Mechanical and antibacterial properties of praseodymium and PLA codoped hydroxyapatite nanobiomaterials

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In this research, chemical precipitation technique used to create a novel Pr/HA and Pr/PLA codoped nanohydroxyapatite composite and characterized using like TEM, SAED, SEM, AFM, XRD and FTIR techniques. Sample binding strengths for Pr/HA and Pr/PLA were extremely similar to 17.65 MPa and 24.65 MPa, respectively. Hv values of 322 and 332 were obtained from the Vickers micro-hardness test on samples of as-developed Pr/HA and Pr/HA/PLA, respectively. Antibacterial activity against K.pneumoniae and S. epidermidis aureus was tested using the synthesized Nano biomaterials. These results show that HA/Pr/PLA with improved antibacterial activity and mechanical qualities may be useful in medical settings.

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1. Introduction

The inorganic phase of the organic extracellular matrix is composed of calcium phosphate crystals in the form of hydroxyapatite (HA). These calcium phosphate crystals fill the interstices between the type polymers that make up the organic extracellular matrix. HA is responsible for the rigidity of the bone, whereas polymer is responsible for the elasticity and tensile strength of the bone [1]. Synthetic calcium phosphate scaffolds, most usually in the form of HA, are used rather frequently in the field of medicine due to the osteo-conductivity and bioactivity that these scaffolds possess [2]. The ability of HA, a naturally occurring component of bone, to adsorb more cell-adhesive plasma proteins such as fibronectin and vitronectin leads in an increase in the number of cells that attach to the surface of the bone. On the other hand, because of its poor mechanical properties, it has only seen extremely limited use. In today's medical practise, scaffolds made of synthetic calcium phosphate are being employed to encourage the body's natural synthesis of bone [3]. Biological components like as growth factors or medications are placed inside of these scaffolds. The most significant issues with these scaffolds are a low solubility for medicines and growth hormones, a short half-life, proper dosage, and the potential for unintended effects such as ectopic bone production [4,5]. Rare earth metals, such as praseodymium ions (Pr^{2+}), have, as of late been the subject of research as a possible safer technique due to their capacity to naturally stimulate growth factor production by cells in order to promote osteogenesis [6]. In addition, the presence of these components has the potential to improve the physical properties as well as the biological responses of scaffolds. The most prevalent form of this bone-dwelling ion is praseodymium (Pr), which functions as a Ca^{2+} substitute. Pr^{2+} is engaged in a broad range of anabolic and catabolic activities that are critical for maintaining cellular homeostasis. This is in addition to its important role in the immune system. At the location of the implantation, the proliferation of bacteria is one of the most prevalent factors contributing to the failure of bone grafting and healing procedures to take effect [7,8]. The use of polymeric nanoparticles with antibacterial properties, such as rare earth elements, and other similar substances, has shown to be beneficial for the treatment of periodontal problems and dental prostheses. However, more in vivo

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research are necessary to assess their biocompatibility, cytotoxicity, and degradation [9,10]. However, these particles have a tendency to agglomerate over time, which diminishes their antibacterial efficacy. Pr nanoparticle-containing polymer matrices have been proven to be useful in wound dressing by limiting bacterial proliferation at the wound site 11,12]. This is an extra benefit of using these types of wound dressings. Poly-lactic-acid (PLA) is a biocompatible polymer and has changeable mechanical qualities as well as a breakdown rate. PLA is used extensively in many different contexts, including as a medicine or bioactive factor carrier [13]. In addition, PLA has been used to encapsulate antibacterial medications, which resulted in a significant reduction of bacterial growth in some clinical studies; nevertheless, this raises concerns of a burst release of the encapsulated antibiotic and may result in a rise in the number of antibiotic-resistant bacterial species. Numerous studies have been conducted on pr ions, mostly because to the fact that they have the ability to eradicate germs [14,15]. The goal of this research was to develop a bio-composite bone scaffold made of Pr-doped HA-PLA that is both antibacterial and hospitable to cells. Because Pr^{2+} promotes bone repair and inhibits bacterial development, it was opted to dope HA in this investigation. On the other hand, PLA was integrated into the scaffold to improve its mechanical qualities.

