Drug in a cell:

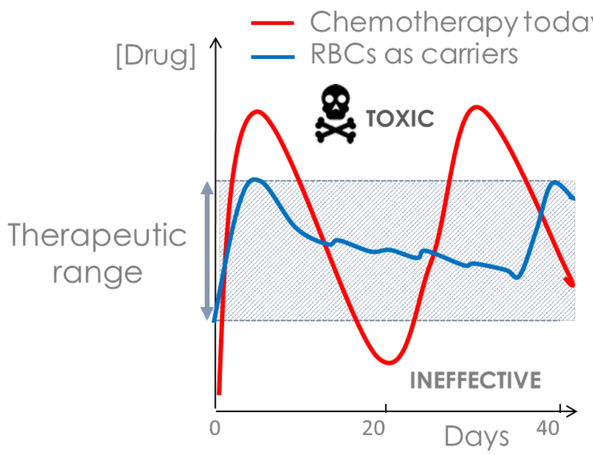
AN INNOVATIVE DEVICE FOR A MORE TOLERABLE CHEMOTHERAPY

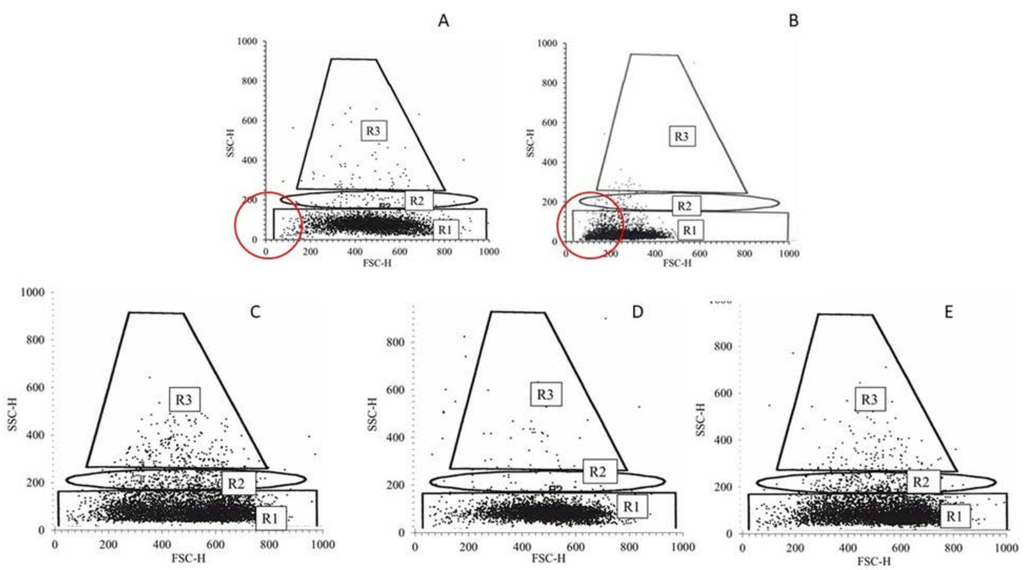
Monica Piergiovanni, Giustina Casagrande, Elena Bianchi, Clara Bernardelli, Maria Laura Costantino

Laboratory of Biological Structure Mechanics (LaBS), Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy -

Email: monica.piergiovanni@polimi.it

The use of Red Blood Cells (RBCs) as drug carriers is a challenging topic with relevant applications in clinical practice [1, 2], especially in the treatment of cancer, where a sustained release of the drug can be a valid alternative to immunotherapy. The use of biological drug carriers – autologous RBCs among few others – will allow for a prolonged residence time in the body and for a reduction in the toxic peak, usually due to standard overdosing procedures.

