cell seeding (HUVECS, HNDF, Fibroblasts …)
- Functional network
- ECM

Kolesky, D. B., Truby, R. L., Gladman, A., Busbee, T. A., Homan, K. A., & Lewis, J. A. (2014). 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. Advanced Materials, 26(19), 3124-3130.

Printing ears

NANO LETTERS
Letter
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3D Printed Bionic Ears

Manu S. Mannoor,[†] Ziwen Jiang,[†] Teena James,[‡] Yong Lin Kong,[†] Karen A. Malatesta,[†] Winston O. Soboyejo,[†] Naveen Verma,[§] David H. Gracias,[‡] and Michael C. McAlpine*[,†]

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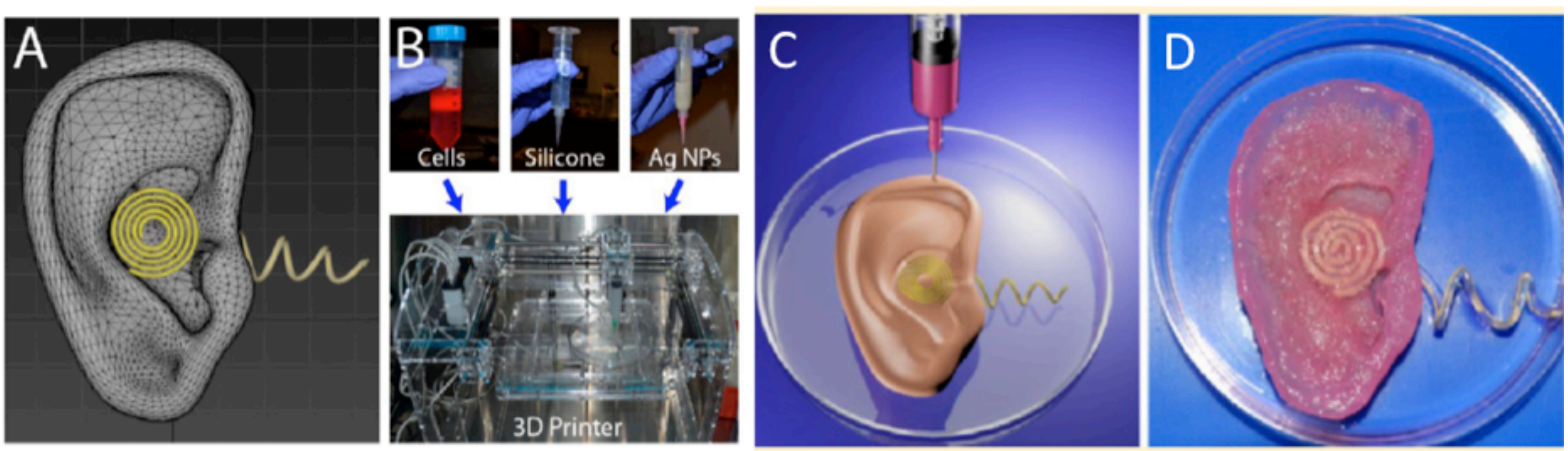
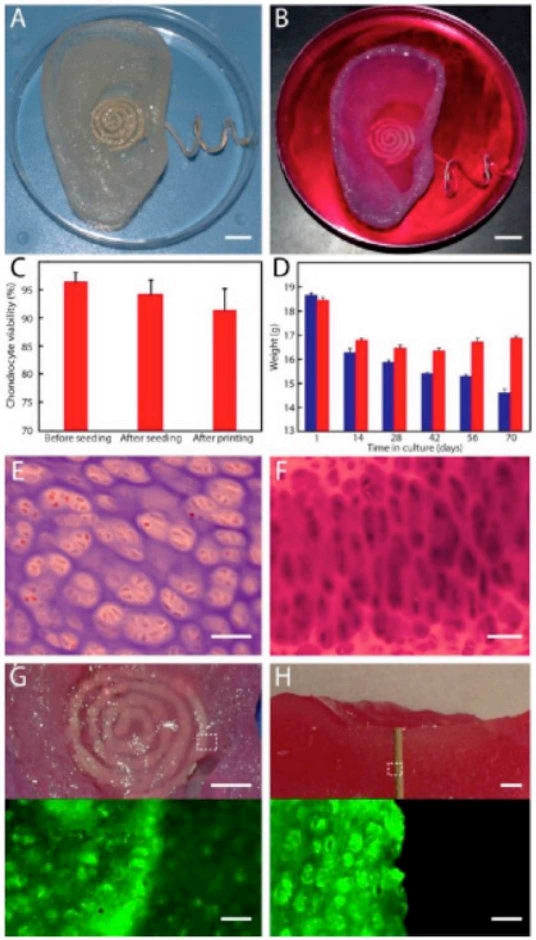
[‡]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland 21218, United States
[§]Department of Electrical Engineering, Princeton University, Princeton, New Jersey 08544, United States



Mannoor, M. S., Jiang, Z., James, T., Kong, Y. L., Malatesta, K. A., Soboyejo, W. O., ... & McAlpine, M. C. (2013). 3D printed bionic ears. Nano letters, 13(6), 2634-2639.

Printing ears



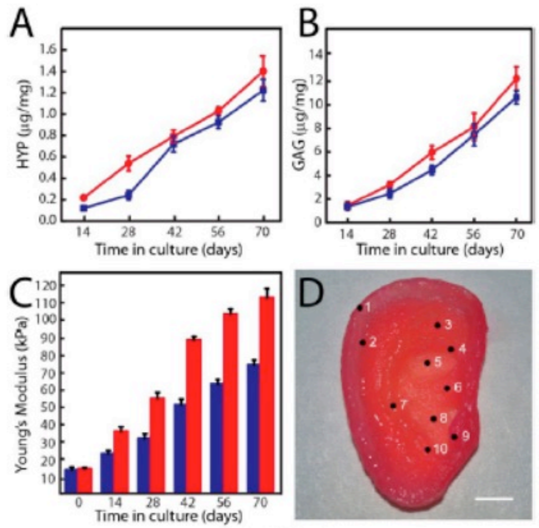


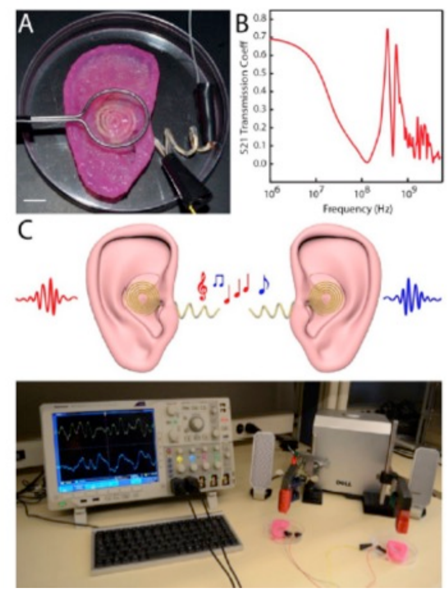
Table 1

Part	Mean Hardness (kPa)
1. Helix	44.85 ± 2.68
2. Scapha	38.93 ± 3.00
3. Fossa	42.40 ± 2.87
4. Crura Antihelix	45.47 ± 3.95
5. Cymba Conchae	41.53 ± 4.36
6. Crus of Helix	46.80 ± 4.72
7. Antihelix	40.67 ± 3.13
8. Cavum Conchae	38.50 ± 1.73
9. Tragus	40.10 ± 2.42
10. Antitragus	39.27 ± 3.26

Figure 3. Biomechanical characterization of the 3D printed neo-cartilage tissue. (A) Variation of HYP content over time in culture with 20% (red) and 10% (blue) FBS. (B) Variation of GAG content over time in culture with 20% (red) and 10% (blue) FBS. (C) Variation of Young's modulus of 3D printed dog bone constructs over time in

- chondrocytes
- alginate hydrogel matrix
- electrically conductive silver nanoparticles (AgNP)

Bionic ear - printing



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Printing hearts ...

Microfluidics19
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FULL PAPER

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Tissue Engineering

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3D Printing of Personalized Thick and Perfusable Cardiac Patches and Hearts

*Nadav Noor, Assaf Shapira, Reuven Edri, Idan Gal, Lior Wertheim, and Tal Dvir**

Adv. Sci. **2019**, *6*, 1900344 1900344 (1 of 10)

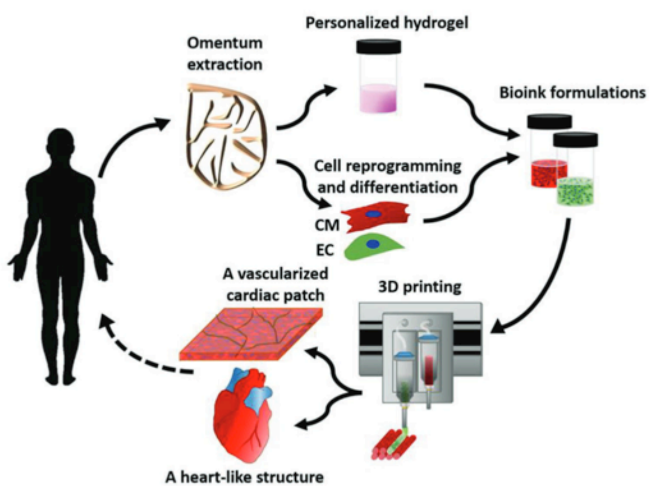


Figure 1. Concept schematic. An omentum tissue is extracted from the patient and while the cells are separated from the matrix, the latter is processed into a personalized thermoresponsive hydrogel. The cells are reprogrammed to become pluripotent and are then differentiated to cardiomyocytes and endothelial cells, followed by encapsulation within the hydrogel to generate the bioinks used for printing. The bioinks are then printed to engineer vascularized patches and complex cellularized structures. The resulting autologous engineered tissue can be transplanted back into the patient, to repair or replace injured/diseased organs with low risk of rejection.

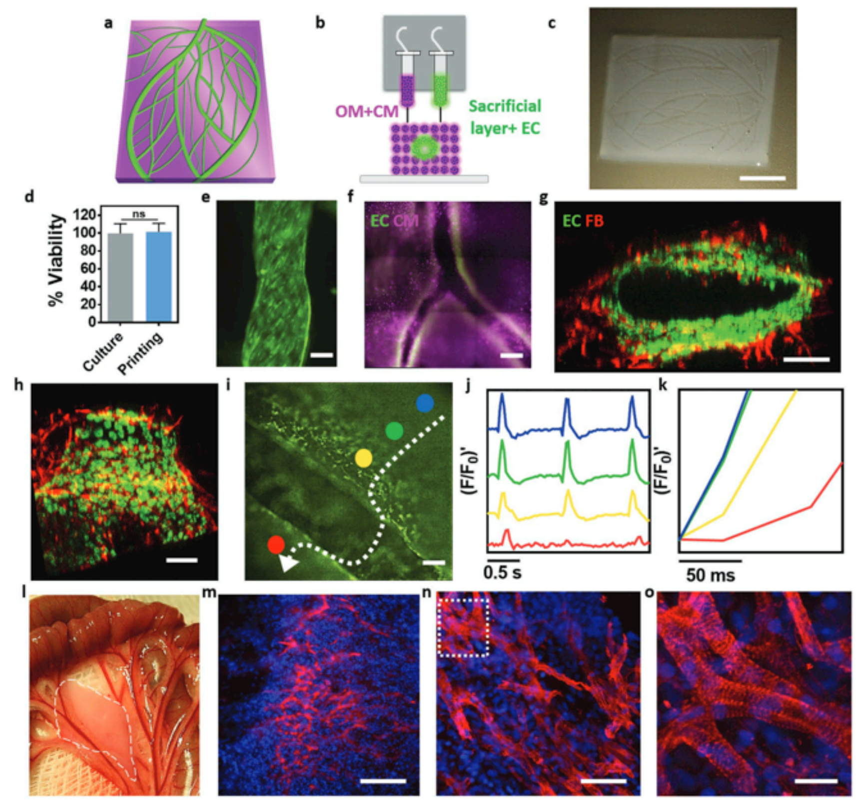


Figure 4. 3D printing of personalized cardiac patches. a) A 3D model of the cardiac patch. b) A side view of the printing concept and the distinct cellular bioinks. c) A printed vascularized cardiac patch. d) Cell viability after printing. e) A printed blood vessel, continuously layered with GFP-expressing ECs. f) A printed iPSCs-derived cardiac patch where the blood vessels (CD31 in green) are seen in-between the cardiac tissue (actinin in pink). g,h) Cross-sections of a single lumen, showing the interactions of GFP-expressing ECs and RFP-expressing fibroblasts. i–k) Calcium imaging within a printed vascularized cardiac patch (separate regions of interest are represented in different colors. The white arrow represents signal direction). i) The lumen of a blood vessel can be easily observed in-between the cardiac cells. j,k) Quantification of calcium transients across a lumen of the vascularized cardiac patch. l) Transplantation of the printed patch in between two layers of rat omentum. Dashed, white line highlights the borders of the patch. m–o) Sarcomeric actinin (red) and nuclei (blue) staining of sections from the explanted patch (panel (o) represents a high magnification of the marked area in (n). Scale bars: (c) = 1 cm, (e,g,h,l,m) = 100 µm, (f) = 500 µm, (n) = 50 µm, (o) = 25 µm.

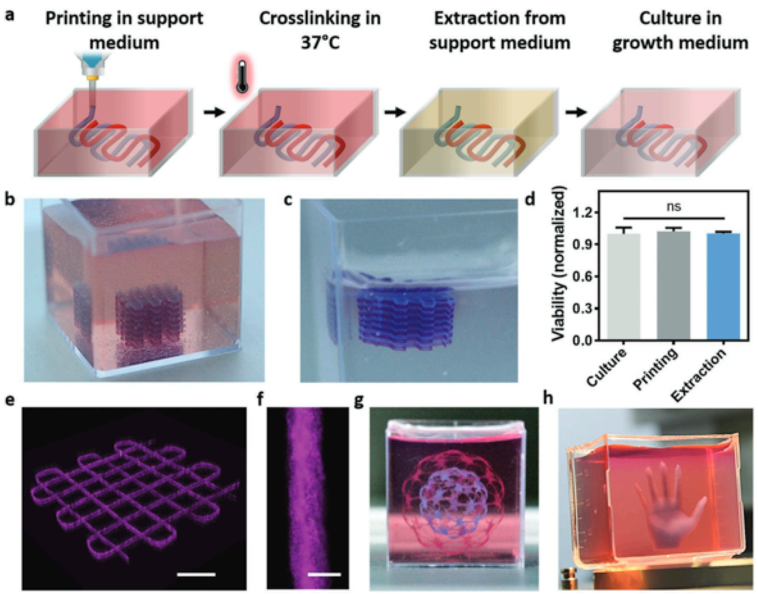


Figure 5. Printing the personalized hydrogel in a supporting medium. a) A scheme of the 3D printing concept. The construct is free-formed printed inside the support followed by incubation at 37 °C to crosslink the personalized hydrogel. Then, the structure can be safely extracted by an enzymatic or chemical degradation process of the support material and transferred into growth medium for culturing. b) A multilayered crisscross construct printed inside the support bath, and c) after its extraction. d) Cell viability before and after printing and after extraction. e) 3D confocal image of a double layered construct, printed in the support medium. f) A single strand of the personalized hydrogel within the support. g,h) Accurate, high resolution thick structures printed from the personalized hydrogel. Scale bars: (b) = 0.5 cm, (e) = 1 mm, (f) = 100 µm, (g,h) = 1 cm.