2. Experimental work

In order to produce the Praseodymium, Polymer Polylactic-acid codoped hydroxyapatite (Pr/PLA/HA), the following precursors were required: calcium nitrate, ammonium hydrogen phosphate, Polymer (PLA), and praseodymium (III) nitrate hexahydrate purchased from sigma Alrich, USA. It was decided to obtain glutaraldehyde from the German company Merck. Analytical-grade sodium hydroxide and phosphate buffer solution (PBS) with a pH of 7.4 were both used in the experiment. Ethanol was used to dissolve controlled quantities of praseodymium nitrate and ammonium hydrogen phosphate in order to produce praseodymium-doped hydroxyapatite nanoparticles. The solution was heated to 40 degrees Celsius and rapidly agitated for a total of 24 hours after the addition of distilled water. A stoichiometric quantity of calcium nitrate was dissolved in ethanol in a separate container with vigorous stirring at a temperature of forty degrees Celsius for twenty-four hours. After a gentle addition of the Ca-containing solution to the P-containing solution, the mixture was allowed to mature at room temperature for 72 hours before being heated to 40 degrees Celsius for 24 hours. In order to get a [Ca+Pr]/P value of 1.67, the component ratios in the Pr/HA sol were modified. After obtaining Pr/HA nanopowders, they were subjected to a treatment at 80 degrees Celsius for six hours. In order to generate PLA coated Pr doped hydroxyapatite bio-composites, Pr doped hydroxyapatite was added to PLA, and as a result, Pr/HA/PLA was produced. The composites included the same amount of PLA, had a pH of 7.4, and had been cross-linked with 0.5% glutaraldehyde in accordance with the procedure that was detailed earlier. Following the completion of the cross-linking procedure, the gel composites were lyophilized with the help of the dryer, resulting in the production of porous composites.

3. Result and discussion

3.1. Functional group analysis

Figure 1 shows the FTIR spectrum of Pr/HA and Pr/PLA/HA. We found the phosphate group symmetric stretching vibration in Pr/PLA/HA to be at 559, 731, 869, and 1087 cm^{-1}. On the other hand, the bands at 3589, 2941, 1671, 1019, 881, and 821 cm^{-1} show the stretching of OH, CH, CO, and OH in the plane. Another sign of CO stretching is the band at 821 cm^{-1}. The 1671 and 1019 cm^{-1} lines in the Pr/PLA/HA mixture make it easy to tell the difference between the grafting method and the presence of an inserted PLA matrix. In the FTIR spectrum of the Pr/PLA/HA scaffold, new bands at 564, 721, 871, and 1082 cm^{-1} were found. The phosphate group in the Pr/HA scaffold caused all of these new bands because it was stretching. The phosphate group's bands become less intense when Pr/HA is added to a polymer mixture. In Pr/HA, the phosphate band is usually at 1087 cm^{-1}, but in Pr/PLA/HA, it has moved to 1076 cm^{-1}. In any case, FTIR research was used to make a hybrid of PLA and Pr/HA.

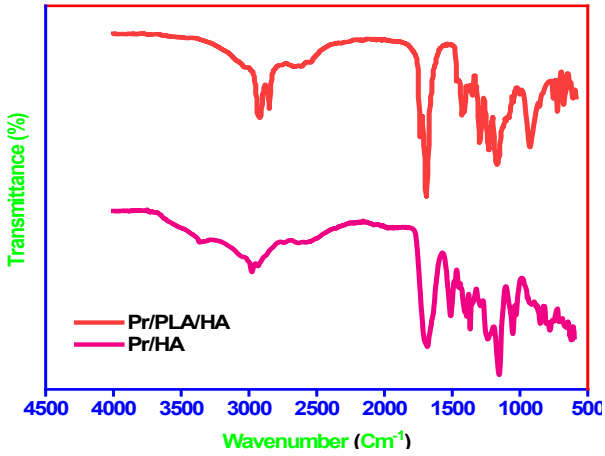


Fig. 1. FTIR of Pr/HA and Pr/PLA/HA.

