Cell Membrane Fragment-Wrapped Parenteral Nanoemulsions: A New Drug Delivery Tool to Target Gliomas

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Supplementary materials

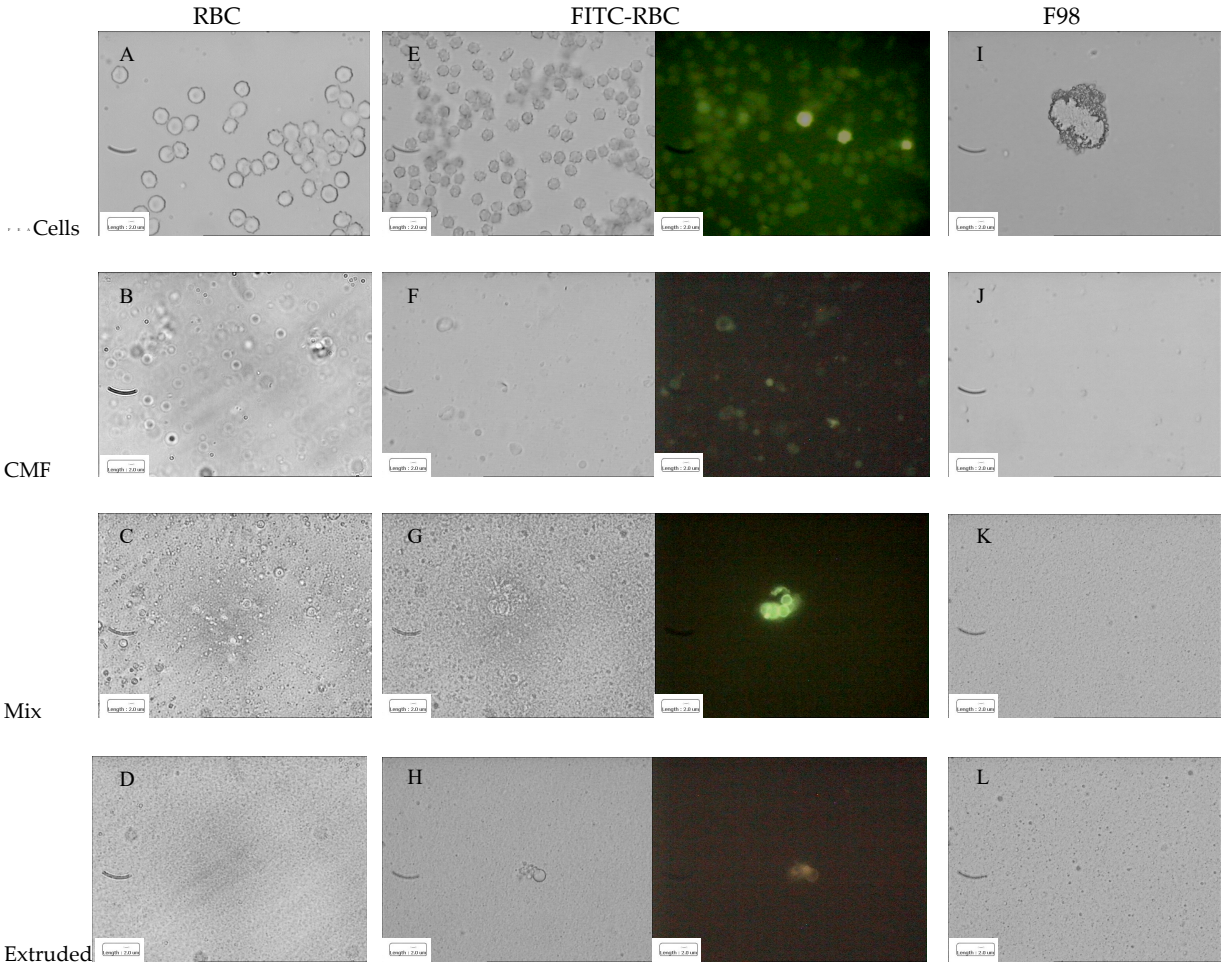
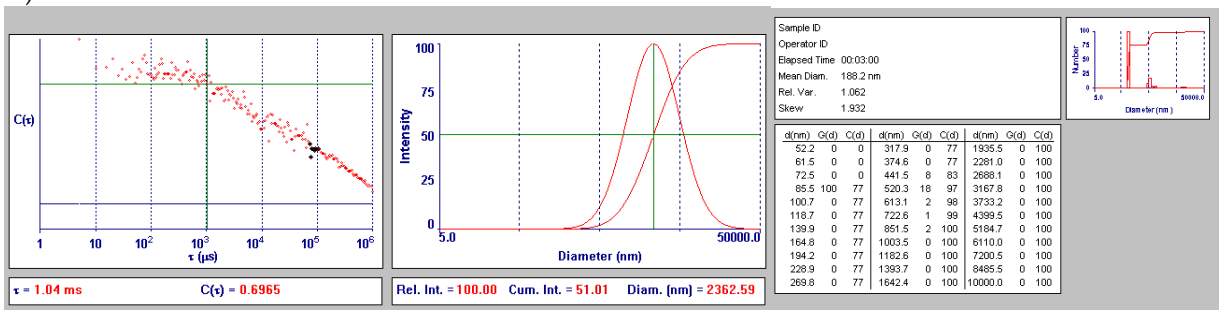


