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# A Novel Micro Free-Flow Electrophoresis 3D printed Lab on a Chip for exosomes separation


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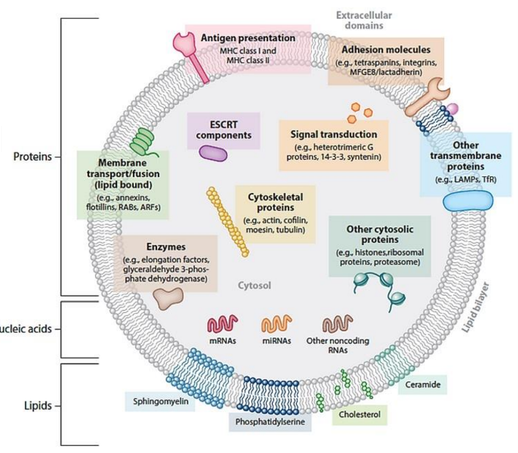
## Research context and motivation

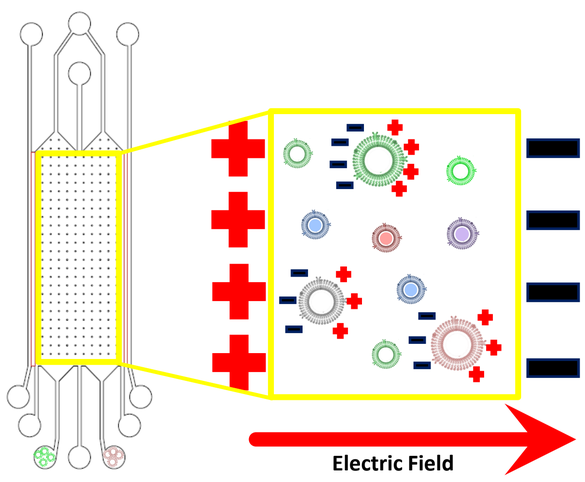
- Exosomes (30-150 nm) are nanosized lipid vesicles, containing DNA, mRNA, miRNA, lipids and short-chain peptides, secreted in the extracellular environment by different type of cells.
- They play a fundamental role in the intercellular communication, thus exosomes can be evaluated as potential biomarkes for non invasive disease diagnosis, prognosis and therapy.
- To date, exosomes separation technologies (ultracentrifugation-based, size-based, immuno-affinity capture-based and exosomes precipitation-based) from complex biological fluids are time-consuming, laborious with low purity efficiency.




- A Lab-on-chip (LOC) approach could offer several advantages to the separation of exosomes for analysis in the clinical practice. Microfluidic devices would allow to integrate in a cost-effective way a whole analytical process reducing its complexity, time of analysis, sample and reagents volumes.

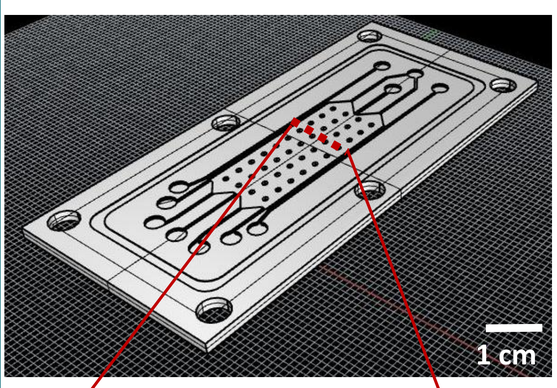
## Addressed research questions/problems

- Our goal is to develop a Lab-On-Chip device based on Micro Free-Flow Electrophoresis approach in order to achieve a rapid and accurate exosome isolation through a simple sample preparation and simple fabrication process.

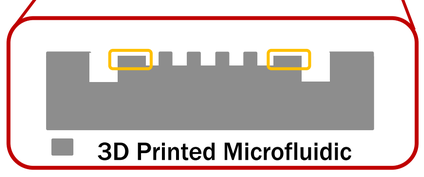



- Field Flow Electrophoresis (FFE) is a continuous, analytical separation technique used to separate a flowing stream of analytes, according to size and charge. A thin sample stream is introduced into a planar separation channel with buffer running in parallel. An electric field is applied perpendicularly across the separation channel, and charged analytes are deflected laterally based on their electrophoretic mobility.

