mRNA-LNPs Microneedle Delivery in 3D Human Dermal Equivalent Model for Preliminary *In-Vitro* Testing

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Background & Rationale:

Human dermal equivalent model as an in vitro platform for microneedle delivery study

Why are advanced models needed for microneedle testing?

1) 2D models inadequately replicate the complex architecture and barrier functions of human skin. Their inability to mimic the extracellular matrix and cellular interactions leads to unreliable predictions of drug delivery efficacy and safety.

2) Animal or *ex vivo* skin is affected by variability and poor translational relevance

3) Need for reliable, reproducible, human-based 3D models

What is an HDE?

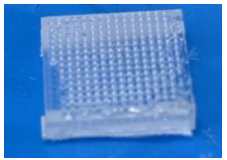
A 3D human dermal model engineered *in vitro* with an endogenous Collagen/Hyaluronic acid-based extracellular matrix produced by human dermal fibroblasts (HDF)

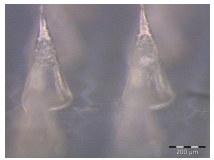
Aim of the study

Human Dermal Equivalent model (HDE) as an in vitro platform for microneedle delivery study

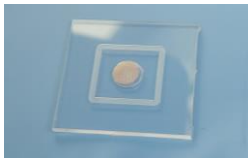
1. **Develop reliable HDE models for mRNA-LNPs-Microneedle testing**
2. **Key Assessments:**
   - **Microneedle insertion efficiency and penetration depth**
   - **Distribution of delivered agents within the dermal matrix**
   - **mRNA expression profiles post-delivery**
   - **Local cellular response and tissue compatibility**

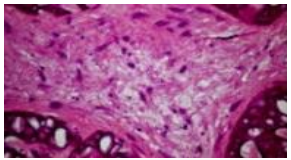
Microneedle patch (MNs)



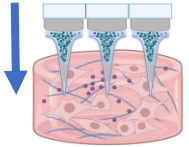


Human Dermal Equivalent model (HDE)

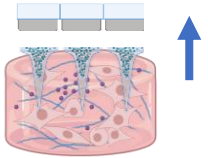




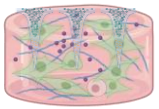
MNs implantation in HDE model



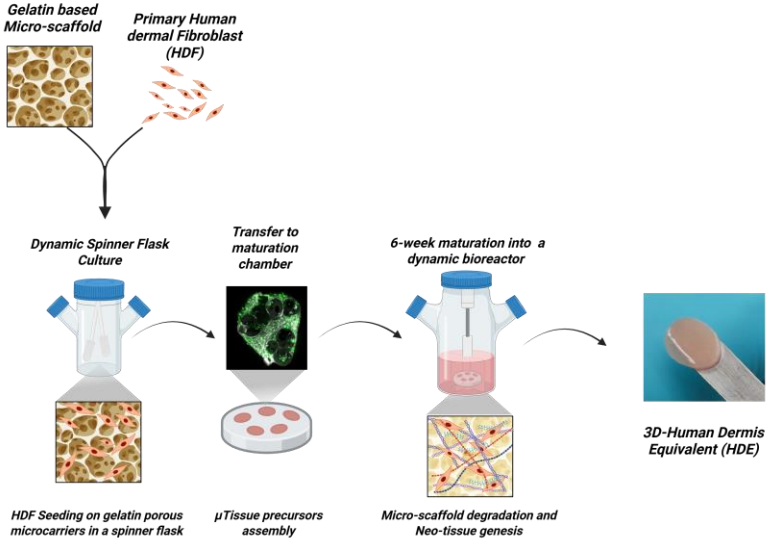
Pillar removal and MNs dissolution, mRNA LNPs MPs release



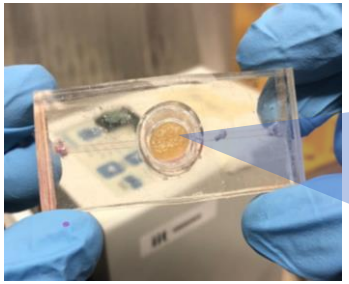
GFP expression

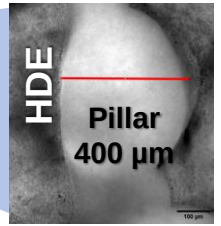


Model Development Workflow: HDE Model Genesis



HDE in a customised optical accessible device





Fibroblasts start assembling the connective tissue around the pillars, exploiting their support function for the tissue.

