National University of Science and Technology POLITENICA Bucharest
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PHD THESIS

Influence of dopant rare-earth ions on the photoluminescence characteristics of hydroxyapatite for biomedical uses

SUMMARY

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Bioceramics are ceramic materials that are biocompatible, biodegradable, and bioactive. They are created to stimulate particular cellular responses at molecular level, making them appropriate to be employed in the repair and regeneration of different materials [1-3]. The expanding demand for photosensitive materials for contemporary optoelectronic and photonic equipment, with uses in numerous disciplines, other than just medical applications, has also increased interest in luminescent materials [4-5]. Due to its osteogenic, good osteoconductivity, and bone regeneration properties, hydroxyapatite is used as a bone replacement and tooth repair [1,6] and due to its porous surface and biodegradable qualities, hydroxyapatite is also employed in chemotherapy and antibiotic medication treatment [7]. In order to modify the characteristics of synthetic hydroxyapatite, it can be substituted using a variety of synthesis methods and substitution ions. However, multiple scientific research explained that controlling and modifying particle size, morphology and topology, could be more effective than the type of dopant use or its concentration [8-16]. Some investigations have also shown that calcium phosphate nanoparticles could be doped with lanthanides to create fluorescent probes [17-19].

The literature review presented in Part I of the thesis (**Analysis of the literature data**) highlighted the structure of natural and synthetic hydroxyapatite and the possibility to use anionic and cationic substitution to enhance specific properties. As reported in **Chapter 1**, various methods of synthesis and various sources of hydroxyapatite were used in scientific research. Even through hydroxyapatite has excellent biocompatibility, it obtains bioactivity when substituting ions are accepted into its unit cell, so it can be tailored to meet specific requirements.

Besides, the use of lanthanides as a dopant for substitution of hydroxyapatite because of their distinct physicochemical and imaging properties, contrast and photostability were presented in **Chapter 2**. Using a lanthanide cation, the persistent luminescence can be enhanced, and the traps and the position of the conduction band can be changed by modifying the biomaterial's design if an optimal composition of the doping elements is found. To overcome the disadvantages of authentic biomaterials, different lanthanide elements like Eu^{3+}, Gd^{3+}, La^{3+}, Ce^{3+} and Tb^{3+}, etc., have been used as doping agents for different types of nanostructures for application in bioimaging field.

In particular, the influence of four rare earth elements was studied in **Chapter 3**:

(i) Ce^{3+}/Ce^{4+}, a common element, that is preferred as a dopant because besides luminescent properties, it has other benefits, as biodegradability and antibacterial properties.

(ii) Eu^{3+}, which has obtained attention in the recent research activity as it shows low toxicity, photostability, chemical and thermal stability and high luminescence quantum yield.

(iii) Tb^{3+}, an activator in a variety of hosts using excitation wavelengths ranging from 300nm to 380 nm. Tb^{3+}-hoped hydroxyapatite has superior spectral characteristics and biocompatibility, thereby can be used as a luminescent biological label.

(iv) Er^{3+}, which is naturally present in bone ribs, can be used in non-toxic biomedical applications, but the use of this element in bioimaging applications is not extensively studied.

