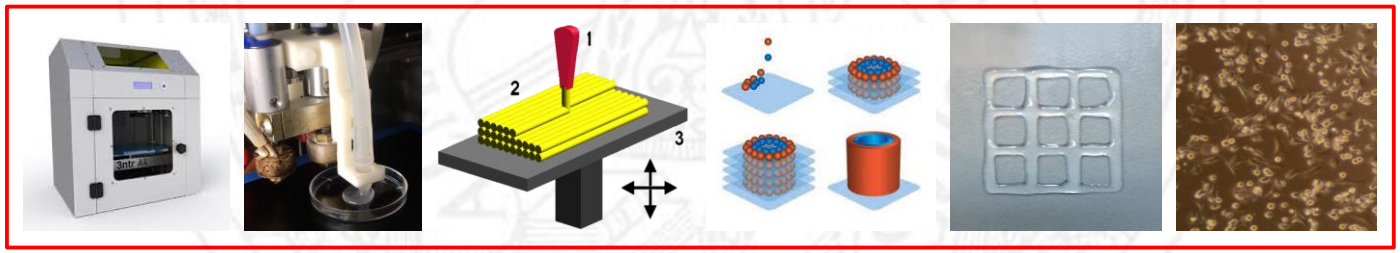
3D@UniPV
Virtual Modeling and Additive Manufacturing for Advanced Materials

CHT Centre for Health Technologies University of Pavia

FONDAZIONE ISTITUTO NEUROLOGICO NAZIONALE "CASIMIRO MONDINO"

3ntr INDUSTRIAL GRADE 3D PRINTERS

Customizzazione di una stampante 3D FDM per l'erogazione di alginato di sodio per bioink



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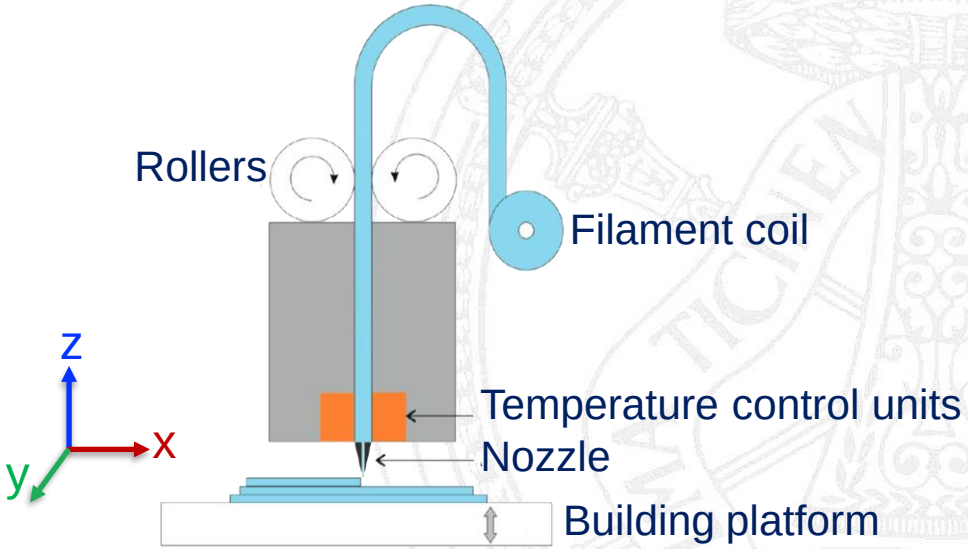
CompMech Group

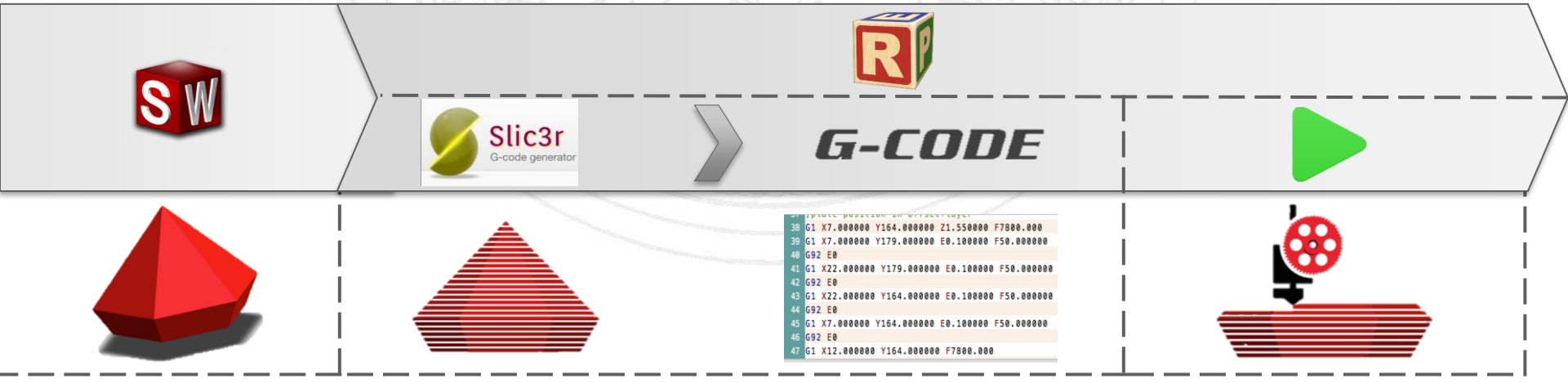
FDM 3D printer: why?

Fused Deposition Modeling is one of the **most common** rapid **prototyping process**

- **Layer by layer** deposition



- **Features**
  - Simple process
  - Economy
  - Create complex objects
  - Using multi-material
  - Simple to adapt



From FDM towards Bioprinting

3D printing material

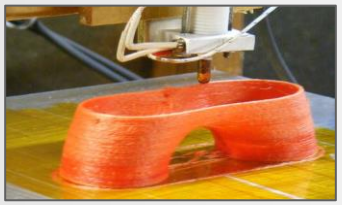
- ✓ Thermoplastic polymer
- ✓ Biocompatible polymer
- ✓ Elastomer
- ✗ High viscous material like **hydrogel** or **silicon**
  - **3D networks**
  - **Biocompatible**
  - **Encapsulate** biological components
  - **Biomedical** and tissue engineering **application**

Bioprinting

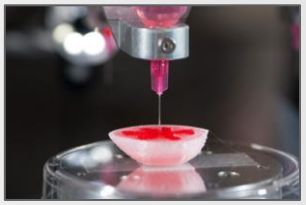
- ✓ Hot technology trend
- ✗ Cytotoxicity
- ✗ Bioplotter versatility and availability

Goal

Customize a commercial **FDM 3D printer** to extrude **hydrogel** suitable for **bioink** and **Bioprinting purpose**

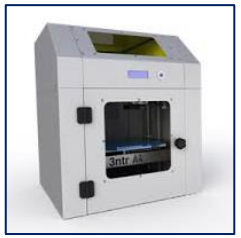


FDM **Bioprinting**

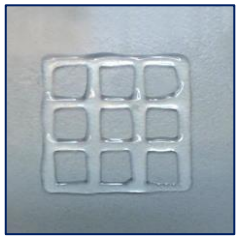


Summary

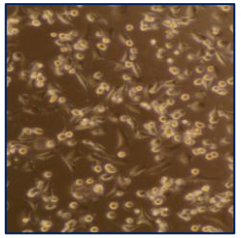
- **FDM printer customization**



- **Sodium alginate extrusion**



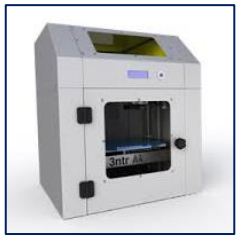
- **Bioink extrusion: proliferation tests**



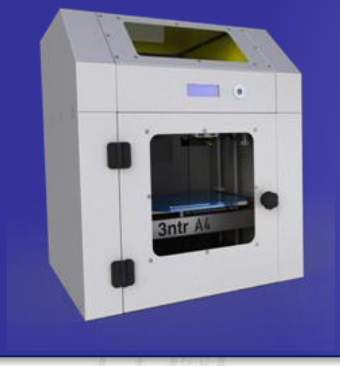
- **Conclusion and future developments**