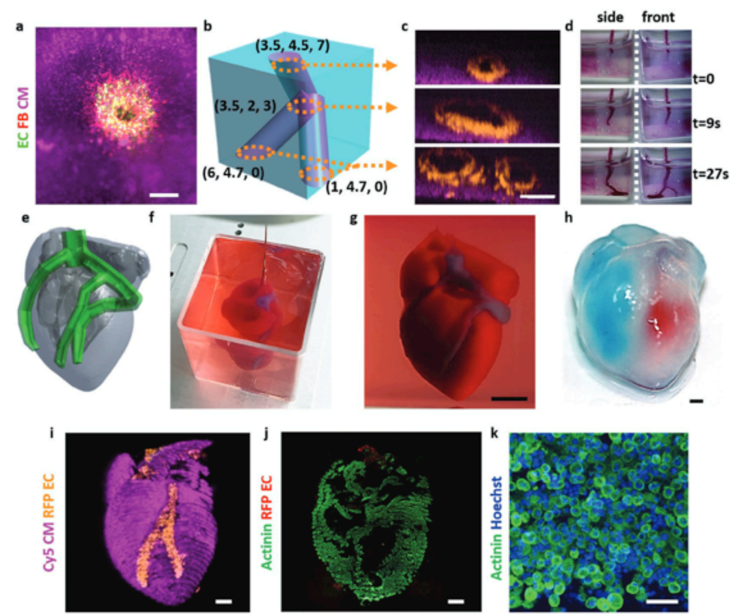
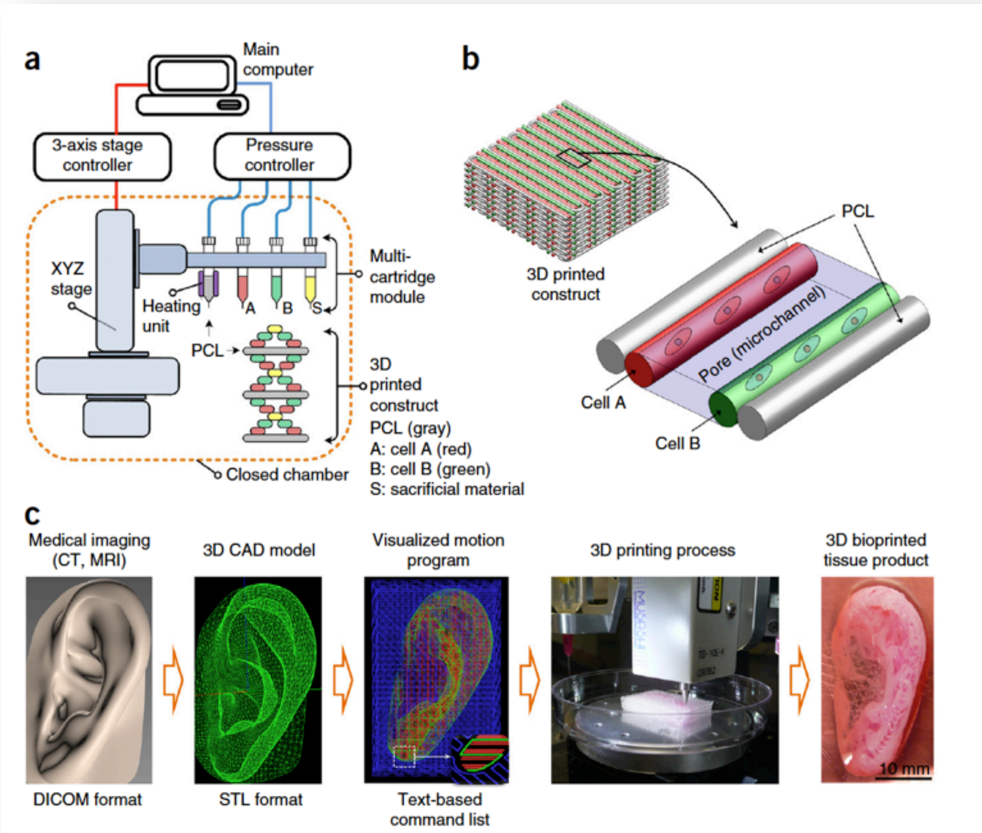


Figure 6. Printing thick vascularized tissues. a) A top view of a lumen entrance (CD31; green) in a thick cardiac tissue (actinin; pink). b) A model of a tripod blood vessel within a thick engineered cardiac tissue (coordinates in mm), and c) the corresponding lumens in each indicated section of the printed structure. d) Tissue perfusion visualized from dual viewpoints. e–k) A printed small-scaled, cellularized, human heart. e) The human heart CAD model. f,g) A printed heart within a support bath. h) After extraction, the left and right ventricles were injected with red and blue dyes, respectively, in order to demonstrate hollow chambers and the septum in-between them. i) 3D confocal image of the printed heart (CMs in pink, ECs in orange). j,k) Cross-sections of the heart immunostained against sarcomeric actinin (green). Scale bars: (a,c,h, i,j) = 1 mm, (g) = 0.5 cm, (k) = 50 µm.

nature biotechnology

tissue constructs with structural integrity

Hyun-Wook Kang, Sang Jin Lee, In Kap Ko, Carlos Kengla, James J Yoo & Anthony Atala



- PCL : Scaffold
- Pluronics 127 : sacrificial layer
- Gelatin/Fibrinogen/HA/Glycérol :
- cell seeded hydrogel

Advantages :

- complex tissues with better integrity for implantation
- vascularization
- resolution : 2μm ??

Disadvantages :

- same as extrusion based system

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Printing liver

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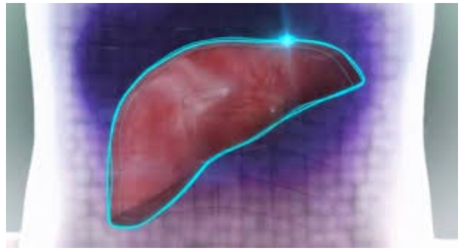

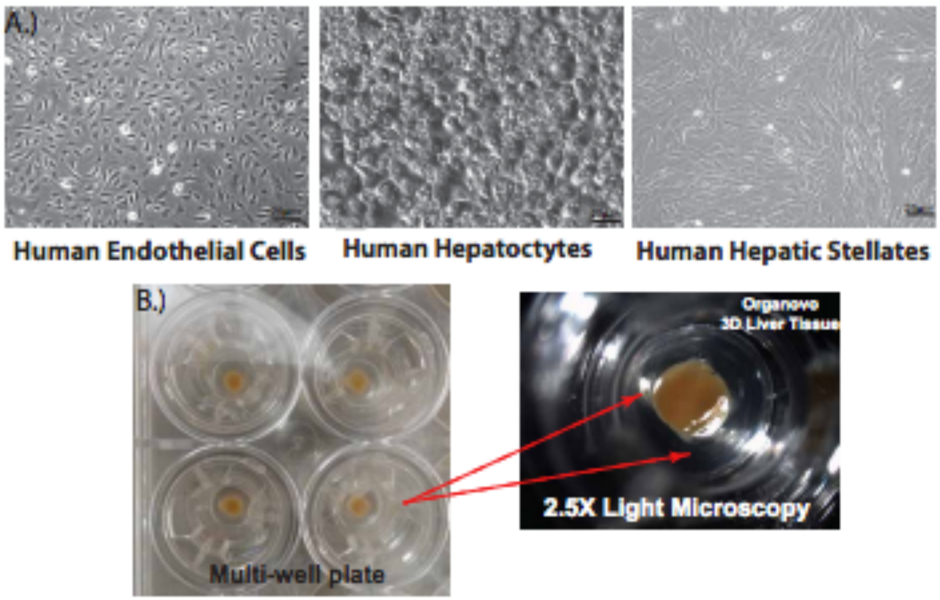

Figure 2. Features of Organovo's 3D bioprinted human liver tissues. A.) Bioprinted 3D human liver tissues include parenchymal and non-parenchymal cell types deposited in distinct compartments. B.) Multi-well format enables compound screening.

Microextrusion printing

Biomaterials 30 (2009) 2164–2174

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Leading Opinion

Organ printing: Tissue spheroids as building blocks☆

Vladimir Mironov[a,*], Richard P. Visconti[a], Vladimir Kasyanov[b], Gabor Forgacs[c], Christopher J. Drake[a], Roger R. Markwald[a]

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[b] Department of Anatomy and Anthropology, Riga Stradins University, Riga, Latvia
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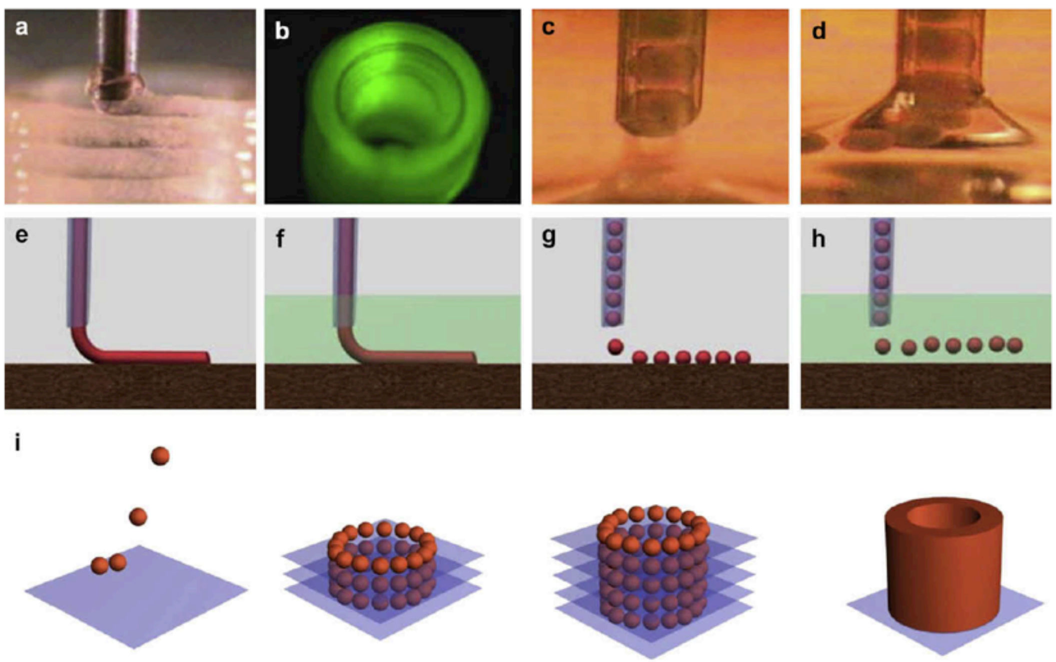

Fig. 2. Principles of bioprinting technology: a) bioprinter (general view); b) multiple bioprinter nozzles; c) tissue spheroids before dispensing; d) tissue spheroids durin dispensing; e) continuous dispensing in air; f) continuous dispensing in fluid; g) digital dispensing in air; h) digital dispensing in fluid; i) scheme of bioassembly of tubular tissu construct using bioprinting of self-assembled tissue spheroids illustrating sequential steps of layer-by-layer tissue spheroid deposition and tissue fusion process.

Printing microtissues / tissue spheroids

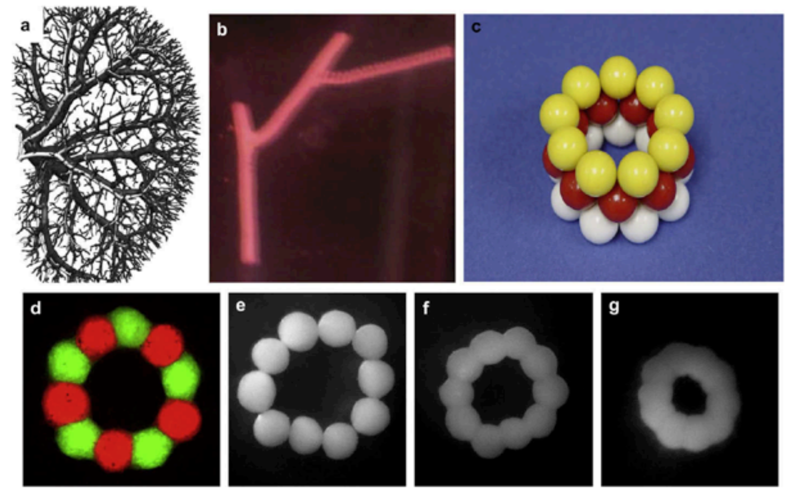

Fig. 5. Bioprinting of segments of intraorgan branched vascular tree using solid vascular tissue spheroids: a) kidney intraorgan vascular tree; b) bioprinted segment of vascular tree; c) physical model of bioassembly of tube-like vascular tissue construct using solid tissue spheroids; d) bioassembled ring-like vascular tissue constructs of tissue spheroids fabricated from human smooth muscle cells. Tissue spheroids are labeled with green and red fluorescent stains in order to demonstrate absence of cell mixing during tissue fusion process; e–g) sequential steps of morphological evolution of ring-like vascular tissue construct during tissue fusion process.

→ synthesis of more sophisticated soft natural-like biomaterials and extracellular matrices (bioprocessible and biomimetic stimuli-sensitive functional hydrogels)

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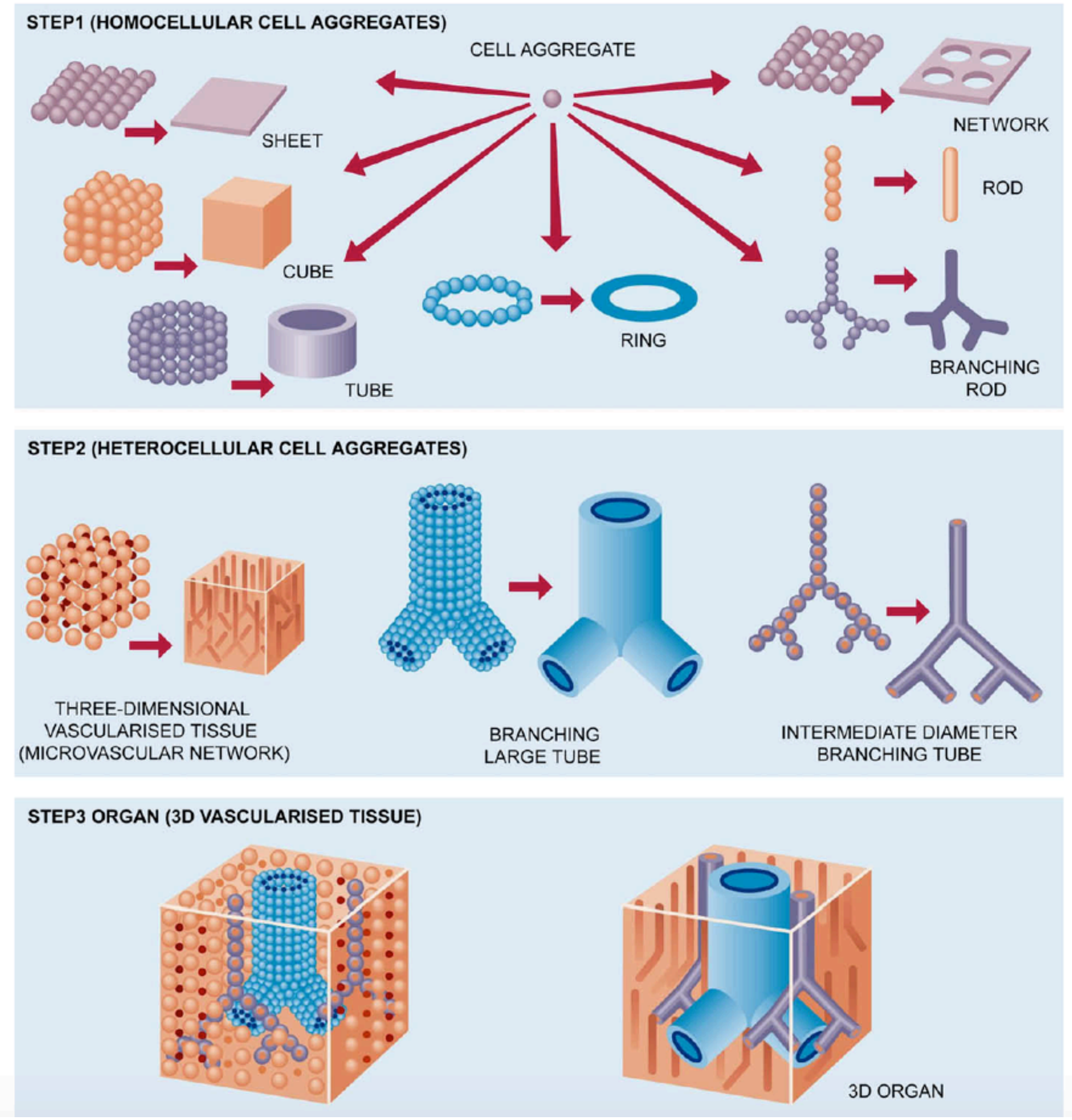
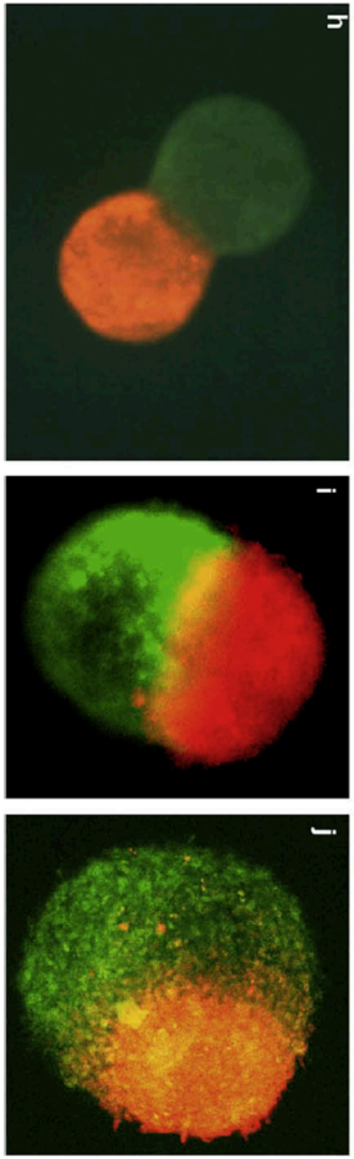
Microextrusion printing





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Scaffold free vs scaffold tissue engineering

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Opinion

The Synergy of Scaffold-Based and Scaffold-Free Tissue Engineering Strategies

Aleksandr Ovsianikov,[1,7,*] Ali Khademhosseini,[2,3,4] and Vladimir Mironov[5,6]

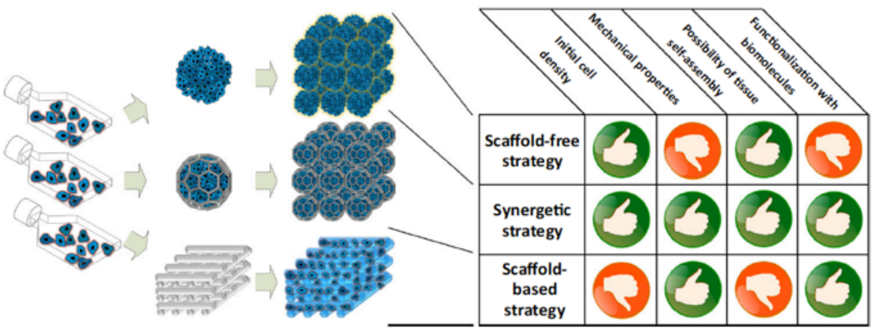


Figure 1. Summary of Different Technological Tissue Engineering Approaches. Expanded cells (left) can be used to (bottom) seed scaffolds to produce tissue-engineered constructs in the scaffold-based strategy; (top) produce spheroids to assemble scaffold-free constructs; and (middle) produce spheroids directly within cage-like microscaffolds, which are later assembled into a 3D construct and create cohesive construct via spheroid fusion in a synergetic tissue engineering strategy.

