2021년 2월
박사학위논문

Effects of SLPI on the attachment and inflammatory response of periodontal ligament fibroblasts cultured on titanium surface for development of periodontio-integrated implant

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치주인대 섬유모세포의 부착과 염증반응에서 SLPI의 효과

2021년 2월 25일

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지도교수 정 문 진

이 논문을 치의학 박사학위 논문으로 제출함

2020년 10월

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2020년 12월

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ABSTRACT IN KOREAN

치주인대접목 임플란트 개발을 위한 티타늄 표면에 배양된 치주인대 섬유모세포의 부착과 염증반응에서 SLPI의 효과

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치주인대는 치아를 지지할 뿐만 아니라 인접 이틀뼈 및 시멘트질의 수복과 재생에도 관여하는 것으로 알려져 있다. 티타늄은 뛰어난 물리적 강도 및 생체적합성으로 인해 뼈유착률이 높아 임플란트 매식체와 교정용 미니스크류로 널리 사용되고 있다. 임플란트는 치주인대의 제거로 인하여 교합력에 대한 완충효과 및 외부 염증에 대한 면역력이 자연치에 비해 낮다.

분비백혈구단백분해효소억제제(Secretory leukocyte protease inhibitor, SLPI)는 상처치유와 세포증식을 촉진하고, 치주염에서 이틀뼈 흡수를 감소시키며 뼈모세포의 전염증성사이토카인 발현을 억제한다. 또한, 티타늄 표면에서 뼈모세포의 부착 및 분화와 광화를 촉진한다. 따라서, 본 연구에서는 치주인대를 구성하는 세포 중 치주인대 섬유모세포를 이용하여 티타늄 표면 부착과 염증반응에 대한 SLPI의 효과 및 작용기전을 규명하고자 하였다.

티타늄 표면에 배양된 치주인대 섬유모세포에 SLPI를 처리한 후 MTT분석, 면역형광염색, western blotting을 시행하여 치주인대 섬유모세포의 부착과 증식에 대한 SLPI의 기능을 확인하였다. 또한, LPS에 노출된 티타늄 표면의 치주인대 섬유모세포에 SLPI를 처리한 후 RT-PCR, western blotting, NO, PGE_2 분석을 통하여 SLPI의 염증억제 효과를 분석하였다.

SLPI는 티타늄 표면에서 치주인대 섬유모세포의 부착과 생존 및 증식을 촉진시켰다. SLPI를 처리한 치주인대 섬유모세포에서 F-actin형성과 paxillin의 발현 그리고 FAK의 인산화가 대조군에 비하여 증가하였다. 그리고, SLPI를 처리한 치주인대 섬유모세포에서 Grb2, Ras의 발현 및 ERK1/2의 인산화가 대조군에 비하여 증가하였다. 또한, SLPI는 LPS를 처리한 티타늄 표면의 치주인대 섬유모세포에서 TNF-α, IL-1β의 RNA와 단백질 발현을 감소시켰다. 또한, iNOS와 COX-2의 발현을 억제하였으며 NO와 PGE_2의 분비를 억제하였다.

따라서, 본 연구 결과를 토대로 SLPI는 티타늄 표면에서 치주인대 섬유모세포의 부착과 증식을 촉진하고, 염증성 사이토카인 및 매개분자의 분비를 감소시킴으로써 티타늄과 치주인대 섬유모세포의 부착을 강화시켜 임플란트 식립 후 뼈유착과정에서 생물학 및 기계적 안정성을 제공하여 임상적 성공률을 높일 수 있는 효과적인 분자일 것으로 생각된다.

