New approach for cardiovascular disease treatment

Adriana Cambón*, Lilia Arellano, Eva Villar-Álvarez, Xaniar Smailzadeh, Silvia Barbosa, and Pablo Taboada.

Grupo de Física de Coloides y Polímeros, Departamento de Física de Partículas, Facultad de Física, Campus Vida, Universidad de Santiago de Compostela, E-15782. Santiago de Compostela (Spain).

* ***adriana.cambon.freire@usc.es***

Abstract

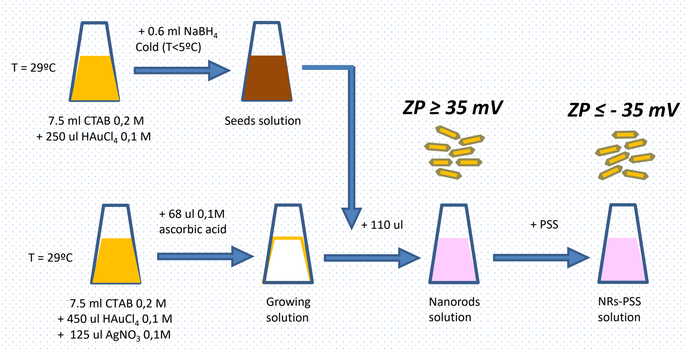
Cardiovascular diseases (CVD) is a general term to enclose diseases that affect the circulatory system and/or the heart. Their underlying pathology is atherosclerosis, an inflammatory disease characterized by the accumulation of lipids, inflammatory cells and fibrous tissue in the arteries internal wall, provoking in some extent their obstruction. Atherosclerosis is still addressed as a simple disease instead of being faced as the complex interplay of different types of cells and cascade signaling pathways, so that **the use of any single imaging or therapeutic agent alone is unlikely to provide a satisfactory outcome.** Hence, other treatment strategies need to be implemented, in particular, new nanomaterials able to target the plaque, to efficiently treat it and that can be easily released by the body without provoking adverse effects. Following this premises, we have designed a ***biocompatible drug delivery vehicle that efficiently load and protect the drug Atorvastatin while a folate receptor in the external shell will target inflamed areas***. Statins reduce the LDL (low density lipoproteins) level by inhibiting the action of the coenzyme that catalyzes the rate-limiting step in cholesterol biosynthesis (coenzyme HMG-CoA reductase). Atorvastatine calcium salt (ATO) is a synthetic statin that has longer half life than natural statins, achieving better results in lowering cholesterol and LDL values in humans. Nevertheless, commercialized ATO formulations, don´t protect the drug from their high trans-membrane permeability, fact that make decrease their bioavailability to values close to 12%. By protecting the drug we pretend to increase its bioavailability, and in consequence, the administered drug could be reduced at least 10-fold times for similar effect while reducing non-desired collateral side effects.. With this purpose we used Atorvastatine calcium salt (ATO-Ca). To avoid the common toxic effects of Folic acid (FA) or ATO in the body at certain concentrations, the vehicle will provide covalent attachment for the FA on the surface and cage structure for ATO protection.

Experimental Work and Results

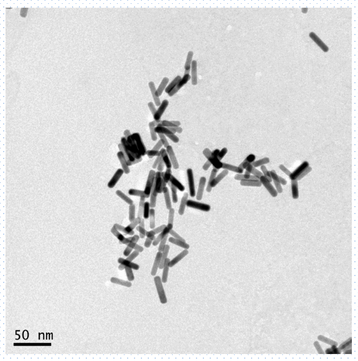
In this study we have designed and optimized a new nano-vehicle able to load high amounts of atorvastine and protect them, that also will target inflamed areas.

With this objective, we have selected **gold nanorods** (NRs) as substrate to create **nanosized capsules** (NRs size is 40 nm long and less than 10 nm wide). The elongated shape will confer the drug delivery system (DDS) with an optimal shape for cell internalization. In addition, AuNRs present other valuable advantages, as biocompatibility, simple synthesis procedures, easily tunable size and shape, facile surface modification, and versatile conjugation with biomolecules

Gold nanorods were synthesized by a seed mediated two-steps protocol. [1]

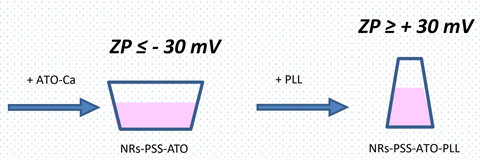


NRs washing was performed by centrifugation to discard all the CTAB in excess in the solution. A double layer of Poly-Styrene Sulfonate (PSS) was added on the surface to minimize CTAB contribution, but also to confer the NRs with a negative surface where to trap the drug. The plasmon bands and size are almost unchanged, while surface charge is reversed if appropriate amount of poly-electrolyte is added.



TEM image shows the NRs morphology and a size of ca. 35 x 5 nm.