Figure S1. Optical microscopy of IL wrapping process with RBC and F98 CMF. A-D: RBC cells, CMF, CMF + IL mix, CMF/IL mix after extrusion and resuspension. E-H: FITC–RBC cells, CMF, CMF + IL mix, CMF/IL mix after extrusion and resuspension. I-L: F98 cells, CMF, CMF + IL mix, CMF/IL mix after extrusion and resuspension. Paired fluorescence and normal-light images are reported for FITC-labelled RBC. Abbreviations: CMF: cell membrane fragments; FITC: fluorescein isothiocyanate; IL: Intralipid® 10%; RBC: red blood cells.

A) F98 CMF



B) RBC CMF

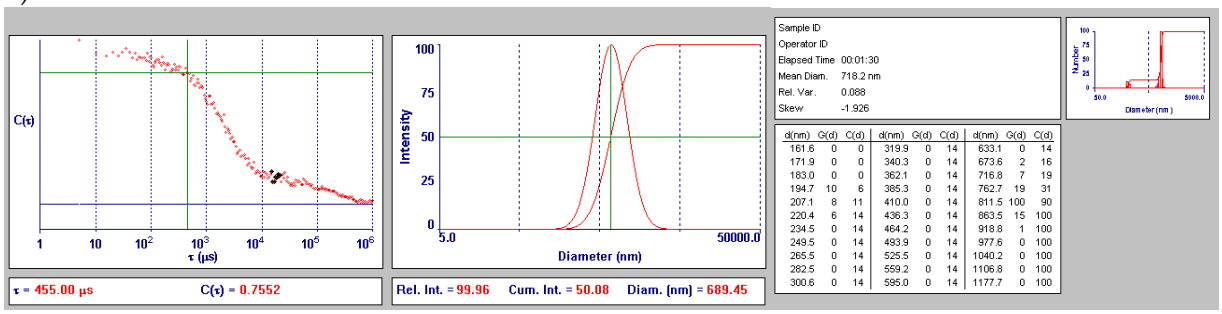


Figure S2. Raw DLS data of F98 (A) and RBC (B) CMF: autocorrelation curve; lognomal size distribution; MSD summary by number of particles. For MSD lecithin was hypothesized as the main component of CMF, with a refractive index=1.459 (Bansal, N.; Truong, T.; Bhandari, B. Feasibility study of lecithin nanovesicles as spacers to improve the solubility of milk protein concentrate powder during storage. *Dairy Sci. Technol.* **2017**, *96*, 861–872, doi:10.1007/s13594-016-0307-0). Abbreviations: CMF: cell membrane fragments; DLS: dynamic light scattering; MSD: multimodal size distribution; RBC: red blood cells.

