Nanoscintillators

Characterization of Micro- and Nanoscale LuPO₄:Pr³⁺,Nd³⁺ with Strong UV-C Emission to Reduce X-Ray Doses in Radiation Therapy

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UV-C emitting nanoscale scintillators can be used to sensitize cancer cells selectively against X-rays during radiation therapy, due to the lethal DNA lesions caused by UV-C photons. Unfortunately, nanoscale particles (NPs) show decreased UV-C emission intensity. In this paper, the influence of different Nd³⁺ concentrations on the UV-C emission of micro- and nanoscale LuPO₄:Pr³⁺ is investigated upon X-ray irradiation and vacuum UV excitation (160 nm). Co-doped LuPO₄ results in increased UV-C emission independent of excitation source due to energy transfer from Nd³⁺ to Pr³⁺. The highest UV-C emission intensity is observed for LuPO₄:Pr³⁺,Nd³⁺(1%,2.5%) upon X-ray irradiation. Finally, LuPO₄ NPs co-doped with different dopant concentrations are synthesized, and the biological efficacy of the combined approach (X-rays and UV-C) is assessed using the colony formation assay. Cell culture experiments confirm increased cell death compared to X-rays alone due to the formation of UV-specific DNA damages, supporting the feasibility of this approach.

1. Introduction
Radiation therapy is still the gold standard for cancer treatment and relies on the high penetration depth of X-rays into tissue.

Nonetheless, the therapeutic window is limited by the harmful side effects of X-rays, leading to tumor recurrence or metastases. Current research is focusing on improving conventional radiation therapy by using radiosensitizers such as caffeine, or gold nanoparticles (NPs).[1–4]

More recently, several approaches using nanoscale scintillators as radiosensitizers have been reported. For instance, Du et al. combined LiLuF₄:Ce³⁺ NPs with UV-responsive Roussin’s black salt for increasing the generation of peroxynitrite and superoxide upon X-ray irradiation.[5] Both agents promote cell death by increasing DNA damage during routine radiation therapy. A similar tactic was described by Zhang et al. and Wang et al.. However, they proposed the use of LiLuF₄:Ce³⁺ NPs with surface modifications such as ZnO or smaller Ag₃PO₄ NPs particles combined with cisplatin prodrug, respectively.[6,7] The novel approach in this study uses UV-C-emitting NPs as a radiosensitizer, which damages the DNA of cancerous cells via an oxygen-independent mechanism.

UV-C photons (200–280 nm) target DNA directly in bacteria and eukaryotic cells, causing lethal lesions.[8] The two major DNA lesions are cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (6-4PPs).[9–11] As a consequence of these lesions, the structure of the DNA helix is changed, and if it remains unrepaired, they interfere with DNA transcription and replication causing the death of the cell or organism.[11,12]

UV radiation is of significant interest within the medical field and is a well-established treatment for skin diseases.[13] Although further applications are plausible, UV therapy is limited to the body surface due to its low penetration depth into tissue. Jüstel and Feldmann proposed to use nanoscale UV-C-emitting scintillators inside the body, e.g., at the tumor site, to overcome the low penetration predicament.[14]

Pr³⁺-doped LuPO₄ emits UV-C radiation at the same wavelength range where DNA shows strong absorption. Therefore, nanoscale LuPO₄:Pr³⁺ in the vicinity of cancer cells could not only allow the use of UV-C in situ but improve the therapeutic effect of conventional radiation therapy by producing additional UV-C photons during X-ray treatment. In 2018, Squillante et al. and Müller et al. showed with cell culture experiments that the combined treatment with UV-emitting LuPO₄:Pr³⁺ and X-rays...
resulted in increased cell death compared to the X-ray treatment alone.\cite{15,16}

Nonetheless, reducing the particle size of scintillators to the nanoscale is challenging due to the loss of optical properties along with low yielding synthesis methods.\cite{17} Previous studies have shown that the UV-C emission of Pr\textsuperscript{3+}-activated LuPO\textsubscript{4} is dependent on the particle size: for NPs of around 5 nm, the Pr\textsuperscript{3+} emission was found to be quenched completely. For detectable Pr\textsuperscript{3+} emission in the UV-C range, 80–100 nm particles were necessary to produce emission.\cite{18,19} The majority of previous research has emphasized that the loss of the emission intensity is due to the nonradiative relaxation resulting from the interaction with surface defects.\cite{20,21,22} Moreover, continuous X-ray irradiation degrades materials and increases surface defects. Therefore, an important property of scintillators is that because of the high surface-to-volume ratio of NPs; surface defects play a more important role here than in their bulk or single-crystal counterparts. Consequently, the emission intensity decreases rapidly.\cite{17,21}