- The device dimensions are 40 mm in width, 85 mm in length with an height of 2.8 mm.




| Feature | Dimensions [mm] |
|---|---|
| Separation chamber | Width 13<br>Length 30<br>Height 0.1 |
| Elliptic pillars | Width 1<br>Height 0.1 |
| Partioning bars | Width 0.5<br>Length 30<br>Height 0.05 |
| Electrodes channels / inlet and outlet channels | Width 0.5<br>Height 0.5 |
| Inlet/Outlet diameter | 3.20 |



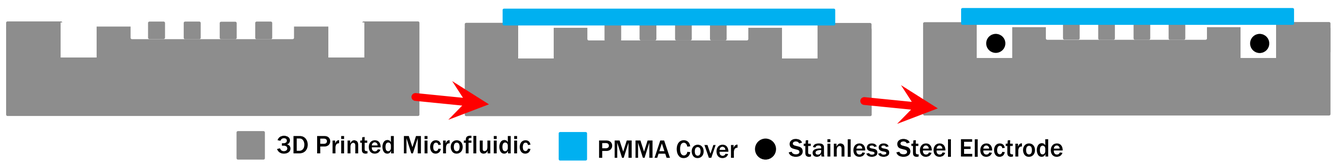

➢ **Partitioning bars** are integrated between the separation chamber and electrode channels to hinder gas bubbles from entering the separation channel and allowing a stream stability during the experiment.

## Submitted and published works


- Canavese G., Ancona A., Racca L., Canta M., Dumontel B., **Barbaresco F.**, Limongi T., Cauda V., Nanoparticle-assisted ultrasound: a special focus on sonodynamic therapy against cancer, Chemical Engineering Journal, 340, (2018) pp.155-172.
- **Barbaresco F.**, M. Cocuzza, F. C. Pirri, S. L. Marasso, A Novel Micro Free-Flow Electrophoresis 3D printed Lab on a Chip for exosomes separation, Micro & Nano Engineering Journal, 2019


## Novel contributions

- **µFFE Fabrication Process**

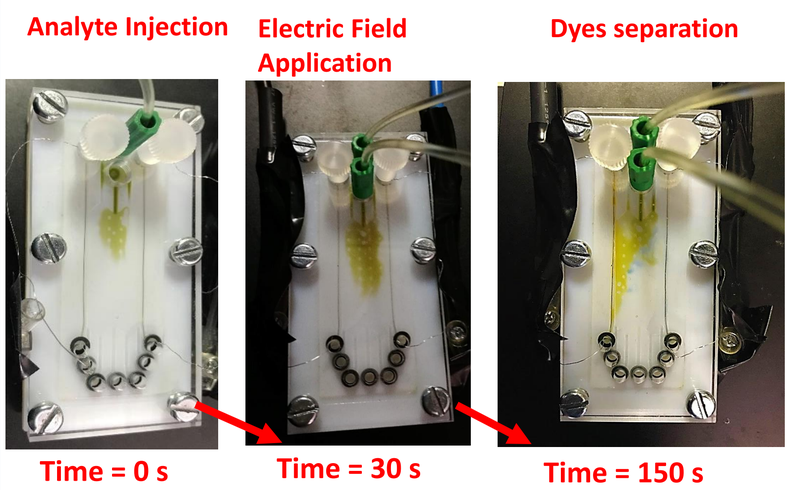



The bottom of the µFFE device is obtained starting from a stereolithographic (STL) file by PolyJet 3D Printing (Objet 30) using the Verowhiteplus RGD835 resin. The top of the device consists of a slice of PMMA 750 µm thick, where inlets and outlets have been milled through a CNC milling machine. The 3D printed part is sealed with the PMMA cover exploiting a thermal process using PEGDA 575 with 1 % IRGA CURE 819. The PEGDA resin is placed along the external channel around the device and then it is left one hour on a hot plate at 70 °C. Finally, stainless steel wire electrodes are manually inserted in the lateral channels.

- **µFFE Bonding**

Leakage tests are performed by filling the device with orange food dye. A uniform and irreversible bonding is then verified.