Table S1. Ion pair characterization. Percentage purity was calculated as the percentage ratio between measured and theoretical drug mg_{eq} for each mg ion pair. Abbreviations: AOT: sodium docusate; mg_{eq}: mg equivalent.

Drug	Ion pair	Molar ratio	mg_{eq} drug / 1 mg ion pair		% purity
			Theoretical	Measured	
Doxorubicin hydrochloride	Doxorubicin–AOT	1:1	0.61	0.56	91.8
Irinotecan hydrochloride	Irinotecan–AOT	1:1	0.64	0.57	89.1
Cisplatin	Cisplatin–AOT	1:2	0.28	0.068	24.3

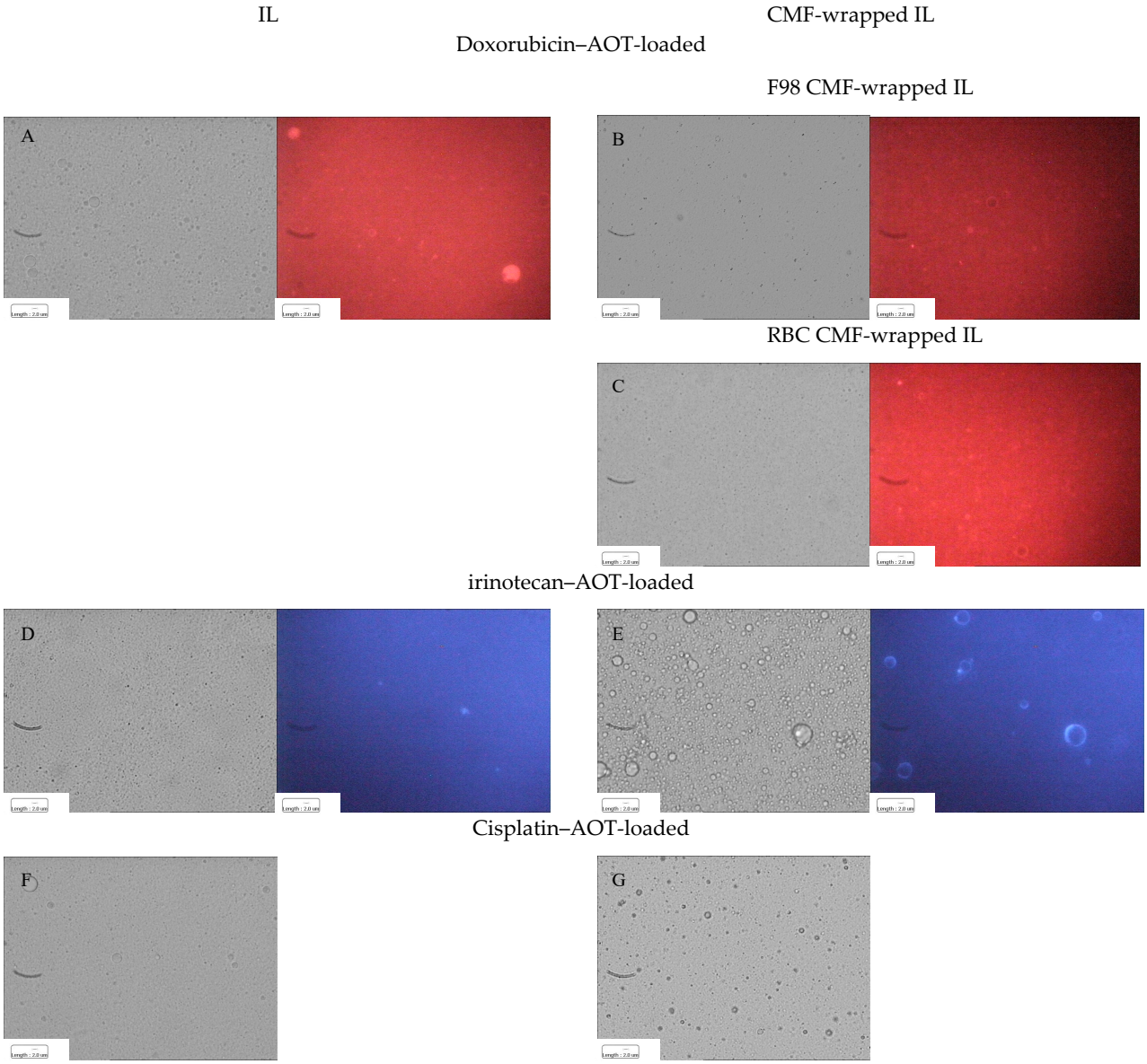


Figure S3. Optical microscopy (florescence and normal light) of drug (doxorubicin–AOT, irinotecan–AOT, cisplatin–AOT) loaded, unfunctionalized and RBC and F98 CMF-wrapped nanoemulsions. A-C: doxorubicin–AOT loaded IL: unfunctionalized, F98 CMF wrapped, RBC CMF wrapped; D-E: irinotecan–AOT-loaded IL: