Supplementary Material

Assessment of hyaluronic acid-conjugated pH-sensitive liposomes for prednisolone delivery to activated macrophages

Andreia Marinho^{ab}, Salette Reis^a, Cláudia Nunes^{*ac}

^aLAQV, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4500-313 Porto, Portugal.

^bLAQV, REQUIMTE, Faculdade de Ciências, Universidade do Porto, R. Campo Alegre s/n, 4169-007 Porto, Portugal.

^cLAQV, REQUIMTE, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.

*Corresponding author: cdnunes@ff.up.pt

1. Simulated synovial fluid preparation

Table S1 list the reagents used for the preparation of simulated synovial fluid (SSF). The reagents were added to 700 mL of water, in the order shown in the table, one by one, after each reagent was completely dissolved. At the end the pH value was adjusted to 7.4.

ReagentAmount for 1L of SSFSodium chloride8.000 gPotassium chloride0.200 gSodium phosphate dibasic1.440 gPotassium phosphate monobasic0.240 gHyaluronic acid3.000 g

Table S1: Chemical composition of simulated synovial fluid (SSF) buffer

2. Cell uptake pathway inhibitors solutions

Table S2 lists the receptors used to study the internalization pathways of the developed liposomal formulations, as well as their respective functions.

Table S2: Selected inhibitors and respective functions applied to mechanistic studies of
liposomal formulations transport by THP-1 macrophages.

	Inhibitor	Concentration	Function	
	Chlorpromazine	10 μg·mL ^{−1}	Clathrin-mediated endocytosis	
Endocytosis	Cytochalasin-D	5 ug·ml ⁻¹	Disrupt actin filaments and	
inhibitors		5 με πε	Macropinocytosis	
	Filipin	1 μg·mL⁻¹	Caveolae-mediated endocytosis	
Energy- dependence	Sodium azide	1 mg·mL ⁻¹	Active transport inhibitor	
	4ºC		Passive diffusion and active	
		-	transport inhibitor	

3. Correlation Values for PDP Leakage from Liposomes in Simulated Synovial Fluid

Table S3 presents the R² values for PDP leakage from liposomes in simulated synovial fluid, providing additional support for the data presented in Figure 1C.

	Zero order	First order	Hixson Crowell	Higuchi	Korsmeyer-Peppas
Lipo:PDP	0.9752	0.6413	0.9689	0.9958	0.9396
LipoHA:PDP	0.9394	0.6014	0.9881	0.9939	0.9212

Table S3: Correlation values (R²) for PDP leakage from liposomes in SSF.