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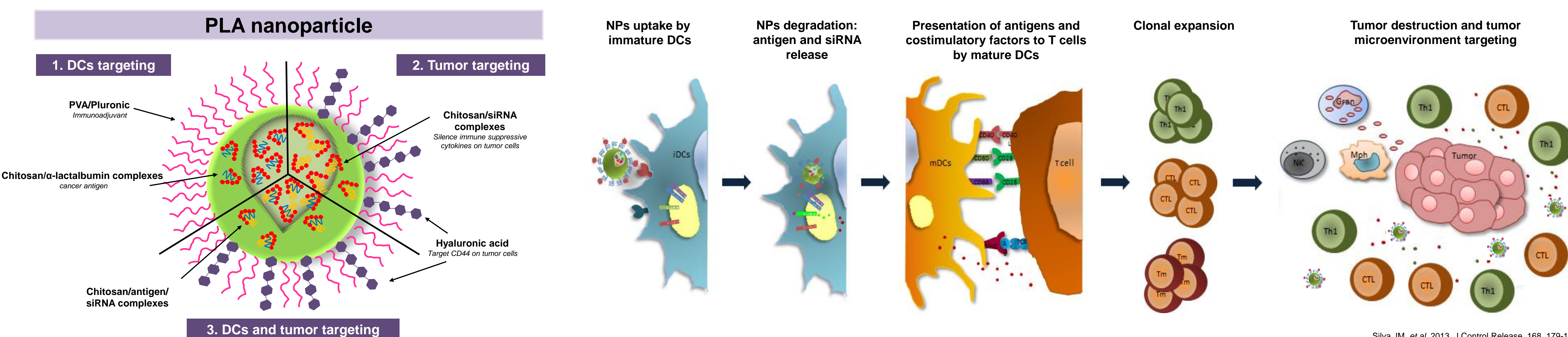
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Introduction and Aim

- ✓ Conventional therapies used for cancer do not target specifically the tumor, leading to several side effects.
- ✓ Cancer vaccines have been used as an alternative therapeutic strategy and have already shown promising results in clinical trials.
- ✓ However, only a small number of cancer vaccines has led to an effective tumor regression, which can in part due to the release of potent immunosuppressor molecules, by dendritic (DCs) cells and tumor cells.

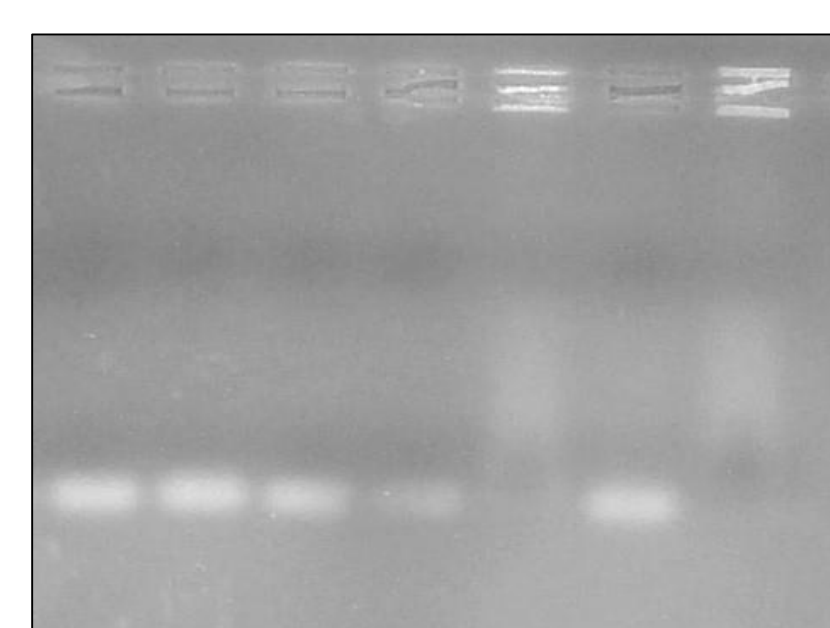
This study aims to develop a nanoparticle-based cancer vaccine to deliver tumor associated antigens (TAA) and/or siRNA to DCs and for immune modulation by silencing immune suppressive cytokines, following parenteral administration. In addition, mannan-associated nanogels will be developed for oral vaccination and their immune adjuvant potential will be assessed in the near future.