unfunctionalized, F98 CMF-wrapped; F-G: cisplatin–AOT-loaded IL: unfunctionalized, F98 CMF-wrapped. Paired fluorescence and normal-light images are reported for doxorubicin–AOT and irinotecan–AOT-loaded formulations. Abbreviations: AOT: sodium docusate; CMF: cell membrane fragments; RBC: red blood cells.

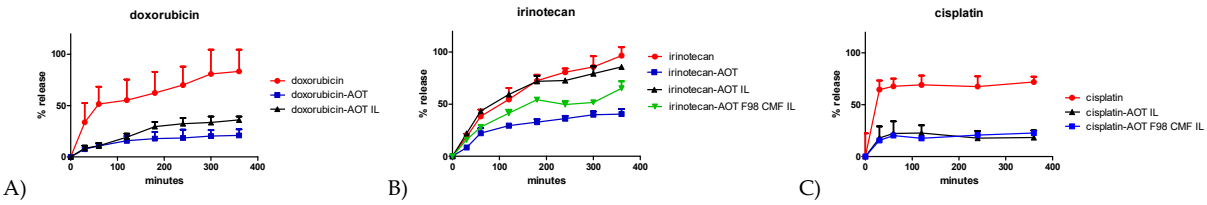


Figure S4. Drug (doxorubicin, irinotecan, cisplatin) release from doxorubicin–AOT, irinotecan–AOT, cisplatin–AOT loaded nanoemulsions. A: Doxorubicin; B: irinotecan; C: cisplatin. Abbreviations: AOT: sodium docusate; CMF: cell membrane fragments.

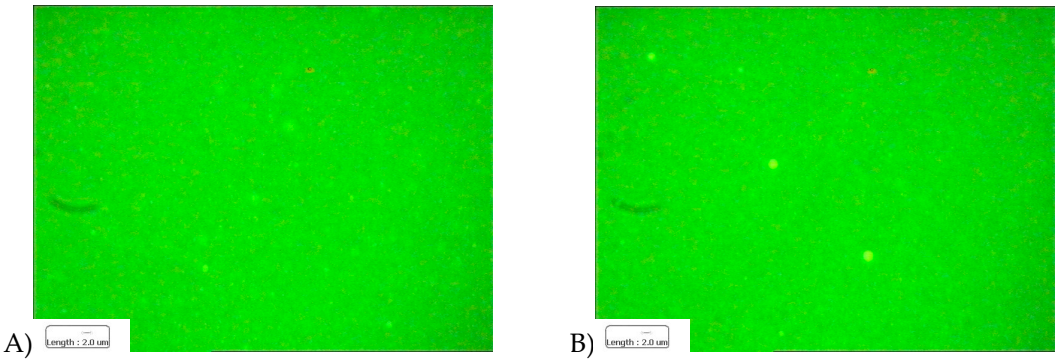


Figure S5. Fluorescence microscopy of 6-cum labelled nanoemulsions. A: IL; B: F98 CMF-wrapped IL. 630x magnification. Abbreviations: 6-cum: 6-coumarin; CMF: cell membrane fragments; IL: Intralipid® 10%.