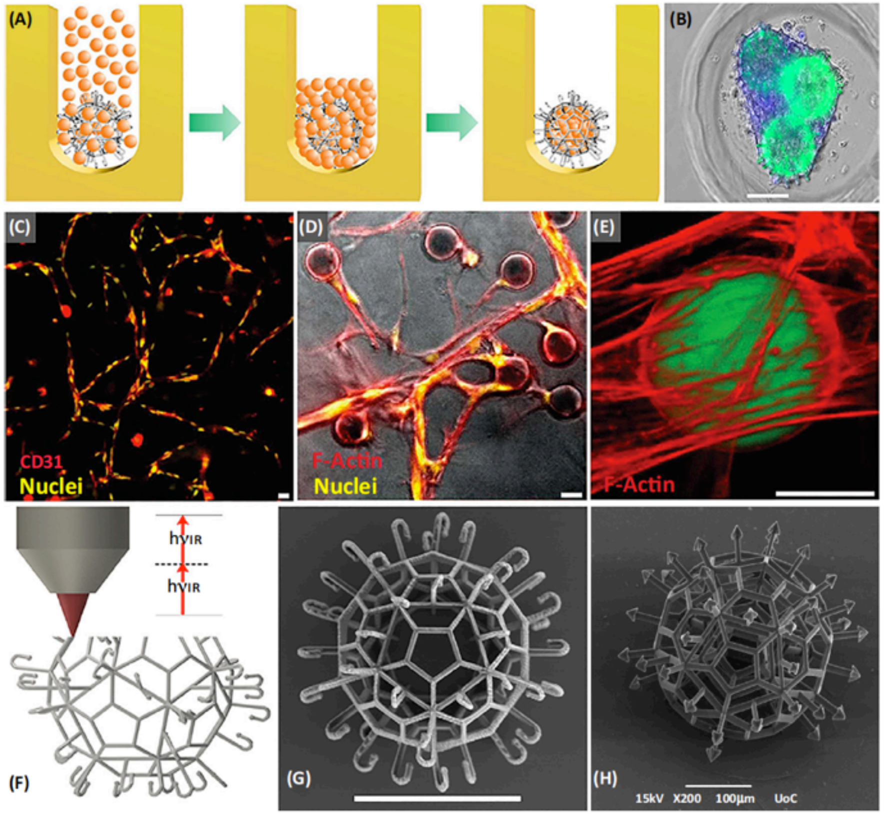
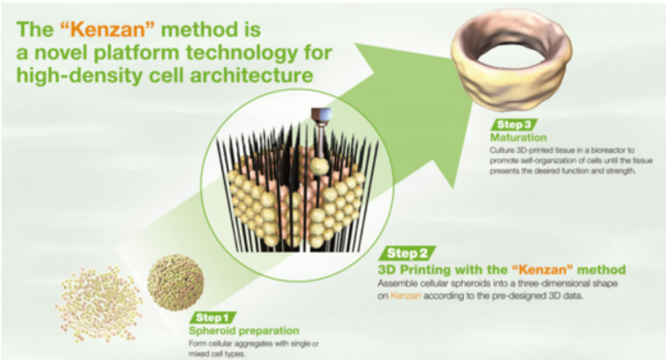


Figure 2. Self-Assembly in the Synergetic Tissue Engineering Strategy. (A) Seeding procedure, from left to right: microscaffold inserted into an antiadhesive microwell, cell seeding, and spheroid formation directly within the microscaffold [27]; (B) fusion of microscaffolds carrying spheroids produced from adipose tissue-derived mesenchymal stem cells [27]; (C–E) formation of a CD31-positive interconnected network within a modular microgel construct [31]; (F) laser 3D printing of a microscaffold by means of two-photon polymerization (2PP) technique; (G) scanning electron microscopy (SEM) image of a microscaffold equipped with hooks – the lockyball [35]; (H) SEM image of an arrow-headed microscaffold [26] [scale bar is 25 µm for images (C–E) and 100 µm throughout the rest of the figure]. Reproduced, with permission, from the indicated references.



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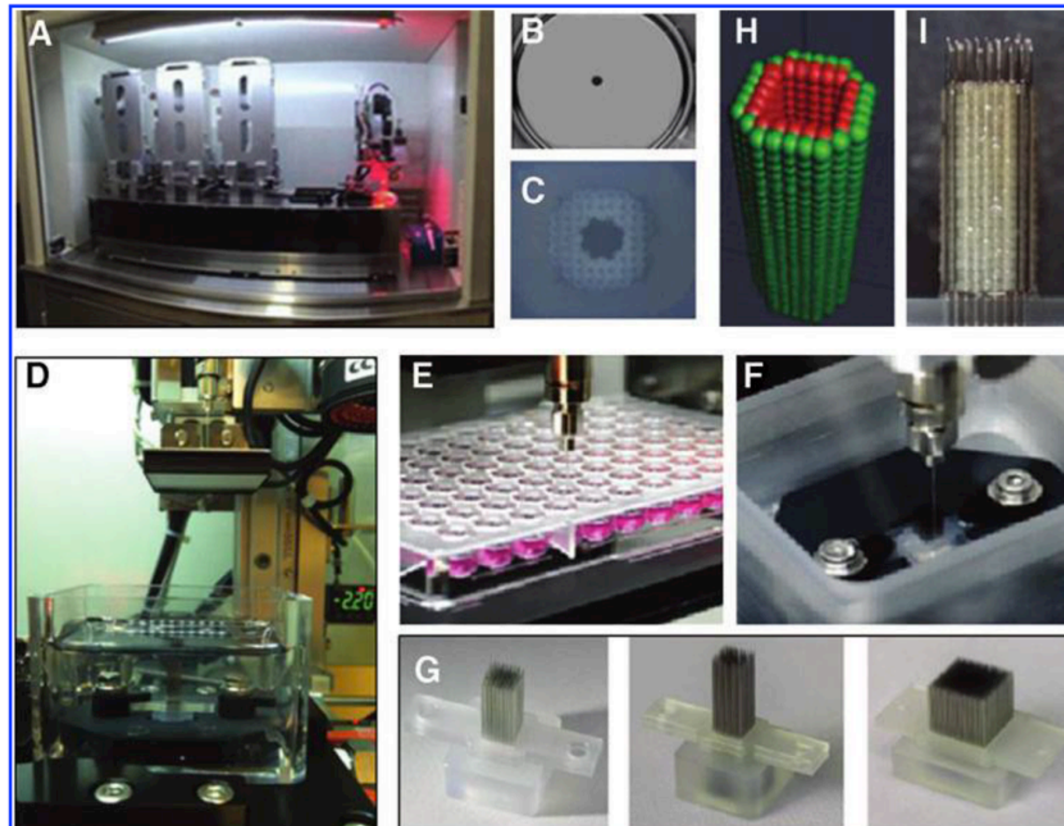
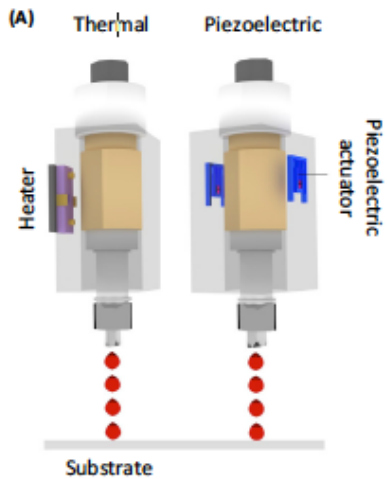


Itoh, M., et al. PLoS One 10, e0136681, 2015.

Inkjet printing



→ Both adapted to non bio and bio materials

- Digitalized printing (controlled volumes)
- 2D deposition
- Moving printing heads
- Thermal / Acoustic / Piezo actuation
- High speed / low price

Materials :

- Liquids (viscosity <10Cp)
- Stability after printing
  → Chemical, pH or UV mechanisms to cross link
  → Toxicity ?

Potential issues: Cell viability ?

- Thermal stress : Heating nozzle (200°C-300°C)

… limited temperature increase (4°C-10°C)

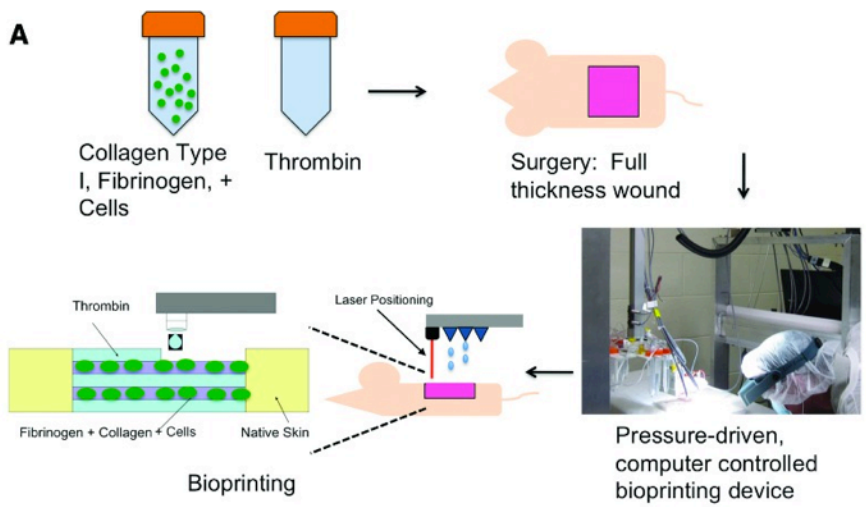
- Mechanical stress (shear stress)
- Nozzle clogging
- Resolution : 50um

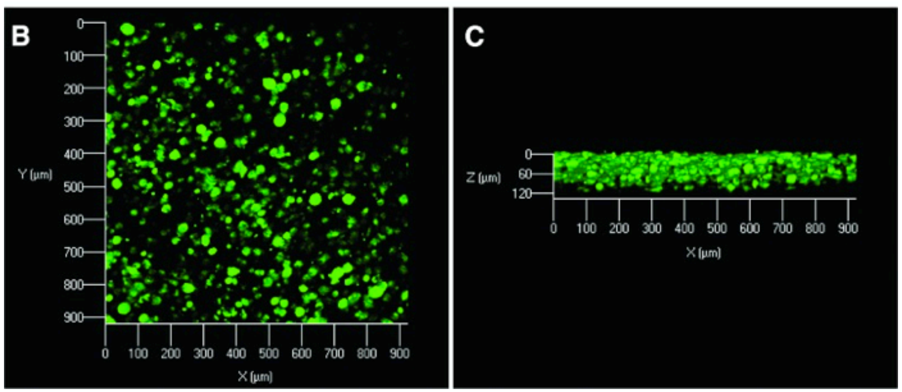
Inkjet printing

Stem Cells Transl Med. 2012 Nov;1(11):792-802. doi: 10.5966/sctm.2012-0088. Epub 2012 Oct 29.

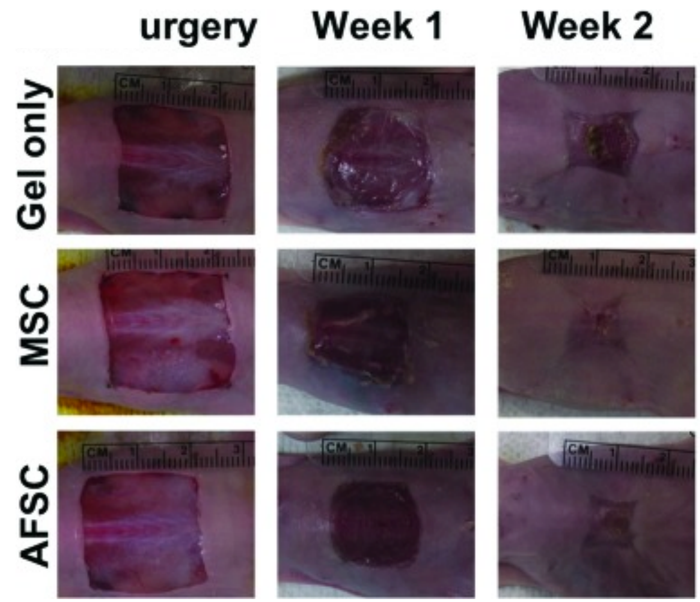
Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds.

Skardal A[1], Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, Soker S.

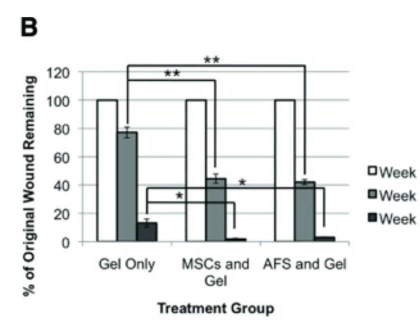




Bioprinting stem cells for treatment of skin wounds. (A): A schematic describing the approach by which amniotic fluid-derived stem (AFS) cells are bioprinted in order to increase healing of a full-thickness skin wound. Wounds containing the deposited gels with green fluorescent protein-tagged AFS cells were harvested 24 hours postprinting and analyzed with confocal microscopy. Images revealed evenly distributed cells in the gels, as viewed from above (B) or from the side (C).



Wound closure rates are increased in AFS cell- and MSC-treated mice. (A): Gross histology images illustrating wound closure in gel-only, MSC, and AFS treatments. (B): Percentage of unclosed wound remaining at surgery, 1 week, and 2 weeks. Significance: *, $p < .05$; **, $p < .01$. Abbreviations: AFS, amniotic fluid-derived stem; AFSC, amniotic fluid-derived stem cell; MSC, mesenchymal stem cell.

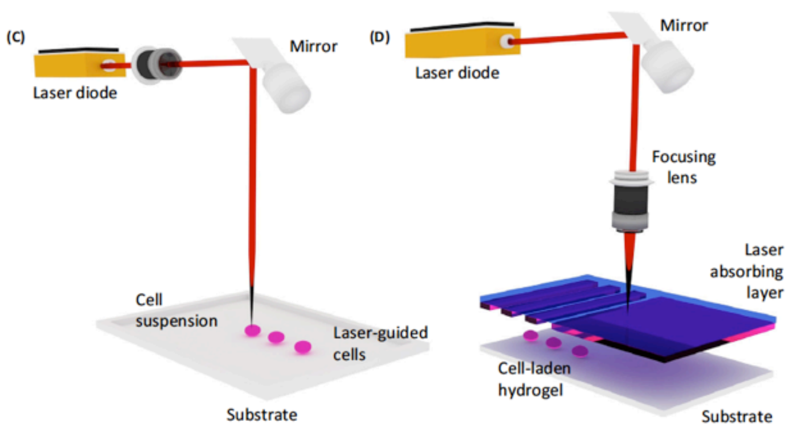


Laser assisted bioprinting (LAB)

Methods in Cell Biology
Volume 119, 2014, Pages 159–174
Micropatterning in Cell Biology Part A

Chapter 9 – Cell Patterning by Laser-Assisted Bioprinting

Raphaël Devillard, Emeline Pagès, Manuela Medina Correa, Virginie Kériquel, Murielle Rémy, Jérôme Kalisky, Muhammad Ali, Bertrand Guillotin, Fabien Guillemot



Laser Induced Forward Transfer

Principle :

- Pulsed laser source
- Ribbon coated with bioink
- Absorbing layer (Ti, Au)

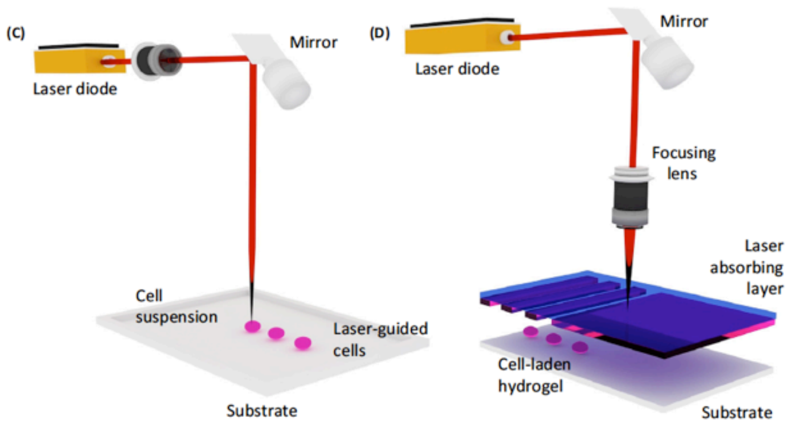
poietis
make tissues real

Laser assisted bioprinting (LAB)

ELSEVIER
Methods in Cell Biology
Volume 119, 2014, Pages 159–174
Micropatterning in Cell Biology Part A

Chapter 9 – Cell Patterning by Laser-Assisted Bioprinting

Raphaël Devillard[*,†], Emeline Pagès[*,†], Manuela Medina Correa[*,†], Virginie Kériquel[*,†], Murielle Rémy[*,†], Jérôme Kalisky[*,†], Muhammad Ali[*,†], Bertrand Guillotin[*,†], Fabien Guillemot[*,†]



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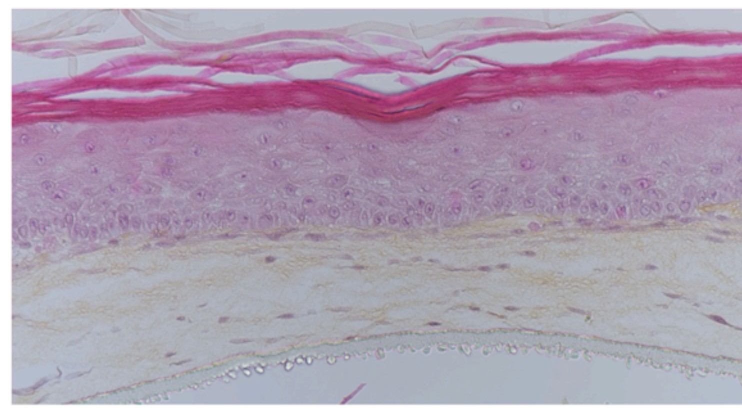
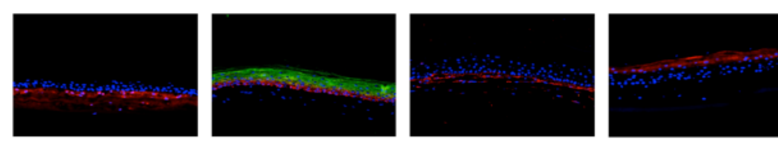


HES staining of Poieskin® skin model at D12 Air-Liquid interface



Collagen I / DAPI staining | Cytokeratin 5 / Cytokeratin 10 / DAPI staining | Collagen IV / DAPI staining | Filaggrin / DAPI staining

Laser assisted bioprinting (LAB)

Biomaterials 31 (2010) 7250–7256

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Laser assisted bioprinting of engineered tissue with high cell density and microscale organization

Bertrand Guillotin[a,*], Agnès Souquet[a], Sylvain Catros[a], Martí Duocastella[b], Benjamin Pippenger[a], Séverine Bellance[a], Reine Bareille[a], Murielle Rémy[a], Laurence Bordenave[a], Joëlle Amédée[a], Fabien Guillemot[a]

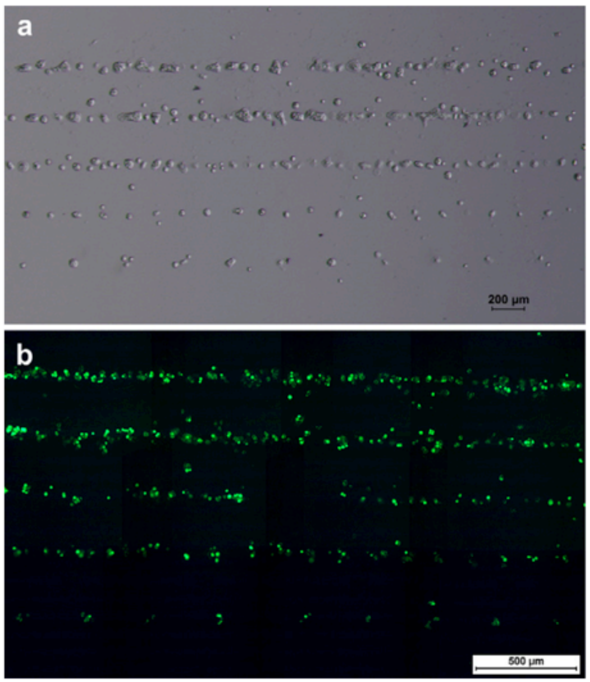


Fig. 3. Cell printing resolution according to the laser scanning speed. Hundred million cells per ml loaded with green fluorescent Calcein-AM (4 µM) were prepared in DMEM. Cells were printed according to five parallel lines of varying scanning speed (from top to bottom): 100; 200; 400; 800 and 1600 mm/s (with laser parameters set at $E = 6$ µJ, $D = 11$ mm). (a) Phase contrast microscope image of cells printed onto glass. Scale bar: 200 µm. (b). Fluorescence microscope image of cells printed onto a 100 µm thick layer of Matrigel. Scale bar: 500 µm.

