Original Article

Effect of iron doped hydroxyapatite nanoparticles on the structural, morphological, mechanical and magnetic properties of polylactic acid polymer

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\begin{abstract}
Iron doped hydroxyapatite nanoparticles (FeHA NPs) were successfully prepared and the solution casting technique was used to fill the polymeric matrix of polylactic acid (PLA) with different mass fractions of FeHA NPs. The absorption spectra of FT-IR spectroscopy demonstrated that FeHA NPs were linked with PLA via hydrogen bond formation between OH of Ca(OH)\textsubscript{2} and oxygen atoms in the ester group in PLA and new peaks appeared. XRD analysis confirmed the presence of FeHA NPs within PLA by showing its characterizing phases and demonstrated that the degree of crystallinity was enhanced for nanocomposite with low content of FeHA NPs. The SEM micrograph showed aggregations of FeHA NPs for nanocomposite with high content. The results of mechanical characterization evidenced that inclusion of FeHA NPs with 10 wt.\% would represent an effective reinforcement associated with a high value in both the elastic modulus and tensile strength. The super-paramagnetic behavior for the PLA nanocomposites, indicated by a very low coercive field, was confirmed by the results of magnetic measurements. The PLA nanocomposites showed a high antibacterial activity against Pseudomonas aeruginosa and Bacillus thuringiensis bacteria. Thus, the prepared PLA nanocomposites could be tailored for many biomedical applications.

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\end{abstract}

1. Introduction

Tissue engineering is a scientific field mainly concerned with the development of tissue and organ repair improving biological substitutes which are able to develop or restore function of tissue. Thus, tissue engineering could avoid the problems associated with tissue damage and should develop tissue substitutes which are able to ‘grow’ with the patient [1,2]. Bone is known as a natural inorganic–organic nanocomposite, in which collagen fibrils and hydroxyapatite (HA) nano-crystallites are well arranged in a hierarchical architecture [3,4]. Bone tissue engineering substitutes are considered as an alternative strategy for regenerating bone and

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https://doi.org/10.1016/j.jmrt.2019.01.017

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treated its defects [3,5]. Many strategies, such as tissue inducing factors, biocompatible scaffolds and cell-based therapies, have been adopted for realizing regeneration of tissue. Predominantly, scaffolds must fulfill many requirements like controlled bioresorbability and biodegradability and suitable mechanical properties, interconnecting pores with adequate size and specific shape, to favor cell adhesion, differentiation and proliferation [6].

Therefore, natural and synthetic materials have been utilized for developing scaffolds with respect to the particular requirement of tissue restoration. Ceramic materials such as tricalcium phosphate and HA are used to produce scaffolds for bone tissue regeneration with suitable efficiency [7]. Although these materials like the inorganic part for natural bone and have osteoconductivity properties, they do not coincide with its mechanical performances and are brittle. Furthermore, ceramic scaffolds are inadequate because they have a higher stiffness. Therefore, an interesting substitution should be performed in the tissue regeneration field through both natural (i.e. alginate, collagen, chitosan and agarose) and synthetic polymers. Polymers have advantages like more processability and versatility. Their mechanical performances and degradation rate, as well as their chemical and physical properties, can be suitably modified through varying the macromolecule composition or the chemical structure [7,8].

Thus, the development of biopolymers-based biocomposite scaffolds composed of biopolymers reinforced with ceramic inorganic micro-/nanofillers has a great importance in the research for bone tissue regeneration [7,9-11]. Synthetic aliphatic polyesters and their copolymers have been widely studied [3,6,7].

PLA is a biodegradable, biocompatible and linear thermoplastic polymer. Also, it has a high-modulus and high-strength and can be used in either the industrial packaging field or the bioabsorbable/biocompatible marketing of medical device. PLA is easily biodegradable through enzymatic and hydrolytic pathways. PLA is used widely in several medical applications such as tissue culture, surgical implantation, degradable surgical sutures, controlled release systems and wound closure. Moreover, it is considered as immune-inert synthetic biopolymer that has excellent mechanical and thermal properties [3,12-17]. Thus, it was chosen in the current study for designing a biocomposite to be used in tissue engineering scaffold.

