Design and development of multi-walled carbon nanotube-liposome drug delivery platforms

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Abstract

The aim of this study is to design and develop delivery platforms made of liposomes and multi-walled carbon nanotubes (MWCNTs). We used different lipids (with different main transition temperature (Tm)) and differently functionalized MWCNTs with organic addends possessing either positive or negative charge. The phospholipids used for the formulations were 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Tm=41ºC) and L-α-phosphatidylcholine, hydrogenated (Soy) (HSPC) (Tm=53ºC). By Differential Scanning Calorimetry (DSC), we studied the interaction between the DPPC and HSPC bilayers and MWCNTs. Liposome-MWCNTs delivery platforms prepared according to the protocol used in the literature. We used dynamic and electrophoretic light scattering in order to investigate the physicochemical characteristics of these mixed nanocarriers. The presence of MWCNTs causes alterations of the size of the conventional HSPC and DPPC liposomes. The ζ-potential values of mixed nanocarriers are near zero. This observation indicates the effective incorporation of MWCNTs into the lipid bilayer of liposomes. Fluorescence spectroscopy has been utilized to exact some qualitative information on the internal nanostructure and nanoenvironment of the lipid/carbon nanotube mixed structures. Finally, we conclude that we successfully prepare and completely characterize mixed nanocarriers composed of lipids and MWCNTs, with low toxicity as indicated by in vitro screening.

Keywords: liposomes; carbon nanotubes; drug delivery; nanocarriers; toxicity

1. Introduction

In recent years, significant research effort has been devoted for the investigation of carbon nanotubes (CNTs) in biology and medicine toward their utility in bio-applications (Bianco et al., 2005; Pagona and Tagmatarchis, 2006; Kostarelos et al., 2009; Liu et al., 2009; Heister et al., 2012, He et al., 2013). Particularly, among the numerous outstanding characteristics of CNTs, their high aspect ratio, chemical stability, robustness, high drug carrier capacity and ability to penetrate cell membranes, render them ideal vehicles for delivering bioactive molecules, such as drugs, DNA and proteins (Peretz and Regev,2012; Mendes et al., 2013). In general, biomolecules are conjugated to CNTs through well-established approaches either covalently, forming chemical bonds with the CNT scaffold, or non-covalently, by adsorption and/or wrapping onto the surface of CNTs or even by encapsulation, filling the empty inner cavity of CNTs (Patigelli et al., 2013). Specifically for medical applications, CNT-based materials need to disperse very well in water. However, pristine CNTs are insoluble in most common solvents as well as in aqueous and biological media due to their hydrophobic surface. In order to overcome this hurdle, functionalization of CNTs is absolutely required, which not only leads to solubility enhancement but also improves biocompatibility and minimize toxicity (Sayes et al., 2006; Lacerda et al., 2008; Bianco et al., 2011; Dumortiev et al., 2006; Liu et al., 2013). Additionally, the size of CNTs plays an important role so that their length is necessary to be short in order to minimize toxicity issues (Kostarelos, 2008). Considering all the above, oxidation of CNTs is the method of choice for engineering the material since it allows the shortening of CNTs and importantly introduces

oxygen-based functionalities suitable to perform post-modification reactions toward the construction of advanced CNT-based biomaterials (Jain et al., 2011).

Current liposomology is a huge and still progressing area. Liposomes belong to colloidal dispersion systems and are characterized as lyotropic liquid crystals (Demetzos 2008; 2015). These particular liposomal lyotropic states are responsible for the mesophases taking place in phase transitions and are related to their thermal stress during phase transitions (Demetzos 2008;2015; Pippa et al., 2015).Liposomes are clinically used delivery systems with many advantages such as biocompatibility and biodegradability (Allen and Cullis, 2013).

Pharmaceutical nanotechnology can provide challenges for producing innovative drugs based on bio-inspired nanostructures that can be employed as *advanced Drug Delivery nano Systems* (aDDnSs). Hence, mixed nanocarriers have attracted the scientific interest the last years with numerous applications in controlled-release of active pharmaceutical ingredients (Demetzos and Pippa, 2015). In particular, Karmchemski et al. designed CNTs liposome conjugates that could deliver a high dose of drug into cells using a low concentration of CNTs and thus CNTs related toxicity reduced significantly. Active targeting and delivery of drugs to desired cells and consequently preventing potential adverse systemic reactions was also achieved by CNT-liposome conjugates. Supramolecular nanotrains composed of CNTs and liposomes are used as smart biomimetic molecular–transport systems with lab-on-a-chip applications (i.e. medical diagnosis, bionic computers and artificial biological networks (Miyako et al., 2012). Pereira and colleagues successfully developed hybrids composed of cationic liposomes and multi-walled carbon nanotubes (MWCNTs) for the simultaneous delivery of siRNA and anticancer drug to cancer cells. The functionalized MWCNTs incorporation did not affect the overall cationic

