Cancer-Cell-Targeted Theranostic Cubosomes

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Supporting Information

ABSTRACT: This work was devoted to the development of a new type of lipid-based (cubosome) theranostic nanoparticle able to simultaneously host camptothecin, a potent anticancer drug, and a squarain-based NIR-emitting fluorescent probe. Furthermore, to confer targeting abilities on these nanoparticles, they were dispersed using mixtures of Pluronic F108 and folate-conjugated Pluronic F108 in appropriate ratios. The physicochemical characterization, performed via SAXS, DLS, and cryo-TEM techniques, proved that aqueous dispersions of such cubosomes can be effectively prepared, while the photophysical characterization demonstrated that these nanoparticles may be used for in vivo imaging purposes. The superior ability of these innovative nanoparticles in targeting cancer cells was emphasized by investigating the lipid droplet alterations induced in HeLa cells upon exposure to targeted and nontargeted cubosomes.

INTRODUCTION

The main goal of nanomedicine is probably the improvement of diagnostic and therapeutic tools available in clinics. Therefore, considerable efforts have been made to develop the physicochemical and biological characteristics of the nanoparticles engineered to encapsulate drugs or imaging agents with pharmaceutical purposes. Since nanoparticles may be formulated using an extensive range of organic and inorganic materials, these efforts resulted in the assembly of a huge number of “soft” and “hard” nanocarriers, such as polymeric, silica, or iron oxide nanoparticles, liposomes, dendrimers, and quantum dots. Recently, the development of single platforms able to simultaneously transport and release pharmacologically active and imaging agents has led to so-called theranostic nanoparticles. Besides, the latter usually contain a targeting ligand as a third component to specifically direct both the therapeutic and diagnostic agents to the pathological site. In principle, these multifunctional nanocarriers should fulfill a number of requisites that include the controlled delivery of poorly water-soluble (multiple) drugs, the selective targeting of malignant cells for minimizing side effects, and the in-progress evaluation of the treatment efficacy via molecular detection.

The accumulation of nanoparticles in tumor tissues passively occurs as a result of aberrant tumor angiogenesis that forms nonmature, leaky capillaries. Indeed, the primary consequences of the defects in tumor vessels are great permeability to colloidal macromolecules or nanoparticles in comparison to physiological vessels and reduced lymphatic drainage of these colloids from the interstitial space of tumors, a phenomenon called the enhanced permeation and retention (EPR) effect. Conversely, nanoparticles may be actively targeted to tumor tissues employing cancer-cell-specific ligands such as folic acid. The popularity of this ligand has mainly grown because cancer cells frequently overexpress folate receptors (which is the case for ovary, breast, brain, kidney, head, neck and other tumors) and because of its high binding affinity, ease of modification, stability during storage, and low cost.

Fluorescent imaging is one of the most powerful techniques in clinical diagnosis for monitoring biological processes in living systems due to its characteristics of high spatial and temporal resolution. Contrast agents such as Gd complexes and fluorodeoxyglucose are widely used in MRI (magnetic resonance imaging) and PET (positron emission tomography). In the last few decades, a profusion of fluorescent probes has been developed by synthesis chemists. However, most of them can hardly be used for in vivo applications because the majority of the conventional fluorophores emit in the UV–vis region.
Indeed, in this portion of the electromagnetic spectrum the absorption of biomolecules (water, hemoglobin, etc.), the autofluorescence of the cells, and the light scattering are high, causing limited tissue penetration as well as low signal-to-noise ratios, resulting in unsuitability for in vivo imaging. Conversely, dyes active in the NIR (near infrared) region of the spectrum (650–900 nm) have attracted much attention because of minimum photodamage to biological samples, deep tissue penetration, and minimum interference from background autofluorescence by biomolecules in living systems.36–38 Different classes of NIR-emitting fluorophores have been developed such as phtaloazines, cyanines, and squaraines. In particular, the latter39 are the decondensation products of electron-rich molecules with squaric acid and are characterized by a sharp and intense low-energy absorption associated with strong fluorescence in solution.

