Preparation and spectroscopic investigations of hydroxyapatite-curcumin nanoparticles-loaded polylactic acid for biomedical application

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ABSTRACT

The main goal of this work is to fabricate a biocomposite material of hydroxyapatite (HA) and polylactic acid (PLA) loaded with different concentrations of curcumin for using in bone tissue scaffold engineering. PLA-HA-curcumin biocomposite was prepared via the precipitation method with PLA/HA ratio of 80/20 wt% and different ratios of curcumin. The structure and surface morphology of the prepared biocomposite were studied using Fourier Transform Infrared spectroscopy (FTIR), x-ray diffraction spectroscopy (XRD), transmission electron microscope (TEM) and scanning electron microscope (SEM). The bioactivity enhancement of the prepared HA-PLA biocomposite was examined after immersion in simulated body fluid (SBF) before and after addition of curcumin. Drug release of the HA-PLA biocomposite prepared with different concentrations of curcumin was studied in phosphate buffer solution. Our data confirm the nanostructure of the prepared HA-PLA biocomposite and show good miscibility and diffusion of curcumin inside the scaffold matrix. Furthermore, curcumin supplementation effectively enhanced the bioactivity of the HA-PLA biocomposite.

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Introduction

Bone replacement and repair are of the most successful applications of tissue engineering. The biodegradable porous scaffold materials act as three-dimensional templates for cell adhesion and subsequent bone regeneration [1,2]. Hydroxyapatite (HA) is a common ceramic bioactive and biocompatible material, which can be used to repair damaged calcified tissues in the human body. Hence, HA is used as a scaffold in human bone replacement [3]. However, HA cannot be directly used as a healing supplementary implant in defected bones due to its low mechanical properties. In order to enhance the regeneration of bone tissue, it is important to synthesis materials with appropriate mechanical properties. These materials should also enhance the cell attachment with the prepared scaffold. To overcome the low mechanical strength of HA, it is important to combine HA, as a filler, in the biopolymer. In general, the human bone matrix is an organic and inorganic composite biomaterial [4]. Biodegradable polymers such as polylactic acid (PLA), polycaprolactone (PCL) and its copolymer poly(lactic-glycolic) acid (PLGA) are used mainly for the biomedical applications [5,6]. PLA is extensively used in the field of biomedical application [7], with its biocomposite with HA constituting the most common scaffold materials in literatures. PLA, when blended with HA,
not only enhances the strength of the resultant biocomposite but also has an effective role in the formation of new bone [8]. Curcumin is one of the main active components that are extracted from the rhizomes of turmeric plants (Curcuma longa L). The extensive uses of curcumin are related to its various medical applications and therapeutic activities [9]. Curcumin has a highly pleiotropic action and can physically interact with an adverse range of molecular targets including growth factors, transcriptional factors, enzymes, cytokines, and genes regulating cell apoptosis and proliferation [10–12].

Various clinical and pre-clinical studies demonstrated that curcumin has an anti-microbial, anti-inflammatory, anti-oxidant, anti-arthritic, and anti-carcinogenic, and wound healing properties [13–15]. The ability of curcumin to enhance wound healing is related to its effectiveness in increasing biosynthesis of extracellular matrix proteins such as collagen [16,17].

The main purpose of this work is to fabricate a biocomposite suitable as a scaffold for bone tissue engineering and increase the effectiveness of the fabricated biocomposite in bone regeneration activity by the addition of curcumin.

Materials and methods

Materials

Calcium Nitrate A.R (Ca(NO₃)₂·4H₂O) was purchased from Winlab, UK. Di-ammonium Hydrogen Phosphate ((NH₄)₂·HPO₄) was purchased from Sisco Research Laboratories pvt. Ltd, India. Polylactic acid (PLA) pellets (Mw = 60,000) and curcumin powder (C₂₁H₂₀O₆, curcumin longa (Turmeric)), were purchased from Sigma-Aldrich, UK. All chemicals were used without further purification.