In this paper, we propose Nd\textsuperscript{3+} as a co-dopant for LuPO\textsubscript{4}:Pr\textsuperscript{3+} to increase UV-C emission: based on the analysis of the energy level diagram of trivalent rare earth ions (Dieke’s diagram), Nd\textsuperscript{3+}-doped LuPO\textsubscript{4} shows a band at 190 nm caused by interconfigurational 5d-4f transitions and therefore it possesses a big spectral overlap with the 4f-5d transitions of Pr\textsuperscript{3+}. Additionally, the use of Nd\textsuperscript{3+} as a co-activator could enhance the production of electron–hole (e–h) pairs due to thermalization processes upon X-ray excitation.\cite{15,16,19,23}

The preliminary investigation of the UV-C emission intensity and the effect of Nd\textsuperscript{3+} doping into LuPO\textsubscript{4} was conducted for the bulk material: we started by preparing LuPO\textsubscript{4} microscale particles co-doped with different ratios of Pr\textsuperscript{3+} and Nd\textsuperscript{3+}. Simultaneously, LuPO\textsubscript{4} particles solely doped either by Pr\textsuperscript{3+} or Nd\textsuperscript{3+} were synthesized to evaluate the effectiveness of the co-doping approach. Two different set ups were used to analyze the particles. One set up utilized a deuterium discharge lamp (λ\textsubscript{max} = 160 nm) whereas the other one was equipped with an X-ray tube with a tungsten target. Based on these results, the co-doped LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+} samples with the highest emission intensities were synthesized as NPs. We also evaluated the unspecific toxicity of these NPs as well as the increased cytotoxicity of the combined treatment with and without X-ray irradiation on human lung cancer cells (A549).

2. Results and Discussion

2.1. Phase Formation and Particle Size Distribution

Crystallinity and phase purity of the synthesized samples were validated using X-ray powder diffraction (XRD). The recorded XRD patterns were consistent with the literature data for LuPO\textsubscript{4} published by Patwe et al., two of them presented in Figure 1.\cite{24} This illustration compares the sample with the lowest dopant concentration, namely, LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(0.1%,0.75%), to LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,3%), the sample with the highest total dopant concentration. All samples crystallized in the xenotime structure adopting the space group I41/amd, and no signals corresponding to impurities or different phases were detected. An overview of the XRD patterns of all samples can be found in Figure S1 in the Supporting Information.

The results of the laser diffraction measurements of the LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+} particles dispersed in water showed a narrow and monomodal particle size distribution and are depicted in Figure S2 in the Supporting Information. No significant difference has been noticed between samples, therefore the average particle size was calculated to be d\textsubscript{10} = 3.13 μm, d\textsubscript{50} = 5.63 μm, and d\textsubscript{90} = 9.81 μm. Only the sample LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,1%) resulted in much smaller size values compared to the other samples. Thus, no optical characterization of this sample was performed.

2.2. Nd\textsuperscript{3+}, An Efficient Sensitizer for Pr\textsuperscript{3+} Photoluminescence in LuPO\textsubscript{4}

The first set of results describes the effect of the Nd\textsuperscript{3+} addition on the emission of LuPO\textsubscript{4}:Pr\textsuperscript{3+}(1%) upon UUV excitation when systematically increasing the Nd\textsuperscript{3+} concentration from 0% to 3% (Figure 2). The Pr\textsuperscript{3+} concentration of 1% was found to yield the highest UV-C emission intensity and therefore, was kept constant.\cite{19}

The resulting emission spectra upon UUV excitation are shown in Figure 2a, and the corresponding emission integrals from 215 to 400 nm are displayed in Figure 2b. Upon excitation at 160 nm, a significant increase of UV-C emission was observed with increasing Nd\textsuperscript{3+} concentration. The emission intensity of the samples was normalized to the most intense one, namely, LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,0.75%), shown in black. This sample showed a 14 times greater emission integral than single-doped LuPO\textsubscript{4}:Pr\textsuperscript{3+}(1%) in the same range, presented in blue. A concentration higher than 0.75% resulted in a decrease
in the emission intensity. This decline of the emission intensity is possibly due to concentration quenching which has been previously described in other Nd\textsuperscript{3+}-activated luminescent materials such as LiGd(BO\textsubscript{3})\textsubscript{3} or Na\textsubscript{3}La(BO\textsubscript{3})\textsubscript{2}.\textsuperscript{(25)} Concentrations above 1.5% are not shown in Figure 2, as the decreasing trends of these samples remained the same.

