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Supporting Information

The amount of Dextran in PLGA nanocarriers modulates protein corona and promotes cell membrane damage

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Summary

Characterization of the PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs by Fourier transform infrared spectroscopy (FTIR)

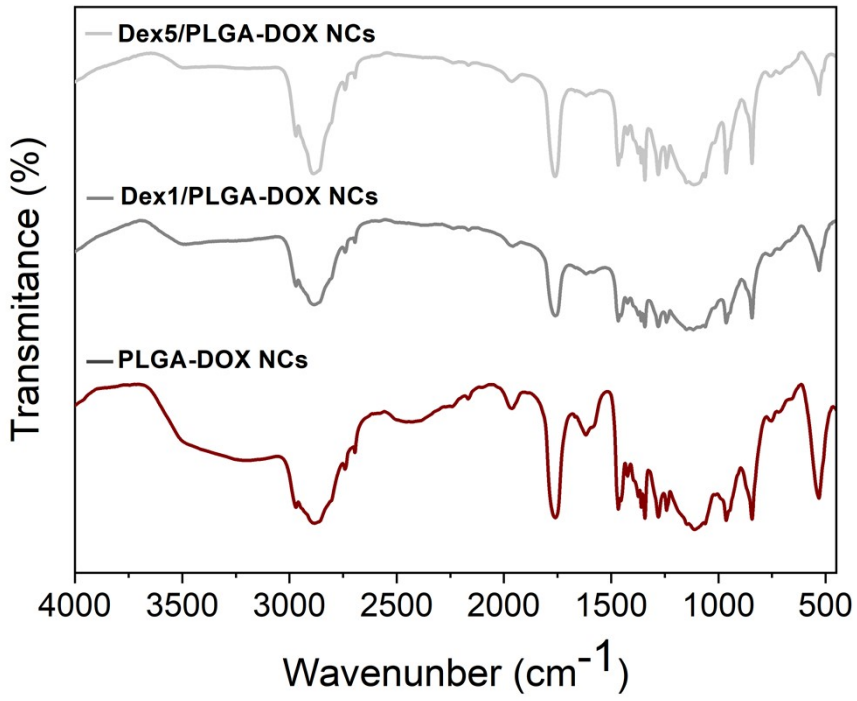

Fig. S1 Infrared spectra of PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs obtained by FTIR. Samples were prepared by drop-casting 20 µl of the samples diluted in deionized water in silicon wafers and dried under reduced atmosphere. The spectrum was collected using an Infrared spectrometer Nicolet 6700/GRAMS Suite, with 128 scans per sample with 4 cm^{-1} resolution from 4000 to 400 cm^{-1}.

Infrared spectra of the NCs were obtained by Fourier transform infrared spectroscopy (FTIR). In all spectra there are a band at 1750 cm^{-1} and less intense bands between 1050 and 1300 cm^{-1} that confirm the formation of ester bonds (O-C-O). The strong band at 2850 cm^{-1} corresponds to alkane aldehyde stretching, which is present in the chemical structure of PLGA. The 3410 cm^{-1} band presented in the FTIR spectrum of the NCs corresponds to the stretching vibration of O-H bonds.

DOX calibration curve

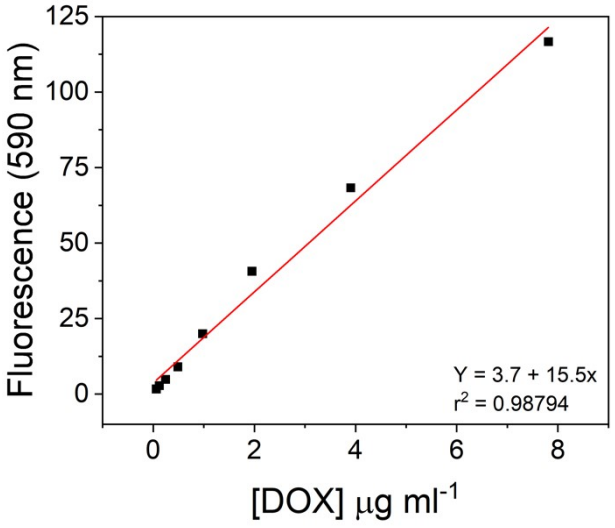

Fig. S2 Calibration curve of DOX in ddH_2O (ex: 480 nm; ems: 590 nm). The linear regression equation was used to calculate the amount of DOX released from the NCs in the cumulative release studies.