Drug loading and protection has been achieved by surface adsorption between several layers. By protecting the drug we pretend to increase its bioavailability, while reducing non-desired collateral side effects. With this purpose we added small aliquot of concentrated Atorvastatine calcium salt (ATO-Ca) in methanol over the NRs-PSS aqueous solution under agitation and let the methanol to evaporate for a couple of hours. The solution was washed by centrifugation, and the supernatant was analyzed by HPLC technique to obtain the non-loaded ATO-Ca amount. ***Drug protection*** has been achieved by surface adsorption of a double poly-l-lysine (PLL) layer on the outer surface. We have chosen PLL because its biocompatible, and its positive charge at the acidic conditions of our system allows adsorption by simple electrostatic interaction. PLL invert the surface charge and increase the size of the system.



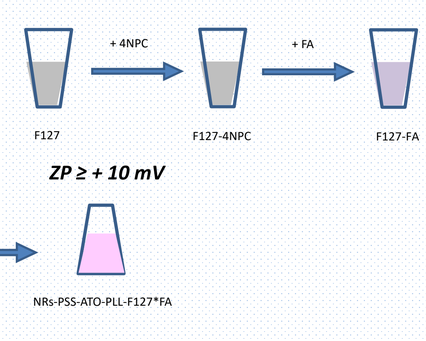
$$Drug\ loaded = drug\ added - drug\ in\ supernatant$$

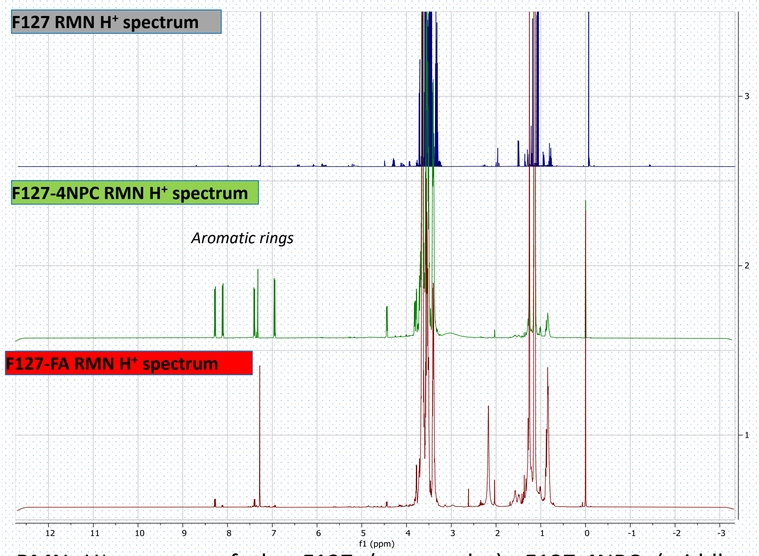
$$EE = 100\ x\ \frac{amount\ of\ drugloaded}{amount\ of\ drug\ added} \qquad DL = 100\ x\ \frac{amount\ of\ drugloaded}{weight\ of\ the\ vehicle}$$

ATO-Ca added	ATO-Ca loaded	EE	ϵ (EE)	% DL	ϵ (DL)
g/l	g/l	%	±	%	±
0,01	0,008	77,0	2,7	9,0	0,0
0,05	0,047	93,3	0,2	42,5	0,2
0,1	0,095	94,4	0,4	105,3	0,8
0,15	0,141	94,1	3,3	149,8	0,6
0,2	0,186	93,1	0,6	143,9	0,6
0,25	0,234	92,9	1,2	108,8	0,1
0,3	0,276	90,5	1,0	159,6	0,1
0,5	0,480	91,4	2,9	302,7	2,8

The exposed formulation increases the ATO-Ca solubility by several magnitude orders at the studied concentrations. ATO-Ca solubility in water is 1,12 x 10^{-6} g/l.

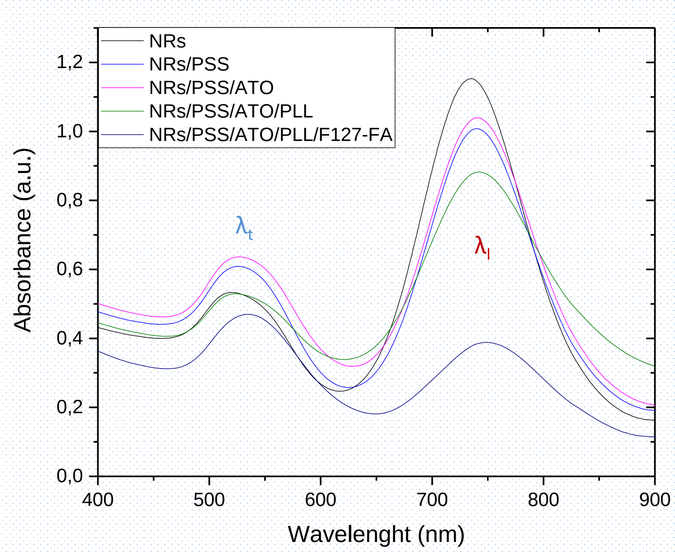
To confer the system with **colloidal stability** while reduce recognition by the body defense system, a polyethylene layer is included as external shell. Pluronic **F127** is a FDA approved triblock copolymer that consist of two lateral PEG chains and a central PPO block, which confers its amphiphilic character. To drive the system to inflamed areas we want to use **folic acid** (FA) as target. Adequate amounts of FA dimmish homocysteine levels in blood, that are an independent risk factor in cardiovascular diseases, favoring platelets agglomeration and clots formation. To avoid size effects because high levels of FA in the bloodstream, FA will be covalently liked to F127. [2-3]