Even through numerous studies were focused on biomedical applications of hydroxyapatite-based materials, the investigation of hydroxyapatite nanoparticles as nanoprobes is less studied, perhaps due to the challenges that may occur in the synthesis process. In modern biomedical research, there is an urgent need to obtain proper biocompatible nanomaterials that have the potential to replace conventional materials. **Part II – Original contributions** aims to define the objectives of the current thesis: synthesis and characterization of rare earth-doped hydroxyapatite composites and the optimization of the properties to enhance the luminous and biological properties of the composites. The novelty of the study consists in the development of new rare earth-doped hydroxyapatite materials obtained by substitution of pure hydroxyapatite with Ce^{3+}/Ce^{4+}, Er^{3+}, Eu^{3+} and Tb^{3+} ions for their photoluminescence properties, and further use in medical imaging. As presented in **Chapter 5**, in order to achieve the objectives of the thesis, the dopant ions were carefully selected. Despite the evidence that rare earth-doped hydroxyapatite can be successfully used in biomedical applications, the use of luminescent probe is not extensively considered. Erbium-doped hydroxyapatite is very less studied in the literature, but it represents a domain of interest for future research. Just a few reports were published to demonstrate the capacity of Europium-doped hydroxyapatite for applications in bioimaging and nanomedicine and the aim of this thesis if to uncover the successful use of the Eu^{3+} ions for luminescent probes. Furthermore, the doping of hydroxyapatite with $Cerium^{3+}/Cerium^{4+}$ has attracted a lot of interest recently, the studies being focused on developing materials with biological properties. This research seeks to investigate the potential applications of composite nanoparticles as a contrast agent for imaging procedures, such as MRIs and CT scans.

Chapter 5 was focused on the synthesis process of Er^{3+}, Ce^{3+}/Ce^{4+}, Eu^{3+} and Tb^{3+} used in combination with hydroxyapatite via precipitation method to obtain photoluminescent probes for cellular imaging. $Ca_{10-x}REE_x(PO_4)_6(OH)_2$ represents the replacement of different rare earth element ions concentrations into hydroxyapatite. To determine the ideal elemental composition, the ion concentration was changed from 0 to 1 during the study. Furthermore, the differences and impacts of all doping ions on the crystalline structure, shape, dimensions, and surface characteristics of hydroxyapatite, as well as the photoluminescence properties of all samples, were investigated.

Luminescence properties of europium, cerium, erbium, and terbium substituted hydroxyapatite were chosen to be investigated in this thesis and data gathered along the research activity demonstrated consistent results. thesis and data gathered along the research activity demonstrated consistent results.

Inductively coupled plasma mass spectrometry (ICP-MS), Fourier Transform Infrared spectrometry (FT-IR), scanning electron microscopy (SEM), bright field transmission electron microscopy (TEM), X-ray diffraction (XRD), and UV-Vis and PL spectroscopy (PL) were used to examine the morphological, structural, and optical properties of the obtained samples. ICP-MS was used to analyze the rare earth ions in doped hydroxyapatite. The results showed that each rare earth ion was present in doped HA powders. The content of dopant ions was found to increase from 0.05 to 1% of ions doped HA.

The synthesis of HA was verified by FTIR and X-ray diffraction tests. The findings from the study indicate that Er^{3+}, Ce^{3+}/Ce^{4+}, Eu^{3+}, and Tb^{3+} ions successfully replaced calcium ions into the crystal structure of hydroxyapatite. The FT-IR spectra of substituted hydroxyapatite with various REE concentrations were found to be identical to the FT-IR spectra of pure HA when the spectra of all rare earth element doped HA powders were compared. In contrast to Tb^{3+}/HA, as REE concentration increases to Er^{3+}, Ce^{3+}/Ce^{4+}, and Eu^{3+} 0.25% molar percentages, the strength of the phosphate bands PO_4^{3-} decreases. A reduction in HA crystallinity is associated with a rise in REE ion concentration. These results were supported by an XRD study.

The average crystallite size of pure hydroxyapatite was 6.07 nm, however for Er/HA, Eu/HA, and Tb/HA, the size of the crystallite gradually decreased when the ion dopant concentration increased. Analyzing the obtained data, can be highlighted that adding Er^{3+} ions into HA lattice leads to a rise of crystallite size from 5.71 to 6.46 nm for a concentration of 0.1 for Er^{3+} ions. If the substitution degree goes up to 1, the crystallite size decreases to 4.74 nm. The increase of lattice microstrain to 1.96% for maximum degree of substitution. A slowly decrease of the lattice parameters can be observed at Eu/HA powders with the enhance of Eu^{3+} content. The crystallite dimensions of the Eu/HA decreases from 5.98 to 5.55 nm, while the microstain increases from 1.54% to 1.67%. A small increase in crystallite size was observed for a higher substitution degree, followed by a decrease. Same trends were also noticed for Tb/HA powders, where the crystallite dimensions decreased from 5.72 to 4.83 nm with the increase of Tb ions concentration. Some differences can be highlighted for Ce/HA: (i) for a substitution degree of 0.25 of Ce^{3+} an increase in crystallite size appeared; (ii) because of the inclusion of cerium ions into the HA lattice, the crystallinities decreased as cerium concentration increased.