The calibration curve of DOX in Fig. S2 was employed to determine the concentration of DOX in the samples collect for the characterization of the drug released from the nanocarriers, where Y is fluorescence and x the concentration of DOX in μg ml^{-1}.

Cell viability after 24 hours of incubation with the NCs

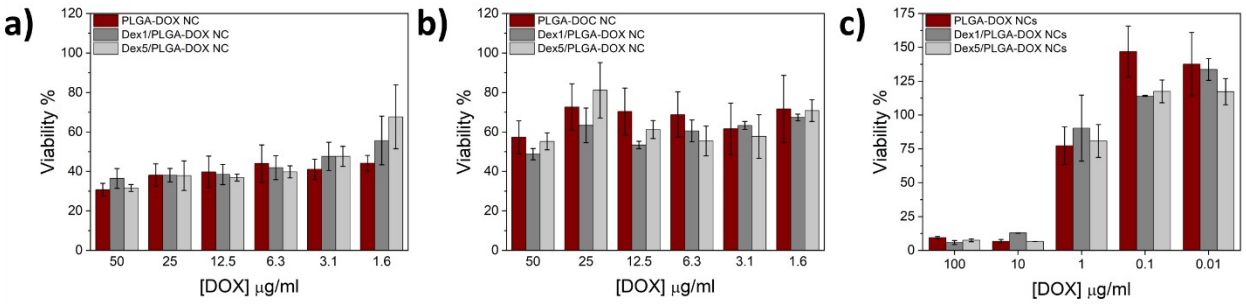

Fig. S3 Evaluation of cellular viability by MTT assay for nanocarriers prepared in the presence and absence of dextran 40. Viability of a) MCF-7, b) H9C2 and c) RAW 264.7 incubated for 24 h at 37 °C with PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs. Statistical analysis was performed using ANOVA with Tukey's post-hoc test. Values represented are mean±SD (n=4).

NCs prepared with and without dextran equally affect cell viability of the three cell lines after 24 h of incubation in all DOX concentrations tested. For RAW 264.7 cells, it is possible to observe a dose-dependence response, which is in accordance with DOX mechanism of toxicity in this cell line.[1,2]

