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Preparation of polyelectrolyte/calcium phosphate hybrids for drug delivery application

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ABSTRACT

Biocompatible and biodegradable polyelectrolyte complex consisting of carboxylmethyl cellulose (CMC) and chitosan (CHI) were studied as a template for calcium phosphate biomimetic mineralization. CMC/CHI/calcium phosphate hybrids were prepared using different concentrations of simulated body fluid (2, 5 and 10 × SBF) for producing hybrids with different organic/inorganic ratio. These hybrids were characterized using X-ray diffraction (XRD), infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The equilibrium swelling extents of the hybrids were found to be dependent on the inorganic % in the hybrids. The release profile of bovine serum albumin as a model drug in simulated intestine solution (pH 7.4) during 24 h has established the efficiency of the hybrids as a sustained delivery system. The hybrids developed in this contribution exhibit a great potential in bone tissue engineering and drug delivery applications.

1. Introduction

Recently, the combination of ceramic particles with polymeric matrices has been extensively investigated as an alternative in bone tissue engineering (Danilchenko et al., 2011; Schweizer & Taubert, 2007). This process attempt to mimic the formation of mineralized tissues which composed of hierarchically neat apatite with collagen. It is reported that, the organized structures of bone have been shown to form by nonclassical mineralization processes controlled by polyelectrolyte rich in acidic moieties (Ye, Wang, Zeiger, Miles, & Lin-Gibson, 2013). Moreover, it is highly desirable that hybrid materials can be used for bone implantation and drug delivery synergistically (Zhang & Zhang, 2002). Using simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma requires more than 7 days for producing a uniform apatite coatings. However, using SBF with high ionic concentrations have been cited as a method to control the surface topography in biomimetic calcium phosphate mineralization and to reduce the time required for biomimetic mineralization process (Costa et al., 2012). Practically, several studies have been made to understand the interactions between charged polymers and calcium phosphate to better control the biomimetic mineralization process. Moreover, a number of acidic molecules such as polyaspartic acid (Deshpande & Beniash, 2008) and polyacrylic acid (Liu et al., 2011) have been shown to promote intrafibrillar mineralization. These acidic polymers exhibit differences in their ability to control the mineralization process. Recently, the kinetics of calcium phosphate aggregation in the presence of carboxylate-bearing polymers has been studied. The results revealed that the polymer structure can alter calcium phosphate aggregation mechanisms, whereas polymer concentration influences the rate of calcium phosphate aggregation (Ye et al., 2013). However, using natural polymers in biomimetic mineralization, especially anionic polysaccharide, are a new approach for those purposes. Attempts have been carried out to enhance calcium phosphate mineralization onto ionic polysaccharides for preparation of hybrid materials resemble natural bone although it have different chemical structure(Barbosa, Granja, Barrias, & Amaral, 2005; Liuyun, Yubao, & Chengdong, 2009). In addition, using natural polymers as scaffolds for tissue engineering prevents the risks which may occur due to morbidity between human cells and scaffolds. The biodegradability of these polymers prevents a second surgical procedure for removing the scaffold after bone healing. However, there are only a few reports on the synthesis of carbohydrate/calcium phosphate hybrid materials. For example, Coleman and coworkers studied the rate of hydroxyapatite growth and mineralization using anionic polysaccharides like alginate and phsphorylated alginate. They found that alginate had no large effect on development of calcium phosphate crystals comparing to phosphorylated alginate which exhibited strong nonspecific binding to the crystals (Coleman, Jack, Perrier, & Grøndahl,

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2013). Sotome et al. (2004) prepared hydroxyapatite/collagene-alginate as bone filler and as a carrier for drugs. This hybrid was implanted into the femur and the results showed high bone formation, stability against enzyme digestion comparing with the same hybrid without alginate. Wang et al. prepared calcium phosphate/carboxymethyl chitosan hybrid materials nanoparticles by precipitation method for doxorubicin hydrochloride delivery. The hybrid materials loaded with drug were decorated by peptide KALA via self-assembly method. The in vitro study of these decorated nanoparticles shows that the cell inhibition significantly enhanced by the presence of peptide (Wang et al., 2013).

Carboxymethyl cellulose (CMC), the major commercial derivative of cellulose, is anionic polysaccharide widely used in pharmaceuticals (Devi & Maji, 2009) and in biomedical fields (Ninan et al., 2013; Rodríguez, Renneckar, & Gatenholm, 2011). A three dimensional carboxymethyl cellulose/hydroxyapatite nanocomposites were investigated for their application as a load bearing synthetic bone graft. The study showed that ionic/polar or electrostatic interactions are the main driving force for formation of load bearing three dimensional nanocomposites via a process similar to matrix mediated biomineralization (Garai & Sinha, 2013).

