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CeF₃-ZnO scintillating nanocomposite for self-lighted photodynamic therapy of cancer

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Abstract We report on the synthesis and characterization of a composite nanostructure based on the coupling of cerium fluoride (CeF₃) and zinc oxide (ZnO) for applications in self-lighted photodynamic therapy. Self-lighted photodynamic therapy is a novel approach for the treatment of deep cancers by low doses of X-rays. CeF₃ is an efficient scintillator: when illuminated by X-rays it emits UV light by fluorescence at 325 nm. In this work, we simulated this effect by exciting directly CeF₃ fluorescence by UV radiation. ZnO is photo-activated in cascade, to produce reactive oxygen species. This effect was recently demonstrated in a physical mixture of distinct nanoparticles of CeF₃ and ZnO [Radiat. Meas. (2013) 59:139–143]. Oxide surface provides a platform for rational functionalization, e.g., by targeting molecules for specific tumors. Our composite nanostructure is stable in aqueous media with excellent optical coupling between the two components; we characterize its uptake and its good cell viability, with very low intrinsic cytotoxicity in dark.

1 Introduction

Self-Lighting PhotoDynamic Therapy (SLPDT [1]) is a promising innovative cancer therapy that has recently gained interest in the scientific community; it holds the promise to extend to deep cancers the traditional photodynamic therapy (PDT [2]), now commonly applied to treat tumors on the skin or just under it. In usual PDT, a laser light stimulates a photosensitizer (PS) molecule, which promote the production of highly cytotoxic singlet oxygen species (¹O₂), causing tissue death through oxidative stress. For this purpose, a broad variety of PS molecules have been considered, i.e., organic dyes and aromatic hydrocarbons, porphyrins, phthalocyanines, and related tetrapyrroles, etc. [3].

From this point of view, SLPDT technique extends the traditional PDT to deep cancer tissues, which cannot be directly reached by light. Recent pilot studies [4, 5] indicate that scintillating nanostructures can potentially be used to activate PDT as a promising deep cancer treatment modality; in this case, scintillator nanostructures play the role of a “local” source of light. When irradiated by X-ray beams as in the traditional radiotherapy of cancer, but at lower doses, they trigger the cascade processes that promote the final production of singlet oxygen [1].

Several classes of nanosystems have been proposed in the literature as effective scintillators for SLPDT, such as nanoparticles, nanotubes, or nanowires [6–9]. Recently, an interdisciplinary collaborating effort proposed an innovative hybrid nanomaterial for X-ray induced PDT [10]. It is based on the stable organic/inorganic interface formed on 3C-SiC@SiO_x core-shell nanowires by functionalization with H₂TPACPP porphyrin. In that case, by the marker kit Singlet Oxygen Sensor Green (SOSG [11]), singlet oxygen generation was demonstrated as a

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61 consequence of irradiation with an X-ray beam at 6 MeV.
62 Consequent cell death due to oxidative stress was con-
63 firmed by clonogenic survival assay during in vitro
64 experiments.

65 The lifetime of singlet oxygen in water is about 3–4 μ s
66 [12]; it corresponds to a mean free path of about 200 nm for
67 Brownian diffusion. This puts a constraint to the local
68 nature of the treatment: the treatment is effective only if the
69 scintillation mechanism is activated in the target tissue.
70 While being efficient scintillators, nanowires are char-
71 acterized by low diffusivity. In this respect, nanoparticles
72 could offer advantages since they diffuse easily and, if
73 functionalized with suitable targeting molecules, could tar-
74 get specific tissues or specific tumor cells.

75 Here we report on the synthesis and the characteriza-
76 tion of a novel composite nanostructure, which we
77 deem could be of use for SLPDT. It is based on a nano-
78 CeF_3 as scintillator material, coupled to nano-ZnO, with
79 size in the range 100–500 nm. CeF_3 is known to be an
80 excellent scintillator material where Ce^{3+} ions are the
81 emitters[13, 14]; it has high density (6.16 g/cm),
82 fast response and high radiation resistance[15, 16] in
83 addition to several advantages provided by the fluoride
84 matrix, such as high quantum yield and high photo-
85 stability[17]. ZnO has the ability to generate reactive
86 oxygen species (ROS) and to have photocatalytic activity
87 [18, 19]. Moreover the oxide surface offers several
88 functionalization possibilities[20] e.g. with targeting
89 molecules that could be used to drive the nanocomposite
90 to determined cancer tissues, such as folic acid[21],
91 transferrin[22] etc. and with PS molecules, such as por-
92 phyrins[10] etc.