Preparation of the biocomposite

Hydroxyapatite nano-powder was prepared via chemical preparation method previously described in [18,19]. Briefly, calcium nitrate and diammonium hydrogen phosphate were dissolved in distilled water then mixed at pH range 11–12 by adding drops of ammonium solution. The precipitate was dried and sintered to obtain the HA nano-powder. PLA was dissolved in chloroform then the required quantity of prepared nano-HA powder was dispersed in the PLA solution using probe sonicator. The ratio PLA/HA was kept as 80/20 wt%. For the curcumin release studies, the desired amount of curcumin powder was dissolved in chloroform solution and added to the biocomposite at different ratios 1, 2, 3, 4 and 5 wt% to the total composite. After complete mixing of all solutions, the solutions were poured into Petri-dishes and dried at room temperature, then cut into pieces for characterizations.

Structural and morphological analyses

Fourier transform infrared (FTIR) absorption spectra of the pure and biocomposite samples were obtained using the FTIR spectrometer Nicolet iS10, USA, in the wavenumber range 4000–400 cm⁻¹ at room temperature. X-ray diffraction (XRD) analysis was performed using X-ray diffractometer, copper Kα (λ = 1.540 Å) (PANalytical X’Pert PRO) within Bragg angle 5–70°. High-resolution transmission electron microscope (HR-TEM) (JEOLJEM-2100, USA) with a CCD camera at an accelerating voltage of 200 kV was used to investigate the average size and size distribution of the nanoparticles. The Morphology of the prepared samples was examined using Field Emission scanning electron microscope (FESEM) (JEOL JSM 6510 LV, USA).

In vitro test

In vitro investigations were performed in 50 ml capacity screw polyethylene bottles, which contains 10 ml of SBF and 1 cm² of each sample (in film form). The SBF solution for bioactivity studies was prepared by dissolving the following chemical reagents: 8.026g-NaCl, 0.352g-NaHCO₃, 0.225g-KCl,
0.230g-K2HPO4.3H2O, 0.311g-MgCl2.6H2O, 0.293g-CaCl2 and 0.072g-Na2SO4 into 700 ml distilled water. The temperature was kept at 37 °C and the pH was adjusted at 7.4 with 6.063 g of tri-hydroxymethyl-aminomethane and 40 ml hydrochloric acid [20]. The weight/volume ratio between samples and SBF was adjusted to be 1 g:10 ml. Sealed bottles were kept at 37°C in a water bath. The samples were soaked in SBF for 14 days before they were taken out for testing and analyses. The SBF was replaced at constant times to keep the Ca2+ and P+5 ion concentrations constant. Formation and morphology of apatite-like structures in prepared samples were examined using FESEM and confirmed by EDX measurements.

**Drug release studies**

Similar pieces from the biocomposite films loaded with different ratios of curcumin 1, 2, 3, 4 and 5wt% were immersed in 10 ml of phosphate buffer solution (PBS) containing 30% ethanol at pH 7.4. The temperature was fixed at 37°C. The samples were then incubated on a shaker at 150 rpm. Following, 2 ml of curcumin solution was withdrawn at different time intervals to measure the release percentage. Calibration curve of curcumin solution was measured using UV spectrometer (T80+, UV/Vis. spectrometer, PG Instrument Ltd.) at \( \lambda_{\text{max}} \) = 431 nm. Using the curcumin calibration curve, the drug content was determined, and the cumulative %drug release against time was calculated using a normal plot according to the equation:

\[
\text{Release(\%) } = \frac{\text{curcumin released}}{\text{Total curcumine loaded}} \times 100
\]

**Results and discussions**

**FTIR analyses**

Figure 1 shows the FTIR spectra of pure curcumin, PLA and their biocomposite with HA. The FTIR spectrum of curcumin shows a sharp absorption band at 3508 cm\(^{-1}\) due to the OH group. The sharp absorption band at 1626 cm\(^{-1}\) is mainly due to the overlapping between C = C and C = O groups. A strong absorption band at 1601 cm\(^{-1}\) is attributed to the stretching symmetric vibrations of the (C = C) aromatic ring. The 1508 cm\(^{-1}\) absorption band is

