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Porous membrane with reverse gradients of PDGF-BB and BMP-2 for tendon-to-bone repair: In vitro evaluation on adipose-derived stem cell differentiation

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ABSTRACT

Polycaprolactone (PCL)/Pluronic F127 membrane with reverse gradients of dual platelet-derived growth factor-β (PDGF-BB) and bone morphogenetic protein 2 (BMP-2) concentrations was fabricated using a diffusion method to investigate the effect of reverse gradients of dual growth factor concentrations on adipose-derived stem cell (ASC) differentiations, such as tenogenesis and osteogenesis. The PDGF-BB and BMP-2 were continuously released from the membrane for up to 35 days, with reversely increasing/decreasing growth factors along the membrane length. Human ASCs were seeded on the membrane with reverse PDGF-BB and BMP-2 gradients. The cells were confluent after 1 week of culture, regardless of growth factor types or concentrations on the membrane. Gene expression (real-time polymerase chain reaction), Western blot and immunohistological analyses after 1 and 2 weeks of ASC culture showed that the membrane sections with higher PDGF-BB and lower BMP-2 concentrations provided a better environment for ASC tenogenesis, while the membrane sections with higher BMP-2 and lower PDGF-BB concentrations were better for promoting osteogenesis. The results suggest that the membrane with reverse gradients of PDGF-BB and BMP-2 may be promising for tendon-to-bone repair, as most essential biological processes are mediated by gradients of biological molecules in the body.

1. Introduction

Tendons are tough bands of fibrous connective tissue that transmit tensile loads from muscle to bone [1]. Tendon tears most often occur at the tendon-to-bone interfacial zone. Surgical methods are often used to reattach torn tendon to the bone. However, surgery often has a poor clinical outcome, with repair failure rates of massive rotator cuff tendon tears exceeding 80%, for instance [2]. This is generally attributed to insufficient restoration of native biochemical and mechanical properties at the injury site, where a multitissue interface comprises a cellular and tissue transition from the tendon itself, to a fibrocartilage region, followed by bone. Cells within each zone actively secrete and assemble an extracellular matrix composed of collagens, proteoglycans and calcified/non-calcified regions, with corresponding mechanical variations across the interfacial zone [3]. The heterogeneous mechanical properties serve to minimize stress and allow load transfer from soft tendon to hard bone tissue in a stable manner [4]. Given the inadequacy of current surgical approaches, biological approaches have attracted increasing research and clinical attention [5], but progress has been limited by the complexities of the tissue transition and mechanical demands. The incorporation of gradient-based strategies into tissue engineering might prove an effective biological solution to this problem. Many essential biological processes are mediated by gradients of biological molecules in the body. It is well recognized that concentration gradients of biological molecules guide tissue formation and regeneration [6–11]. Growth factors are polypeptides that can either stimulate or inhibit cellular proliferation, differentiation, migration, adhesion and gene expression. Growth factor effects are concentration dependent, often in a complex nonmonotonic way [12]. Due to their control of many biological processes, growth factors are widely used in the regeneration of many tissue types, such as musculoskeletal, neural, hepatic and vascular systems [13]. Clinical therapies administer these factors either systemically or via direct injection into the tissue site of interest. However, the short half-lives, relatively large size, slow tissue penetration and potential toxicity at the systemic level have hindered many applications of these bioactive compounds [14]. One option to enhance the in vitro and in vivo efficacy of growth factors is to incorporate them into substrates in order to maintain their stability and control their release kinetics. Growth factors can

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be incorporated directly into a polymeric scaffold to be used for tissue formation either during, or after, scaffold fabrication [15–19].

In this study, we fabricated a polycaprolactone (PCL)/Pluronic F127 porous membrane with reverse gradients of dual growth factor concentrations (i.e. platelet-derived growth factor-β (PDGF-BB) gradient from the left to right side on the membrane, and bone morphogenetic protein 2 (BMP-2) gradient from the right to left side) for tendon-to-bone repair. Both growth factors were immobilized onto the surface of the membrane by specific interactions between Pluronic F127 and heparin (hydrogen bonding) and subsequent interactions between heparin and growth factors (ionic interaction), which can preserve the biological function of growth factors without denaturation [20]. Fig. 1 illustrates the schematic diagram of the membrane with reverse gradients of PDGF-BB and BMP-2 concentrations, and the successive binding of the growth factors onto the PCL/Pluronic F127 membrane. PDGF-BB and BMP-2 are well recognized as effective promoters for tendon [21–23] and bone [24–26] regeneration, respectively. The growth factors immobilized on the membrane surface can be released with concentration gradients in a sustained manner. As an initial attempt, the tendon-to-bone repair potential of the membrane with reverse gradients of PDGF-BB and BMP-2 was investigated by examining the in vitro differentiation behaviors of adipose-derived stem cells (ASCs) along the growth factor concentration gradients of the membrane.