Summary

- **FDM printer customization**
- Sodium alginate extrusion
- Bioink extrusion: proliferation tests
- Conclusion and future developments



FDM printer customization



Customized 3ntr A4v2 FDM printer to extrude hydrogel

Hardware modification

Elements set-up detection

- *Peristaltic pump*
- *Silicon tube*
- *Preloaded Syringe*
- *Nozzle*
- *Adapter*

Software implementation

MATLAB function implementation to generate simple and monolayer shape and G-code

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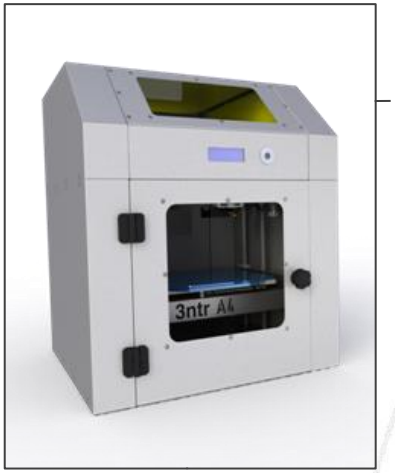
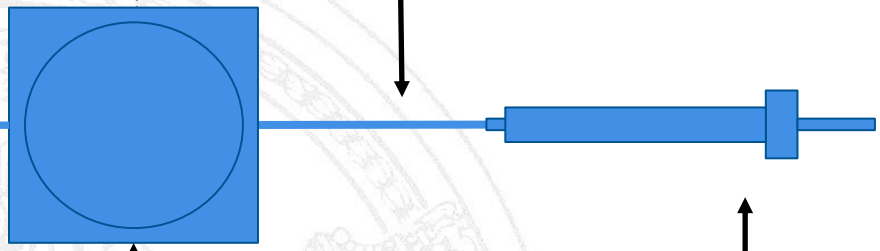
INDUSTRIAL GRADE 3D PRINTERS

Hardware modification

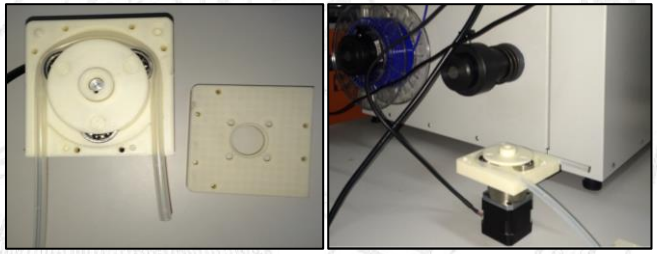
3ntr A4v2



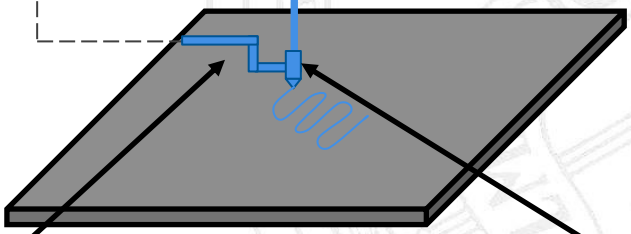
b) Silicon tube



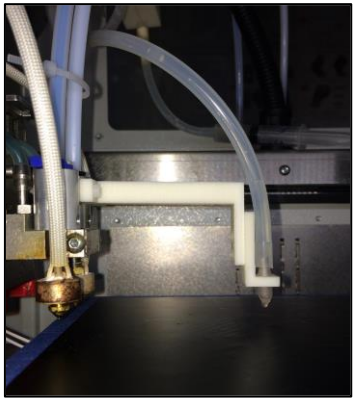
a) Peristaltic pump



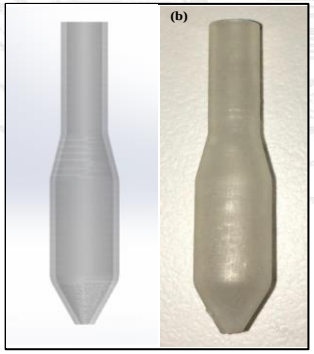
c) Preloaded syringe



e) Adapter



d) Nozzle



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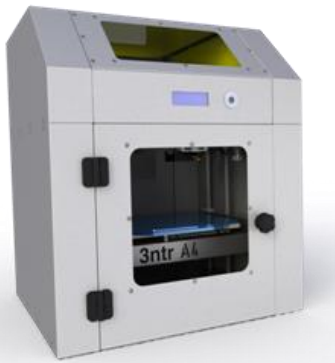
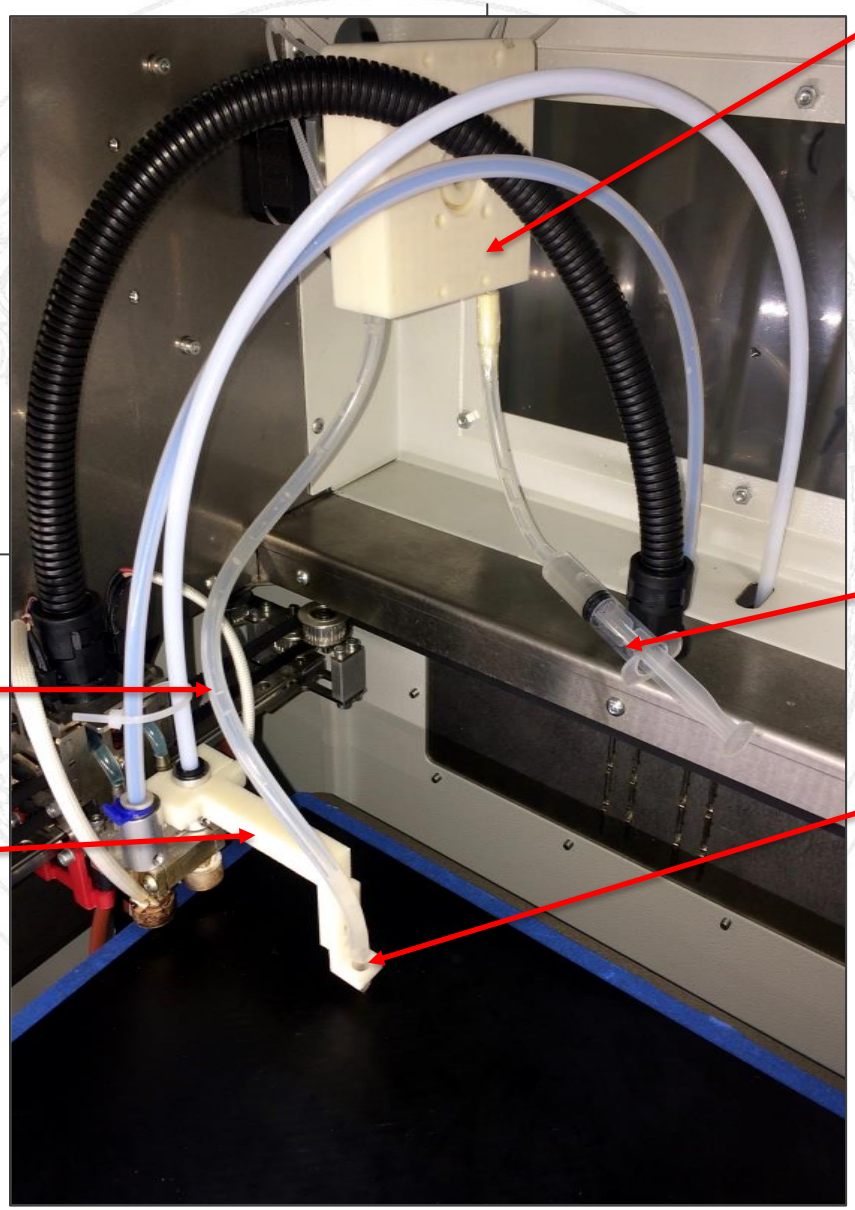
Set-up overview