surface charge of the final nanostructures formed and this is of great importance for the delivery of nucleic acids (Pereira et al., 2015). Lysine modified single-walled carbon nanotubes-liposomes conjugate loaded with the toxic anticancer drug doxorubicin was developed to ameliorate the anticancer effectiveness with a dual targeting mechanism (Zhu et al., 2015).

The aim of the current study is to design and develop innovative delivery platforms of therapeutic agents made of liposomes and MWCNTs. In this context, we employed different lipids, showing different main transition temperature (Tm) and different MWCNTs, properly modified to carry different surface functional groups. The phospholipids used for the formulations were 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Tm=41ºC) and L-α-phosphatidylcholine, hydrogenated (Soy) (HSPC) (Tm=53ºC). Firstly, Differential Scanning Calorimetry (DSC) experiments were applied on lipid: MWCNTs bilayers, to evaluate the cooperativity of different materials, based on the system thermotropic behavior. Series of light scattering and imaging techniques were used to elucidate their physicochemical characteristics and morphology of the prepared hybrid systems respectively. The in vitro toxicity of liposome-MWCNTs delivery platforms was also evaluated.

2. Materials and Methods

2.1. Materials

The phospholipids used for the chimeric formulations were 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine(DPPC) and L-α-phosphatidylcholine, hydrogenated (Soy) (HSPC). There were purchased from Avanti Polar Lipids Inc., (Albaster, AL, USA) and used without further purification. Multi-walled carbon nanotubes (MWCNTs) were obtained from Nanostructured and Amorphous Materials, Inc.,

(www.nanoamor.com; outer diameter 8-15 nm, length 500 nm; purity 95%). Chloroform and all other reagents used were of analytical grade and purchased from Sigma–Aldrich Chemical Co.

2.2. Preparation of MWCNTs

*2.2.1. Preparation of oxidized MWCNTs **1a***

In flask containing pristine 50 mg of MWCNTs, a freshly prepared piranha solution (50 mL, 4:1 v/v, 96% H_2SO_4 / 30% H_2O_2) was added. The reaction mixture was stirred upon heating at 70 ^oC for 7 hours. After that period, the highly acidic mixture was carefully added to 1 L of deionized water and the suspension was filtered through a PTFE membrane filter (pore size 0.22 μm). The collected oxidized MWCNTs **1a** were washed onto the filter with deionized water, until the pH of the filtrate was neutral.

*2.2.2. Preparation of oxidized MWCNTs **1b***

In a flask containing pristine 50 mg of MWCNTs, oleum (25 mL, 30% SO_3) was added and the mixture was stirred under a nitrogen atmosphere for 18 hours. Then, a 1:1 mixture of SO_3/HNO_3 (25 mL) was slowly added into the above suspension with stirring in an ice bath to keep the temperature close to ambient conditions and then the resulting dispersion was heated at 65 ^oC for 2 hours. After cooling to room temperature, the highly acidic mixture was carefully treated with deionized water (150 mL) and the suspension was filtered over a PTFE membrane filter (pore size 0.22 μm). The collected oxidized MWCNTs **1b** were washed onto the filter with deionized water to remove acidic residues, until the pH of filtrate was neutral.

*2.2.3. Preparation of anionic MWCNTs materials **2a** or **2b***

Oxidized MWCNTs (15 mg of **1a** or **1b**) were dispersed in deionized water (10 mL) through sonication for 15 min. Then, aqueous $NaHCO_3$ (24 mg in 5 mL H_2O) was added and the mixture was stirred for 18 hours at room temperature. Finally, the suspension was filtered over a PTFE membrane filter (pore size 0.22 μm) and the collected material **2a** or **2b** were washed extensively onto the filter with deionized water until the pH of filtrate was neutral.

