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Photoluminescence and decay characteristics of PEGylated long lasting nanophosphors for tissue engineering applications

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ABSTRACT

$SrAl_2O_4:Eu^{2+}$, Dy^{3+} phosphor, as a substance with long lasting luminescent properties, can continue to emit light for several hours after excitation has been stopped. Drawing on such photoluminescent properties, we could unlock its enormous potential in the field of tissue engineering. For biological applications, $SrAl_2O_4:Eu^{2+}$, Dy^{3+} phosphor should be modified to be water-resistant and biocompatible while at the same time retaining its luminescent properties. Here, we report sol-gel synthesis of $SrAl_2O_4:Eu^{2+}$, Dy^{3+} nanophosphors in which varied metoxy poly (ethylene glycol) (mPEG) concentrations were applied for coating the nanophosphors upon modification of the nanophosphors with phosphoric acid. Subsequently, there were nanophosphors characterization tests done to determine the photoluminescent properties and the afterglow signals, and the optimum coating levels were, in turn, determined. In particular, the 10mg/ml PEG concentration levels show adequate intensity in face of excitement spectra in ranges of visible light (390 nm). This modified nanophosphor could be applied as a source of electromagnetic wave to regulate cell signaling in the engineering of the eye tissue.

Keywords: *Photoluminescence; PEGylation; Nanophosphors; Tissue engineering.*

1. INTRODUCTION

Photoluminescent biomaterials in biomedical and biological fields have widely attracted interests due to their wide applications in biological labelling , biosensing, cellular imaging , immunology, drug delivery, and cancer therapy [1, 2, 3, 4].Some type of photoluminescent biomaterials include organic fluorophores, fluorescent organic dye, fluorescent proteins, quantum dots, lanthanide chelates, and persistent luminescent nanoparticle. Most of the fluorophores and fluorescent organic dyes cannot be used in vivo because of their poor photo stability, poor signal-to-noise ratio from auto-fluorescence, strong photobleaching and cytotoxicity [5]. Furthermore, the aggregation of toxic ions released from quantum dots remains to be a significant concern, especially for long-term use in vivo [6].

The persistent luminescent nanoparticles based on alkaline earth aluminates show a high chemical stability, high quantum yields, no radioactive radiation and low toxicity, and their optical properties can be tuned by variation of lanthanide dopants and the host matrix [7, 8, 9]. Among them SrAl2O4:Eu2+,Dy3+ phosphor was firstly obtained in 1996 by Matsuzawa as a new phosphor which shows longer-lasting phosphorescence [10].

This long lasting phosphorescent show considerable usage in the form of luminescent paints, ink, warning signals and sensors [11, 12]. Furthermore, these kind of nanoparticles are potential candidates in the fields of biomedical application ranging from biological staining such as cell imaging to contrast agents. Significant efforts have been committed to introduce new bio-application for the SrAl2O4:Eu2+,Dy3+ phosphor Recently Loveridge et al. [13] suggested a dental restorative composite with this phosphorescent material. Due to long lasting persistence of SrAl2O4:Eu2+,Dy3+ phosphor which can last several hours after being excited, teeth show more natural visual effects. In another attempts, Sun and et al. [14] introduced strontium aluminate nanoparticle for super-long time in vivo imaging by repeatedly exciting luminescent nanoparticle.

In order to mimic the in vivo situation for tissue regeneration, the first step is considering primary principles like biocompatibility, degradation rate, and mechanical match of the scaffold and the second step is cell signaling regulation [15]. Previous studies showed that cell behaviors like attachment, proliferation and differentiation are directly influenced by a variety of stimulations, which are considered as a cell signals regulation [16]. These stimulations can be physical, chemical and mechanical [17, 18]. Among them ultrasound and electromagnetic wave might be a new hope in cell signaling regulation since this feature was recorded on a cellar level [19, 20]. To address the above situation, we developed a hypothesis that the application of the nanoparticles in tissue engineering could show remarkable results in cell signaling. SrAl2O4:Eu2+,Dy3+ phosphor can be considered as an electromagnetic wave cell signaling agent specially in the eye tissue engineering. Our first concern regarding the use of SrAl2O4:Eu2+,Dy3+ nanoparticles in eye tissue engineering was surface modification of the nanoparticles to enhance biocompatibility while keeping the luminescent properties intact.