To further detangle the effect of Nd\textsuperscript{3+} ions on the photoluminescence of Pr\textsuperscript{3+} in LuPO\textsubscript{4}, the excitation and emission spectra of the most intense sample, i.e., LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,0.75%) were compared to the excitation and emission spectra of single-doped LuPO\textsubscript{4}:Pr\textsuperscript{3+}(1%) and LuPO\textsubscript{4}:Nd\textsuperscript{3+}(0.75%) upon VUV excitation.

Figure 2c (bottom panel) illustrates the excitation and emission spectra of Pr\textsuperscript{3+} in LuPO\textsubscript{4}, the observed 5d-4f emission bands of Pr\textsuperscript{3+} are separated in four distinctive peaks due to crystal field splitting. These peaks correspond to the transitions from the lowest [Xe]4f\textsubscript{5}d\textsuperscript{1} crystal field component of the [Xe]4f\textsuperscript{5}d\textsuperscript{1} configuration to the 1\textit{I}_\text{6}\textsubscript{7/2}, 1\textit{I}_\text{11/2}, and 1\textit{I}_\text{13/2} multiplets, i.e., the lowest ground state terms of the [Xe]4f\textsuperscript{4} configuration of Nd\textsuperscript{3+}. The excitation spectrum of the 4f\textsubscript{2}5d\textsubscript{1}-4f\textsuperscript{3} luminescence was recorded by monitoring the emission band at 190 nm. The spectrum possesses a maximum at around 147 nm. Our spectra are in accordance with previous results reported in the literature.\textsuperscript{(27,28)}

The emission spectrum of co-doped LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,0.75%) is a superposition of the emission bands of Pr\textsuperscript{3+} as well as of Nd\textsuperscript{3+}, as illustrated in the top panel of Figure 2c: In other words, it is composed of a broad band peaking at 190 nm and dominated by four distinctive peaks in the UV-C region. It is worth mentioning that the two weaker Nd\textsuperscript{3+} bands peaking at 240 and 279 nm are no longer visible after the addition of Pr\textsuperscript{3+}. The excitation spectrum of co-doped LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+}(1%,0.75%) monitored at 235 nm reveals the combined excitation spectra of Nd\textsuperscript{3+} (red) and Pr\textsuperscript{3+} (blue).

From the results above, some key findings emerge: first, the emission maximum of Nd\textsuperscript{3+} at 190 nm showed spectral overlap with the Pr\textsuperscript{3+} excitation spectrum. Second, by comparing the excitation band of LuPO\textsubscript{4}:Pr\textsuperscript{3+},Nd\textsuperscript{3+} with the excitation band of LuPO\textsubscript{4}:Pr\textsuperscript{3+}, it is clear to see that the absorption of VUV radiation at 160 nm (purple arrow) was strongly improved by the addition of Nd\textsuperscript{3+}, whereas the absorption of Pr\textsuperscript{3+} at 160 nm is minimal. Third, the increase of Pr\textsuperscript{3+} concentration from 0.5% to 2.0% results in a decreased intensity of the Nd\textsuperscript{3+} emission at 190 nm (red line) as shown in Figure S3 in the Supporting Information. These results demonstrate that the
UV-C emission intensity of the Pr\(^{3+}\) and Nd\(^{3+}\) co-doped samples improve upon excitation at 160 nm even if a rather low Nd\(^{3+}\) concentration is used due to better absorption and energy transfer from Nd\(^{3+}\) to Pr\(^{3+}\).

### 2.3. Nd\(^{3+}\) as a Co-Activator for UV-C Emission of LuPO\(_4\):Pr\(^{3+}\)

This section focuses on the effect of Nd\(^{3+}\) on the UV-C emission intensity of LuPO\(_4\):Pr\(^{3+}\) upon X-ray excitation (50 kV, 2 mA, tungsten target). The results are displayed in Figure 3. The emission spectra were recorded from 200 to 800 nm (Figure 3a), and the emission intensity was integrated throughout the same range (Figure 3b). As observed upon VUV excitation, the emission intensity of the samples increased with increasing Nd\(^{3+}\) concentration. Nonetheless, the highest emission intensity was reached with a Nd\(^{3+}\) concentration of 2.5% (dark purple), instead of 0.75%. LuPO\(_4\):Pr\(^{3+}\),Nd\(^{3+}\)(1%,2.5%) possessed 18- and 52-percentage points higher emission intensity integral than LuPO\(_4\):Pr\(^{3+}\),Nd\(^{3+}\)(1%,0.75%) and LuPO\(_4\):Pr\(^{3+}\)(1%), respectively.