![Figure 1. FTIR of pure curcumin, pure PLA, PLA-HA scaffold and PLA-HA-cur biocomposite scaffold.](image-url)
attributed to the (C = O), while the absorption band at 1272 cm\(^{-1}\) is due to the enol group (C–O)\(^{21,22}\). The spectrum of PLA shows a sharp absorption band at 1737 cm\(^{-1}\) attributed to the stretching vibration of the carbonyl group (C = O) in the – CO–O – group of the PLA. Another sharp absorption band at 1165 cm\(^{-1}\) is related to the stretching vibration of – C–O – in the – CH–O – group in the chain of PLA polymer. Triple mountainous-like peaks at 1110, 1072 and 1025 cm\(^{-1}\), assigned as the stretching vibrations in –CO–O–group in the polymer chain\(^{23}\). The spectrum of PLA-HA biocomposite shows the appearance of characteristic bands of HA inorganic phase with polymer matrix peaks.

In comparison with the pure PLA, PLA-HA biocomposite has two strong peaks appear at 563 and 602 cm\(^{-1}\) characteristic for the bending modes of PO\(_4\)\(^{3-}\). The sharp band at 1384 cm\(^{-1}\) is assigned as N-O stretching mode of NO\(_3^-\). The stretching mode of PO\(_4\)\(^{3-}\) is assigned at 1031 cm\(^{-1}\). These characteristic peaks indicate the presence of HA in the composite\(^{18}\). The spectra for the PLA-HA-curcumin scaffold show the same characteristic peaks of PLA and HA without disturbance. This stability of the biocomposite may be attributed to the formation of ion-dipole bond between oxygen in the ester group of PLA and the calcium in HA\(^{24}\). This confirms the tight bonding between the HA and the PLA matrix. The curcumin peaks appear in the biocomposite matrix without any shift due to its loading without chemical interaction with the scaffold materials. This physical interaction is important for the drug release mechanism.

**X-ray diffraction (XRD) analysis**

XRD diffraction spectra in Figure 2 show the presence of characteristic peaks of the different composition of the biocomposite. XRD of pure HA shows the characteristic diffraction peaks represented at 2\(\theta\) = 25.95°, 31.51°, 39.70°, 46.78°, and 48.00°\(^{18}\). The PLA spectrum shows the most intense peaks of PLA, especially at 2\(\theta\) = 17° and 19°. The PLA-HA and PLA-HA-curcumin biocomposite spectra confirmed the existence of crystalline HA phase in the biocomposite. All diffraction peaks of HA in the biocomposite appear at almost the same angles and intensity before and after the addition of curcumin. This confirms that the presence of PLA and curcumin do not affect the structure of HA crystals. In the spectra of curcumin and PLA-HA-curcumin biocomposite, the characteristic peaks confirm the presence of curcumin in the crystalline form as indicated by the main peaks at 2\(\theta\) = 7.96, 8.90, 12.26, 14.54, 17.24°\(^{21}\). It appears that some peaks of curcumin are absent in the spectrum of the biocomposite scaffold, which suggests the good diffusion of curcumin inside the scaffold matrix. Thus, the curcumin peaks are too broadened, and the XRD pattern is dominated by the structure of the PLA-HA components.

**Morphology of the biocomposite**

Figure 3 shows the images obtained by the transmission electron microscope of the prepared biocomposite. The figure shows the formation of nano-hydroxyapatite surrounded by PLA and curcumin with good homogeneity. The appeared agglomeration can be due to the ion-dipole bond between ester group of PLA and the calcium in HA\(^{24}\). This confirms the tight bonding between the HA and the PLA matrix. The curcumin peaks appear in the biocomposite matrix without any shift due to its loading without chemical interaction with the scaffold materials. This physical interaction is important for the drug release mechanism.