Magnetic nanoparticles (NPs) have many outstanding properties, such as high coercivity and magnetic susceptibility [18], and possess some attractive possibilities due to dimensions that make them comparable to various biological entities such as virus, gene and protein. Furthermore, these NPs can be potentially utilized in variety of fields such as sensors, catalyst, drug delivery, bioseparation and magnetic separation [19]. Mainly, their magnetic properties can be affected through applying an external magnetic field gradient. This indicates that it would be possible to transport the magnetic NPs themselves and tagged biological units magnetically [6]. So, many papers reported on the preparation of polymer filled with magnetic NPs to be used various applications [18-21].

In the tissue engineering field, magnetic force-based tissue engineering for designing tissue-engineered tubular and sheet-like constructs through utilizing magnetic NPs were proposed by Ito et al. [22,23]. Dobson et al. reported the main principles to prepared a novel magnetic force mechanical conditioning bioreactor for tissue engineering via binding magnetic NPs to the surface of cells and the possibility to control and manipulate cell function by applying an external magnetic field has been investigated [24]. From a magnetic point of view, NPs with size below 30 nm show superparamagnetic behavior, implying their ability to be magnetized through applying a magnetic field without remanence once the field is turned off [25]. Thus, the magnetic scaffolds may produce higher magnetic field gradients, that are able to provide considerable magnetic attractive forces, and via magnetic driving scaffolds may attract and take up bioagents or cells bound to the magnetic NPs and in vivo growth factors [25]. Santis et al. prepared magnetic polymer-based nanocomposite scaffolds through incorporation of superparamagnetic PVP-coated Fe3O4 NPs in a PCL polymer and their mechanical and biological properties had been assessed [26].

From all the above-mentioned applications, Tampieri et al. [27] prepared a novel biocompatible superparamagnetic-like phase (FeHA) through doping Fe3+/Fe2+ ions within hydroxyapatite for bone tissue engineering and studied their microstructural, physico-chemical and magnetic properties. Gloria et al. [6] incorporated FeHA NPs into poly(1-caprolactone) matrix.

The main objective of this work was to develop and design magnetic nanocomposite scaffold for medical applications, such as bone tissue engineering, composing of a PLA biopolymer matrix filled with FeHA NPs. Up to our knowledge, the preparation of the PLA/FeHA nanocomposites has never been reported before. The structural, morphological, mechanical and magnetic properties of these substrates were properly discussed and evaluated. Further, the antibacterial activity for PLA/FeHA nanocomposites was determined. These magnetic nanocomposites can be utilized as a fixed ‘station’ that can be manipulated in situ through applying external magnetic fields, which can also control specific processes at cell level by releasing bioactive factors, in turn, attached to magnetic nanocarriers.

2. Experimental work

2.1. Materials

The used chemicals were PLA pellets (Mw = 60,000 g mol−1, Sigma–Aldrich, St. Louis, MO, USA), calcium nitrate .A.R (Ca(NO3)2·4H2O) (WINLAB, UK) and diammonium hydrogen phosphate (NH4)2HPO4 (SISCO Research Laboratories Pvt. Ltd, India). Both iron (II) chloride tetrahydrate (FeCl2·4H2O, purity 98%) and iron (III) chloride hexahydrate (FeCl3·6H2O, purity 99%) were obtained from Sigma–Aldrich, St. Louis, MO, USA. The bidistilled water and chloroform were used as solvents.

2.2. Preparation of magnetic hydroxyapatite (FeHA) nanopowder

For the preparation of FeHA nanopowder, certain amount of calcium nitrate (Ca(NO3)2·4H2O) was dissolved in bidistilled water with vigorous stirring (2 h). At room temperature, the pH was adjusted at about 10 by adding drop by drop NH4OH with constant stirring. 1.31 g of (FeCl2·4H2O) was dissolved in 20 ml
of bidistilled water, and 2.7 g of FeCl$_3$·6H$_2$O also dissolved in 10 ml of bidistilled water. The two solutions as sources for Fe$^{2+}$ and Fe$^{3+}$ ions were simultaneously added to the basic suspension of calcium nitrate. The total amount for iron ions with respect to calcium ions was adjusted to: Fe/Ca = 20 mol%. (NH$_4$)$_2$HPO$_4$ solution was added drop by drop with stirring for 7 h at the same pH until turbidity clears in the solution because of the formation of FeHA powder [6]. Then, the suspension was left aging at room temperature for 24 h. The precipitate was separated by centrifugation and washed with bidistilled water. The final product was crushed using agate mortar and pestle to obtain resultant powder.

