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Mineralization of pristine chitosan film through biomimetic process

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ABSTRACT

The biomineralization of pristine chitosan film without any prior surface treatment was evaluated by immersing the film in simulated body fluid (SBF) at 37 °C for 3 weeks. The film was prepared by solvent casting method using chitosan of known degree of deacetylation (DD). The formation of the hydroxyapatite (HA) phase on the film surface after immersion was studied periodically by X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), energy dispersive X-ray analysis (EDX) and scanning electron microscopy (SEM) methods. The electron micrographs showed the morphology of the deposited apatite as small globules appearing uniformly throughout the films surfaces. The Ca/P ratio of the apatite was found to increase with increase in immersion time and approaching towards the stoichiometric value of the HA phase. The mineralized chitosan film could be of promising support to hard tissue regeneration.

1. Introduction

Biomimetic mineralization of polymers is a promising method for the synthesis of advanced scaffold materials due to its ability to form hydroxyapatite (HA) layer on the surfaces of implants for hard tissue engineering applications [1]. The HA, ($Ca_{10}(PO_4)_6(OH)_2$) is a major mineral component of the calcified tissues (i.e. bones and teeth) with higher osteointegration properties (ability to bond with bone directly) [2]. Hence, there is a significant interest in understanding and expounding the mechanisms of biomineralization as the structures of the biocomposite materials formed by mineralization are highly controlled from nanometer to micrometer, resulting in complex architectures that provide multifunctional properties.

Chitosan and collagen are the widely used organic polymers in conjunction with calcium and phosphate salts to give structural support to bones [3]. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), which is usually obtained by partial deacetylation of chitin extracted from the shells of crustaceans [4]. Characteristics of chitosan such as hydrophilicity, biodegradability, antibacterial action, and affinity for proteins and lipids have made them a suitable candidate for various biomedical applications such as soft tissue engineering, drug delivery, regenerative medicine, and biopharmaceutical as listed in Table 1 [5–9]. Moreover, chitosan posses better mechanical properties than other natural polymers and hence, the most appropriate material for bone tissue engineering [10].

Extensive research has been undertaken to develop various polymer based composite biomaterials, among which chitosan–HA composites have attracted much attention as new bone substitute material due to the presence of HA which can accelerate the formation of bone like apatite on the surface of the implants [11]. The preparation of chitosan–HA composite by biomimetic method is a simple and viable method which gives a controlled hierarchical structures and tailor made properties [12]. Studies on the early stages of "*in vitro*" calcification on chitosan films in stimulated body fluids (1.5× SBF) showed that the acetylation treatment of chitosan films influenced the initial calcium distribution on the chitosan and produced more organized calcium deposits which were mostly of calcium phosphates (apatites) [13,14]. So, various treatments including glutaraldehyde cross linking, phosphorylation, calcium hydroxide and calcium chloride treatment have been promoted to enhance the *in vitro* biomineralization of chitosan [15–18]. A calcium silicate treatment has also shown to result in the formation of apatite layer on the surface of the chitosan microparticles [19]. These treatments mainly create a number of calcium phosphate precursor sites over the chitosan surface and subsequent immersion of the chitosan in SBF eventually results in a favourable condition for the nucleation and growth of HA. The present study is focussed on the morphological and phase analysis of the SBF mediated mineralization of the chitosan film of known degree of deacetylation (DD) without any surface pre-treatments.

2. Materials and methods

2.1. Materials

Chitin with 6–8% nitrogen content was procured from S.D. Fine chemicals Mumbai, India. Chemicals such as sodium

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Table 1
Chitosan: properties and biomedical applications.

Properties	Applications	
Biocompatible	Tissue engineering	Cartilage
Biodegradable		Bone
Hydrophilic		Spine
Anti-bacterial		Skin
Anti-fungal		Blood vessel
Anti-viral	Molecular delivery	Drug
Affinity for proteins		Protein
Affinity for lipids		Growth factors
Anti-coagulant	Molecular immobilization	Fat
Wound healing		Enzyme
Bone healing	Biopharmaceutical	Wound dressing
Hemostatic		Cosmetics
Dental plague inhibition		Food and nutrition
Tumor inhibition		Surgical sutures
Spermicidal	Therapy	Cancer
Immunoadjuvant		Gene
Anti-cholesteremic		Analgesia
Anti-static and soil repellent		Ophthalmology

hydroxide (NaOH), acetic acid (CH_3COOH), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4{\cdot}3H_2O$), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$), trishydroxymethyl aminomethane ($(HOCH_2)_3CNH_2$) and hydrochloric acid (HCl) of analytical grade were procured from Merck, India.

