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Sustainable pectin fascinating hydroxyapatite nanocomposite scaffolds to enhance tissue regeneration

Murugan Sumathra[a], Dharman Govindaraj[a], Murugaraj Jeyaraj[b], Abdulla Al Arfaj[c], Murugan A. Munusamy[c], Selvaraj Suresh Kumar[d], Mariappan Rajan[a,*]

[a] Biomaterials in Medicinal Chemistry Laboratory, Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai 625021, India
[b] National Centre for Nanoscience and Nanotechnology, University of Madras, Guindy Campus, Chennai 25, India
[c] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
[d] Department of Microbiology, School of biological Science, Madurai Kamaraj University, Madurai 625021, India

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ABSTRACT

The preparation of biocompatible nanomaterials is one of the emerging areas and it is continuously developing with the use of various contrived methods to accomplish the formation of nanoscale materials. Nevertheless, unfortunately, many of these strategies utilize harmful organic solvents, which make the pertinence of nanoparticles in medicinal applicationsimpractical. In this study, the morphology-focused hydroxyapatite (HAP) was prepared using pectin extracted from the citrus fruit peel (*Citrus limonum*) and it is used for the synthesis of nano HAP by varying the concentration of pectin as a template. The chemical structure, crystallinity, and morphology were determined by FTIR, XRD, and SEM, respectively. To increase the biocompatibility of HAP, pectin aided HAP (tHAP) and HAP/pectin composites were synthesized with different concentrations of pectin. The compatibility of HAP/pectin was carried out in a human osteoblast cell line. The physic-chemical and biocompatibility showed, HAP/pectin, and HAP/pectin composites are promising materials for bone tissue engineering applications.

1. Introduction

Pectin is a heterogeneous polysaccharide occurring in the cell walls and intracellular layers of earth plants (Yokoi et al., 2002). Pectin can be represented as a single extracellular matrix which is a complex structure that is formed continuously through the body of the plant. It can be categorized as one of the anionic polysaccharides in the cell wall that consists of “smooth” α-D- galacturonic acid with 1–4 linkages region monomer (Schols et al., 1996). While, the polysaccharide of galacturonic acid has consisted of various sugars such as galactose, rhamnose, arabinose, xylose and glucose. Pectin richly found in naturally available resources, particularly agricultural wastes are principally serviceable, which have the potential application in meditative applications because they are, biocompatible, biodegradable, nontoxic, easily available and ready to support the designing of medicinal devices (Lin Shu et al., 2004). Recently, the pectin was extracted from peels of banana, pomegranate, orange, mango, apple (Henrique et al., 2016; Majdoub et al. 2001; Oliveira et al., 2016). On the other hand, pectin-rich fruit peels waste is generated from food industries (Rishabha et al.al., 2014, Fishman et al., 2008).

The pectin was shown to be a valuable product component in the design a biorefinery products from the sources citrus peel compared to commercial pectin (Ciriminna et al., 2008; Banerjee et al., 2016). Recent studies have been indicated the pectin influence the pathogenic adhesion as an anti-inflammatory agent, anti-coagulant, and wound healing substances to the tissue site (He et al., 2012; Shanahan 2004; Filomena et al., 2012). Researcher two combinations of pectins ie., Low Methoxy Pectin (LMP) and High Methoxy Pectin (HMP) were extracted from the natural sources. LMP enhance the synthesis of nanomaterials with specific size and morphology (Gobi et al., 2014). The citrus cell wall is a high amount of LMP present and the peels are mostly dumped as solid waste. Valorization of citrus peels to recover pectin has the potential to increase the economic viability of a biorefinery utilizing thus waste resource (Kanimozhi et al., 2014).

The synthesis of nanomaterials by the greener way is emerging technology for the recent research. Nanoparticles with particular and morphologies are often promptly synthesized by difference technique such hydrothermal, co-precipitation, freezing, reverse microemulsion,

* Corresponding author.
E-mail address: rajanm153@gmail.com (M. Rajan).

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ultrasonic irradiation and template-assisted synthesis (Yan et al., 2001). Meanwhile, these ways utilized harmful solvent and chemicals agents which are prospective threatening to the environmental and biotic systems (Govindaraj et al., 2015). Hence, in this present study was synthesized nano HAP by using pectin as a green template extracted from citrus peel. HAP is bone mimic materials and it enhances growth of new bone formation. In addition, the addition polymers association with HAP, polymer will enhance the biocompatibility, mechanical activity of HAP for bone tissue engineering (Govindaraj et al., 2015; Camarero-Espinosa et al., 2016; Ottaviani et al., 2012; Cox et al., 2015; Dubnika et al., 2012; Tadros et al., 2014; Linshu et al., 2003; Luqman et al.al., 2015; Uddin et al., 2012). The HAP/polymer composite materials have attracted more attention because of the specific advantageous properties, such as flexibility and osteoconductivity (Dubnika et al., 2012; Tadros et al., 2014; Linshu et al., 2003).

Different types of sustainably derived polymer matrices have been used in bone tissue engineering, including gelatin, collagen, chitosan, polycaprolactone and cellulose as natural polymers. The incorporation of pectin as the polymer matrix is improve the bone implanted materials has been broadening for the latest category of biocomposite material (Munarin et al., 2015; Gentilini et al., 2013). The mechanical performance of nanocomposites is extremely prompt by the indigenous properties of the nHAP (Feng et al., 2015). Since pectin improves the proliferation of osteoblast, it has been recently attracting much attention as a novel bone biomaterial (Lisheng et al., 2016). This work was established the enhancement of morphology, purity, and crystalline behavior of HAP nanoparticles by the various concentration of pectin extracted from the viable source of citrus peel and HAP-pectin composites was also performed for bone engineering applications.