Gate strategy for *in vitro* cellular uptake studies

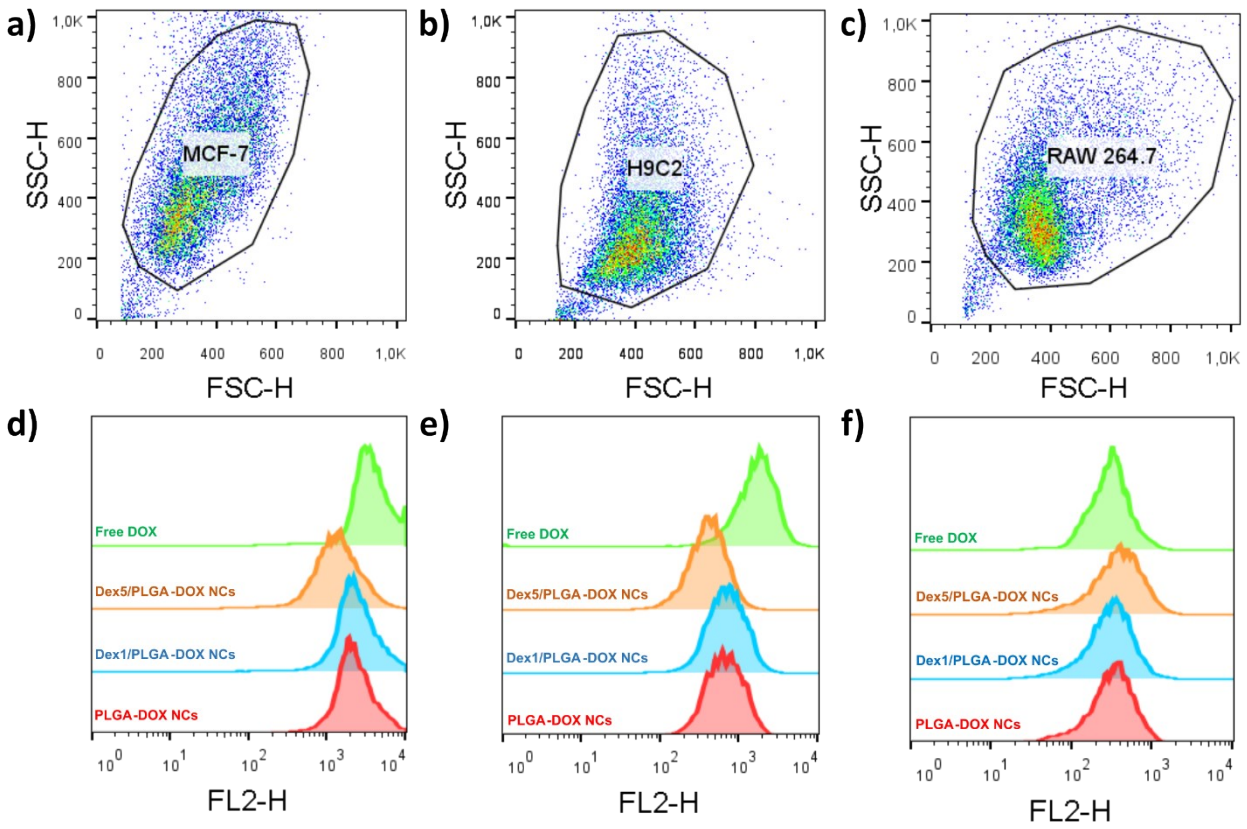

Fig. S4 *In vitro* cellular uptake of PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs by flow cytometry analysis. Gating strategy for a) MCF-7, b) H9C2 and c) RAW264.7. Representative histograms of flow cytometry of d) MCF-7 and e) H9C2 incubated for 4 h at 37 °C with DOX, PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs and f) RAW 264.7 incubated for 2 h at 37 °C with DOX, PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs. Excitation was measured using FL2 (590/30) and data analysis were performed using FlowJo v10 Software.

NCs uptake by RAW 264.7 after 4 hours of incubation and inhibition studies

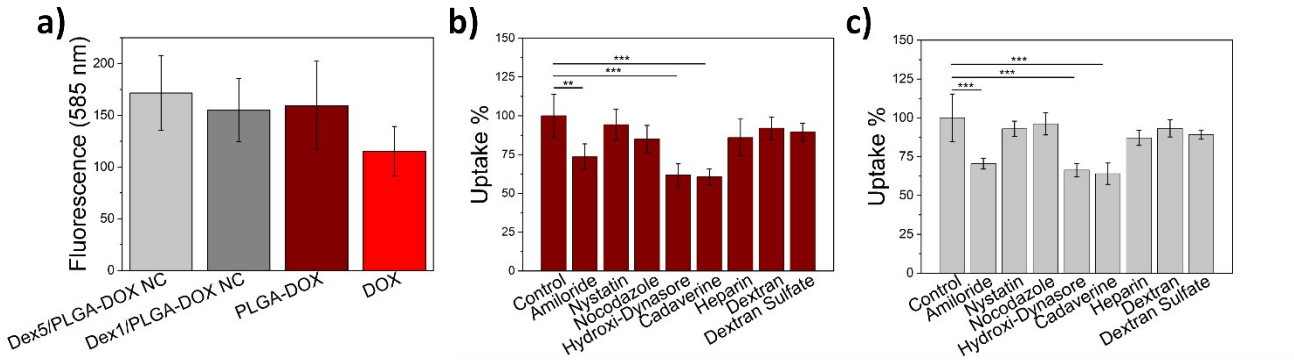

Fig. S5 *In vitro* cellular uptake of PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs by flow cytometry analysis. Comparison of cell fluorescence of a) RAW 264.7 incubated for 4 h at 37 °C with DOX, PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA NCs. Inhibition studies to evaluate the mechanism involved in the uptake of b) PLGA-DOX and c) Dex5/PLGA NCs by MCF-7. The cells were incubated for 30 minutes with amiloride, nystatin, nocodazole, hydroxi-dynasore, cadaverine, heparin, dextran and dextran sulfate (100 µg ml^{-1}, 40 µg ml^{-1}, 10 µg ml^{-1}, 100 µmol l^{-1}, 100 µmol l^{-1}, 10 units ml^{-1}, 2 mg ml^{-1} and 5 µg ml^{-1}, respectively) prior exposure to the NCs. DOX dosage was 12.5 µg ml^{-1} for all analysis. Statistical analysis was performed using ANOVA with Tukey's post hoc test. Values represented are mean ± SD (n=3/4).