2. Materials and methods

2.1. Materials

PCL (Mw 80,000; Aldrich), tetraglycol (glycofurol; Sigma) and Pluronic F127 ($EG_{99}PG_{65}EG_{99}$, Mw 12,500; BASF) were used to fabricate porous membranes. GMP-grade PDGF-BB and BMP-2 were used as growth factors to enhance the tenogenic and osteogenic differentiation, respectively, and were purchased from R&D Systems. All other chemicals were of analytical grade and were used as received. Water was purified (>18 mΩ) using a Milli-Q purification system (Millipore Co.).

2.2. Fabrication and characterization of PCL/F127 membrane

PCL/F127 membranes with selective permeability and hydrophilicity were prepared using an immersion/precipitation method [27]. Briefly, PCL pellets were dissolved in hot tetraglycol (90 °C, 12 wt.%) and Pluronic F127 powders (5 wt.%, PCL base) were added to the PCL solution. The hot PCL/F127 solution was cast in a mold (60 mm × 80 mm × 0.4 mm) and then directly immersed in excess water for 1 h at room temperature. The PCL/F127 membrane was produced after washing of the membrane in excess water to remove residual solvent, followed by vacuum drying (Fig. 2).

Surface and cross-section structures of the PCL/F127 membrane were estimated using scanning electron microscopy (SEM; S-3000, Hitachi, Japan). The average pore sizes of the membrane were measured using an image analysis program (*i*-solution, IMT, Korea). The porosity of the membrane was measured using a specific gravity bottle based on the Archimedes principle, as described elsewhere [28]. The mechanical strength of the membrane was measured using an ultimate tensile test machine (AG-5000G, Shimadzu, Japan) equipped with a 10 kgf load cell. The maximum tensile strength was obtained from the stress–strain curve of the sample (ASTM D638 [29]; crosshead speed of 1 mm min^{-1}).

2.3. Preparation and characterization of membrane with reverse growth factor gradients

PDGF-BB and BMP-2 were incorporated onto the PCL/F127 membrane (50 mm × 10 mm × 0.4 mm) via heparin immobilization. For heparin immobilization, the PCL/F127 membrane was immersed in a heparin solution (1 mg ml^{-1} in 2 wt.% NaCl solution) at 4 °C for 3 h. The heparin-immobilized membrane was rinsed successively with a 2 wt.% NaCl solution and water, and then freeze-dried. The amount of heparin immobilized on the membrane surface was determined by toluidine blue assay [30]. To produce reverse growth factor gradients on the membrane, the growth factor solutions (150 μl each of PDGF-BB or BMP-2 dissolved in phosphate-buffered saline (PBS, pH 7.4) at a concentration of 2 μg ml^{-1}) were dropped onto both ends of the heparin-immobilized membrane simultaneously, one end with PDGF-BB solution and the other end with BMP-2 solution. The growth factor solutions on the membrane were allowed to stand in a refrigerator (4 °C) for 1 h, which is the amount of time needed for the growth factor solution on each end to be diffused out into the opposite end of the membrane. The membrane was washed five times with PBS, and the amount of both growth factors immobilized on the membrane along the membrane length was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Duoset®, R&D systems) [19] (see Fig. 1). The membrane was divided into 10 sections along the membrane length (each section, 5 mm × 10 mm), and the amounts of both PDGF-BB and BMP-2 immobilized on the membrane sections were separately quantified.

To investigate the release behavior of the growth factors, both growth factor-immobilized membrane sections were incubated in 1 ml PBS supplemented with 1% bovine serum albumin (Sigma)

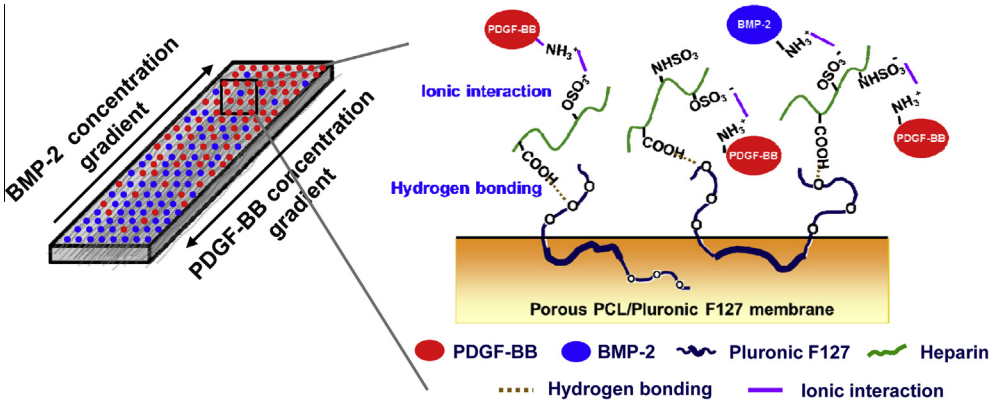

Fig. 1. Schematic diagram illustrating the formation of PCL/Pluronic F127 membrane with reverse gradients of PDGF-BB and BMP-2 and the successive binding of heparin and growth factors onto the membrane surface.