Particle size and surface properties of the nanophosphors are the key factors that affect their in vivo efficiency. Phosphors show promising behavior when synthesized on a nano-sized scale [21]. Much effort has been done to synthesize SrAl2O4:Eu2+,Dy3+ phosphor nanoparticles like solid-state reaction [22], chemical precipitation [23], hydrothermal co-

precipitation [24], combustion [25], microwave combustion [26] and sol-gel [27]. Among these techniques, sol–gel technique appears to be a promising due to easier composition control, more homogeneity and relatively low processing temperature [28, 29].

The surface properties of this nanoparticles are the main key factors that determine their interactions in terms of their biologic fluid and their luminescent properties.So far, PEGylation has only been used in coating rare earth doped nanoparticle to increase surface hydrophilicity and enhance biocompatibility [30, 31]. The shell-forming PEG around the nanoparticles guarantees the biocompatibility. Different methods were adopted to obtain PEGylation by using PEG or its derivatives to covalently attaching, entrapping, or adsorbing PEG chains onto the surface of a nanoparticle [32]. In case of photoluminescent nanoparticles, the PEG coating could influence the photoluminescent properties. To our knowledge, the effect of different mPEG concentration on luminescent properties of SrAl2O4: Eu+2, Dy3+ nanoparticles have not be revealed.

In this study, we report the preparation of SrAl2O4: Eu+2, Dy3+ nanoparticle by applying the sol-gel method. After that the nanophosphors were modified by phosphoric acid and coated by different mPEG concentration to increase their water-resistance and biocompatibility besides keeping photoluminescent properties. Finally, the coated nanophosphor particles were systematically characterized by XRD, TEM, dynamic light scattering (DLS) and fluorescence spectrometry and the coating thickness effects on excitation and emission mechanisms of this modified nanophosphor were also evaluated. The second part of this study will be published relating to the application of these modified nanophosphors in the eye tissue engineering.

2. EXPERIMENTAL SECTION

2.1. Synthesis of SrAl2O4: Eu2, Dy3 phosphors.

Analytical graded reagents aluminum nitrate (Al(NO3)3), strontium nitrate (Sr(NO3)2), dysprosium nitrate (Dy(NO3)3) and europium nitrate (Eu(NO3)3) were dissolved in distilled water based on molar ratio (Sr0.97Eu0.01Dy0.02)Al2O4. The doping rates of Eu+2 and the Dy+3 codopants were selected to achieve the longest and intense afterglow according to Matsuzawa et al. suggestion [10]. Then certain amounts of PEG 2000 as dispersers and ammonium bicarbonate (NH4HCO3) solution (0.1 M) containing 1 wt.% PEG 600 were added dropwise while stirring until pH levels reached 5.1. The transparent solution was stirred for 2 hours at a temperature of 80 °C. Then citric acid was added with the molar ratio 1:1. The mixture was heated at 40 °C gelation. The gel was dried to remove water. The resulting gel was dried and fired in an electrical furnace at a firing rate of 10 °C/min until 1200 °C was reached, and then kept at this temperature for 16 h in an active carbon atmosphere [29].

2.2. PEGylation.

In the first step, the surface was modified with phosphoric acid (H3PO4) in ethanol to improve the water-resistance property [33]. 0.5 ml H3PO4 (85%,w/w) were added into 10 ml anhydrous ethanol and were mixed thoroughly. As prepared solution was dropped into the nanoparticle suspension under constant violent stirring for 24 h at room temperature. In a typical synthesis 0, 5, 10, 15, 30 gram of mPEG 4000 was added into 10ml deionized water to form a transparent solutions. After agitation for 15 min the nanophosphors were dispersed in solutions. Then the samples were taken in an ultrasonic bath for 45 min. Samples were washed with distilled water and centrifuged, and finally dried under air for 24 hours.

