

Molecular weight influence of superficially exposed hyaluronic acid on nanoparticles cell internalization kinetics

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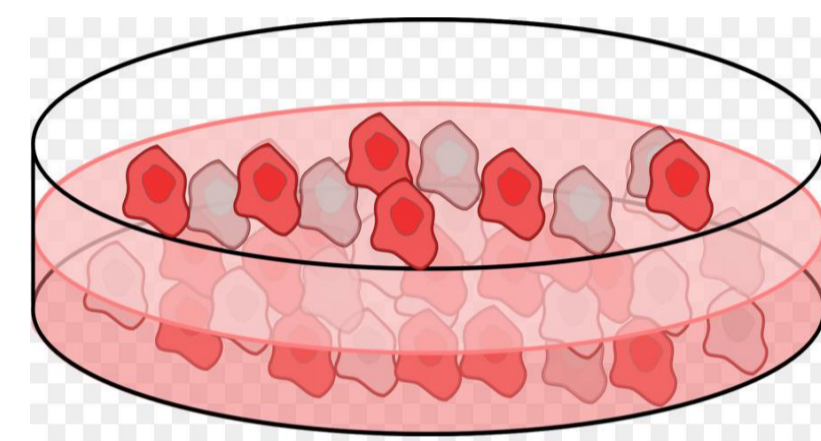
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INTRODUCTION

- ❖ In the engineering of nanodevices intended for active tumor targeting, hyaluronic acid (HA) plays a significant role due to its tropism for CD44 and RHAMM receptors [1,2]. Here, it has been investigated the molecular weight influence of HA on cell internalization kinetics
- ❖ Biodegradable poly(lactic-co-glycolic acid)-based nanoparticles (NPs) have been decorated with HA at three different molecular weights: 200, 800 and 1437 kDa (NP formulations were named HA2, HA8 and HA14, respectively)

EXPERIMENTAL

- ❖ NPs were produced by *nanoprecipitation* by forcing a PLGA/F68/F127 solution in acetone (5 mL, 3% w/v; 1:0.5:0.5 weight ratio) through a syringe at 333 $\mu\text{L}/\text{min}$ by a Syringe Pump
- ❖ The solution was precipitated into 40 mL of an aqueous phase (W1), containing F127 and F68 as surfactants (1:1 w/w ratio; 0.5% w/v) and HA (3.75, 0.81 and 0.5 mg/mL for HA2, HA8 and HA14 NPs). The organic solvent was evaporated overnight. The obtained NPs was washed by centrifugation (10.000 rpm, 10 min) and stored at -80°C
- ❖ Fluorescent NPs were prepared by adding Nile Red (NR) to the organic phase (0.01% w/w NR:polymers)



- ❖ NPs were characterized for their morphology, size and z-potential (ZP)
- ❖ NPs architecture was studied by differential scanning calorimetry (DSC) (10-80°C, 5°C/min)

Cell culture and uptake kinetics

- $2 \cdot 10^4$ cells /well
- Healthy cell line: L929: passage 15–23
- Tumor cell line: HS578T (+ 10 $\mu\text{L}/\text{mL}$ bovine insulin)
- Penicillin 100 U/mL + Streptomycin 100 $\mu\text{g}/\text{mL}$ as preservatives
- Medium: DMEM + 10% FBS; ml/well
- Incubation at 37°C (5% CO_2) with NP suspension (1mg/mL) for 72 h
- At scheduled time points, cells were rinsed twice with PBS and lysed with 0.1 mL of lysis buffer
- Cell lysates were diluted with 0.4 ml of PBS and analysed by spectrofluorimetric assay ($\lambda_{\text{ex}} = 572 \text{ nm}$) \rightarrow NP-associated fluorescence

