Laboratori Nazionali di Legnaro – INFN

Work Package 1: activities 2025

V. Di Marco, D. Maniglio

June 18th, 2025

Outline

Brief review of WP1 activities in 2025:

I. (Di Marco)

II. (Maniglio)

Brief review of WP1 activities in 2025

Done

MS1.0	Optimization of Ag-111 production and Ag/Pa separation
MS1.1	Routine production of Ag-111 at the LENA facilities, purification and quality control
MS1.2	Synthesis of improved stable Ag chelators and radiochemical characterization
MS1.3	Development of the macromolecule for active targeting
MS1.4	Bioengineering of 3D scaffolds for in vitro tissue mimicking

This talk (Di Marco)

This talk (Maniglio)

Brief review of WP1 activities in 2025

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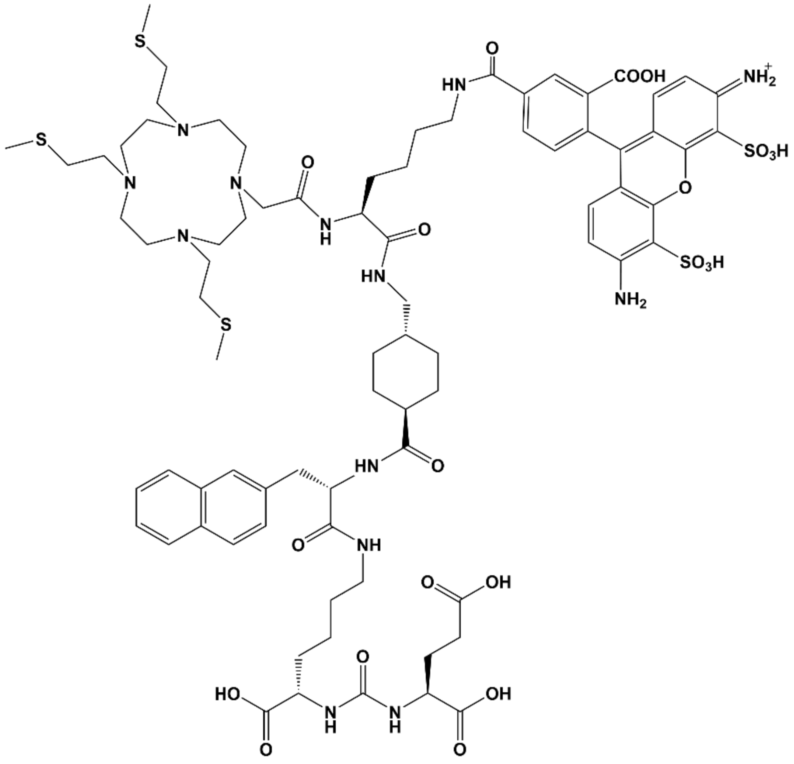
Cooperation agreement in due course with

A.C. Eder, University of Freiburg, Department of Nuclear Medicine, Germany

Brief review of WP1 activities in 2025



Chemical Formula: $C_{79}H_{108}N_{13}O_{21}S_5^{+}$
Exact Mass: 1734,64
Molecular Weight: 1736,10

Expected shipping date from Germany: July 2025

Brief review of WP1 activities in 2025

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Brief review of WP1 activities in 2025

Just arrived (17/06/2025): results of the ICP-MS analysis of $^{111}Ag/^{110}Pd$ separations

Resin/sample	Pd (ppb) $^{111}Ag/^{110}Pd$
LN1-1	4765
LN1-2	2230
LN1-3	3300
LN1-4	43
LN2-5	481
LN2-6	292
LN2-7	385
LN2-8	23
TK200-9	3
TK200-10	1.5
TK200-11	11
TK200-12	0.26

Data are in good agreement with previous analyses (March, 2025) performed on $^{nat}Ag/^{nat}Pd$ separations

Pd (ppb) $^{nat}Ag/^{nat}Pd$
2.6
2.4
0.5
2.1

Brief review of WP1 activities in 2025

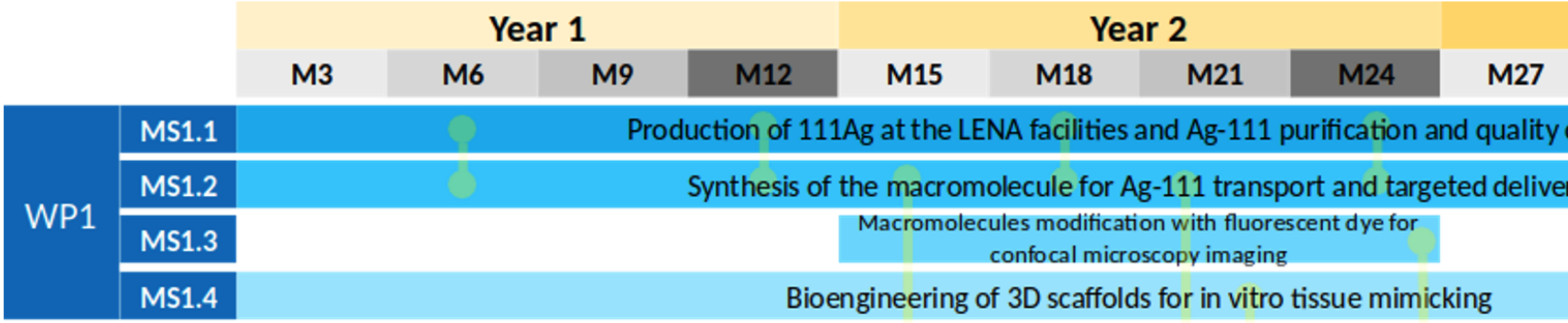
Month	Starting date	# Activity [MBq]	# Activity [mCi]	Experiment
January	27/01/2025	55,5	1,5	SPECT imaging with phantoms
February	10/02/2025	20	0,5	Vitro (UMR-106 foci)
March	31/03/2025	60	1,6	Vitro (MDA-MB-231 uptake and clonogenic assay)
April				
May	12/05/2025	18	0,5	Vitro (LNCaP clonogenic assay) + γ imaging
June	16/06/2025	60	1,6	Vitro (MDA-MB-231 foci assay)
July	30/06/2025	18	0,5	Vitro (LNCaP foci) + γ and β imaging
August				
September		18	0,5	Vitro 3D (LNCaP) + γ and β imaging
October				Imaging test with phantoms (Ag-111 vs Lu-177)
November		18	0,5	Vitro + γ and β imaging (extra tests)
December		60	1,6	Vitro (extra tests)

