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In vitro biocompatibility evaluations of hexanoyl chitosan film

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Abstract

The present contribution reports for the first time some *in vitro* biocompatibility evaluations of hexanoyl chitosan (H-chitosan) for possible utilization in biomedical applications. The evaluations comprised the cytotoxicity testing and the attachment, proliferation, and spreading of L929, mouse connective tissue, fibroblast-like cells that were cultured on the surface of H-chitosan film in comparison with those on chitosan film. These films were fabricated by solution-casting technique. Some thermal, physico-chemical, and morphological characteristics of H-chitosan film were also investigated. H-chitosan film exhibited two steps in the loss of its mass at 242 and 299 °C, respectively, while chitosan film exhibited only one at 297 °C. The water contact angle on the surface of H-chitosan film was 76°, while that on the surface of chitosan counterpart was 71°, a result indicating the more hydrophobicity of H-chitosan film in comparison with the chitosan counterpart. Indirect cytotoxicity evaluation of H-chitosan film using L929 revealed non-toxicity of the film to the cells. Lastly, both the attachment and the proliferation of L929 cells on H-chitosan film were inferior to those on tissue-culture polystyrene plate (TCPS). The attachment of the cells on H-chitosan film was better than that on the chitosan counterpart at a short seeding time (i.e., <5 h), while the proliferation of the cells on H-chitosan film was better than that on the chitosan counterpart after 2 and 3 days in culture.

Keywords: Chitosan; Hexanoyl chitosan; Biocompatibility; Fibroblast

1. Introduction

Chitin or poly(*N*-acetyl-D-glucosamine) is one of the most abundant polysaccharides commonly found in shells of various insects and crustaceans as well as cell walls of various fungi. Chitosan is a partially *N*-deacetylated derivative of chitin. In the food industry, chitosan in its blends with gelatin was developed as edible films (Arvanitoyannis, Nakayama, & Aiba, 1998), while the blends between chitosan and poly(vinyl alcohol) (PVA) was suggested to be suitable materials for food packaging (Arvanitoyannis, 1999). In biology, chitosan is structurally similar to glycosaminoglycans (GAGs), such as condroitin sulfate and hyaluronic acid, in the extracellular matrix (ECM) of connective tissues. As a result, chitosan has been heavily explored as a suitable functional material in biomedical applications (Ma, Wang, He, & Chen, 2001; Muzzarelli et al., 2001; Qi, Xu, Jiang, Hu, & Zou, 2004), due mainly to its biocompatibility, biodegradability, and non-toxicity. Despite the vast applicabilities of this polymer, utilization of chitosan is somewhat limited by its poor solubility in common solvents and its physical properties that are rigid and brittle, a direct result of the strong intra- and inter-molecular hydrogen bonding.

Chitosan can be functionalized rather easily through its hydroxyl and/or amine groups (Jayakumar, Prabaharan, Reis, & Mano, 2005). Some chemical modifications such as acylation (Hirano, Ohe, & Ono, 1976; Zong, Kimura,

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Takahashi, & Yamane, 2000), alkylation (Yalpani & Hall, 1984), and phthaloylation (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002; Nishimura, Kohgo, Kurita, & Kuzuhara, 1991) reactions can be carried out. Most of the organically soluble derivatives of chitosan are used to formulate materials for biomedical applications, such as drug delivery (Tien, Lacroix, Szabo, & Mateescu, 2003) and wound dressing (Pielka et al., 2003). Recently, chitosan derivatives bearing cyclodextrin cavities were developed as novel adsorbent materials (Prabaharan & Mano, 2006). Among such derivatives, acylated chitosans are soluble in various common organic solvents, such as chloroform, benzene, pyridine, and tetrahydrofuran (THF) (Zong et al., 2000). *N*-acylated chitosan has been fabricated as membranes (Seo, Ohtake, Unishi, & Iijima, 1995), films (Xu, McCarthy, Gross, & Kaplan, 1996), and fibers (Hirano, Usutani, Yoshikawa, & Midorikawa, 1998). Among the various acylated chitosans, *N*-hexanoyl chitosan (H-chitosan) was found to exhibit the best blood compatibility (Lee, Ha, & Park, 1995). In addition, H-chitosan was found to be anti-thrombogenic and resistant to hydrolysis by lysozyme (Hirano & Noishiki, 1985). Even though H-chitosan is a very interesting derivative of chitosan to be used in biomedical applications, its biological properties with living cells have not yet been available in the open literature.