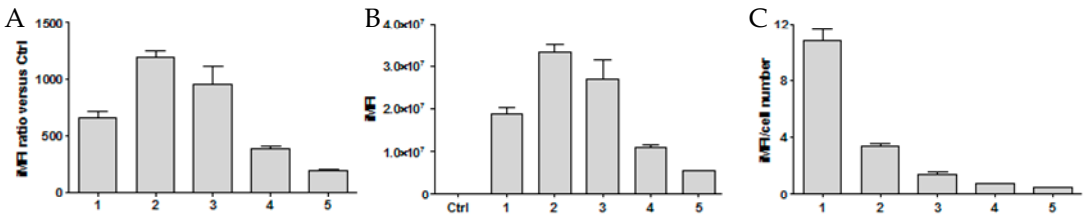
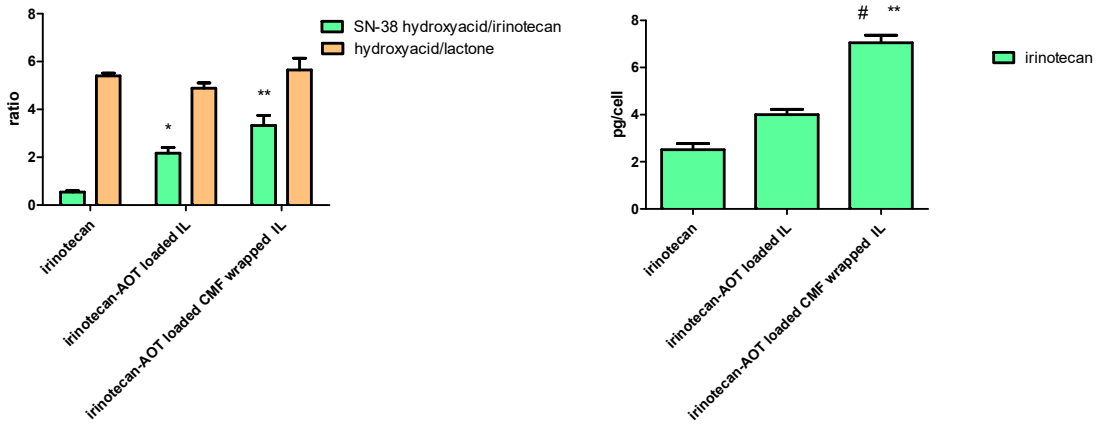


Figure S6. Flow cytometry internalization of 6-cum labelled IL in F98 cells, with different IL/cell ratio. A: iMFI ratio vs ctrl (untreated cells); B: iMFI; C: iMFI/cell number. Abbreviations: 6-coumarin; IL: Intralipid® 10%; iMFI: integrated mean fluorescence intensity. Conditions: 1) 1 µL IL *vs* 1 x 10^5 cells ; 2) 1 µL IL *vs* 5 x 10^5 cells; 3) 1 µL IL *vs* 1 x 10^6 cells; 4) 1 µL IL *vs* 5 x 10^6 cells; 5) 1 µL IL *vs* 1 x 10^7 cells.



A) B)

Figure S7. Irinotecan lactone/hydroxyacid transition, SN38 conversion and internalization within 2D-cultured F98 cells. A: Cell medium; B: cells. Abbreviations: AOT: sodium docusate; CMF: cell membrane fragments; IL: Intralipid® 10%; SN38: 7-ethyl-10-hidroxy-campthotecin. Statistical analysis: * $p<0.05$ *vs* irinotecan; ** $p<0.01$ *vs* irinotecan; # $p<0.05$ vs irinotecan-AOT loaded IL.