Among the various nanocarriers engineered for theranostic nanomedicine, cubosomes have been recently only proposed.40 Basically, these are aqueous dispersions of lipid-based bicontinuous liquid-crystalline phases typically stabilized by Pluronics.41,42 Therefore, the inner nanostructure of the nanoparticles is constituted of curved, triply periodic non-intersecting bilayers folded on an infinite periodic minimal surface of cubic symmetry and organized to form two disjointed continuous water channels.3,44

Throughout this article it will be shown that cubosome nanoparticles coloaded with both an NIR fluorescent probe and an anticancer drug can be prepared and effectively stabilized by mixtures of F108 and folate-conjugated F108 Pluronics that give these nanoparticles superior targeting ability toward HeLa cancer cells with respect to traditional cubosomes.

### EXPERIMENTAL SECTION

**Chemicals.** Monoolein (MO, 1-monooleylglycerol, RYLO MG 19 PHARMA, glycerol monooleate, 98.1 wt %) was kindly provided by Danisco A/S, DK-7200, Grinsted, Denmark. Pluronic F108 (PEO10−

**NMR measurements were performed on a Bruker Avance 300 MHz (7.05 T) spectrometer at an operating frequency of 300.13 MHz at 25 °C. A standard variable-temperature control unit with an accuracy of ±0.5 °C was used. Chemical shifts for 1H NMR are reported in parts per million (ppm), with coupling constants reported in Hertz (Hz). The following abbreviations are used for spin multiplicity: s, singlet; d, doublet; t, triplet and m, multiplet. A selection of the characteristic 1H signals are reported in the synthesis of F108-CDI, F108-NH2, and F108-FA Pluronics.

**Synthesis of Folate-Conjugated Pluronic F108.** Synthesis of CDI-Activated Pluronic F108 (F108-CDI). To a solution of Pluronic F108 (2.01 g, 0.014 mmol) in dry acetonitrile (10 mL), a solution of a great excess of N,N′-carbodimide (CDI, 0.26 g, 1.6 mmol) in a solution of triethylamine (0.78 mL, 0.78 mmol) was added. The reaction mixture was kept stirring for 24 h at room temperature under a nitrogen atmosphere. The solvent and unreacted ethylenediamine were removed under vacuum. The crude product was precipitated with diethyl ether and collected as a beige powder. Yield: 93% (1.890 g, 0.13 mmol); mp: 54 °C. 1H NMR (300 MHz, DMSO-d6, 298 K): δH 1.03 (d, J = 6 Hz, 3H × 50, −CH3 of PPO), 3.47−3.54 (m, 3H × 50, 4H × 264, −CH3−

**Synthesis of NH2-Terminated Pluronic F108 (F108-NH2).** A solution of Pluronic F108-CDI (1.010 g, 0.068 mmol) in dry acetonitrile (10 mL), 2.4 times ethylenediamine was added. The reaction mixture was kept stirring for 24 h at room temperature under a nitrogen atmosphere. The solvent and unreacted ethylenediamine were removed under vacuum. The crude product was precipitated with diethyl ether and collected as a beige powder. Yield: 81% (0.813 g, 0.055 mmol); mp: 56 °C. 1H NMR (300 MHz, DMSO-d6, 298 K): δH 1.03 (d, J = 6 Hz, 3H × 50, −CH3 of PPO), 2.65−2.75 (m, 2H × 2 protons adjacent to the terminal amine), 2.96 (q, J = 6 Hz, 2H × 2 protons adjacent to the amide bond), 3.47−3.54 (m, 3H × 50, 4H × 264, −CH3−CH(CH2)−O− of PPO and −CH2−CH2−O− of PEO), 7.17 (t, J = 6 Hz, 3H × 2, amidic NH), 7.60 (1H, CH=O of the amide bond), 8.25 (1H, unreacted amide bond). IR (solid state, cm−1): ν = 2885 (s), 1716 (w).
Dynamic Light Scattering (DLS). Particle size and ζ-potential determinations of the nanoparticles were performed with a ZetaSizer Nano ZS (Malvern Instruments, Malvern, U.K.) at a temperature of 25 ± 0.1 °C. Samples were backscattered by a 4 mW He–Ne laser (operating at a wavelength of 633 nm) at an angle of 173°. Diluted samples (1:50) were housed in disposable polystyrene cuvettes of 1 cm optical path length with water as the solvent. At least two independent samples were taken, each of which was measured three to five times. The autocorrelation function (ACF) of scattered light intensity was converted to the ACF of the scattered electric field. From this last quantity, the software supplied by the producer evaluates the intensity-weighted size distribution function through cumulant analysis. The hydrodynamic diameter distribution is mean and variance indistinguishable from those recovered through intensity-weighted size distribution functions were monomodal with the G factor.