The morphologies of pure HA and REE/HA in scanning electron microscopy confirmed the impact of doping ions compared to pure HA. It is visible from SEM images of pure HA that the particles are spherical and range in size from 5 to 9 nm. The size of the nanoparticles decreases as the concentration of dopant ions increase when europium and erbium ions are added to HA matrix. The size of the crystallites decreased as the ion concentration increased in cerium doped hydroxyapatite as well, with a higher crystallite size for Ce(IV) ions compared with Ce(III) ions at the same concentration. Regarding the SEM images of Tb/HA, small agglomerations of particles were formed with the increase of terbium content.

The HRTEM images allows to differentiate well crystalized particles, and to measure distance to the Miller indices which match to hydroxyapatite with hexagonal symmetry. The present results indicate that the 10% degree of doping is the limit for terbium ions to be accepted into hydroxyapatite lattice.

For all samples, a typical trend has been observed when analyzing the absorption spectra of REE-doped hydroxyapatite samples – as the amount of dopant ions rises, the intensity of the absorption peaks tends to rise, displaying a strong UV absorption. The photoluminescence spectra of rare earth substituted hydroxyapatite show high fluorescent emission peaks, confirming that the photoluminescent properties were enhanced due to the utilization of rare earth elements.

The MTT test was implemented to assess the overall biocompatibility of synthesized rare earth-doped hydroxyapatite powders using human mesenchymal amniotic fluid stem cells (AFSC). The GSH-Glo Glutathione Test was utilized to evaluate oxidative stress, and a fluorescent microscope and a RED CMTPX fluorophore, a tool for monitoring live cells throughout time in a single cell, were used to assess viability.

The MTT assay demonstrated that even at greater concentrations and with absorption values that were close to, lower than, or higher than those of the control sample, the produced nanoparticles had no harmful effects. At 72 hours, the tested samples substantially enhanced cellular proliferation relative to the control, stimulating cellular metabolism. In the presence of REE-doped hydroxyapatite, glutathione, a marker of oxidative stress, can reduce cellular damage brought on by free - radical, peroxides, heavy metals, and lipid peroxides.

AFSC cells exhibited similar behavior to control cells for REE-doped hydroxyapatite nanomaterials, proving that the examined samples don't generate cellular stress, thus confirming the biochemical results. We can draw the conclusion that the synthesized samples could be used as antioxidants to prevent cell damage brought on by oxidative stress as well as fluorescence probing samples for cellular imaging. Fluorescent images showed that AFSCs have a normal morphology, are viable, and do not have dead cells or fragments.

Hydroxyapatite and its derivates have substantial significance in many fields of science. The data presented provides evidence of the ongoing interest in using rare earth elements for substitution for advanced biological performance.

The nanomaterials produced in the current thesis, which are made of hydroxyapatite doped with four rare earth elements at different concentrations, show an increase in photoluminescent properties with dopant ion concentration, resulting in them being attractive candidates for advancement and use in biological system imaging. Corroborating the structural, morphological, and optical properties with cytotoxicity assessments and fluorescence behavior prove that by co-precipitation method can be used to obtain new doped hydroxyapatite using various concentrations of rare earth ions (until 1%), which can serve as promising nanomaterials for medical applications.