Advancement in the creation of 2.5D constructs

Annual meeting of WP1

September 13rd, 2024

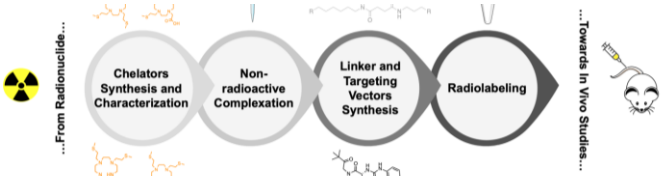
Planned Activities



WP1 - Radiochemistry & Pharmacology

1-2 - Radiopharmaceutical

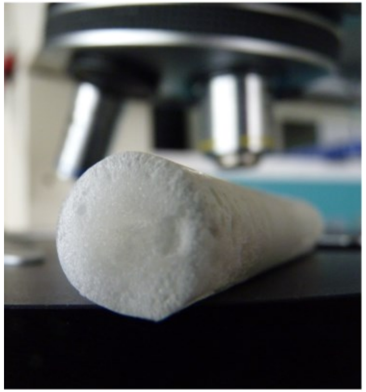
Produced radionuclides must be inserted in a **macromolecular context** able to bind and transport them into the tumor tissue where they should **selectively** recognize and associate with cancer cells. The resulting research involves **studying chelators, spacers and targeting agents** to develop a **stable radiopharmaceutical** for ^{111}Ag complexation. In addition, **radiochemistry experiments** focused on the dissolution of the irradiated target where ^{111}Ag is obtained are required to optimize the radioisotopic purification.



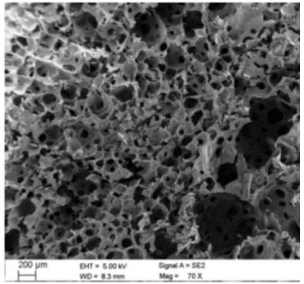
Radiopharmaceutical design & development framework

1-3 - Bioengineering

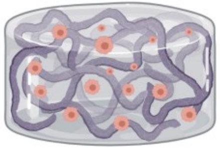
One of the goals of the new ISOLPHARM project is to extend this kind of studies to targeted radionuclide therapy and to more realistic **dynamic cell cultures** in 3D tissue-mimicking scaffolds, employing the bio-engineering expertise of the collaboration.



3D printed scaffold.

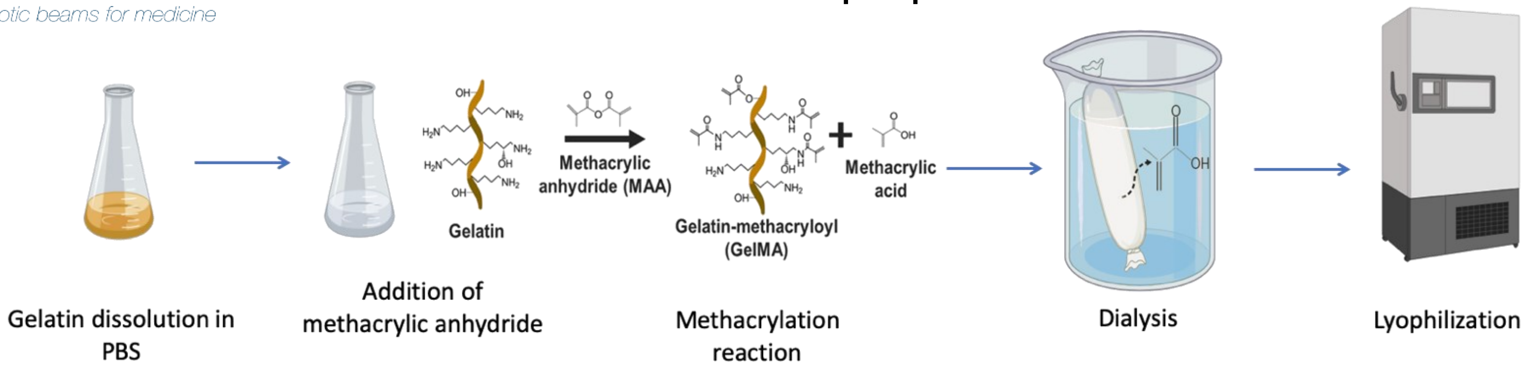


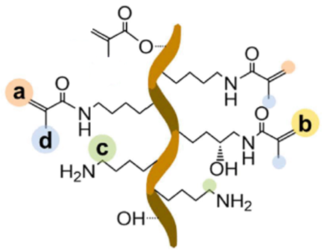
Scaffold porosity as detected by SEM.



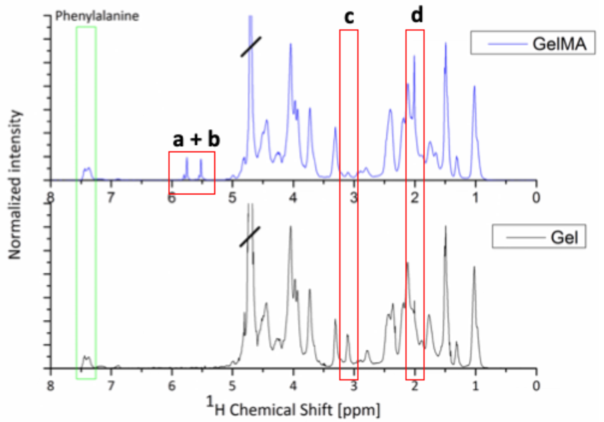
Natural polymer hydrogel embedding cells and supporting them viable and functional

The materials: base bioink preparation





a + b $C\underline{H_2}=C(CH_3)CONH-$
c $NH_2C\underline{H_2}CH_2CH_2CH_2-$
d $CH_2=C(C\underline{H_3})CO-$