- Human cell-based
- Reproducible and modifiable
- Predictive of the penetration of the drug delivery system and early tissue response

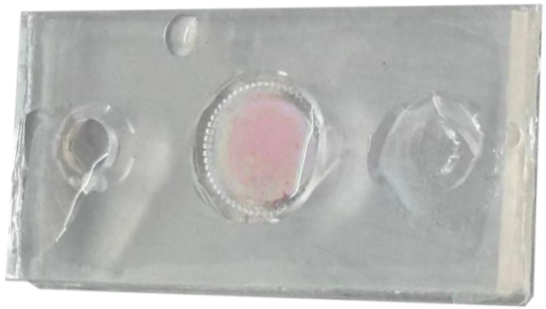
In collaboration with Dr Roberta Passariello, Dr Giorgia Imparato and Dr Atefeh Malek Khatabi for HDE and chip fabrication, and design.

Bioengineering **2022**, *9*, 233. https://doi.org/10.3390/ bioengineering9060233 . *Biomaterials*, ***2019***, *192: 159-170. Journal of tissue engineering and regenerative medicine*, ***2017***, *11.8: 2276-2285*

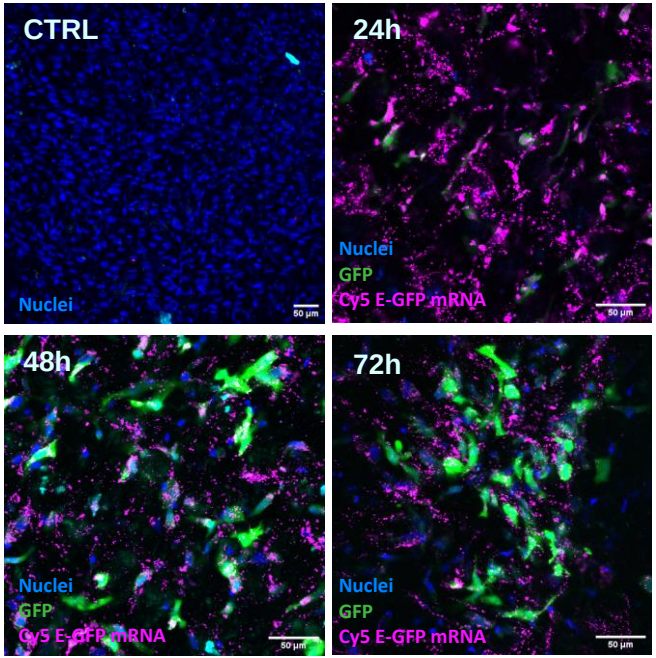
HDE model validation for mRNA delivery

Transfection with Cy5 E-GFP mRNA with the gold standard lipofectamine

We evaluated the transfection after the different incubation time points (24h, 48h, and 72h) by monitoring the ***GFP expression*** in the live HDE model with a confocal microscope.



HDE in a customised optical accessible device



25×/0.45 NA objective **objective lens, Hoechst stain for nuclei (blue), green fluorescent protein (GFP) for protein expression (green), Cy5 E-GFP mRNA, scale bar 50 um**

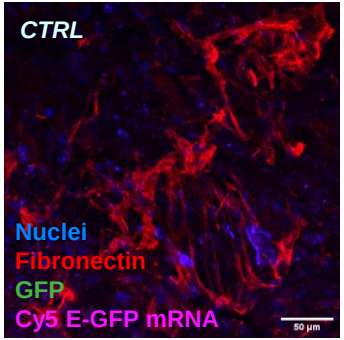
HDE model validation and characterization:

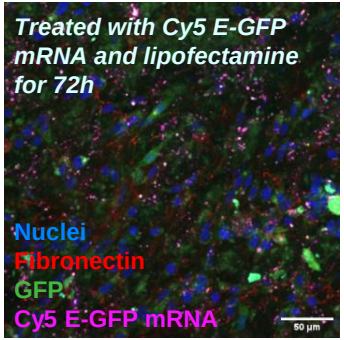
Second harmonic generation (SHG) and Immunofluorescence for HDE matrix staining

We performed the immunofluorescence to stain the HDE matrix. In detail, we labelled **fibronectin (red)** and exploited second-harmonic **(SHG grey)** generation to observe the collagen matrix.