2.3. Characterization of SrAl2O4: Eu2, Dy3 phosphors.

The crystal phase of the nanophosphors and mPEG-nanophosphors was recorded through powder X-ray diffraction (XRD) using a Siemens- Brucker D5000 diffractometer at 40kV and 40mA with Cu Kα radiation (λ=1.540600 Å). For qualitative analysis, data was collected XRD in the interval $10^\circ \leq 2\Theta 70^\circ$ at scan speed of 2°/min. The samples morphology was characterized by Transmission electron microscopy (TEM) Zeiss, EM10C at an accelerating voltage of 80 kV. Samples were prepared by evaporating a drop of a diluted nanoparticle dispersion in dichloromethane on a carbon-coated Cu 200 TEM grid. The hydrodynamic diameters of the nanoparticles were measured by a Malvern Zeta Sizer Nano S-90 dynamic light scattering (DLS) instrument. Prior to the experiment, the particles were diluted in ethanol (0.1 mg/ml).The photoluminescent properties, including fluorescence excitation spectra, emission spectra and afterglow decay curves at right angle were acquired on a Perkin–Elmer LS-55 fluorescence spectrophotometer with a 230 V pulsed Xenon source for excitation. The emission fluorescence spectra were measured using original reacting mixtures at the room temperature. Prior to the afterglow decay measurements, the samples were irradiated by 390 UV for 10 minutes.

3. RESULTS SECTION

The structural properties of the host lattice (SrAl2O4) play an important role in luminescent properties. Figure 1 shows the XRD patterns of the SrAl2O4: Eu+2,Dy+3 nanophosphor. It is seen that the monolithic phase of SrAl2O4 is predominant (JCPDS No. 34-0379). SrAl2O4 crystal shows two crystallographic polymorphism: monoclinic phase and hexagonal phase according to synthesis methods and treatment heated [34]. The monoclinic phase is stabilized at room temperature and shows luminescent properties as the host structure when doped with rare earth ions as the activator [35]. However; Cordoncillo et al [36] shown that in the present of Eu2+ in the structure the long-lasting phosphorescence is not developed in neither hexagonal nor monoclinic phases.

As hexagonal pattern overlaps the monoclinic pattern, the hexagonal polymorph will be difficult to detect by XRD. In hexagonal phase the middle peak should be the highest and the

pure monolithic left peak should be the highest. It shows that despite sol-gel route, pure monolithic phase was not achieved. In Figure 1 Sharp and strong diffraction peaks indicate good crystallization and Sr–Al–O impurities are not seen or are very weakly in the XRD. In addition the XRD pattern of uncoated and coated was almost the same that shows the modification reactions did not destroy the bulk structure of the nanoparticles.

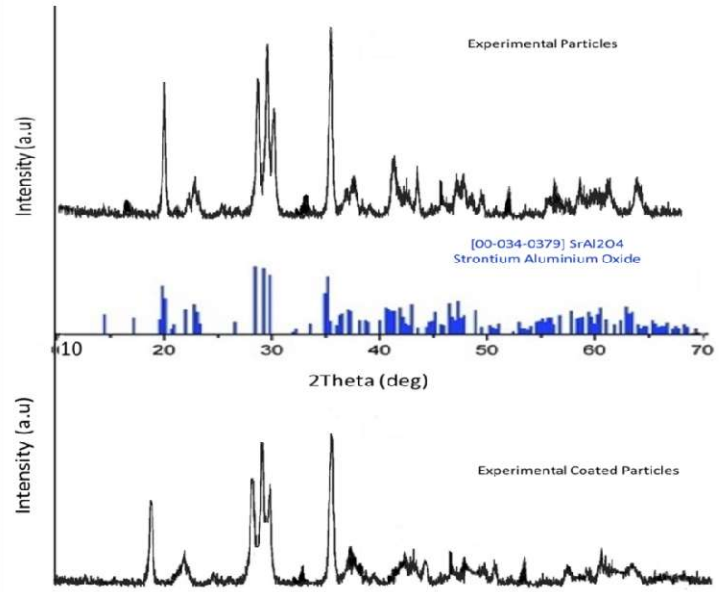

Figure 1. XRD spectra of the monolithic SrAl2O4: Eu+2,Dy+3 nanophosphor.

