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Characterization of a bioactive Jagged1-coated polycaprolactone-based membrane for guided tissue regeneration

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ABSTRACT

Objective: The aim of the present study was to develop a Jagged1-coated polycaprolactone (PCL) membrane and to evaluate the response of human periodontal ligament cells (hPDL) on this membrane *in vitro*.
Methods: Membranes were prepared from PCL and PCL-incorporated hydroxyapatite (PCL/HA). The membranes' surface roughness, surface wettability, and mechanical properties were examined. An indirect affinity immobilization technique was used to coat the membranes with Jagged1. Membrane cytotoxicity was evaluated using LIVE/DEAD and MTT assays. The morphology of the cells on the membranes was observed using scanning electron microscopy. hPDL alkaline phosphatase (ALP) enzymatic activity and mineral deposition were examined using an ALP assay and Alizarin Red S staining, respectively. Notch target gene mRNA expression was determined using real-time polymerase chain reaction.
Results: The PCL/HA membranes exhibited a significantly reduced surface contact angle, decreased maximum tensile strain, and ultimate tensile stress. However, the surface roughness parameters were significantly increased. The PCL and PCL/HA membranes were not cytotoxic to hPDL *in vitro*. hPDLs attached and spread on both membrane types. Further, indirect affinity immobilized Jagged1 on the membranes upregulated hPDL Notch target gene expression. After culturing in osteogenic medium, Jagged1-immobilized PCL/HA membranes significantly enhanced hPDL ALP enzymatic activity.
Conclusion: Indirect immobilized Jagged1 PCL/HA membranes could be further developed as an alternative guided tissue regeneration membrane to promote osteogenic differentiation in periodontal defects.

1. Introduction

Guided tissue regeneration (GTR) is a successful clinical procedure to regenerate periodontal tissues (Villar & Cochran, 2010). Various membrane materials have been employed and investigated both experimentally and clinically (Bottino et al., 2012). However, the results from a systematic review and meta-analysis indicate that GTR exhibits high variability between studies (Needleman, Worthington, Giedrys-Leeper, & Tucker, 2006). This suggests that clinically available membranes need improvement to efficiently promote periodontal tissue regeneration.

GTR membranes act as a physical barrier to prevent gingival epithelial cell downgrowth, reducing long junctional epithelium formation in periodontal defects (Bottino et al., 2012). To increase periodontal tissue healing, the development of drug/bioactive agent-containing membranes has been investigated. Various agents such as antimicrobials, growth factors, and small molecules have been added into the membrane to facilitate and/or enhance periodontal tissue regeneration (Bottino et al., 2012; Sam & Pillai, 2014). Many studies have demonstrated an increased effect by the combination of these agents with traditional GTR membranes (Sam & Pillai, 2014).

A systematic review assessing the clinical efficacy of non-resorbable and bioresorbable GTR membranes indicates that the use of non-resorbable membranes results in better clinical outcomes compared with bioresorbable collagen (Parrish, Miyamoto, Fong, Mattson, & Cerutis, 2009). However, the second surgery that is required to remove the non-

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resorbable membrane is a disadvantage. Polycaprolactone (PCL) has been introduced as a candidate biomaterial for tissue regeneration because its physical characteristics (*e.g.* strength and biodegradability) are easily manipulated. In addition, PCL exhibits good biocompatibility (Shi et al., 2014). Moreover, PCL biocompatibility and safety have resulted in its approval for clinical use, which suggests the potential of PCL as a base material for GTR membranes.

A disadvantage of a conventional GTR membrane is its lack of osteoconductive and osteoinductive properties. However, hydroxyapatite (HA) has demonstrated both osteoconductive and osteoinductive effects. HA supports osteoblast attachment and proliferation. The calcium and phosphate ions released when HA dissolves enhanced the osteogenic differentiation of osteoblasts (An, Ling, Gao, & Xiao, 2012). Previous reports indicate that HA incorporated in PCL scaffolds promotes osteogenic differentiation in various cell types, including human mesenchymal stem cells and human osteoblast-like cells (Chuenjitkuntaworn et al., 2010; Osathanon et al., 2014).

Jagged1, a canonical Notch ligand, stimulates Notch signaling and enhances the osteogenic differentiation of human periodontal ligament stem cells and human bone marrow derived mesenchymal stem cells as indicated by significantly increase alkaline phosphatase (ALP) expression and mineralization (Dishowitz et al., 2014; Osathanon, Nowwarote, Manokawinchoke, & Pavasant, 2013; Osathanon, Ritprajak et al., 2013). Thus, the aim of the present study was to develop a Jagged1-coated PCL-based membrane as a bioactive GTR membrane and to evaluate the response of human periodontal ligament cells (hPDL) on this membrane *in vitro*.