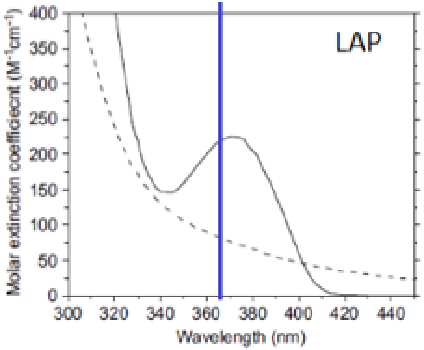


$$DoF_{lysine}\% = \left(1 - \frac{\int lysine\ GelMA}{\int lysine\ unmodified\ gelatin}\right) * 100$$

DoF*= 70%

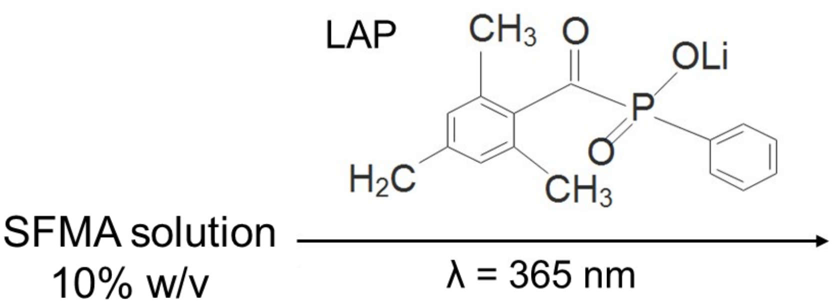
New protocols experimented by Daniel
10 days → 3 days

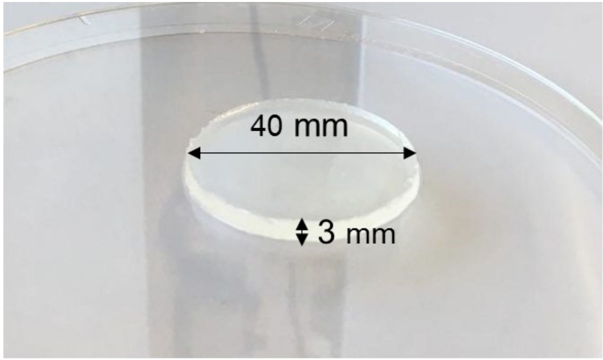
Photoinitiator



Lithium Phenyl-2,4,6-trimethylbenzoylphosphinate (LAP)

- Low cytotoxicity
- High solubility in water
- High absorption at 365 nm (ε = 218 $M^{-1}cm^{-1}$)





How to turn bioink to a 3D scaffold?

DLP (Digital Light Processing)

Matches Photolithography and SLA advantages:

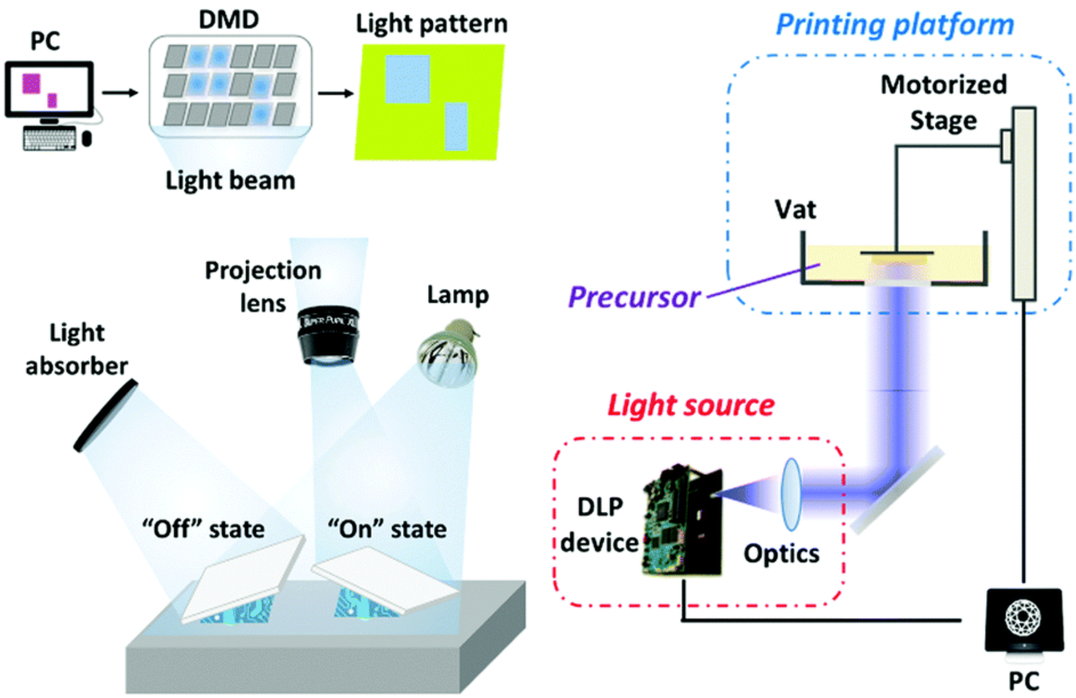
- **Maskless**
- **Each layer is printed at once**

Working principle:

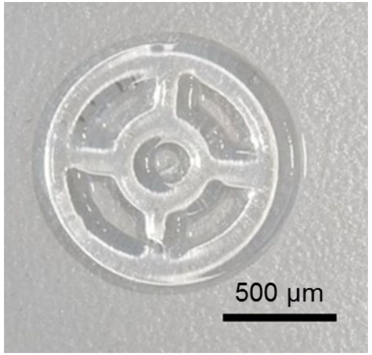
- Projector with LED source for illumination
- DMD (Digital Micromirror Device): digital programmable photomask to control the crosslinkable area
- No CAD modeling but any raster image

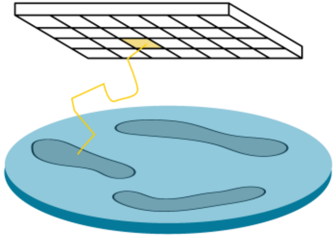
Biomaterial requirements:

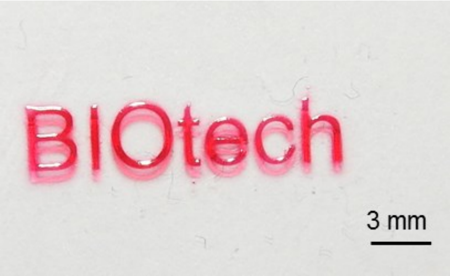
- Photopolymerizable
- Photoinitiator with low toxicity



Complex patterns with DLP







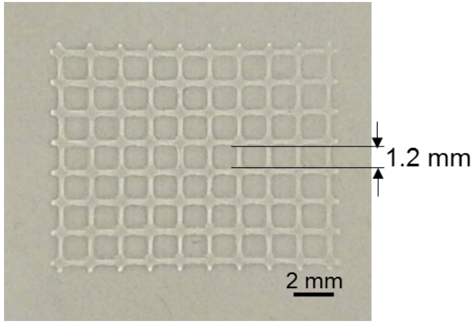
It is optimal for 2D ½ scaffolds design for Beta imaging

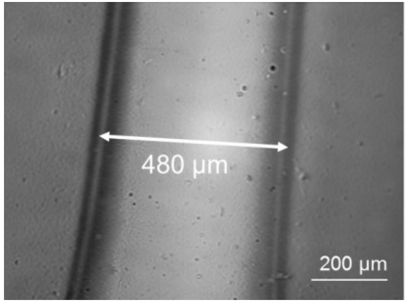
Staining used: Betanin

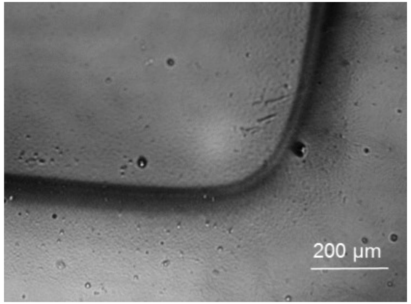
Printed meshes with DLP

Lower limit of printability

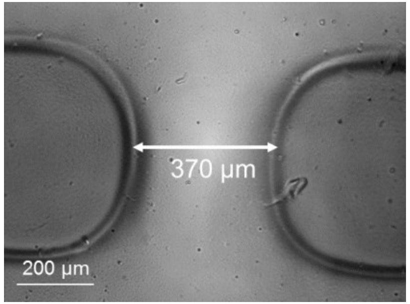
Expected dimensions:
Lines = 400 µm
Holes = 1200 µm

