Drug in a cell:

AN INNOVATIVE DEVICE FOR A MORE TOLERABLE CHEMOTHERAPY

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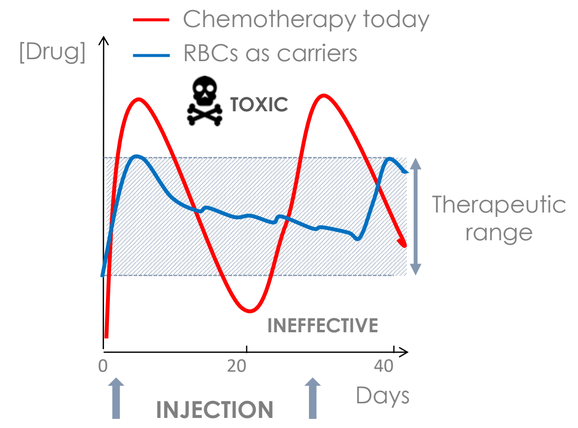
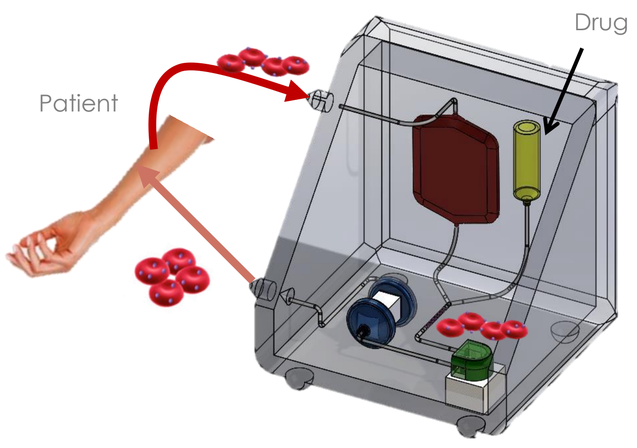
To modulate the pharmacokinetics and bio-distribution of chemotherapeutic agents is a promising strategy to improve the therapeutic efficacy minimizing the adverse reactions in cancer treatment. The use of Red Blood Cells (RBCs) as drug carriers is a challenging topic with potentially relevant clinical applications, including the prolongation of the time a drug is present in the body in its active form, and the reduction of toxicity due to a delayed release by RBCs [1, 2].