Figure 2(a, b) shows the bright-filed TEM images of the synthesized SrAl2O4:Eu2Dy3 nanophosphors which are not highly dispersible in water before PEGylation. The image clearly shows an enhanced tendency for integration in smaller particles to bigger ones as a result of an increase in the surface energy. At further magnification (Figure 2c) the average particle size is approximately 50nm and there are no uniformity in size; and the particles are irregularly shaped. For the coated samples (Figure 2d), it is hard to detect PEG layers by TEM. PEG molecules show a rather poor contrast in TEM due to their low electron density, in addition, preparation techniques may destroy the PEG coating.

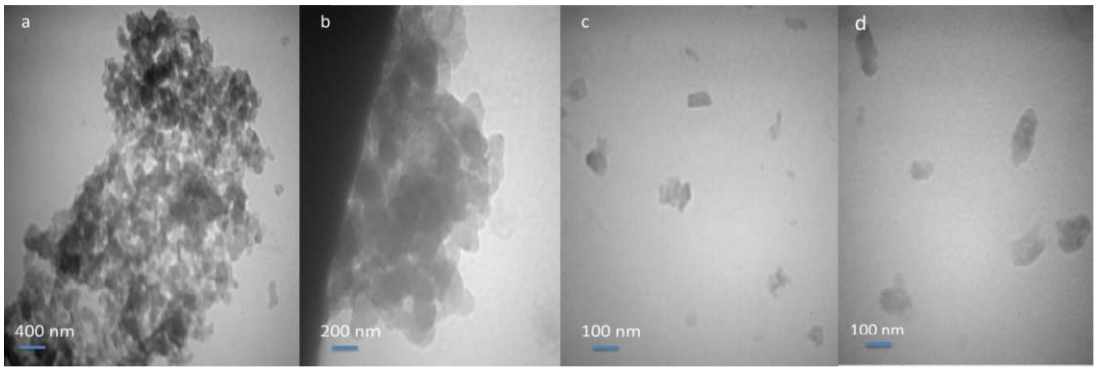

Figure 2. (a, b) TEM Image of the monolithic SrAl2O4: Eu+2,Dy+3 nanophosphor cluster; (c) TEM Image of the magnified monolithic SrAl2O4: Eu+2,Dy+3 nanophosphor; (d)TEM Image of PEG coated monolithic SrAl2O4: Eu+2,Dy+3 nanophosphor.

The dynamic light scattering (DLS) measurements carried out in ethanol (Figure 3) show that PEGylation significantly change the average value of the sample sizes from 90 nm to 120 nm; this particle size is larger than particle size in TEM measurements. Since DLS instrument uses Brownian movement of the solvent particles (hydrodynamic diameter) and assumes particle as a spherical particle, which explains this gap. As shown in Figure 3, the particle size increases firstly with the increased PEG concentration up to 0.15mg/ml PEG but after exceeding the concentration level to over 0.15mg/ml the line becomes constant; after 0.35 g/ml the detected size of particles decreases. According to our PEGylation method and aggregation of PEG molecules around the particles, PEG was non covalently immobilize in outer shells, and due to the high concentration of PEG, the nanoparticle coating became unstable. Photoluminescent (PL) excitation and emission spectra of the nanoparticles at room temperature are shown in Figure 4. The excitation spectra (Figure 4(a)) show that SrAl2O4:Eu2+, Dy3+ nanophosphor can be excited by a broad range of light, including prominent peaks at 270 and 330, 365 and 390 nm. Since a typical human photoreceptor cells will respond to wavelengths from about 390 to 700 nm [37], the 390nm excitation spectra will provide the expected peak in our second part of the study. Emission spectra shows a broad band peak at 512 nm, which results in transition of 4f6 5d1 to 4f7 of excited Eu2+ ions in the matrix [38]. The peak shows a blue shift from 520 nm which is a typical emission bands of the Eu2+ [10, 39]. A similar blue shift was reported in sol-gel SrAl2o3: eu2+, dy3+ preparation technique [29, 40]. Swati and et al. [11] shown nanophosphor emission peaks have a blue shift due to a decrease in grain size below a critical diameter in comparison with the bulk form. In