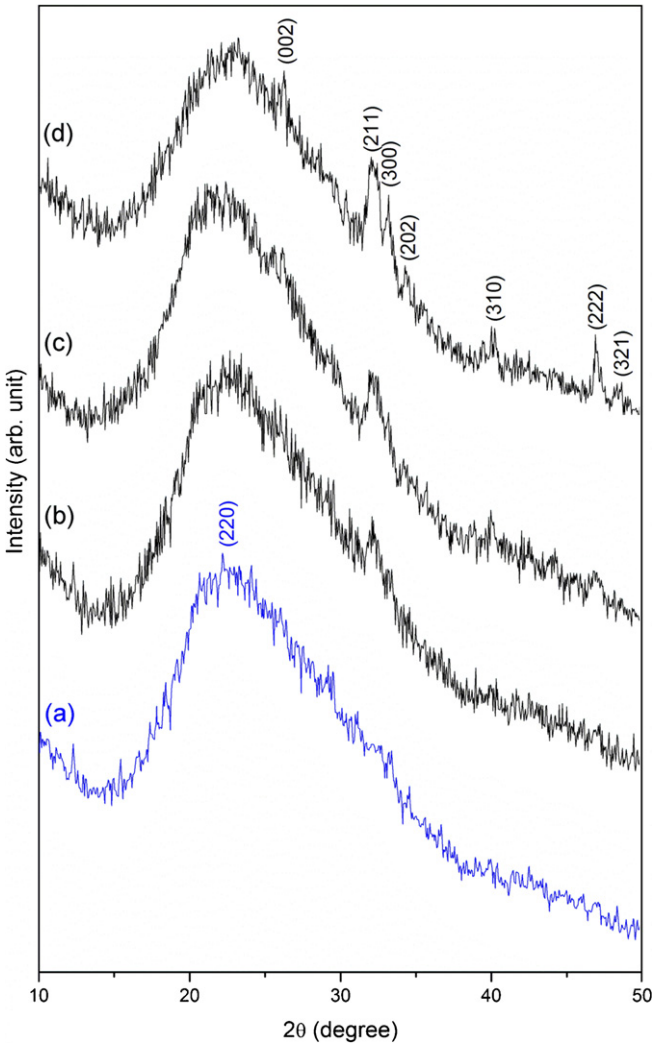

Fig. 1. XRD patterns of chitosan films (a) before immersion, (b) after 1 week, (c) after 2 weeks and (d) 3 weeks of immersion in the 1.5× SBF.

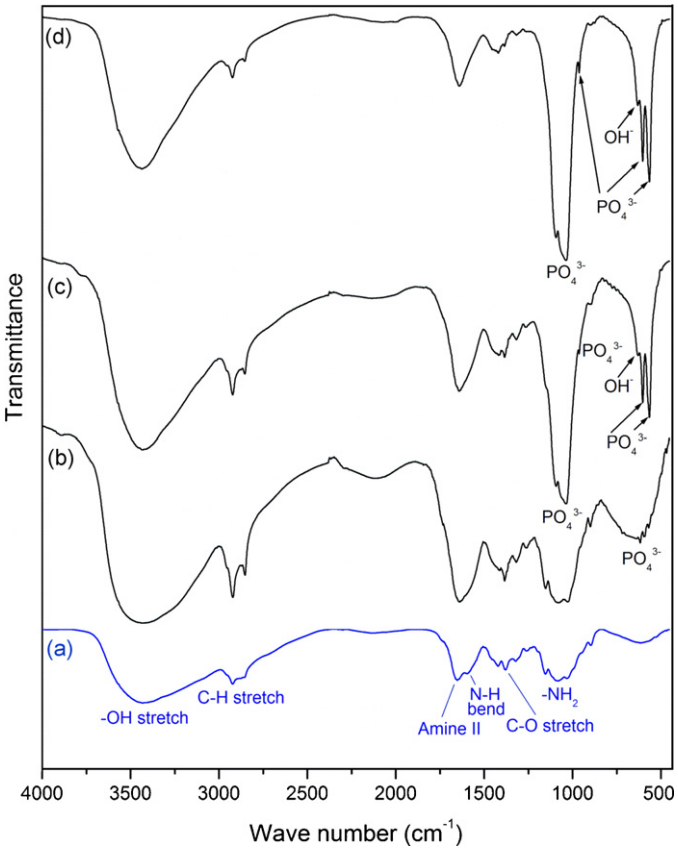

Fig. 2. FT-IR spectra of chitosan films (a) before immersion, (b) after 1 week, (c) after 2 weeks and (d) 3 weeks of immersion in the 1.5× SBF.

2.2. Preparation of chitosan film

Chitosan was prepared from deacetylation of chitin according to the method reported by Lima and Airoldi [20]. The chitosan film was prepared by solvent casting method by taking 1 g of deacetylated chitosan powder dissolved in 100 ml of 0.3 M acetic acid and stirred using a magnetic stirrer until the powder get fully dissolved into the solvent. The casting was made by pouring the chitosan solution into Teflon coated glass mould and subsequently kept in an oven at 50 °C in order to dry. The film was removed from the mould and neutralized with 1 wt.% NaOH solution for 30 min and washed thoroughly with distilled water and dried subsequently. The DD of the chitosan film was calculated to be 85% by the method followed by Sabnis and Block [21].

2.3. Preparation of SBF solution

SBF containing nearly 1.5× times of the inorganic ion concentration of human blood plasma was prepared according to the method proposed by T. Kokubo and Takadama [22]. Briefly, NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, Na_2SO_4, $(HOCH_2)_3CNH_2$ were dissolved in deionized water. The solution pH value was adjusted with 1.0 M HCl and the final pH of the solution was made to 7.3.