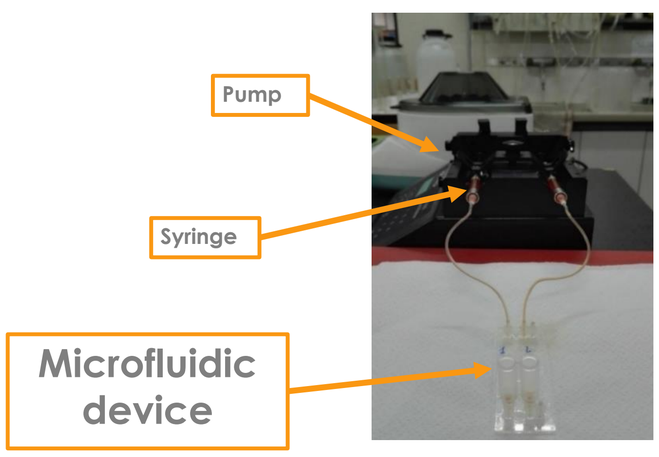


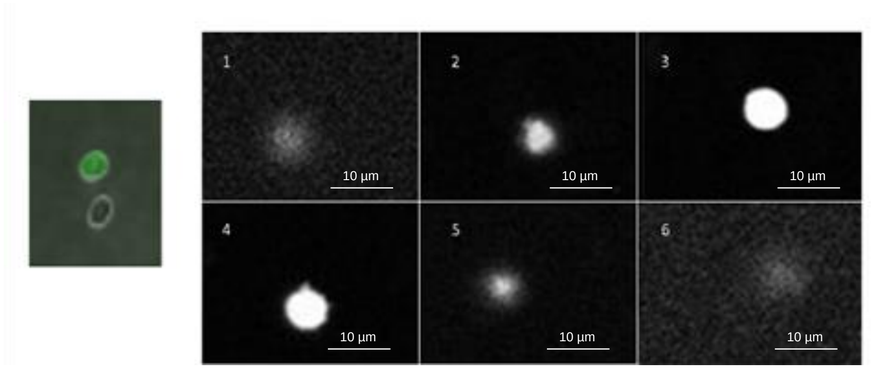


We demonstrated that fluid shear stress is able to temporary open the pores naturally present in RBCs membrane, allowing the diffusion of a molecule from its original solution to the RBCs cytoplasm [3,4].
The novelty of the method illustrated here is the possibility to use autologous RBCs in a device properly designed to be directly connected to the patient, minimizing the handling of blood by operator.

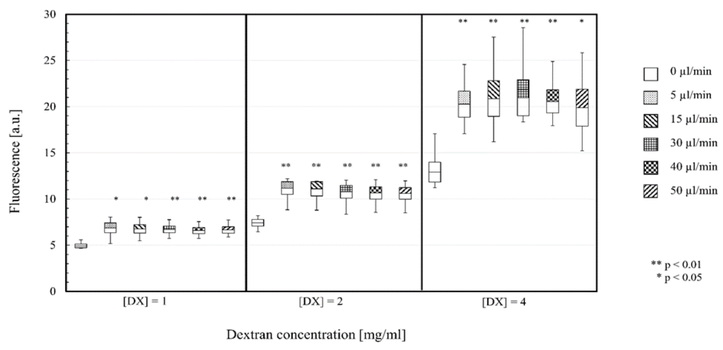
Preliminary tests were performed on human blood using a microfluidic channel with optimized dimensions. By setting the proper molecule concentration and fluid flow rate, it is possible to achieve more than 80% efficiency in the encapsulation. The presence of fluorescent probe molecules (FITC-Dextran, molecular weight 40 ÷ 150 kDa) inside the cells was confirmed both by confocal microscopy and by cytofluorimetric analysis, demonstrating also that RBCs maintained their physiological shape after microfluidic solicitation. A delicate balance needs to be maintained in order to achieve adequate encapsulation in relatively short times and avoid mechanical hemolysis of the cells.

