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Carboxymethyl cellulose/silica hybrids as templates for calcium phosphate biomimetic mineralization

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ABSTRACT

Multiphase hybrid materials were synthesized using carboxymethyl cellulose (CMC) as bioactive polymer, silica gel as matrix assisted networks and calcium phosphate as inorganic mineral phase. These hybrids were investigated with infrared spectroscopy, X-ray diffraction, scanning electron microscopy/energy dispersive X-ray spectroscopy and transmission electron microscopy. Biomimetic crystal growth nucleated from the CMC/silica hybrids was suggested as amorphous calcium phosphate with an evidence that hydroxylapatite, the mineralized component of bone, may be formed at high CMC content. This study provides an efficient approach toward bone-like hybrids with potential bone healing applications.

1. Introduction

More than 2.2 million bone grafting process is carried out every year for regenerative bone surgery [1]. However, due to the limitations associated with these bone surgery, searching for new alternative biocompatible regenerative scaffolds for bone defects becomes a clinical challenge. Bone tissue engineering is emerging as a novel solution that can be used to promote near-natural materials for using in implantology and traumatology. Last decade, significant efforts have been devoted to develop bone graft substitutes from biomaterials that can augment or regenerate injured bone [2–6]. The ideal biomaterials are required to be bioactive, biodegradable, have sufficient mechanical integrity for implantation in load bearing defects, and have a highly porous and interconnected architecture for bone and vascular ingrowth.

Low-bioactive materials need firstly surface modifications by incorporation of functional groups to be mineralized by the biomimetic method. Some reports revealed that the introduction of compounds have bioactive functional groups such as proteins [7,8], charged polysaccharides [9,10] and amino acids [11] as initiations for calcium ions nucleation can promote hydroxyapatite formation when immersed in a biological fluid containing ion concentrations nearly equal to those of human blood plasma [12]. A recent study investigated the effects of anionic polysaccharides like alginate and phosphorylated alginate on the rate of hydroxyapatite growth and mineralization. The results of the study showed that alginate had no large effect on development of calcium phosphate crystals comparing to phosphorylated alginate which exhibited strong nonspecific binding to the crystals [3]. Carboxymethyl cellulose (CMC) has gained increasing interest in the recent years as a candidate material for bone tissue engineering due to its biocompatibility, biodegradability and anionic properties [13–17]. In most cases, CMC was mechanically mixed with pre-prepared hydroxyapatite for hybrid material formation. The additions of nanohydroxyapatite to carboxymethyl cellulose/chitosan film had improved the mechanical properties, swelling behavior and bioactivity of CMC/chitosan films [14]. However, few attempts have been made for studying bioactivity and biomematic mineralization of ionic polysaccharides like chitosan [18], and carboxymethyl cellulose [5] in simulated body fluids.

Silica has numerous favorable properties such as biocompatibility, adjustable surface area, and easy modification with a large number of functional molecules. In addition, the effect of silicon on bone formation has been studied early and the results revealed that silicon deficiency in animal trials results in abnormal bone formation with reduced precipitation of bone apatite [19]. Also, there is evidence that the presence of polysilanol groups of the amorphous silica may promote the role of collagen as a template for intrafibrillar apatite precipitation. The fragility and difficult processing in the form of 3D scaffold architecture of sol–gel derived bioactive

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Table 1
Sample codes for the prepared CMC/silica hybrids.

Samples	CMC%
Pure silica*	0
CMC/silica^{A}	5
CMC/silica^{B}	10
CMC/silica^{C}	15
CMC/silica^{D}	20

* Prepared with the same condition at 8 ml TEOS.

Table 2
Composition of double concentration simulated body fluid.

	Phosphate part* [gm]	Calcium part [gm]
Tris	1.2110	1.2110
$Na_2SO_4{\cdot}10H_2O$	0.0644	–
$NaHCO_3$	0.1411	–
K_2HPO_4	0.0696	–
NaCl	–	3.1980
KCl	–	0.0880
$MgCl_2{\cdot}6H_2O$	–	0.1220
$CaCl_2{\cdot}2H_2O$	–	0.1470

* Every part dissolves in 100 ml double distilled water.

glasses restricted their application for bone regeneration. Several attempts have been carried out to combine organic polymers like polysaccharides with silica [20–26] to improve the mechanical properties [22,24], washing durability [27] and thermal stability [28] of the produced hybrid. Porous bioactive glass microspheres contained chitosan as biomolecular template initiate the precipitation of apatite crystals on their surface when immersed in SBF for 24 h at 37 °C [22]. The hydrogen bonding between the polysaccharides and the inorganic mineral has a positive effect in avoiding phase separation and producing transparent free standing hybrids [29].