## Adopted methodologies

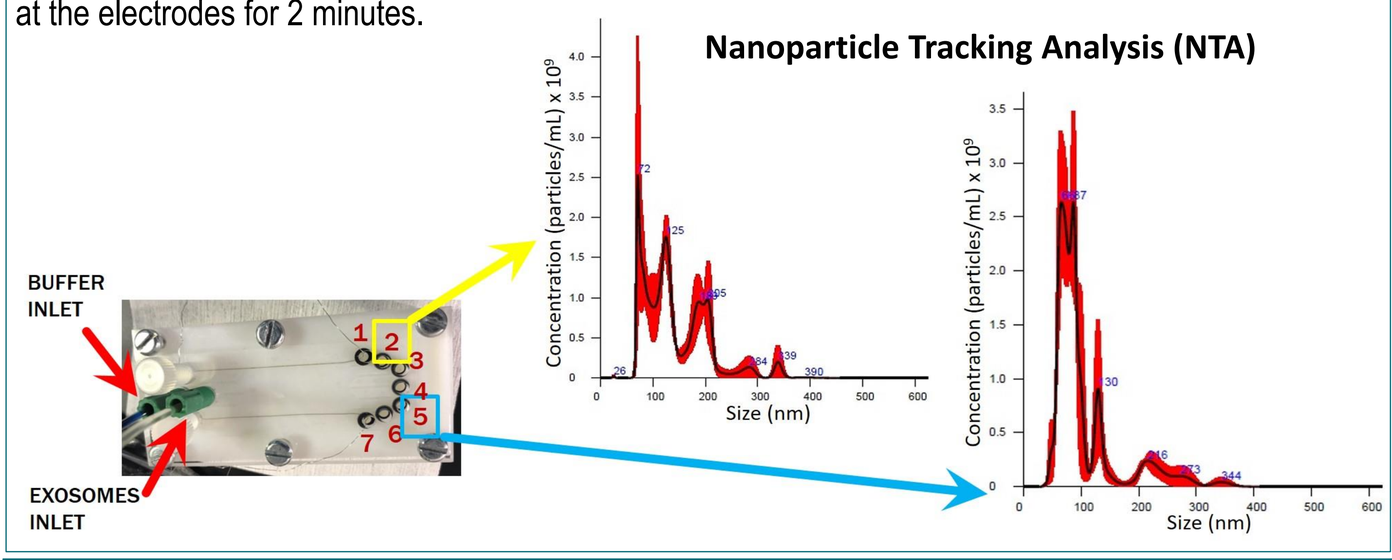



- **Dyes Separation test**

HEPES 20 mM (pH 7) as buffer solution and Loading Buffer 6X as analyte solution have been pumped into the device using a syringe pump at 2 µL/min and 1 µL/min respectively. By applying 200 V at the electrodes for 3 minutes, it has been possible to observe the separation between the different dyes into the main chamber.

- **Exosomes Separation Test**

Exosomes collected from Fetal Bovine Serum are separated in the device main chamber by setting 1 V at the electrodes for 2 minutes.




## Future work

- Design a new enhanced geometry
- Test the µFFE device for the separation of exosomes from different biological fluids

## List of attended classes

- 01REIRR - Terapie avanzate (nanomedicina, terapia genica e cellulare) in chirurgia (29/06/2018,4 cfu)
- 01LXBRW - Life Cycle Assessment (LCA) (02/07/2018, 5 cfu)
- 02LCRKG – Fisica di superfici ed interfacce (05/10/2018, 3 cfu)
- 01QORRV - Writing Scientific Papers in English (27/06/2018, 3 cfu)
- 01RISRV - Public Speaking (01/09/2018, 1 cfu)
- 02LWHRP - Communication (04/09/2018, 1 cfu)
- 01TGSIY - Advanced diagnostics for reactive flows (didattica di eccellenza vp) (09/04/2019, 4 cfu)
- 01SYBRV - Research integrity (04/06/2019, 1 cfu)
- 01SWQRV - Responsible research and innovation, the impact on social challenges (05/06/2019, 1 cfu)
- 01SWPRV - Time management (06/06/2019, 1 cfu)
- 02RHORV - The new Internet Society: entering the black-box of digital innovation (06/06/2019, 1 cfu)
- 01TGKI - Physical Chamistry of Materials for Nanotechnologies (21/06/2019, 7 cfu)
- 01SDDKI - Additive manufacturing polimerico (19/07/2019, 4 cfu)

POLITECNICO DI TORINO

PhD program in
**Electrical, Electronics and Communications Engineering**