93 As already proven in the literature, a co-precipitated
94 mix of CeF_3 and ZnO nanoparticles shows a strong
95 enhancement of the ZnO luminescence, both using an UV
96 excitation or an X-ray source, due to the FRET optical
97 coupling with CeF_3 [23]. In this work, we prepare
98 nanostructures that contain both phases in the same
99 nanocomposite. We detail the synthesis procedure, and
100 we provide morphological and optical characterizations
101 of the nanocomposite; in particular, its UV-excited
102 fluorescence spectrum proves the effective optical cou-
103 pling between CeF_3 and ZnO. By Energy dispersive X-
104 Ray (EDX) microanalysis, we demonstrate their uptake
105 into human lung adenocarcinoma epithelial cell line
106 (A549), while both by CellTiter-Glo and vital staining
107 with methylene blue assays, we prove the good cyto-
108 compatibility of the composite nanostructures. The A549
109 cell line was chosen because this kind of neoplasia is one
110 of most common yet fatal cancers, often with metastasis
111 diffusing throughout the body. When diagnosed and
112 treated by conventional methods, the 5-year survival rate
113 can be only 17.4 %.

2 Materials and methods 114

2.1 Chemicals for the NCs synthesis 115

116 Cerium nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), ammonium
117 fluoride (NH_4F), sodium hydroxide (NaOH), and poly-
118 ethylene glycol (PEG-400) are purchased from Sigma
119 Aldrich® S.r.l. Italy, while zinc acetate dihydrate (Zn
120 $(\text{O}_2\text{CCH}_3)_2 \cdot \text{H}_2\text{O}$) is purchased from Carlo Erba Reagents S.
121 r.l. Italy. All reagents are used as received without further
122 purifications.

123 Deionized (DI) water is produced by a two stage sys-
124 tem (Millipore, Elix plus MilliQ) providing a resistivity
125 greater than 18 $\text{M}\Omega\text{-cm}$ with a good stability over several
126 hours.

2.2 Nanostructures characterization 127

128 Scanning Electron microscopy (SEM) analyses were per-
129 formed using a Field-Emission SUPRA40 Zeiss SEM
130 equipped with a GEMINI FESEM detection column and an
131 oxford instruments EDX microanalysis setup.

132 Transmission electron microscopy (TEM) investigations
133 were performed with a Field-Emission JEOL JEM-2200FS
134 TEM microscope, equipped with High angle annular dark
135 field detector and EDX microanalysis, operated at 200 kV
136 in conventional and Scanning (STEM) mode. For the
137 analysis, the nanostructures were dispersed in ethanol,
138 sonicated and transferred by drop-casting on carbon-coated
139 copper grids.

140 Raman spectra were acquired using a Jobin-Yvon
141 T64000 triple monochromator spectrometer in subtractive
142 mode, employing 1800 g/mm gratings. Raman light was
143 collected by a confocal microscope in backscattering
144 depolarized geometry with a 50 \times objective. Excitation at
145 532 nm was provided by a power controlled DPSS laser,
146 with maximum power to the sample of 1 mW. Typical
147 integration times ranged from 60 to 600 s.

148 UV-visible absorption spectra in the range 200–900 nm
149 have been measured with a Jasco V550 double-source,
150 double-beam spectrophotometer equipped with a photo-
151 multiplier detector (Hamamatsu, R928) in ambient
152 conditions.

153 Excitation and emission spectra were performed in
154 ambient conditions using a Perkin-Elmer LS50 lumines-
155 cence spectrometer. Excitation spectra were collected by
156 monitoring the emission at 325 nm and 375 nm using
157 excitation wavelengths between 200–300 nm. Emission
158 spectra were measured exciting at 240 nm and recording the
159 emission between 250 and 450 nm.

160 2.3 In vitro tests

161 2.3.1 Cell cultures

162 Dulbecco's Modified Eagle Medium, fetal bovine serum,
163 antibiotics for cell culture and all reagents were purchased
164 from Gibco Life Technologies (MO, USA); disposable
165 sterile plastics from Costar (Cambridge, MA). A549 human
166 adenocarcinoma alveolar basal epithelial cells were pur-
167 chased from American Type Culture Collection (ATCC,
168 Manassas, VA, USA) and cultured in DMEM. All media
169 were supplemented with 10% FBS, penicillin (100 U/ml),
170 streptomycin (100 µg/ml), and L-Glutamine (2 mM), and
171 cells were incubated at 37 °C with 5% of CO₂ in a water
172 saturated atmosphere.

173 2.3.2 Direct contact cytotoxicity test

174 Thirty-thousand cells/ml were seeded onto 96 multiwell
175 plates and allowed to attach. A stock suspension of NCs
176 was freshly prepared. The DMEM in the wells was then
177 replaced with fresh medium containing NCs at increasing
178 concentrations (0, 5, 10, 25, and 50 µg/ml) and the incu-
179 bation continued at 37 °C and 5% CO₂ in a humidified
180 atmosphere for 24 and 72 h. Cell viability was analyzed
181 using either a CellTiter-Glo luminescent cell viability assay
182 (Promega, WI, USA), a homogeneous method of deter-
183 mining the number of viable cells based on the quantitation
184 of cellular ATP, or cell counting (data analysis performed
185 by ImageJ software) by optical microscopy (stereomicro-
186 scope Nikon SMZ25) after vital staining with Methylene
187 Blue.