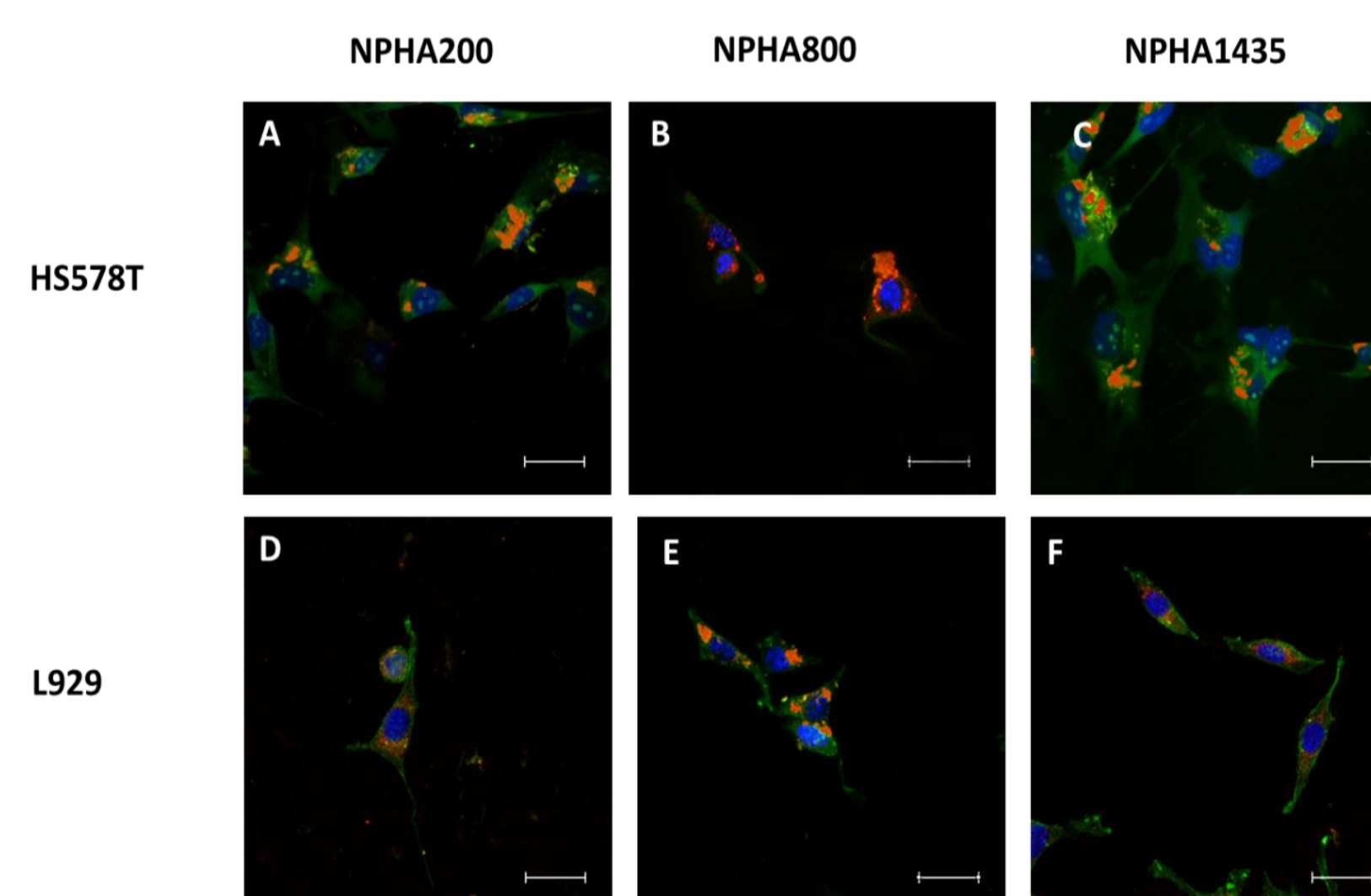
RESULTS AND DISCUSSION

NP size and Z-potential (ZP)