3ntr A4v2



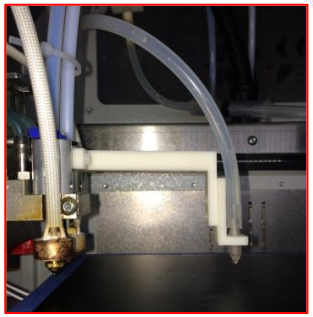


Peristaltic pump

Preloaded syringe

Silicon tube

Adapter



Nozzle

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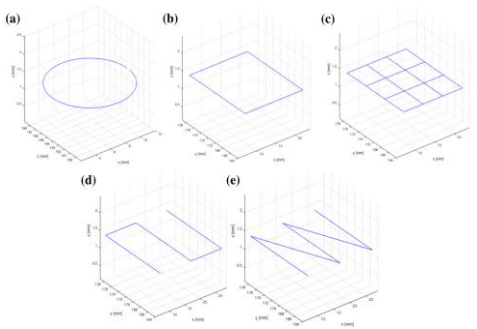
Software implementation

Functions implemented to realize given monolayer shape and G-code

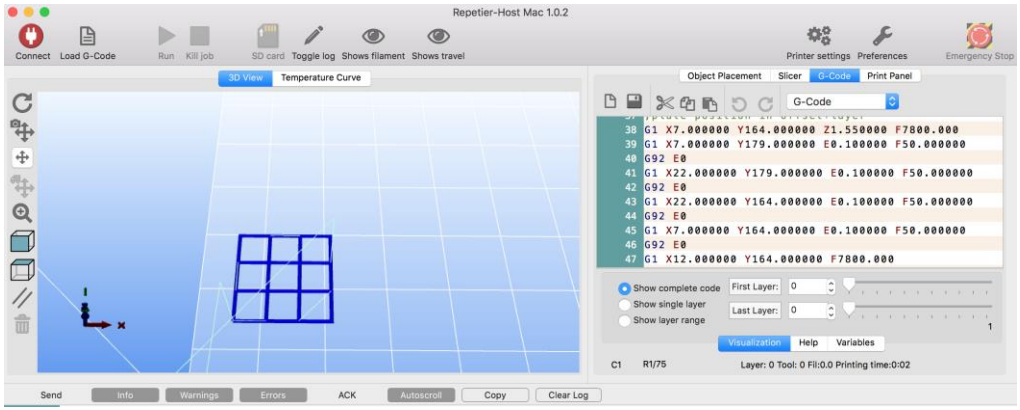
- Object **position**
- Geometry **size**
- **Equivalent filament** to be **extruded** for each change of coordinate mm (E)
- **Feed rate** mm/min (F)



GcodeBioprinting.txt

```
M107
;init gcode
;[mm] mode
G21
;fan off
M107
;absolute mode
G90
;homing
G28 Z0
;relative mode to avoid collisions when homing
G91
;increase z clearance (15 mm)
G92 X1 Y150 F120000
G1 Z15
;absolute mode
G90
;move y e x in Petri position
G1 X10.000000 Y160.000000
G1 Z1.300000
;set extrude coordinate to 0
G92 E0
```

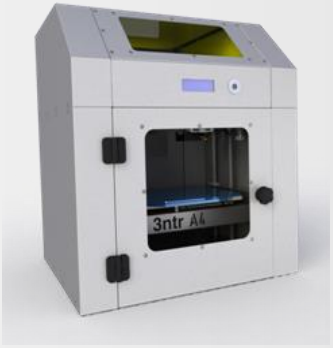
1. Setting parameters
2. Choose shape
3. Generate G-code



4. Load G-code and start printing

Summary

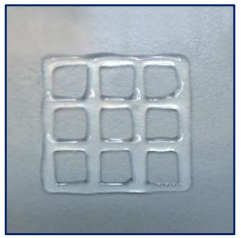
✓ **Customized 3ntr A4v2 FDM printer to extrude hydrogel**



- **Hardware modification**
- Set-up FDM printer assembling components
- **Software implementation**
- MATLAB functions implementation to generate monolayer shape and g-code

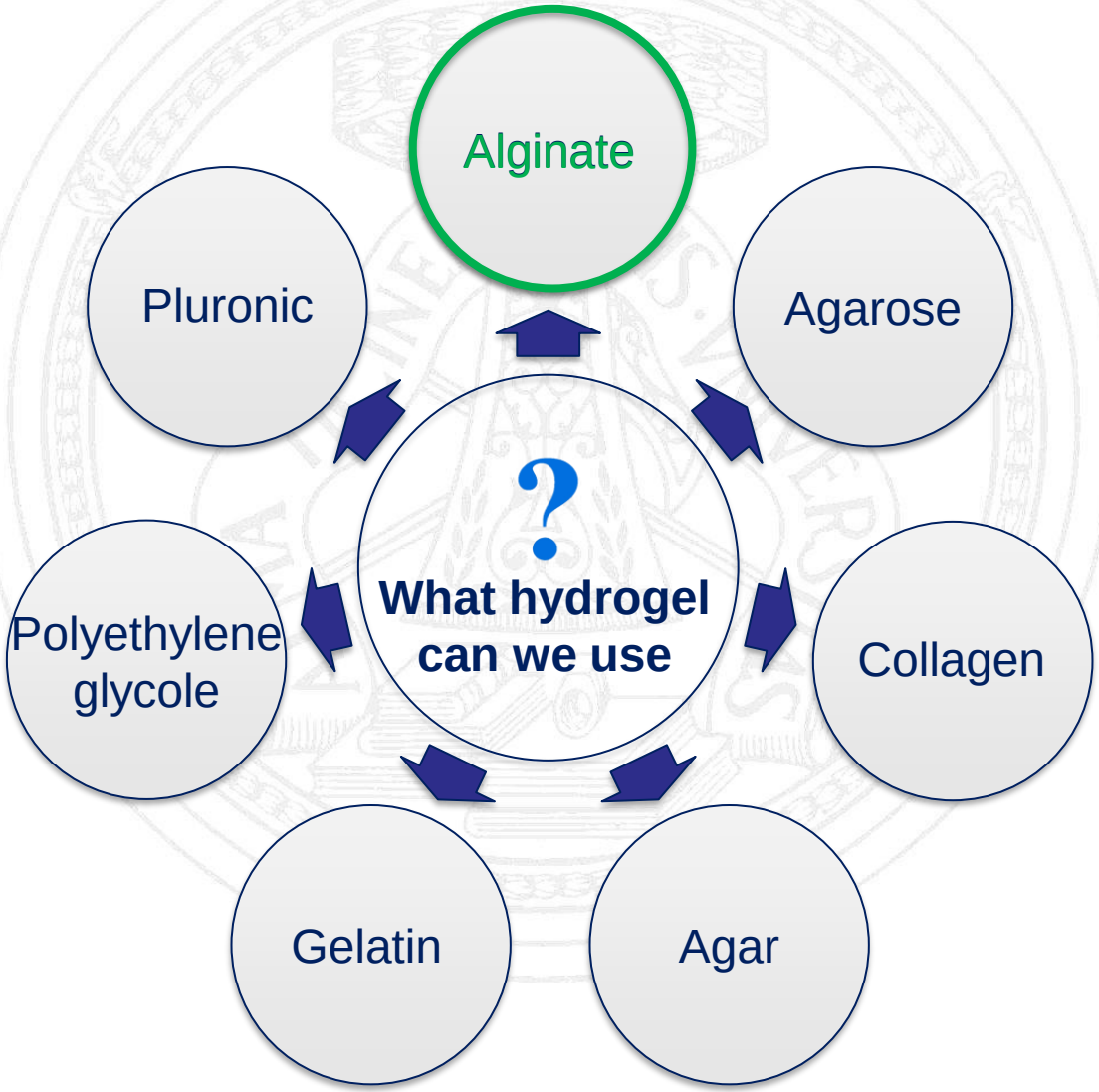
Summary

- FDM printer customization
- **Sodium alginate extrusion**
- Bioink extrusion: proliferation tests
- Conclusion and future developments



Hydrogel: what?

