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Bioactive and metal uptake studies of carboxymethyl chitosan-*graft*-D-glucuronic acid membranes for tissue engineering and environmental applications

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ABSTRACT

Carboxymethyl chitosan-*graft*-D-glucuronic acid (CMCS-*g*-D-GA) was prepared by grafting D-GA onto CMCS in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and then the membranes were made from it. In this work, the bioactivity studies of CMCS-*g*-D-GA membranes were carried out and then characterized by SEM, CLSM, XRD and FT-IR. The CMCS-*g*-D-GA membranes were found to be bioactive. The adsorption of Ni^{2+}, Zn^{2+} and Cu^{2+} ions onto CMCS-*g*-D-GA membranes has also been investigated. The maximum adsorption capacity of CMCS-*g*-D-GA for Ni^{2+}, Zn^{2+} and Cu^{2+} was found to be 57, 56.4 and 70.2 mg/g, respectively. Hence, these membranes were useful for tissue engineering, environmental and water purification applications.

1. Introduction

The development of modern mineralized biopolymer-based membranes for tissue engineering applications is one of the most challenging tasks in the materials science field. Chitosan is a biopolymer that offers a great potential in this field [1]. Chitosan is a linear polyaminosaccharide composed of (1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine, commonly derived at a low cost from chitin, a waste material of the seafood industry. It is a semi-crystalline polymer whose degree of crystallinity depends on the degree of *N*-deacetylation that usually varies between 50 and 100%, with its minimum at an intermediate degree of deacetylation. Due to the presence of amino groups in its molecules, chitosan is a cationic polyelectrolyte soluble in aqueous acidic media. It also chelates transitional metal ions. When dissolved, chitosan forms viscous solutions that can be gelled to produce a great variety of gel configurations by evaporating the solvent, increasing the pH, crosslinking, ionotropic gelation or freeze-drying [2]. As a biofunctional material chitosan offers a unique set of characteristics, including biocompatibility, biodegradability to harmless products and non-toxicity, as well as bioactivity that comprises antibacterial, hemostatic, fungistatic, antitumoral, anticholesteremic, and importantly, osteoconductive properties [3]. It evokes a minimal foreign body reaction and fibrous encapsulation upon implantation [4], and its biodegradation proceeds by lysozyme-catalyzed hydrolysis of acetylated residues. These highly favorable biological and chemical properties make chitosan, hitherto underutilized, an attractive starting material for many diverse biomedical and bio-related applications [5–7].

Chitosan and its derivatives have great potential application in the areas of biotechnology, biomedicine, food ingredients, and cosmetics. Different forms of chitosan-based biomaterials have recently been developed for biomedical purposes, including scaffolds [8–11], composites [12–14], tubes [15], sponges [16], coatings [17], microspheres [18], wound dressings [19], salts [20] and membranes [21,22], of which, notably, those combined with hydroxyapatite are especially useful for potential applications in bone repair and regeneration.

Chitosan and its derivatives are capable of adsorbing a number of metal ions as its amino groups can serve as chelation sites. Due to their high nitrogen content and porosity, chitosan-based sorbents have exhibited relatively high sorption capacities and kinetics foremost heavy metals [23–27]. However, lack of specificity towards several highly toxic heavy metals limits the use of chitosan as an effective sorbent.

Carboxymethyl chitosan (CMCS), a natural amphoteric polyelectrolyte derived from chitosan has attracted considerable interest in a wide range of biomedical applications, such as wound dressings,

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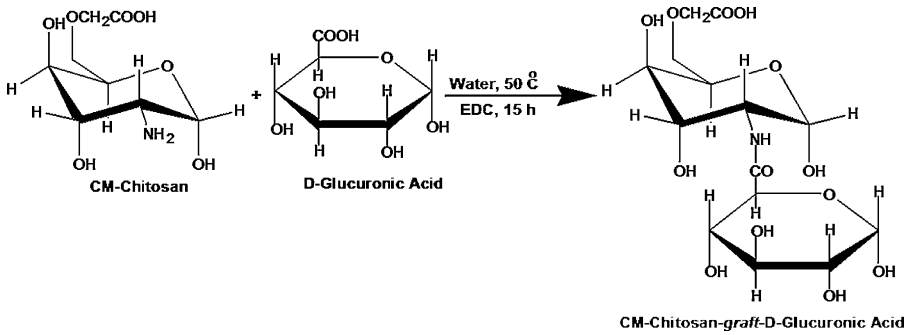

Fig. 1. Synthesis of CMCS-*graft*-D-GA.