The different optimal concentrations may be explained by the fact that X-rays are ionizing radiation that interacts with the whole LuPO\(_4\) particle, whereas VUV excitation at 160 nm (7.75 eV) mainly excites the surface due to its low penetration depth.\(^{[19]}\) Consequently, an inhomogenous distribution of Nd\(^{3+}\) ions (donor clusters) in the particle and on the particle surface leads to quenching at lower Nd\(^{3+}\) concentrations, as observed upon VUV excitation. The increased UV-C emission intensity of the LuPO\(_4\):Pr\(^{3+}\),Nd\(^{3+}\) particles at a high dopant concentration is likely related to an efficient energy transfer from the LuPO\(_4\) matrix to the Nd\(^{3+}\) ion which has been previously described in the literature.\(^{[29]}\) Furthermore, high Nd\(^{3+}\) concentration increases the chance of harvesting energy absorbed by the host material and Nd\(^{3+}\) and at the same time the probability of energy transfer between Nd\(^{3+}\) and Pr\(^{3+}\) increases.\(^{[30]}\) The proposed energy transfer mechanism is illustrated in Figure 3c.

Figure 3b compares single-doped LuPO\(_4\):Pr\(^{3+}\)(blue) to the most intense sample LuPO\(_4\):Pr\(^{3+}\),Nd\(^{3+}\)(1%,2.5%) in dark purple. From this comparison, it is clear that the addition of Nd\(^{3+}\) did not result in peak broadening or shift of the 5d-4f emission.

![Figure 3](image_url)
bands of Pr$^{3+}$ in the UV-C range. However, a reduced intensity of the peaks observed at around 600 nm can be clearly recognized (inset). These peaks have been attributed to the 1D$^2 \rightarrow$ 3H$^4$ and 3P$^0 \rightarrow$ 3H$^6$ transitions of the Pr$^{3+}$ ion.[21,31,32]

Several studies have observed that the emission of the 1D$^2 \rightarrow$ 3H$^4$ transition of Pr$^{3+}$ disappears upon increasing the Pr$^{3+}$ concentration.[19,31,32] In this case, the Pr$^{3+}$ concentration was kept at 1% in all samples. However, this effect could still be a result of high doping concentrations in the host material (1% Pr$^{3+}$ and 2.5% Nd$^{3+}$).

### 2.4. Strong UV-C-Emitting Nanoscale Scintillators

The nanoscale scintillators were synthesized by using a modified sedimentation nucleation method previously used for Pr$^{3+}$-doped LuPO$_4$ NPs as illustrated in Figure S4 in the Supporting Information.[19,33] The resulting particles were characterized as described in the Experimental Section and representative results for the nanoscale sample LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$(1%,2.5%) (blue) are presented and compared to microscale LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$ (red) with the same dopant concentrations in Figure 4.

Figure 4a displays the diffraction patterns of microscale and nanoscale LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$. The position and relative intensities of the measured XRD peaks are in good agreement to the published data for bulk LuPO$_4$.[24] Nonetheless, as expected for a small mean particle size, the peaks are slightly broadened compared to the bulk material.

Figure 4b illustrates the particle size distribution of the LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$ samples in water. A $d_{50} = 5.6 \mu$m for the bulk material and $d_{50} = 190$ nm for the nanoscale sample were calculated. However, there was a significant difference between this last value and the observed particle size in the transmission electron microscopy (TEM) images (Figure 4c). The TEM photographs show smaller particles, most of them spherical, some are slightly square-shaped (inset). These small particles are between 20 and 80 nm and agglomerate to bigger clusters of around 190 nm, which explains the bigger particle size calculated in the dynamic light scattering measurements. Further studies to stabilize the particles will be undertaken by introducing steric repulsion.