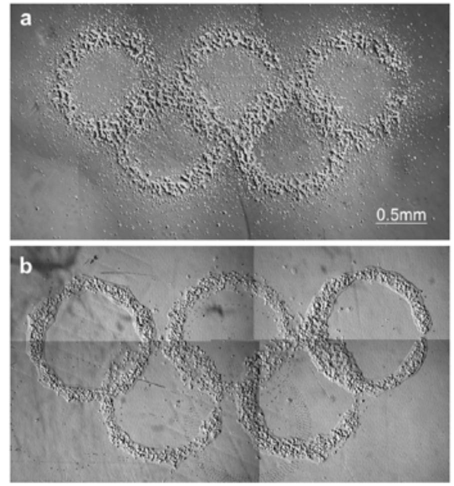


Fig. 1. Cellularized 2D pattern resolution according to viscosity. Fifty million cells per ml were suspended in DMEM supplemented with 10% glycerol (a), plus 1% (w/v) alginate (b). Satellite droplets (splashing) are virtually absent when 1% (w/v) alginate is added to the bioink. Phase contrast microscope image of cells printed onto glass. Magnification 25×.

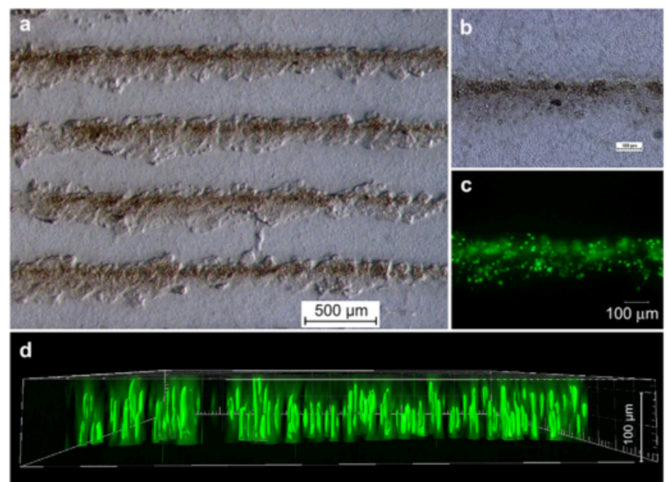
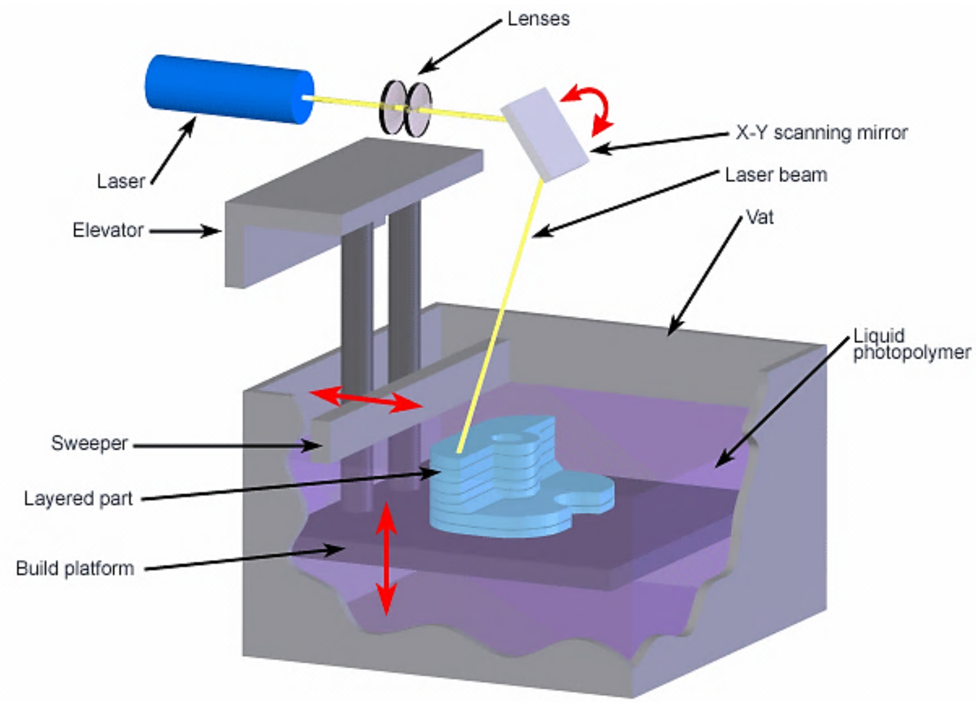
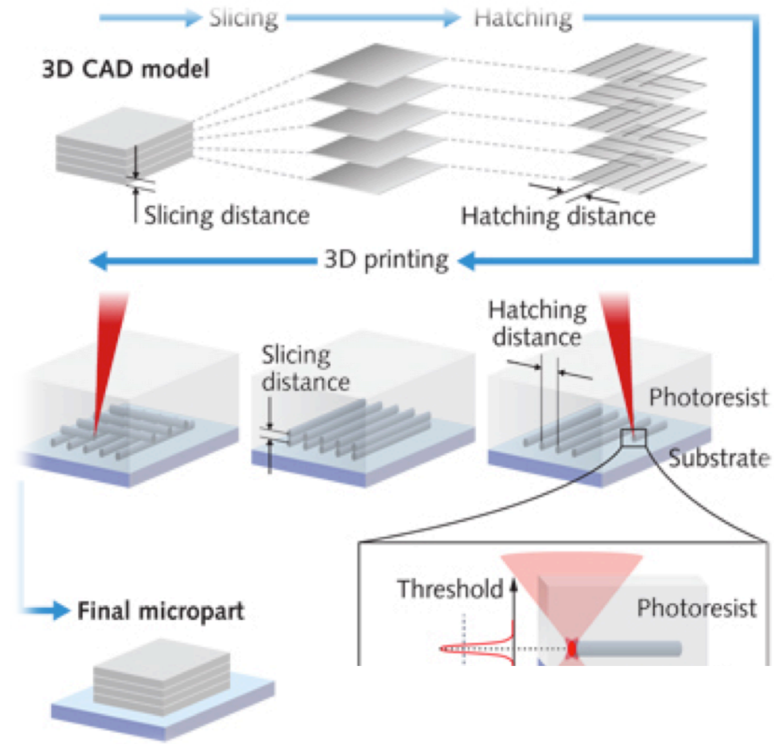


Fig. 6. Geometrically controlled cellularized soft free form bioprinting. Endothelial cell line Eahy926 has been added to the bioink (as detailed in Fig. 5) at a concentration of 6 10^7 cells/ml. The cell containing bioink was then printed on a layer of fibrinogen (90 mg/ml), according to computer designed parallel lines (2 cm in length, 500 µm pitch). (a) Phase contrast optical microscope image, scale bar: 500 µm. (b) Phase contrast optical microscope image, scale bar: 100 µm. (c) Live/dead assay staining of Fig. 6b, fluorescence microscope image, scale bar: 100 µm. (d) Orthogonal view of the surface representation of the z-stack of Fig. 6c.

Advantages :
High cell viability
Single cell resolution
Nozzle free technique

Bioprinting … increasing resolution with Stereolithography

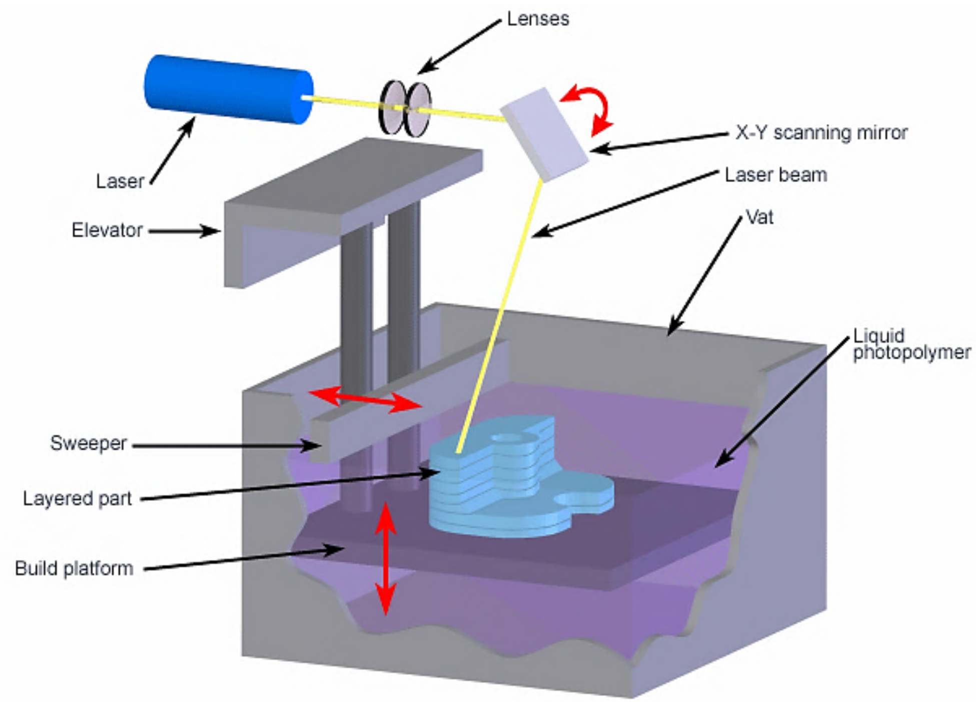




Specifications :

- parts are built from a liquid photopolymer
- each layer is created by a UV laser that cures one cross section at a time.

Bioprinting … increasing resolution with Stereolithography



Resolution x,y
SLA: spot size > 30um
DMD-PP : pixel size > 50um
Resolution z
Down to 1μm

Materials :
Photosensitive polymers
(synthetic / natural)
ex : PEG DA, GelMA, …

Advantages :
Speed
Simple, low cost
No viscosity limitation
Large area

Limits:
Cell viability
Photoinitiator compatibility
Multimaterials (single material at a time)

Stereolithography (SLA) &
Digital micromirror-based projection printing

PAPER www.rsc.org/loc | Lab on a Chip

Three-dimensional photopatterning of hydrogels using stereolithography for long-term cell encapsulation†

Vincent Chan,[ad] Pinar Zorlutuna,[ad] Jae Hyun Jeong,[b] Hyunjoon Kong[b] and Rashid Bashir*[acd]

Received 16th March 2010, Accepted 11th June 2010
DOI: 10.1039/c004285d

→Long term cell culture
→ Mutlimaterial ?

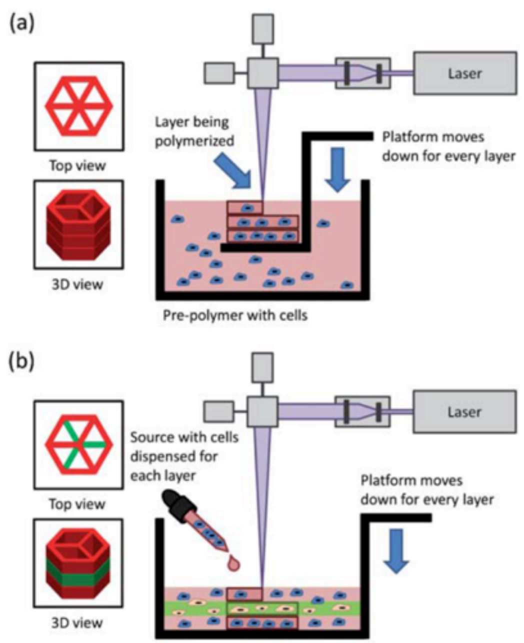


Fig. 1 A schematic representation of the SLA modifications. (a) In the top-down approach, the layout consists of a platform immersed just below the surface of a large tank of pre-polymer solution. After the layer is photopolymerized, the platform is lowered a specified distance to recoat the part with a new layer. (b) In the bottoms-up approach, the pre-polymer solution is pipetted into the container one layer at a time from the bottom to the top. This setup was modified especially for cell encapsulation applications, which required: (1) reduction in total volume of photopolymer in use and (2) removal of photopolymer from static conditions that cause cells to settle.

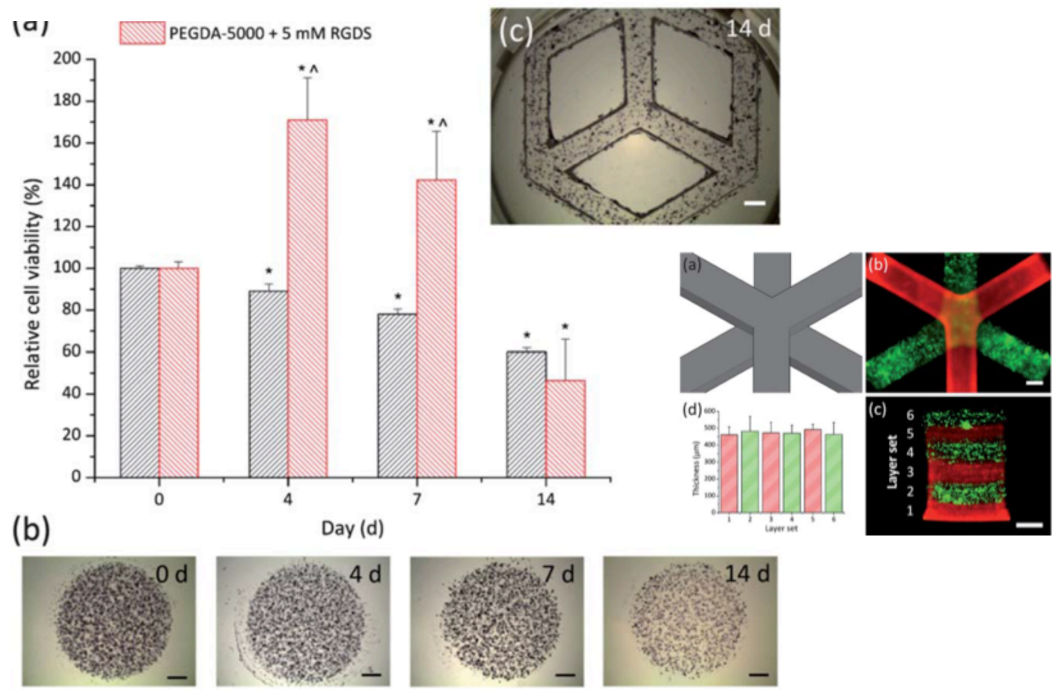
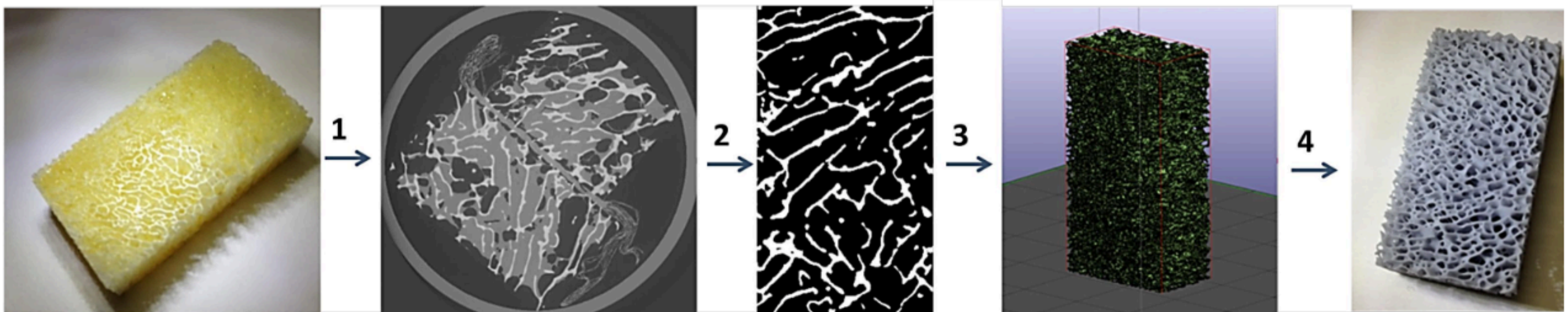


Fig. 5 NIH/3T3 cell viability over 14 days in PEGDA hydrogels with M_w 5000 using single-layer and multi-layer approaches. (a) OD (490 nm) values quantified with MTS assay were normalized to day 0. All values are mean ± standard deviation of $n = 3$. (*) denotes statistical difference compared to 0 day of same approach. (^) denotes statistical difference compared to different approach of same day. (b) MTT staining that shows living cells in a patterned multi-layer PEGDA hydrogel with M_w 5000 Da after 14 days. (c) MTT staining that shows living cells in single-layer PEGDA hydrogel disks with M_w 5000 Da after 0, 4, 7, and 14 days. There was a noticeable difference in intensity after 14 days. Scale bars are 1 mm.