*2.2.4. Preparation of functionalized MWCNTs materials **4a** or **4b***

A suspension of oxidized MWCNTs (15 mg of **1a** or **1b**) in thionyl chloride (8 mL) was stirred at 75 °C for 24 hours under N_2 atmosphere. Then, the reaction mixture was evaporated to dryness to remove the excess of thionyl chloride and the intermediate MWCNT-based acyl chloride was obtained. Subsequently, *N-tert*-butoxycarbonyl-2,2′-(ethylenedioxy)bis-(ethylamine) **3** (200 mg) in dry THF (10 mL) was added and the reaction mixture was heated at reflux for 48 hours. After cooling to room temperature, the solution was diluted with THF, filtered over a PTFE membrane filter (pore size 0.22 μm) and the collected solid materials washed extensively with MeOH and CH_2Cl_2 in order to provide the Boc-protected functionalized MWCNTs **4a** or **4b**.

*2.2.5. Preparation of functionalized MWCNTs materials **5a** or **5b***

A solution of BOC-protected functionalized MWCNTs **4a** or **4b** (7 mg) in CH_2Cl_2 (7 mL) was treated at room temperature with TFA (10 mL) for 18 hours. After that period, the highly acidic solution was evaporated to dryness, followed by addition of fresh dichloromethane with sonication and filtration through a PTFE membrane filter (pore size 0.22 μm) to afford MWCNT-based materials **5a** or **5b** after extensive washing with MeOH and CH_2Cl_2.

2.3. Preparation of pure and mixed lipid/MWCNTs bilayers

Pure lipid and mixed bilayers were prepared using the technique described elsewhere (Pippa et al., 2015, See also Supporting Information for details).

2.4. Preparation of Liposome-MWCNTs delivery platforms

HSPC and DPPC pure liposomes were prepared according to thin-film hydration method. Liposome-MWCNTs delivery platforms prepared according to the protocol used in the literature (Karchemski et al., 2012; Zhu et al., 2015). The weight ratio was at 10:1 (liposome-MWCNTs).

2.5.Methods

2.5.1.Differential Scanning Calorimetry (DSC) and Light Scattering Techniques (Dynamic and Electrophoretic-DLS, ELS)

Differential scanning calorimetry involves applying a linear heating or cooling (or isothermal signal to a sample and reference, and then measuring the temperature and the energy associated with thermal events such as melting, crystallization, or lipid phase transitions. The protocols used for the thermodynamic (by DSC) and physicochemical (by DLS, SLS and ELS) characterization of the nanostructures in aqueous media are described in our previous investigations (Pippa et al., 2013a,b, 2015, See also Supporting Information for details).

2.5.2. Fluorescence Spectroscopy

Fluorescence spectroscopy has been utilized in an attempt to extract some qualitative information on the internal micro-environment of the mixed lipid/MWCNTs

nanostructures in HPLC grade water (Pippa et al., 2013a,b. See also Supporting Information for details).

2.5.3. cryoTEM

In order to confirm the size and morphology of the materials, samples containing the hybrid nanostructures composed of the liposomes and CNTs were studied by cryogenic transmission electron microscopy (cryo-TEM). A droplet of a water suspension containing the original materials was vitrified by plunge freezing, using the automatic system FEI Vitrobot™. Vitrified grids were then introduced in the TEM using a cryo-transfer holder from Gatan™. TEM images were obtained in a Tecnai T20 (FEI company) operated at 200KV, coupled with a Veleta CCD camera. The electron dose was reduced to avoid as much as possible the irradiation damage of the specimens.

2.6. In vitro screening

The MCF10 cell line was used for all the *in vitro* experimental procedures. The cells were cultured in a 5% CO_2 at 37 °C. At confluence they were harvested and re-seeded in a 96 well plate at a density of 10000 cells per well. The nanostructures were inoculated for three days with the cells. Then the cell viability was measured using a MTT assay described in Rubistein et al., 1990 and Galani et al., 2014.

3. Results and Discussions

3.1. Preparation of MWCNTs-based materials

The preparation of MWCNT-based materials **2** and **5** is illustrated in Scheme 1. Two different oxidization routes were followed, allowing the shortening of MWCNTs with the simultaneous incorporation of carboxylic acid units at their tips. The first