fact, Eu 2+ ions in nanophosphors show stronger crystal filed effect around Eu2+. Hence, the de-conformation causes a shifting of the emitted wavelength.

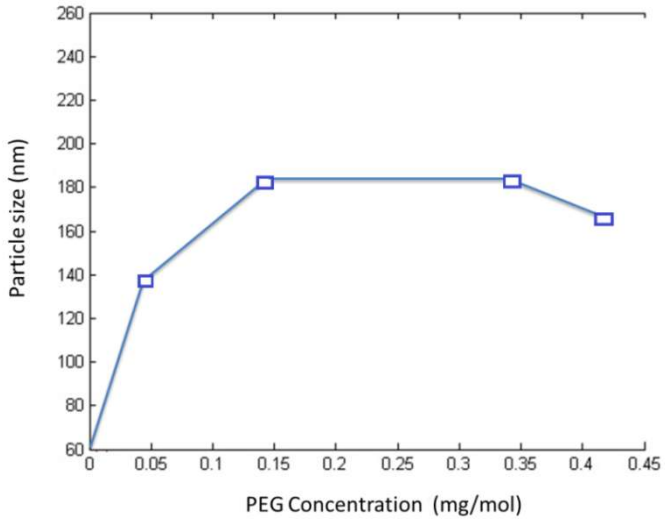

Figure 3. Partticle size with variation of PEG concentration.

The fluorescence spectra of uncoated Sral203: EU2+Dt3+ nanoparticles coated with different concentration of mPEG have been measured under excitation of 390 nm, as shown in Figure 4(b). Considering the effect of mPEG coating the scattering of the excitation light influences the profile of emission but the profile of emission does not show significant difference between uncoated and coated samples, although PEGylation reduce light intensity.

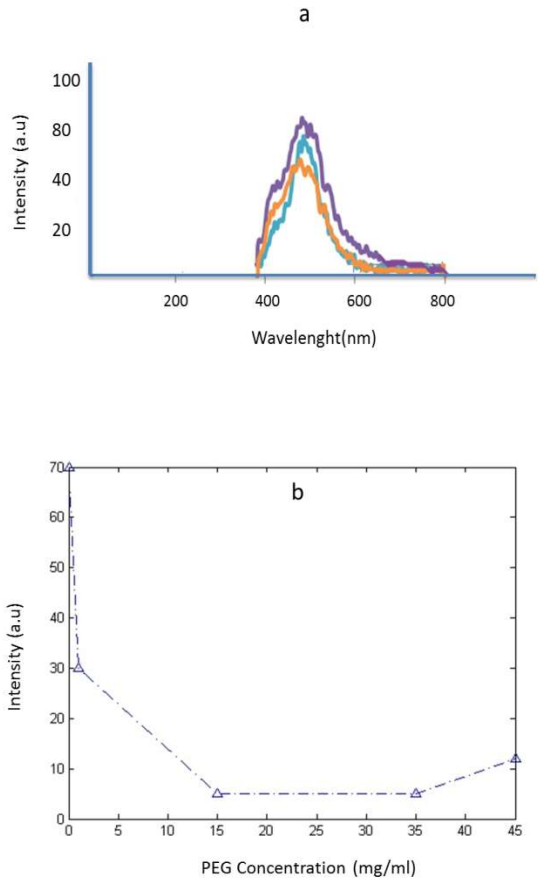

Figure 4. (a) Excitation spectra of pure and PEG-coated SrAl2O4:Eu2+, Dy3+ of; (b) Emission intensity of the coated SrAl2O4:Eu2+, Dy3+with the variation of PEG concentration.

