Published in final edited form as:

Opt Mater (Amst). 2018 June ; 80: 197–202. doi:10.1016/j.optmat.2018.04.033.

Fabrication and characterization of UV-emitting nanoparticles as novel radiation sensitizers targeting hypoxic tumor cells

Michael R. Squillante^{a,*}, Thomas Jüstel^c, R. Rox Anderson^b, Charles Brecher^a, Daniel Chartier^a, James F. Christian^a, Nicholas Cicchetti^a, Sara Espinoza^c, Daniel R. McAdams^a, Matthias Müller^b, Brooke Tornifoglio^a, Yimin Wang^a, and Martin Purschke^b

^aRadiation Monitoring Devices, Inc., Watertown, Massachusetts, USA

^bWellman Center for Photomedicine, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, USA

^cFachhochschule Münster, Institute of Optical Technologies, Münster, Germany

Abstract

Radiation therapy is one of the primary therapeutic techniques for treating cancer, administered to nearly two-thirds of all cancer patients. Although largely effective in killing cancer cells, radiation therapy, like other forms of cancer treatment, has difficulty dealing with hypoxic regions within solid tumors. The incomplete killing of cancer cells can lead to recurrence and relapse.

The research presented here is investigating the enhancement of the efficacy of radiation therapy by using scintillating nanoparticles that emit UV photons. UV photons, with wavelengths between 230 nm and 280 nm, are able to inactivate cells due to their direct interaction with DNA, causing a variety of forms of damage. UV-emitting nanoparticles will enhance the treatment in two ways: first by generating UV photons in the immediate vicinity of cancer cells, leading to direct and oxygen-independent DNA damage, and second by down-converting the applied higher energy X-rays into softer X-rays and particles that are more efficiently absorbed in the targeted tumor region. The end result will be nanoparticles with a higher efficacy in the treatment of hypoxic cells in the tumor, filling an important, unmet clinical need.

Our preliminary experiments show an increase in cell death using scintillating LuPO₄:Pr nanoparticles over that achieved by the primary radiation alone. This work describes the fabrication of the nanoparticles, their physical characterization, and the spectroscopic characterization of the UV emission. The work also presents *in vitro* results that demonstrate an enhanced efficacy of cell killing with x-rays and a low unspecific toxicity of the nanoparticles.

Keywords

UV emitting nanoparticles; Scintillating nanoparticles; Tumor hypoxia; Radiation dose enhancement; LuPO₄

*Corresponding author. Radiation Monitoring Devices, Inc., 44 Hunt St., Watertown, MA 02472, USA., msquillante@rmdinc.com (M.R. Squillante).

1. Introduction

Radiation therapy is one of the primary therapeutic techniques for treating cancer [1]. Nearly two-thirds of all cancer patients receive radiation therapy in the form of neutrons, X-rays, gamma rays, or charged particles during their illness, with an average of 29 radiation treatment episodes. Other treatments, like chemotherapy, gene therapy, and surgery, are often combined with radiation to treat cancer, though there are still significant shortcomings in treatment efficacy for many patients, especially those with hypoxic tumor areas.

Radiation therapy relies on reactive oxygen species, and has difficulty in achieving complete tumor control within hypoxic regions of solid tumors. Cellular hypoxia is associated with radio resistance, resulting in the survival of some cancer cells, which can lead to recurrence and relapse.

In solid tumors, neoplastic and stromal cells frequently experience dramatically reduced oxygenation for a variety of reasons, including severe structural abnormalities of tumor microvessels, deterioration of effective diffusion geometries, and tumor-or treatment-associated anemia. As a result, areas with very low-to-zero oxygen partial pressures occur in solid tumors. These microregions of extremely low O_2 partial pressures are heterogeneously distributed within the tumor mass and may be located adjacent to regions with normal oxygenation. Cells within these hypoxic regions are resistant to radiotherapy [2–5], as well as a wide variety of chemotherapeutics, immunotherapies, and photo-dynamic therapies [6–8].

1.1. Ultraviolet radiation and cancer therapy

There is longstanding research into the photochemistry of cytotoxicity under ultraviolet irradiance, primarily in the field of skin cancer biology and bacterial sterilization. Recent research has confirmed that many of the photochemical UV-induced DNA damage mechanisms are oxygen independent in the intracellular matrix, as they are enabled by direct depositions of photon energy to form DNA base dimer lesions, rather than by a free radical oxidative process [9,10].