188 2.3.3 Statistical analysis

189 Data were analyzed using Prism 6 (GraphPad, La Jolla, CA,
190 USA). All values are reported as Mean ± Standard Devia-
191 tion of six repeated experiments. Differences between group
192 means were evaluated with one-way or multivariate
193 ANOVA statistical tests with Tukey's post hoc tests. No
194 statistically significant (*p*-values < 0.05) differences were
195 found.

196 2.3.4 SEM imaging

197 For SEM observation of A549 cultured for 72 h with the
198 NCs, the samples were first washed with PBS at 37 °C and
199 then fixed using 2.5% glutaraldehyde in 0.1 M sodium
200 cacodylate buffer for 30' at room temperature. Next the
201 samples were dehydrated with a sequence of treatments in
202 alcohol at increasing concentrations. Finally, the samples
203 were covered with a thin gold layer deposited by sputtering.
204 Cell distribution and morphology were characterized using

a Field-Emission SUPRA40 Zeiss SEM equipped with a 205
GEMINI FESEM detection column, operated at 3 keV. 206

207 3 Results

208 3.1 Synthesis procedure

209 CeF₃-ZnO nanocomposites are synthesized using a two-step 209
wet chemistry synthesis procedure. During the first step, 210
CeF₃ nanoparticles are prepared using a method similar to 211
that reported by Chen[24]. 1 mmol of cerium nitrate hex- 212
ahydrate (Ce(NO₃)₃·6H₂O) is dissolved in DI milliQ water. 213
Polyethylene glycol (PEG-400) is added as a surfactant to 214
the Ce(NO₃)₃·6H₂O solution; the mixture is left to stir at 215
room temperature for 10 min. Then, a second solution with 216
3 mmol of ammonium fluoride (NH₄F) in DI milliQ water is 217
added dropwise into the first solution. After stirring for 30 218
min, the mixture is heated for 2 h at 90 °C. CeF₃ nano- 219
particles are then collected through centrifugation, washed 220
with DI milliQ water for several times and dried overnight 221
at 45 °C in the oven. 222

223 The second step of the synthesis is based on a seed 223
mediated growth: nano-ZnO grows onto the CeF₃ seeds by 224
a method similar to that of Haldar[25]. In our case, 10 mg of 225
CeF₃ seeds are suspended in DI milliQ water, then is added 226
the zinc acetate dihydrate (Zn(O₂CCH₃)₂·H₂O) and the 227
solution is stirred for a few minutes at 350 rpm at room 228
temperature. At the same time, a sodium hydroxide (NaOH) 229
solution is prepared keeping the stoichiometric ratio of 2 230
with the zinc precursor. The reaction is triggered adding 231
carefully and dropwise this solution to the CeF₃ seeds 232
suspension. The synthesis is carried out at 50 °C for 1 h 233
while stirring at 350 rpm. The final products are finally 234
washed several times with DI milliQ water before being 235
collected through centrifugation. 236

237 3.2 Nanocomposites morphology and elemental analysis

238 We investigated the morphology of CeF₃ seeds produced by 238
the first step of the synthesis by SEM. Measurements 239
highlight their spherical shape accordingly with previous 240
works present in literature[26, 27]; SEM images and the 241
size distribution are shown in Supplementary Information 242
Figure SI01. The detailed structural and compositional 243
properties of the CeF₃ nanoparticles have been analyzed by 244
TEM; results are reported as Supplementary Information 245
Figure SI02. The High Resolution TEM study shows that 246
the particles are crystalline and the lattice corresponds to 247
hexagonal CeF₃ (JCPDS card 8–45). 248

249 The SEM and TEM analysis of the CeF₃-ZnO nano- 249
composite sample (Fig. 1) reveal conjugate nanostructures 250
with a size in the range 100–500 nm. They form clusters 251