2. Materials and methods

2.1. Materials and reagents

PCL (Mw 80,000 g/mol), Bovine serum albumin (BSA), Alizarin Red S, β-glycerophosphate, and dexamethasone (Dex) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, and penicillin–streptomycin–amphotericin B solution were purchased from Gibco (Gibco BRL, Carlsbad, CA, USA). The *p*-nitrophenol phosphate was obtained from Invitrogen, USA. Recombinant human Jagged-1/Fc was purchased from R&D systems (Minneapolis, MN, USA) and recombinant protein G was purchased from Zymed, USA. Human IgG Fc fragment was purchased from Jackson Immunoresearch Laboratory, USA. BCA assay kit was purchased from ThermoFisher Scientific, USA. Isol-RNA Lysis was purchased from 5Prime (Gaithersburg, MD, USA). Reverse transcriptase reaction kit was obtained from Promega (Madison, WI, USA). FastStart® Essential DNA Green Master was purchased from Roche Applied Science (IN, USA).

2.2. PCL fabrication

The PCL membrane fabrication technique was modified from a previously described method (Chuenjitkuntaworn, Osathanon, Nowwarote, Supaphol, & Pavasant, 2016; Osathanon et al., 2014). Briefly, 1 g PCL was dissolved in 10 mL chloroform. The PCL incorporated hydroxyapatite (PCL/HA) membrane was prepared by mixing HA powder with PCL solution at 1% w/v. The membranes were made by pouring solution into a mold and allowed to dry for 24 h. Alkaline treatment was performed using NaOH. The membrane was placed in 70% v/v ethanol for 30 min and washed with sterilized deionized water before use.

2.3. Physical property analysis

The membrane surface morphology was evaluated by a scanning electron microscope (SEM; Quanta 250, FEI, Hillsboro, OR, USA). The surface chemical composition was determined using energy dispersive X-ray (EDX) analysis (JSM5401LV, JEOL, Tokyo, Japan). The surface wettability was analyzed using static contact angle measurement (DSA 10, Kruss, Hamburg, Germany). Briefly, 10 µl deionized water was dropped on the specimen surface. The contact angle was measured 20 times per sample. Four samples per membrane type were tested. The membrane ultimate tensile strength, maximum strain, and Young's modulus were analyzed using a Lloyd LRX universal testing machine (Lloyd Instruments Ltd., West Sussex, UK). Eight samples per membrane type were used for the physical tests. The surface roughness evaluation was performed using a contact profilometer (Talyscan 150, TaylorHobson, Leicester, UK). The average surface roughness (R_a) and the root mean square roughness (R_q) were calculated from five samples per membrane type.

2.4. Jagged1 immobilization

An indirect affinity immobilization method was performed as previously described (Manokawinchoke, Nattasit et al., 2017; Osathanon, Ritprajak et al., 2013). Briefly, 50 mg/mL recombinant protein G was incubated on the membranes for 16 h. Next, background blocking was performed. The membranes were incubated with 10 mg/mL BSA for 2 h. The surfaces were then washed three times with sterile PBS and incubated with 10 nM recombinant human Jagged-1/Fc (R&D systems) for 2 h. An equal amount of human IgG, Fc fragment (hFc) was used as the control. The membranes were washed three times with culture medium and cells were immediately seeded on the immobilized surface.

2.5. Human periodontal ligament cell (hPDLs) isolation and culture

The hPDL isolation protocol was approved by the Ethics Committee, Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2016-007). Periodontal tissue was obtained from surgically extracted impacted third molars of healthy adult subjects. The isolation protocol was performed as previously described (Manokawinchoke, Sumrejkanchanakij, Pavasant, & Osathanon, 2017; Osathanon et al., 2015). Briefly, periodontal tissue was scraped from the middle third of the root surface and cultured in DMEM (Gibco BRL, Carlsbad, CA, USA) containing 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B at 37 °C in a humidified 5% carbon dioxide atmosphere. The medium was changed every 48 h. After reaching confluence, the cells were subcultured at a 1:3 ratio. The experiments were performed using hPDL cell lines isolated from 4 different donors.

For osteogenic induction, the cells were maintained in osteogenic medium (growth medium supplemented with 50 µg/mL ascorbic acid, 100 nM dexamethasone, and 10 mM β-glycerophosphate). The medium was changed every 48 h.

2.6. Cell viability

A standard cell line toxicity test was employed. A mouse fibroblast cell line (L929) was maintained in DMEM (Gibco BRL, Carlsbad, CA, USA) containing 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B at 37 °C in a humidified 5% carbon dioxide atmosphere. To directly test toxicity, cell viability was evaluated using a LIVE/DEAD® staining kit per the manufacturer's protocol (Sigma-Aldrich). For indirect toxicity analysis, an MTT assay was performed (Manokawinchoke, Nattasit et al., 2017). Briefly, after incubating the cells in MTT solution for 30 min, the formazan crystals were solubilized using 1 mL of a glycine buffer and dimethysulfoxide mixture. The absorbance was measured at 570 nm.

2.7. Cell morphology evaluation

Cell morphology was evaluated using SEM (Quanta 250, FEI, Hillsboro, OR, USA). Sample preparation was performed as previously