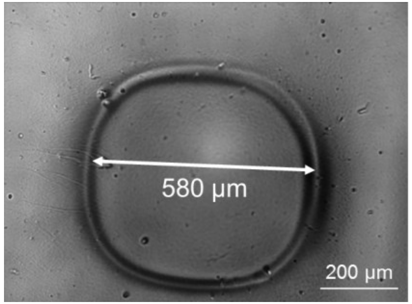


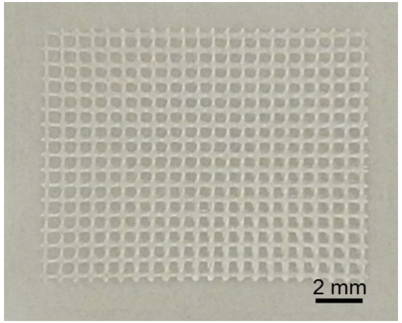




Expected dimensions:
Lines = 240 µm
Holes = 480 µm

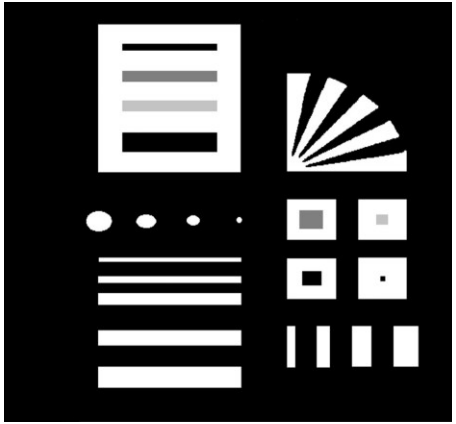


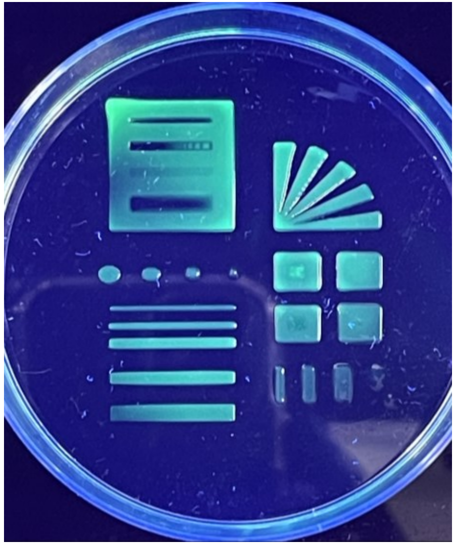


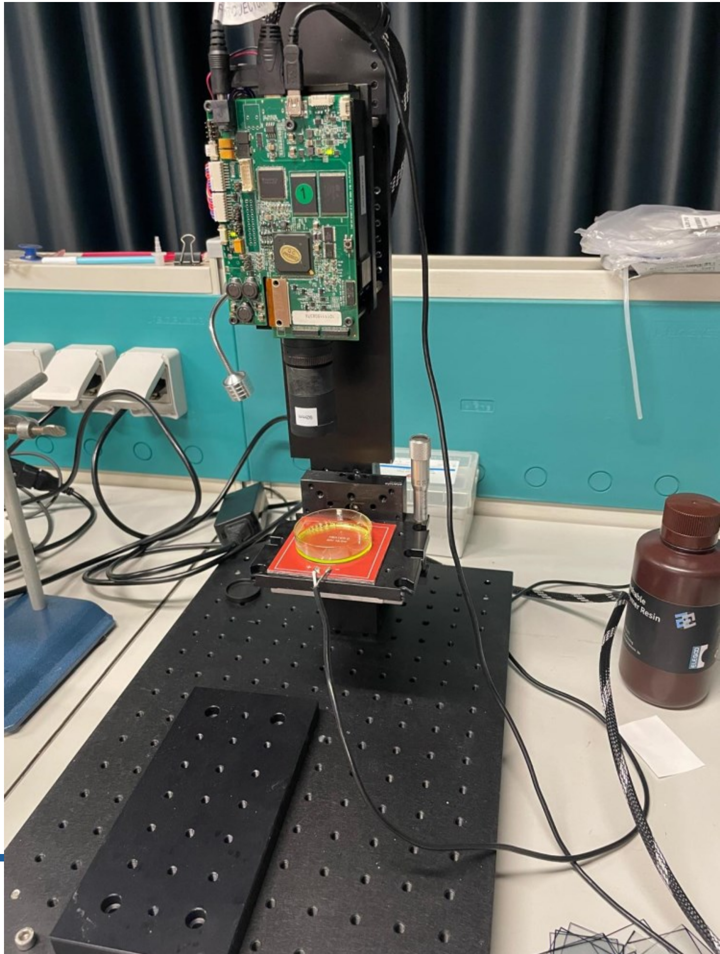


Geometries suitable to 2D β-imaging detector

Assessing large image printing

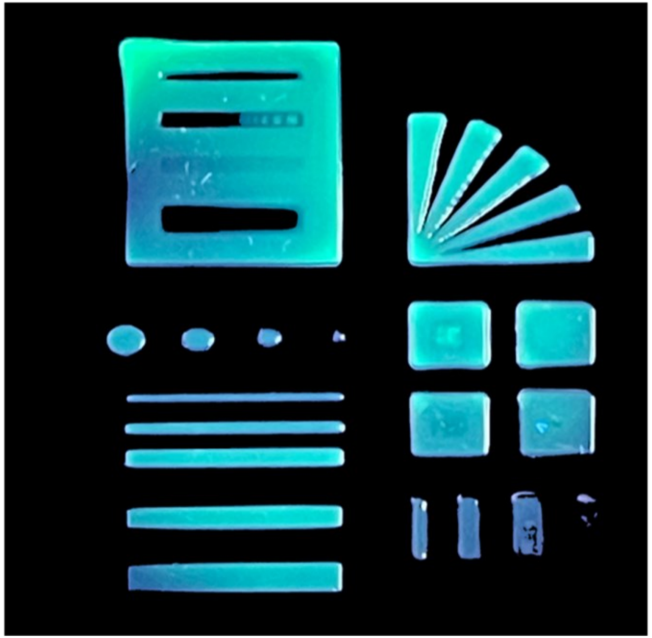




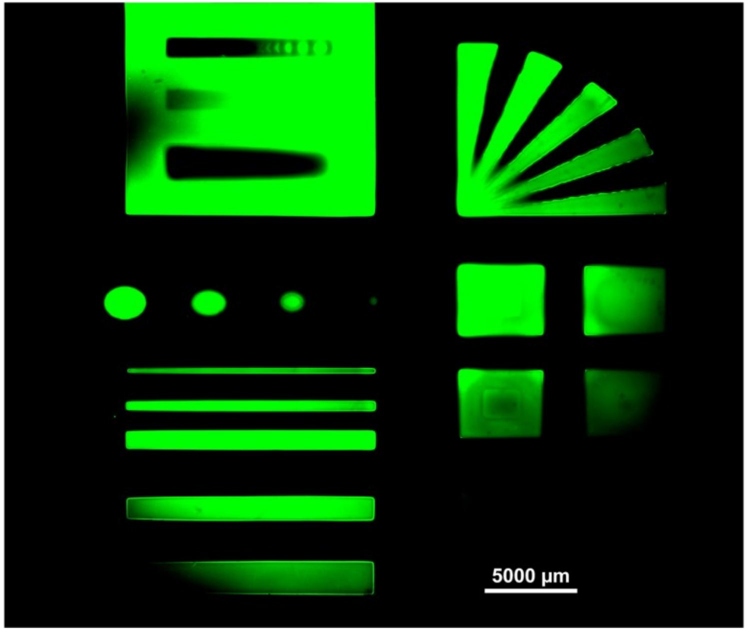


GelMA 20% + fluorescein dye + 1% LAP

Quality of layout