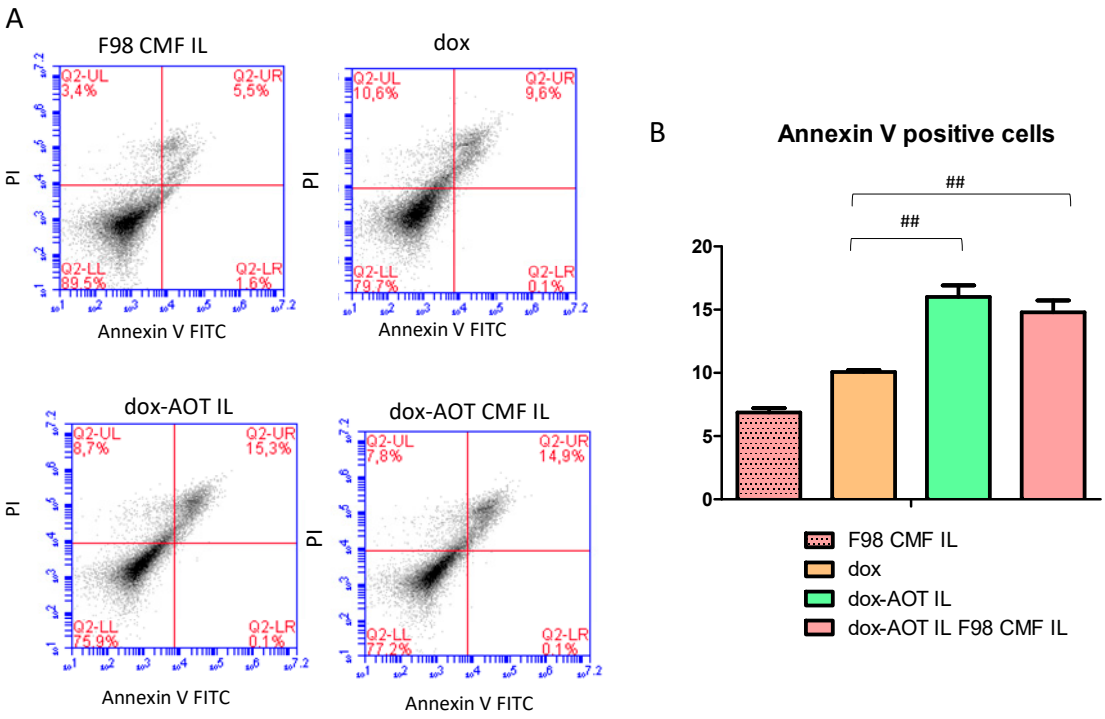


Figure S8. Apoptosis in F98 cells treated with F98 CMF-wrapped IL (F98 CMF IL), free doxorubicin (dox), doxorubicin–AOT-loaded IL (dox-AOT IL), and doxorubicin–AOT-loaded F98 CMF-wrapped IL (dox-AOT F98 CMF IL). (A) The flow cytometry profiles of a representative experiment in Annexin V/PI-stained cells at 24 hours are shown. Q1-LL = live (annexin V−/PI−), Q1-UL = necrosis (annexin V−/PI+), Q1-LR = early stage of apoptosis (annexin V+/PI−), Q1-UR = late stage of apoptosis (annexin V+/PI+), and (B) histograms reporting cytofluorimetric analysis of annexin V/PI staining in F98 treated sublines. Early and late apoptosis were expressed as means ± SEM of three independent experiments. Abbreviations: AOT: sodium socusate; CMF: cell membrane fragments; dox: doxorubicin; IL: Intralipid® 10%; propidium iodide (PI). Statistical analysis: ## $p < 0.01$ *vs* F98 CMF IL treated cells.