oxidation pathway involves treatment of pristine MWCNTs with a freshly prepared solution of sulfuric acid and hydrogen peroxide in 4:1 ratio at 70 ^{o}C, yielding oxidized MWCNTs **1a**. In the second oxidation route followed, pristine MWCNTs were treated with oleum, affording oxidized MWCNTs **1b** (Ziegler et al., 2005; Chen et al., 2006). Then, the anionic carboxylates **2a** and **2b** were obtained upon treatment with aqueous $NaHCO_3$. The acquisition of functionalized MWCNT-based materials **4a** and **4b** was realized by initially activating **1a** and **1b** to the corresponding MWCNT-based acyl chlorides and subsequently reacting them with *N*-tert-butoxycarbonyl-2,2′-(ethylenedioxy)bis-(ethylamine) **3**. The BOC-protecting moiety in **4a** and **4b** was cleaved after treatment with trifluoroacetic acid, liberating free amine groups in MWCNT-based materials **5a** and **5b**, respectively. The amino loading of functionalized MWCNT-based materials **5a** and **5b** was calculated by the Kaiser test and found to be 110 and 150 μmol g^{-1}, respectively.

All MWCNT-based materials were fully characterized with the aid of complementary spectroscopic, thermal and microscopy techniques. Briefly, Raman spectroscopy was employed to prove the successful oxidation of pristine MWCNTs. In the graph of Raman spectra (Supporting Information section, Figure S1), oxidized MWCNTs **1a** displayed an increased D/G ratio as compared with that of pristine MWCNTs (I_D/I_G = 1.26 for **1a**; I_D/I_G = 1.1 for pristine MWCNTs). Furthermore, the intensity of the D-band of material **4a** did not change (I_D/I_G = 1.26) as compared to the one based on **1a**, since the coupling of BOC-amine does not further disrupt the skeleton of MWCNTs. Similar findings observed in the Raman spectra of **1b** and **4b** (Supporting Information section, Figure S2), hence verifying the success of their preparation. ATR-IR studies further confirmed the effective coupling of BOC-amine **3** in oxidized MWCNTs **1a** and **1b**. The latter displayed the characteristic strong carbonyl vibration due to the

presence of carboxylic acid groups at 1714 cm^{-1}, which disappeared while a new band evolved for materials **4a** and **4b** due to carbonyl amide vibration at 1645 cm^{-1} (Supporting Information section, Figures S3 and S4). The functionalization degree of oxidized MWCNTs **1a** and **1b** as well as of functionalized MWCNTs-based materials **4a** and **4b** was evaluated by thermogravimetric analysis (TGA). Pristine MWCNTs are thermally stable up to 900 °C under an inert N_2 atmosphere, while oxidized MWCNTs **1a** and **1b** showed a weight loss of about 5% and 8.5% at 550 °C, respectively, due to decomposition of the carboxylic acid species at the tips of the nanotubes, as well as the defects generated on the carbon skeleton. For materials **4a** and **4b**, a weight loss of 20% and 16% was observed at 550 °C (Figure 1), respectively. On this basis, the number of attached organic units onto MWCNTs was calculated as 1 per every 92 carbon atoms for **4a** and 120 carbon atoms for **4b**.

3.2. The thermotropic behavior of mixed lipid/MWCNTs systems

Thermal methods, in particular DSC, are well established within the liposome field, and are most frequently used to measure the phase behavior of the phospholipid bilayers, from which information on phospholipid conformation and liposome-biomaterials interactions. In addition, knowledge of the transition behavior of phospholipids is essential for the rational development of drug delivery systems and manufacturing protocols. By DSC we studied the interaction between the DPPC and HSPC bilayers and MWCNTs. On heating, $L_{\beta'}$ gel state phospholipids undergo the pre-transition to "rippled" gel ($P_{\beta'}$) state. The pre-transition usually occurs between 5-10ºC below the main transition with a lower enthalpy and may be attributed to rotation of the polar head groups or the co-operative movement of the hydrocarbon chains, prior to melting. The pre-transitions of DPPC (at 36.5°C) and HSPC (at