Stouwdam and et al. [41] shown the luminescent of core-shell nanoparticles is more efficient than of nanoparticles that lack the shell, since non-radiative processes at or near the surface of the nanoparticles are much reduced. But our finding indicate that luminescent was not concerned with energy transfer between the modified layer and nanoparticles. Photons encounter nanoparticles were reflected and absorbed and the absorbed part would cause the luminescent emission. The emission photons must transmit the coating and get to the surfaces. In this transition numerous reflection, absorption, and refraction occurred at the surface.

According to Figure 4(b) with increasing the mPEG concentration the luminescent intensity decrease. It is worth mentioning that, the luminescent intensity decrease dramatically in sample with 15 mg/ml mPEG concentration due to the thin protective coating on the surface of the nanoparticles that partly defends the excited light and scatters the emitted light. The luminescent intensity of the coated Sral2o3:Eu2+,Dy+3 nanohosphor keeping constant in mPEG concentration exceeding 15mg/ml since the particle size was remain Constance. The above investigation indicated that In spite of mPEG inertness and transparency to the excitation and emission light mPEG coating affected remarkably the luminescent properties as a result of the PEG conformation at the surface that depends on the PEG molecular weight (MW), surface density of the PEG coating, and PEG chain architecture [42].

In order to clear the influence of the PEG layer on the emission intensity various excitation power density were measured for uncoated sample and the prefer coating that achieved by 10mg/ml PEG concentration. In uncoated sample with increasing the excitation power density from 165 to 490 W/cm2 the emission intensity increased linearly (shown in Figure 5a).

But in the coated samples with increasing the excitation power density the emission intensity did not show a constant trend (Figure 5b). There was not significant different between 330 and 495 W/cm2 power density. It seems that the light reaching the nanoparticle not only was a function of the coating thickness, but also the surface state might change it.

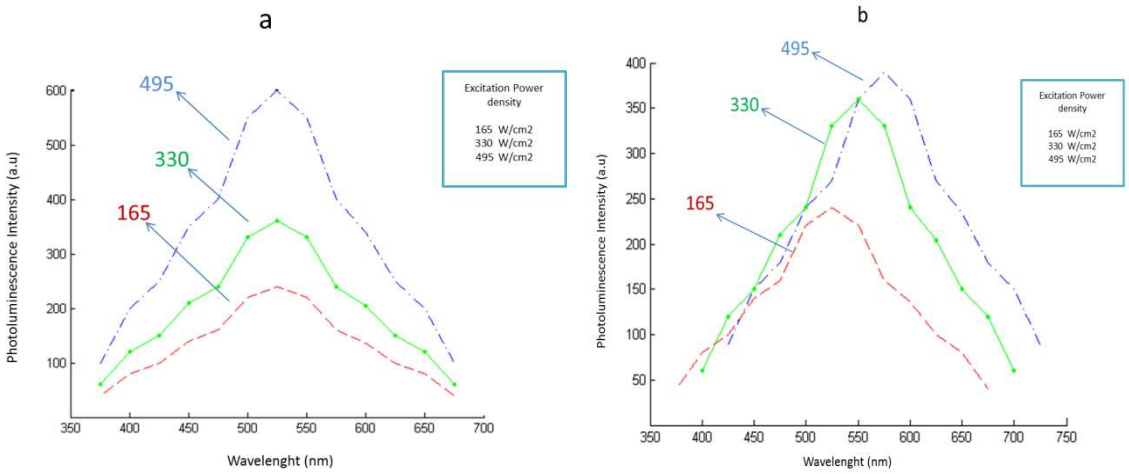

Figure 5. (a) Increasing emission intensity of SrAl2O4: Eu+2,Dy+3 nanophosphor by increased excitation power densit ;(b) No significant Increasing emission intensity of PEG-coated SrAl2O4: Eu+2,Dy+3 nanophosphor by increased excitation power density.

