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Electrospun anti-adhesion barrier made of chitosan alginate for reducing peritoneal adhesions

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ABSTRACT

In this study, novel anti-adhesion mat made of chitosan alginate was fabricated via electrospinning. The structure and morphology of electrospun membrane were examined using a field-emission scanning electron microscope and confocal laser scanning microscope. The degree of disintegration of the electrospun mat was assessed. The results showed that the degree of disintegration in DMEM for chitosan alginate mat was 64% of that of conventional calcium alginate mat after 5 days. In addition, the tissue anti-adhesion potential was evaluated with in vitro cell adhesion model and in vivo rat model. About 40% of the animals treated with chitosan alginate exhibited no tissue adhesion between injured peritoneum and cecum. The result demonstrated that chitosan alginate was effective in reducing the formation of tissue adhesion.

1. Introduction

In order to prevent postoperative adhesions, various pharmacological and barrier approaches to treat adhesion have been developed. Recently, the potential therapeutic agents has increased considerably, including steroidal, and non-steroidal anti-inflammatory compounds, tissue plasminogen activator (tPA), and the agents that interfere with specific cytokines or vascular permeability (Cashman, Kennah, Shuto, Winternitz, & Springate, 2010; Yeo & Kohane, 2008). One common approach to prevent adhesion is to place a physical barrier between injured site and the adjacent tissues. Various materials, made of animal tissues, biological materials and synthetic polymers, have been reported to be effective in reducing adhesion both in animal models and in clinical practices.

Seprafilm™ (Genzyme, Cambridge, MA) is a hydrophilic membrane composed of sodium hyaluronate and carboxymethylcellulose. This barrier has shown clinical efficacy to reduce adhesions during the post-operative healing phase (Chuang et al., 2008). However, many surgeons reported that Seprafilm is breakable and sticky (Ward & Panitch, 2011). In addition, hyaluronate degrades quickly and disappears from the injured site soon after application (Way, Hsieh, Chang, Hung, & Chiu, 2010). Furthermore, Seprafilm would increase adhesion in the presence of bacterial peritonitis in a murine model (Kayaoglu, Ozkan, Hazinedaroglu, Ersoy, & Koseoglu, 2005). Interceed™ (Gynecare, Somerville, NJ) is a fabric composed of oxidized regenerated cellulose (ORC) that can significantly reduce adhesions effectively in some animal models and clinical studies. Interceed does seem to provoke a large leukocyte response and can cause mesothelial cell sloughing in mice. However, blood infiltration makes the product ineffective in preventing adhesions, thus surgeons must ensure that all blood is cleared from the surgical field prior to using Interceed™ (Kayaoglu et al., 2005).

To improve the shortcomings of the aforementioned commercial products, anti-adhesion barrier must be more efficient in preventing the occurrence of adhesion. Therefore, many researchers attempt to design novel biomaterials with less complications and higher efficiencies (Bölgen, Vargel, Korkusuz, Menceloğlu, & Pişkin, 2007). In the literature, in vivo studies in animals have demonstrated that N,O-carboxymethylchitosan (NOCC) is safe and efficacious as an anti-adhesion barrier (Diamond et al., 2003). A modified chitosan–dextran gel was used to prevent peritoneal adhesions in a rat model, and significantly reduced the formation of intra-abdominal adhesions without adversely affecting wound healing (Lauder, Garcea, Strickland, & Maddern, 2011). Although chitosan/alginate mixtures have been patented for post-surgical adhesion barrier (Yeo et al., 2006), no product of this material was commercialized up to date.

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Chitosan and alginate have been proposed for many biomedical applications for their excellent biocompatibility. Chitosan is a natural-based aminopolysaccharide with a wide variety of useful biological properties, including excellent biocompatibility, biodegradability, hemostatic activity, nontoxicity, antimicrobial activity, and free radical scavenging activities (Anraku et al., 2011; Pillai, Paul, & Sharma, 2009). Hence, chitosan has been investigated for using in a wide variety of biomedical applications, including drug delivery system, and tissue engineering (Bhattarai, Gunn, & Zhang, 2010; Li, Ramay, Hauch, Xiao, & Zhang, 2005; Muzzarelli, 2009, 2010). Alginate is a naturally anionic polysaccharide consisting of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units. Alginate can form hydrogels, beads, porous sponges, and microfibers. Alginate exhibits excellent biocompatibility, nontoxicity, non-immunogenicity, and biodegradability (George & Abraham, 2006). Furthermore, chitosan and alginate can inhibit lipid peroxidation of phosphatidylcholine and linoleate liposomes (Tomida et al., 2010).