3.2. Structural analysis

Figure 2 shows the XRD shapes of Pr/HA and Pr/PLA/HA. The XRD patterns of Pr/PLA/HA using the JCPDS (09-0432) card for apatite show that the first one is crystalline in a very clear way. The polymer mixture that was made is amorphous, which can be proven by the fact that it doesn't have any solid structures and instead has a wide peak. Also, finding wide planes in the 2-theta range of 25^0 to 32^0 for Pr/PLA/HA suggests that there may be a phase that is less solid.

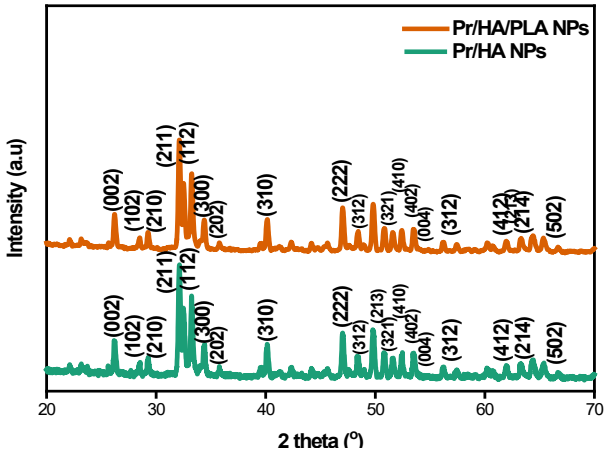
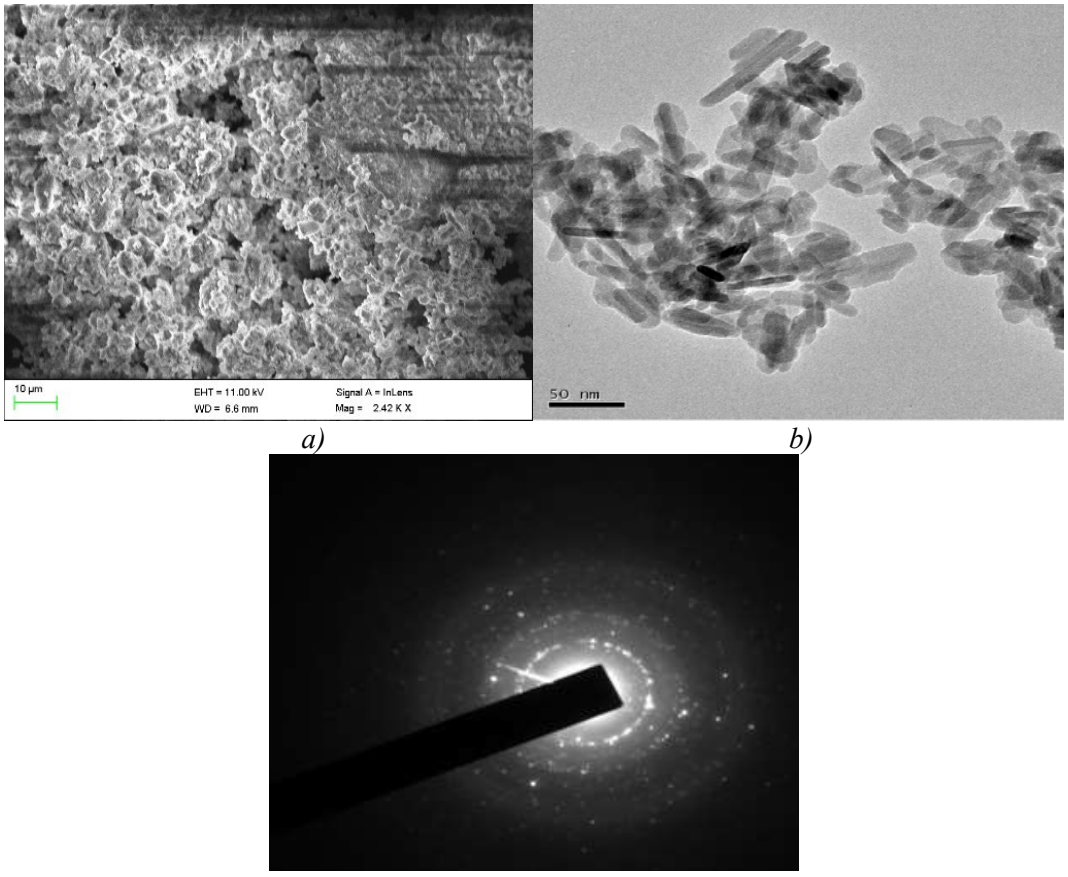