The threshold task of this article was to study the development of a novel CMC-silica-calcium phosphate multiphase biocomposite. CMC could be used as assistant reagent for silica gel formation at low silica precursor concentration, and as a material for improving mechanical properties and a template for biomimatic mineralization of bone like inorganic. Moreover, silica gel was used as a network matrix for preparing of bioactive water insoluble CMC/silica hybrids. Additionally, the study aims to understand the growth of bioactive calcium phosphate crystals as a function of CMC concentration.

2. Materials and methods

2.1. Materials

Carboxymethyl cellulose sodium salt (>99.5%) with high viscosity was purchased from Fluka Biochemika. The viscosity of 4% CMC in water at 25 °C is 1000–1500 mPa s. Tetraethyl orthosilicate (TEOS), (99.9%) was purchased from Sigma Aldrich. The other chemicals, such as calcium chloride dihydrate, dibasic potassium phosphate, Magnesium chloride hexahydrate and other simulated body fluid chemicals were analytical grade and used as received without further purification.

2.2. CMC/silica hybrids preparation

Organic-inorganic hybrids with different weight ratios of CMC:TEOS (*w:w*) were synthesized via a sol–gel process. As shown in Table 1, a given amount of CMC was dissolved in doubly distilled water at room temperature under continuous stirring for 24 h. Then, 4 ml TEOS and 4 ml acetic acid solution (acetic acid:water 2:1 *v*/*v*) were added to form a mixture of pH 4 with a total volume 32 ml. The former was used as a silica precursor and the latter as a catalyst. Subsequently, the mixture was stirred at 60 °C for 2 h to ensure a complete hydrolysis of TEOS molecules. The resulting homogenous solution was cast in 15 ml Falcon tube in a water bath at 40 °C for 48 h to form a homogenous gel. The unreacted residue was extracted from the hybrid materials via washing for three days with distilled water and subsequently dried at 40 °C for 24 h in a vacuum oven.

2.3. Biomimetic mineralization of calcium phosphate on the hybrids

All solutions were prepared just before use. The in vitro bioactivity of the CMC/silica hybrid disk samples (10 mm × 10 mm) was carried out by immersing in double concentration simulated body fluid (2 × SBF). This solution was prepared in two parts as shown in Table 2, to accelerate the hydroxyapatite formation [30].

CMC/silica hybrids in swellable form were incubated in 50 ml 2 × SBF (25 ml from each part) in Falcon tubes for 5 days. The solution was renewed after centrifugation every 24 h and the pH was checked on samples regularly. pH values were maintained at constant physiological pH (7.4) over the entire course of the mineralization to minimize problems associated with SBF preparation and stabilization [31]. Finally, the samples were washed with doubly distilled water and dried at room temperature for further analysis.

2.4. Cyclic water absorbance of CMC/silica hybrid

Water absorbance for the hybrid samples was done with double distilled water. Discs of the hybrid materials with a defined weight were immersed in 20 ml of water. The weights of the hydrated samples were recorded over various time intervals after removal of the surface liquid using Whitman filter paper until reach equilibrium. The percent of water uptake was calculated by the following equation:

$$\text{Water absorption\%} = \left[\frac{W_t - W_0}{W_0}\right] \times 100$$

where W_0 is the initial weight and W_t the final weight of the hybrid samples at time *t*.

The study was continued for 4 times to observe the stability of the hybrids toward reabsorption and drying.

2.5. Characterization methodology

2.5.1. 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}.

2.5.2. XRD

X-ray diffraction (XRD) patterns were recorded with an Empyrean Powder Diffractometer (Cu K_α, 0.154 nm) between 3 and 70° 2θ with a step size of 0.01° s^{-1}. Samples were mounted on a silicon support.

2.5.3. Compressive strength test

Hybrids (diameter: 20 mm; height/diameter ratio: 4/1) were tested using a LLOYD universal testing machine with a 500 N load cell by simultaneously determining force and corresponding length