Small-Angle X-ray Scattering (SAXS) Experiments. Small-angle X-ray scattering was recorded with a S3-MICRO SWAXS camera system (HECUS X-ray Systems, Graz, Austria). Cu Kα radiation of wavelength 1.542 Å was provided by a GenX X-ray generator, operating at 50 kV and 1 mA. A 1D-PSD-50 M system (HECUS X-ray Systems, Graz, Austria) containing 1024 channels of width 54.0 μm was used for the detection of scattered X-rays in the small-angle region. The working q range (Å⁻¹) was 0.003 ≤ q ≤ 0.6, where q = 4π sin(θ)λ⁻¹ is the scattering wave vector. For the analysis, thin-walled 2 mm glass capillaries were filled with the cubosome dispersions. The diffraction patterns of cubosomes were recorded for 2 h. The lattice parameter a of the cubic phase was determined using the relation a = d[(h² + k² + l²)½] from linear fits of the plots of 1/d versus (h² + k² + l²)½, where d = 2π/q (q is the measured peak position) and h, k, and l are the Miller indices. Water channel radii were calculated using the relation Rg = [Acr/2πff]^1/2λC − Lg, where λ is the lipid length value (17 Å), a is the lattice parameter obtained from the SAXS analysis, and Acr and ff are the surface area and the Euler characteristic of the IPMS geometries (Pn3m, Acr = 1.919, ff = −2). To minimize scattering from air, the camera volume was kept under vacuum during the measurements. Silver behenate (CH₃(CH₂)₂₀−COOAg) with a d spacing value of 58.38 Å was used as a standard to calibrate the angular scale of the measured intensity.

Photophysical Measurements. Cubosome solutions were diluted with Milli-Q water. UV–vis absorption spectra were recorded at 25 °C with a PerkinElmer Lambda 45 spectrophotometer. Quartz cuvettes with an optical path length of 1 cm were used. Corrected fluorescence emission and excitation spectra (450 W Xe lamp) were obtained with an Edinburgh Instruments FLS920 modular UV–vis–NIR spectrophotometer equipped with a Hamamatsu R928P P photomultiplier tube (for the 500–850 nm spectral range) and an Edinburgh Instruments Ge detector (for the 800–1600 nm spectral range). The same instrument connected to a PCS9000 PC card was used for the TCSPC (time-correlated single-photon counting) experiments (excitation laser λ = 405 nm). Corrections for instrumental response, inner filter effects, and phototube sensitivity were performed. All fluorescence anisotropy measurements were performed on an Edinburgh FLS920 equipped with Glan-Thompson polarizers. Anisotropy measurements were carried out by an L-format configuration, and all data were corrected for polarization bias using the G factor.

Cell Culture and Treatments. Human carcinoma cell line HeLa (ATCC collection) was grown in phenol red-free Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, USA) with high glucose, supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U mL⁻¹), and streptomycin (100 μg mL⁻¹) (Invitrogen) in a 5% CO₂ incubator at 37 °C. Cells were seeded in 35 mm dishes, and experiments were carried out 2 days after seeding, when cells had reached 90% confluence. Cubosomes (1:500, 4 μL in 2 mL of free-serum medium) were added to the cells and incubated at 37 °C for 2, 4, and 24 h. For live cell imaging, after replacing the particle suspension with fresh serum-free medium, cells were loaded with fluorescent probes, and after the incubation time, the probes were washed out before the imaging session. Cells were supravitaly stained with the following probes: 300 nM Nile red (NR) (9-diethylamino-6H-benzo[a]phenoxazine-S-one) for 15 min and 650 nM Hoechst 33258 (HOE) for 30 min. Vehicles were DMSO for NR and water for Hoechst. Stock solutions were 1000-fold concentrated not to exceed the 0.1% concentration of vesicles in the medium. Nile red was from Fluika (Buchs, SG, Switzerland); Hoechst was from Sigma-Aldrich (St. Louis, MO, USA).