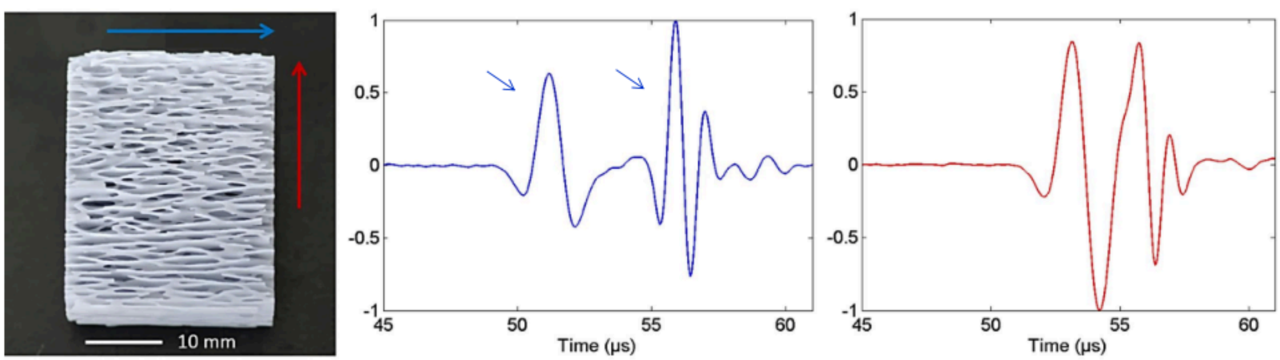
Bioprinting … increasing resolution with Stereolithography

Reconstruction of the bone microstructure from the a bone micro-tomography



1-μCT of the horse femoral epiphysis; 2- adjusting threshold while removing contribution from interstitial spaces filled with air or marrow; 3-3D data set converted into a STL file (standard format for stereolithography) , 4- printing of the sample (real sample)

Modified structure : control ever the bone porosity

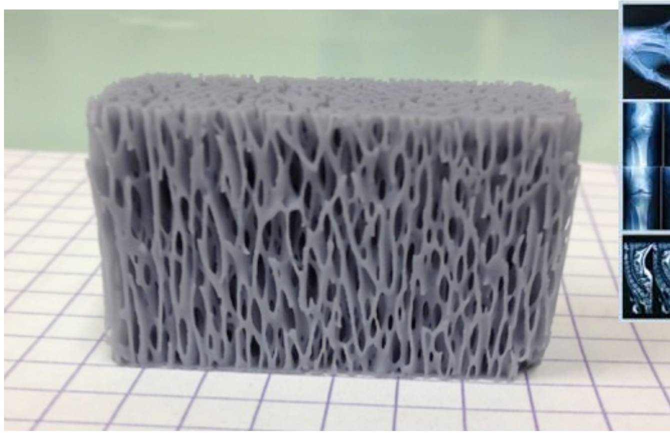


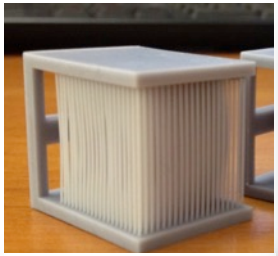
Sub-volume of the numerical structure was extracted and stretched four-fold along one dimension.
→ new structure: enhanced structural anisotropy, increased thickness along the stretched axis

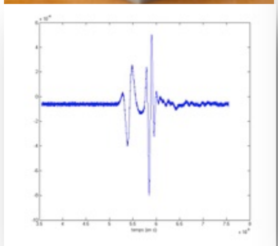
L. Malaquin, P. Juskova, J.L. Viovy

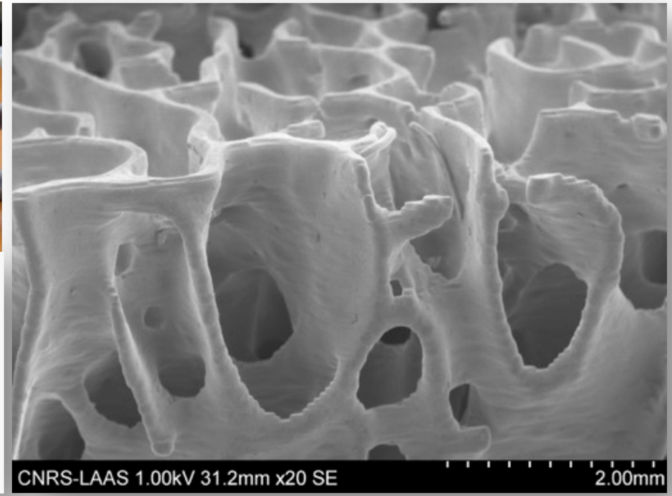
Bioprinting … increasing resolution with Stereolithography

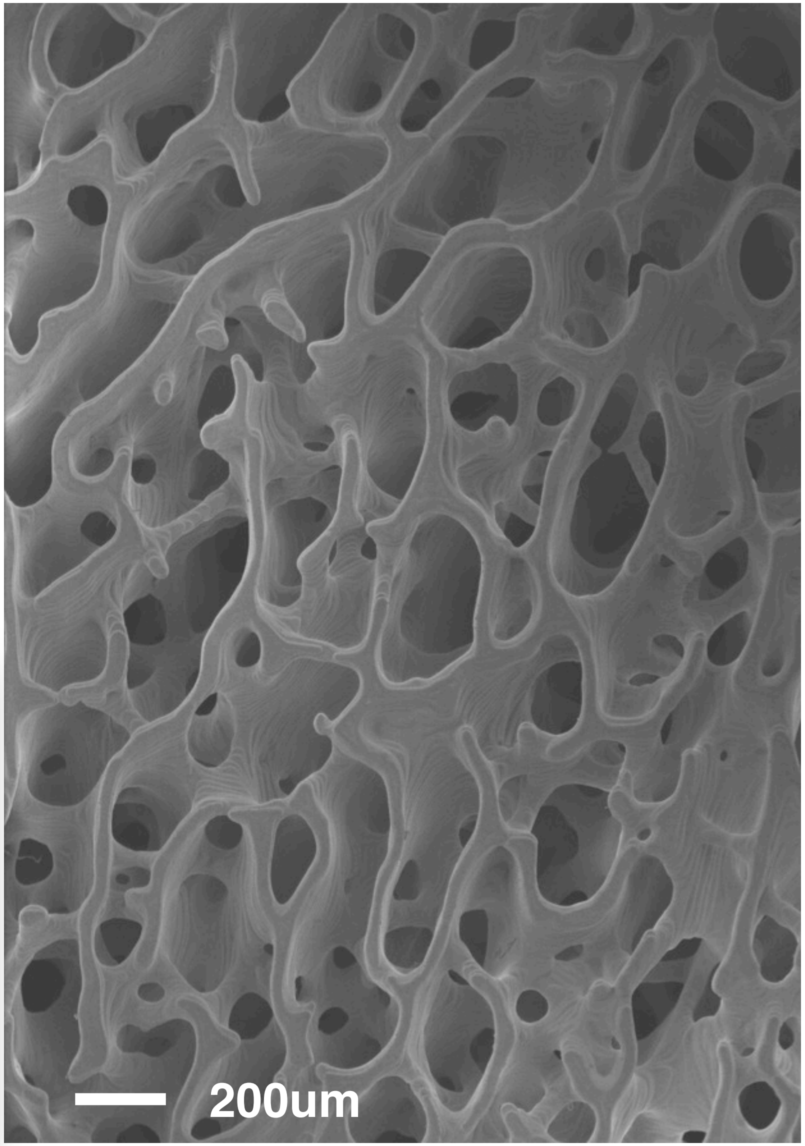
- Trabecular Bones







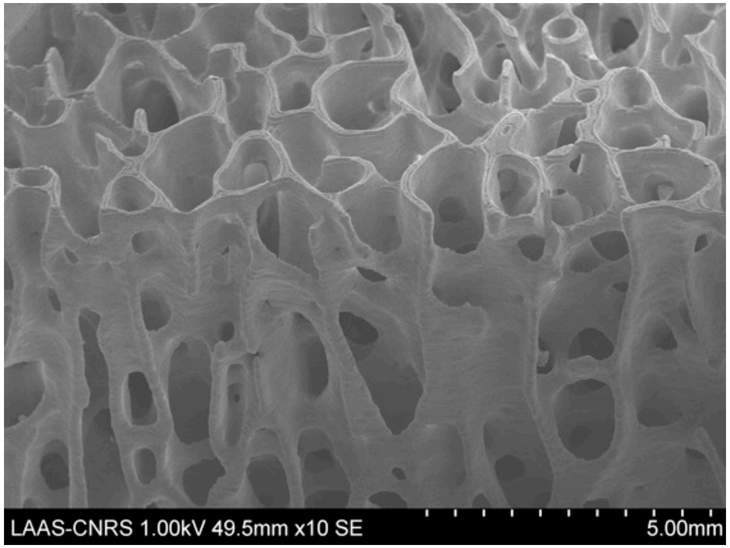


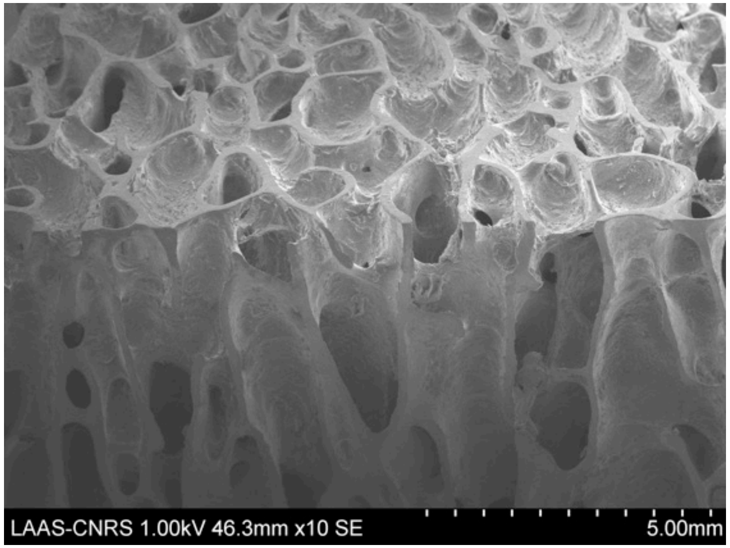


Echography for osteoporosis analysis (LAAS, Curie Inst., Langevin Inst.)

(1) Mézière, F.; Juskova, P.; Woittequand, J.; Muller, M.; Bossy, E.; Boistel, R.; Malaquin, L.; Derode, A. Experimental Observation of Ultrasound Fast and Slow Waves through Three-Dimensional Printed Trabecular Bone Phantoms. J. Acoust. Soc. Am. 2016, 139, EL13–EL18.

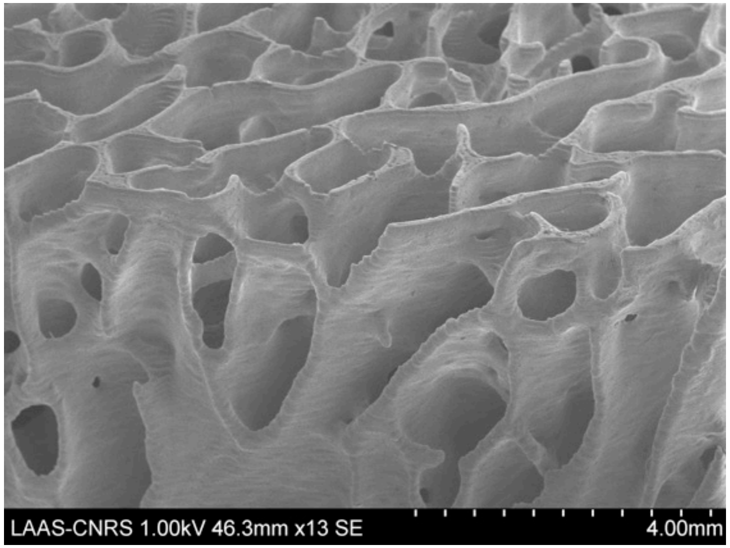
Fabrication of trabecular bones

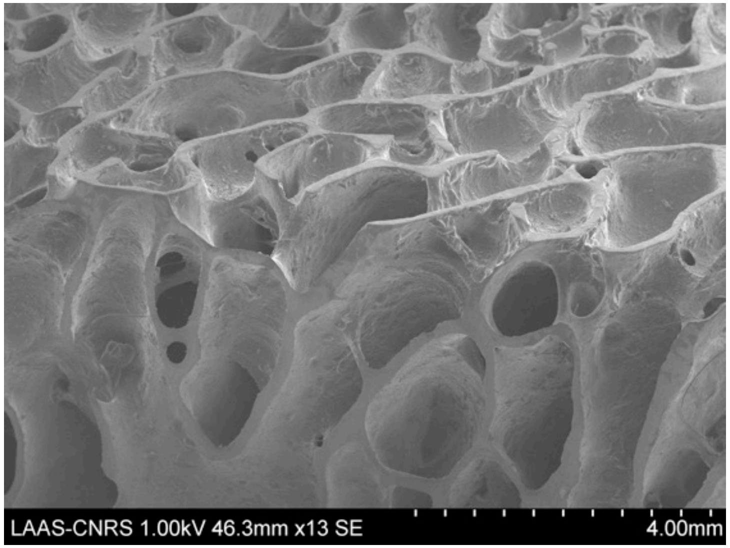




Bone’s phantom

Bone

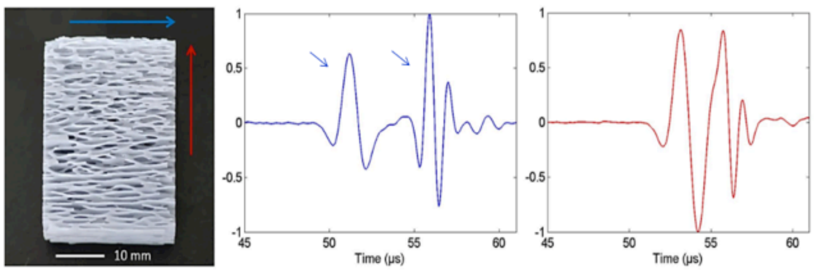




Fabrication of trabecular bones

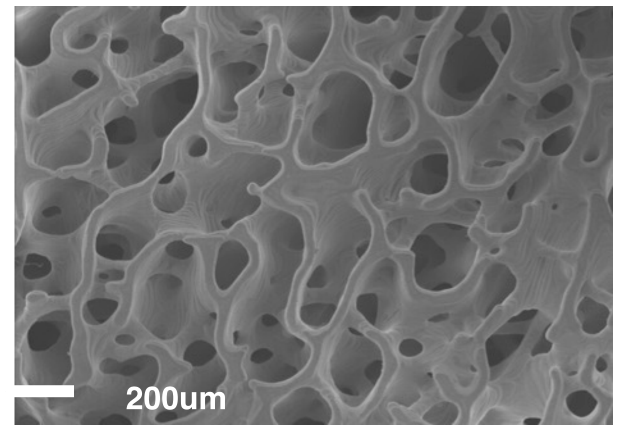
In vitro scaffolds for Mesenchylmal stem cell culture
(F. Deschaseaux, StromaLab Toulouse, France)

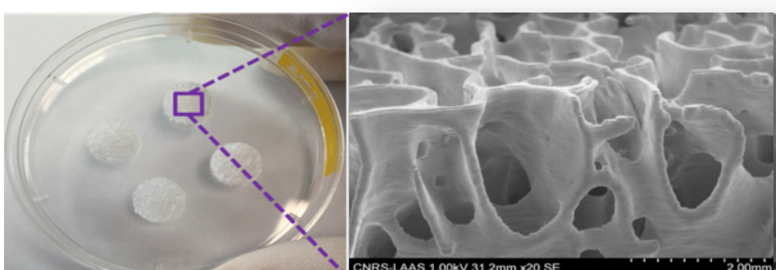
Modified structure : control ever the bone porosity



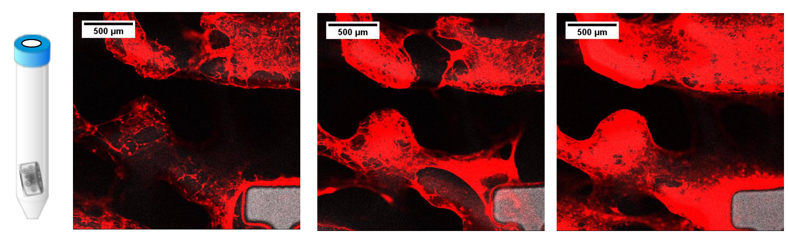
Printed elongated structure (left) and transmitted signals resulting from the propagation of a 1 MHz pulse along (middle) and across (right) the direction of anisotropy.