Ⅰ. INTRODUCTION

The periodontal ligament (PDL) is located between two mineralized tissues, bone and cementum, and is involved in the repair and regeneration of alveolar bone and cementum (McCulloch, 1993). The periodontal ligament fibroblast (PDLF) not only expresses alkaline phosphatase (ALP) and osteocalcin (OC) but is also involved in the formation of mineralization nodules and production of interleukin-6 (IL-6), an osteoclast differentiation-inducing factor, by interleukin-1beta (IL-1β) (Shimizu et al., 1992; Basdra and Komposch, 1997; Okada et al., 1997). PDL stem cells express bone regulator factors on the titanium (Ti) surface (Lin et al., 2011).

Ti is widely used as an implant fixture for the replacement of missing teeth and orthodontic mini-screws because of its high success rate of osseointegration and excellent biocompatibility (Kokubo et al., 2010). Unlike natural teeth, this osseointegrated implant is less resistant to the progression of periodontal disease as it does not have the PDL that acts as a protective barrier against the spread of inflammation and bacteria to teeth and alveolar bone (Schou et al., 1993).

Bacterial infection or lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, causes inflammation by inducing the secretion of nitric oxide (NO) and prostaglandin E_2 (PGE_2) act as inflammatory mediators and are involved in the production of cytokines such as tumor necrosis factor-α (TNF-α) and IL-1β. This peri-implantitis promotes the differentiation of osteoclasts and resorption of bone matrix, resulting in the failure of osseointegration after implant placement (Morandini et al., 2011; Pivodova et al., 2011).

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor that promotes wound healing and cell proliferation (Thompson and Ohlsson, 1986) and exerts anti-inflammatory effects during the early response in LPS-stimulated odontoblasts (Choi et al., 2009). In addition, it reduces the expression of inflammatory cytokines TNF-α and IL-1β and the resorption of alveolar bone in periodontitis (Lee et al., 2016) and suppresses the destruction of cartilage and bone

via inhibition of TNF-α and NO production in arthritis (Song et al., 1999). Recent studies have reported that SLPI promotes the adhesion of osteoblasts to the Ti surface by increasing the formation of F-actin, expression of paxillin, and focal adhesion kinase (FAK) phosphorylation (Jeong et al., 2015b), and regulates genes involved in osteoblast differentiation and mineralization (Choi et al., 2016).

Focal adhesions (FAs) associated with cell adhesion are complexes composed of signaling proteins such as integrin, FAK, and paxillin, which connect the cytoskeleton to the extracellular matrix and are involved in cell migration, growth, differentiation, and survival (Giancotti and Ruoslahti, 1999; Pivodova et al., 2011). FAK regulates the signal transduction pathways involved in cell adhesion, proliferation, and migration (Avizienyte et al., 2002; Mitra et al., 2005). The growth factor receptor-bound protein 2 (Grb2) induces cell proliferation by activating Ras, which leads to the stimulation of extracellular regulated kinase 1 and 2 (ERK1/2) that act as adapter proteins connecting the epidermal growth factor receptor (EGFR) signaling pathway (Giubellino et al., 2008).

To date, studies are underway examining strategies for the improvement of implant placement and durability, such as changes in implant shape and design (Chen et al., 2005; Lee et al., 2005), surface modification to control cell behavior (Oates et al., 2007), nanostructured surface coatings (Catledge et al., 2002; Wang et al., 2005; Hedia, 2007), and the use of biomolecules such as growth factors (Park et al., 2006). Recently, various studies have been conducted on periodontio-integrated implants for the regeneration of periodontal tissues to compensate for the fundamental limitations of such osseointegrated implants (Gault et al., 2010; Lee et al., 2017; Garg and Deepa, 2018; Kaoru et al., 2018). The application of this periodontio-integrated implant is capable of providing high mechanical stability in the long term due to early osseointegration and formation of periodontal tissue similar to natural teeth. In addition, studies have been carried out on the discovery of biological molecules capable of regulating peri-inflammation after the implant and the function of cells involved in osseointegration processes such as osteoblasts (Chen et al., 2013; Choi et al., 2015; Jeong et al., 2015b; Choi et al., 2016; Jeong

and Jeong, 2016). However, there is no research on the effective biological molecules that promote PDLF activity on the Ti surface.

