Carbohydrate Polymers 114 (2014) 230–237

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Evaluation of the ability of xanthan gum/gellan gum/hyaluronan hydrogel membranes to prevent the adhesion of postrepaired tendons

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ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 21 July 2014
Accepted 22 July 2014
Available online 19 August 2014

Keywords:
Tendon
Anti-adhesion
Xanthan gum
Gellan gum
Hyaluronan

ABSTRACT

After tendon-repair surgery, adhesion between the surgical tendon and the synovial sheath is often presented resulting in poor functional repair of the tendon. This may be prevented using a commercially available mechanical barrier implant, Seprafilm, which is composed of hyaluronan (HA) and carboxymethyl cellulose hydrogels. In a rat model, prepared membranes of various compositions of gellan gum (GG), xanthan gum (XG) and HA as well as Seprafilm were wrapped around repaired tendons and the adhesion of the tendons was examined grossly and histologically after 3 weeks of healing. Certain formulations of the XG/GG/HA hydrogel membranes reduced tendon adhesion with equal efficacy but without reducing the tendon strength compared to Seprafilm. The designed membranes swelled rapidly and blanketed onto the tendon tissue more readily and closely than Seprafilm. Also they degraded slowly, which allowed the membranes to function as barriers for extended periods.

1. Introduction

The tendon adhesion that occurs after tendon-repair surgery is a physiological phenomenon that results in both the inflammatory response triggered at the site of surgery and the loss of physical separation between the repaired tendon and the surrounding tissue (Potenza, 1962). Postsurgical adhesion in the tendon interferes with the normal gliding function of tendons and nerves and causes pain and greatly restricts motion; if these effects are severe and prolonged, ischaemia and nerve injury can develop. Tendon healing is a slow process that lasts for months, during which the continuity and strength of the tendon fibre are re-established (Hagberg, Tengblad, & Gerdin, 1991). Moreover, tendon healing frequently results in the formation of scars that restrict the full range of motion of tendons, and the repaired tendon fails to recover its original strength.

Commercial medical products that exhibit antiadhesive properties are currently available; in these products, films, gels, membranes, or fluids are used. One of the products available is Dynavics, which is a clear and absorbable gel designed for coating tissues that are injured as a result of tendon or peripheral-nerve surgery. A Seprafilm adhesion barrier (Genzyme, Cambridge, MA, USA) is a bioresorbable membrane composed of chemically modified sodium hyaluronate (HA) and carboxymethyl cellulose (CMC), which is rapidly degraded and cleared after placement in the body. In clinical practice, Seprafilm has been observed to hydrate to form a lubricious gel coating within 24–48 h when applied on the surface of tissues. The barrier is resorbed from the site of application within 7 days and, thus, a second operation for removing the barrier is not required (Genzyme). The safety and efficacy of Seprafilm have been demonstrated, and the barrier has been used for preventing adhesion after abdominopelvic surgery (Altuntas, Tarhan, & Delibas, 2002; Vrijland et al., 2002; Becker et al., 1996; Tsapanos, Stathopoylou, Papathanassopoulou, & Taingounis, 2002; Beck et al., 2003) and cardiovascular surgery (Ballore, Orru, & Nicolini, 2000; Kudo et al., 2004). In a preclinical study, Seprafilm was used to prevent adhesion after tenolysis of the flexor digitorum tendon in chickens (Karakurum, Buyukbebeci, Kalender, & Gulec, 2003). The tendons covered with Seprafilm were observed to exhibit a substantially improved gliding excursion profile and a diminished incidence of adhesion when compared with controls. However, the use of these products is limited by shortcomings such as their high cost and rapid degradation, their nondeformability, and the difficultly involved in wrapping the barriers around a tendon, and

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http://dx.doi.org/10.1016/j.carbpol.2014.07.049
0144-8617/

the inability of the barriers to function in certain circumstances, such as when bleeding or infection occurs and after anastomotic surgery. These shortcomings adversely affect patient management and intervention after surgery and increase healthcare costs.

Xanthan gum (XG), a polysaccharide secreted by the bacterium *Xanthomonas campestris*, belongs to a family of substances known as hydrocolloids. XG can form a gel and bind many times its weight in water, which makes XG a valuable food additive and rheology modifier in cosmetic products. One particularly noteworthy property of XG is its ability to substantially increase the viscosity of a liquid when added at 0.5 wt%. XG is extremely stable over a wide temperature and pH range. XG is also used in numerous nonfood products and applications because it is nontoxic and exhibits high thickening capacity (Sharma & Maffulli, 2005; Chang, Haung, Yang, Kuo & Lee, 2012).

Gellan gum (GA) is a linear, anionic polysaccharide secreted by *Pseudomonas elodea*. GA is also a food additive that functions as a stabiliser, thickening agent, and gelling agent in a wide variety of foods. Recently, GA has been investigated for its use as a drug-delivery agent, cell carrier, guided bone-regeneration material, and wound-dressing material in biomedical engineering because of the biocompatibility and low cytotoxicity of GA.