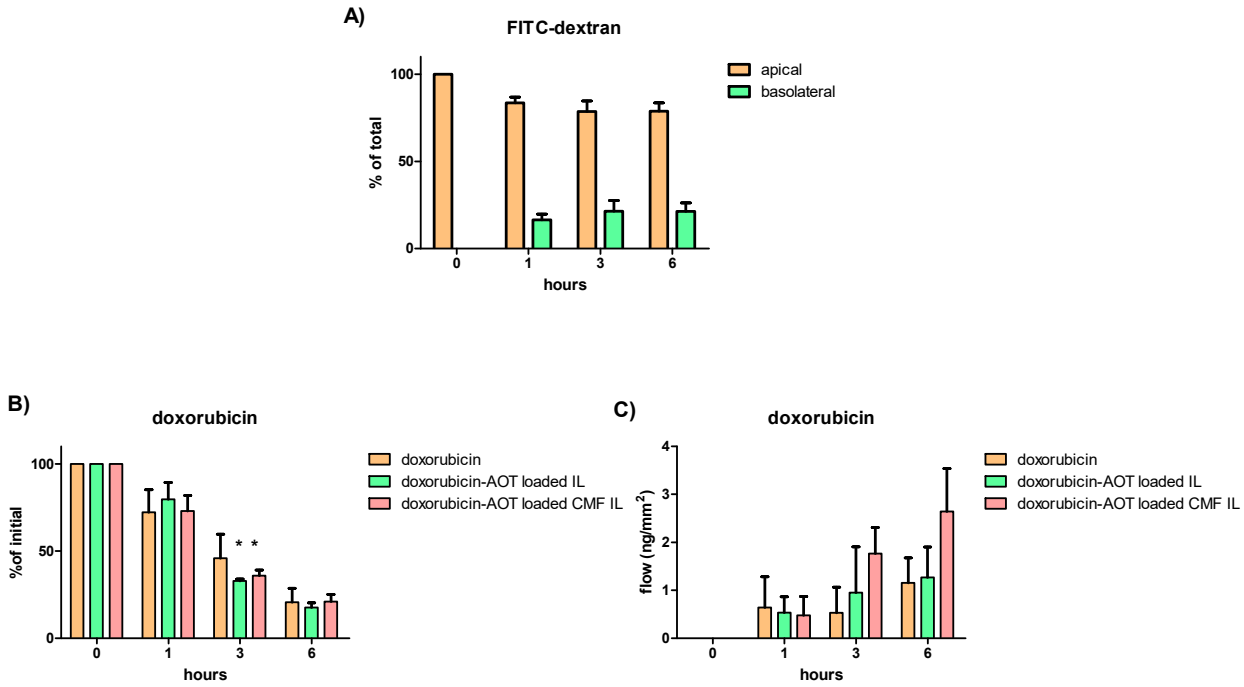
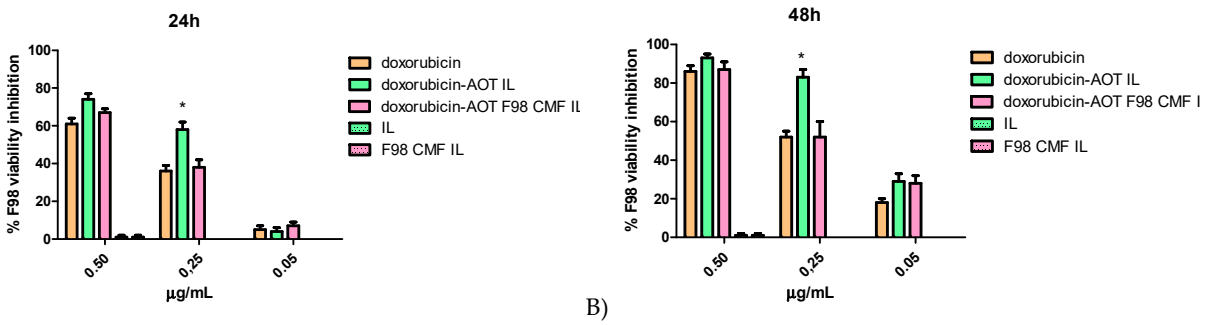