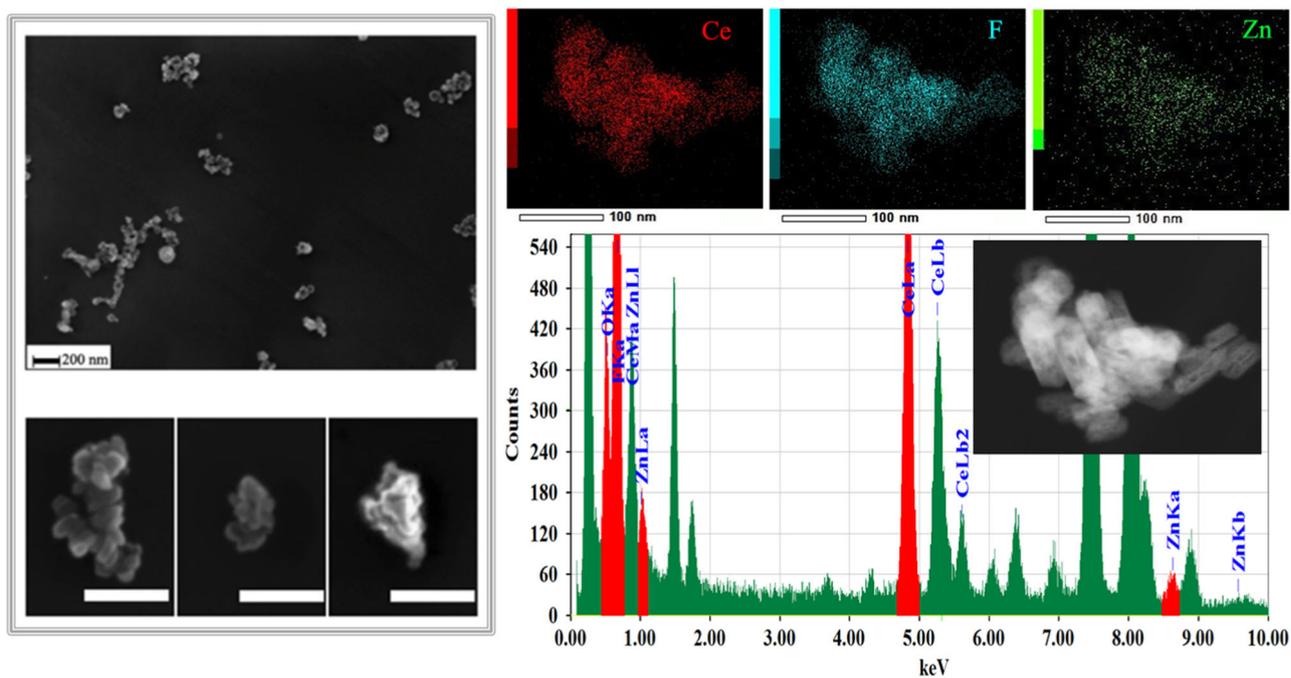


Fig. 1 (Left) representative large-area SEM image (top) and three magnified details (bottom) showing typical CeF₃-ZnO nanocomposites. Scale bars are 200 nm long. (Right) STEM-EDX spectrum

acquired on an isolated composite and corresponding elemental maps of Cerium (*L edge*), Fluorine (*K edge*), and Zinc (*sum of L and K edges*)

252 with an irregular pocked surface; a typical SEM image of
 253 the ensemble behavior of CeF₃-ZnO nanocomposites sus-
 254 pension dried on flat polished silicon is reported in Fig. 1
 255 (top left), together with a detail of single composite
 256 nanostructures (bottom left).

257 STEM-EDX spectroscopy and elemental mapping
 258 (Fig. 1 right) shows that the conjugates, encompassing
 259 several CeF₃ particles, contain Zinc and Oxygen. High
 260 Resolution studies (reported as Supplementary Information
 261 Figure S103) confirm that the ZnO domains within the
 262 nanostructures are crystalline with standard hexagonal
 263 structure (JCPDS card 36–1451), and that the CeF₃ and
 264 ZnO lattices are in close contact.

3.3 Raman spectroscopy

265
 266 The composition of the sample was also investigated using
 267 Raman spectroscopy. In Fig. 2, we report the Raman
 268 spectrum in the range 250–750 cm⁻¹ of the CeF₃ seeds
 269 (Panel a), of ZnO reference nanoparticles (Panel b), and of
 270 the CeF₃-ZnO nanocomposite (Panel c). We focus our
 271 attention on the low-energy peaks corresponding to the
 272 lattice vibrations of CeF₃[28] and ZnO[29]: the correspon-
 273 dent Raman bands are shown in Table 1. We find that the
 274 spectrum of the nanocomposite is consistently fitted by a
 275 linear combination of the spectra from CeF₃ and ZnO (Panel
 276 c, black line).

3.4 Nanocomposite optical properties

277
 278 The luminescence response of the CeF₃-ZnO nanocompo-
 279 site was compared with that of pristine CeF₃ nanoparticles.
 280 Fig. 3 reports the emission spectrum of CeF₃ (red dash line)
 281 and ZnO (blue dash-dot line) nanoparticles, as well as for
 282 the CeF₃-ZnO nanocomposite (green thick line) generated
 283 exciting at λ = 240 nm, below the band-edge wavelength of
 284 ZnO and where CeF₃ has absorbance peaks.

285 As Supplementary Information, we report with further
 286 details the characterization of the excitation and emission
 287 spectra at different emission/excitation wavelengths for all
 288 the aforementioned samples (figure S104).