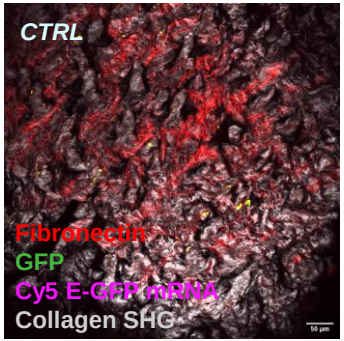
20×/0.45 NA objective **water lens, Hoechst stain for nuclei (blue), Fibronectin(red), green fluorescent protein (GFP) for protein expression (green), Cy5 E-GFP mRNA(magenta) , Collagen SGH, scale bar 50 um**

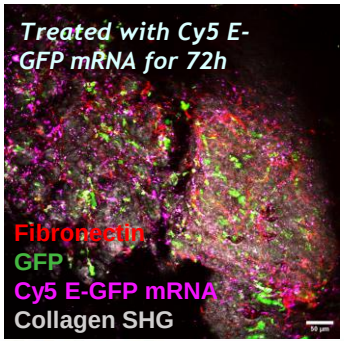
Immunofluorescence

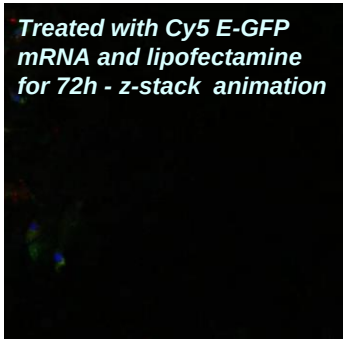




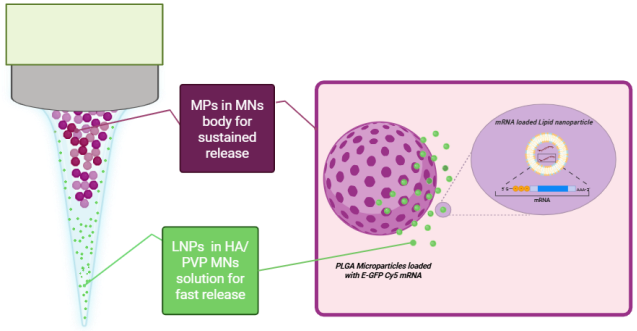
SHG

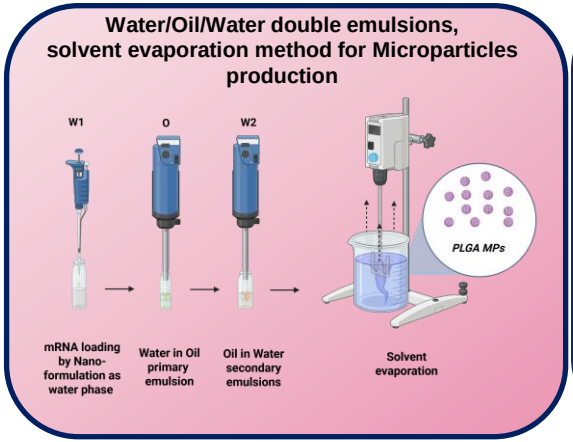


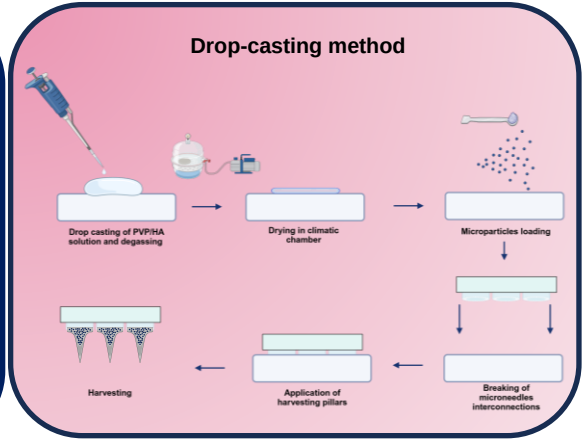




Multi-compartimental Microneedle assembly: Matryoshka mRNA MNs







UNIVERSITÀ DEGLI STUDI DI MILANO

IEO Istituto Europeo di Oncologia

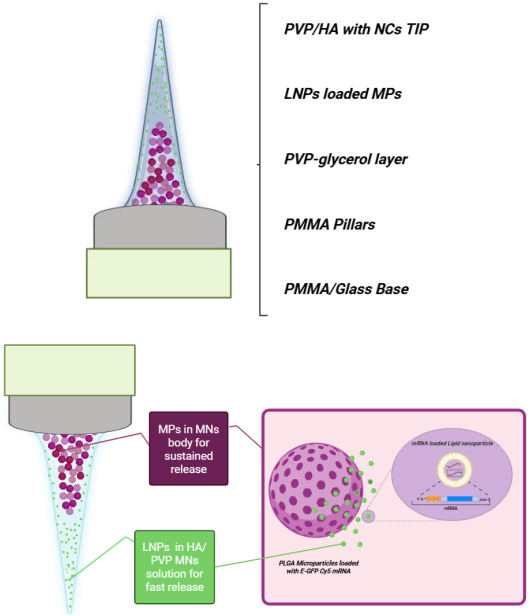
In collaboration with Dr. Stefano Persano from UniMI and IEO, partners who provide E-GFP mRNA-loaded Nanoformulations

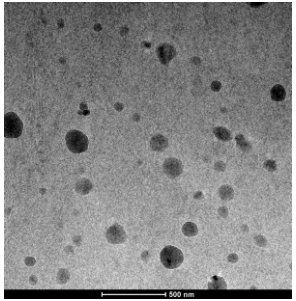