The $\zeta$-potential of the nanoscale suspensions in deionized water ($pH = 6.7$) was also determined. LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$(1%,2.5%) nanoscale scintillators possessed a $\zeta$-potential of about $-40.7$ mV ($\pm 3.2$), and the resulting $\zeta$-potential distribution is presented in Figure S5 in the Supporting Information. A high negative $\zeta$-potential is a key indicator of the stability of LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$ NPs as a colloidal suspension. This finding was further confirmed, since no precipitation of the NPs was observed in suspensions within 6 h (Figure 4d).
When the nanoscale crystals were excited by X-rays, the emission spectra of all samples showed the same four characteristic peaks of Pr$^{3+}$-doped LuPO$_4$ as observed for the microscale particles as depicted in Figure 5a. For a better comparison with previous literature, the emission spectra of the samples were normalized to the emission maximum of YPO$_4$:Bi$^{3+}$ from Philips.$^{19,34}$ The resulting integrals are presented in Figure 5b. The emission integral of the NPs is 55% of the YPO$_4$:Bi$^{3+}$ standard.$^{19}$ This confirms that the herein described samples emit 33% more UV-C radiation than earlier samples with only 22%, published by us in previous papers and compared to the same reference material.$^{19}$

2.5. Radiation Hardness

High energy radiation degrades materials by creating color centers in the host structure, reducing the emission intensity. Although LuPO$_4$ has high radiation resistance, damage still occurs on the surface, especially if the size of the particles is small. Nonetheless, radiation-resistant NPs with stable UV-C emission are critical for the proposed medical application. Therefore, the emission intensity at 235 nm of the nanoscale LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$(1%,2.5%) samples, as well as their microscale counterparts were studied under continuous irradiation with X-rays (Figure 6).

The emission intensity of the microscale particles decreased within the first hour of irradiation by 6% of the initial intensity. Afterward, it remained almost constant until the end (92.8%) of the experiment ($t = 5$ h), as shown in Figure 6a (red). The intensity of the nanoscale scintillators decreased exponentially, losing around 20% of its initial intensity after 5 h (blue).

The extent of quenching of the 5d-4f luminescence of Pr$^{3+}$ has been previously reported as strongly temperature-dependent. Therefore, an on/off cycle of 5 min was added just before the end of the measurement (4.5 h). In both cases,

**Figure 5.** Optical characterization of the nanoscale (blue) and microscale (red) LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$(1%,2.5%) particles: a) emission spectra recorded upon X-ray excitation (50 kV; 2 mA) and b) their emission intensity integrals from 200 to 800 nm. In both cases, the integrals of the emission spectra were set in relation to the normalized integral of YPO$_4$:Bi$^{3+}$ (black, 100%).

**Figure 6.** Radiation hardness of the microscale (red) and nanoscale (blue) co-doped LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$ particles. a) Kinetic scan at 235 nm over 5 h irradiation (50 kV, 2 mA). b) Reflectance spectra of the microscale samples before (black) and after irradiation (red), and c) reflectance spectra of the nanoscale samples before (black) and after irradiation (blue). Inset: Microscale (red) and nanoscale (blue) LuPO$_4$:Pr$^{3+}$,Nd$^{3+}$(1%,2.5%) sample after 5 h irradiation.
the emission intensity did not recover to its initial value. This observation indicates that the measured radiation hardness is not significantly affected by temperature.

Figure 6b,c depicts the diffuse reflectance spectra of the micro- and nanoscaled particles before and after irradiation with X-rays, respectively. The maximum of the lowest energy absorption is located at 708 nm, and it remained the same after irradiation—nonetheless, the absorption of the samples in the range from 250 to 550 nm increases. The increased absorption is ascribed to the formation of oxygen defects, and it can be recognized by a pale reddish color of the samples on the insets in Figure 6a. To ensure that the crystal structure of the LuPO₄:Pr³⁺,Nd³⁺ particles remained unchanged after 5 h of irradiation, the samples were analyzed with XRD. The measured XRD patterns (Figure S6, Supporting Information) showed no extra peaks and were almost identical to the patterns recorded before X-ray irradiation (Figure 4a) confirming the xenotime structure of LuPO₄.

2.6. Cytotoxicity of UV-Emitting NPs

In order to assess the biological effects of the UV-emitting NPs, A549 cells were incubated with different NP concentrations of LuPO₄:Pr³⁺,Nd³⁺(1%,0.75%) and LuPO₄ and irradiated with an X-ray dose of 6 Gy. Using optical microscopy as well as TEM imaging, no uptake of the particles by the cells during the 30 min incubation period was observed (Figure 7). The viability of the cells after the combined treatment was analyzed using the colony formation assay.

Figure 8a depicts the surviving fractions obtained from the colony formation assay after the combined treatment. The left-hand side of the graph illustrates the nonirradiated samples. The nonspecific toxicity of the LuPO₄:Pr³⁺,Nd³⁺(1%,0.75%) and LuPO₄ NPs is reflected by the surviving fractions at 0 Gy. The surviving fractions of the LuPO₄:Pr³⁺,Nd³⁺(1%,0.75%) and LuPO₄-treated samples did not show a significant nonspecific toxicity for any of the tested NP concentrations.