2.3. Preparation of magnetic polyactic acid/iron-doped hydroxyapatite nanocomposites

Magnetic nanocomposites were prepared through embedding FeHA NPs into a PLA polymer matrix through the casting method. Firstly, PLA pellets were dissolved at room temperature in chloroform under stirring. Then, the prepared FeHA NPs were added to the PLA solution during stirring with different weight ratios PLA/FeHA NPs wt.% (90/10, 80/20 and 70/30) were prepared at pH = 7. A probe sonicator device was also used to optimize the dispersion of NPs within the PLA matrix. The solutions of prepared nanocomposite samples were poured into Petri-dishes and kept in oven at 40°C for three days to evaporate the solvent and the obtained samples with thickness ~0.09 mm were stored in highly evacuated desiccator.

2.4. Techniques

Fourier transform-infrared spectrometer FT-IR (Nicolet iS10, USA) was used in the wavenumber range 4000–400 cm$^{-1}$ to study the chemical structure. PANalytical X’Pert PRO XRD system using Cu Kα radiation (λ = 1.540 Å, the operating voltage of tube 30 kV) was used to obtain the XRD spectra in the range 5–80° for the prepared biocomposite samples and investigate its different crystalline structures. Scanning electron microscope (SEM) (JEOL JSM 6510 LV, USA) with accelerating voltage 20 kV was used to investigate the morphological properties of the nanocomposite sample with high content (70/30 wt.%) of FeHA NPs at magnification 400×. Surface of the nanocomposite film was coated with a thin layer of gold (4 nm) through the vacuum evaporation technique in order to reduce sample charging effects as a result from the electron beam. Energy Dispersive X-ray Diffraction (EDX) unit coupled with a scanning electron microscope was used to depict the elemental analysis and the chemical composition and confirm the presence of FeHA within the polymeric matrix of this sample. The size of prepared FeHA NPs was studied through transmission electron microscopy (TEM) (JEOL TEM-1230, JEOL Ltd., Tokyo, Japan). The mechanical properties for the prepared samples were performed through the tensile strength method where a computer controlled Lloyd LRX5K mechanical testing machine (Lloyd Instruments Ltd, United Kingdom) was used at room temperature with speed 2 mm/min in the tensile tests. For each run, 3 samples were studied and the average values were reported. Magnetic properties for the prepared nanocomposites had been studied at constant temperature (310 K) through vibrating sample magnetometer (VSM) (Model: LakeShore 7410) analyzing their dependence on moment and field. The antibacterial activity for PLA and PLA nanocomposites was obtained against two types of bacteria gram-positive (Bacillus thuringiensis) and gram-negative (Pseudomonas aeruginosa) through the nutrient agar disk diffusion way with the inhibition zones determination (mm). Shortly, sterile paper discs (6 mm) were fertile in sample solutions overnight and then left to dry in sterile conditions for 24 h. The bacterial suspensions were obtained through making a saline suspension of isolated colonies selected from 18 to 24 h of nutrient agar plating. Then, suspensions were set to resemble the tube of 0.5 McFarland turbidity standards through the aid of spectrophotometry at 600 nm, which equals (1.5 × 10$^8$ colony-forming units/ml). The nutrient agar surface was quite inoculated by a sterile swab, which was soaked in the bacterial suspension. The fertile discs were put on the inoculated agar and incubated for 24 h at 37°C. Finally, the diameters for the growth inhibition zones were obtained.

3. Results and discussion

3.1. FT-IR

FT-IR analysis was performed to depict the structural changes within the PLA/FeHA nanocomposite samples. Fig. 1 indicates the FT-IR spectra for PLA biopolymer before and after embedding FeHA nanofiller with different mass fractions. It can be observed that the characteristic bands of PLA are also retained in the filled samples and Table 1 lists the position of characteristic peaks for PLA and their assignments [15,28–30]. From the spectra of PLA/nanocomposites, the embedding process of FeHA within PLA matrix induces largely changes and effects for the functional groups of PLA. From the spectrum of PLA/FeHA (90/10 wt.%) nanocomposite, the absorbance of OH increases and CH$_2$ stretching vibrations decreases and the shape of C=O becomes more organized with less broadness. This implies that the low content of FeHA induces structural rearrangements within the nanocomposite matrix and its distribution within PLA becomes more...
organized. This observation leads to an improvement for the crystalline regions within PLA which is depicted by XRD results. Furthermore, the absorbance of peak at 1045 cm\(^{-1}\) has a maximum value as compared with the other nanocomposite spectra and the intensity of amorphous peak at 873 cm\(^{-1}\) is decreased.