Sub-volume of the numerical structure was extracted and stretched four-fold along one dimension.
→ new structure: enhanced structural anisotropy, increased thickness along the stretched axis



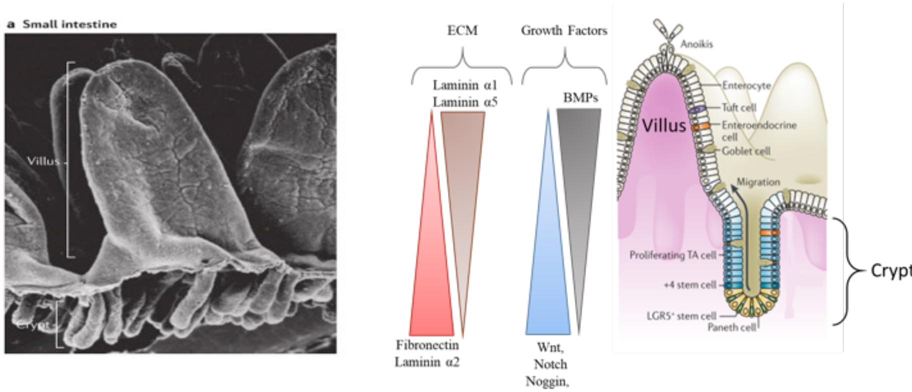


One week | Two weeks | Three weeks



MSCs inside the bone scaffolds with Cell trace YELLOW staining
Bone scaffold

In Vitro models of intestinal epithelium

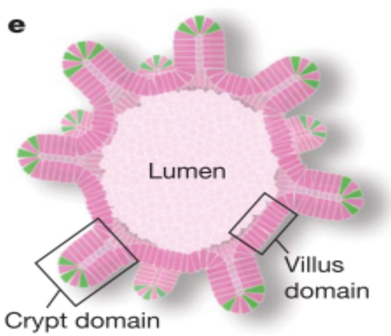


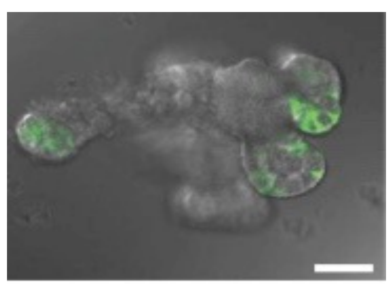
Van der Flier L.G. and Clevers H., Annue. Rev. Physiol, 2009
Tian H. et al, Nature, 2011, Clevers H., Cell, 2013, Barker N., Nature, 2014

Intestinal epithelium compartments

- Villi (differenciated and proliferating cells)
- Crypts (2 Stem Cell populations)

Finding alternative to Organoids or 2D cell culture → in vitro standard models



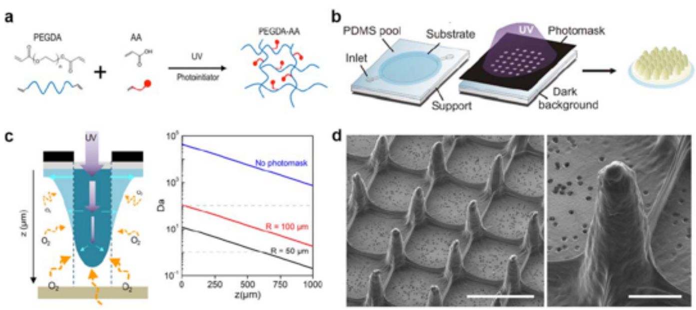


- Growth factor gradients (Wnt, BMP…)
- Biomechanical forces (rigidity, shear stress)
- Absence of co-culture
- Variability
- Matrigel dependent

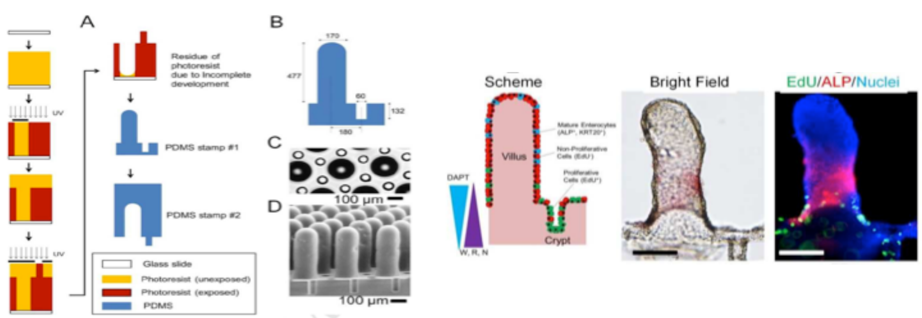
Collaborations :
A. Besson, J. Creff (PhD) CBI Toulouse
A. Ferrand, D. Hamel PhD) IRSD Toulouse
S. Descroix (Institut Curie) Paris

In Vitro models of intestinal epithelium

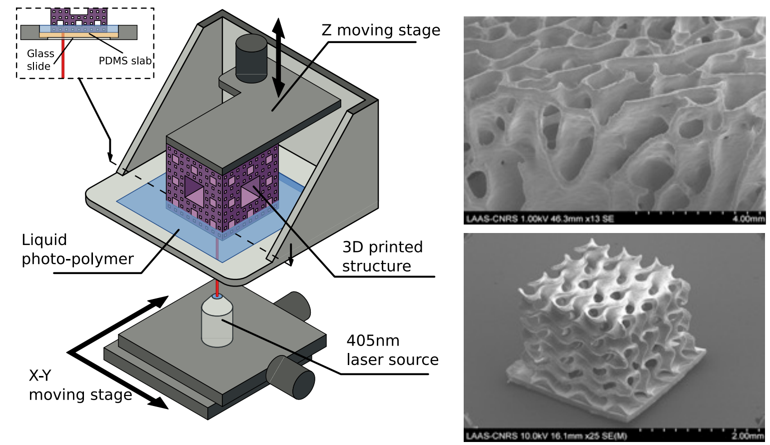
- Fabrication scaffold using molding or photolithography based approaches



Albert G Castaño et al., Biofabrication 2019, 11 (2)



Y. Wang et al. Biomaterials. 2017, 128 (44).



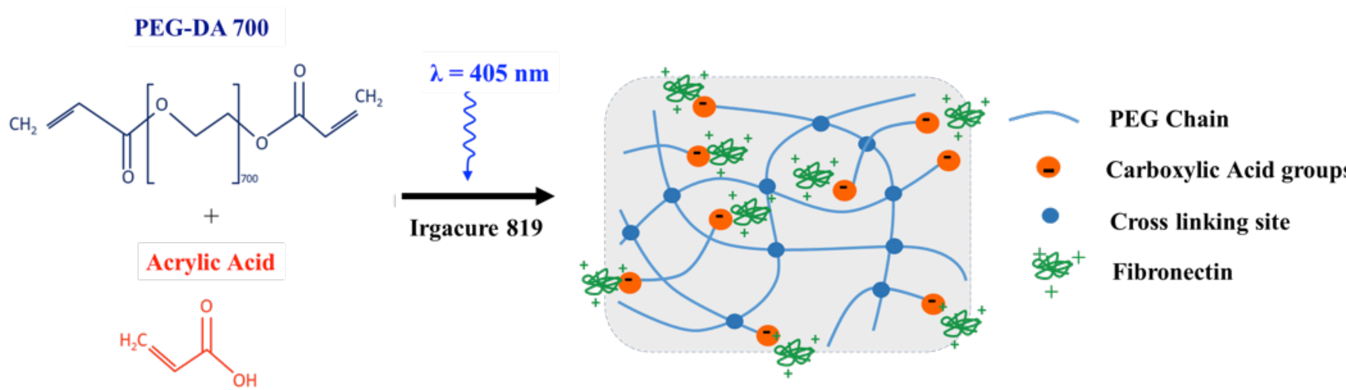
- 3D complex architecture
- Rapid prototyping / Flexibility
- Material ?
- Resolution ?
- Production throughput ?

A. Accardo, A.; et al. Addit. Manuf. 2018, 22, 440–446.

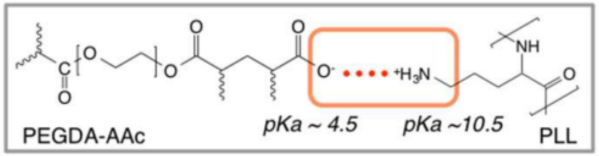
Developing a photosensitive hydrogel materials

PEG-DA:

- Biocompatible and photosensitive
- Controlled mechanical properties
- Optical transparency
- PEG-DA alone: very weak cellular adhesion → addition of acrylic acid

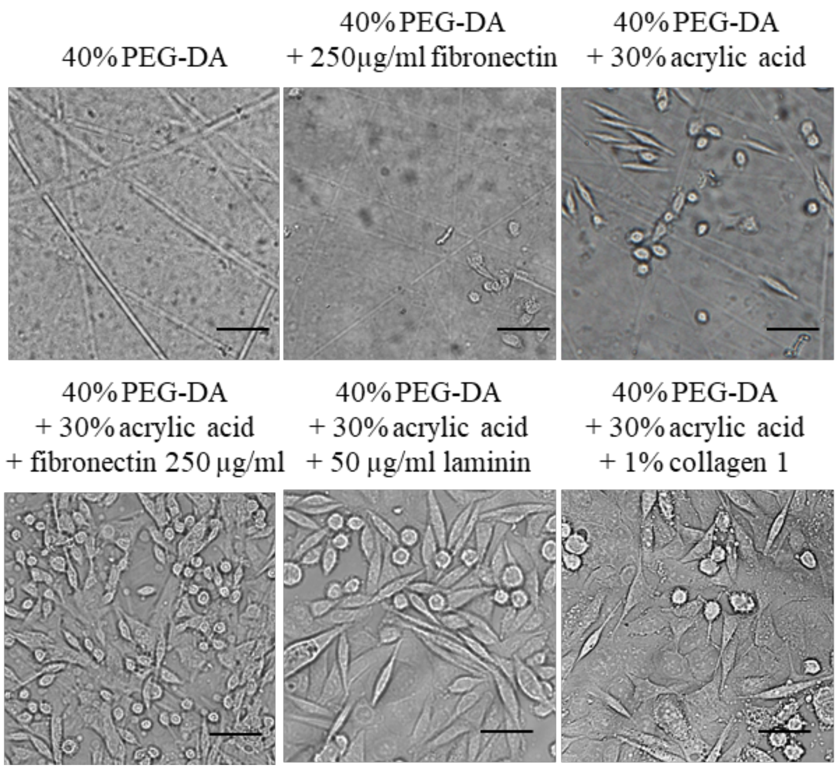


PEG-DA : poly(ethylene)glycol diacrylate
AA : Acrylic Acid
Irgacure 819 / LAP = photoinitiator



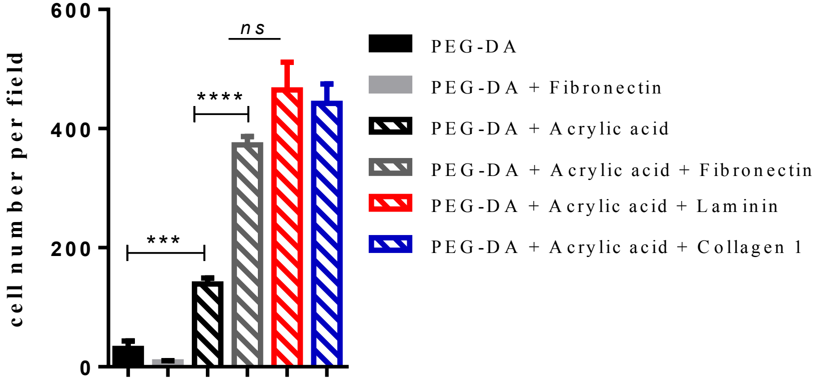
Chen L., Langmuir, 2015

Developing a photosensitive hydrogel material



Cell culture validation

- SW480/Caco2 colorectal cancer cells on 2D surfaces
- Caco 2 cell lines
- Optimum found for PEG-DA 700 at 40% (v/v) + 30% acrylic acid
- Cell morphology and number analysed at D6

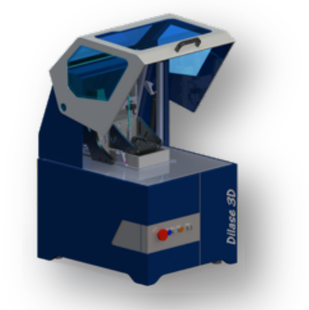


High resolution 3D printing with stereolithography

High-resolution 3D printing

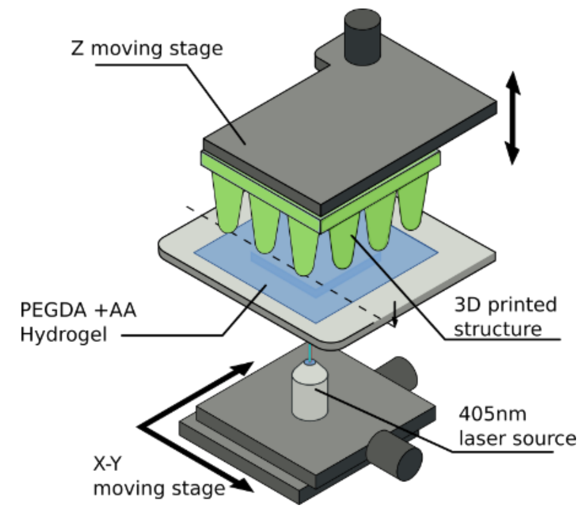
- Stereolithography
- Printer: Dilase 3D (LAAS-KLOE), additive fabrication
- Multi Resolution system

www.kloe.com
Montpellier , France

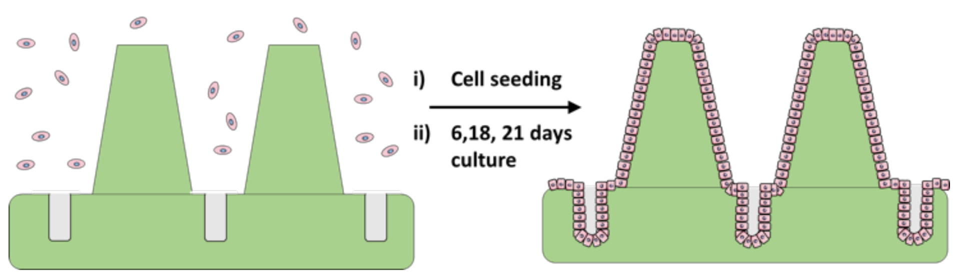


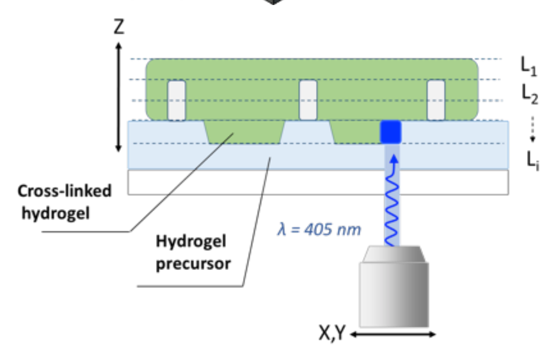
Dilase 3D (SLA):
- Resolution X,Y : 3 / 20-65 um
- Resolution Z : 5-100um
- Laser Wavelength : 375/405 nm (50 mW)
- Samples size (10 x 10 x 5 cm - X,Y,Z)
- Laser speed : 100mm/s
- Roughness < 2μm