The right-hand side of the graph depicts the surviving fractions after the irradiation with an X-ray dose of 6 Gy. The surviving fraction for X-rays alone was about 15%, which is in accordance with the literature for A549 cells.[35,36] The NP concentration of 1.0 mg mL⁻¹ resulted in a decrease of the surviving fraction to 9%. Using a particle concentration of 2.5 mg mL⁻¹, the surviving fraction decreased by 1 point to 8%. For an NP concentration of 5.0 mg mL⁻¹, the surviving fraction decreased further to 2% for the cells treated with LuPO₄:Pr³⁺,Nd³⁺(1%,0.75%). The surviving fractions after treatment with undoped LuPO₄ were 11%, 11%, and 8% for 1.0, 2.5, and 5.0 mg mL⁻¹, respectively. The higher surviving fractions of the samples treated with undoped LuPO₄ are ascribed to

![Figure 7. TEM image of A549 cells after 3 h of incubation with LuPO₄:Pr³⁺,Nd³⁺(1%,2.5%).](image)

![Figure 8. a) Clonogenic survival of A549 cells treated with different concentrations of LuPO₄:Pr³⁺,Nd³⁺(1%,0.75%) and LuPO₄ before (left) and after (right) the irradiation with 6 Gy. Error bars represent the standard error of mean. b) Detection of CPDs for A549 cells. The concentration of LuPO₄:Pr³⁺,Nd³⁺ and LuPO₄ NPs was 5.0 mg mL⁻¹, the X-ray dose was 9 Gy, and the peak wavelength, as well as radiant exposure of the UV lamp, was 254 nm and 100 J m⁻², respectively. Error bars represent the standard error of mean.](image)
the absence of UV emission. The difference compared to the untreated control is expected due to down-conversion of the hard X-rays to soft X-rays by LuPO₄.

2.7. UV-Specific DNA Damage

The cytotoxic effect of UV radiation is due to the formation of mutagenic DNA damage. The majority of these lesions are CPDs and 6-4PPs. The CPDs are the more abundant lesions whereas the 6-4PPs may have a higher mutagenic effect.[11] The formation of CPDs after UV exposure was used to investigate whether the increased cytotoxicity of the combined treatment is due to the generation of UV emission by the LuPO₄:Pr³⁺,Nd³⁺ NPs. To this end, A549 cells were treated with LuPO₄:Pr³⁺,Nd³⁺, LuPO₄, and X-rays solely as well as with the combined approach. The amount of CPDs was assessed immediately after the treatment. The results were compared to the number of CPDs produced after UV irradiation with a germicidal UV lamp as a positive control. Because of the low sensitivity of the immunofluorescence assay, the experiment was conducted using a X-ray dose of 12 Gy.

Figure 8b illustrates the amount of detected CPDs normalized to 0 and 100 for untreated cells and cells exposed to the UV lamp, respectively. Cells treated only with LuPO₄:Pr³⁺,Nd³⁺, LuPO₄, or X-rays showed a nonsignificant amount of CPD products of about 8% with respect to the positive control. Further, the combined treatment with LuPO₄:Pr³⁺,Nd³⁺ showed a significant formation of UV-specific CPDs of 31%. On the other hand, for the cells treated with undoped LuPO₄ and X-rays, the number of produced CPDs was as low as for particles and X-rays only. After normalization to the amount of CPDs formed after an exposure of 100 J m⁻², the yield of CPDs of the combined approach with LuPO₄:Pr³⁺,Nd³⁺ and X-rays equaled a radiant fluence of 31 J m⁻².

3. Conclusions

LuPO₄ microscale particles co-doped by Pr³⁺ and Nd³⁺ were obtained without impurity phases. These particles exhibited VUV emission at 190 nm which can be tailored with the Pr³⁺ concentration and improved UV-C emission upon VUV as well as X-ray excitation. Ultimately, the combinations exhibiting the highest UV-C yield, were synthesized as NPs. Pr³⁺ and Nd³⁺ co-doped LuPO₄ nanoscale scintillators (~20–80 nm) possessed 55% of the emission intensity integral of the reference (YPO₄:Bi³⁺). These particles are stable in water and radiation resistant. The use of LuPO₄:Pr³⁺,Nd³⁺ nanoscale scintillators upon X-ray excitation resulted in an improved cell inactivation of more than 90% compared to X-rays alone. Our findings support the feasibility of the combined approach and might open new ways for the treatment of cancer. However, the delivery of the particles to the tumor side as well as the uptake of the particles has to be investigated in further experiments. Efficient particle delivery could be achieved by utilizing the passive mechanism known as enhanced permeability and retention effect, which exploits the leaky vasculature of fast-growing tumors. Another approach is the modification of the particles with tumor-targeting antibodies. Furthermore, the uptake of the particles could be enhanced by encapsulating the particles in liposomes to avoid clearance by the immune system.