Figure S9. Transwell experiments with hCMEC/D3 monolayer. A) FITC-dextran permeation across the hCMEC/D3 monolayer. B) Doxorubicin in the apical chamber over time. C) Doxorubicin flow into the basolateral chamber over time. Abbreviations: AOT: sodium docusate; CMF: cell membrane fragments; FITC: fluorescein isothiocianate; hCMEC/D3: human cerebral microvascular endothelial cells; IL: Intralipid® 10%. Statistical analysis: * $p<0.05$ vs doxorubicin.



A) B)

Figure S10. MTT assay at 24 and 48 hours, in F98 cells, of doxorubicin-AOT loaded, unfunctionalized and F98 CMF-wrapped IL. A: 24 hours; B: 48 hours. Abbreviations: AOT: sodium docusate; IL: CMF: cell membrane fragments; Intralipid® 10%; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Statistical analysis: * $p<0.05$ *vs* doxorubicin.

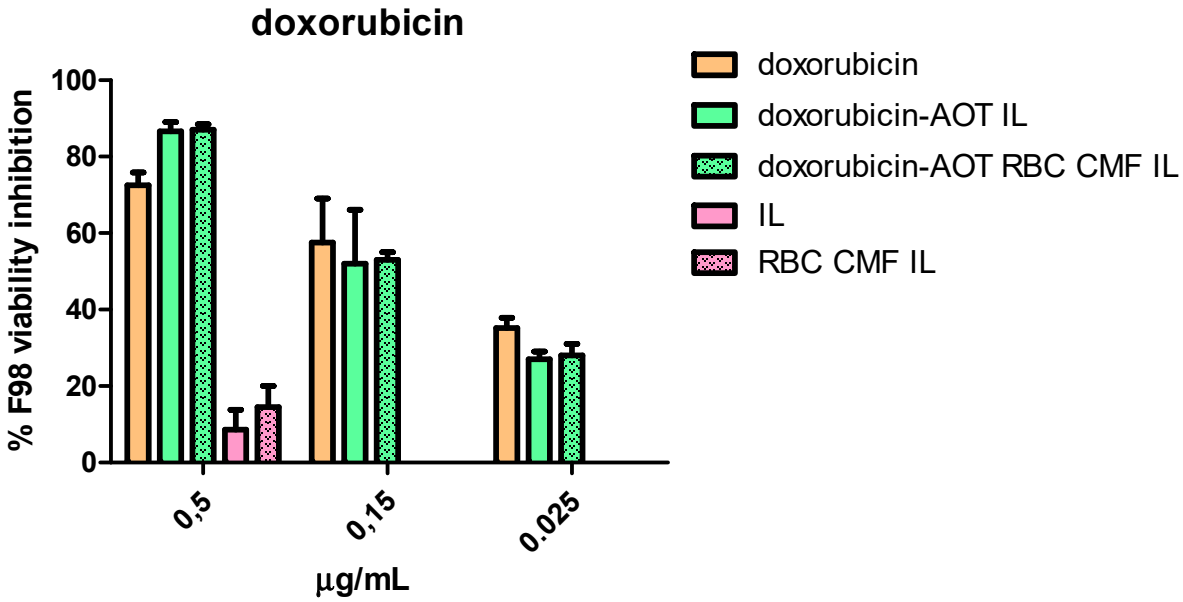


Figure S11. MTT assay at 72 hours, in F98 cells, of doxorubicin-AOT loaded, unfunctionalized and RBC CMF-wrapped IL. Abbreviations: AOT: sodium docusate; IL: CMF: cell membrane fragments; Intralipid® 10%; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RBC: red blood cells.