Ultraviolet (UV) radiation spans the range of wavelengths from below 200 nm–400 nm, and is divided into three groups: UVC (200–290 nm), UVB (290–320 nm), and UVA (320–400 nm) [11]. The biological effects of UVC and UVB have been studied extensively, and it has been generally concluded that both types of UV light directly damage DNA, causing toxic lesions such as cyclobutane pyrimidine dimers (CPDs), 6–4 pyrimidine-pyrimidone photoproducts and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-Oxo). UVC creates an order of magnitude more DNA lesions per unit intensity than UVB, pointing towards its choice for this study [12].

UVC photons cause damage directly to nucleic acid causing the formation of covalent bonds between certain adjacent bases in the DNA. The formation of such bonds prevent the DNA from being unzipped for replication. UVC exhibits the highest oxygen-independent cytotoxicity and presumably improves the efficacy of killing hypoxic cancer cells because the reactions do not require oxygen to disrupt the DNA, but cannot penetrate deeply into

tissue, and thus has only been used to treat skin diseases. In tissue, UVC penetrates less than a few microns before being completely absorbed, depositing a significant amount of energy directly to DNA damage mechanisms.

Nanoscintillators in close proximity to or inside of a cell can convert X-ray radiation energy into UV photons, thus reducing radiation resistance and preventing relapse.

1.2 High atomic number sensitizers

Another development in radiotherapy research is the use of nano-particles as radio-sensitizers that down-convert the high energy X-rays to lower energy X-rays and electrons. Nanoparticles containing high atomic number (Z) elements have much higher radiation stopping power than the low Z elements that make up tissue. This can enhance the efficacy of the high-energy X-rays by down-converting their energy into secondary photons and electrons whose energy deposition rates are much higher. The energy absorbed is converted into Compton rays, characteristic x-rays, photoelectrons, Auger electrons, and pair production to the form of an electron and a positron. Gold nanoparticles, for example, have shown great promise by increasing radiotherapy toxicity *in vitro*, demonstrating a roughly 60% increase in cell death [13]. Unfortunately, the increased cytotoxicity resulting from the use of high-Z nanoparticles is also oxygen dependent [14].

1.3 UV-emitting nanoparticles

It is possible to make UV emitting materials from high atomic number elements. In this way it is possible to take advantage of the enhancement created by high atomic number materials and the enhancement created by UV photons. There are three important parameters, atomic number, emission spectrum, and emission intensity. The ideal material would have high values of all of these. Fig. 1 shows the absorption spectrum of DNA with a peak at 260 nm due to the purine and pyrimidine bases.

The chart in Fig. 2 shows a number of scintillating materials. The chart plots the density of the materials and their germicidal effectiveness, which is a function of the emission spectrum of the material. The better the overlap of the emission spectrum to the absorption spectrum of DNA, the higher the germicidal effectiveness. Lutetium phosphate has one of the best combinations of atomic number and emission spectrum, and also has a good radiation yield of 15,000 photons per MeV energy absorbed.

2. Material and methods

2.1. Fabrication of $\text{LuPO}_4\text{:Pr}$ nano-sized powder

Nanoparticles of LuPO_4 were synthesized by coprecipitation by slowly mixing lutetium nitrate and praseodymium acetate solutions with $\text{NH}_4\text{H}_2\text{PO}_4$. The mole fraction of praseodymium was varied between 0.5% and 5%. A fine precipitate was formed after reaction. The powder was washed and dried at atmospheric pressure and room temperature. A portion of the powder was further processed under hydrothermal conditions in a pressure vessel. During the wash and dry process, the excess water in the solution was removed via filter paper through a Buchner funnel; the precipitate was then washed three times with

deionized water followed by a final wash with 2-propanol. The precipitates were then thoroughly dried in air at 150 °C for 12 h. During the hydrothermal process, the precipitate was allowed to stay in the solution for 12 h at 150 °C before the wash and dry process described above. Finally, both powder batches were calcined in a reducing atmosphere for 4 h at 950 °C or 2 h at 1100 °C. The powder synthesis processes flow diagram is shown in Fig. 3.