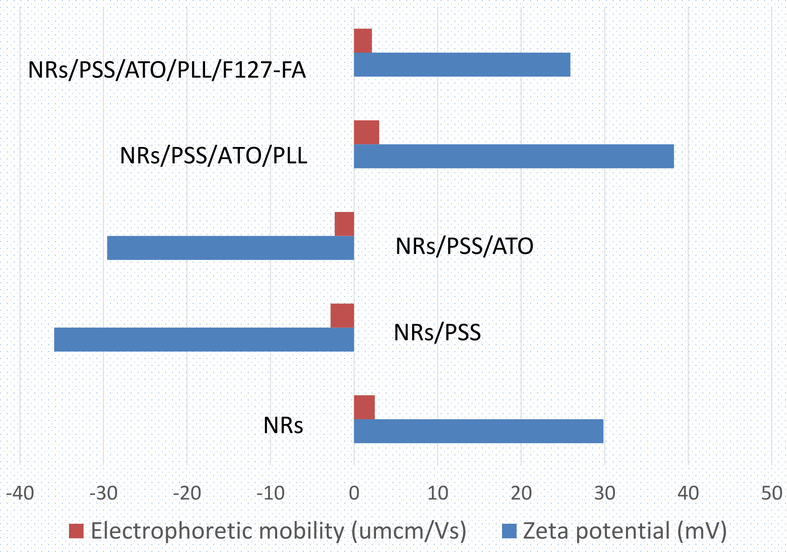


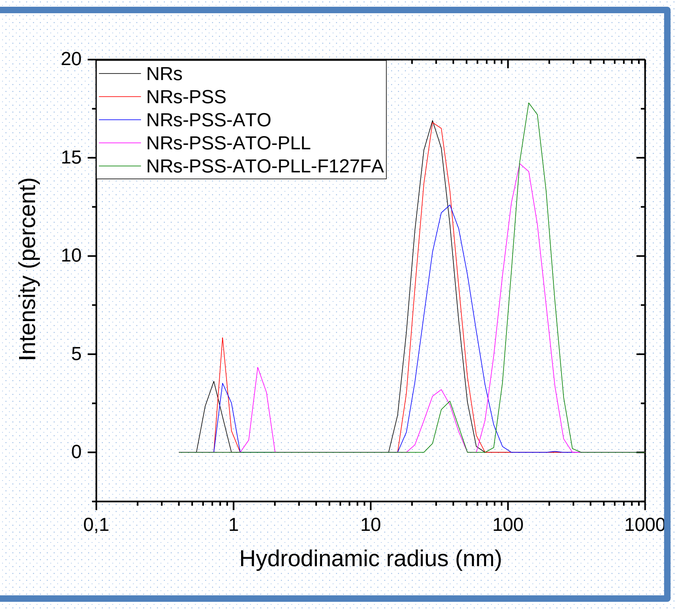


RMN H^+ spectra of the F127 (upper plot), F127-4NPC (middle spectra) and F127-FA (lower spectra).

Characterization of each step in the system formulation was performed by UV-vis spectroscopy, zeta potential and dynamic light scattering.







Conclusions

We have designed a biocompatible organic-inorganic platform by using gold NRs as substrate. By layer deposition we have loaded high amount of the drug atorvastatine, increasing its solubility by several magnitude orders. By a final layer of pluronic F127 covalently linked to FA we dotted the system with a targeting moiety for inflamed areas, that twill help to drive and accumulate the system in the affected zones. All the employed layers were constructed by using biocompatible materials. Further assays will be performed to characterize the behaviour of the proposed system.

References

[1] Villar-Alvarez, E., et al., *Combination of light-driven co-delivery of chemodrugs and plasmonic-induced heat for cancer therapeutics using hybrid protein nanocapsules.* 2019. **17**(1): p. 1-19.

[2] Wang, Y., et al., *Difunctional Pluronic copolymer micelles for paclitaxel delivery: Synergistic effect of folate-mediated targeting and Pluronic-mediated overcoming multidrug resistance in tumor cell lines.* International Journal of Pharmaceutics, 2007. **337**(1): p. 63-73.

[3] Zhou, Z., et al., *Synthesis and Micellization of Linear–Dendritic Copolymers and Their Solubilization Ability for Poorly Water-Soluble Drugs.* Macromolecules, 2009. **42**(20): p. 7936-7944.

Acknowledges

This work was supported by the Agencia Estatal de Investigación (AEI) through Project MAT2016-80266-R and Xunta de Galicia (Grupo de Referencia Competitiva ED431C 2018/26; Agrupación Estratégica en Materiales-AEMAT ED431E 2018/08). A.C. specially thanks Xunta de Galicia for her postdoctoral fellowship.