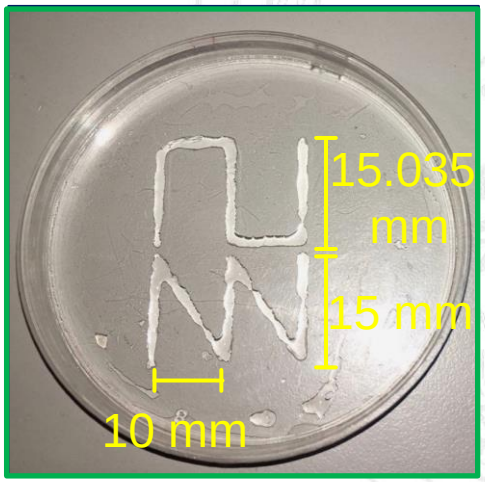
Modify FDM printer to **extrude hydrogel** for Bioprinting purpose

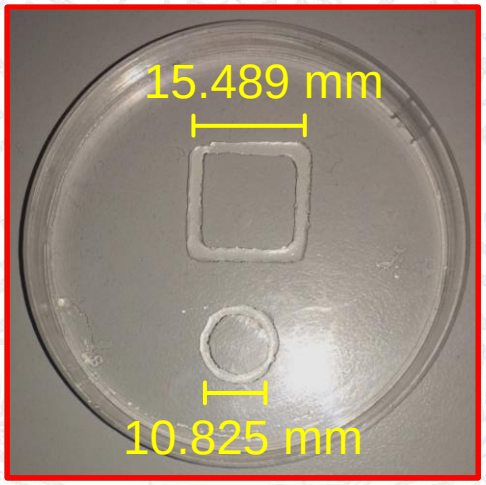


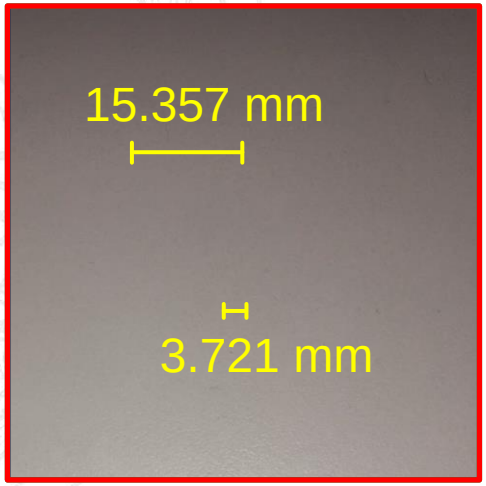
First experience: alginate extrusion

2% sodium **alginate** geometries extruded to evaluate printability:
fixed E and F at 0.2 mm and 90 mm/min

- Stripes (linear or oblique connection)
  - Length: 15 mm
  - Distance: 10 mm
- Circle
  - Diameter: 10 mm
- Square
  - Side: 15 mm
- Grid
  - Side: 15x15 mm
  - Cell: 5x5 mm







✓ Good **shape** accuracy.

✗ No perfect **size** accuracy. → **Increase** sodium alginate **concentration**

✗ **Air bubbles** during printing. → **Remove** before printing

Resolution and repeatability tests

2%, **4%** and **6%** sodium **alginate** geometries extruded using customized FDM printer to evaluate**:**

Resolution

Extrude different size standard geometries

- *Circle*
- *Square*
- *Grid*

Repeatability

Extrude 5 times standard geometries

- *Circle*
- *Square*
- *Grid*

Resolution and reapatability tests

2%, **4%** and **6%** sodium **alginate** geometries extruded using customized FDM printer to evaluate**:**