47.9°C) are explained in term of structural changes in the lamellar lattice. The pre-transition is a thermal phenomenon in which the lipid bilayer reorganizes from one-dimensional lamellar to a two-dimensional monoclinic lattice consisting of lipid lamellae distorted by periodic "ripples". Heating $P_{\beta'}$state phospholipids results in co-operative "melting" of the hydrocarbon chains to give liquid–crystalline phase (Lc state), which is the main phase transition temperature and (Tm) corresponds to 41.5°C for DPPC and 53.5°C for HSPC. This transition is a co-operative thermal event because the intermolecular distance is approximately 2nm and impacts on adjacent molecules (Koynova and Caffrey, 1998; Torchilin and Weissig, 2003). In Table 1, the calorimetric heating and cooling profiles of DPPC with MWCNT-based materials **2a**, **2b**, **5a** and **5b** are presented. The main transition temperature, which corresponds to the mobility of the acyl chains of phospholipids remains unaffected for all the mixed lipid bilayers. The transition enthalpy ΔHm decreased slightly with the presence of **2a** and **2b**, while the transition enthalpy ΔHm decreased significantly with the presence of **5a** and **5b**, respectively. This observation indicates that only the MWCNTs in **5a** and **5b** increase the intermolecular distance between the lipid molecules (and less energy requested for melting) but do not interact strongly with the DPPC lipid chains and for this reason the Tm remain more or less unaffected. The cooperativity, which corresponds to$\Delta T_{1/2}$values (half width at half peak height of the transition), remained unaffected in the presence of **5a** and **5b**, too (Table 1 and Figure 1). The presence of **2a** and **2b** caused reduction of the cooperativity of DPPC lipids, as indicated by the increase of$\Delta T_{1/2}$ values. Generally, according to the recent literature, when nanotubes are parallel to the membrane and located at its center, only small structural differences are observed compared to pure lipid membrane (Baoukina et al., 2013; Parathasarathi et al., 2012). On the other hand, the pre-transition effect

decreased and this observation indicates strong interactions between the polar groups of DPPC/HSPC lipid and all examined MWCNT-based materials. Namely, the pre-transition enthalpy (ΔHs values) was lower when the MWCNTs are included at the lipid bilayer system in comparison to pure DPPC lipid bilayers, showing that interaction of MWCNTs with polar groups is enhanced by **5a** and **5b** inclusion (Table 1 and Figure 1). The pre-transition temperature (Ts) decreased one degree ºC in the presence of **5a** and **5b** indicating the change of the orientation of polar groups and biophysical defects –different in nature materials in the bilayer. Regarding the cooling scans, small hysteresis (1.5ºC) was observed for temperature at which the thermal event (T_{onset}) starts and Tm was observed (Koynova and Caffrey, 1998; Torchilin and Weissig, 2003).

Another chemical composition of lipid was also used in order to investigate how the MWCNTs can affect the thermotropic behavior of model lipid membranes. Table 2 and Figure 2 summarize the calorimetric values, measured in all the fully hydrated mixed HSPC/**2a**; **2b**; **5a** and **5b** mixed lipid bilayers in the heating and cooling cycles. The incorporation of MWCNTs into HSPC bilayers led to more or less the same thermotropic profiles, in relation to those obtained with DPPC thermograms. This observation indicates that the influence of these additives on the main transition is not influenced by the nature and the physicochemical/thermotropical properties of the lipid. For this reason, we can predict the localization of these MWCNTs within the liposomes.

3.3. Liposome-MWCNTs innovative delivery platforms of therapeutic agents: physicochemical evaluation

Liposome-MWCNTs delivery platforms prepared according to the protocol used in the literature as described in the experimental section. We employed dynamic and electrophoretic light scattering in order to investigate the physicochemical characteristics of these mixed nanocarriers. The presence of MWCNTs causes alterations of the size of the conventional HSPC and DPPC liposomes. Namely, the presence of **2a** and **2b** reduce the size of HSPC/DPPC mixed nanocarriers, while the presence of **5a** and **5b** caused an increase of the hydrodynamic diameter (Dh) of the mixed nanocarriers. On the other hand, the **5a** and **5b** led to more homogeneous liposomal population, as low polydispersity index values revealed. We should highlight that in all cases the size of the prepared mixed nanocarriers less than 300nm, allowing their use as drug delivery vectors for biomedical applications. The ζ-potential values of mixed nanocarriers are near zero (Table 3). This observation indicates the effective incorporation of MWCNTs into the lipid bilayer of liposomes and absence of surface charge into mixed liposomal surface. In other words, the ζ-potential remains unaffected after the incorporation of MWCNTs into lipid bilayer.

Furthermore, fluorescence spectroscopy has been utilized to exact some qualitative information on the internal lipid structure and environment of the lipid/MWCNTs nanocarriers. Changes occurring in the lipid/MWCNTs bilayer were monitored by fluorescence spectroscopy measurements of incorporated pyrene. The pyrene was used as a hydrophobic/lipophilic probe, able to incorporate itself in the hydrophobic region of the lipid bilayer. The micropolarity of the hydrophobic regions in HSPC and DPPC liposomes was also determined for comparison purposes. For HSPC/**2a** and HSPC/**2b** nanostructures the micropolarity was increased. This increase may be correlating to the way that the carbon nanotubes are incorporated into the lipid bilayer (lipid tails region), which apparently results in minimum changes in the micropolarity.