artificial bone and skin, bacteriostatic agents, and blood anticoagulants, due to its unique chemical, physical, and biological properties, especially its excellent biocompatibility [28–30]. It has also demonstrated that good tolerable pH and ion sensitivity of chitosan in aqueous solutions are due to abundant –COOH and $–NH_2$ groups in carboxymethyl chitosan [31]. Glucuronic acid is a carboxylic acid. Its structure is similar to that of glucose. Glucuronic acid is highly soluble in water. In the animal body, glucuronic acid is often linked to the xenbiotic metabolism of substances such as drugs, pollutants, bilirubin, androgens, estrogens, mineralocorticoids, glucocorticoids, fatty acid derivatives, retinoids, and bile acids. These linkages involve O-glycosidic bonds, and this linkage process is known as glucuronidation [32]. Glucuronidation occurs mainly in the liver, although the enzyme responsible for its catalysis, UDP-glucuronyltransferase, has been found in all major body organs, e.g., heart, kidneys, adrenal gland, spleen and thymus [33]. Graft copolymerization onto chitosan will be a key point, which will introduce desired properties and enlarge the field of the potential applications of chitosan by choosing various types of side chains [6]. Grafting of D-glucuronic acid into carboxymethyl chitosan derivative may, improve the biological properties and it will also be useful for drug delivery applications. Before using this material for drug delivery and other biological applications, the bioactivity and metal uptake property studies are required. So, in this paper we are reporting the bioactivity and the metal uptake (Ni^{2+}, $Zn^{2+}$and Cu^{2+}) behavior of CMCS-*g*-D-GA in detail for tissue engineering and environmental applications.

2. Experimental

2.1. Materials

CMCS was received from Koyo Chemicals Ltd. D-Glucuronic acid and EDC were received from Wako Pure Chemicals.

2.2. Synthesis of CMCS-*g*-D-GA

CMCS-*g*-D-GA was prepared by the following method [34]. In brief, CMCS (1 mole) was grafted with D-GA (0.25 mole) by using EDC catalyst (0.25 mole) in water at 50 °C for 15 h at pH 6.0. After 15 h the reaction mixture was precipitated in excess of ethanol and washed several times with methanol. The synthesis method of CMCS-*g*-D-GA is shown in Fig. 1.

2.3. Preparation of CMCS-*g*-D-GA membranes

CMCS-*g*-D-GA membranes were prepared by dissolving 1 g of CMCS-*g*-D-GA 1% acetic acid solution. The samples were dried in Petri dish at room temperature for 3 days and were neutralized with NaOH solution.

2.4. Preparation of SBF solution

The SBF solution was prepared by following method [35], and contained 15 ml of each of the following: 2.74 mol l^{-1} NaCl, 0.06 mol l^{-1} KCl, 0.05 mol l^{-1} $CaCl_2$, 0.03 mol l^{-1} $MgCl_2$, 0.0895 mol l^{-1} $NaHCO_3$, 0.02 mol l^{-1} K_2HPO_4 and 0.01 mol l^{-1} Na_2SO_4. These were added to each 200 ml volumetric flask along with 25 ml of 0.4 mol l^{-1} Tris(hydroxymethyl)methylamine amine and 0.36 mol l^{-1} of HCl. The pH of the solution was adjusted to 7.4 by adding a few drops of HCl with the remainder of the volume being distilled water.

2.5. Bioactivity studies of CMCS-*g*-D-GA membranes

The bioactivity studies of CMCS-*g*-D-GA membranes were carried out by biomimetic method with 1.5× SBF solutions. All the membranes were previously subjected to $CaCl_2$ treatment for 3 days at 37 °C in order to enhance the bioactivity properties. The $CaCl_2$ treated CMCS-*g*-D-GA membranes were vertically suspended in plastic jars with cotton threads and 30 ml of 1.5× SBF solutions was added. The SBF solution was replaced each day. Samples were retrieved after 7, 14 and 21 days of soaking at 37 °C. The retrieved samples were thoroughly rinsed with distilled water and dried at 65 °C before performing various characterization studies.

2.6. Metal uptake studies of CMCS-*g*-D-GA membranes

2.6.1. Effect of pH test

25 ml of aqueous solution of metal ions with different initial pH values were added to 50 mg CMCS-*g*-D-GA membranes. After stirred at 200 rpm for 8 h at room temperature, the mixtures were filtered. The metal ions concentration in the filtrate and initial concentration were determined by atomic adsorption spectrophotometer and the adsorption capacities were calculated as follows:

$$q = \frac{(C_o - C)V}{m}$$

where q is adsorption capacities of chitosan derivatives (mg metal ion/g adsorbent), V is volume of metal ion solution (l), C_o is concentration of metal ion before adsorption (mg l^{-1}), C is concentration of metal ion after adsorption (mg l^{-1}), and m is the weight of chitosan derivatives (g).

2.6.2. Effect of initial concentration of metal ions

25 ml of aqueous solution with metal ions with different initial concentrations adjusted to the desired pH with hydrochloric acid (or nitric acid) or sodium hydroxide aqueous solution were added to 50 mg CMCS-*g*-D-GA samples. After stirred for 8 h at room temperature, the mixtures were filtered. The metal ions concentration in the filtrate and initial concentration were determined by atomic adsorption spectrophotometer.