Fig 2. XRD analysis of Pr/HA and Pr/PLA/HA.

3.3. FESEM/EDAX analysis

Figure 3 (a) for a picture of the surface shapes of Pr/PLA/HA. Pr/HA was added to the polymer PLA so that the end goods would have sides that are both smooth and porous. These traits could be seen in the samples that had just been made. When the alloys are already made, their open sides make them useful for a number of medical purposes. Some very clear pictures of electron microscopes (HRTEM) are shown in Figure 1.4(b). They show that Pr/PLA/HA crystals tend to form in one of two planes that are not parallel to the HA crystal lattice. An experiment called selected area electron diffraction (SAED) was done on needle-shaped particles of Pr/PLA/HA with three different Ca/P ratios. The results are shown in Figure 1.3(c). The crystal structures of the particles were studied to come to these conclusions. This is clear because diffraction rings can be seen in Pr/PLA/HA samples.



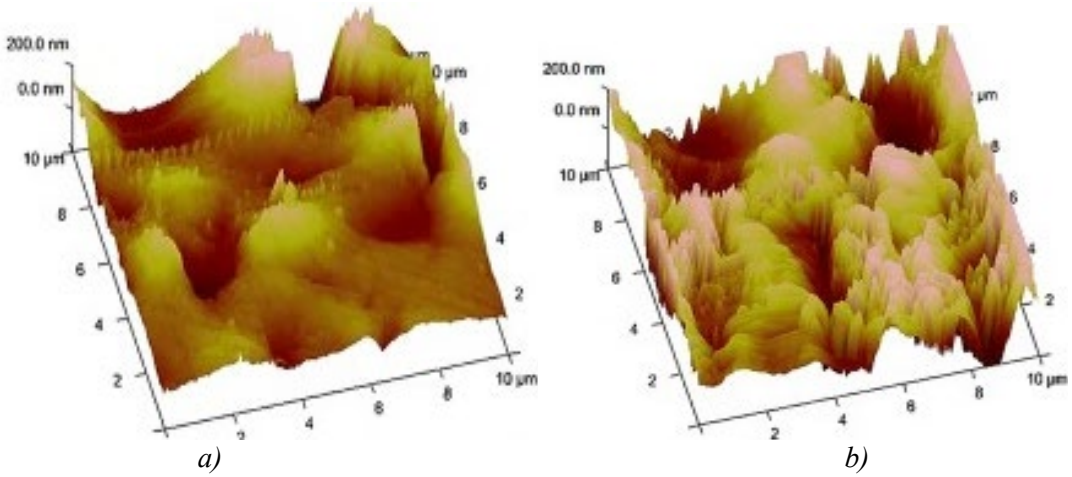
a) *b)*

c)

Fig. 3. SEM, TEM and SAED analysis of Pr/PLA/HA.