In the present contribution, H-chitosan film was prepared with solution-casting technique. The main purpose was to investigate the biological properties of H-chitosan by examining for its cytotoxicity and the attachment, proliferation and spreading of L929, mouse connective tissue, fibroblast-like cells that were cultured on H-chitosan film. Biological properties of solution-cast chitosan film were also determined for comparison purposes. In addition, some thermal, physico-chemical, and morphological characteristics of H-chitosan film were also investigated.

2. Experimental

2.1. Materials

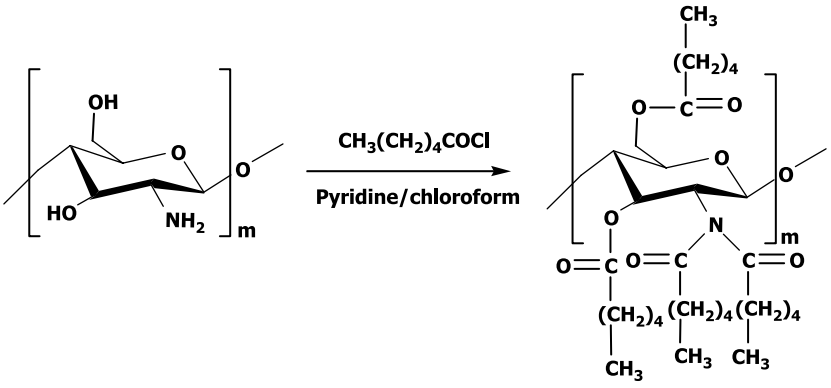
Hexanoyl chitosan (H-chitosan) was synthesized in our laboratory via a heterogeneous acylation reaction of chitosan, prepared from shells of *Penaeus merguiensis* shrimps [Surapon Foods Public Co. Ltd. (Thailand)], with hexanoyl chloride in a mixture of anhydrous pyridine and chloroform based on the method described by Zong et al. (2000) (see Scheme 1). The degree of deacetylation (DD) of the feed chitosan was determined based on an infrared spectroscopic method (Sabnis & Block, 1997) to be about 88%, while the viscosity-average molecular weight $\overline{M}_v$ was evaluated from the intrinsic viscosity $[\eta]$ based on the Mark–Houwink equation [i.e., $[\eta] = K\overline{M}_v^a$, where K and a assume the values 6.59×10^{-3} ml g^{-1} and 0.88 (Wang, Bo, Li, & Qin, 1991), respectively] to be about 5.76×10^5 g mol^{-1}. The intrinsic viscosity was measured in a mixture of 0.2 M acetic acid and 0.1 M sodium acetate at 30 °C. The as-prepared H-chitosan had a degree of substitution (DS) of the hexanoyl groups on chitosan molecules of about 3.0 (i.e., Calcd.: C 63.23, H 8.96, N 3.04; found: C 63.23, H 9.17, N 3.00). It should be noted that the DS for fully substituted H-chitosan is 4.0 (Zong et al., 2000).

2.2. Sample preparation

H-chitosan film was prepared by casting 1% w/v H-chitosan solution in chloroform on a polytetrafluoroethylene plate and the plate was maintained at room temperature to evaporate as much chloroform from the film as possible. The as-prepared film was further "dried" *in vacuo* for another 24 h. Chitosan film, used as the reference material, was prepared by casting 2% w/v chitosan solution in 1% acetic acid aqueous solution on a stainless steel plate and let dried at 40 °C for 24 h. The as-prepared chitosan film was further neutralized with 1 M NaOH solution, excessively washed with distilled water, and dried *in vacuo* at room temperature for 24 h. The obtained H-chitosan and chitosan films were cut into circular disc specimens, of which diameter was about 14 mm.

2.3. Physical characterization

Thermogravimetric analysis (TGA) of H-chitosan and chitosan films was carried out by a Perkin-Elmer Pyris



Scheme 1. A synthetic route of the perfectly substituted H-chitosan.