Silva JM. *et al.* 2013, J Control Release, 168, 179-199

Results

1. Cs-siRNA complexation assay with 3 different chitosans derivatives



Well 1: free-siRNA
Well 2: GCs:siRNA (5:1)
Well 3: GCs:siRNA (15:1)
Well 4: GlutCs:siRNA (5:1)
Well 5: GlutCs:siRNA (15:1)
Well 6: HCCs:siRNA (5:1)
Well 7: HCCs:siRNA (15:1)

Three chitosan derivatives were tested for their ability to complex the nucleic acid. Contrarily to glycol (GCs) chitosan, glutamate (GlutCs) and hydro-chloride (HCCs) derivatives complexed the siRNA when the ratio Cs:siRNA was 15:1. GlutCs was chosen as the chitosan derivative for future assays.

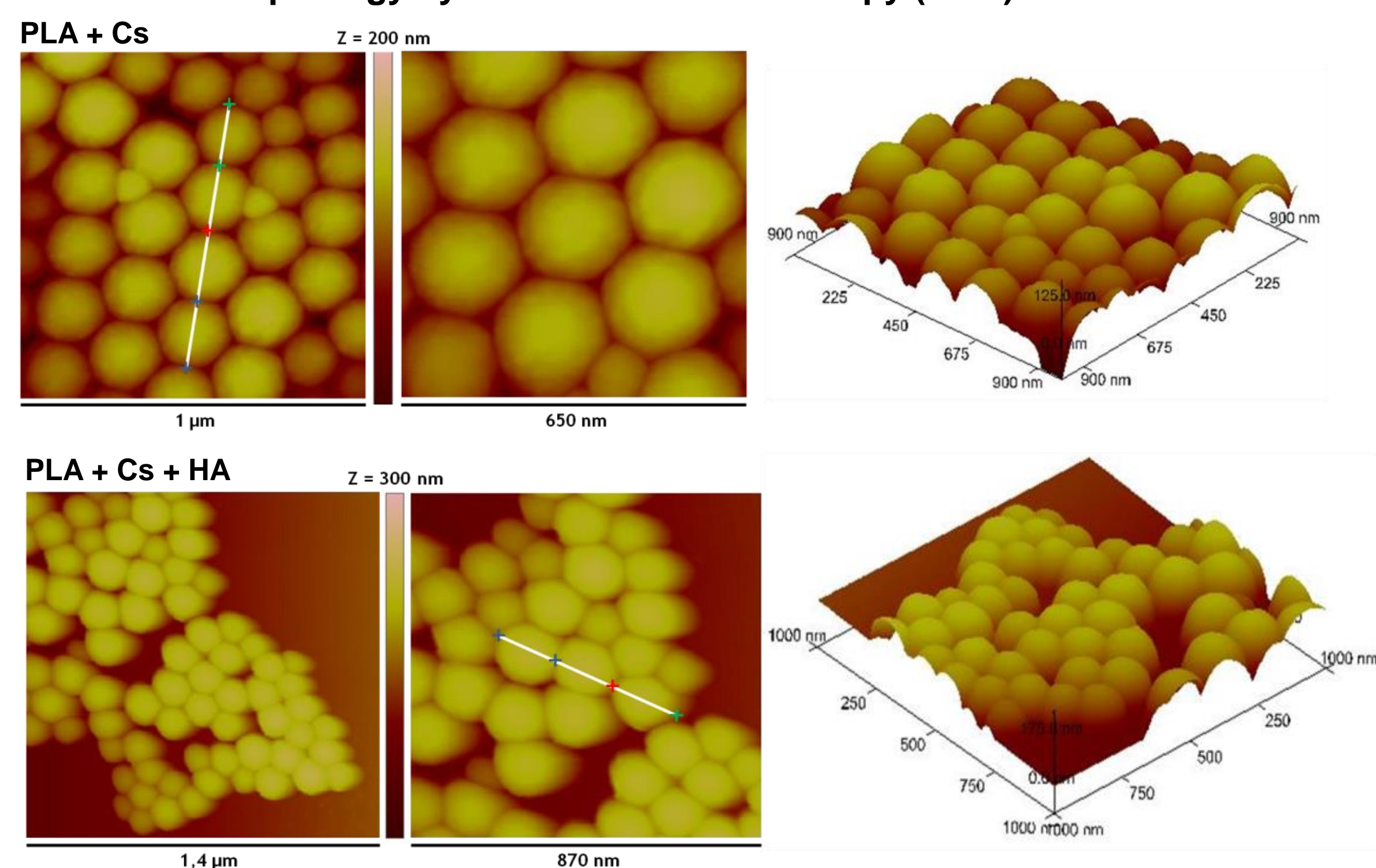
2. Physicochemical characterization by Dynamic Light Scattering (DLS) and Laser Doppler Electrophoresis (LDE), and Entrapment Efficiency (E.E.) and Loading Capacity (L.C.) by HPLC for α -Lac and PicoGreen® for siRNA