Fluorescence Microscopy. Light microscopy observations were made with a Zeiss (Axioskop) upright fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with 10×, 20×, and 40×/0.75 NA water-immersion objectives and an HBO 50 W L-2 mercury lamp (Osram, Berlin, Germany). Twelve-bit-deep images were acquired with a monochrome-cooled CCD camera (QICAM, Qimaging, Canada) with variable exposure. For the observation of the HOE
probe, the filters were ex 360 ± 20 nm and em 460 ± 25 nm. For NR, the filters were ex 470 ± 20 nm and em 535 ± 40 nm for nonpolar lipids and ex 546 ± 6 nm and em 620 ± 60 nm for total lipids. The adopted filters allowed a virtually complete separation of the emissions and the simultaneous observation of NR and HOE probes. Image alignments were obtained with Image Pro Plus software.

**Lipid Droplet Staining and Quantification.** Cells were seeded in 35 mm dishes and cultured in serum-containing media. After proliferation, cells were stained with NR and HOE. NR is an ideal probe for the detection of lipids, as it exhibits high affinity, specificity, and sensitivity to the degree of hydrophobicity of lipids. The latter feature results in a shift of the emission spectrum from red to green in the presence of polar and nonpolar lipids, respectively.46

For this reason, in staining living cells with NR, cytoplasmic membranes are stained red, whereas neutral lipids of lipid droplets are stained green. Fluorescent images of NR-stained cells were filtered at 480 nm by the internal monochromator of the microscope interfaced with an imaging microscope. The iod (integrated optical density) per cell was calculated using Image ProPlus software (Media Cybernetics, Silver Springs, MD).

**Confocal Microscopy.** Measurements were performed on mounted slides by a Leica TCS SP5 SM (Leica Microsystems, Mannheim, Germany) inverted confocal microscope. For Hoechst (nuclear stain), excitation was provided by a 485 nm 40 MHz pulsed laser diode (Picoquant, Berlin, Germany), whereas the 458 nm laser line of a cw Ar laser was used to excite the dye. In both cases, the typical excitation average power was in the 100–200 μW range, when measured after the objective. Hoechst emission was collected at 420–480 nm by the internal monochromator of the microscope interfaced with a photomultiplier detector. Dye emission was filtered at wavelengths >680 nm by an interferential filter (Semrock, Rochester, NY, USA) and collected by one avalanche photodiode detector (Picoquant, Berlin, Germany). The confocal pinhole was set to a 1 Airy size; a 100×1.4 NA objective was used for all measurements. The line-scanning speed was 400–700 Hz in standard acquisition mode.

### RESULTS AND DISCUSSION

**Cubosomes Characterization.** Here, the use of monolein (MO)-based cubosomes as nanoparticles for the active targeting of cancer cells was investigated. For this purpose, the surfactant used to stabilize cubosomes (Pluronic F108, F108) was conjugated to folic acid37 (Scheme 1), a targeting ligand which displays a high affinity for the folate receptor overexpressed on the tumor cell membrane. Briefly, F108, initially activated with CDI, was reacted with ethylenediamine to obtain amine-terminated F108, and the resulting F108-NH2 was finally reacted with NHS, DCC, Et3N, and folic acid (see Supporting Information for characterization details). The purity of F108-FA and then the absence of unreacted F108-NH2 were confirmed by a thin-layer ninhydrin assay. Furthermore, after the pH value was decreasing to 2, there was no precipitation of unreacted folic acid.48

Subsequently, mixtures of F108 and its folate-conjugated counterpart were used to stabilize the aqueous cubosome dispersions. At first, the physicochemical characterization of cubosome nanoparticles stabilized with different F108/F108-FA ratios was performed by SAXS and DLS. The results are summarized in Table 1. As can be seen from the SAXS data, nanoparticles retained the Pn3m structure found in traditional cubosomes (i.e., those prepared with 100% F108), with no significant variations in the lattice parameter, even after the complete substitution of F108 with F108-FA. Conversely, the DLS analysis indicated that the amount of F108-FA used to stabilize nanoparticles strongly affected the mean particle size, which increased from 133 nm (100% F108) to 168 nm (100% F108-FA). Although a slight increase in the PDI was detected upon decreasing the F108/F108-FA ratio, independently by the pH value was decreasing to 2, there was no precipitation of unreacted folic acid.48

Table 1. Space Group, Lattice Parameter (a), Water Channel Radius (rW), Mean Diameter (Dm), Polydispersion Index (PDI), ζ Potential, and Stability of the Cubosome Nanoparticles Stabilized with Different F108/F108-FA Ratios (Wt %)46