Gate strategy for in vitro annexin V binding assay

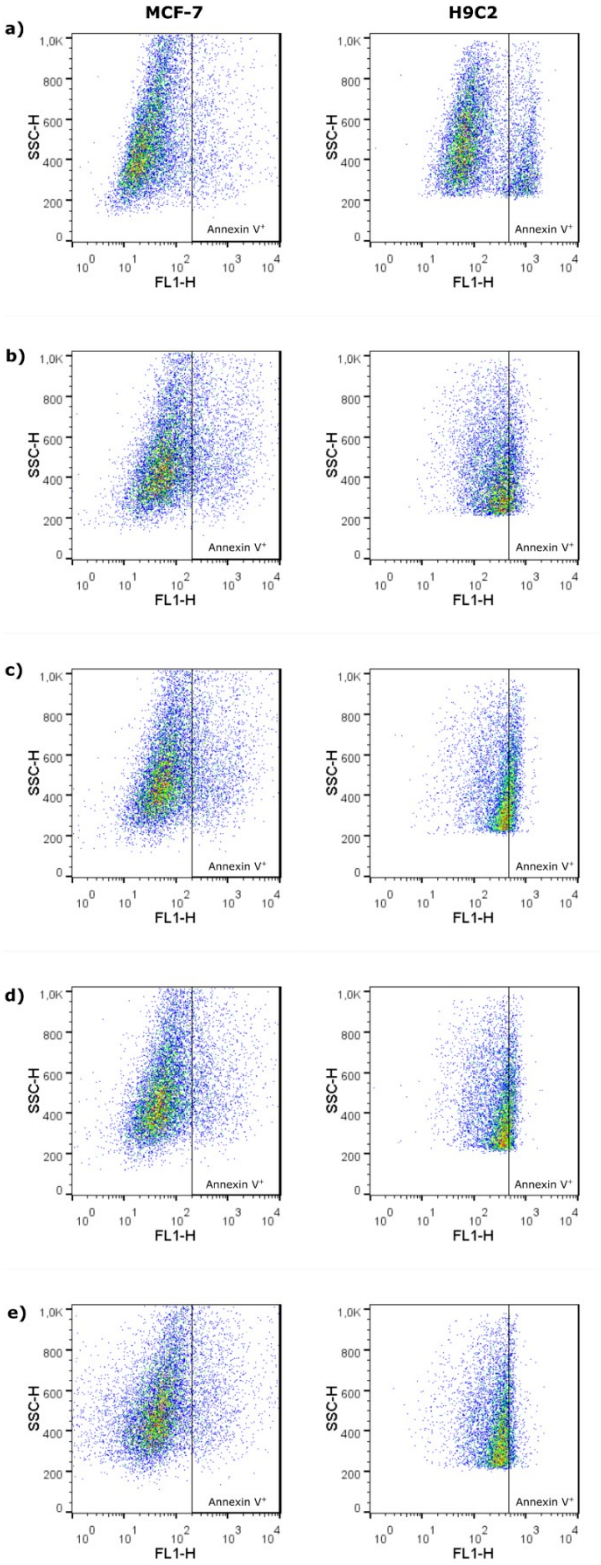

Fig. S6 *In vitro* evaluation of cellular viability by annexin V binding assay. Gating strategy for a) live cells control and cells incubated for 24 h at 37 °C with b) Dex5/PLGA NCs, c) Dex1/PLGA-DOX, d) PLGA-DOX and e) DOX. Excitation was measured using FL1 (530/30) and data analysis were performed using FlowJo v10 Software.

Raw image of SDS-PAGE gel of the proteins recovered from protein corona

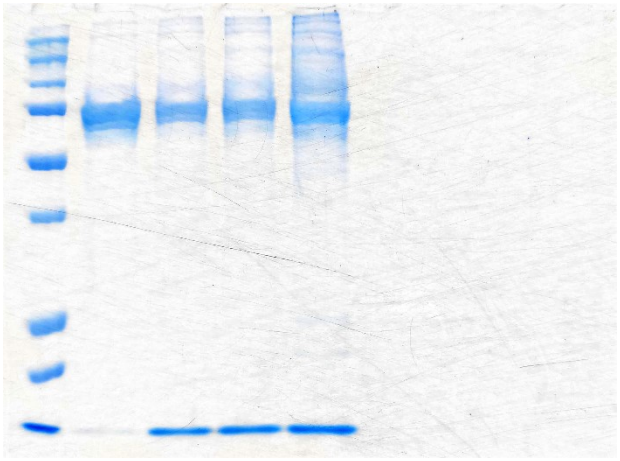

Fig. S7 Raw image for SDS-PAGE gel image in Fig. 2h of the proteins recovered from protein corona formed on PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA-DOX NCs.