With continuous increasing the FeHA NPs content, the broadness and absorbance of OH and CH\(_2\) stretching peaks in the wavenumber region 3750–2600 cm\(^{-1}\) are largely increased. The increase in OH absorbance in this region is due to the existence of Ca(OH)\(_2\) of FeHA. This broad band implies the complete loss for water dehydration of Ca(OH)\(_2\) [31,32] and Fe\(^{3+}\) incorporation with high Lewis acid strength leads to improved adsorption of water [32].

Also, the absorbance of C=O peak gradually decreases and its broadness increases associated with a new shoulder at 1640 cm\(^{-1}\) its absorbance increases with increasing the content of FeHA NPs. This depicts that there is a strong interaction/coordination between carbonyl group (C=O) of PLA and Fe of FeHA nanoparticles where the C=O group of PLA adsorbs Fe colloid surface preferably through the non-bonding electrons of C=O [33–35]. This new shoulder can be attributed to the overlapping between C=O peak and OH bending of FeHA that appears at 1640 cm\(^{-1}\) [31,36].

Besides, the absorbance peak at 1045 cm\(^{-1}\) is greatly decreased, disappeared at PLA/FeHA (70/30 wt.%), and the absorbance of amorphous peak at 873 cm\(^{-1}\) is increased. From these observations, the amorphous regions of nanocomposite samples are increased as indicated by XRD analysis. From the spectra for nanocomposite samples in the wavenumber region 630–530 cm\(^{-1}\), there are two new peaks appearing at 610 and 564 cm\(^{-1}\) that can be attributed to the triply degenerate bending of O–P–O mode of the apatitic structure [32,36]. The characteristic peaks for iron oxides in the wavenumber region 600–400 cm\(^{-1}\) [37–39] may be occluded by those of HA because of the low amount of iron present in the nanocomposite samples. From the IR spectra for nanocomposite samples, FeHA is linked with PLA via Hydrogen bond formation between OH of Ca(OH)\(_2\) and oxygen atoms in the ester groups of PLA. This type of linkage has great effect on mechanical properties and interface behavior.

### 3.2. XRD

**Fig. 2** shows the XRD spectra of the virgin PLA and PLA/FeHA nanocomposite samples. All spectra exhibit two peaks at 16.52 and 18.73° which are characteristic for PLA polymer. From the spectrum for PLA/FeHA (90/10 wt.%), the intensity of these peaks has a maximum value. This observation implies that the addition of FeHA with low content induces structural rearrangements and the distribution of FeHA NPs is more organized within PLA [40]. A correlation between the degree of crystallinity and the peak intensity in XRD patterns was proposed by Hodge et al. [41]. They observed that the intensity of XRD decreases as the degree of crystallinity decreases and amorphous nature increases [42]. Thus, the incorporation of NPs with low content results in an improvement for the intermolecular interaction between the PLA chains. Thus, the degree of crystallinity for PLA/FeHA (90/10 wt.%.) sample is increased. From the spectra of nanocomposites (80/20 and 70/30 wt.%), the intensity of these peaks is largely decreased implying that the high content of FeHA NPs induces a decrease in the degree of crystallinity, indicating that the FeHA NPs distribution within the PLA matrix became random that caused an increase for the amorphous regions [43,44].