Based on previous studies demonstrating that SLPI inhibits the excessive inflammatory response via regulation of gene expression associated with inflammation and promotes the activity of osteoblasts on the Ti surface, thus SLPI may be involved in the adhesion of PDLF to the Ti surface and the regulation of the inflammatory response. Therefore, the purpose of this study was to investigate the function of SLPI in the inflammatory response and adhesion of PDLF on the Ti surface before the modeling of periodontio-integrated implants.

Ⅱ. MATERIALS AND METHODS

Ⅱ-1. Titanium samples

Two types of Ti discs, 20 ㎜ and 48 ㎜ in diameter and 2 ㎜ in thickness, were used. Polished commercially pure titanium (Cp-Ti) discs were prepared using a previously described method (Jeong et al., 2015b).

Ⅱ-2. Cell culture and treatment of LPS and SLPI

The periodontal ligament fibroblasts (PDLF) were plated on the Ti discs and incubated in a CO_2 incubator. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (WelGENE, Korea) containing 10% fetal bovine serum (WelGENE) and 1% antibiotic antimycotic solution (WelGENE). The PDLF were treated with LPS (Sigma, USA) (200 ng/㎖) (LPS/PDLF) followed by SLPI (R&D systems, USA) (1 ㎍/㎖) treatment (LPS/SLPI/PDLF).

Ⅱ-3. Cell adhesion and proliferation assay

The PDLFs plated on the Ti discs (2×10^5 cells/㎖) were incubated in serum-free DMEM with or without 1 ㎍/㎖ SLPI (R&D systems) for 10, 30, and 60 min for the adhesion assay and 12, 24, and 48 h for the proliferation assay. Viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay, as described previously (Jeong et al., 2015b).

Ⅱ-4. Western blotting

Total protein was extracted from PDLFs using NP-40 lysis buffer. A total of 30 ㎍ of the proteins were subjected to electrophoresis, and the separated proteins

were transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, GER). The membrane was blocked with 5% bovine serum albumin (BSA; Bioshop, USA) or 5% skim milk (Bioshop) for 1 h at room temperature and blotted with the primary antibodies for 16 h at 4℃. Antibodies included 1:1,000 of anti-rabbit phosphorylated FAK (pFAK, Cell signaling, USA), 1:1,000 of anti-rabbit FAK (Upstate, USA), 1:2,500 of anti-rabbit phosphorylated ERK1/2 (pERK1/2, Cell signaling), 1:2,500 anti-mouse ERK1/2 (Upsate), 1:40,000 of anti-mouse paxillin (Transduction Laboratories, USA), 1:1,000 of anti-mouse Grb2 (Upstate), 1:1,000 of anti-rabbit Ras (Upstate), 1:1000 of anti-rabbit TNF-α, 1:1000 of IL-1β (Abcam, England), 1:1000 of anti-rabbit iNOS, 1:1000 of anti-rabbit COX-2 (Cell signaling), and 1:2,500 of anti-mouse β-actin (Santa Cruz Biotechnology, USA). After washing, the membrane was blotted with 1:5,000 of either HRP-conjugated goat anti-rabbit or mouse-IgG (Enzo Life Sciences, USA). The membrane was developed by using an ECL solution (Merck Millipore) in an X-ray film (Fuji Film, Japan). The density of the expressed bands was measured using the Science Lab Image Gauge (Fuji Film).