289 The emission spectra of CeF₃ and ZnO nanoparticles
 290 are in agreement with the literature[26, 27, 30, 31]; for
 291 nanostructured CeF₃ we find a broad emission peak cen-
 292 tered at 325 nm, while for ZnO nanoparticles we observe
 293 the near-band-edge emission peak at 365 nm, as well as
 294 the broad defect emission band in the visible range of
 295 wavelengths. We compare the emission spectrum of the
 296 nanocomposite with a linear combination of the spectra of
 297 CeF₃ and ZnO nanoparticles. The broad emission peak of
 298 CeF₃ in the nanocomposite spectra has 80 % intensity of
 299 that of pristine nanostructured CeF₃; 37 % of the lost
 300 spectrum area is found as a shoulder at higher wavelength,
 301 peaked at 375 nm, e.g., out of the CeF₃ emission peak, as
 302 shown in the inset of Fig. 3. The detailed comparison of

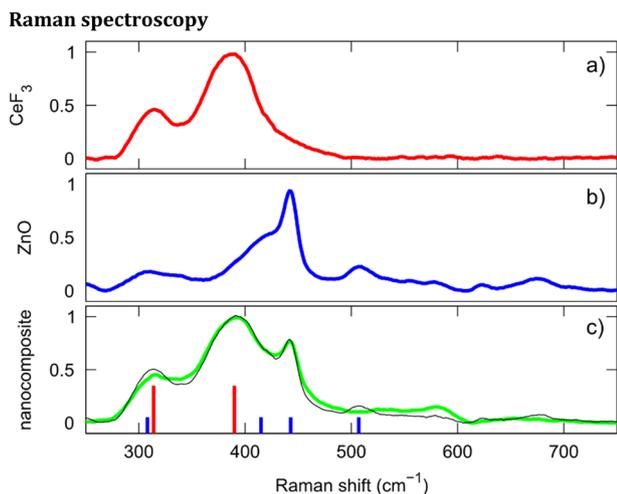


Fig. 2 Normalized Raman spectra of CeF₃ **a** and ZnO **b** nanoparticles and of the CeF₃-ZnO nanocomposites **c**. Panel **c** reports also the linear combination of the spectra of CeF₃ and ZnO nanoparticles (*black line*). The vertical lines (in **c**) are in correspondence of the Raman modes, reported in Table 1, of CeF₃ (*red lines*) and ZnO (*blue lines*), respectively

Table 1 Raman bands of the CeF₃ seed nanoparticles and ZnO reference nanoparticles extracted from spectra in Fig. 2

Wavenumber (cm ⁻¹)	CeF ₃ nanoparticles	ZnO nanoparticles
$\tilde{\nu}_1$		308
$\tilde{\nu}_2$	314	
$\tilde{\nu}_3$	390	
$\tilde{\nu}_4$		415
$\tilde{\nu}_5$		443
$\tilde{\nu}_6$		507

303 the spectra areas is reported as Supplementary Information
304 Figure SI05.

305 Finally, a fourfold increase is found in the 200–300 nm
306 region of the excitation spectrum of the nanocomposite with
307 respect to that of pure ZnO (Supplementary Information
308 Figure SI04) measured at emission of 375 nm.

309 3.5 Cells culture and uptake

310 Proofs of cellular uptake was obtained via SEM-EDX
311 analysis of A549 cells grown on glass substrate in presence
312 of nanoparticles in the culture medium (10 μg/ml). EDX
313 spectroscopy identifies the presence of Ce and F elements,
314 normally not present in these cells; combined with SEM
315 microscopy, sub-micron clusters of nanostructures inter-
316 nalized by cells can be identified. In Fig. 4a we show a SEM
317 image of a typical A549 cell overlaid with EDX maps of Ce

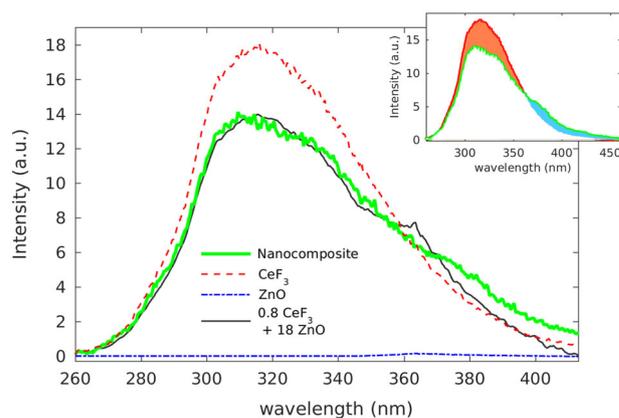


Fig. 3 The luminescence emission of the CeF₃-ZnO nanocomposite excited at 240 nm (*green thick line*), where CeF₃ has its excitation peak, is compared with the analogous emission from pristine CeF₃ (*red dash line*) and ZnO (*blue dash-dot line*) nanoparticles, as well as with a linear combination of the two (*dark thin line*); the differences are discussed in the text. Inset shows the comparison of the spectra of CeF₃ and of the nanocomposite; the area lost on the emission peak of CeF₃ (*orange*) is partially recovered as a broad shoulder above 360 nm (*cyan*), indicative of energy transfer to ZnO in the nanocomposite. All spectra are measured on samples of equal weight concentration (0.05 mg/ml) and corrected to account for differences in the instrumental configurations