From **Fig. 3**, the most diffraction patterns of pure FeHA spectrum are just assigned between apatite (JCPDS card no. 14-0215) and hydroxyapatite (JCPDS card no. 09-0432) [31,45]. However the peaks of (112) and (300) phases, at 2θ ~ 32.14 and 32.90°, do not exist, which are just consistent with apatite. This depicts the substitution of Fe ions into the HA lattice via ion exchange reaction [46] that results in large amounts of OH channels and Ca channels out of wider c-planes [45]. Thus, it can be concluded that the reduction for Fe ions trapped into the lattice of HA, which exists at the heterogeneous gas-solid interface [27], is homogeneous within the whole bulk and can ensure a balanced distribution for Fe\(^{3+}\) and Fe\(^{2+}\) within the structure and the kinetics for both formation of magnetite and crystallization of apatite occur. From these results, XRD patterns for prepared FeHA NPs show the Bragg reflection peaks of magnetite at 2θ ~ 36, 43.50, 57.50 and 64° [6,27,47,48] (**Fig. 2**).
Also, some of FeHA peaks appear in the diffraction patterns of nanocomposite samples which are an indicator for the existence of FeHA phases within the polymeric matrix of PLA.

3.3. **SEM, EDX and TEM**

Fig. 4 shows the SEM micrograph for the nanocomposite sample of high content of FeHA, PLA/FeHA (70/30 wt.%), and its EDX image. It can be observed that the aggregates of FeHA NPs are observed on the nanocomposite surface and distributed uniformly within the polymeric matrix in general as shown in Fig. 4a. These aggregations of NPs were due to the existence of NPs with high content within the polymeric matrix, where the mobility of polymeric chains increases due to the increase in the content of amorphous regions as indicated in XRD results.

Therefore, the polymeric chains relax and this assists NPs to move, allowing crystals to agglomerate. From Fig. 4b, EDX image confirms the presence of FeHA NPs within PLA matrix by showing the spectral lines HA components (such as Ca and P) and magnetite which are in agreement with the literature [6, 27, 47, 48]. Fig. 5 shows the TEM micrograph for the prepared FeHA NPs which are inorganic spherical-like HA particles with size range 3–12 nm and are composed of fine sub-crystallites of magnetite. The aggregation in TEM micrograph is due to the magnetic feature of FeHA NPs [21] that form dark regions.

3.4. **Mechanical testing**

The mechanical properties of the biocomposite samples with different concentrations between PLA and FeHA NPs were tested by universal testing machine to study the effect of FeHA NPs on the mechanical properties of PLA biopolymer. Fig. 6 shows the stress–strain curve of 90/10, 80/20, 70/30 wt.% bio-composites compared with the pure PLA. The results depict that the incorporation of FeHA NPs with high concentrations decreases the mechanical parameters including tensile stress, elastic modulus and strain to failure of the biocomposites. It is clearly obvious that elastic modulus of biocomposites decrease with increasing FeHA nanofiller concentration as shown in Table 2. This decrease in the mechanical properties is due to the orientation of FeHA NPs in tensile test sample under...
the tensile strain. This orientation and pulling of reinforcing nanofillers are continued until the breaking limit of film. Also, it can be attributed to the high flexibility acquired by the chains of the PLA nanocomposites with high content of FeHA NPs where the amorphous content increases as confirmed by XRD analysis. In addition, this decrease can be due to agglomeration of NPs above the optimum loading value, as shown in SEM micrograph, where the NPs behave as ‘weak points’ for the PLA matrix [33,49,50]. Gloria et al. [6] reported that the reduction of the mechanical properties for the nanocomposites with high content of NPs because of the formation of stress concentration at the polymeric matrix/nanoparticle interface and discontinuities in the stress transfer, which may be due to the difference in ductility between the inorganic nanofillers and the polymeric matrix (Table 3).

On the other hand, these results show that the adhesion between the nanofiller and the polymeric matrix has been significantly enhanced in the small concentration of FeHA NPs, PLA/FeHA (90/10), via grafting of polymer from the surface of FeHA NPs [49]. The significant increase in mechanical strength with the addition of only 10 wt.% FeHA NPs is an excellent indication of the advantages of NPs as opposed to micro-sized particles which typically decrease the strength of the composites. This enhancement can be ascribed to the fine dispersion of FeHA NPs which will carry much of the load transfer through the polymer matrix [50–52]. Also, this can be ascribed to the strong interfacial adhesion between FeHA NPs and PLA within nanocomposite matrix, where it significantly limits the movement of PLA chains [49,52].

Thus, the results of mechanical tests show the good effect of small ratios of FeHA on the mechanical properties of the biocomposites which have a great attention in some biological applications like composite scaffold for bone tissue engineering.