Electrospinning (ES) is a valuable and versatile fabrication method for creating micrometer or nanometer-sized fiber from various types of naturally and synthetic materials for biomedical or tissue engineering applications (Agarwal, Wendorff, & Greiner, 2008; Lee, Jeong, Kang, Lee, & Park, 2009; Muzzarelli, 2011). In the electrospinning process, fiber mats were formed by applying a high voltage to polymer solutions flowing through a syringe. The fibrous mats were collected on a grounded substrate (Rutledge & Fridrikh, 2007). Electrospun fibrous mats possess high surface areas, high porosities, and variable fiber diameters (Agarwal, Wendorff, & Greiner, 2009). Currently anti-adhesion barrier made of electrospun mat was little studied. Only poly(lactide-co-glycolide) (Zong et al., 2004), poly(ε-caprolactone) (Bölgen et al., 2007), and polylactide-poly(ethylene glycol) triblock copolymer (PELA) (Yang, Chen, Xiong, Xiong, & Wang, 2009) have been reported. Both polymers are synthetic and non-bioactive.

Many methods have been employed to reduce the formation of adhesions. However, no single approach has been wholly satisfactory, because the anti-adhesion barrier often imposes several limitations of their final properties. Chitosan film can be too brittle to handle and will degrade very slowly (Paulo et al., 2009). Chitosan gel is still defective because it is highly flowable (Zhang, Xu, & Zhou, 2006). In our recent studies, we have first fabricated chitosan alginate fibrous mat with core-sheath structure via electrospinning (Chang, Lee, Wu, Yang, & Chien, 2012). Such a mat exhibited higher flexibility, hydrophilicity, and degradability than pure chitosan film. The goal of this study is to employ this chitosan alginate mat as a novel anti-adhesion barrier to prevent peritoneal adhesions. In the literature, alginate is not adhesive to cells and serum proteins (Jeong, Krebs, Bonino, Khan, & Alsberg, 2010), and chitosan can reduce the formation of adhesion layer (Zhang et al., 2006). The morphology and structure of fibrous mats were investigated by field-emission scanning electronic microscope (FE-SEM) and confocal laser scanning microscope (CLSM). The anti-adhesion ability and cytocompatibility of the ES mat was assessed. In addition, the in vivo performance of the ES mat for peritoneal operation was evaluated histologically using rat models.

2. Experimental

2.1. Materials

Sodium alginate (Mw 220 kDa) and chitosan (Mw 200 kDa) were purchased from Acros, USA. 1-Ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), fluorescein isothiocynate (FITC), rhodamine B isothiocynate (RITC), glycerol, ethanol, 3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), acetic acid, and 3,3′-diaminobenzidine (DAB) were purchased form Sigma, USA. All chemicals were used without any further purification.

2.2. Preparation of polymer solutions

The 1.5 wt% alginate solution was prepared by dissolving sodium alginate powder in 50 wt% glycerol at room temperature for 1 day under stirring to form a homogeneous solution. Chitosan was dissolved in an aqueous solution containing 5 wt% acetic acid at 25 °C under stirring for 1 day to form a homogeneous solution of 1 wt%. Afterwards, the chitosan solution was mixed with equal volume of ethanol as a coagulant solution.

2.3. Electrospinning

The electrospinning system was consisting of a power supply (ES30P, Gamma High Voltage Research, USA), a syringe pump (LSP04-1A, Baoding Longer Precision Pump Co., Ltd., China), and a coagulating bath. The alginate solution was loaded into a 5 ml syringe with a needle spinneret (OD 1.07 mm, ID 0.77 mm). In this study, the flow rate was 0.5 ml/h, the applied voltage was 14 kV, and the distance between the needle and the coagulating bath was 70 mm. The resulting fibers were labeled as chitosan alginate. For comparison, alginate solution was electrospun into 80 wt% ethanol aqueous solution containing 10 wt% $CaCl_2$. The resulting fibers were labeled as calcium alginate. All these resulting fibers were immersed in ethanol for 1 h and then rinsed with ethanol to remove residual glycerol. Afterwards, the samples were dried at 60 °C for 1 d.

2.4. Characterization

The morphology of the ES mats was examined using a field-emission scanning electronic microscope (FE-SEM, JSM-6500F, JEOL, Japan), and a confocal microscope (LSM 510 META, Carl Zeiss Inc., USA). According to our previous work, the structure of the resulting fiber can be observed using fluorescence-labeled polymers (Lee, Chang, Lai, Yang, & Chien, 2011; Lee, Ryu, Je, & Kim, 2011). Briefly, alginate was labeled with FITC (alginate-FITC) and chitosan was labeled with RITC (chitosan-RITC). These labeled polymers were mixed with neat polymers to prepare ES mat as described in previous section. Cryomicrotome sections of the ES mat were then examined using a confocal microscope.

2.5. In vitro disintegration of ES mat

The ES mats disintegrate gradually during in vitro tests. To investigate the disintegration behavior, the mass loss of the ES mat was determined under static conditions. Briefly, the dried samples were immersed in 10 ml of Dulbecco's modified Eagle medium (DMEM) solution at ambient temperature. At a specific time, samples were washed with deionized water three times, and dried at 60 °C to constant weight and the fiber morphology was examined using SEM. The mass loss of each samples were determined according to the following equation:

$$\text{Mass loss}\ \ (\%) = 100\% \times \frac{W_{\text{before}} - W_{\text{after}}}{W_{\text{before}}}$$

where W_{before} and W_{after} are the weights of dry sample before and after swelling, respectively. Each experiment was repeated six times and the average value was recorded.