In collaboration with Dr Alessandro Attanasio, Dr Daniela Orefice, Dr Atefeh Malek Khatabi, Dr Emilia Cioffi for Microneedle and microparticles fabrication and characterisation

Method For Producing Shaped Polymeric Microparticles", Italian Patent n. IT 102013902145293, PCT n. WO 2014/167495 and n. EP 2983880

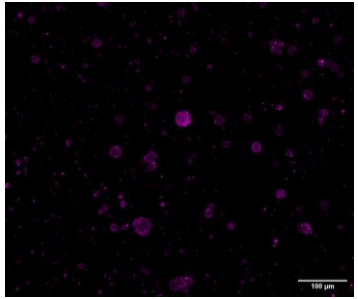
Frontiers in Bioengineering and Biotechnology, *7*, 296., *Materials* 13.8 (2020): 1807., *Progress in biomaterials* 9 (2020): 153-174

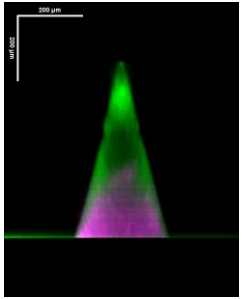
Matryoshka mRNA LNPs MNs characterization

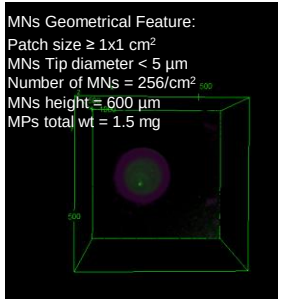


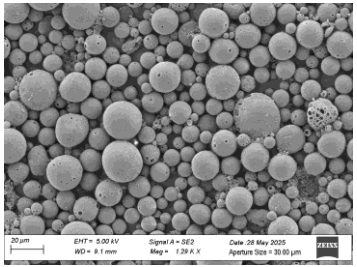


LNPs images acquired by Cryo-TEM, LNPs mean size 80-90 nm obtained by microfluidic device

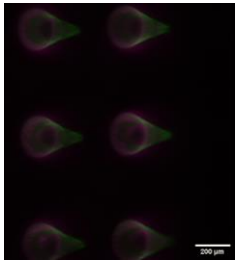


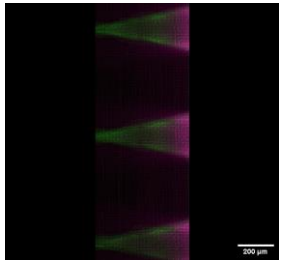






A) Confocal image and B) SEM image of **E-GFP Cy5 mRNA LNP loaded MPs, MPs mean size 10 um** with 10×/0.45 NA objective air LS900 Zaiss