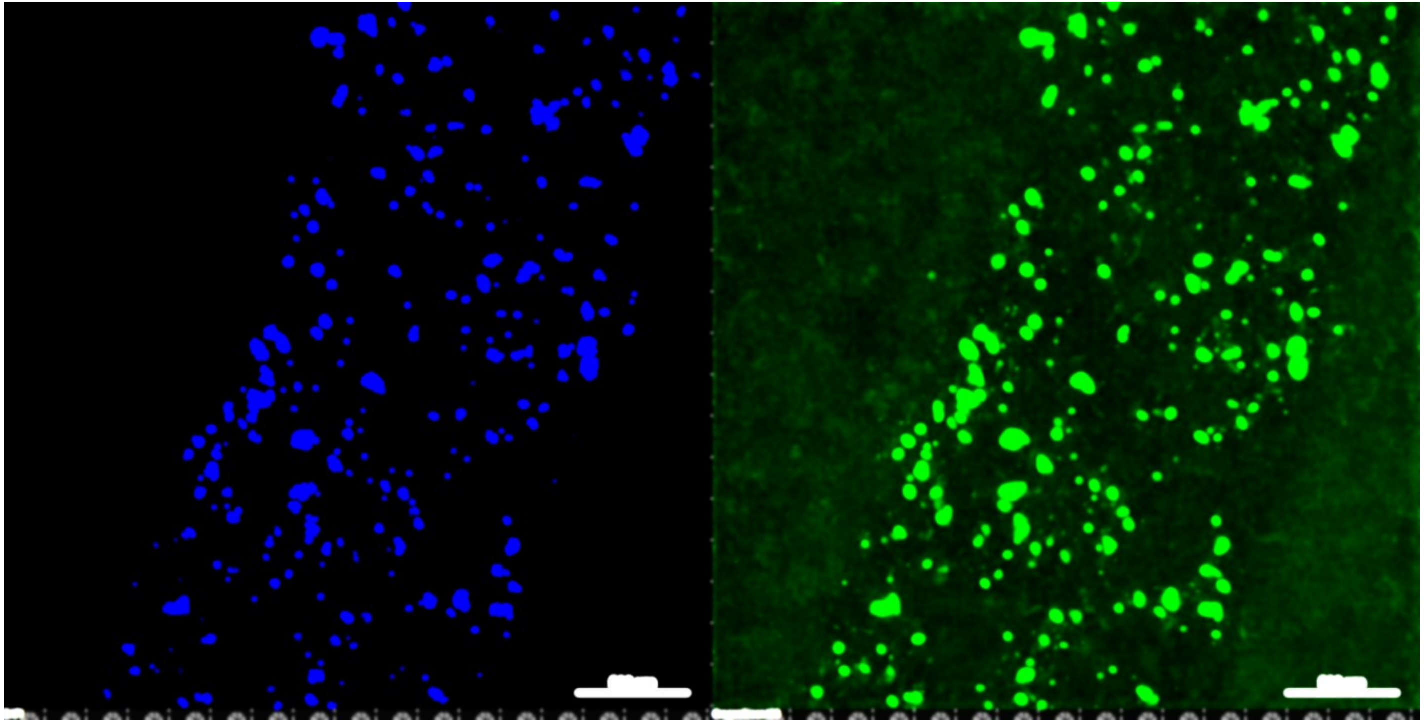


UV Transilluminator



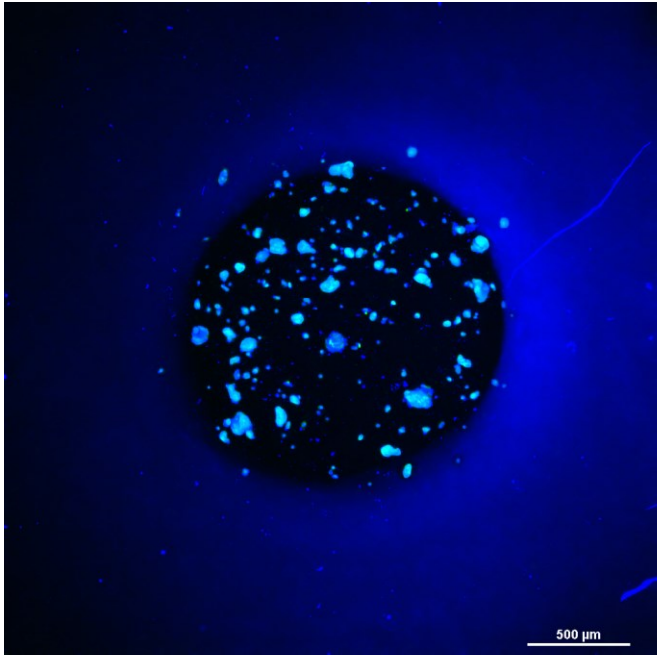
Confocal microscopy

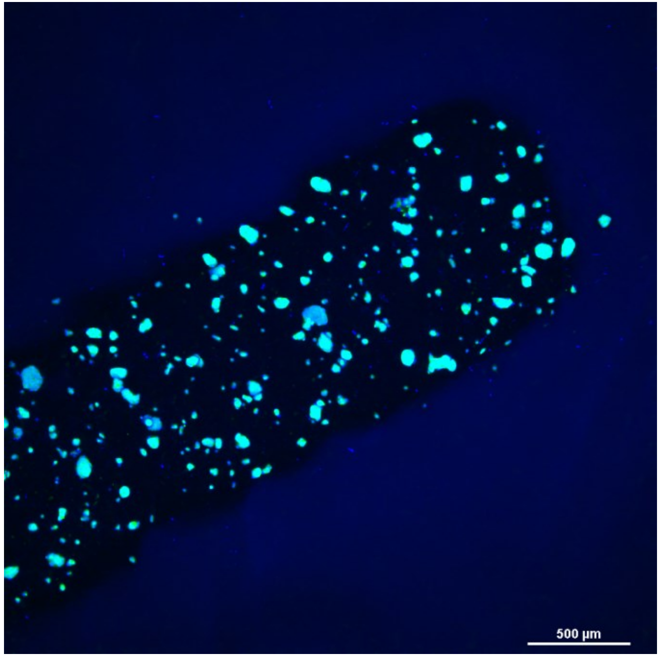
Embedding cells



Stripes and cells day 1

Circles and stripes day 4





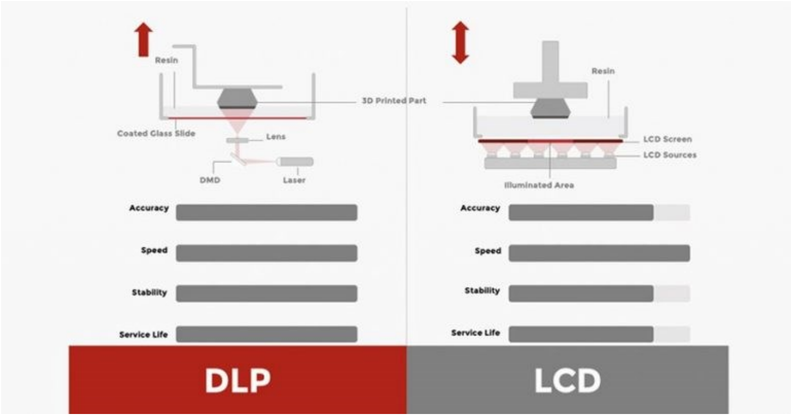
Strategies for developing $2D^{1/2}$

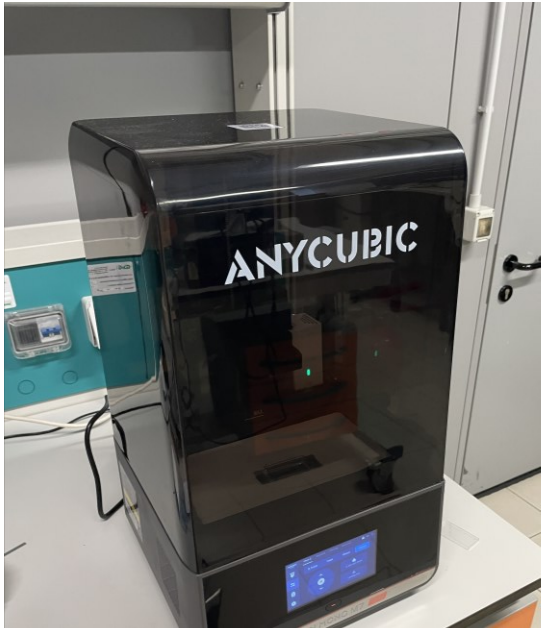
- Finding optimal projection support
  (sterile – thin interlayer material – transportable – size compatible with gamma camera)