NMR spectrum of PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA-DOX NCs

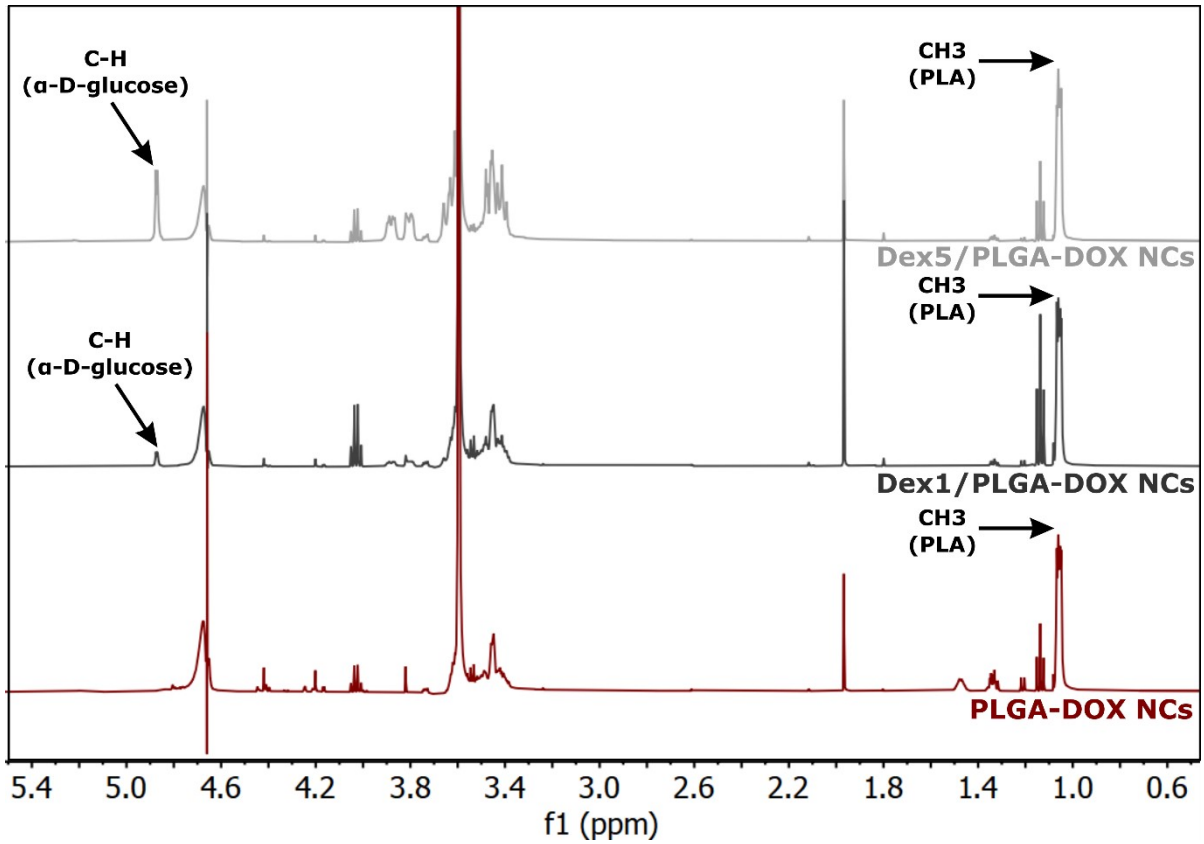

Fig. S8 NMR spectra of PLGA-DOX, Dex1/PLGA-DOX and Dex5/PLGA-DOX NCs

The ^{1}H-NMR spectra of PLGA-DOX, Dex1/LGA-DOX and Dex5/PLGA-DOX NCs show peaks at δ 1.20 ppm, assigned to the methyl groups of lactic acid monomers, and δ 4.88 ppm, corresponding to CH of (1→6)-α-D-glucose monomers of dextran molecule. The mole fraction of (1→6)-α-D-glucose monomers and PLA

monomers calculated by equation 1 was 8.2 and 1.7 mol% for Dex5/PLGA-DOX and Dex1/PLGA-DOX NCs, respectively.

REFERENCES

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