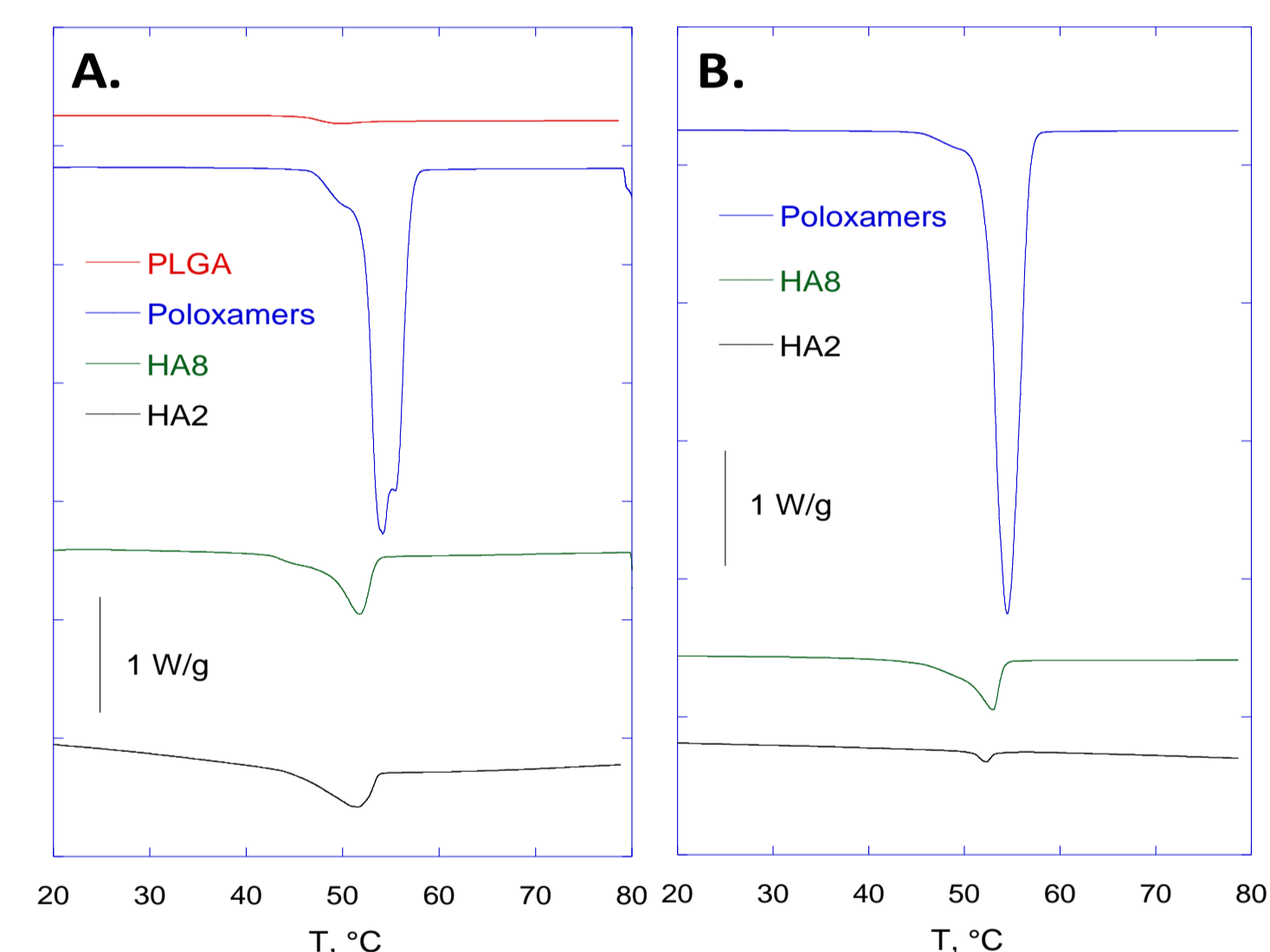
Formulation	HA MW(kDa)	d(nm)	ζ potential (mV)
NPHA200	200	191,3 ($\pm 1,08$)	-46,8 ($\pm 1,16$)
NPHA800	800	169,7 ($\pm 1,1$)	-44,65($\pm 1,30$)
NPHA1437	1437	185,5 ($\pm 0,67$)	-46,3 ($\pm 1,25$)