Resolution

Extrude different size standard geometries

- *Circle*
- *Square*
- *Grid*

Repeatability

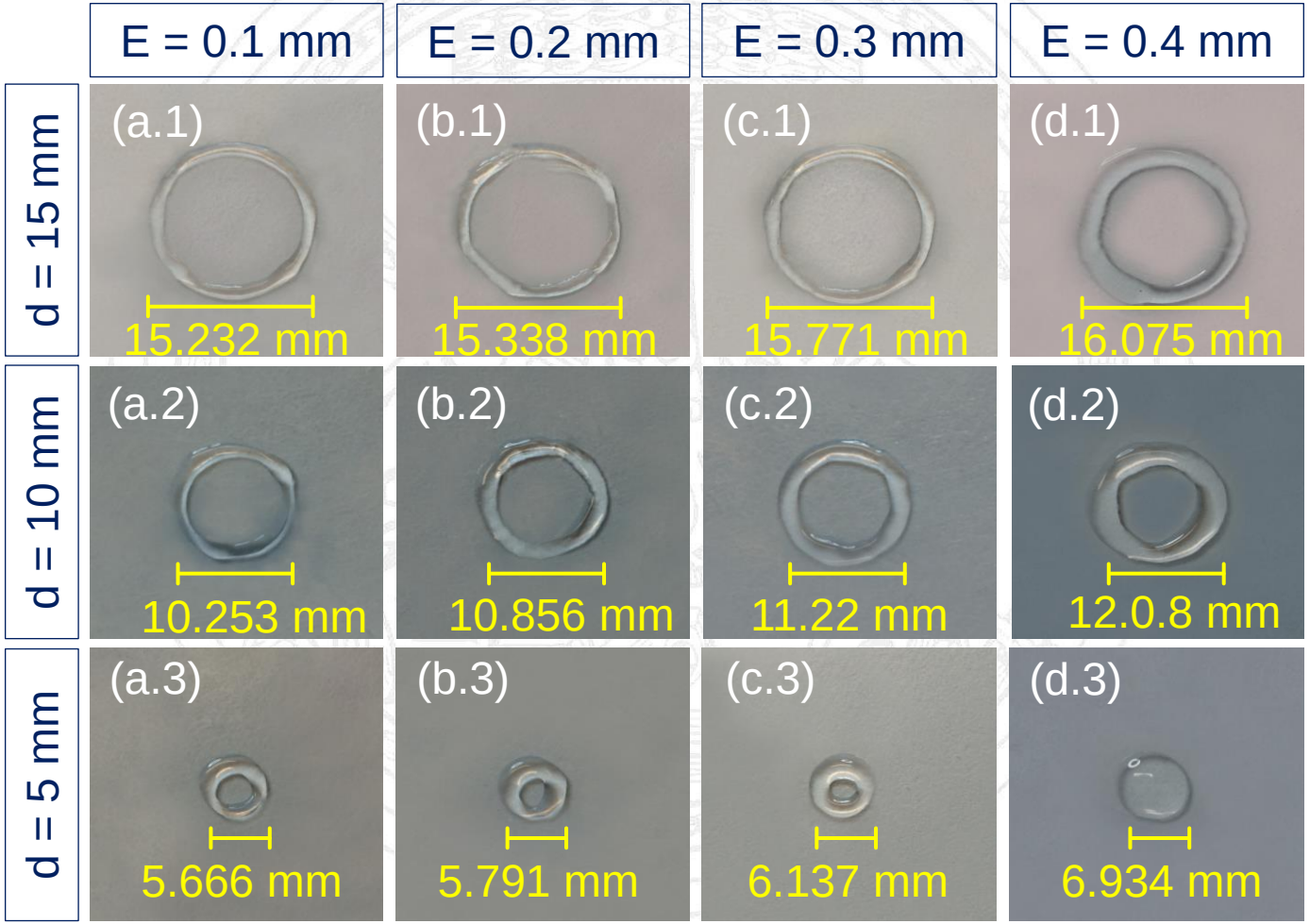
Extrude 5 times standard geometries

- *Circle*
- *Square*
- *Grid*

Resolution test: results I

2% sodium **alginate circle:**

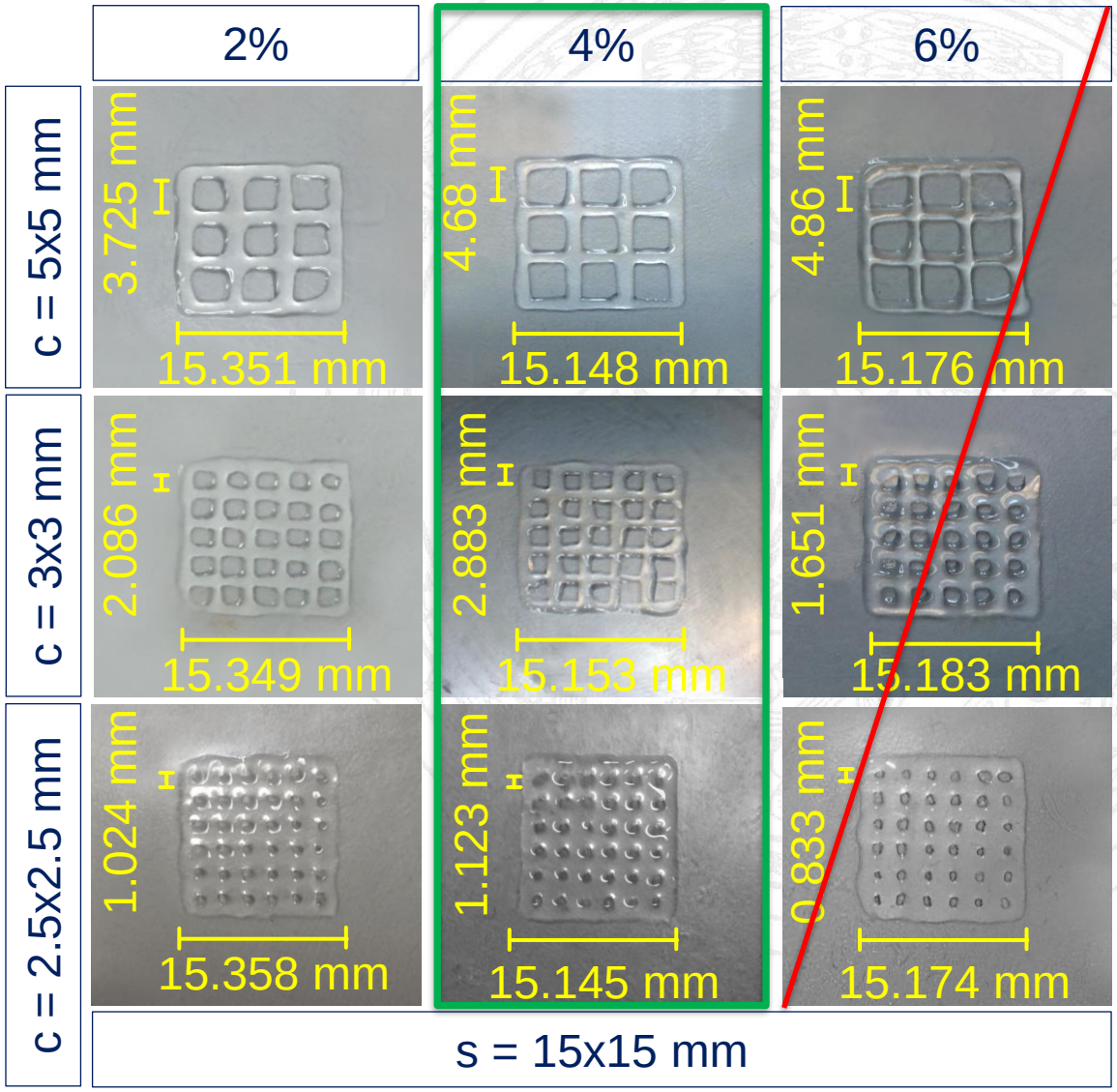
changing E and fixed F at 50 mm/min



- ✓ **Increasing *E* increases** extrusion **thickness**
- ✓ **Low *E*** value **increases resolution**

Resolution test: results II

Different **size grid:** *fixed E and F at 0.1 mm and 50 mm/min*



- ✓ **Increasing** alginate **concentration** increases **shape** resolution **accuracy**
- ✓ **4%** alginate **good** resolution and **shape** and **size accuracy**
- ✗ **6%** alginate **good resolution**, but it is **not able** to be extruded because of **air bubble**

Resolution and reapatability tests

2%, 4% and **6%** sodium **alginate** geometries extruded using customized FDM printer to evaluate**:**