Chitosan (CHI) is the nature unique cationic polysaccharide composed of β-(1,4)-linked glucosamine that is produced via the alkaline deacetylation of chitin, the second- most abundant natural polymer after cellulose (Chicatun et al., 2011). CHI has numerous and plentiful amino and hydroxyl groups in the macromolecular chains which provide advantageous for conducting medications reactions and for providing distinctive biological functions (Danilchenko et al., 2011). Accordingly, CHI has been studied for a wide range of biomedical applications such as drug delivery (Hu, Yang, & Hu, 2011; Wang et al., 2010) and tissue engineering as a scaffolding material (Rinaudo, 2008) because its degradation accompanied without inflammatory reactions or toxic products (Chen et al., 2009). Moreover, CHI has the ability to promote growth and mineral rich matrix in cell culture and osteoconductivity (Tanase, Popa, & Verestiuc, 2012).

Polyelectrolytes are mixtures of positively and negatively charged polymers blended at the molecular level (Sun, An, Zhao, Shangguan, & Zheng, 2012). Polyelectrolytes were reported as stimulated materials for inorganic biomimetic mineralization like $CaCO_3$, $BaSO_4$ and hydroxyapatite (Sun et al., 2012). Polyelectrolytes have been developed to be used as guide bone tissue regeneration. CMC can interact strongly with CHI due to the structural similarity. Consequently, CMC/CHI polyelectrolyte was widely investigated in the field of biomaterials (Chen & Fan, 2007; Jiang et al., 2008; Liuyun et al., 2009).

The current article focuses on the development of biodegradable porous scaffolds made from naturally derived polymers. CMC and CHI were chosen because their composition exhibits virtually no endotoxicity, cytotoxicity, and no antigenic properties upon implantation. The biomimetic mineralization process of polyelectrolyte could be enhanced using high concentrations of simulated body fluids (SBF). The study also addresses the morphology, crystal phase of mineralized calcium phosphate on the swellable CMC/CHI polyelectrolyte's. Moreover, the release profile of bovine serum albumin, a model for macromolecules and drugs, as a function in inorganic ratio was investigated.

2. Materials and methods

2.1. Materials

Carboxymethyl cellulose sodium salt (>99.5%) with high viscosity (4% in water at 25 °C are 1000–1500 mPa S) was purchased from Fluka Biochemika. Chitosan, microcrystalline molecular weight: 100 000 to 300 000 and deacetylating grade 70 to 85% was supplied from Acros. Comassie brilliant blue (G-250) and bovine serum albumin (BSA) were supplied by Sigma-Aldrich, St. Louis, USA. All other reagents were of analytical grade and used as received without further purification.

Table 1
Compositions of 2×, 5× and 10 × SBF.

		2×	5×	10×
Phosphate part* [gm]	Tris	1.2110	3.0275	6.055
	$Na_2SO_4.10H_2O$	0.0644	0.161	0.322
	$NaHCO_3$	0.1411	0.3528	0.7055
	K_2HPO_4	0.0696	0.174	0.348
Calcium part [gm]	Tris	1.2110	3.0275	6.055
	NaCl	3.1980	7.995	15.99
	KCl	0.0880	0.22	0.44
	$MgCl_2.6H_2O$	0.1220	0.305	0.61
	$CaCl_2.2H_2O$	0.1470	0.3675	0.735

* Every part dissolves in 100 mL double distilled water.

2.2. CMC/CHI polyelectrolyte complex preparation and mineralizstion

CMC (100 mg) was dissolved in 10 mL deionized water and CHI (100 mg) was dissolved in 10 mL 2% acetic acid solution. The two solutions were mixed 1:1 by adding the CMC drop wise into the stirring CHI solution. The formed polyelectrolyte was washed by deionized water to remove acetic acid residue and freeze dried.

Simulated body fluids with different concentrations were prepared as shown in Table 1, to accelerate the calcium phosphate formation (Ohtsuki, Kokubo, & Yamamuro, 1992). The biomimetic mineralization process by immersing 5 gm of swellable CMC/CHI polyelectrolytes in 50 mL (25 ml from each of calcium and phosphate part) 2×, 5× and 10 × SBF in Falcon tubes for 72 h. The samples were attached by polystyrene thread to prevent the formation of calcium phosphate by precipitations. SBF was renewed after centrifugation every 24 h and the pH was checked on samples regularly. pH values were maintained at 7.4 over the entire course of the mineralization to minimize problems associated with SBF preparation and stabilization (Bohner & Lemaitre, 2009). Finally, the samples were washed with double distilled water for 5 days and dried at room temperature for further analysis.

2.3. Characterization methodology

2.3.1. ATR-FTIR

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was done on a Thermo Nicolet FT-IR Nexus 470 with a diamond crystal. Spectra were recorded from 500 to 4000 cm^{-1} with a resolution of 2 cm^{-1}.

2.3.2. XRD

X-ray diffraction (XRD) patterns were recorded using a Philips apparatus PW 105 diffractometer (Philips Analytical, Cambridge, UK) using Ni-filtered Cu radiation (CuK_α, 0.154 nm) at an operating voltage of 40 kV.

2.3.3. SEM and EDX

Scanning electron microscopy was done on a JEOL JXA-840A Electron probe microanalyzer with tungsten filament (30 kV). For EDX experiments an Oxford INCAx-sight SN detector with a resolution of 128 eV at 5.9 keV was used.