<table>
<thead>
<tr>
<th>F108/F108-FA</th>
<th>space group</th>
<th>a (Å)</th>
<th>rW (Å)</th>
<th>Dm (nm)</th>
<th>PDI</th>
<th>ζ potential (mV)</th>
<th>stability (weeks)</th>
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</thead>
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<tr>
<td>100/0</td>
<td>Pn3m</td>
<td>99 ± 2</td>
<td>22 ± 1</td>
<td>133 ± 1</td>
<td>0.11</td>
<td>−28 ± 2</td>
<td>&gt;24</td>
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<tr>
<td>90/10</td>
<td>Pn3m</td>
<td>95 ± 1</td>
<td>20 ± 1</td>
<td>135 ± 3</td>
<td>0.13</td>
<td>−20 ± 1</td>
<td>&gt;24</td>
</tr>
<tr>
<td>80/20</td>
<td>Pn3m</td>
<td>97 ± 2</td>
<td>21 ± 1</td>
<td>139 ± 3</td>
<td>0.11</td>
<td>−29 ± 1</td>
<td>&gt;24</td>
</tr>
<tr>
<td>70/30</td>
<td>Pn3m</td>
<td>100 ± 1</td>
<td>22 ± 1</td>
<td>142 ± 1</td>
<td>0.13</td>
<td>−29 ± 1</td>
<td>18</td>
</tr>
<tr>
<td>60/40</td>
<td>Pn3m</td>
<td>93 ± 1</td>
<td>19 ± 1</td>
<td>151 ± 1</td>
<td>0.13</td>
<td>−23 ± 1</td>
<td>18</td>
</tr>
<tr>
<td>50/50</td>
<td>Pn3m</td>
<td>99 ± 1</td>
<td>22 ± 1</td>
<td>159 ± 2</td>
<td>0.12</td>
<td>−25 ± 1</td>
<td>2</td>
</tr>
<tr>
<td>40/60</td>
<td>Pn3m</td>
<td>96 ± 1</td>
<td>20 ± 1</td>
<td>168 ± 1</td>
<td>0.13</td>
<td>−25 ± 1</td>
<td>2</td>
</tr>
<tr>
<td>30/70</td>
<td>Pn3m</td>
<td>102 ± 1</td>
<td>23 ± 1</td>
<td>145 ± 2</td>
<td>0.14</td>
<td>−23 ± 1</td>
<td>&gt;24</td>
</tr>
<tr>
<td>20/80 + drug</td>
<td>Pn3m</td>
<td>102 ± 1</td>
<td>23 ± 1</td>
<td>141 ± 1</td>
<td>0.12</td>
<td>−25 ± 1</td>
<td>&gt;24</td>
</tr>
</tbody>
</table>

*Errors are reported as mean ± SD.

Figure 1. SAXS patterns of cubosome dispersions stabilized by F108 and F108-FA Pluronics. Relevant peaks are indicated.

SAXS patterns of cubosome dispersions is typically related to the very small nanoparticle size (in which the peak width is inversely proportional to the crystalline domain size). Therefore, the quasi-Bragg peaks’ appearance in the sample containing 100% F108-FA represents an additional proof of the increased size of the nanoparticles.
Concerning the measured ζ-potential values (reported in Table 1), they were found to be in the range of −20 to −30 mV, almost equivalent to those of nontargeted cubosomes (−28 mV), with some fluctuations that appeared independently of the F108/F108-FA ratio. Nevertheless, the stability over time was found to depend greatly on the F108/F108-FA content because samples prepared with a higher percentage of F108-FA exhibited a reduced shelf life, as demonstrated by the observed phase separation (Table 1).

The role of Pluronics in this kind of formulation consists of stabilizing the obtained nanoparticles against coalescence or coagulation basically via mixing and entropic effects. Therefore, the reported experimental findings suggest that the conjugation of the folate to the terminal end of the PEO moieties greatly modifies the original stabilizing properties of F108, thus producing the observed alteration in cubosome formulations when F108 is gradually replaced by F108-FA. Indeed, it is likely that such a conjugation destabilizes the delicate balance between the PEO−solvent and PEO−PEO interactions, on the basis of the mixing effects.2 There-
lipids, when long-chain unsaturated fatty acids, such as oleic acid, are available from exogenous sources inside the cells.56 Indeed, after entering the cells, unsaturated fatty acids are rapidly activated, esterified, and stored as triglycerides inside the lipid droplets. Therefore, detecting changes in LD formation and size may be useful in differentiating between targeted and traditional cubosome uptake.