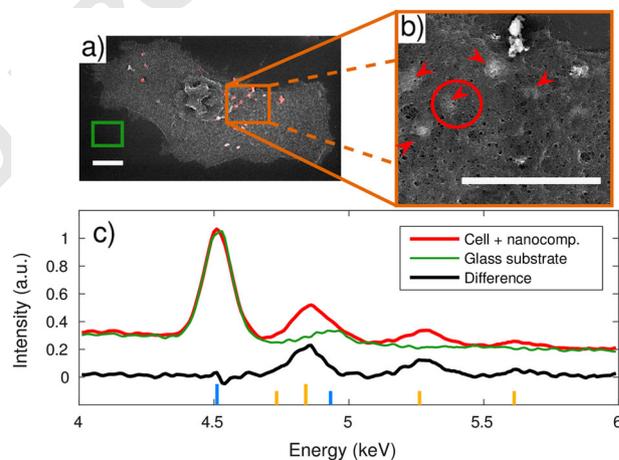


Fig. 4 SEM and EDX spectroscopy performed at 15 keV demonstrate uptake of the nanostructure by the cells. **a** SEM image of an A549 cell cultured in the presence of nanocomposite, overlaid with EDX map of Ce and F elements (*red*). **b** Magnified image shows sub-micron nanostructures inside the cell (*red arrowheads*). *White bars* are 5 μm long. **c** EDX spectra of an internalized nanostructure (*red circle* in Panel **b**), compared with that of the glass substrate (*green rectangle* in Panel **a**). Their difference has peaks corresponding to the L lines of Ce (*yellow*), while Ti K lines (*blue*) come only from the substrate

and F (in red) that identifies the localization of the nano-
structures. A close inspection at higher magnification
(Fig. 4b) shows that brighter aggregates are indeed attached
to the outer side of the cell membrane; other clusters of
nanostructures -such as the ones marked by red arrows- are
clearly surrounded by the cell membrane.

Fig. 4c reports the EDX spectrum collected in correspondence of the region highlighted by the red circle in Fig. 4b (red line); we focus on the 4–6 KeV energy range. We take into account the contribution coming from the glass substrate by subtracting a spectrum measured outside the cell (green line, acquired in the green rectangle in Fig. 4a). The difference curve shows peaks at energies corresponding to the L lines of Cerium (vertical yellow lines in the figure) as a confirmation of the presence of composite nanostructures inside the cell.

3.6 Cytotoxicity evaluation

The cytocompatibility of CeF₃ nanoparticles and CeF₃-ZnO nanocomposites has been evaluated by measuring the viability of A549 cells exposed to increasing concentrations of nanostructures, in the range 0–50 µg/ml, at 24 and 72 hours of culture. Viability has been assessed by CellTiter-Glo assay (Fig. 5) and by counting viable cells marked by methylene blue staining (reported as Supplementary Information, Figure SI07). Both assays shows consistent results; even at the highest concentration, cell proliferation does not decrease in the presence of nanoparticles.

SEM observations (Fig. 6) support this finding: cells appear spread and well attached to the substrate, displaying

lamellopodia and filopodia characteristic of good cellular adhesion. Cell membranes possess the typical morphology of healthy living cells both in absence and in presence of nanoparticles, and no morphological changes characteristic of cellular degradation or programmed cell death, such as membrane blebs and rounded apoptotic bodies, were observed. Cytoplasmic protrusions, identifiable as microvilli for size and shape[32], have been identified as the structures of contact between the cell membrane and the nanostructure clusters. The contact, mediated by cellular extension (Fig. 6d), is established with all the tested nanostructures during uptake, as typical of some endocytosis pathways [33, 34].

4 Discussion

Electron microscopy (SEM/TEM/HRTEM, and STEM-EDX spectroscopy) demonstrates that the two-steps synthesis effectively produces nanocomposites with size in the range 100–500 nm, where nanocrystalline domains of CeF₃ and ZnO are in close contact (Fig. 1).