Formulation	Z-Ave (nm)	Pdl	ZP (mV)	E.E. (%)	L.C. (μ g/mg)
PLA + Cs	208.7 \pm 2.1	0.074 \pm 0.036	-1.55 \pm 0.99		
PLA + Cs + α -Lac	203.8 \pm 5.7	0.081 \pm 0.013	-3.80 \pm 0.96	92.0 \pm 1.5	23.0 \pm 0.4
PLA + Cs + siRNA	207.2 \pm 6.7	0.077 \pm 0.034	2.96 \pm 1.76	88.2 \pm 2.1	22.1 \pm 0.5
PLA + Cs + HA	214.7 \pm 8.2	0.099 \pm 0.014	-3.12 \pm 0.75		
PLA + Cs + HA + α -Lac	210.4 \pm 4.2	0.100 \pm 0.040	-2.16 \pm 0.97	99.5 \pm 0.1	0.99 \pm 0.0
PLA + Cs + HA + siRNA	205.8 \pm 7.5	0.125 \pm 0.057	-2.75 \pm 1.51	99.3 \pm 0.1	0.99 \pm 0.0

n > 3, Mean \pm SD

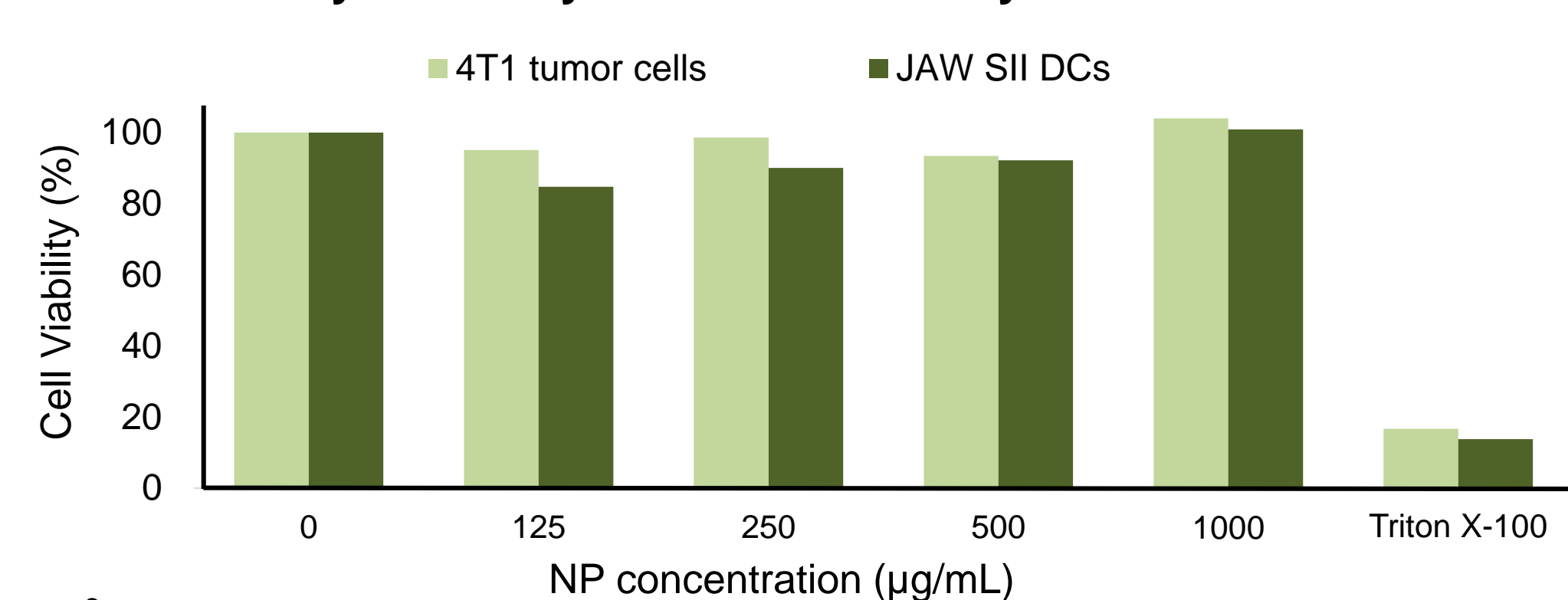
NPs presented a mean diameter close to 200 nm with low polydispersity index (Pdl) values, ZP close to neutrality. E.E. and L.C. were calculated indirectly by the determination of the amount of antigen that remained free in the supernatants collected during the washing and centrifugation steps. Both methods suggested that this nanoplatform is able to entrap antigens and siRNA at high extension.

3. Surface morphology by Atomic Force Microscopy (AFM)



AFM images indicated that NPs are spherical with a smooth surface and present a mean diameter close to 200 nm, which corroborates results obtained by DLS.