3.4. AFM analysis

In Figure 4, that the composites of Pr/HA(a) and Pr/PLA/HA(b) have some crests and valleys in their 3D surface texture. This could make the layer break down in SBF solution. The Pr/HA nanomaterials, on the other hand, have a surface that is fully flat and smooth. There were a lot fewer pits in the Pr/PLA/HA nanocompistes, and their coats were spread out. Bioresistivity of the Pr/PLA/HA layer has gone up because it now has needed mineral ions like silver and cerium in it. This type of covering might also make the graft material last longer in long-term medical uses.



a) *b)*

Fig. 4. AFM analysis of a) Pr/PLA and b) Pr/PLA/HA.

3.5. Mechanical properties

In Figure 5(a) how the binding strengths of the samples that were made changed over time. The test showed that the Pr/PLA material could hold things together with 17.65 MPa of force. It was also shown that composite coats stuck better when polymers (PLA) were added to them. Binding strengths in the sample were very close to 24.65 MPa. Based on these results, PLA was able to make the coats stick together better. At 24.65 MPa, the Pr/PLA/HA composite has a high bond strength, which makes it a good choice for large loads. It was clear that this could improve the mechanical qualities of Metal codoped HA/PLA. Through Vickers micro-hardness (Hv), we can find out important details about a materials ability to hold its weight. We did the Vickers micro-hardness test (Hv) on samples of as-developed Pr/HA and Pr/HA/PLA in Figure 1.5(b). It was possible to get Hv levels of 322 and 332 Hv. Instead, the Hv value from the sample with PLA added is a little higher (332 Hv) than the value from the sample with Pr/HA added. It's important that the material became much stiffer after PLA was added to it. There is a chance that the composite's uniform and more compact layer shape plays a part in the hardness value in some way.

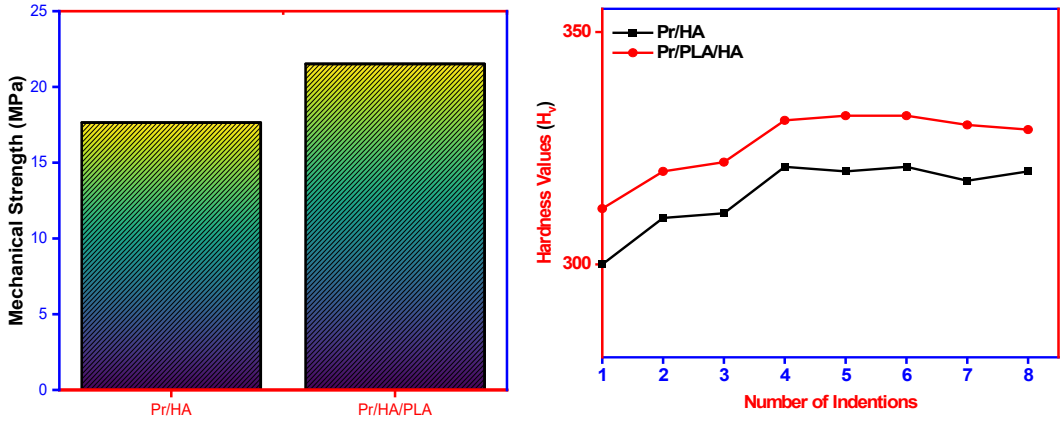


Fig. 5. Mechanical strength and Vickers Micro-hardness Evaluation of a) Pr/PLA and b) Pr/PLA/HA.