- ✓ NPs with a mean size < 200 nm and strongly negative ZP values were obtained

NP morphological analysis

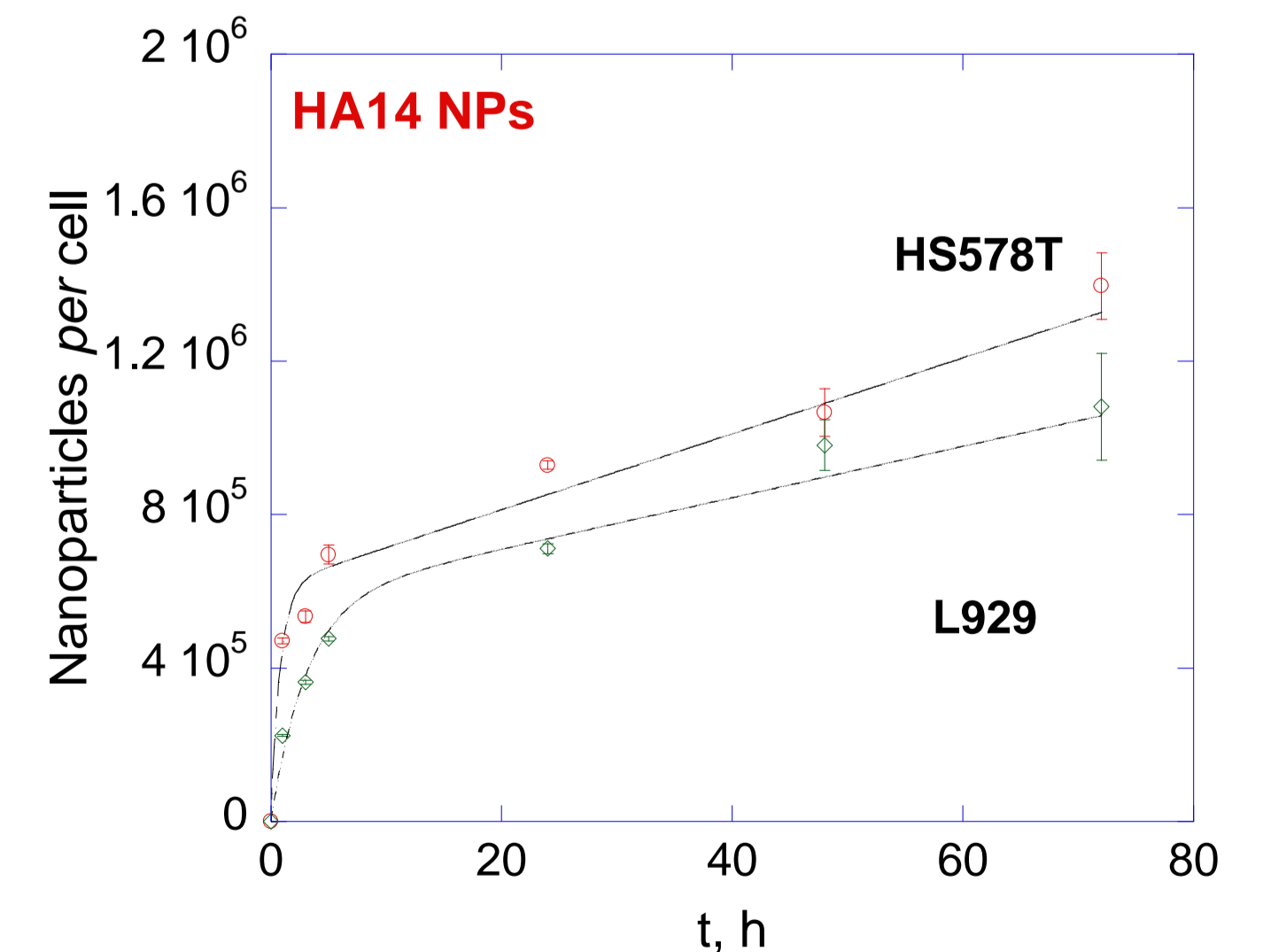
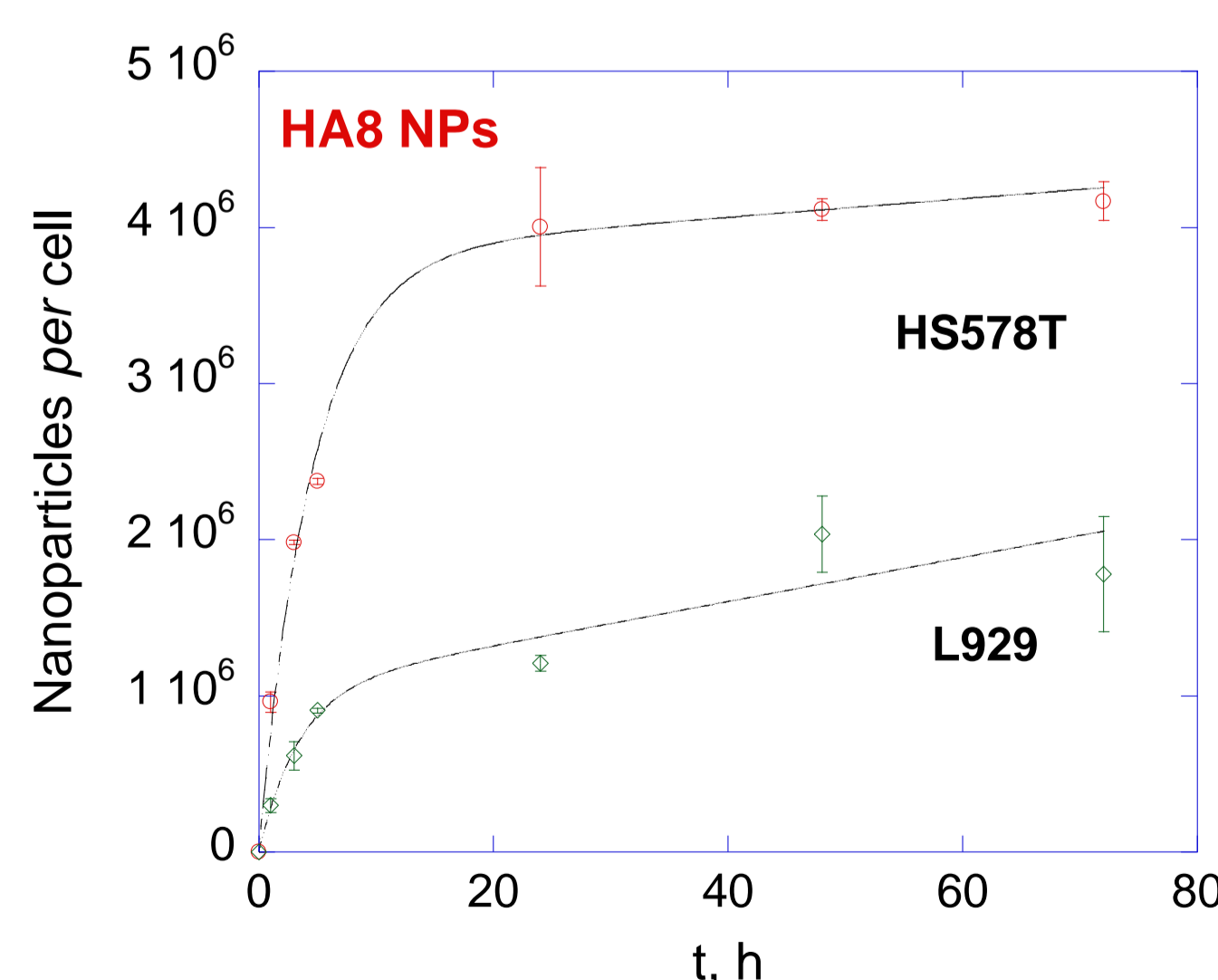
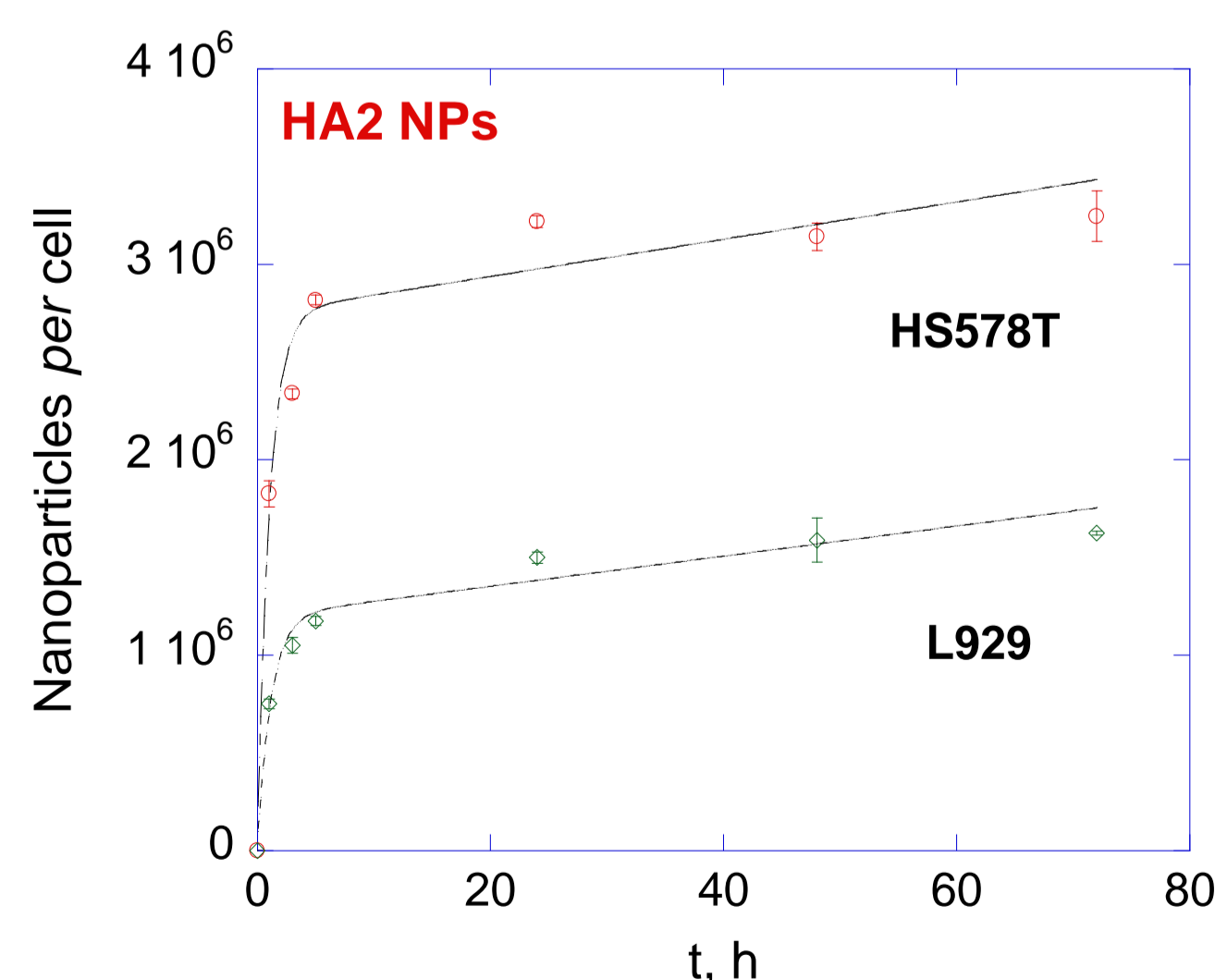


NP architecture



- ✓ The results of thermal analysis show that, for PLGA and poloxamers, glass transition temperature (T_g) is lower than that of PLGA, suggesting a plasticizing effect of poloxamers in the organic blend

Cellular uptake kinetics



- ✓ Results of cell internalization showed that uptake by cancer cells was promoted for all three formulations, with an approximately 2, 2.3 and 1.3-fold increase for HA2, HA8 and HA14 NPs, respectively

CONCLUSIONS

HA8 NPs were internalized faster and more effectively than HA2 and HA14 NPs, confirming that the differences in HA chain length can affect the binding and the internalization rate.

[1] Li H. et al. . Expression of hyaluronan receptors CD44 and RHAMM in stomach cancers: relevance with tumor progression. Int J Oncol. 2000.

[2] Oldenburg D. et al. CD44 and RHAMM are essential for rapid growth of bladder cancer driven by loss of Glycogen Debranching Enzyme (AGL). BMC Cancer. 2016.