Transitioning hybrid lipid polymer Nanoparticles from lab to bulk scale production with microfluidic

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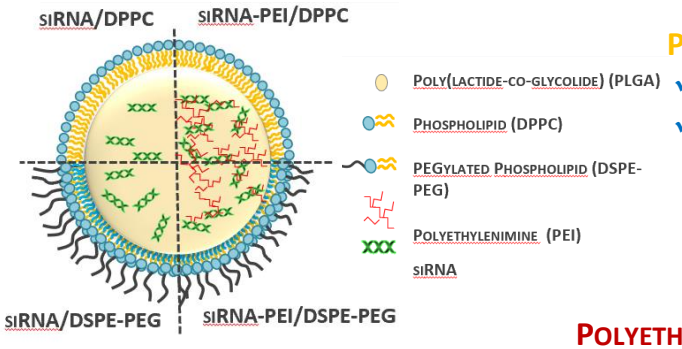
ACROSS DISCIPLINES

hNPs from lab to bulk scale

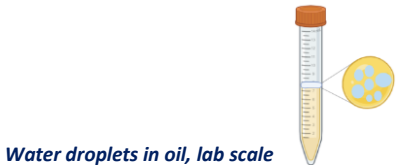


d'Angelo et al., J. Aer. Med. Pulm. Drug Deliv. , 31:170-181, 2018
Conte et al., ACS Appl. Mater. Interfaces 14, 7565−7578, 2022

Poly(lactide-co-glycolide) (PLGA)
- ✓ Protection of the entrapped siRNA
- ✓ Sustained release

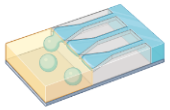
Phospholipid (DPPC)
- ✓ Enhanced biocompatibility
- ✓ Tuning NP interactions with biological barriers

Polyethylenimine (PEI)
- ✓ Delivery platform for nucleic acid therapeutics
- ✓ Assisting siRNA entrapment, release and cell uptake