3.6. Antibacterial properties

Synthesized Polynano composites against klebsiella pneumoniae (Gram-negative) and Staphylococcus epidermidis (Gram-positive) to killed bacteria. These are the most common types of bacteria that cause illnesses after surgery on implants. Different amounts (50 to 80 mL) were used in these tests. When Pr/HA/PLA materials were mixed together as a medicine, it had the most powerful antibiotic effect. The agar disc diffusion method was used (at 37°C for oneday), and Fig. 6(a) and 6(b) shows Pr/HA and Pr/HA/PLA composite coated sample killed K.pneumoniae and S. epidermidis at 50 to 80 mL. The images showed that the zone that stopped K. pneumoniae germs from spreading was 23 mm wide and the zone that stopped S. aureus strains from spreading was 19 mm wide. The sample that was covered with a Pr/HA/PLA mixture at the highest concentration (80 mL) had the best antibacterial action. The Pr/HA/PLA hybrid layer was found to be slightly more effective against K.pneumoniae bacteria than against S. epidermidis bacteria when it came to killing bacteria. The bacterial species' cell walls have changed shape. This is the cell wall of gram-positive Some strains of K.pneumoniae, which are gram-negative, had cell walls that were only 1.5 to 10 nm thick. This was because their cell walls had two layers of peptidoglycan. There was a change in the thickness of the cell walls because there was a double coat of peptidoglycan. Researchers have thought in the past that Pr^{2+} and PLA might have a role in killing only S. epidermidis, especially types of the bacteria that are immune to antibiotics. Testing the synthesised mixture against K.pneumoniae and S. epidermidis showed that it was much more effective at killing bacteria than the control material.

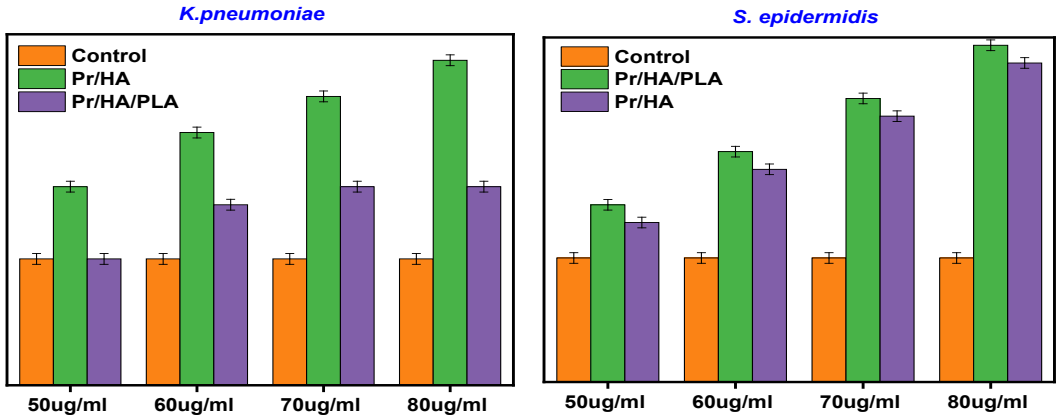


Fig. 6. Antibacterial activities of a) Pr/HA and b) Pr/HA/PLA.

4. Conclusion

Applying a polymer composite (PLA) coating to a bioimplant may boost its antimicrobial and mechanical properties. The coating on the synthesised composites alloy was examined using FTIR and XRD, and the presence of functional groups and phase purity were confirmed. The surface morphology of the coated alloy indicates that the coating was applied uniformly. Both microorganisms were more effectively inhibited by the Pr/HA that had been encapsulated with PLA. In tests measuring adhesive strength and hardness value, Pr/HA coated with PLA came out on top. Compared to untreated, Pr/HA/PLA showed better antibacterial activity and mechanical qualities, as shown above. Extreme synthesis conditions, which might alter the polymer's characteristics, are required for the production of nanobiomaterials that prevent bacterial attachment and growth. This work introduced a unique Pr codoped HA-PLA nano biomaterials with antibacterial action against. K.pneumoniae and S. epidermidis; this antibacterial activity enhanced with increasing when capped with PLA. Similarly, our prior research shown that both nanobio materials improved mechanical property and stability. Our findings indicate that HA/PLA/Pr is a highly antibacterial option for use in bone tissue engineering.

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