4. Experimental Section

Synthesis of Microscale Particles: In preliminary experiments, it was estimated that 1% Pr³⁺ results in the highest emission intensity in the UV-C range.[9] Therefore, the Pr³⁺ concentration was kept constant (1%) and different Nd³⁺ ion concentrations were chosen: 0%, 0.25%, 0.5%, 0.75%, 1%, 1.5%, 2%, 2.5%, and 3%. All samples were prepared via a modified suspension method as published earlier.[9]

Briefly, LuO₂ (99.99%, Alfa Aesar), PrO₂ (99.99%, Treibacher Industrie AG), and Nd₂O₃ (99.99%, Alfa Aesar) were suspended in stoichiometric amounts (12 mmol) in water (15 mL). After 30 min, H₂PO₄ (85%, Merck KGaA) was added to the reaction mixture and left stirring for 24 h. Afterward, the suspension was dried overnight at 90 °C. Furthermore, NH₄H₂PO₄ (3.5 wt%) (Reag. Ph Eur, Merck KGaA) was added to the white powder blend. LiF (2 wt%) (99.9%, Sigma-Aldrich) was used as a fluxing agent. The mixture was annealed for 4 h at 1000 °C in a CO atmosphere to prevent oxidation of Pr³⁺. The resulting powder samples were washed once with distilled water and dried at 90 °C. The obtained powders were sieved through a nylon sieve (mesh size = 95 µm) to remove agglomerates.

Synthesis of Pr³⁺,Nd³⁺ Co-Doped LuPO₄ NPs: The nanoscale scintillators were synthesized with a sedimentation nucleation method, introduced by Vistovsky et al. and modified by other groups.[18,19,37] LuCl₃·7H₂O (99.99%, Jiaton), Pr(NO₃)₃ (99.9%, Sigma-Aldrich), and Nd(NO₃)₃ (99.9%, Sigma-Aldrich) (18 mmol) were dissolved in water (81 mL). The doping levels of Pr³⁺ were 0.1% and 1% as well as 0.75% and 2.5% Nd³⁺. NaH₂PO₄ (Reag. Ph Eur, Merck KGaA) was dissolved in water (100 mL), and the pH was increased to 12. The NaH₂PO₄ solution was added dropwise to the first mixture under continuous stirring for 3.5 h and then left to stir for another 30 min. The suspension was centrifuged and washed several times with distilled water until pH = 7 was reached. A final washing step was performed with acetone before the samples were dried at 60 °C under vacuum. The remaining powder was transferred to a corundum crucible and annealed for 2 h at 1000 °C in a reducing CO atmosphere. The synthesis method is illustrated in Figure S4 in the Supporting Information. The last step of the synthesis was modified by adding a washing step with HNO₃. The resulting nanoscale and microscale samples (3 g) were suspended in 12.5% HNO₃ solution (80 mL) and stirred vigorously for 6 h at 60 °C.

Physicochemical Characterization: The particle size analysis of the microscale samples was performed via laser diffraction technique using a La-950-V2 organic particle sizer (Horiba, Japan). The particle size of the NPs and ζ-potential were investigated using a Zetasizer Nano ZS (Malvern, UK). The crystal structure and phase formation were determined via XRD using a MiniFlex II diffractometer (Rigaku, USA). The morphology of the synthesized NPs was analyzed with a JEOL-2100 TEM (Jeol, USA) with a LaB₆ cathode and an acceleration voltage of 200 kV.

Spectroscopic Characterization: UV/Visible emission and radiation hardness were recorded with a FL5980 spectrometer (Edinburgh Instruments, UK) adapted with a water-cooled X-ray tube Neptune 5200 (Oxford Instruments, UK). The parameters were set to an acceleration voltage of 50 kV and a cathode current of 2 mA. The sample was placed 2 cm below the X-ray tube. The X-ray tube was equipped with a tungsten target with X-ray emission lines at 59.32 keV (Kα₁), 57.98 keV (Kα₂), 67.24 keV (Kβ₁), 69.10 keV (Kβ₂), and 66.95 keV (Kβ₃). The X-radiation was not filtered.