Resolution

Extrude different size standard geometries

- *Circle*
- *Square*
- *Grid*

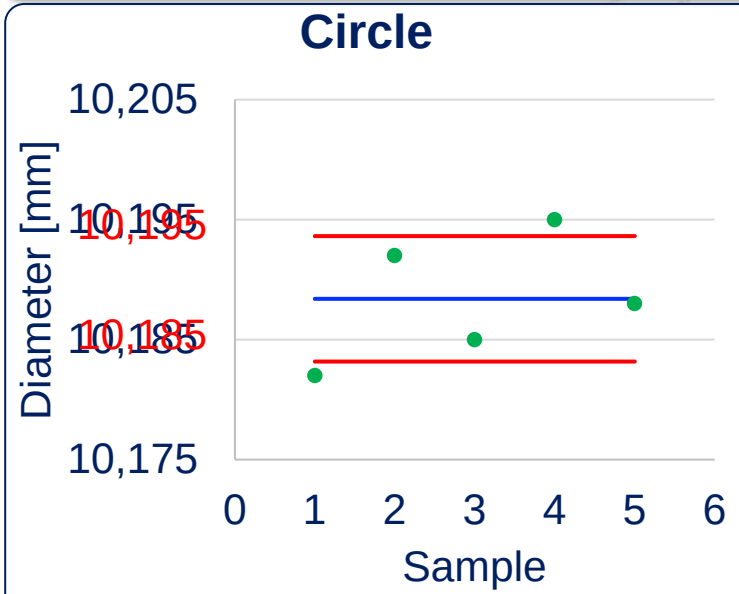
Repeatability

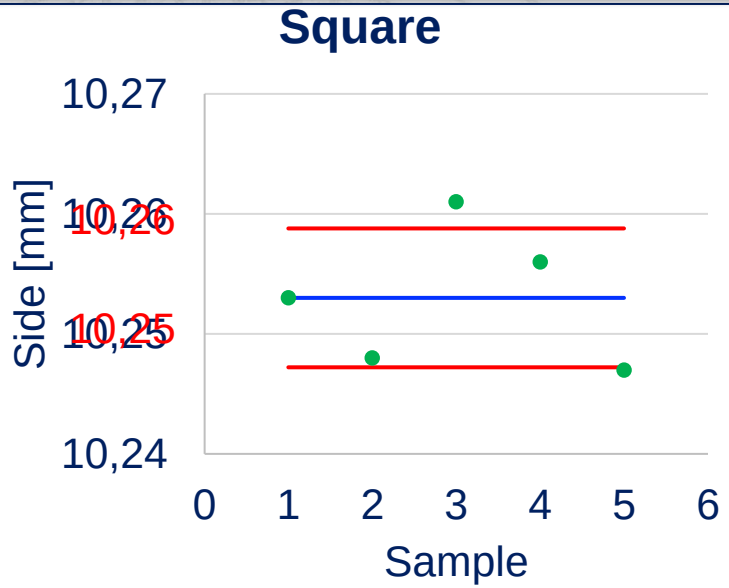
Extrude 5 times standard geometries

- *Circle*
- *Square*
- *Grid*

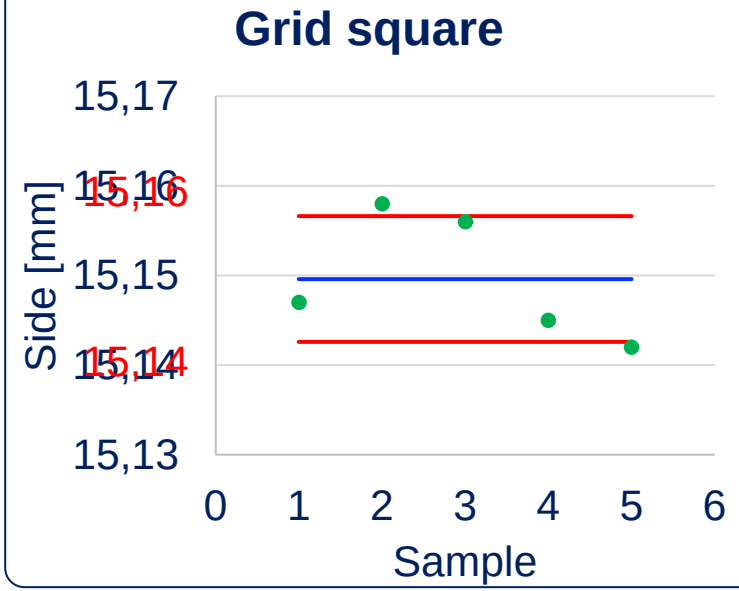
Repeatability test: results

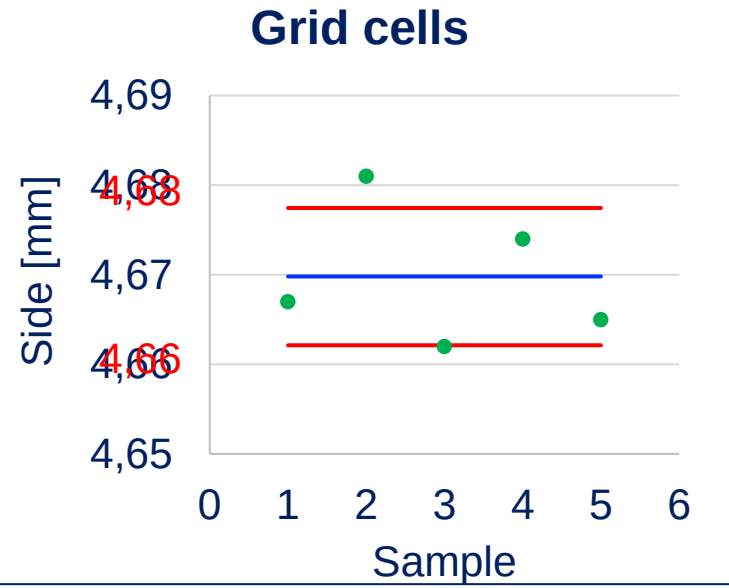
4% sodium **alginate** geometries: *fixed E and F at 0.1 mm and 50 mm/min*





✓ Customized system gives good **shape** and **size** **repeatability**

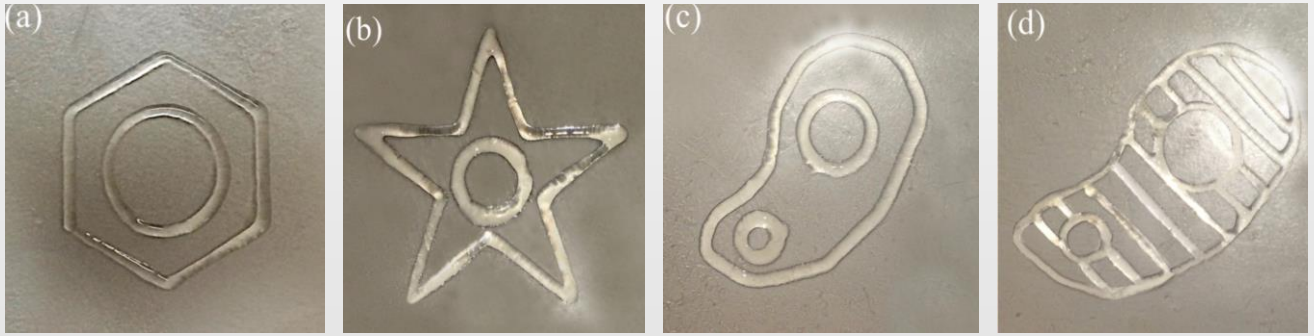




Using SolidWorks and Sli3r

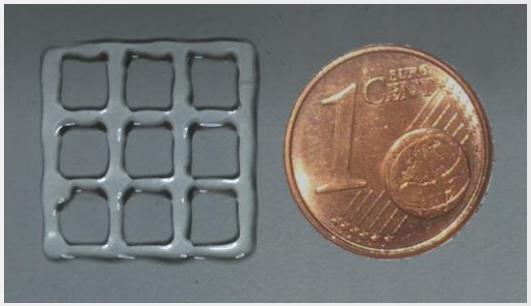
Using **CAD** and **slicing tools** to printing **complex** and asymmetric **geometries**

- Create CAD model
- Setting parameters and slicing
- Generate G-code and start printing



Summary

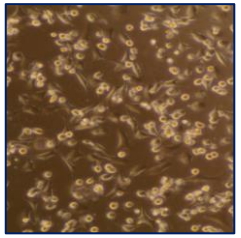
✓ **Sodium alginate extrusion using customized FDM**



- Customized **system** is **able** to **extrude** viscous materials like sodium **alginate**
- Customized system gives **good shape resolution** and **size repeatability**
- **4%** alginate is **better** concentration for **printability**
- Using **SolidWorks** and **Slic3r** to realize **complex and asymmetric geometries**

Summary

- FDM printer customization
- Sodium alginate extrusion
- **Bioink extrusion: proliferation tests**
- Conclusion and future developments



Bioink composition

Bioink

Hydrogel		Cells	
Sodium alginate	Gelatin	HeLa Cervical cancer tumor immortal cell line	SH-SY5Y Human neuroblastoma cell line

? Why changing composition

For increasing cell **proliferation into** sodium **alginate structures**

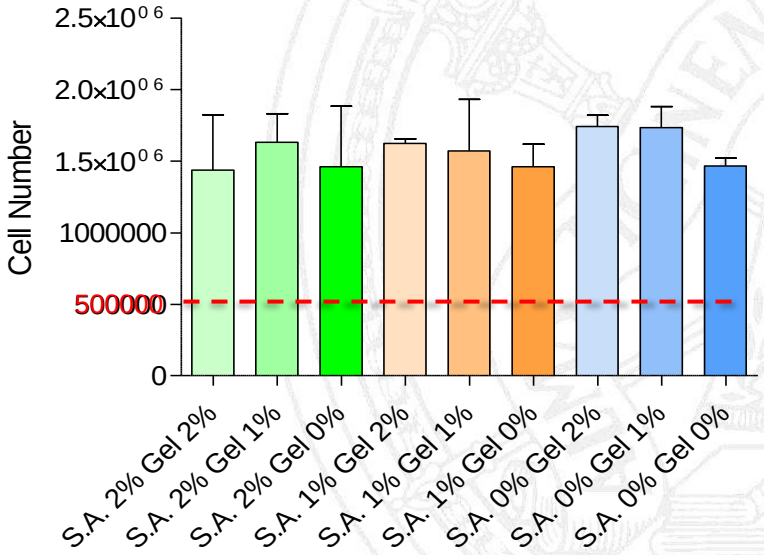
Printing test

Sodium alginate and gelatin ⟷ Pure sodium alginate

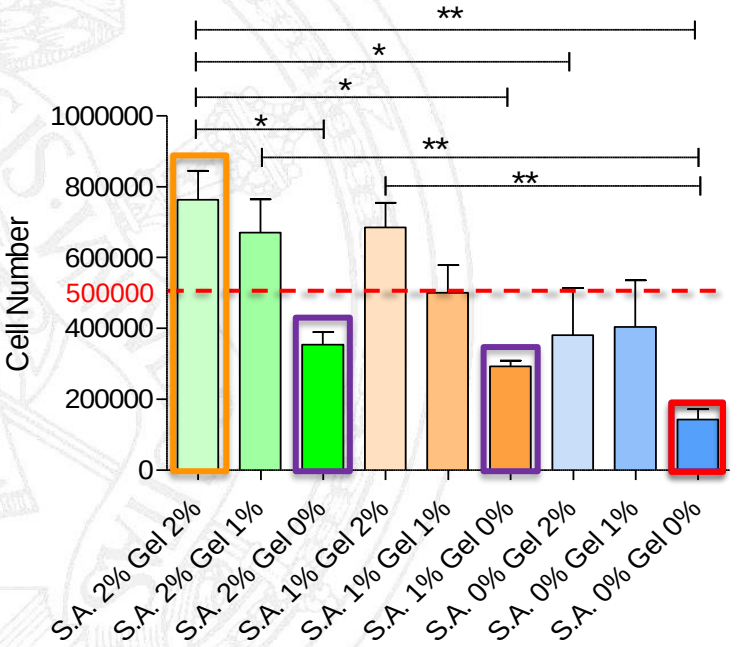
Bioink evaluation and proliferation test: results