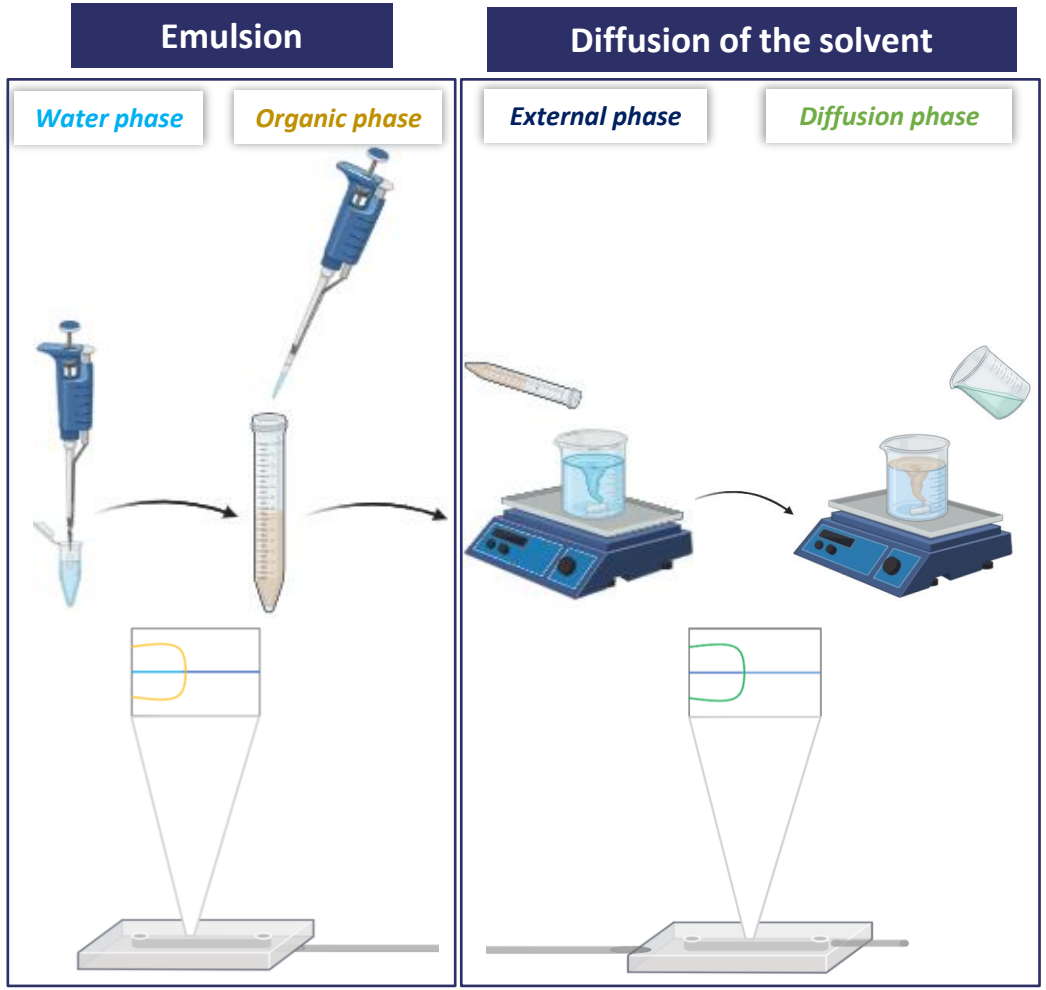
Water droplets in oil, lab scale

Limitations of conventional technique:
- ✓ Poor scalability
- ✓ High batch-to-batch variability.



Microfluidic chip, partial cross-section

Advantages of Microfluidic platform:
- ✓ High scalability
- ✓ Good control of droplet/bubble size and modelling the flow process



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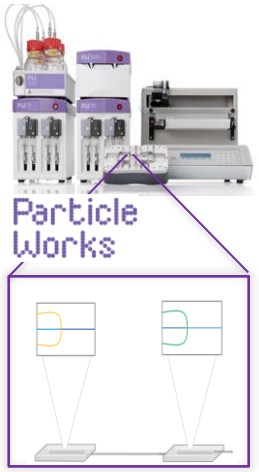
hNPs from lab to bulk scale: *results*

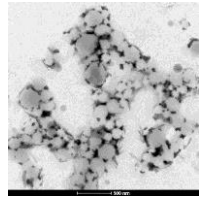
Characterization of DPPC@PLGA hNPs produced with ANP



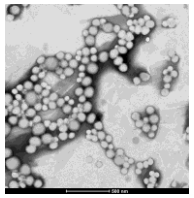
	TFR	FRR O:W:D	PVA:PLGA W:W	Centrifuge condition RPM/min	Size nm± SD	PDI mean± SD	ζpot mV± SD	Yield %± SD
DPPC@PLGA_PVA8_10000_CTR	-	-	1:8	10000/20	162.3±3.6	0.20±0.01	-29.2±0.6	40.5±5.4
DPPC@PLGA_ANP_7000_1X	1X	2:1:10	-	7000/20	166.5±19.5	0.21±0.08	-32.3±5.2	37.1±2.7
DPPC@PLGA_ANP_7000_2X	2X	2:1:10	-	7000/20	331.3±39.4	0.31±0.1	-29.9±1.5	34.3±7.2
DPPC@PLGA_PVA2_ANP_7000_1X	1X	2:1:10	1:2	7000/20	204.6±3.6	0.11±0.02	-27.3±3.1	24.5±0.1
DPPC@PLGA_PVA4_ANP_7000_1X	1X	2:1:10	1:4	7000/20	205.1±0.6	0.17±0.01	-33.5±4.6	n.d.
DPPC@PLGA_PVA6_ANP_7000_1X	1X	2:1:10	1:6	7000/20	184.4±1.6	0.10±0.03	-36.1±2.5	n.d.
DPPC@PLGA_PVA6_ANP_10000_1X	1X	2:1:10	1:6	10000/20	168.4±3.7	0.15±0.05	-35.8±4.5	39.4±2.6
DPPC@PLGA_PVA8_ANP_10000_1X	1X	2:1:10	1:8	10000/20	162.7±10.2	0.11±0.02	-34.3±7.1	36.6±2.8
DPPC@PLGA_PVA8_ANP_10000_2X	2X	2:1:10	1:8	10000/20	176.4±7.3	0.14±0.02	-24.7±2.3	38.5±4.2