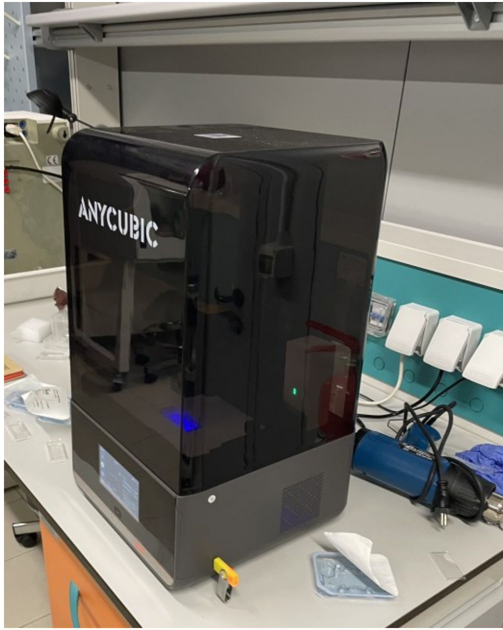
- Adapting twin commercial 3D printers for $2D^{1/2}$ bioprinting to feed WP2 activities

Anycubic modification

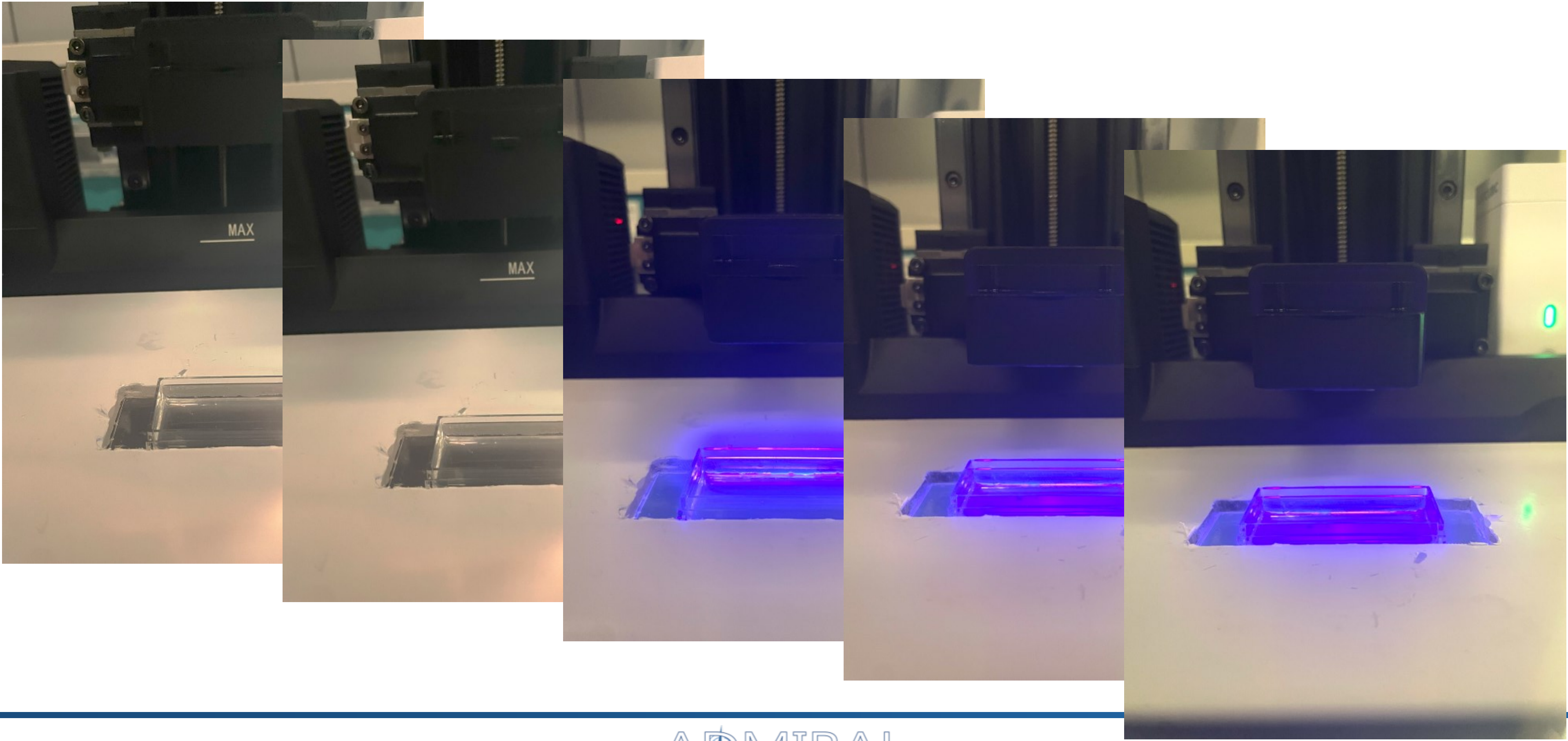
- LCD projector technology (not DLP)
- 405 nm wavelength, compatible with LAP photoinitiator
- Higher resolution for 400 €
- Needs modification