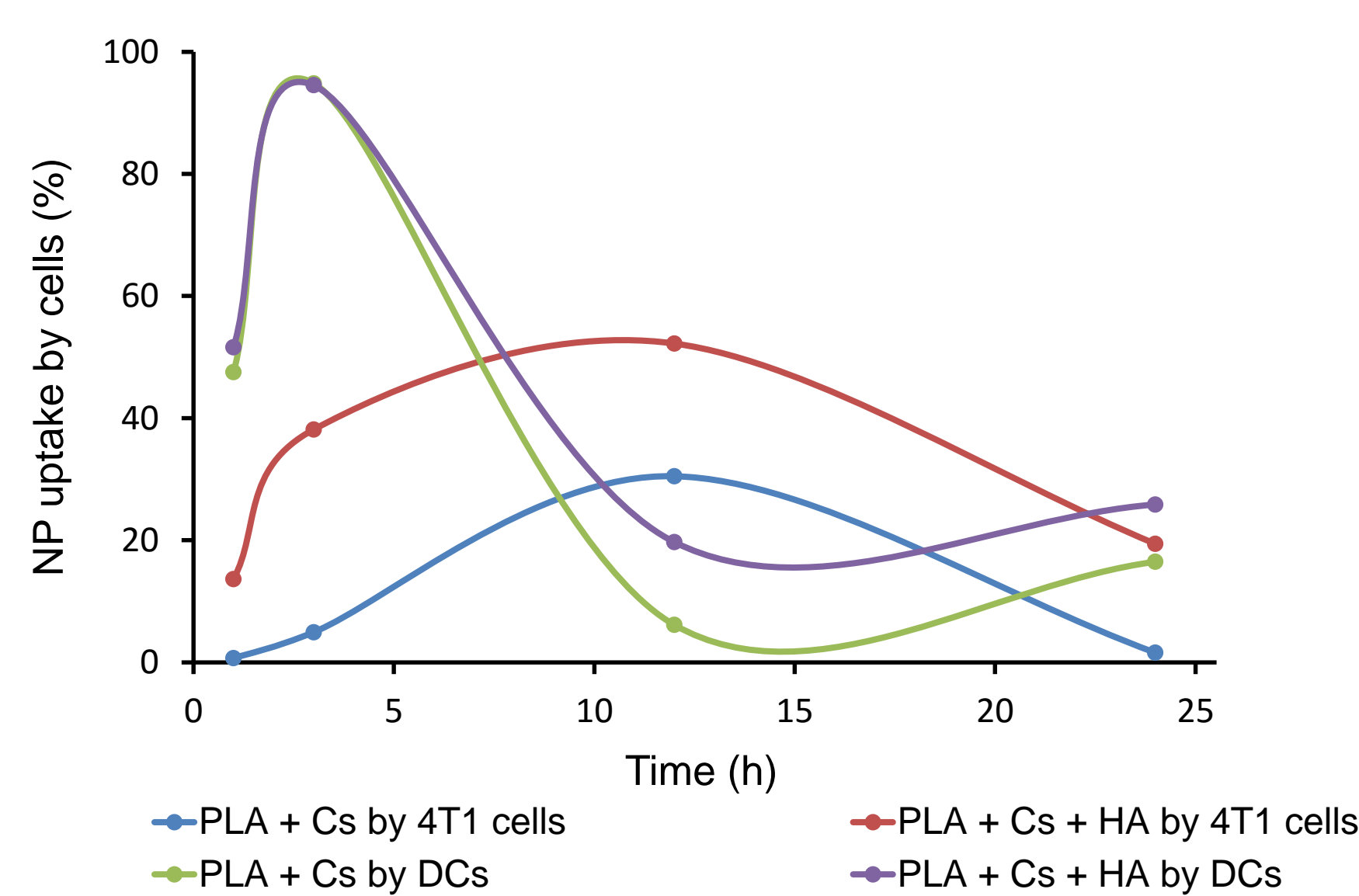
4. Cell viability at 72 h by AlamarBlue® assay