Viability and proliferation test using **2% and 1%** sodium **alginate** and **gelatin** and **500.000 cells/ml**

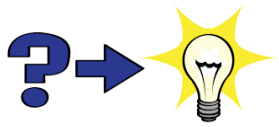
Total cells (death + living)



Living cells



- ✓ Nor alginate nor gelatin affect cell proliferation
- ✓ **2%** alginate/gelatin shows **better proliferation**
- ✓ Samples **without gelatin** shows a **major death rate**



- ➢ Too **many cells seeded** for each sample.
- ➢ **Alginate** creates a **3D microstructure.**

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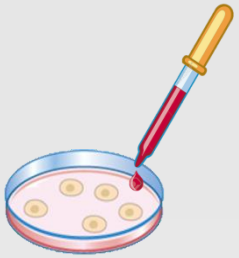
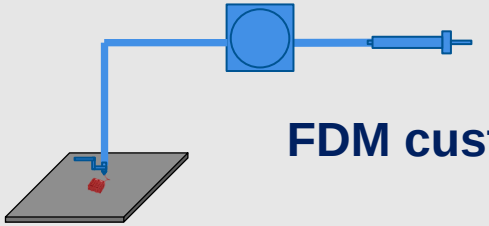
4% alginate/gelatin bioink extrusion: results

Viability and proliferation test using **4%** sodium **alginate** and **gelatin** and **75.000 cells/ml** extruded using



Standard protocol

- ✓ **4% alginate/gelatin** bioink has a good proliferation rate
- ✓ Bioink has **better** proliferation than **2%** and **1%** for **both cells lines**



FDM customized system

Sterile	• Prepare bioink
Sterile	• Load bioink into syringe
Sterile	• Bring sample into Proto-Lab for printing
Not sterile	• Start printing
Sterile	• Culture and cell viability evaluation

Customized system not affect cell viability

Summary

- FDM printer customization
- Sodium alginate extrusion
- Bioink extrusion: proliferation tests
- **Conclusion and future developments**

Conclusion

- ✓ **Literature review**
- ✓ **FDM technology process study**
- ✓ **FDM printer customization**
  - ✓ **Hardware modification: printing and assembly components**
  - ✓ **Software modification: MATLAB functions implementation**
- ✓ **Resolution and repeatability tests**
- ✓ **Active participation in biological experiments**

Customized system is **conformed** to **extrude** viscous solution like **hydrogel (monolayer)**

Good shape **resolution** and size **repeatability**

Using **CAD** and **slicing tools** to printing **geometries**

Alginate creates a 3D microstructure **increasing cell proliferation**

4% sodium **alginate** gives **better printability** and **good proliferation**

Customized **system not affect cell viability**

Future developments

Hardware

- **Sterility** condition improvement

  New FDM **3ntr model** with sterile chamber or bring it under sterile hood

- **Vary** the **nozzle size** and **shape** to extrude higher viscous material
- **Dual extrusion**

  Modify adapter and **realize** nozzle and 3D **printing**

Bioink

- **Multilayer 3D constructs**

  Alginate **crosslinking** with calcium chloride

- **Biological tests**
  - **Triplicate** 4% alginate/gelatine proliferation tests
  - Expression of neural phenotype of SH-SY5Y
  - Differentiation of iPSCs in 3D structures

3D@UniPV
Virtual Modeling and Additive Manufacturing for Advanced Materials

CHT
Centre for Health Technologies
University of Pavia

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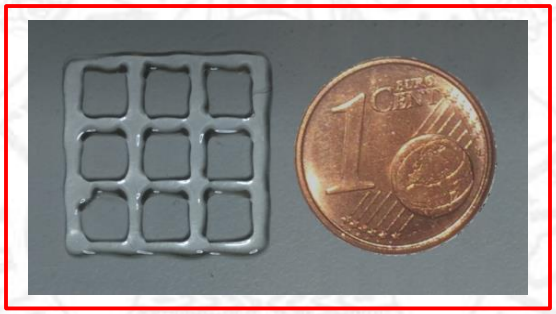
3ntr
INDUSTRIAL GRADE 3D PRINTERS

Thanks for your attention

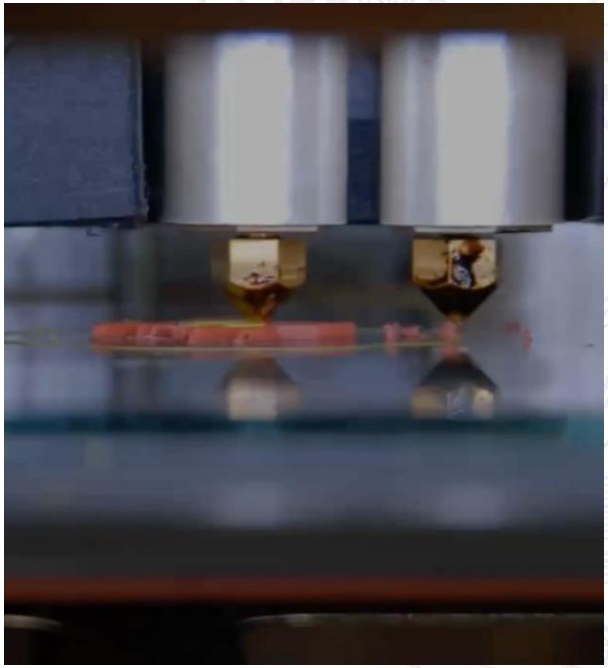


Thanks to all of our collaborators:
Eng. Davide Ardizzoia (3ntr-Jdeal-Form, Oleggio)
Doc. Cinzia ferrari (Exp. Surg. Lab, UniPV)
Prof. Cristina Cereda, Doc. Matteo Bordoni, Doc. Valentina Fantini (Ist. Mondino, Pavia)