Following all surgical incisions, adhesions develop as a part of the normal healing process that occurs in response to tissue trauma. Adhesions occur within the first 3–5 days after surgery and are composed of sticky, fibrous scar tissues. If the development of adhesions can be interrupted, several potential complications associated with surgery can be avoided. In this study, for use as antiadhesive membranes, we prepared XG/GA/HA (XGH) hydrogel membranes featuring various formulations of XA, GA, and HA. We used a rat model to evaluate the ability of these membranes to reduce adhesions in repaired Achilles tendons and to compare this with the efficacy of Seprafilm, an FDA-approved antiadhesive barrier that contains chemically modified HA.

2. Material and methods

2.1. Fabrication of XGH hydrogel membranes

Four membranes featuring distinct weight ratios of HA, XG, and GG were prepared and their ability to prevent postsurgical tendon adhesion was evaluated (Table 1). XG, GG, and HA were dissolved in 10 mL of deionised water and heated to 85–90 °C to generate a transplant solution. The solution was then poured into a glass dish and evaporated in an oven at 37 °C for 24 h to obtain a dry membrane, which was crosslinked using a solution of 15 mM 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysuccinimide (EDC/NHS); the crosslinking was performed for 6 h at room temperature, and the crosslinked membranes prepared using distinct formulations (denoted as A, B, C, and D in Table 1) were washed with 95% ethanol three times to remove any residual unreacted EDC/NHS and then dried at room temperature.

Table 1
Various compositions and weight ratios (%) of the XGH hydrogel membranes in different formulation's groups.

Formulation content (100%)	HA	GG	XG
A	33.3	33.3	33.3
B	38.4	30.8	30.8
C	42.8	28.6	28.6
D	40	26.7	33.3

HA: hyaluronon; GG: Gellan Gum; XG: Xanthan Gum

2.2. Characterisation of XGH membranes

2.2.1. Water-content measurement

The water content (WC) of the XGH membranes was determined by swelling the membranes in a pH 7.4 phosphate-buffered solution (PBS) at room temperature. After the membranes were equilibrated with PBS, the wet membranes were blotted using filter paper to remove the water adherent to the membrane surface. The WC of the membranes was calculated as

$$\mathrm{WC}(\%) = (W_w - W_d)/W_d \times 100\%$$

where *W*w and *W*d are the weights of the wet and dry membrane, respectively. The experiment was conducted three times and the WC mean and standard deviation were calculated.

2.2.2. Mechanical-strength measurement

The Seprafilm sample swelled considerably in solution and, thus, could not be mounted firmly onto the load-cell used in measurements; therefore, we conducted the mechanical-strength test under dry conditions and compared the strengths of Seprafilm and the other membrane samples. The membranes were cut into pieces (1 × 6 cm) and the tensile strengths of the membranes were measured up to the point at which they broke. The mechanical parameters of the membranes were calculated and recorded automatically by using a material-testing system (MTS; Eden Prairie, USA) at a crosshead speed of 5 mm/min.

2.2.3. In vitro degradation test

The *in vitro* degradation of the prepared membranes was tested by incubating the membranes in 10 mL of PBS (pH 7.4) in a vial and placing the vial on a shaker set at 40 rpm and 37 °C. At predetermined times, the membrane was removed from the incubation medium, washed with distilled water, dried, and weighed, after which another 10 mL of fresh PBS was added into the vial and the degradation test was continued. The degradation profiles were obtained as the cumulative weight losses of the membranes. The surface microstructure of the membranes was examined using a scanning electron microscope (SEM); all the samples were dried and sputter-coated with gold before being examined under the SEM (JEOL, JSM-5300, Japan).

2.3. Cell-viability assay

The cytocompatibility of the prepared membranes was measured using the MTT assay. The membranes were sterilised by applying ^{60}Co gamma irradiation at a dose of 15 kGy and then were placed in 24-well bacterial-grade dishes, with each well containing 5×10^4 L929 fibroblasts; 2 mL of culture medium was added to each well and the samples were incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. After incubation, 20 μL of the MTT solution (5 mg/mL) was added to each well and the cells were incubated for an additional 3 h. The formazan precipitate formed was dissolved in 200 μL of DMSO, and the solution was mixed vigorously to dissolve the dye. The 570-nm absorbance of the sample in each well was measured using a multiplate reader. To generate a standard curve, the spectrophotometer (Multiskan Co., Thermo Scientific, USA) was first calibrated to zero absorbance by using a cell-free culture medium. All the experiments were repeated three times, and the absorbance values measured in the MTT assay were converted to cell numbers/well.

2.4. Animal studies

In this study, we used eighteen 8-week-old male Sprague-Dawley rats that weighed 250–300 g. The rats were anaesthetised