### 3.5 Magnetization analysis

As shown in Fig. 7, the zero remanence and coercivity of the hysteresis loops of the biocomposites nanomagnetic materials demonstrate the superparamagnetic behavior of all biocomposite samples at room temperature (310 K) with no hysteresis. Thus, these nanocomposite samples do not maintain any residual magnetism when the external magnetic field is removed, which assists cell separation and magnetic resonance imaging (MRI). The absence of remanent magnetization is probably because of the rapid Néel relaxation, implying that these NPs are superparamagnetic at room temperature [18]. This behavior was also observed in poly(1-caprolactone)/FeHA NPs [6].

The saturation magnetization (MS) values are found to be proportional to the FeHA NPs content. The (MS) value of the pure bare NPs was 90 emu g⁻¹, whereas those for 90/10, 80/20 and 70/30 were 19.18, 21.37 and 26.76 emu g⁻¹, respectively. This improvement in the MS value could be attributed to the increased mass fractions for the FeHA NPs on the magnetic nano-biocomposite [53]. These observations prove PLA/FeHA NPs with unique properties to be utilized in vivo applications, since their functionality can be remotely fine-tuned through controlling both the time of the magnetic field exposure and the amount of NPs.

#### 3.6 Antibacterial analysis

The images of antibacterial activity for the prepared samples are indicated in Fig. 8. From this figure, the PLA nanocomposite samples had comparatively high and nearly similar antibacterial activity against Gram-negative and Gram-positive bacteria as compared to that of pure PLA, where the inhibition zones were increased with increasing the NPs content within PLA matrix. This result can be because of the high surface area for the PLA nanocomposites that enable them to reach easily the nuclear content of bacteria [31]. Although the strong antibacterial activity for the prepared PLA-FeHA NPs, more studies are important to analyze their bactericidal effects on different on various types of bacteria for potential growth of their biological applications.

### Table 2 – Elastic modulus, tensile strength and strain to failure determined from stress–strain curve relations for PLA and its nanocomposite samples.

<table>
<thead>
<tr>
<th>Samples (wt.%)</th>
<th>Elastic modulus (MPa)</th>
<th>Tensile strength (MPa)</th>
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<tbody>
<tr>
<td>PLA</td>
<td>302</td>
<td>17.93</td>
</tr>
<tr>
<td>90/10</td>
<td>330</td>
<td>18.51</td>
</tr>
<tr>
<td>80/20</td>
<td>289</td>
<td>17.6</td>
</tr>
<tr>
<td>70/30</td>
<td>260</td>
<td>15.77</td>
</tr>
</tbody>
</table>

### Table 3 – The bacterial inhibition zone (mm) of PLA and PLA/FeHA nanocomposite samples.

<table>
<thead>
<tr>
<th>Bacteria type</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLA (90/10 wt.%)</td>
</tr>
<tr>
<td>B. thuringiensis</td>
<td>18</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>20</td>
</tr>
</tbody>
</table>

**Fig. 7 – Field-dependent magnetization of PLA/FeHA nanocomposites at 310 K.**
4. Conclusions

The FeHA NPs were successfully prepared and embedded within the PLA biopolymer with different contents through the solution casting method. FT-IR spectra confirmed the complexation between functional groups of PLA and FeHA NPs through change in the intensity of absorption peaks and appearing of new peaks, such as peaks at 1640, 610 and 564 cm$^{-1}$. XRD results showed that the degree of crystallinity of PLA nanocomposites with low content of FeHA NPs enhanced and decreased for those of high content. Also, the XRD spectrum of FeHA NPs showed the crystalline phases for magnetite and HA. SEM micrograph confirmed the existence of FeHA NPs aggregations for nanocomposite with high content and EDX showed the spectral lines for FeHA components and PLA. The size range for the prepared NPs was 3–12 nm as in TEM micrograph. The mechanical properties for PLA with high content FeHA NPS were reduced. The magnetization analysis showed that the values of saturation magnetization were found to be proportional to the content of FeHA NPs. The antibacterial activity of PLA/FeHA NPs samples depicted a strong antibacterial activity against Gram-negative and Gram-positive bacteria. Thus, all previously mentioned results for nano-biocomposites of PLA with low content of FeHA NPs, high degree of crystallinity and excellent mechanical properties, will make these nano-biocomposites a potential candidate for medical applications such as bone tissue engineering and traditional bone-defect repair.

Conflicts of interest

The authors declare no conflicts of interest.

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