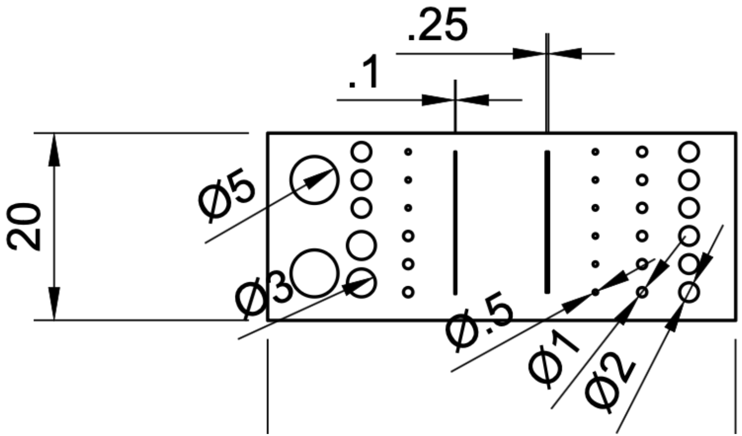


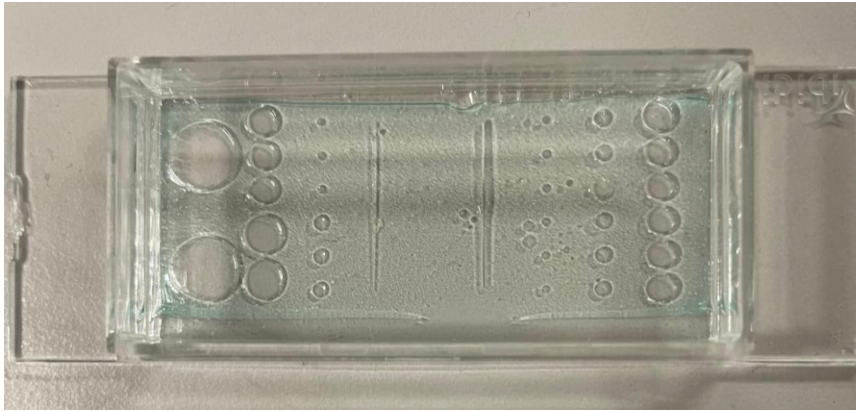


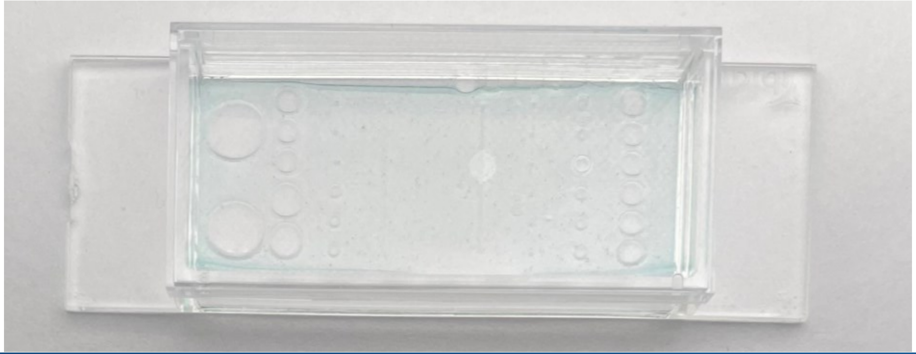
The printing process



Resulting printed scaffolds





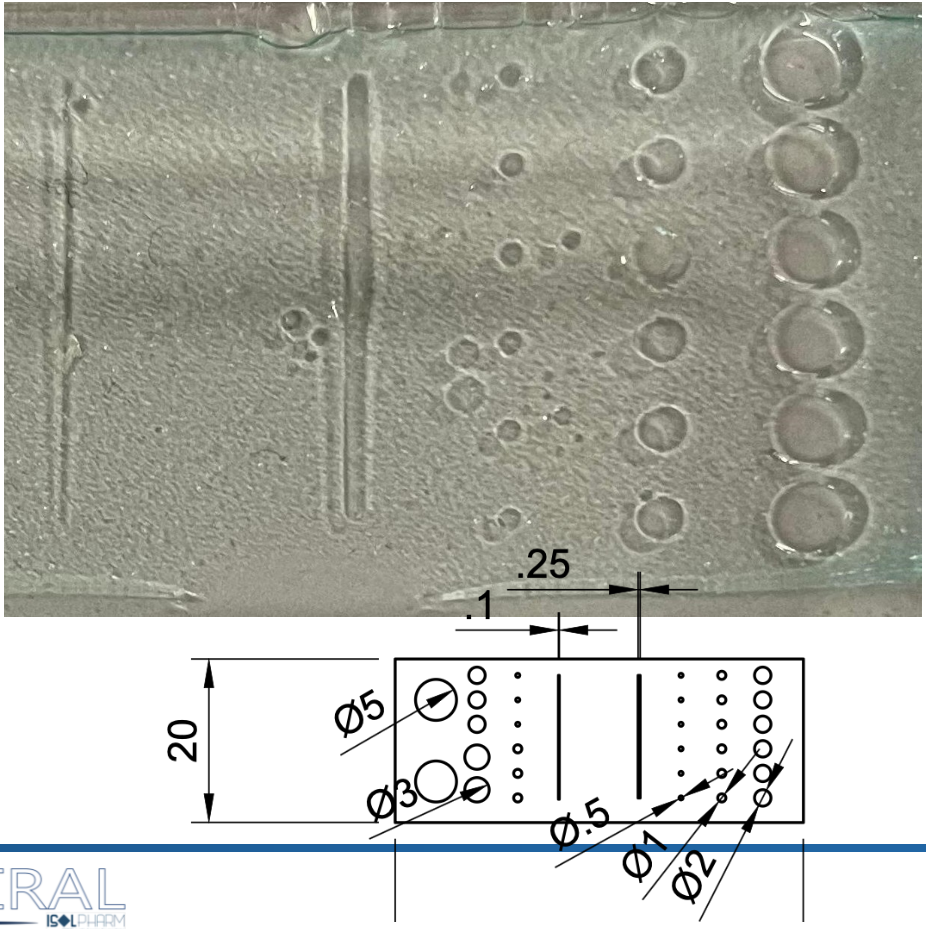


See more in detail

High aspect ratio structures are difficult to be printed (0.2 mm limit)

The correct insertion of cells is reasonably made by pipette

There are also some considerations on the volume to be complemented



Thank you