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The effects of tetracycline-loaded silk fibroin membrane on proliferation and osteogenic potential of mesenchymal stem cells

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ABSTRACT

Background: The main objective of this study was to investigate the effect of tetracycline-loaded silk fibroin membranes (TC-SFMs) on the proliferation and the osteogenic differentiation of human mesenchymal stem cells.

Materials and methods: Four groups (0, 1, 5, and 10% concentration) of TC-SFMs were prepared for the experiments. We investigated cumulative tetracycline (TC) release profile for 7 d. Human gingiva-derived mesenchymal stem cells (GMSCs) were isolated from our previous study and seeded to the TC-SFMs. WST-8 assay (Cell Counting Kit-8; SigmaAldrich Co, St. Louis, MO), staining of Phalloidin-FITC, and scanning electron microscope analyzed the cellular attachment and viability. Staining of Alizarin Red S (Sigma-Aldrich Co.) and osteogenic marker (osteocalcin) analyzed osteogenic differentiation. Additionally, quantitative polymerase chain reaction measured the expression of osteogenic lineage genes, including bone gamma-carboxyglutamic acid protein, bone sialoprotein, runt-related transcription factor 2, and collagen type I α1 according to TC concentration (0.05, 0.1, 0.25, and 0.5 mg/mL).

Results: The release of TC from TC-SFMs plateaued and neared completion in 24 h. Significantly higher viability was noted achieved in 1% and 5% TC-SFMs. The morphology of GMSCs on TC-SFMs at 0% and 1% concentration showed spindle shapes, but cells in 10% TC-SFMs appeared spheroid. During Alizarin Red S staining at 21 d of osteogenic differentiation, calcium and osteocalcin formation were significantly lower in the 10% TC-SFM group than in the 0, 1, and 5 groups. Compared with the control group, bone gamma-carboxyglutamic acid protein showed significantly low expression rate at TC concentration $\geq$0.05 mg/mL. Bone sialoprotein was low at TC concentration $\geq$0.1 mg/mL. Likewise, runt-related transcription factor 2 and collagen type I α1 were low at TC concentration of 0.5 mg/mL.

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Conclusions: Within the limits of this study, 1% and 5% TC-SFMs showed higher proliferation and osteogenic potential of GMSCs than 10% TC-SFM. Therefore, the use of 1% to 5% range of TC may be more suitable to silk fibroin membrane for stem cell tissue engineering.

1. Introduction

Biocompatible scaffold is one of the fundamental factors in tissue engineering. Therefore, various scaffolds have been developed such as membranes, meshes, plates, screws, plugs, or rods [1]. Among them, membranes are useful for guided-tissue regeneration (GTR) or guided-bone regeneration (GBR) procedures in dentistry [2]. Various materials have been used to GBR membranes, such as expanded polytetrafluoroethylene (e-PTFE), collagen, and copolymers of lactic acid [3–5]. However, these conventional membranes possess many structural, mechanical, and biofunctional limitations, and the ideal membrane for use in periodontal regenerative therapy has yet to be developed [6]. For example, e-PTFE is nonresorbable and therefore has an insufficient degradation profile. Almost all bioresorbable collagens have insufficient sustained strength and degradation profile to avoid membrane collapse and perform barrier function.

Recently, many materials have been tested for overcoming the limitation of conventional membranes. Silk is a very good candidate. Silk biomaterials have low immunogenicity. Silkworm silk has been used commercially in biomedical sutures for decades, and in textiles for centuries [7]. In its natural form, it is a filament core protein, silk fibrin, and a glue-like coating of a family of sericin proteins [8]. Previous studies of silk fibers found that sericin proteins are the major cause of adverse biological responses [9–11]. Thus, silk fibroin, degummed (desericinated) silk, has low immunogenicity and seems to be a proper biomaterial for tissue engineering [7,12,13].

Many studies paid attention to additional features of the silk fibroin such as controlled drug delivery [14–16]. Silk fibroin has unique properties for implantable drug delivery, including biodegradability, biocompatibility, control, stabilizing effects on incorporated drugs, diversity of material formats, aqueous, and ambient-processing options [15]. Drug-loaded silk fibroin shows release rates dependent on diffusion of the drug through the silk, degradation of the silk polymer carrier, or a combination of both [15]. Degradation rate can be regulated by β-sheets content in silk fibroin materials. Thus, silk fibroin may control the drug release rate according to the manufacturing methods. Tetracycline (TC) is an effective antibiotic against various pathogens in periodontal disease [17–19] and a low dose of it suppresses host-derived matrix metalloproteinases in the periodontal lesion [20]. Results from these reports suggest that greater bone formation may be obtained with drug-loaded biodegradable membranes [5,21].

The main objective of this study was to investigate the effect of TC-loaded silk fibroin membranes (TC-SFMs) on proliferation and osteogenic differentiation of human gingiva-derived mesenchymal stem cells (MSCs). To our knowledge, this is the first to elucidate the effect of TC-SFMs on proliferation and osteogenic differentiation of human gingiva-derived mesenchymal stem cells (GMSCs).

2. Materials and methods

2.1. Isolation of human GMSCs

In our previous study, human GMSCs were isolated from the connective tissue of gingiva [22]. Cells were cultured with alpha modified minimal essential medium (α-MEM, Gibco, Grand Island, NY) containing 15% fetal bovine serum (Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma–Aldrich Co, St. Louis, MO), 200 mM L-Glutamine (Sigma–Aldrich Co), and 10 mM ascorbic acid 2-phosphate (Sigma–Aldrich Co) in culture dishes (Corning, Tewksbury, MA). The cells were incubated at 37°C humidified incubator with 5% CO_2 and 95% air and fed every 3–4 d.

2.2. Fabrication of TC-SFMs

Silk fibroin solution was prepared according to a published protocol [23]. Silkworm cocoons (Rural Development Administration, Suwon, Korea) were sliced and degummed twice, then washed with distilled water. We dissolved the degummed cocoon in $CaCl_2$:H_2O:ethanol (1:8:2) in volume. We obtained the silk fibroin solution after dialysis against distilled water for 4 d to remove the dissolution agents, and then loaded TC (Sigma–Aldrich Co) into the fibroin solution by weight per volume percentages (0, 1, 5, and 10%). Silk fibroin solutions were cast on a polystyrene dish (SPL Life Sciences, Pocheon, Korea). All TC-SFMs were prepared to 10 mm × 10 mm squares and membranes were sterilized by ethylene oxide gas before experiments.

2.3. In vitro release of TC from TC-SFMs

TC-SFMs (0, 1, 5, and 10%) were immersed in a glass vial containing 5 mL phosphate-buffered saline (PBS; Welgene, Daegu, Korea). We protected the sealed vials from light and placed them in an incubator at 37°C. In 1 wk, we took 100 μL samples from the vials and replaced them with fresh PBS at 3 h, 6 h, 24 h, 3 d, 5 d, and 7 d. We assayed the samples spectrophotometrically at 352 nm on a microplate reader (BioTek, Winooski, VT). Standard concentration was measured at 352-nm absorbance using standard TC solutions (10, 50, 100, 500, and 1000 μg/5 mL).

2.4. Cytoskeletal morphology of GMSCs attached to the silk fibroin membrane

GMSCs (passage 5) were seeded with 0% TC-SFMs at a density of 1 × 10^5 cells per membrane and incubated overnight.