The composition of the nanostructure is confirmed by Raman spectroscopy (Fig. 2). No other chemical species or spurious phases besides CeF₃ and ZnO have been

Fig. 5 Viability histograms, evaluated by CellTiter-Glo assay, of A549 cells cultured for 24 and 72 hours in absence or in presence of different concentrations of CeF₃ nanoparticles and CeF₃-ZnO nanocomposites. No cytotoxicity was observed within 72 hours, in the 0–50 µg/ml concentration range. Statistical analysis does not reveal any significant difference between the groups

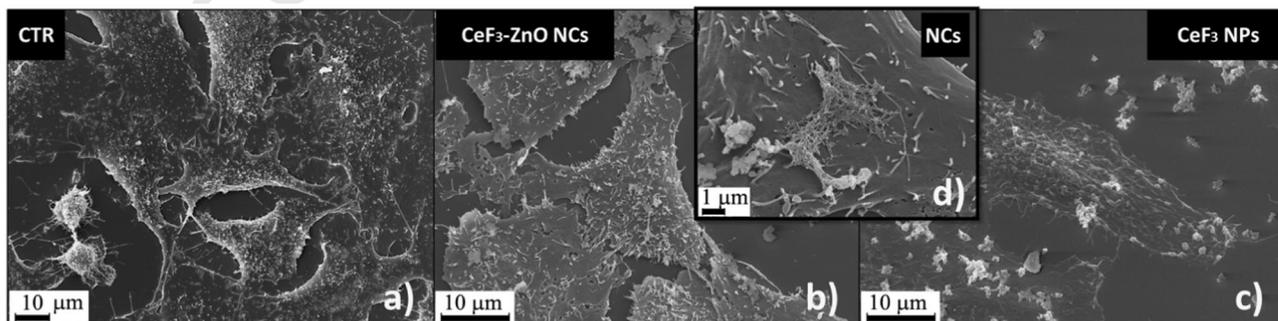
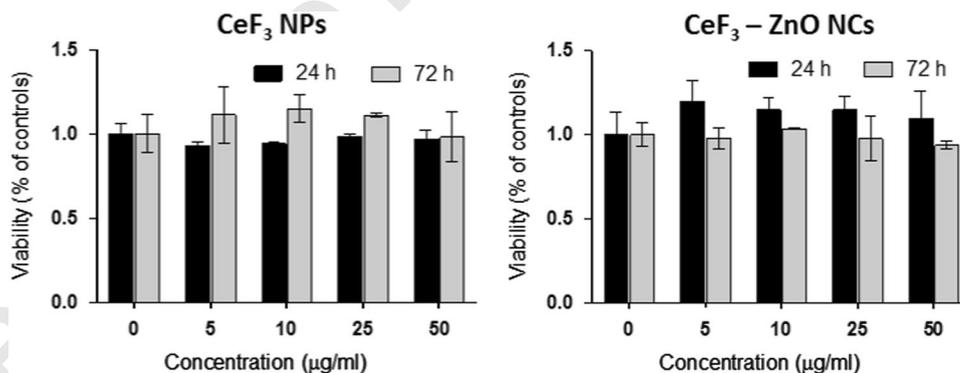


Fig. 6 SEM microphotographs of A549 cells cultured for 72 h with 0 µg/ml (a), 50 µg/ml of CeF₃-ZnO NCs (b) and 50 µg/ml of CeF₃ NPs (c). Detail of a representative A549 cell with CeF₃-ZnO NCs, showing the microvilli assisting cellular uptake (d)

369 synthesized by our approach, at least within the sensitivity
 370 of this technique, as they would result in extra peaks in the
 371 spectrum. For instance, CeO_2 has a single peak at 464 cm^{-1}
 372 in this energy window[35], while ZnF_2 have vibrations at
 373 253, 350 and 522 cm^{-1} . None of these peaks appear in our
 374 data[36]. UV-visible absorption spectroscopy provides a
 375 complementary evidence that the nanocomposite contains
 376 both nanostructured ZnO and CeF_3 (Supplementary Infor-
 377 mation, Figure SI06). We find the four absorbance peaks of
 378 nanostructured CeF_3 below 250 nm, as well as the optical
 379 absorption band edge of ZnO at 350 nm.

380 While these techniques are sensitive to morphology and
 381 chemical composition, nothing can be inferred regarding the
 382 optical coupling between CeF_3 and ZnO, which is a central
 383 point in view of the foreseen application to SLPDT. This
 384 issue is addressed by luminescence spectroscopy.

385 The luminescence spectrum of the nanocomposite
 386 (Fig. 3) excited at 240 nm shows that the emission con-
 387 tribution of CeF_3 , a broad peak centered at 325 nm, is
 388 altered by a shoulder at 375 nm which is compatible with
 389 the ZnO near band-edge emission (see Fig. 5 of ref.[37]).
 390 We calculate that the emission at 375 nm in the nano-
 391 composite is 18 times stronger than in pristine ZnO nano-
 392 particles excited at 240 nm; this means that when the nano-
 393 structure is excited where CeF_3 has maximum absor-
 394 bance/excitation, ZnO near band-edge emission is excited
 395 with efficiency *much higher* than in pristine ZnO
 396 nanostructures.