DISPOSABLE CARTRIDGE TESTING

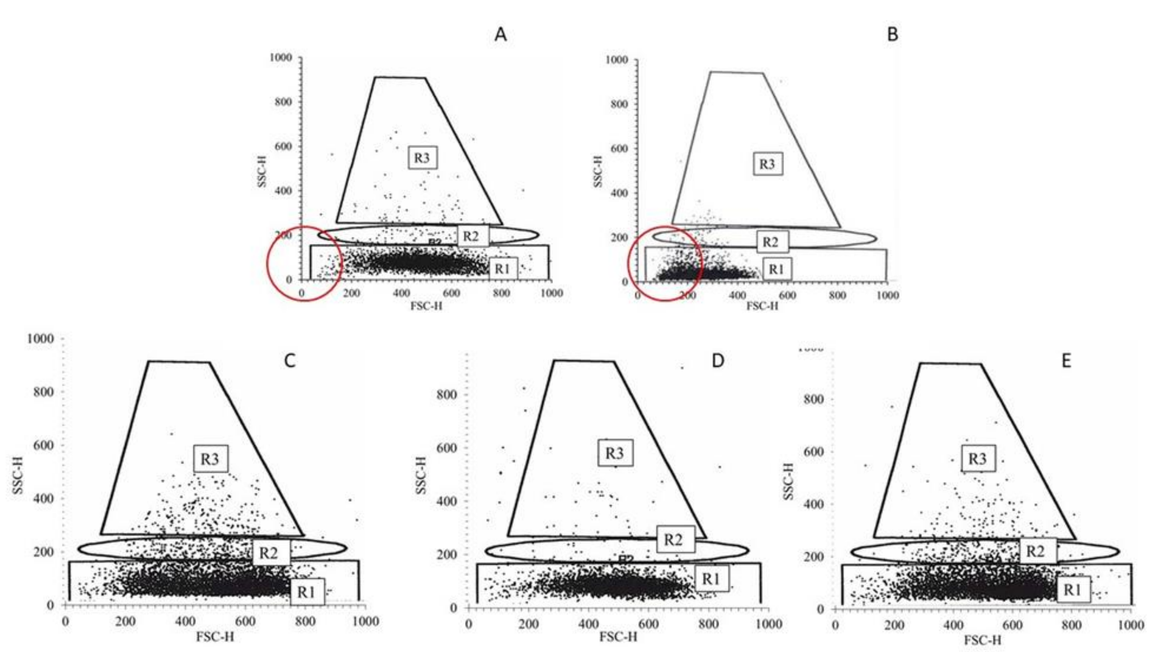




Left: confocal images of fluorescent RBCs after microfluidic solicitation.
Right: confocal images of FITC- Dextran encapsulated in a RBC. z-stack: 0.8 µm (top-bottom direction).



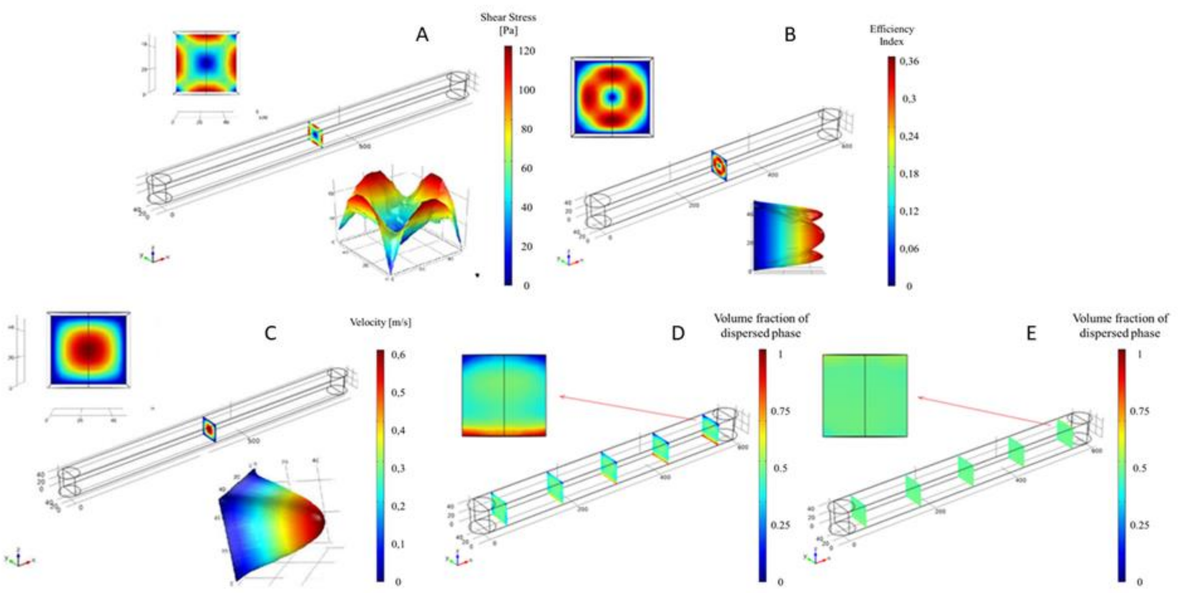
Fluorescence intensity for encapsulation tests in the microchannels.
Data are represented as mean, SD, minimum and maximum.



