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Silk fibroin membrane used for guided bone tissue regeneration

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ABSTRACT

With the aim to develop a novel membrane with an appropriate mechanical property and degradation rate for guided bone tissue regeneration, lyophilized and densified silk fibroin membrane was fabricated and its mechanical behavior as well as biodegradation property were investigated. The osteoconductive potency of the silk fibroin membranes were evaluated in a defect rabbit calvarial model. Silk fibroin membrane showed the modulated biodegradable and mechanical properties via ethanol treatment with different concentration. The membrane could prevent soft tissue invasion from normal tissue healing, and the amounts of new bone and defect closure with silk fibroin membrane were similar to those of commercially available collagen membrane.

1. Introduction

Rapid bone defect filling with normal bone is a challenge in orthopedics and dentistry. Bone graft therapy is an established treatment that has become one of the most reliable options to restore bone tissue. Various bone graft products are available in the form of bulk, plate, fiber and particle. However, some problems of operating feasibility and subsequent manage of wound still remain to be solved [1–3]. For example, bone implants in their particle form have poor operating characteristics, which cause problems together with blood during surgery. Implanted bone graft is usually surrounded by redundant fibrous connective tissue due to inflammatory exudate and/or foreign body stimulation. The phenomenon of ectopia osteogenesis could also be found in inappropriate sites because of the shift of bone graft particles or exceeding the release of loaded-drugs on the bone graft. So far, much attention has been focused on guided bone healing and regeneration in the number of surgical and restorative procedures. Among these efforts, the research and development of novel membranes for guided bone regeneration (GBR) is an area of increasing interest [4].

GBR membranes, used in the interface between soft tissue and restoration areas, aim to prevent faster-growing connective tissue from migrating into the defect, and create a protected space over the bone defect therefore allowing the reconstruction of new bone tissue in a confined space after surgery [5]. In general, available GBR membranes can be divided into two types from the point view of materials stability in the body, i.e. nonresorbable and resorbable membrane [6]. Presently, resorbable GBR membrane draws more attention because it can avoid the second surgery [7,8]. Materials for resorbable GBR membranes mainly include either tissue-derived collagens or synthetic polymers. Compared with synthetic polymers, collagen seems to be a more preferred candidate for GBR membranes due to its good biocompatibility, biodegradability and excellent cell affinity. But the high cost, undefined sources and poor mechanical properties limited the use of collagen-based GBR membranes [9,10]. As a result, resorbable polymers are major commercial materials for GBR membranes, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(lactic-*co*-glycolic acid) (PLGA), and so on [11,12].

Silk fibroin (SF), derived from natural *Bombyx mori* silkworm, is becoming more of a focus in the field of biomaterials because of its biocompatibility, morphologic flexibility, modulated mechanical properties and low cost [13–15]. Silk fibroin can be shaped into various forms, including filament, film, sheet, and scaffolds, and even from soft material to super rigid material due to its special pristine feature and superior machinability [16]. So far, SF has been investigated and used widely in tissue engineering, drug release, and optical apparatus and others in the biomedical material field [17–21].

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In this study, SF membrane as for guided bone regeneration was fabricated via lyophilization, densification and ethanol treatment. And then the obtained SF membrane was characterized by scanning electron microscopy (SEM), Fourier Transform infrared spectroscopy (FTIR), and universal mechanic test machine. In vitro biodegradability of SF membrane was evaluated in PBS with and without protease XIV. The osteoconductive potency of the SF membrane was further investigated in rabbit calvarial defect model with porcine collagen membrane and osteoguide membrane (non resorbable, biocompatible polymer PCL membrane) as controls.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Hangzhou Huipu Chemical Agents Co. Ltd., China, including Na_2CO_3, LiBr, phosphate buffer saline (PBS, 0.05 M, pH 7.4) and ethanol. *B. mori* silkworm cocoons were obtained from Huzhou Academy of Agricultural Science, China. Protease XIV was purchased from BSZH Co. Ltd. China. Porcine collagen membrane, osteoguide membrane and the bone graft of Osteon™ particles were friendly gift from Genoss Co. Ltd., Suwon, Korea.

2.2. Preparation of silk fibroin solution and membrane

A SF solution was prepared from *Bombyx mori* silk fiber according to previously established protocols with minor modifications [22]. First, *Bombyx mori* silk fiber was immersed in 0.5% (w/v) Na_2CO_3 for 30 min at 100 °C to extract its sericin component. The degummed silk fiber (SF) was dissolved in 9.3 M LiBr solution at 37 °C for 4 h and then dialyzed with distilled water at room temperature for 3 d. The distilled water was replaced every 12 h. The resulting mixture was centrifuged at 8000 r.p.m. at 4 °C for 20 min to remove impurities to yield a homogeneous SF solution. The concentration of the SF solution was determined by the Bradford protein assay on a DNA/Protein Analyzer (DU530, Beckman Coulter).