List of Publications

Publications in ISI Journals

1. **Paduraru Andrei**, Oprea, Ovidiu, Musuc Adina Magdalena, Vasile Bogdan, Iordache Florin, Andronescu Ecaterina, Influence of terbium ions and their concentration on the photoluminescence properties of hydroxyapatite for biomedical applications, Nanomaterials, 2021, no. 11, pp. 2442, DOI:0.3390/nano11092442 **(IF= 5.076) – Q1**

2. **Paduraru Andrei**, Musuc Adina Magdalena, Oprea Ovidiu, Trusca Roxana, Iordache Florin, Vasile Bogdan, Andronescu Ecaterina. Synthesis and characterization of photoluminescent ce(iii) and ce(iv) substituted hydroxyapatite nanomaterials by co-precipitation method: cytotoxicity and biocompatibility evaluation, Nanomaterials, 2021, no. 11, pp. 1911, DOI: 10.3390/nano11081911 **(IF= 5.076) – Q1**

3. **Andrei Viorel Paduraru**, Ovidiu Oprea, Adina Magdalena Musuc, Bogdan Stefan Vasile, Anton Ficai, Ecaterina Andronescu, Photoluminescent nanomaterials based on europium doped hydroxyapatite. Romanian Journal of Materials 2021, 51 (3), 353 – 360 (**IF= 0.628**)

4. Diana Georgiana Filip, Vasile Adrian Surdu, **Andrei Paduraru**, Ecaterina Andronescu. Current Development in Biomaterials—Hydroxyapatite and bioglass for applications in biomedical field: A Review. Journal of Functional Biomaterials**,** 2022.11. 13(4):248, DOI: 10.3390/jfb13040248 **(IF= 4.901) – Q2**

5. Ecaterina Andronescu, Daniela Predoi, Ionela Andreea Neacsu, **Andrei Viorel Paduraru**, Adina Magdalena Musuc, Roxana Trusca, Ovidiu Oprea, Eugenia Tansa, Otilia Ruxandra Vasile, Adrian Ionut Nicoara, Adrian Vasile Surdu, Florin Iordache, Alexandra Catalina Birca, Simona Liliana Iconaru, Bogdan Stefan Vasile, Photoluminescent Hydroxyapatite: Eu3+ Doping Effect on Biological Behaviour, Nanomaterials, 2019, 07. 9(9):1187, DOI: 10.3390/nano9091187 **(IF= 5.076) – Q1**

6. **Andrei Paduraru**, Diana Georgiana Filip, Adina Magdalena Musuc, Ovidiu Cristian Oprea, Roxana Trusca, Vasile Adrian Surdu, Bogdan Stefan Vasile, Ecaterina Andronescu, Structural and morphological characterization of erbium doped hydroxyapatite for medical imaging**,** Scientific Bulletin, Series B, Chemestry and Materials Science, ISSN 1454-2331. **(IF= 0.35)**

Participation on Conferences

1. **Paduraru Andrei**, Musuc Adina Magdalena, Oprea Ovidiu, Trusca Roxana, Vasile Bogdan, Andronescu Ecaterina. New cerium substituted hydroxyapatites for biological fluorescence labeling, *SICHEM 2020 Conference*, 17-18 September 2020, Bucharest, Romania

2. **Paduraru Andrei**, Musuc Adina Magdalena, Oprea Ovidiu, Trusca Roxana, Vasile Bogdan, Andronescu Ecaterina. Hydroxyapatite dopped with cerium for medical imaging. *National Scientific Conference of Academy of Romanian Scientists (AOSR)*, 20-27 November 2020

3. **Paduraru Andrei**, Musuc Adina Magdalena, Oprea Ovidiu, Trusca Roxana, Vasile Bogdan, Iordache Florin, Andronescu Ecaterina. Terbium-doped hydroxyapatite, *Scientific Conference of Academy of Romanian Scientists (AOSR),* 25-26 June 2021

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