FDM technology: our modification for hydrogel

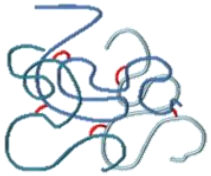
Classical **FDM**

Customization for **hydrogel**



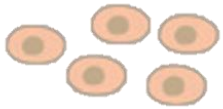
Acceleration 20x

Cell viability and proliferation test



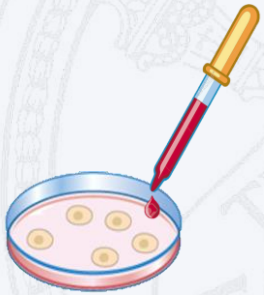
Hydrogel

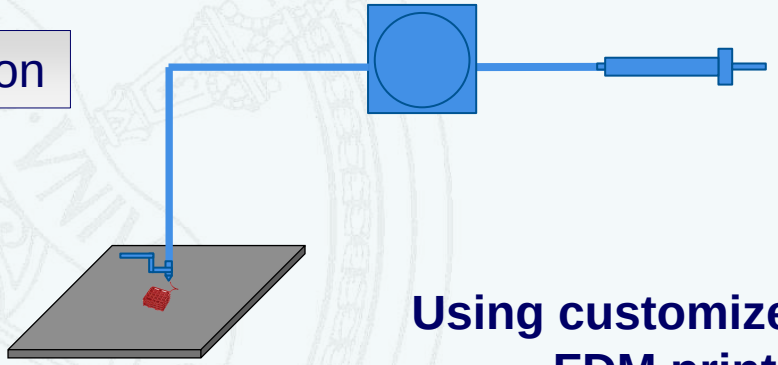
Cells



Standard protocol

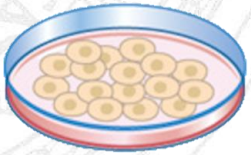
Bioink extrution

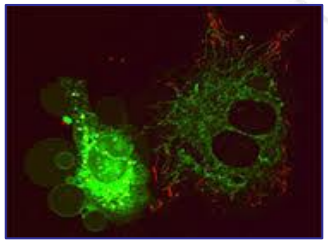




Using customized FDM printer

Cell proliferation





Viability and proliferation evaluation

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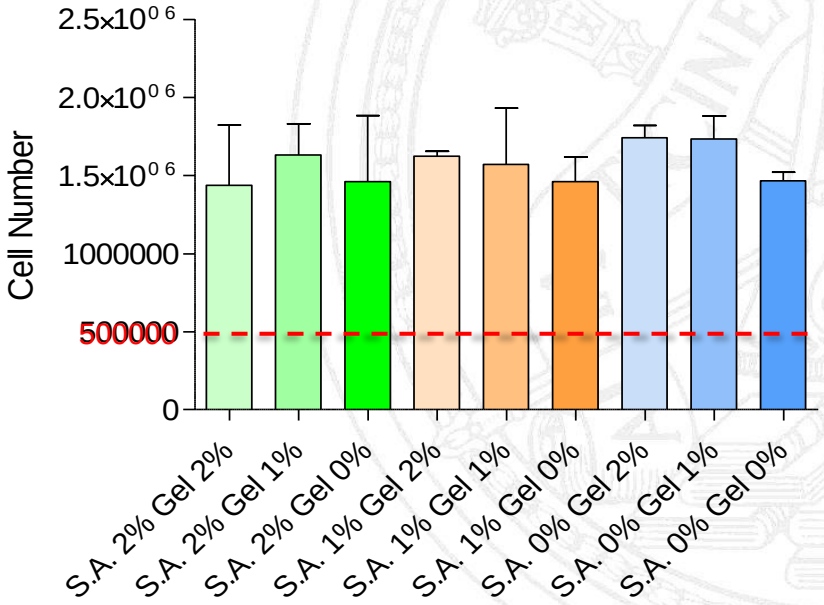
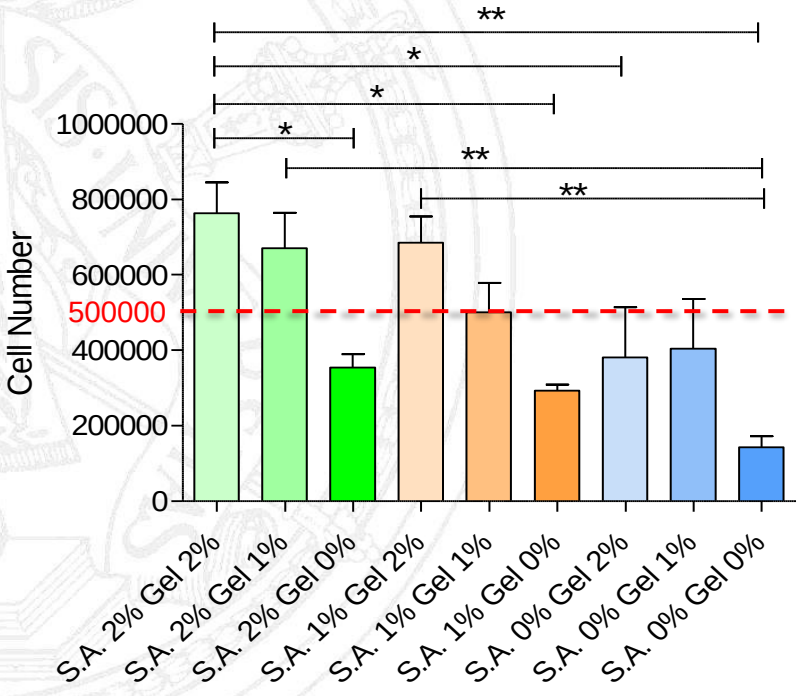
Preliminary proliferation test

Evaluate cell proliferation using different **combination** of **1% and 2% alginate/gelatin** and **500.000 HeLa cells/ml**

Total cells (death + living)



Living cells



✓ **Nor** the sodium **alginate** or **gelatin affect** cell **proliferation**.

✓ **2% alginate 2% gelatin** sample shows the **better** cell **proliferation**.

✗ Samples **without alginate** shows a **major death rate** above all 0% alginate 0% gelatin sample .

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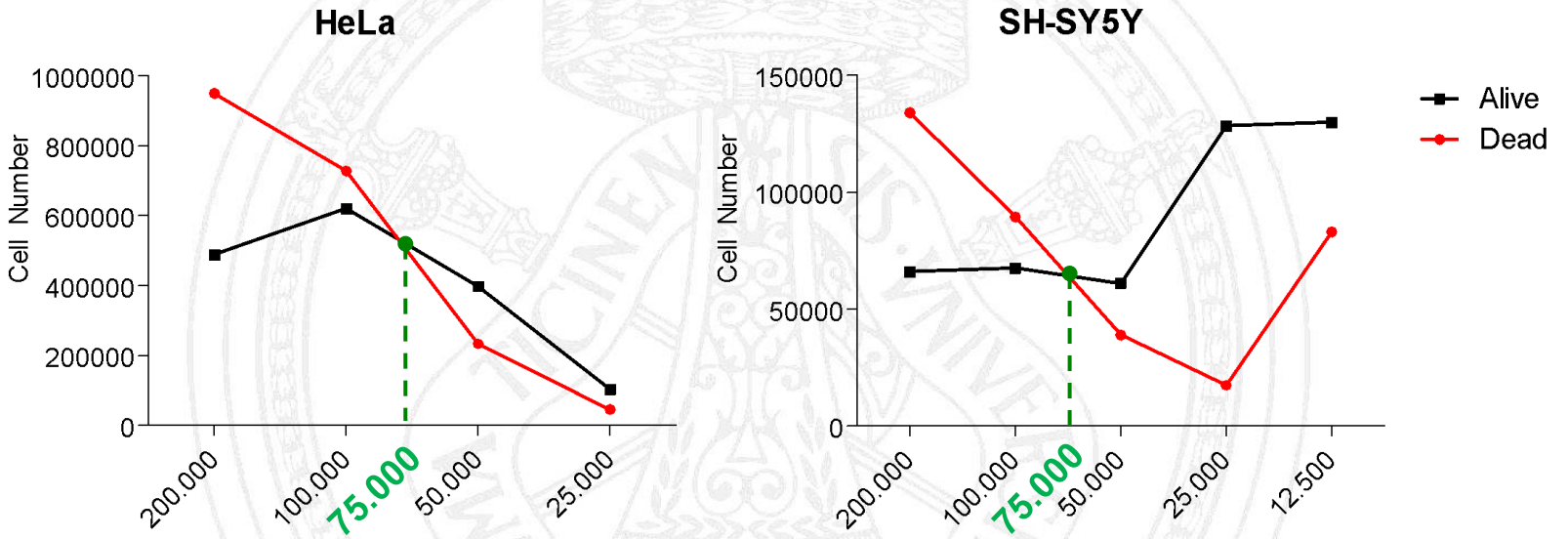
Standard protocol: hypothesis

- Too **many cells seeded** for each sample → Find **cell number** which **maximize proliferation**



- **Alginate** creates a **3D microstructure** and **cells grow better layer by layer** → **Increase** alginate **concentration**

Remind that **4%** alginate is **better** for **printability**

Proliferation test using **4%** sodium **alginate** and **gelatin** and **75.000 cells/ml**