397 We interpret these results as due to optical coupling
 398 between CeF_3 and ZnO. This is rationalized in terms of the
 399 energy levels of CeF_3 and ZnO as represented in Fig. 7. A
 400 similar scheme was proposed in ref.[23] for a physical mix
 401 of nanoparticles, and explained in term of FRET optical
 402 coupling. In physical blend of CeF_3 and ZnO nanoparticles,
 403 a factor 4 increase in ZnO X-ray luminescence was
 404 observed[23]: for our nanostructure, we expect higher

enhancement under X-ray irradiation due to the close con-
 tact between the two materials at the level of the single
 composite nanostructure.

Moreover, from the position of the ZnO near band-edge
 emission shoulder (375 nm), basing on the literature [37]
 reporting the dependence of the ZnO near band-edge
 emission on the size of nanoparticles, we deduce a
 typical size of 18–20 nm for ZnO nanocrystals in the
 nanocomposite. For comparison, a shoulder at 365 nm as
 found in pristine ZnO nanoparticles corresponds to a size of
 5–6 nm.

The use of these nanostructures for SLPDT Therapy
 requires them to be in contact with cell membranes or be
 internalized inside cells. Furthermore, they have to be
 cytocompatible in the absence of light stimuli; upon uptake,
 nanostructures must not compromise cell viability until
 singlet oxygen is generated. To test if this happens, we
 cultured A549 human lung adenocarcinoma epithelial cells
 with CeF_3 -ZnO nanocomposites and we investigated the
 interactions between them and cells in vitro by CellTiter-
 Glo assay, by counting viable cells marked by methylene
 blue staining and by SEM microscopy.

We chose a lung cancer cell line because this kind of
 neoplasia is one of most common yet fatal cancers, often
 with metastasis diffusing throughout the body. When
 diagnosed and treated by conventional methods, the 5-year
 survival rate can be only 17.4 % (“Surveillance, Epide-
 miology and End Results Program”, National Cancer
 Institute, Retrieved 15 July 2014).

Using SEM microscopy and EDX spectroscopy, we
 proved that the nanocomposites are internalized into the
 cells. Both assays highlight no cytotoxicity on A549 cells
 within 72 h, in the 0–50 $\mu\text{g}/\text{ml}$ concentration range. Statis-
 tical analysis of the results of in vitro viability tests does not
 reveal any significant difference between the nanocompo-
 sites and pristine CeF_3 nanoparticles. We conclude that the
 nanocomposites do not affect neither cellular adhesion nor
 proliferation, and that they did not elicit any cytotoxic
 activity.

5 Conclusions

We present a novel composite nanostructure, where we
 coupled CeF_3 seed nanoparticles to act as scintillators to
 excite ZnO, a material known to produce ROS. The
 synthesis of the nanocomposite follows a two-step proce-
 dure. In the first step, CeF_3 seed nanoparticles are synthe-
 sized in water; in the second step, ZnO nanostructures are
 grown onto the seed surface, resulting in the formation of
 nanostructures with size in the range 100–500 nm. In vitro
 tests with alveolar basal epithelial cells from human ade-
 nocarcinoma (A549 cell line) demonstrate that this

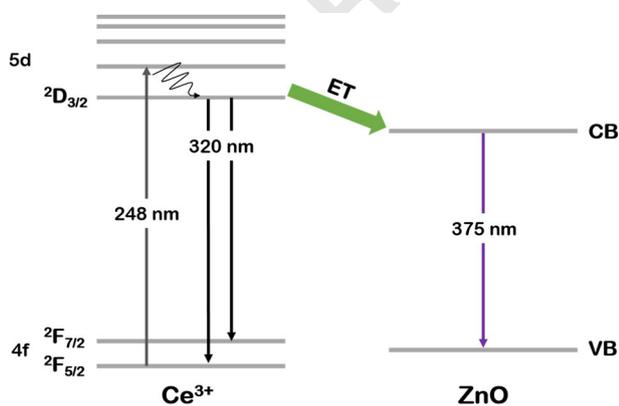


Fig. 7 Scheme of the energy transfer postulated for the CeF_3 -ZnO composite nanostructure. Electronic structures are taken from literature both for CeF_3 [38] and for ZnO[31]

455 proposed SLPDT nanocomposite shows good cellular
456 uptake and has very low cytotoxicity. We characterized the
457 UV-excited luminescence properties of the nanostructure,
458 demonstrating the optical coupling and the energy
459 transfer between its two components. We speculate that
460 ZnO domains in the nanostructure can be excited by
461 the UV emission of CeF₃ also when the nanostructure is
462 irradiated with highly penetrating X-rays from medical
463 facilities.

464 Within this respect, we believe that this composite
465 nanostructure is of potential interest for the Self-Lighted
466 Photodynamic Therapy.

467 Compliance with ethical standards

468 **Conflict of interest** The authors declare that they have no com-
469 peting interests.

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