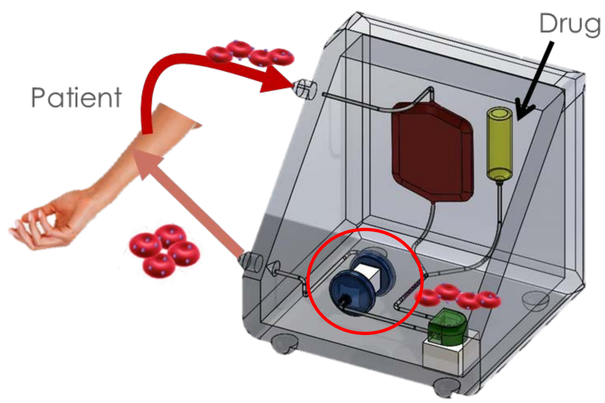


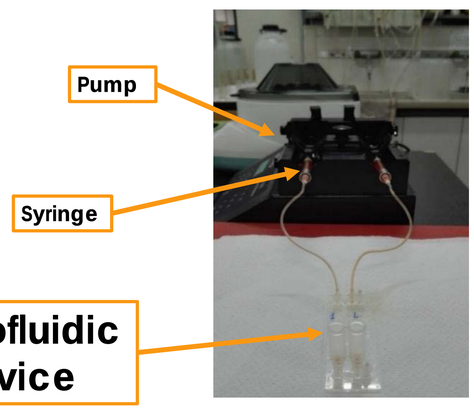


Flow cytometer analyses of different encapsulation conditions, each dot corresponds to a single event (cell or debris). Top panel, high number of fragments, characterized by small dimensions) can be seen in the red circles (mainly in B), showing the effect of channel length (A: Lβ15; B: LLβ15). Bottom panel, physiological cytograms corresponding to different flow rates (C: Mβ0, D: Mβ15, E: Mβ40). Three different zones can be identified in the cytograms: R1 corresponds to normal (biconcave shaped) RBCs, R3 corresponds to echinocytes, while R2 is a transition region.

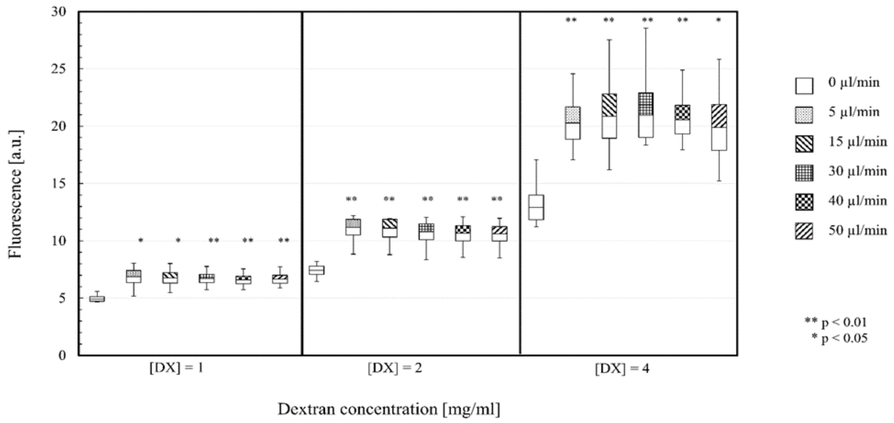
Previous work performed in our laboratory [3,4] proved that the fluid shear stress is able to temporary open the pores naturally present in RBCs membrane and allow for the diffusion of a molecule from the solution to the RBC cytoplasmic fluid. Thus, part of the drug can be "hidden" inside the cells and gradually released when the RBCs naturally break up.

DISPOSABLE CARTRIDGE TESTING





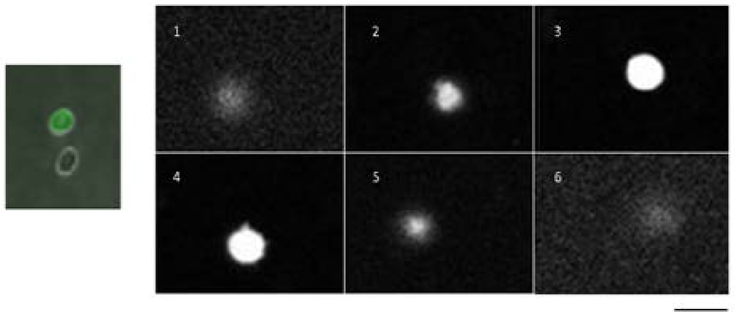
Experimental encapsulation tests were performed on human blood obtained from donors using a microfluidic channel with optimized dimensions. By setting the proper molecule concentration (FITC-Dextran, molecular weight 40 ÷ 150 kDa) and fluid flow rate, it is possible to achieve more than 80% efficiency in the encapsulation. Confocal microscopy images confirmed the presence of the fluorescent molecule inside the cells, while cytofluorimetric analysis confirmed that the cells maintained their physiological shape. A delicate balance needs to be maintained in order to achieve adequate encapsulation in relatively short times and avoid mechanical hemolysis of the cells.