2. Experimental sections

2.1. Materials

Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), diammonium hydrogen phosphate $(NH_4)_2HPO_4$, ethanol, toluene, and liquid ammonia were received from Sigma-Aldrich, Egmore, Chennai, India. Analytical grade chemicals were used throughout experiments without any refining. The double distilled (DD) water was for purification.

2.2. Preparation of cell wall material

Citrus (*Citrus limonum*) were procured from Madurai, Tamil Nadu, India. The fruits were washed with DD water, and rinsed with acetone and divided into pulps and peels. Peels were disconnected into a piece of roughly 1 cm^2. The peel samples were dehydrated at room temperature. The dehydrated samples were ground into fine particles using an electric mixer and keep in a separate container at 4 °C for next experiments. A small piece of peels was heated with 1:2 proportion of toluene: ethanol for 5 h and filtered using a vacuum pump. The residue was extensively washed with 60% aqueous ethanol to eliminate contaminations, pigments, and sugars up to the time of filtrate were uncoloured. Then it was dehydrated in an oven at 40 °C for 24 h to get cell wall material or alcohol insoluble residue.

2.3. Extraction of Pectin from the Cell Wall Material

The dried cell wall material was suspended in double distilled water (solid: liquid ratio, 1:25, w/v) then the suspension was stirred at 60 °C for 4 h to form the slurry. The resultant slurry was cooled to room temperature and filtered through Whatman filter paper no.1. The residue was resuspended in double distilled water (solid: liquid ratio-1:25, w/v), and filtered as given above. The resultant supernatant was precipitated with four volumes of ethanol and obtained gelatinous precipitate was filtered and dried at 40 °C and used for further experiments.

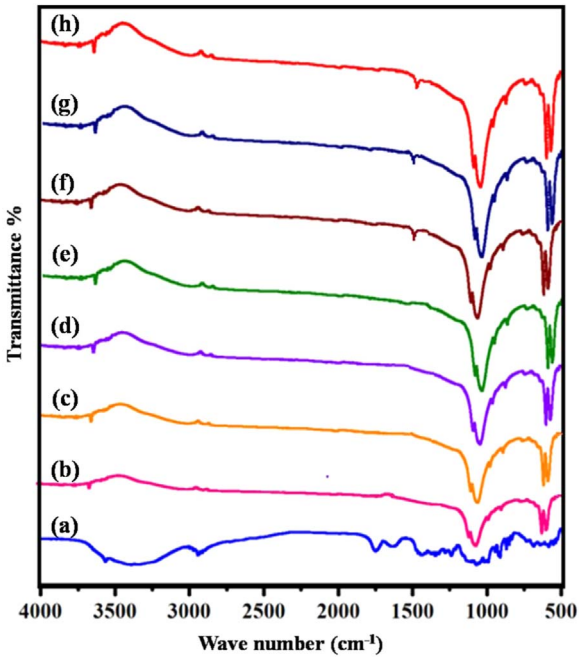

Fig. 1. FTIR spectra of pectin, HAP and HAP/pectin composite (a) extracted pectin; (b) pure HAP;(c–E) 0.01, 0.05, 0.15% (w/v) of t-HAP; (f–h) 0.01, 0.05, 0.15% (w/v) of HAP/Pectin composite.

2.4. Synthesis of HAP Nanoparticles using Citrus Peel Derived Pectin as a Green Template

Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), and ammonium hydroxide (NH_4OH) were used as Ca^{2+}, PO_4^{3-}, and -OH precursors. The aqueous solutions were made by dissolving them in deionized water. The experimental method is outlined in Fig. 1. The $CaCl_2{\cdot}2H_2O$ (0.05 M) was blended with 0.01% (w/v) extracted pectin polysaccharide, and the solution was heated at 60 °C and stirred for about 4 h to ensure that the cooperative interaction and self-assembly action was completed. Subsequently, 0.03 M ($(NH_4)_2HPO_4$) was added gradually to the above blended solution(Ca/P=1.6). The pH of the reaction solution was sustained at pH 10.0 by using aqueous ammonia and the stirring was elongate to about 24 h. A precipitate was acquired which was treated with ultrasonic irradiation for 1 h at 45 °C followed by heating in a micro oven at 150 W for about 10 min. The resultant product was washed thrice with water followed by ethanol and then dried at 40 °C. The as–synthesized composite was sintered for 12 h to remove the template pectin polysaccharide followed by sintering at 850 °C in a muffle furnace to obtain HAP nanoparticles. To study the effect of pectin concentration on the synthesis of tHAP, the above experimental procedure was repeated with various concentrations of pectin, such as 0.05% and 0.15% (w/v).

Similarly, the synthesis of HAP nanoparticles was carried out in the absence of pectin for comparison purposes.

2.5. Synthesis of HAP-Pectin composites

First, pectin (0.01%, w/v) was dissolved in 100 mL double distilled water. Next, 0.499 g synthesized HAP was dispersed in 50 mL distilled water by sonication and added dropwise to the hot solution of pectin followed by stirring for 3 h. The resulting solution was stirred for 24 h. Finally, the white-colored mixture was transferred to 12 well plates, frozen at −80 °C, and lyophilized using a freeze drier (instrument make) to form the scaffold. The above experimental procedure was repeated with various concentrations of pectin, such as 0.05% and 0.15% (w/v) are given in Table 1.