7 mL of 5% (w/v) SF solution was added onto a 10-mm plastic disk at room temperature and frozen at −80 °C for 6 h and then freeze-dried in a freeze-dryer for 48 h to obtain the lyophilized porous SF sponge. The SF sponge was compacted at 10Mpa for 10 min and then was modified at 50 and 90% (V/V) ethanol solution for 2 h respectively. Obtained SF film was named as L5 and L9, corresponding to the concentration of ethanol solution respectively. The SF membrane without ethanol treatment was used as control, which was designated as Ctrl in the following experiment.

2.3. Specimen characterization

The micromorphology of SF membranes was further observed using a field emission scanning electron microscope (FE-SEM, S4800, Hitachi) with an accelerating voltage of 1 kV. The chemical structure of SF membranes were characterized using Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet 5700, Thermo Electron) in the range of 800–2200 cm^{-1} with a resolution of 4 cm^{-1}.

2.4. The mechanical property

To evaluate the mechanical properties of samples, SF membranes were made into 1 cm × 4 cm rectangle pieces, and their elastic modulus and tensile strength were measured with a universal mechanic test machine (Instron 3367, America). Samples in the wet status were obtained after the rectangle pieces were immersed in distilled water at 25 °C for 1 h. All experiments were repeated for three times per group.

2.5. In vitro degradation

2 cm × 2 cm square pieces of SF membranes were weighed and incubated at 37 °C in PBS solution in the presence or absence of 0.1u/ml protease XIV respectively. The samples (n = 3 per groups) were incubated in PBS solution (bath ratio 1:100) for 1, 4, 7, 10, 13, and 16 days under slow shaking. All degradation solution was replaced with a fresh solution every day. At the designated time points, the degraded products were collected rinsed in deionized water, dried at 100 °C, and then weighed. Quantitative changes were expressed as the percentage of weight retained relative to the initial dry weight.

2.6. Animals and implantation

To evaluate the properties of SF membranes in vivo, ten 9–10 months old New Zealand white male rabbits were used. All procedures including animal selection, management, preparation and surgical procedures were performed in accordance with the Institutional Animal Care and Use Committee in Genoss Co. Ltd., Suwon, Korea. All surgeries were performed under sterile conditions. All rabbits were anesthetized with an intramuscular injection of a mixture of zolazepam hydrochloride (0.3 mL/kg; Virbac Laboratories) and xylazine hydrochloride (0.3 mL/kg; Rompun). Surgical area of cranium was anesthetized with 2% lidocaine and the scalps were shaved and disinfected. The cranium was exposed through a mid-line skin incision. The peritoneum was retracted laterally and bilaterally, and 8-mm-diameter defects were made in the parietal bones by means of a standardized trephine cutting bur under physiological saline solution irrigation. Four defects were made per one animal with an inter defect distance of 3 mm to exclude any influence to different groups. The defects were filled with bone graft of OSTEON III™ with an average size of 0.5–1.0 mm [23]. The sterilized SF membranes of L5 and L9 were used with the size of 15 × 15 mm^2 as GBR membranes to cover the defect windows. Porcine collagen and osteoguide membrane were also used as controls. All membranes were fixed on the rabbit cranium using titanium pin for membrane fixation. Surgical procedures are presented in Fig. 1. Finally the skin was closed with 4.0 absorbable monofilament sutures. The rabbits were sacrificed at 6 and 12 weeks respectively. Five animals were used in every group.

2.7. Animals micro-CT evaluations

Microcomputerized tomography (μ-CT) analysis was performed with a micro-CT (Skyscan 1173, Belgium) referring to a protocol as published previously [24,25] to evaluate new bone area (the area of newly formed bone in the defect) and total bone area (total content of residual bone graft and new bone) after 6 W and 12 W implantation under the condition of aluminum filter with 130 kV, 30 μA, and 12.14 μm. Thirty CT pictures per each group were evaluated with 7.99 mm, circular region of interest. The taken images were reconstructed with axial, sagittal, and coronal plane. For the regions with bone loss but excluding implant site, percent bone volume, bone surface/volume ratio, trabecular thickness, trabecular separation, trabecular number, trabecular bone pattern factor, structure model index and degree of anisotropy were measured and the values from both groups were compared using the *t*-test [26].

2.8. Histological observation

For the histological observations, the harvested rabbit cranium tissue specimens were embedded in paraffin and 4 μm sections obtained from the center of the calvarial defects. Dewax section was rinsed in alcohol and water orderly. The two central most sections in each block were selected and stained with Masson Trichorme. The prepared histology slides were observed under a light microscope with an optical microscope (BX51, OLYMPUS) equipped with a CCD (Charge-Coupled Device) camera and images were captured digitally.