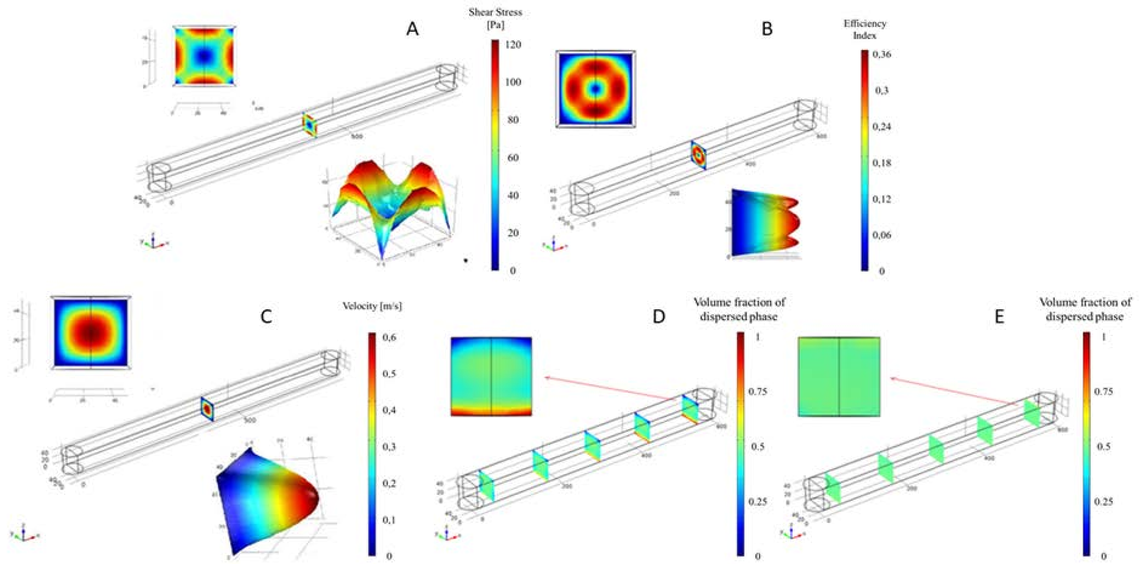
Fluorescence intensity for encapsulation tests in the microchannels. Data are represented as mean, SD, minimum and maximum.

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*

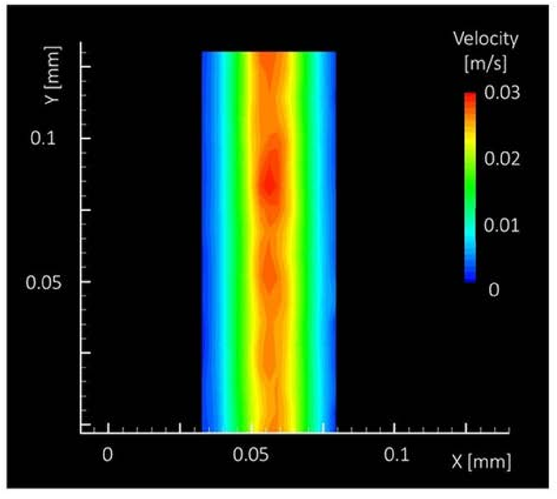
Regression coefficient B and standard error of the multivariate linear regression for the estimation of the parameters effect on the geometric mean of the fluorescence. Results from all experiments were considered. In Italic the statistically significant parameters.

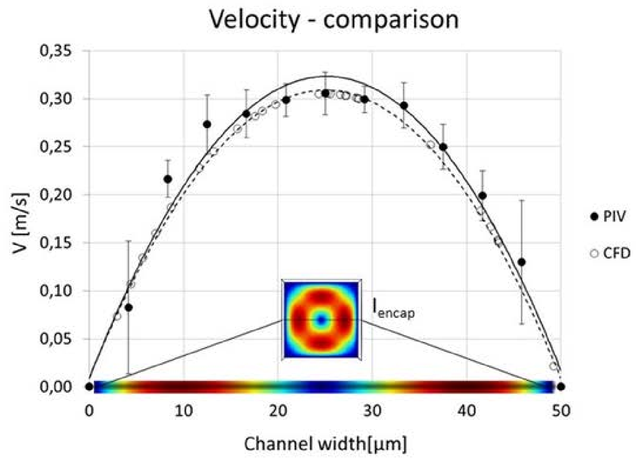


Confocal microscopy. Left panel: fluorescent images of RBCs, numbers refer to top-bottom direction. Right panel: confocal images of FITC dextran encapsulated in a RBC from sample Mγ30. Scale bar is 10 µm and the z-distance between the images is 0.8 µm.



Results of the CFD model on a representative cross section for a simulation at 30 µl/min and Ht = 5%. Shear stress (A), efficiency Encapsulation index (B) calculated with Eq. 5, velocity magnitude (C). Volume fraction of the dispersed phase for low flow rate (D. 0.25 µl/min) and high flow rate (E. 30 µl/min) on a flow section close to the outlet.





On the left, colour map of micro-PIV velocity for a flow rate of 30 µl/min and Ht = 1%. On the 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.

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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).