Materials :
- UV–Vis photosensitive materials

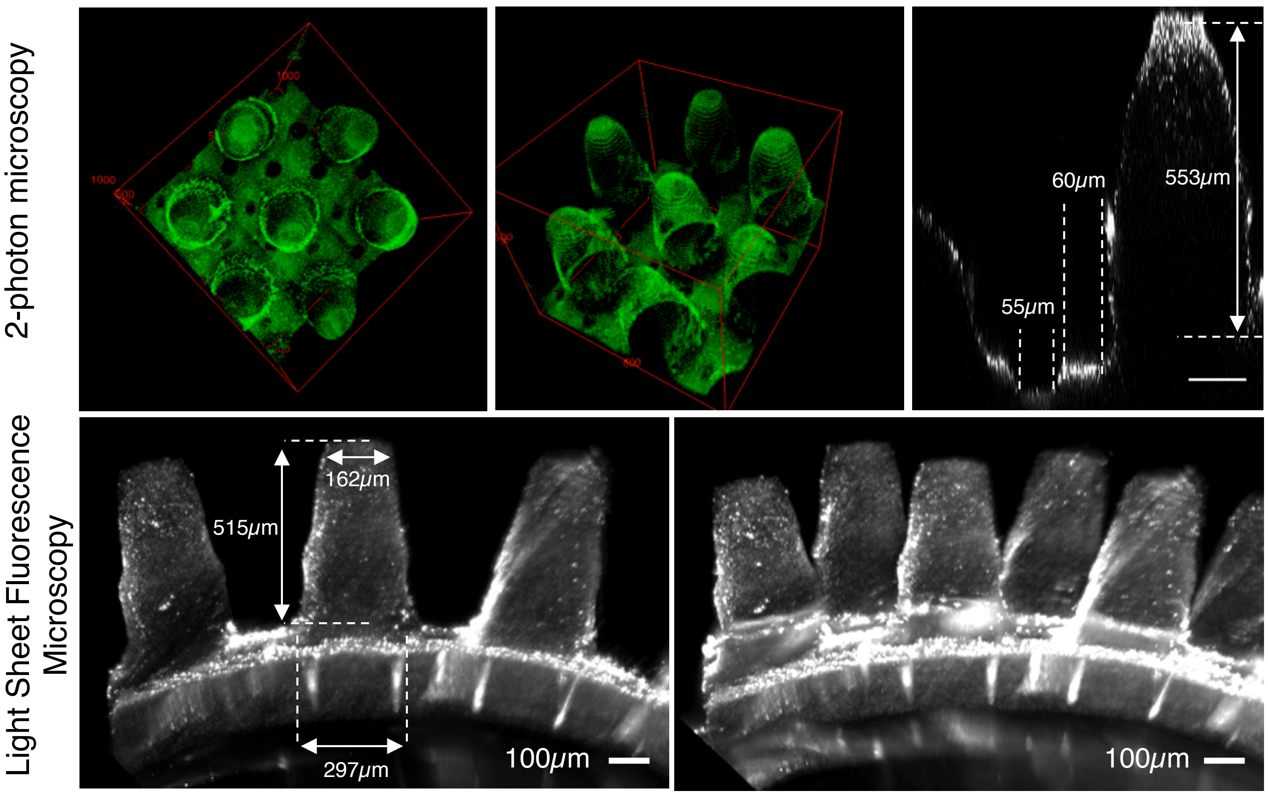


A. Accardo, A., et al. Addit. Manuf. 2018, 22, 440–446.



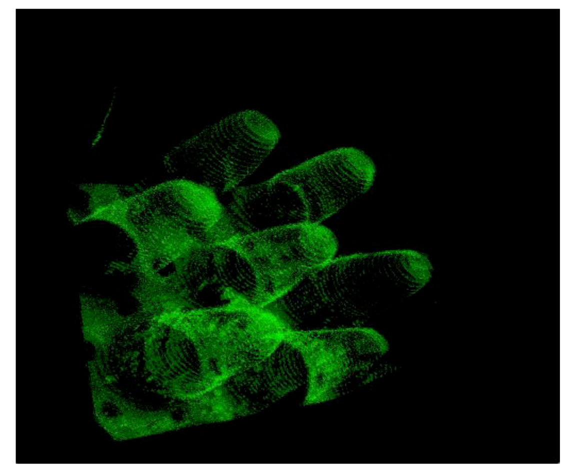


High resolution 3D printing with stereolithography



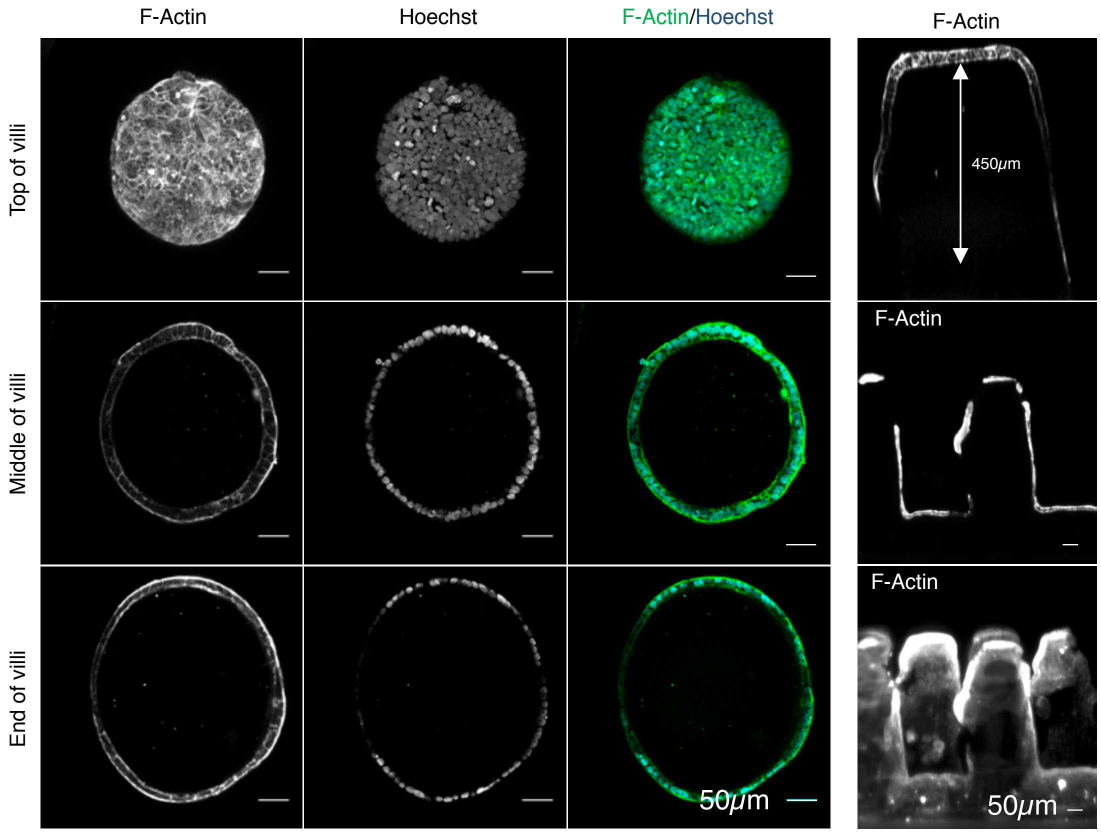
→ Slight swelling of the hydrogel material (10%)

→ Young modulus :90 ± 9 kPa for (40% PEGDA, 30% acrylic acid, 250 µg/mL fibronectin)

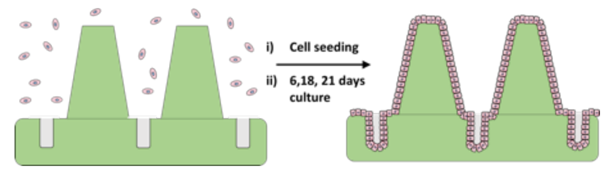


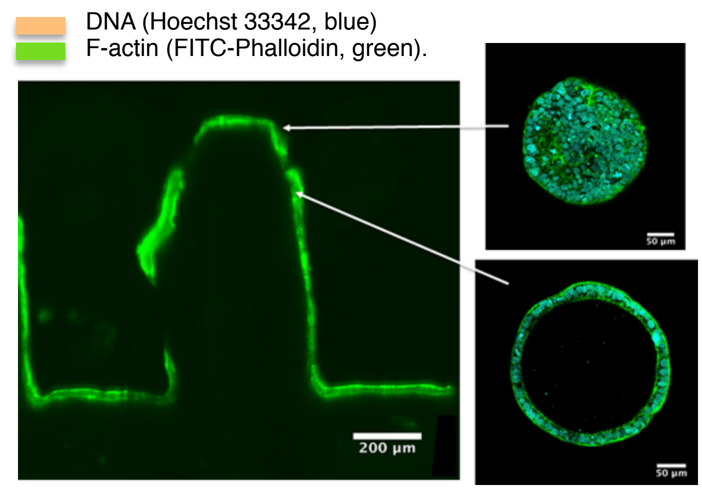
J. Creff, R. Courson et al . Biomaterials 2019, 221, 119404

In-vitro models of intestinal epithelium

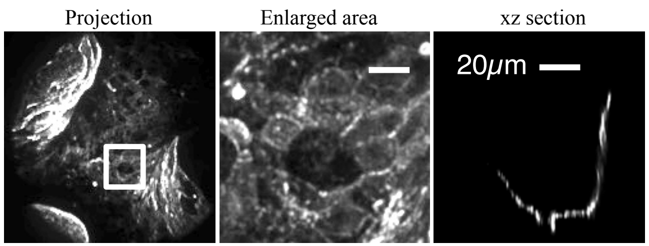


Caco2 cell line - 6 days of culture

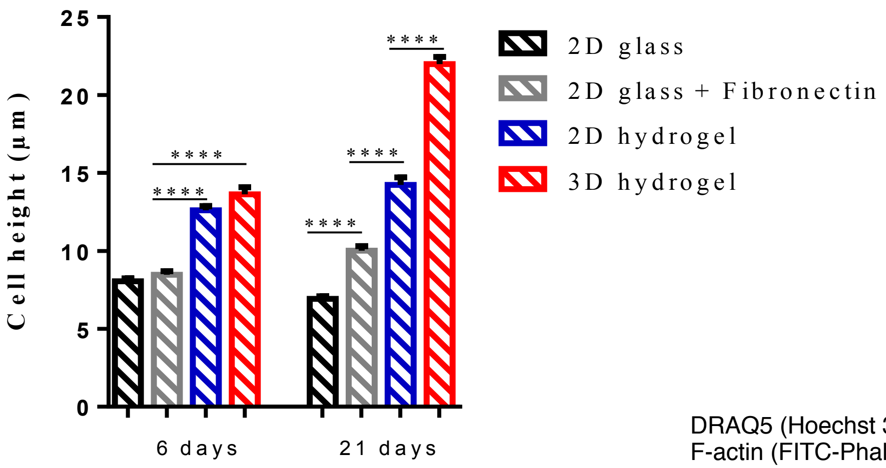


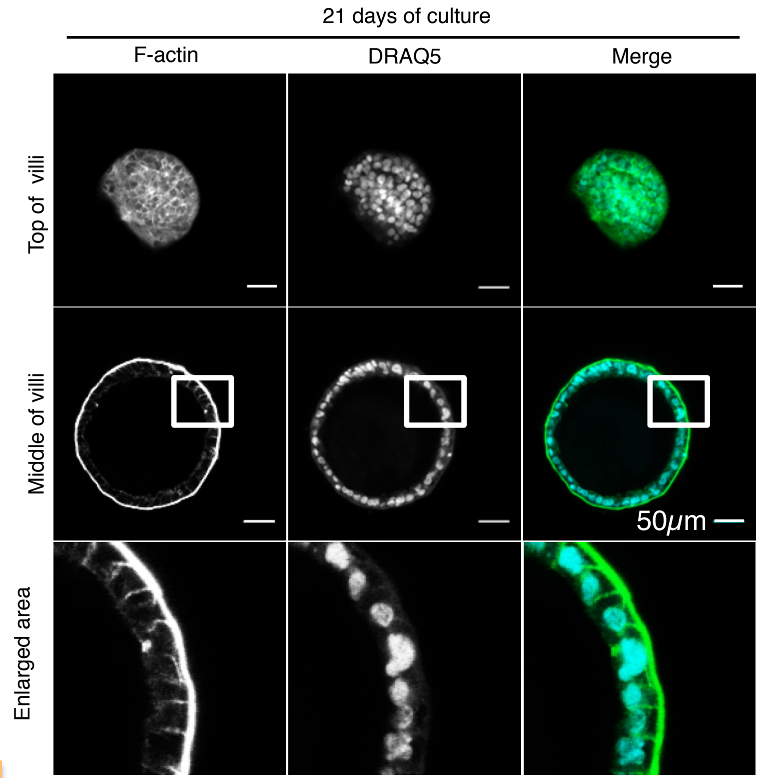


Influence of architecture on cell differentiation



→ Hydrogel materials promotes initiation of cell polarization
→ 3D architecture favors long term cell polarization





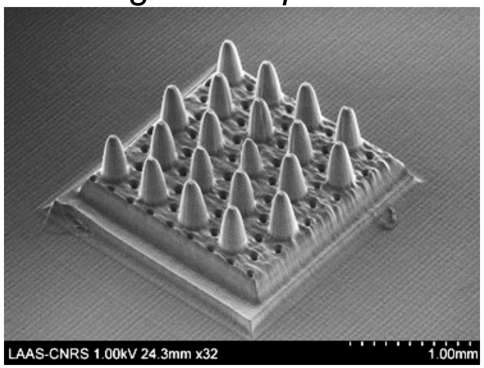
DRAQ5 (Hoechst 33342)
F-actin (FITC-Phalloidin)

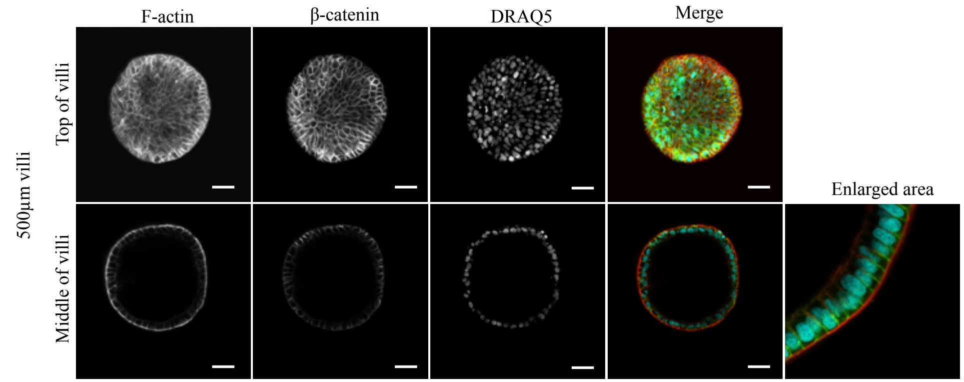
J. Creff, R. Courson et al . Biomaterials 2019, 221, 119404.

Influence of architecture on cell differentiation

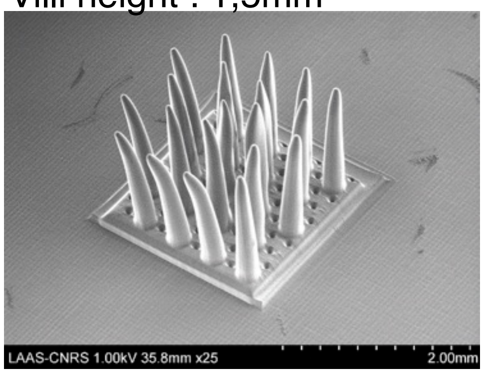
Caco2 cells , 21 days of culture

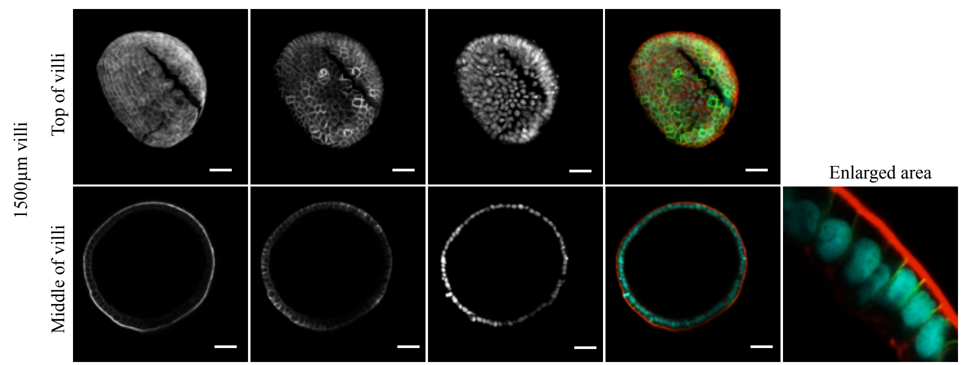
Villi height : 500 μm



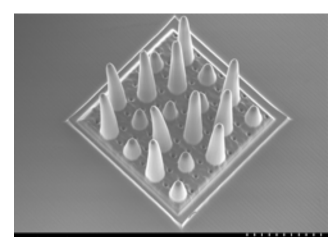


Villi height : 1,5mm





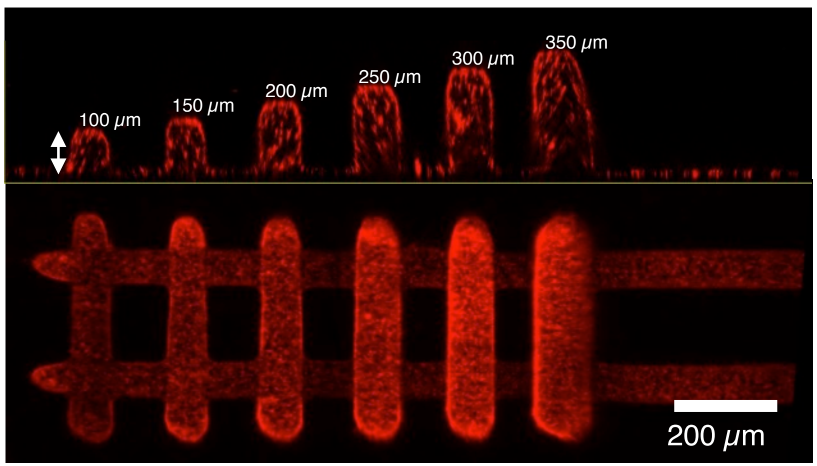
→ Similar results
→ Same differentiation at D21
→ Status at D3, D6, D12 ?