As mentioned above, since the penetration depth of UV light is small, it is critical for the $\text{LuPO}_4\text{:Pr}$ nanoparticles to enter the tumor in the vicinity of the hypoxic region so that the UV light can interact with tumor cells and damage their DNA. In order for the nanoparticle to enter into the tumor, the size of the particle is critical. During the current research, powder processing parameters, such as the powder calcination time and temperature, were examined in order to obtain nanoparticles below 100–200 nm in diameter. X-ray diffraction (XRD), scanning electron microscopy (SEM), as well as transmission electron microscopy (TEM), were used to examine the particles after different processing conditions (temperature and time). Fig. 4 shows SEM images of the resulting powders after calcination at two different temperatures, e.g. 950 °C and 1100 °C. The SEM examination indicates that the primary particles after calcination are around 80 nm in diameter. However, some agglomeration occurred after calcination. The extent of the agglomeration in the powder increases with calcination temperature, with less agglomeration observed in the batches calcined at 950 °C for 2 h. A further TEM examination also confirmed the above observation with a spherical particle diameter of around 100 nm, and some agglomerations after calcination as shown in Fig. 5.

The synthesized $\text{LuPO}_4\text{:Pr}$ nano-particles were studied with XRD to confirm the phase structure after 950 °C for 2 h and 1100 °C for 2 h calcination, respectively. The diffraction patterns were compared with that of the standard ICDD data of LuPO_4 (shown on the bottom of Fig. 6). The XRD results indicate the synthesized powder is a single tetragonal phase for both tested calcination temperatures.

The relationship between the calcination time and particle size was also studied. Particle sizes of the uncalcined $\text{LuPO}_4\text{:Pr}$ powders and those calcined at 950 °C for 10 min, 30 min, and 120 min were analyzed by dynamic light scattering (DLS). The particle size analysis results suggest a bi-modal distribution profile for all the batches regardless of calcination time, with one of the primary particles in the submicron range (from 100 nm to 700 nm) and another of the agglomerations of particles in the 1.5 μm –7.0 μm range. The analysis suggests increases in size for both primary particle and the agglomeration clusters with additional dwelling time at 950 °C, with the size of primary particles increasing from 200 to 700 nm and that of the agglomerated clusters increasing from 2 μm to 7 μm . In order to limit the particle size and agglomeration, a shorter calcination dwelling time will be used during future processing.

The radioluminescence emission of the synthesized $\text{LuPO}_4\text{:Pr}$ powders calcined under different conditions were examined with 50 keV x-ray excitation. The obtained spectra displayed the typical emission band pattern of Pr^{3+} due to the interconfigurational transition $[\text{Xe}] 4f^1 5d^1$ to $[\text{Xe}] 4f^2$ ($^3\text{H}_{4,5,6}$, $^3\text{F}_2$ ground state terms). Moreover, the spectra exhibit some

emission lines in the visible range at 487, 618, 650, and 736 nm, which are typical intraconfigurational $[\text{Xe}]4f^2\text{-}4f^2$ transitions of Pr^{3+} .

2.2. In-vitro cell study

The effect of UVC emission generated by X-ray irradiation was examined on human foreskin fibroblasts (HFF1) pretreated with nano-particles. The $\text{LuPO}_4\text{:Pr}^{3+}$ NPs were dispersed in complete DMEM containing 0.01% Darvan C (Vanderbilt Minerals, LLC, Norwalk, CT). The amounts of powder tested were 1.0, 2.5, 5.0, and $7.5 \text{ mg} \times \text{ml}^{-1}$. The nanoparticle dispersion was treated with ultrasound for 15 min and thoroughly mixed before being applied on cells.

Cells were irradiated with 320 kVp X-rays in 12.5 cm^2 cell culture flasks with the biological irradiator X-RAD 320 (Precision X-Ray, North Branford, CT). Tube current was 12.5 mA. The beam was hardened using a 2-mm thick beam-conditioning Al-filter.

3. Results

3.1. Nanoparticle properties

The emission spectra of the $\text{LuPO}_4\text{:Pr}^{3+}$ NPs are shown in Fig. 7. The spectra were recorded with a commercially available fluorescence spectrometer FLS980 (Edinburgh Instruments, UK). This spectrometer was adapted by in-lining it with a 1–2 mm thick Pb foil. An X-ray source, a water-cooled X-ray tube Neptune 5200 (Oxford Instruments, US) with a W target was attached. Radioluminescence was mostly recorded upon excitation for operation of the X-ray tube at 50 kV and 2 mA.