Figure 6 is afterglow decay measurements of the sample after excited for 30 min by 390 nm. The decay time is defined as the time between the end of the excitation and the moment when the light intensity drops below 0.32 mcdm-², roughly 100 times the sensitivity of the dark adapted human eye [43]. The results indicated that the decay process in uncoated and coated samples contained the rapid-decaying process and the slow-decaying lasted over 11h after the excited cut-off. The initial intensity of the uncoated SrAl204 nanoparticles is high and has a long afterglow. The coated nanoparticles decayed more rapidly than uncoated nanoparticles.

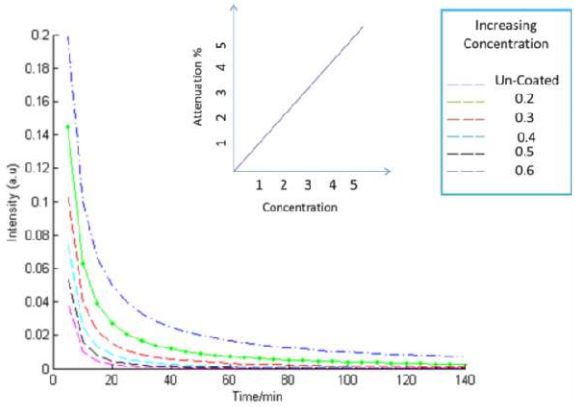

Figure 6. Decay time in uncoated and increased concentration coated SrAl2O4: Eu+2,Dy+3 nanophosphor.

Room Temprature; 27 °C, Excitation Time; 30 min

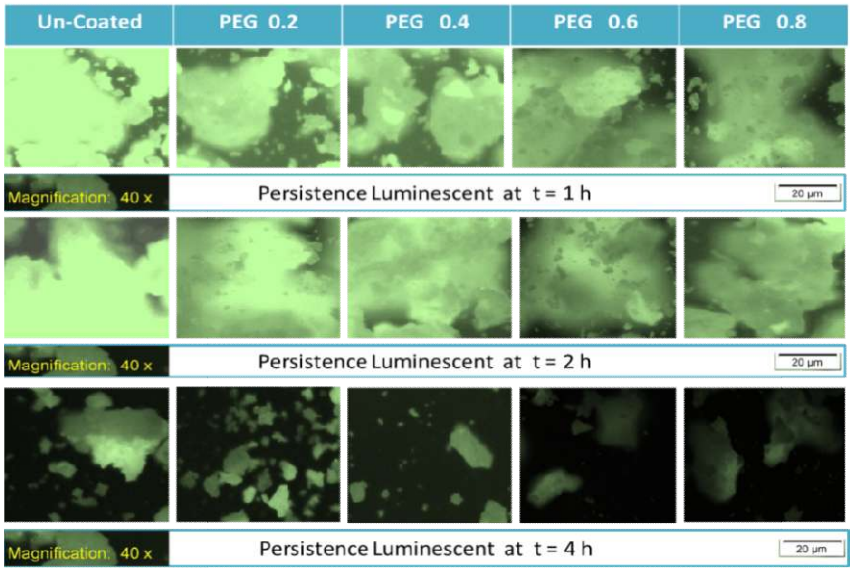

Figure 7. Microscopic phosforescence photograph of SrAl204:Eu2+,Dy3+ nanophosphor and variation of PEG coated SrAl204:Eu2+,Dy3+ nanophosphor with different persistence luminescent.

Furthermore, as described above, coating thickness ranges effect on the afterglow decay curve. Photograph of the nanophosphors in the same situation for afterglow decay curve clearly shows persistence luminescent for the coated and uncoated samples (Figure 7).

4. CONCLUSIONS

We have developed a modified Sral203:Eu2+,Dy3+ nanophosphor which can effectively become activated by visible light and exhibit a persistent luminescent afterglow. The as-prepared MPEG-nanophosphors with a size of ~ 90nm and 10 mg/ml MPEG concentration proves to show the best result. These modified nanophosphors may find many important bio-applications. For the time being, the modified nanophosphor is being developed in our lab as a cell regulate signaling in eye tissue engineering.

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