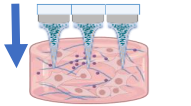


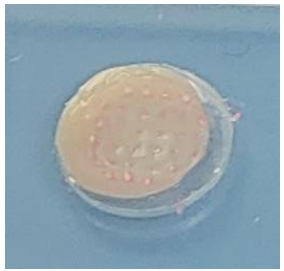


3D confocal reconstruction of Matrioska MNs acquired with 10×/0.45 NA objective air LS900 Zaiss **exploiting LNP DiO labelled (green)** and **Cy5mRNA (magenta)** from signal PLGA MPs loaded with E-GFP Cy5 mRNA LNPs

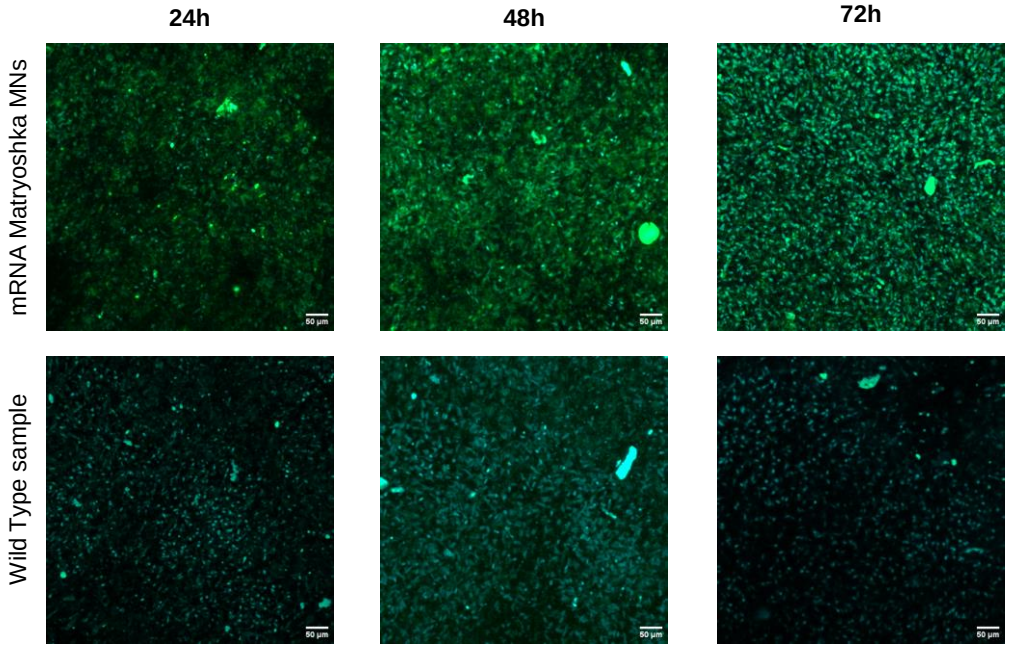
Live imaging of HDE for mRNA Matryoshka MNs delivery Testing

Objective: Assess tissue morphology, microneedle penetration traces, and residual mRNA expression days post-application