The spectrum matches the bulk spectrum well. The desired emission between 225 and 275 nm is observed in all samples. Neither the calcining temperature nor the percent Pr^{3+} doping content had a significant effect on the emission spectrum shape or intensity.

Fig. 8 shows the distribution of particle size for nanoparticles prepared under different calcination conditions. Depending on the various processing parameters, the particles ranged from tens of nanometers to thousands of nanometers, indicating significant agglomeration, as was also seen in the SEM images.

3.2. MTT assay

The enhancement due to the $\text{LuPO}_4\text{:Pr}$ nanoparticles was evaluated for synergistic or additive cell kill in *in vitro* experiments at the Wellman Center for Photomedicine at the Massachusetts General Hospital. The data were obtained as follows: HFF1 fibroblasts were incubated with and without $\text{LuPO}_4\text{:Pr}$ prior to irradiation with 2 Gy of X-rays. The viability was measured 48 h after irradiation using a metabolic activity assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, MTT). Fig. 9 shows the result.

The assay indicates some unspecific toxicity due to the $\text{LuPO}_4\text{:Pr}^{3+}$ nanoparticles without irradiation. However, the combined treatment with nanoparticles and radiation shows a significant increase in cell death, which is an improvement in cell kill compared to the highest used concentration or radiation alone.

Another experiment was performed to separate the effects of UV illumination and X-ray energy down conversion. Any heavy-metal containing material should, by itself, enhance cell death by down converting the radiation energy. To separate the effect of the down conversion and UV emission, LuPO_4 nanoparticles were prepared, which did not have praseodymium dopant. Undoped LuPO_4 has no scintillation emission in the 250 nm range. The results are shown in Fig. 10. As expected, the LuPO_4 by itself decreased cell survivability. The praseodymium doped sample, however, resulted in more cell death. Since the radiation down conversion effect should be nearly the same in both samples the data supports the hypothesis that UV emission does enhance cell killing. A slight decrease in survivability is seen in the cells treated with the doped sample with no irradiation, which may be due to cytotoxicity. The chemical and biological reactions of LuPO_4 and $\text{Lu-PO}_4\text{:Pr}$ should, in principal, be the same. This observation needs to be investigated further to understand the cause. This effect, however, is too small to account for the enhancement observed with the doped nanoparticles under 2Gy of irradiation.

4. Discussion

These initial results are promising, showing a statistically significant increase in loss of viability of about 30% in the combined treatment. The data, however, also indicates a nonspecific toxic effect of the nanoparticles themselves which needs to be addressed by optimizing the nanoparticle concentration, size, and morphology, and most likely, coating them with a biocompatible material. These preliminary results are very encouraging, but not conclusive. A much more detailed study will be carried out in future research.

The successful cell killing studies demonstrate that there is an enhancement due to the emission of the UV photons. There is still much work to be done to optimize the materials and the procedures. Particles are heavily agglomerated, and the size varies. Future studies will investigate methods to better control particle size, and particle size uniformity, to eliminate most or all of the agglomeration. Following the availability of better nanoparticles testing will begin in a preclinical study using mice.

5. Conclusions

In this research, we combined traditional X-ray treatment with localized UVC radiation emitted by nanoparticles. Unlike X-rays, UVC damages the DNA directly via an oxygen-independent mechanism and can thus improve the efficacy of the therapy within hypoxic tumor areas.

The impact of localized UVC emission under X-ray irradiation was tested on human foreskin fibroblasts (HFF1) pretreated with nano-particles. Our preliminary experiments show an increase in UVC damage and cell death by using scintillating $\text{LuPO}_4\text{:Pr}$ nanoparticles over that achieved by the primary radiation alone. Preincubation of cells with UVC emitting nanoparticles, followed by X-ray irradiation, resulted in a significant viability loss compared to radiation alone.

Acknowledgements

The authors gratefully acknowledge the support of The U.S. National Cancer Institute under STTR Grant “UV-emitting nanoparticles as novel radiation sensitizers targeting hypoxic tumors”, No. 1-R41-CA206645-01A1.

The authors also acknowledge the advice and encouragement of Dr. Ali Sayer and Dr. Howard Schlossberg of the U.S. Air Force Office of Scientific Research.