The microfluidity was also increased. The same results were observed for the mixed systems composed with DPPC and the systems prepared by the addition of **5a** and **5b**.

3.4. Morphological evaluation of lipid/MWCNTs systems

For obtaining information about these samples we have used cryo-TEM imaging, which is a very precise technique, since it permits the observation of the materials in their original environment, avoiding the drying of the water suspensions, which can cause modifications in the inner structure of the liposomes, as well as the interaction between liposomes and MWNTs. Moreover, the formation of a thin layer of amorphous ice during vitrification, also serves as a protection to minimize the damage of these electron beam-sensitive materials under the electron beam microscope. In addition, it is worth mentioning that lipids scatter electrons very weakly, and the imaging of their structural details has very low contrast. For improving the contrast, we used an aperture under phase contrast imaging conditions (Arenal and Stephan, 2015; Arenal et al, 2010). Figures 4 and 5 correspond to cryo-TEM micrographs showing different HSPC and DSPC liposomes as well as the presence of the CNTs. The liposomes are clearly visible in these images. Their size and morphology are the standard ones for such materials (Kuntscheet et al., 2011). As for CNTs concern, their structural characteristics (length and diameter) are the same as compared to the original ones.

3.5. *In vitro* screening

To study the toxicity of the aforementioned systems we inoculated human mammary fibroblasts (mcf10A) with various nanosystems at the following concentrations: 500, 250, 125, 60, 30, and 10 μM (Figure 4). After three days in the presence of the mixed systems the cell viability was measured using the MTT assay. From the measured

values the IC_{50} curve for each system was calculated. The results showed low toxicity of the vast majority of the lipid/MWCNTs nanocarriers, even at high concentrations i.e. 500μM (Figure 4). The exact IC_{50} values were not easy to calculate with accuracy, since all the lipid/MWCNTs exhibited low toxicity even at high concentrations, and it was calculated after curve fitting extrapolation. The IC_{50}values for HSPC/MWCNTs vesicles and DPPC/MWCNTs nanocarriers are greater than 500 μMin all cases (Figure 4).

4. Conclusions

In this investigation we successfully designed and developed multi-walled carbon nanotube-liposome drug delivery platforms. For this purpose, MWCNTs were oxidized via two different oxidation procedures and the oxidized MWCNTs were treated, in order to induce different surface charges onto MWCNTs-based materials. Then, we studied the cooperativity between the functionalized MWCNTs and DPPC and HSPC, two different lipids (with different main transition temperature (Tm)) by DSC. Strong interactions between the functionalized MWCNTs and the polar groups of phospholipids were observed in some cases, while in some other cases the nanotubes were oriented parallel to the membrane and located at the center of lipid bilayers. Liposome-MWCNTs delivery platforms prepared according to the protocol used in the literature and several light scattering were used in order to investigate the physicochemical characteristics of these mixed nanocarriers. The presence of MWCNTs causes alterations of the size, size distribution and surface charge of the conventional HSPC and DPPC liposomes. The cryo-TEM images visualized the MWCNTs-liposome drug delivery platforms. The results from *in vitro* screening

experiments showed low toxicity of the vast majority of the lipid/MWCNTs nanocarriers, even at high concentrations. The last observation indicates than the prepared systems are suitable and safe vectors for encapsulation of active pharmaceutical ingredients. Finally, to the best of our knowledge, this contribution represents the first report on preparation and physicochemical characterization of kind of MWCNTs and lipids. We herein provide a novel hybrid system with a versatile and tunable structure fully characterized that could have potential interest for drug delivery platforms with added value in loading and release of bioactive compounds.

Acknowledgments. The cryo-TEM studies were conducted at the Laboratorio de Microscopias Avanzadas, Instituto de Nanociencia de Aragon, Universidad de Zaragoza, Spain. The Government of Aragon, and the European Social Fund are gratefully acknowledged. R.A. gratefully acknowledges the project "Construyendo Europa desde Aragon" 2014-2020 (grant number E/26). R.A. and R.F.-P. gratefully acknowledge the support from the Spanish Ministry of Economy and Competitiveness (MINECO) through project grant MAT2016-79776-P (AEI/FEDER, UE). The research leading to these results has received funding from the EU H2020 program ETN Grant Agreement 642742.

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