Within this scope, HeLa cells, used as model cancer cells since they express high levels of folate receptors,57 were exposed at different times to both types of cubosomes coloaded with NR and HOE probes to identify LDs and nuclear morphology, respectively. The IOD per cell value of nontreated control cells was set to 100%, and values of cubosome-treated cells were expressed as a percentage of control. Results are reported in Figure 4a,b.

At a short incubation time (2 h) with targeted cubosomes, the size and number of LDs, detectable as punctuated green fluorescent cytoplasmic structures dispersed throughout the cytoplasm, did not show significant differences in comparison to treated nontargeted cubosomes and untreated control cells. On the contrary, statistically significant differences were observed after 4 h of incubation. In those cells, a noteworthy increase in the IOD per cell of 1.6-fold \( (p < 0.05) \) was estimated with targeted cubosomes, in comparison to the 2 h treatment and 1.8-fold \( (p < 0.001) \) increase in comparison to that of nontargeted cubosomes. In contrast, the latter did not show significant differences in comparison to the 2 h treatment. At a longer exposure time (24 h), both nanoparticles were effectively internalized. However, treatment with targeted cubosomes caused a 1.2-fold increase \( (p < 0.05) \) in the IOD/cell in comparison to that of nontargeted nanoparticles (2.9- and 3.5-fold increases \( (p < 0.0001) \) in traditional and FA-containing cubosomes, respectively, versus the control). At this time, LDs appeared as fluorescent bright spots with a globular shape that were more numerous and larger in a remarkable way (1 to 2 μm diameter). Importantly, neither a sign of morphological damage nor chromatin condensation (Hoechst positive nuclei) was observed after both types of nanoparticle treatment (data not shown).

**Confocal Microscopy.** HeLa cells treated with dye-unloaded (control) or dye-loaded cubosomes were imaged by confocal microscopy after fixation with 4% paraformaldehyde for 10 min at room temperature (Figure 5). Note that chromatin was also stained with the Hoechst marker to identify the positions of cell nuclei (Figure 5b,e). Since the dye is infrared-emitting, fluorescence collection at >680 nm was obtained by using a single-photon avalanche photodiode with 30 and 5% photon sensitivities at 700 and 900 nm, respectively. An inspection of control cells reveals that some autofluorescence is produced in the IR region of fluorescence collection upon excitation at 458 nm (Figure 5c). However, dye-treated cells display significant differences in the morphology and distribution of emitters (Figure 5f). In fact, the dye-treated cells display a bright, cell-wide emission background together with large emitting perinuclear spots, while both features are nearly absent in control cells. (Note that panels c and f share the same fluorescence pseudocolor scale, i.e., the intensity differences are real.) Overall, these findings are consistent with an internalization of the dye mediated by cubosome nanocarriers.
CONCLUSIONS

Recent works suggested the possibility of using cubosomes in theranostic nanomedicine. Here, the potential use of cubosomes as theranostic nanoparticles also having targeting properties was explored. The physicochemical investigations proved that these nanoparticles, still retaining their inner nanostructure, can be effectively stabilized by mixtures of Pluronic F108 and folate-conjugated F108. Furthermore, it was demonstrated that camptothecin, a potent anticancer drug, and an NIR-emitting fluorescent probe (a squaraine) can be successfully loaded within the cubosomes. Finally, the cellular uptake of these innovative nanocarriers was monitored on HeLa cells. Following the indication given by the cell lipid droplets in terms of their increase in number and growth in size, the superior performance of the cubosomes stabilized through the F108 and folate-conjugated F108 Pluronics mixtures was confirmed. Indeed, even if at longer treatment times (24 h) both targeted and nontargeted cubosomes were effectively internalized and differences were partially leveled out, results collected at short incubation time (4 h) clearly evidenced that the conjugation of Pluronic with folate speeds the cubosomes uptake as a result of receptor–ligand interactions.

On the whole, the results presented here validate cubosomes as a useful platform for theranostic nanomedicine.

ASSOCIATED CONTENT

Supporting Information
Detailed description of the folate-conjugated Pluronic F108 synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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