References

- [1]. World Cancer Report, 2014 World Cancer Report by Stewart BW, Published by IARCPress, International Agency for Research on Cancer, Lyon, France, 2014.
- [2]. Moeller BJ, Richardson RA, Dewhirst MW, Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment, *Canc. Metastasis Rev* 26 (2) (2007) 241–248.
- [3]. Justel T and Feldmann C, “Radiation Therapy and Medical Imaging Using UV Emitting Nanoparticles U.S. Patent Application No. 10/596,440,” 2007.
- [4]. Kwatra D, Venugopal A, Anant S, Nanoparticles in radiation therapy: a summary of various approaches to enhance radiosensitization in cancer, *Transl. Cancer Res* 2 (4) (2013) 330–342.
- [5]. Jaiswal I, Oxygen effect and reoxygenation, [Online]. Available: <http://www.slideshare.net/ishajaiswal169/radiobiology-oxygen-effect-reoxygenation>, .
- [6]. Brown JM, The hypoxic cell: a target for selective cancer therapy - eighteenth Bruce F. Cain Memorial Award lecture, *Canc. Res* 59 (23) (1999) 5863–5870.
- [7]. Cosse J-P, Michiels C, Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression, *Anticancer. Agents Med. Chem* 8 (7) (2008) 790–797. [PubMed: 18855580]
- [8]. Fuchs J, Thiele J, The role of oxygen in cutaneous photodynamic therapy, *Free Radic. Biol. Med* 24 (5) (1998) 835–847. [PubMed: 9586814]
- [9]. Vaupel P, Thews O, Hoekel M, Treatment resistance of solid tumors, *Med. Oncol. (Lond.)* 18 (4) (2001) 243–259.
- [10]. Sinha RP, Hader D-P, UV-induced DNA damage and repair: a review, *Photochem. Photobiol. Sci* 1 (4) (2002) 225–236. [PubMed: 12661961]
- [11]. Besaratinia A, Yoon J, Schroeder C, Bradforth SE, Cockburn M, Pfeifer GP, Wavelength dependence of ultraviolet radiation-induced DNA damage as determined by laser irradiation suggests that cyclobutane pyrimidine dimers are the principal DNA lesions produced by terrestrial sunlight, *Faseb. J* 25 (9) (2011) 3079–3091. [PubMed: 21613571]
- [12]. Jiang Y, Rabbi M, Kim M, Ke C, Lee W, Clark RL, Mieczkowski PA, Marszalek PE, UVA generates pyrimidine dimers in DNA directly, *Biophys. J* 96 (3) (2009) 1151–1158. [PubMed: 19186150]
- [13]. Ravanat J-L, Douki T, Cadet J, Direct and indirect effects of UV radiation on DNA and its components, *J. Photochem. Photobiol. B Biol* 63 (1) (2001) 88–102.
- [14]. Kong T, Zeng J, Wang X, Yang X, Yang J, McQuarrie S, Roa A, Chen W, Xing JZ, Enhancement of radiation cytotoxicity in breast-cancer cells by localized attachment of gold nanoparticles, *Small* vol. 4, (9) (2008) 1537–1543. [PubMed: 18712753]

Fig. 1.
UV absorbance spectrum of DNA (from <https://agctsequencing.wordpress.com/2012/04/>).

Fig. 2.

Plot of the X-ray stopping power and cell killing ability (germacidal effectiveness) of UV emitting materials.

Fig. 3.
Block diagram of LuPO₄:Pr powder coprecipitation process.

Fig. 4.
SEM of synthesized Lu₂PO₄ powder.

Fig. 5. TEM of synthesized Lu₂PO₄ particles (left) (spherical primary particle); (right) agglomeration of the primary particles after calcination.

Fig. 6. (Top) Phase structure of the synthesized LuPO₄:Pr powder after calcination; (bottom) ICDD standard XRD reference pattern for LuPO₄.

Fig. 7.
Emission spectra of $\text{LuPO}_4:\text{Pr}$.

Fig. 8.

Left: Particle size analysis of the LuPO₄:Pr particle with different calcination dwelling time.

Right: Left Box: data corresponding to range from 0 to 800 nm; Right Box: full range of data from 0 to 7000 nm.

Fig. 9.
MTT metabolic activity assay.

Fig. 10.

Comparison of the effect of cell survivability of Pr doped LuPO_4 to undoped LuPO_4 with and without 2Gy of X-ray irradiation.