The VUV-emission and excitation were recorded with a fluorescence spectrophotometer FLS920 (Edinburgh Instruments, UK) flushed with N₂. The emission of the samples was recorded upon excitation at 160 nm with a deuterium discharge lamp DS-775. All samples were normalized to a YPO₄:Bi³⁺ standard from Philips.[34] Excitation spectra were corrected by the spectrum of a sodium salicylate standard (99.5%, Merck Millipore).
Integration sphere. Excitation was provided by a Xenon arc lamp, and BaSO₄ (99.98%, Sigma-Aldrich) was used as a white standard.

Cell Culture: Experiments were performed using the lung cell line A549 (ATCC-CCL-185), which originates from explanted cancerous lung tissue. Cells were cultivated in cell culture flasks at 37 °C in a humidified atmosphere (95% air/5% CO₂) in Dulbecco’s Modified Eagle Medium (DMEM) (Gibco) supplemented with 1% Penicillin/Streptomycin solution (Gibco) and 10% fetal bovine serum (Gibco). Cells were used between passage 3 to 30 and were plated 24 h prior to the experiment. Directly before the experiment, NPs were dispersed in DMEM with 0.01% Darvan C (Vanbrill Minerals) as a dispersing agent. The dispersion was treated in an ultrasonic bath for 15 min and thoroughly mixed before applied on the cells. For X-ray treatment, cells were irradiated with the biological irradiator X-RAD 320 (Precision X-Ray) at a dose rate of 450 Gy min⁻¹. Acceleration voltage and tube current were 320 kV and 12.5 mA, respectively. Further, a 2 mm Al-filter was used to harden the X-ray beam.

TEM Imaging of A549 Cells: Cells were incubated with and without particles for 3 h. Following incubation, cells were fixed in Karnovsky fixative (K2 buffer). After washing the cells with the wash buffer, cells were treated with 1% OsO₄ at 4 °C on an orbital shaker for 2 h. Subsequently, cells were gently collected and centrifuged to form a pellet. Warm agar was added to the pellet to form an agar-cell pellet. The agar-cell pellet was then dehydrated, in gradient alcohol series, infiltrated with propylene oxide/Epon 812 gradient mix, and embedded in Epon 812 (Tousimis, Rockville, MD). Semi-thin sections (80 nm) were cut and stained with Toluidine Blue. Ultrathin sections (0.5 nm) were cut using a Reichert-Jung Ultracut E microtome (Vienna, Austria), collected on uncoated 100-mesh copper grids, stained with 2% uranyl acetate and lead citrate, (2.66% lead nitrate, 3.52% sodium citrate) and examined on a Philips CM-10 TEM (Eindhoven, The Netherlands). Digital TEM images were taken by AMT-XR41M 4.0 Megapixel Cooled sCMOS camera (Advanced Microscopy Techniques).

Clonogenic Cell Survival: Clonogenic cell survival of treated and untreated cells was investigated using the colony formation assay. Therefore, 3×10⁴ cells were seeded in tissue culture flasks (12.5 cm²) 24 h prior to the experiment. For the experiment, cells were incubated with the NPs for 15 min followed by X-ray irradiation with dose of 6 Gy. Immediately after the X-ray treatment, cells were washed gently with DMEM to remove the NPs. The cells were harvested and cultured in triplicates in 6-well plates. After 14 days, DMEM was discarded, and colonies were fixed using 10% phosphate-buffered formalin solution (pH = 6.9, Fisher Chemical) per well. After 1 h, the cells were stained for 24 h using 1 mL crystal purple solution (1 mg mL⁻¹) (ACS grade, MP Biomedicals). Colonies of more than 50 cells were counted and normalized to the untreated control.

Detection of CPDs: The formation of CPDs was detected using an immunochemistry staining assay (OxiSelect (CPD), Cell Biolabs). Analyses were performed according to the manufacturer’s protocol. Briefly, cells were seeded in 96-well plates to be 80–90% confluent 24 h before the experiment. For the experiment, cells were incubated with NPs, followed by irradiation with X-rays (D = 9 Gy). A germicidal lamp with a peak wavelength of 254 nm was used as a positive control. After irradiation, cells were washed, fixed, and incubated with the monoclonal antibody for CPDs. Horseradish peroxidase was used as a secondary antibody. CPDs were quantified measuring the absorbance at 450 nm using an ELISA plate reader.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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