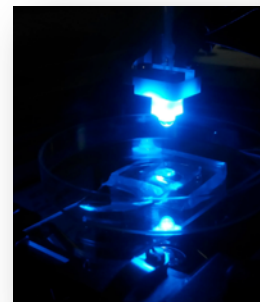


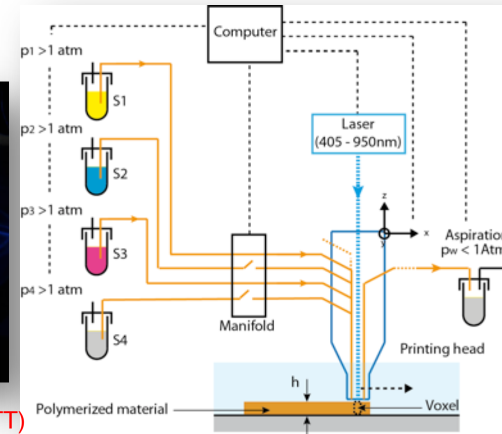
Multimaterial Printing with SLA

- **"LAMP" Light Assisted Microfluidic Printing (patent pending)**

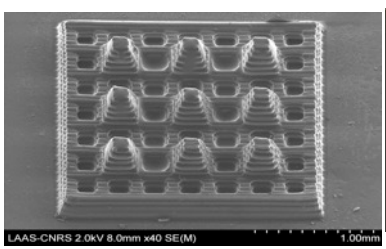
S Assié Souleille , J. Foncy , L. Boyer, X. Dollat, J.L. Viovy

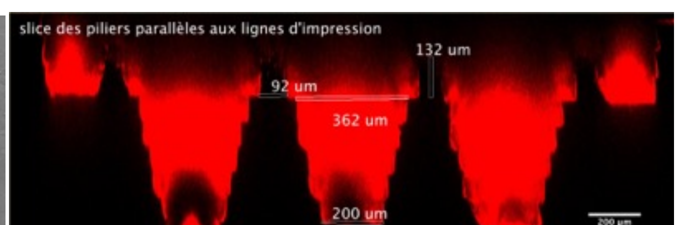






Patent filed (LAAS CNRS – TTT)

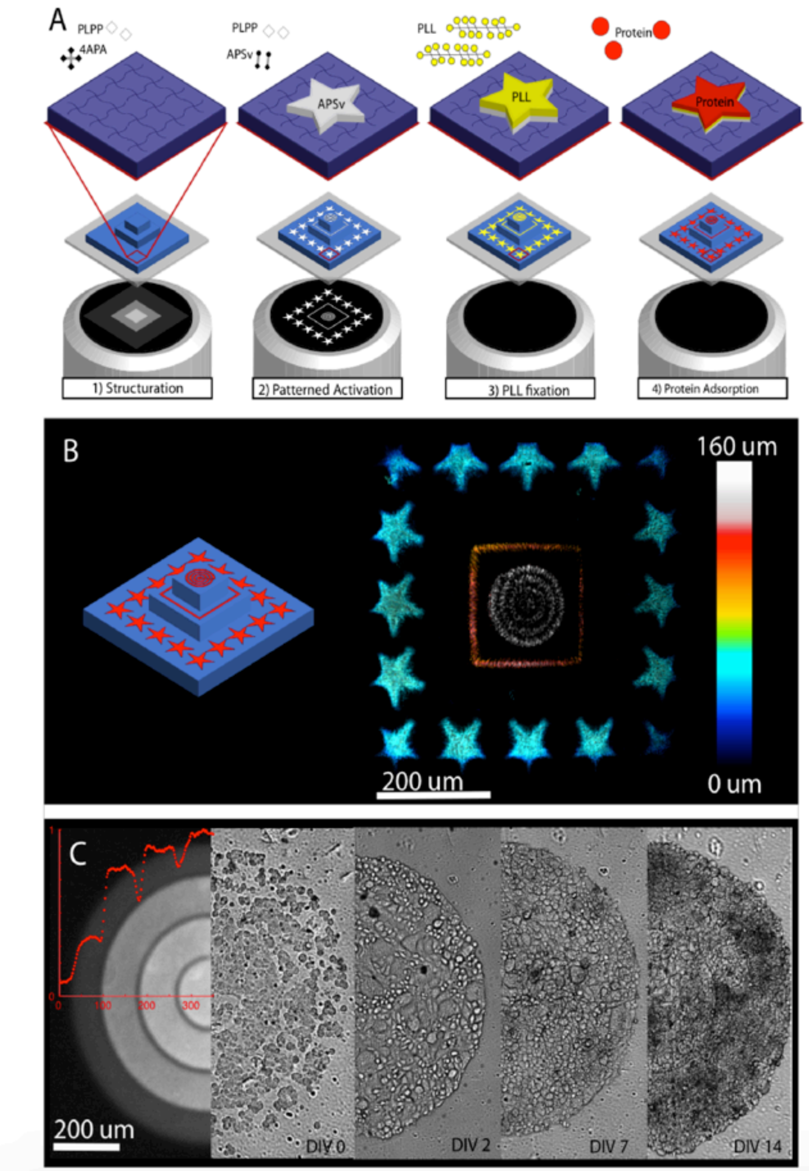




Technical specifications :

- Multimaterial (Microfluidic injection)
- Matrix or Cell printing
- Targeted Resolution X,Y : 20um
- Targeted Resolution Z : 5-500um
- Laser Wavelength : 405 nm (50 mW)
- Samples size (10 x 10 x 5 cm - X,Y,Z)
- Targeted Speed : 0,5mm^3 /min

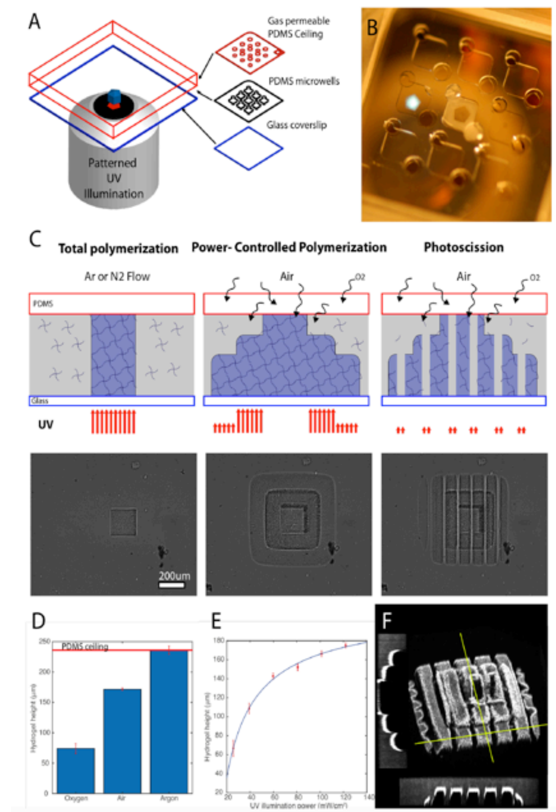
Multimaterial printing



A generic widefield topographical and chemical photopatterning method for hydrogels.

Aurélien Pasturel[1,2,3], Pierre-Olivier Strale[3], Vincent Studer[1,2,*]

[1] University of Bordeaux, Interdisciplinary Institute for Neuroscience, Bordeaux, France.
[2] CNRS UMR 5297, F-33000 Bordeaux, France.
[3] ALVEOLE, Paris, France.
*vincent.studer@u-bordeaux.fr



Bioprinting tissues … what's next ?

Box 2 Next steps for 3D bioprinting

For 3D bioprinting to realize its potential, advances are needed in several aspects of the technology and in our understanding of the biology and biophysics underlying regenerative processes *in vivo*. **Table 2** details some of the specific areas where further research is needed.

Table 2 Issues to be addressed

Area	Focus for future research
Bioprinter technology	Compatible with physiologically relevant materials and cells Increased resolution and speed Scale up for commercial applications Combining bioprinter technologies to overcome technical challenges
Biomaterials	Complex combinations or gradients to achieve desired functional, mechanical and supportive properties Modified or designed to facilitate bioprinter deposition, while also exhibiting desired postprinting properties Use of decellularized tissue-specific ECM scaffolds to study ECM compositions, and/or as printable material
Cell sources	Well-characterized and reproducible source of cells required Combinations of cell phenotypes with specific functions Greater understanding required of the heterogeneous cell types present in the tissues Direct control over cell proliferation and differentiation with small molecules or other factors
Vascularization	Well-developed vascular tree required for large tissues May have to be engineered in the bioprinted construct Capillaries and microvessels required for tissue perfusion Suitable mechanical properties for physiological pressures and for surgical connection
Innervation	Innervation is required for normal tissue function May be inducible after transplantation using pharmacologic or growth factor signaling Simulation before transplantation could be achieved using bioreactors
Maturation	Time required for assembly and maturation Bioreactors may be used to maintain tissues *in vitro* Provide maturation factors as well as physiological stressors Potential for preimplantation testing of constructs

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature biotechnology

Towards 4D printing

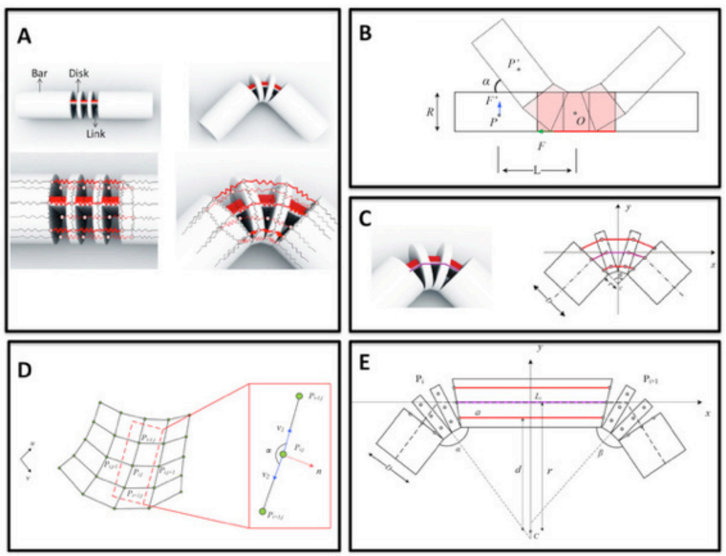
Active Printed Materials for Complex Self-Evolving Deformations

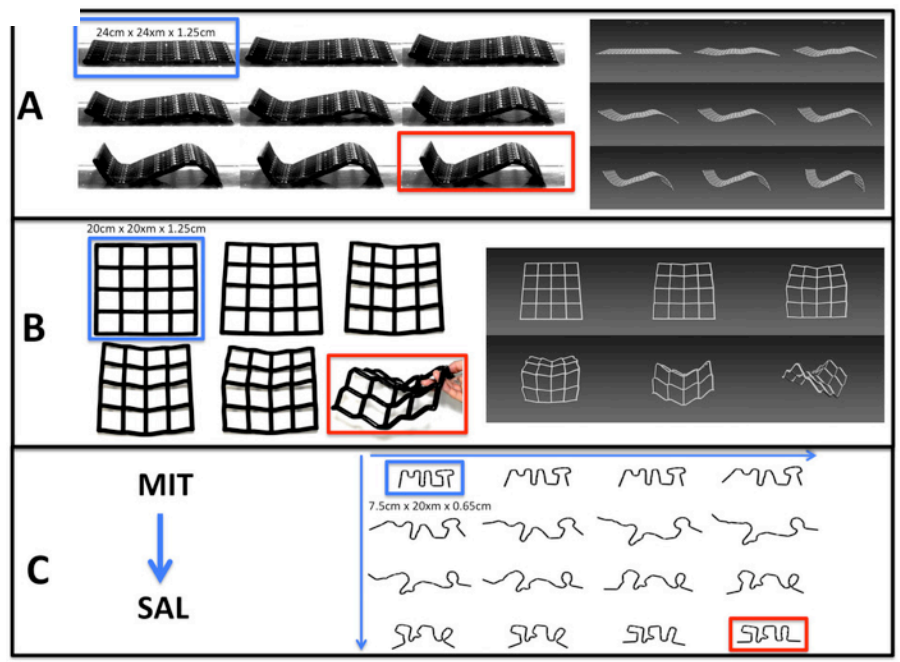
Dan Raviv, Wei Zhao, Carrie McKnelly, Athina Papadopoulou, Achuta Kadambi, Boxin Shi, Shai Hirsch, Daniel Dikovsky, Michael Zyracki, Carlos Olguin, Ramesh Raskar & Skylar Tibbits

Affiliations | Contributions | Corresponding author

Scientific Reports **4**, Article number: 7422 | doi:10.1038/srep07422

4 ing movie

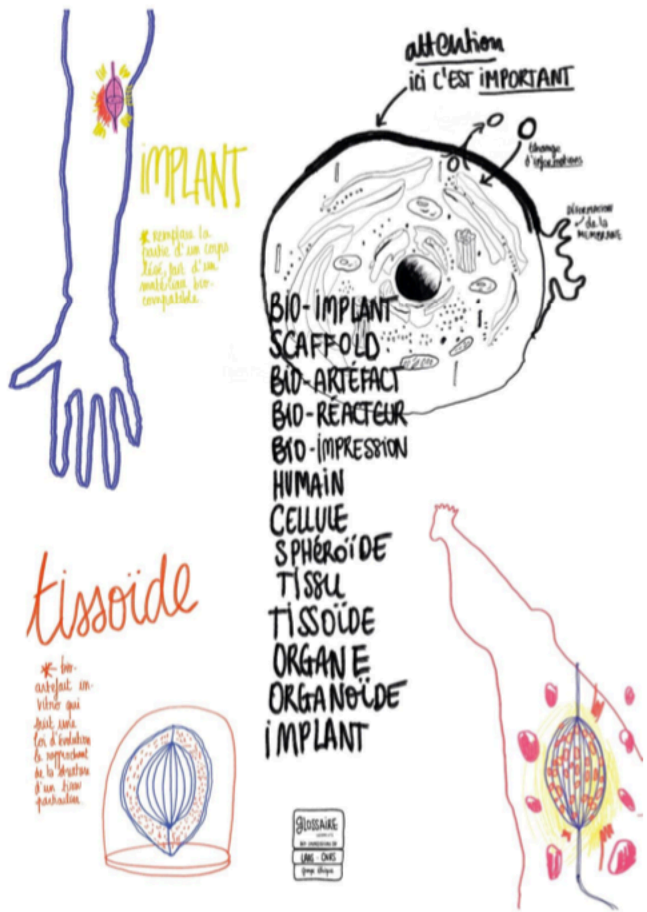




BIO-PRINT WORDS MATTERS

BPWM - *projet de glossaire collaboratif autour des pratiques de bio-impression 3D*

Elise Rigot PhD thesis
erigot@laas.fr



Poster et livret : glossaire, outil pour définir collectivement les pratiques de bio-impression 3D, A3, Groupe Ethique du LAAS, juin 2019

Deux constats

Un : Un foisonnement de termes dans les publications scientifiques aux usages qui diffèrent

Deux : L'imaginaire de l'humain augmenté comme (seul) horizon de la bio-impression 3D

Acknowledgments

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<u>**R. Courson**</u>
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<u>**L. Boyer**</u>
<u>**V. Conedera**</u>
<u>**F. Mesnilgrente**</u>
<u>**L. Bary**</u>
<u>**H. Granier**</u>
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C. Villard

MULTIFAB Platform (LAAS CNRS)
https://www.laas.fr/projects/MultiFAB/

ITAV
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J. Rouquette

micro resist technology
M. Vogler
M. Thesen

CBI Centre de Biologie Intégrative - Toulouse
A. Besson
T. Mangeot

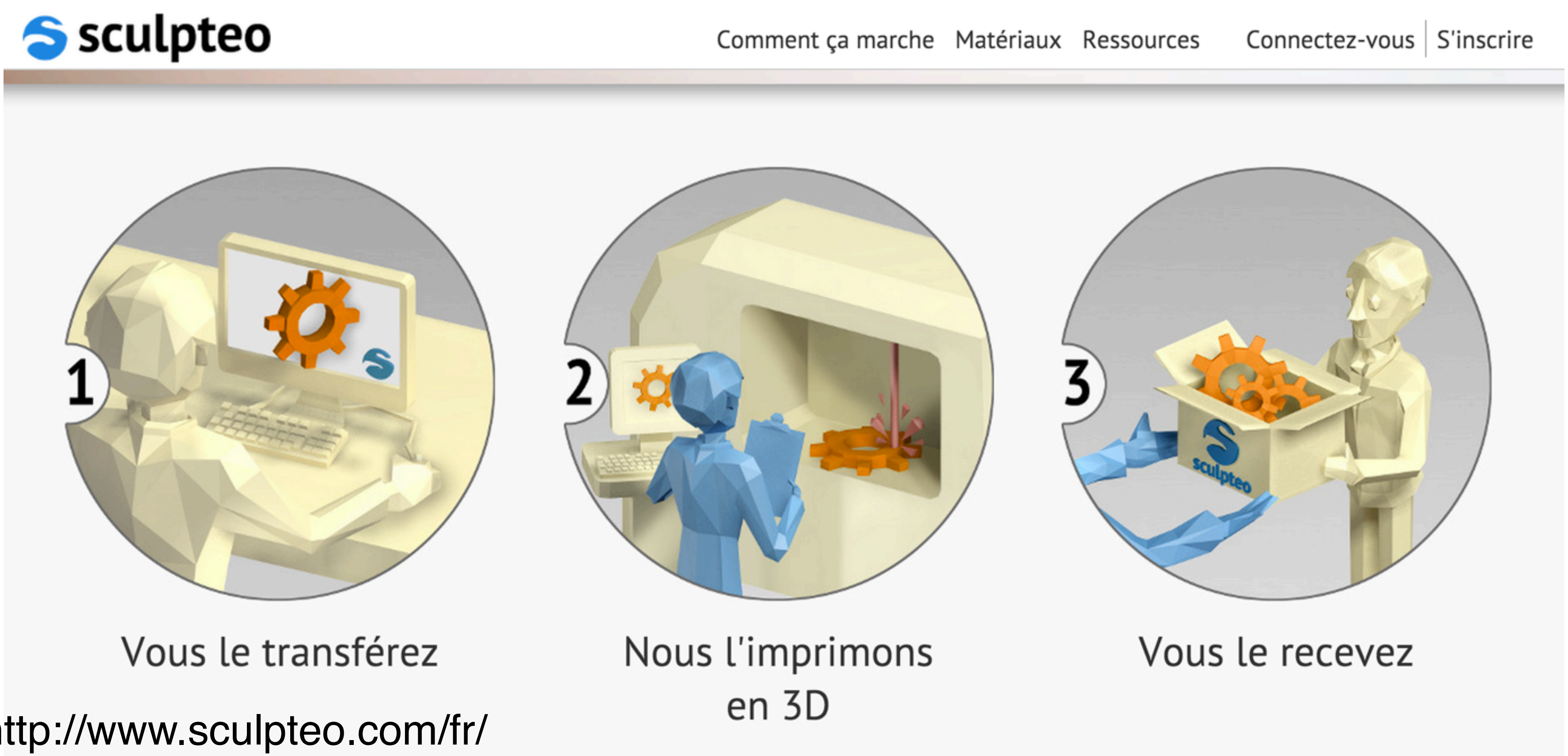
UNION EUROPÉENNE
La Région Occitanie Pyrénées - Méditerranée
INSTITUT CARNOT CIRIMAT

HORIZON 2020 HoliFAB
European Commission
HOLIFAB project

RENATECH French national nanofabrication network
ANR
cnrs

Thank you !

3D printing … where ?



http://www.sculpteo.com/fr/

<u>Many providers available !!</u>