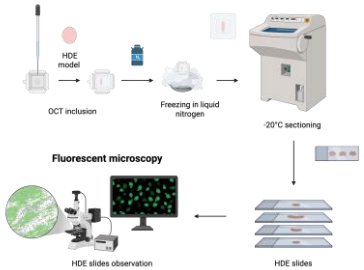


mRNA Matryoshka MN patch insertion in the HDE model

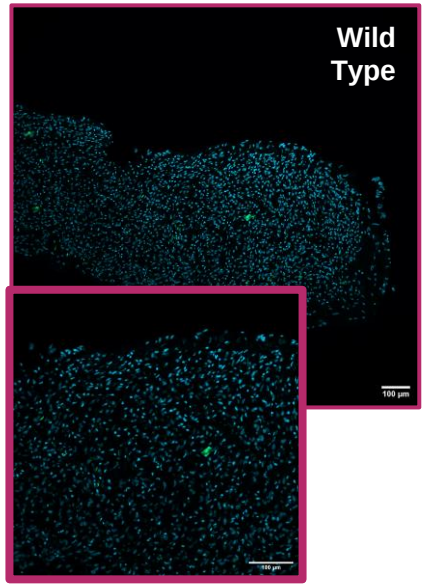


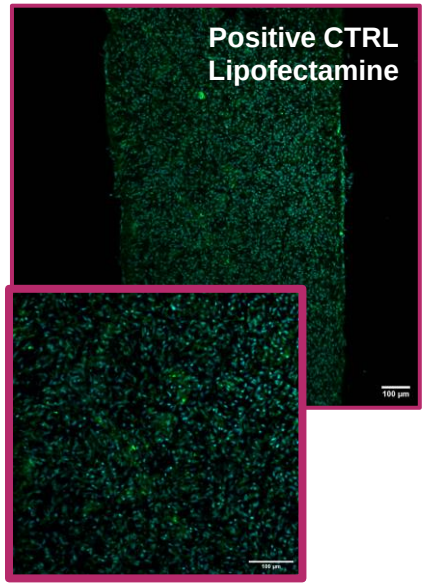
20×/0.45 NA air objective l**ens, Hoechst stain for nuclei (blue), green fluorescent protein (GFP) for protein expression (green), scale bar 50 um**

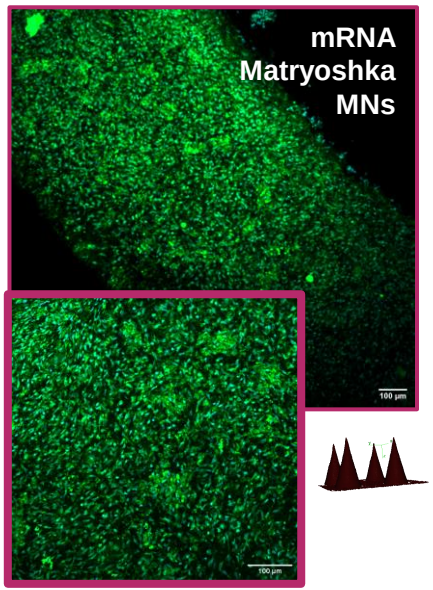
Cryosectioning of the HDE model 10 days after mRNA delivery.



Objective: Assess tissue morphology, microneedle penetration traces, and residual mRNA expression at 10 days post-application



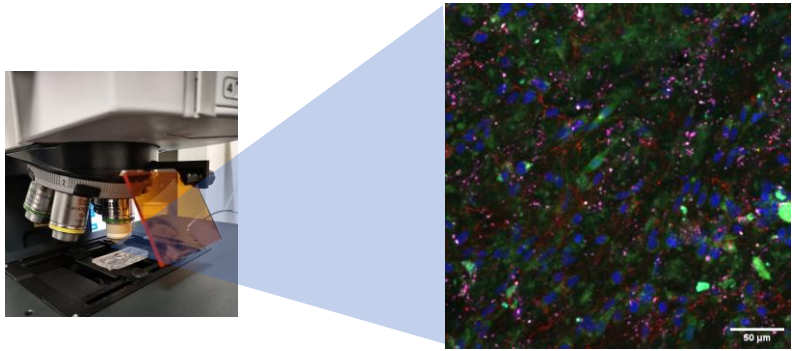


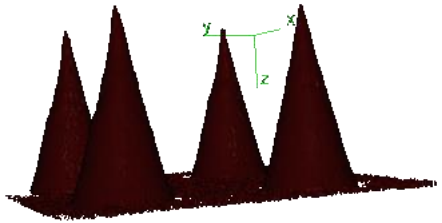


Cryo-sections (30 µm) of the HDE model, 10 days post mRNA delivery. Nuclei stained with Hoechst (blue); GFP expression (green). Acquired with a 10×/0.45 NA objective air lens. Scale bar: 100 µm. Magenta boxes indicate areas magnified in the upper panels.

Conclusion

- The HDE model proves to be a robust and reliable platform for transdermal formulation delivery studies.
- Microneedles efficiently deliver mRNA formulations, achieving detectable protein expression.
- Future developments will focus on integrating immune cells into the HDE model to assess the immunological response to mRNA vaccine delivery.
- Addition of keratinocytes will further improve the physiological relevance of the model.





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- Eng. Emilia Cioffi
- Ph.D. **Roberta Passariello**
- **Ph.D Valentina Mollo**
- **Ph.D Fabio Formiggini**
- Ph.D. Giorgia Imparato

200 µm


Bio-Logic Materials group


IIT@CABHC of Naples


In **collaboration with: Dr Stefano Persano** from UniMi and IEO for mRNA and LNP-mRNA synthesis

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