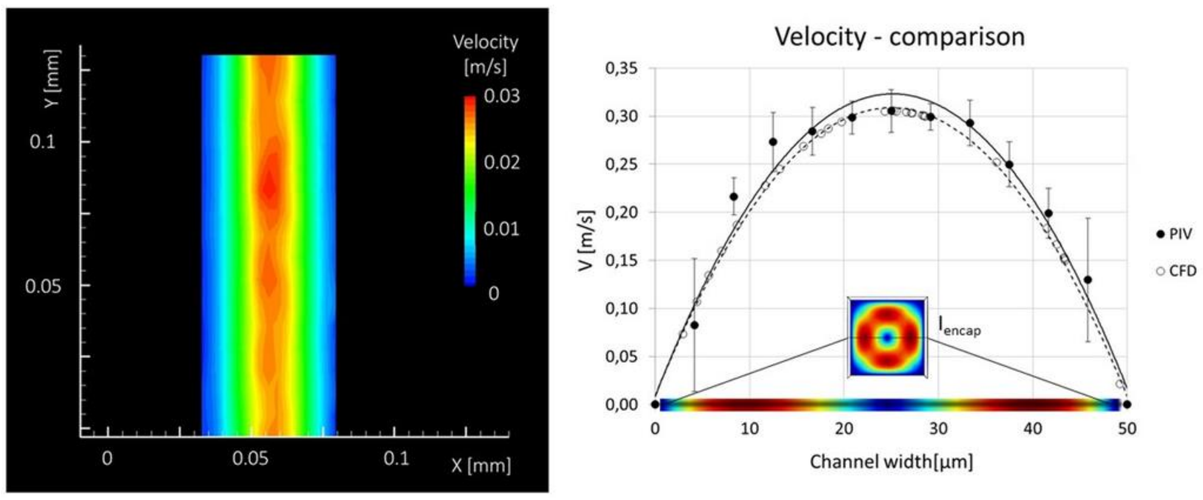
Flow cytometer analyses of different encapsulation conditions.
Top: red circles highlight the presence of fragments to investigate the effect of channel length (A: Lβ15; B: LLβ15).
Bottom: cytograms of cells solicited at different flow rates (C: Mβ0, D: Mβ15, E: Mβ40). The majority of the RBCs maintain its physiological biconcave shape (region R1), even if it is possible to distinguish the presence of echinocytes (region R3). R2 is a transition region.

Parameter	***B***	***Standard error***
Constant	21.101	14.608
Gender	*9.004*	*1.753*
MCV	*-8.026*	*1.634*
[DX]	*3.102*	*0.171*
RDW	1.944	0.809
MCHC	0.898	0.430
Haemoglobin	0.559	0.710
Channel length	0.187	0.308
Flow rate	*0.048*	*0.013*

Regression coefficient B and standard error of the multivariate linear regression for the estimation of the parameters effect on the geometric mean of the fluorescence.
Results from all experiments were considered.
Statistically significant parameters are reported in *Italic*



CFD model on a representative cross section for a simulation at 30 µl/min and Ht = 5%.
A: Shear stress; B: Efficiency encapsulation index calculated with Eq. 5; C: Velocity magnitude; D: Volume fraction of the dispersed phase for low flow rate (0.25 µl/min); E: Flow rate on a flow section close to the outlet.



Left: colour map of micro-PIV velocity for a flow rate of 30 µl/min and Ht = 1%.
Right: velocity comparison of the micro-PIV (solid line, data are shown as mean and SD on 100 image pairs) and CFD (dashed line) for the same fluid dynamic conditions. l_{encap} is also reported at varying length.

Based on these promising results, the validation of the prototype is currently ongoing by performing *in vitro* loading and release test on chemotherapy drugs.

REFERENCES

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[3] Casagrande G, Arienti F, Mazzocchi A, Taverna F, Ravagnani F, Costantino ML, Application of Controlled Shear Stresses on the Erythrocyte Membrane as a New Approach to Promote Molecule Encapsulation, Artif Organs., 2016, 40(10), 959–70.
[4] Piergiovanni M, Casagrande G, Taverna F, Corridori I, Frigerio M, Bianchi E, Arienti F, Mazzocchi A, Dubini G, Costantino ML, Shear-Induced Encapsulation into Red Blood Cells: A New Microfluidic Approach to Drug Delivery, Ann Biomed Eng (2019).