n = 3

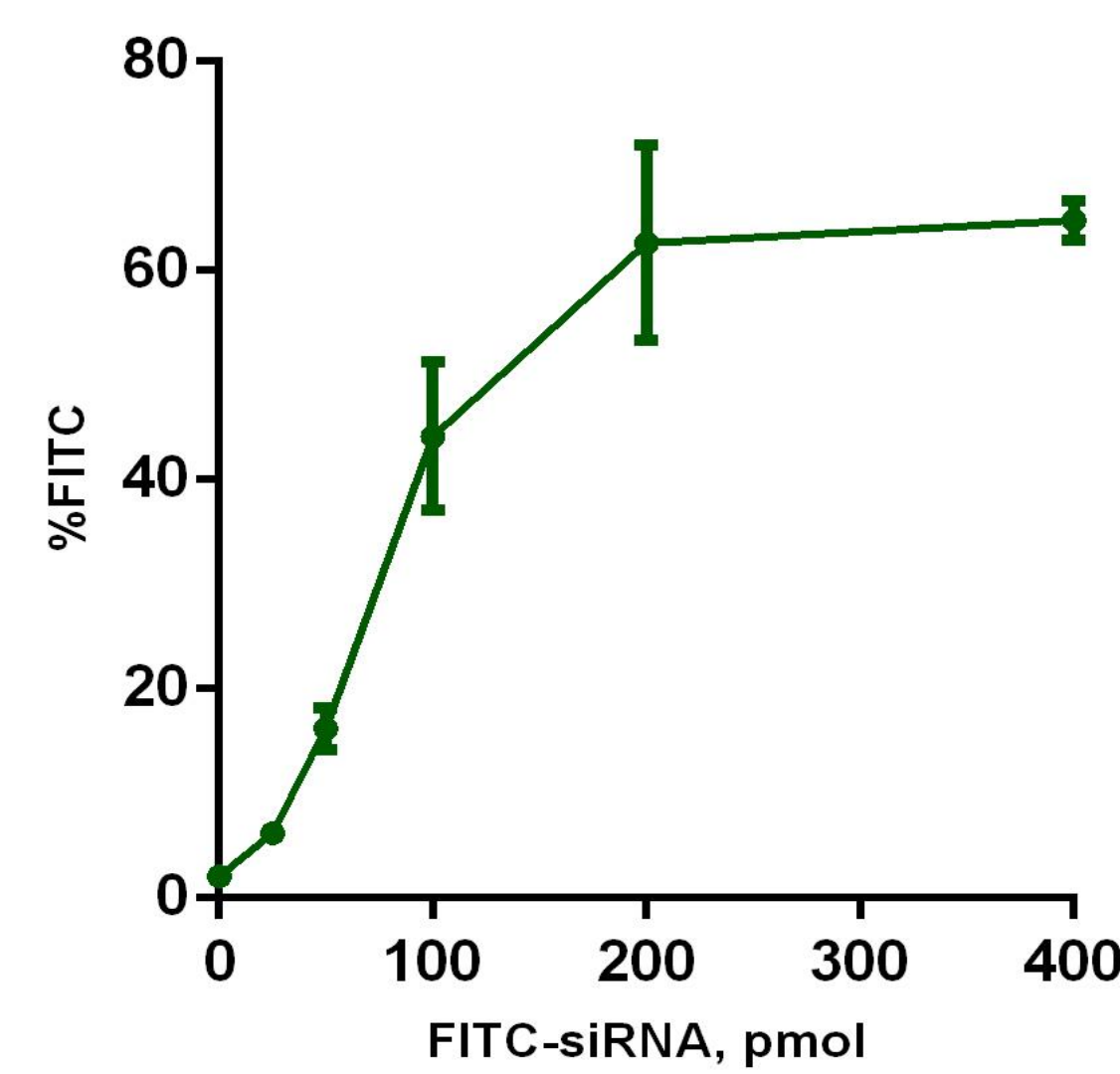
NPs did not present any cytotoxic effect after 72 h of incubation, neither on breast cancer 4T1 cells nor on JAW SII DCs, even at high concentrations.

5. NP uptake by 4T1 cells and DCs by Flow Cytometry



Labeled-NPs were quickly and efficiently taken up by DCs, being not affected by the modification with HA. Although tumor cells were also able to internalize non-modified-NPs, those modified with HA were taken up at a higher extend.

6. Nanobody-based specific delivery of therapeutic siRNA to CXCR4+ T cells by flow cytometry



n = 3, Mean \pm SD

CXCR4+ T-cells were incubated with increasing amounts of FITC-labelled siRNA and 4M5.3 complexes (siRNA binding fragment/VHH anti-CXCR4). Cell-surface binding was assessed by flow cytometry, demonstrating a dose-dependent detection of FITC fluorescence.

Conclusions

Promising nanoplatforms for vaccine delivery and cancer immunotherapy have been developed:

- ✓ Highly reproducible formulation process of NPs with capacity to co-entrap tumor-associated antigens and immunomodulators;
- ✓ Size and surface charge of polymeric PLA NPs may prevent their premature capture by macrophages;
- ✓ Formulated PLA-based nanoparticulate systems do not affect DC nor tumor cell viability, being efficiently taken up by those cells
- ✓ PLA NPs physicochemical properties may predict the promising application of this nanoplatform to target overexpressed CD44 receptors in tumor cells.

References and Acknowledgements

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