TEM Images

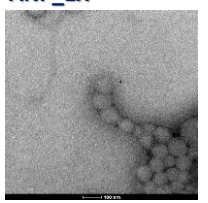
DPPC@PLGA_PVA Ctrl



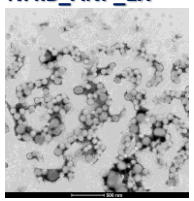
DPPC@PLGA_PVA NFkB_Ctrl



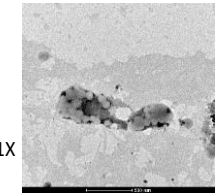
DPPC@PLGA_PVA ANP_1X



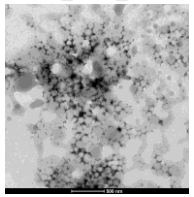
DPPC@PLGA_PVA NFkB_ANP_1X



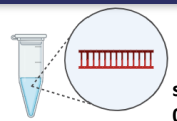
DPPC@PLGA_PVA ANP_2X



DPPC@PLGA_PVA NFkB_ANP_2X



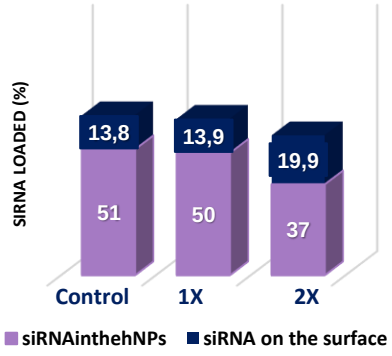
siRNA loaded hNPs produced by ANP



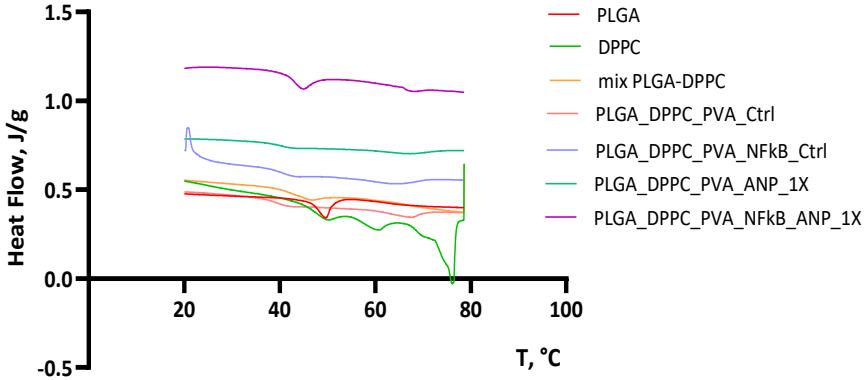
siRNA Nf-KB
0.2nmol/10mg

TFR	Size nm± SD	PDI mean± SD	ζpot mV± SD	Yield %± SD
-	227.2±29.3	0.25±0.05	-27.7±4.2	40.1±8.3
1X	174.3±8.8	0.12±0.02	-27.1±3.5	35.6±5.6
2X	158.2±3.3	0.16±0.03	-33.2±0.2	35.1±5.4

Encapsulation efficency (% siRNA)



Thermoanalytical analysis



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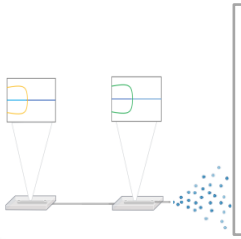
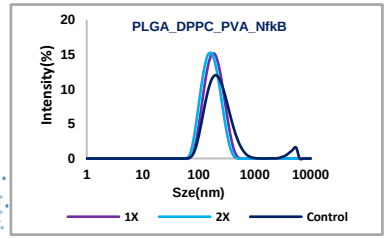
hNPs from lab to bulk scale: *conclusion and future perspectives*

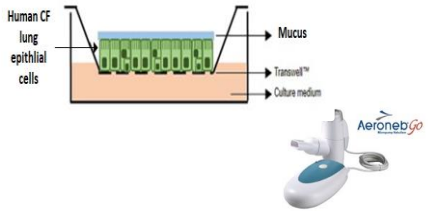
Conclusion





Overall characterization of hNPs produced with the ANP system support the successful transition of the emulsion solvent diffusion technique through the use of a two chip method

Ongoing studies

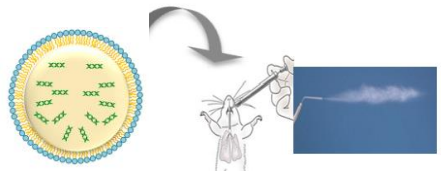


Next Generation Impactor (NGI)

Further studies will be carried out to:

- **Increase siRNA loading and validate the stability of the siRNA upon encapsulation with the ANP;**
- **Evaluate if the hNPs production with microfluidic may affect the ability to deposit in the airways and interact with the physiological environment**

Future studies



The optimized hNPs produced by microfluidic will be tested for tolerability and therapeutic efficacy in murine models.

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Università degli Studi di Napoli Federico II

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