Figure 4 shows the FESEM images of the surface morphology of the prepared biocomposite matrix with no higher visible agglomeration of the HA particles. Figure 5 shows the surface morphology of the PLA-HA and PLA-HA-curcumin biocomposites after in vitro bioactivity test. The microimages show the precipitation of apatite-like structure on the biocomposite surface with precipitation increasing after the addition of curcumin. The higher perception of bone-like apatite structure in curcumin containing biocomposite may be due to the effect of curcumin
in increasing mineralization and nucleation of the apatite crystals [25]. The EDX analysis (Figure 6) shows that the precipitated crystals are apatite-like structures with Ca:P ratio of 1.665 which is comparable to the Ca:P ratio in human bone (1.67) [26].

**Curcumin release studies**

To study the percent of curcumin release from the curcumin-loaded biocomposite, the calibration curve of curcumin is shown in Figure 7. The following equation calculated from the calibration curve of curcumin used to calculate the release percent at different times, and the release behavior is shown in Figure 8.

\[
\text{Concentration (mgm}^{-1}\text{)} = 0.02306 \times \text{Absorbance - 0.007}
\]

As shown in Figure 8, the relation plotted between drug release percent and time for different-loaded curcumin samples shows a nonlinear relationship, which confirms that curcumin release is a biphasic process [27]. The biphasic
Figure 4. FESEM images of (a) PLA-HA scaffold and (b) PLA-HA-curcumin biocomposite scaffold.

Figure 5. FESEM images of (a) PLA-HA scaffold and (b) PLA-HA-curcumin biocomposite scaffold after immersion in SBF.

Figure 6. EDX analysis of PLA-HA-curcumin biocomposite scaffold after immersion in SBF.
release behavior is described by an initial burst release phase (fast release) and then a sustained successive release phase [28]. The burst release from the biocomposite sample was about 50% of the loaded curcumin during the first 2 h. This burst curcumin release may be due to the existence of curcumin on or near the biocomposite surface. The curcumin is then released slowly and reach its maximum value after 7 to 9 h of release in the saline medium. Percent of release is then reduced gradually with 2–4% from its maximum value after 25 h of immersion in the saline medium. The long-term release and value of the maximum curcumin release from the biocomposite was attributed to the low degradation rate of polylactic acid in the water saline medium [29].

Taking into account the controlled release profile of PLA-HA-curcumin scaffolds, the long-term anti-inflammatory effect can be expected, when they are applied as drug-loaded scaffolds for bone

**Figure 7.** Calibration curve of curcumin solution.

**Figure 8.** Curcumin release profile from the PLA-HA-curcumin biocomposite scaffold.
repair and regeneration [28]. These results agree with the previous curcumin release experiments by Suwantong et. al [30], and Mai et. al [22].

Conclusions
PLA-HA-curcumin biocomposite scaffolds were prepared via the precipitation method with PLA/HA ratio of 80/20 wt% and different ratios of curcumin. The FTIR analysis shows a good stability of the biocomposite due to the ion-dipole binding between PLA and HA and show no chemical interaction between curcumin and scaffold materials, which is important for the drug release mechanism. XRD analysis suggested good miscibility and diffusion of curcumin inside the scaffold matrix, which appeared also in FESEM micro-images. Curcumin addition increased the surface bioactivity of the HA-PLA biocomposite through the formation of the bone-like structure after immersion in SBF. All results confirm that HA-PLA-curcumin biocomposite has a high degree of biological activity, which makes it very suitable for the field of biomaterials and other various medical applications.

Highlights

- PLA-HA-curcumin biocomposite scaffolds with PLA/HA ratio of 80/20 wt% and different ratios of curcumin were prepared via the precipitation method.
- Spectroscopic analysis proves the formation of ion-dipole between PLA and HA which reveal good stability of the biocomposite.
- Addition of curcumin increased the surface bioactivity of the HA-PLA biocomposite through the formation of the bone-like structure after immersion in SBF.
- The in vitro and drug release behavior studies confirm that the fabricated biocomposite is appropriate for medical applications.

Disclosure statement
No potential conflict of interest was reported by the authors.

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