Ⅱ-5. Reverse transcription and polymerase chain reaction (RT-PCR)

Total RNA was extracted from PDLFs using the RiboEX^{TM} reagent (GeneAll, Korea) according to the manufacturer's instructions. Total RNA (1 ㎍) was used to synthesize complementary DNA (cDNA) with RT Premix (GeNet Bio, Korea). PCR was performed in a thermocycler (Takara Bio Inc., Japan) using a PCR premix (GeneAll) and 1 ㎕ cDNA. The following primers (Bioneer, Korea) were used for RT-PCR analysis: (1) TNF-α forward 5′-ACG GCA TGG ATC TCA AAG AC-3′, reverse: 5′-CGG ACT CCG CAA AGT CTA AG-3′ (2) IL-1β forward, 5′-CAG GCA GGC ACT ATC ACT CA-3′, reverse: 5′-GGC CAC AGG TAT TTT GTC GT- 3′, and (3); GAPDH forward, 5′-CCA TGG AGA AGG CTG GG-3′, reverse: 5′-CAA AGT TGT CAT GGA TGA CC-3′. The annealing temperature for each primer and number of cycles were as follows: (1) TNF-α 58℃ (35 cycles), (2) IL-1β 59℃ (36 cycles), and (3) GAPDH 60℃ (30 cycles). The density of the

expressed bands was measured using a Science Lab Image Gauge (Fuji Film).

Ⅱ-6. Immunofluorescence staining

The cells (1×10^5 cells/㎖) on the Ti discs were incubated in serum-free DMEM with or without 1 ㎍/㎖ SLPI for 30, 60 min, and 3 h. The cells were fixed with 4% paraformaldehyde for 10 min and treated with 0.15% Triton X-100 for 10 min. The cells were blocked with 1% BSA for 1 h and incubated with anti-mouse paxillin (1 ㎍/㎖, Transduction Laboratories) for 16 h at 4℃. After washing, the cells were incubated with the goat-anti-mouse IgG conjugated with FITC (ThermoFisher Scientific, USA) for 1 h at room temperature. For F-actin staining, the cells were incubated with 50 ㎍/㎖ of FITC-conjugated phalloidin (Sigma) for 40 min at room temperature. The cells on the Ti discs were mounted using a mounting medium containing DAPI (Vector Lab, USA). The actin-labeled cells and paxillin contacts were observed and imaged by fluorescence microscopy (Carl Zeiss, GER). The number of paxillin contacts per cell at each time point was counted. The data show the mean of 50 cells counted at each time point.

Ⅱ-7. NO and PGE_2 assay

NO production was measured in the supernatants of the cells cultured on the Ti surface by the standard Griess reaction. The Griess reagent was prepared by mixing equal volumes of 1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride. A total of 100 ㎕ of this reagent was mixed with the 100 ㎕ of the cellular supernatant and incubated at room temperature for 15 min. The absorbance of the chromophore formed was measured at 540 nm using an automated microplate reader (BioTek Instruments, USA). NO concentrations were calculated using the $NaNO_2$ standard curve. The concentration of PGE_2 was measured in the cellular supernatants on the Ti surface using a PGE_2 enzyme-linked immunosorbent assay kit (R&D Systems) according to the manufacturer's instructions.

Ⅱ-8. Statistical analysis

All experiments were performed in triplicates. All data are reported as mean and standard deviation determined using SPSS 25.0 (SPSS, USA). Significant differences ($*p < 0.05$) were determined using the independent samples t-test.

Ⅲ. RESULTS

Ⅲ-1. SLPI increases the adhesion and proliferation of PDLF on the Ti surface

MTT analysis was conducted to investigate the initial effect of SLPI on the adhesion of PDL fibroblasts (PDLF) on the Cp-Ti surface. The results showed that the adhesion of PDLF and SLPI-treated PDLF (SLPI/PDLF) exhibited the same increasing pattern from 10 min to 60 min. SLPI/PDLF showed a significant increase in the adhesion, approximately 1.2 times at 30 min and 60 min compared to PDLF (Fig. 1).

In PDLF, the phosphorylation of FAK remained unchanged until 30 min after cell adhesion and increased from 60 min. In SLPI/PDLF it increased from 10 to 60 min. The phosphorylation of FAK in SLPI/PDLF was significantly increased by 1.3 times after 10 and 30 min compared to that in PDLF (Fig. 2A and B).

Paxillin expression in PDLF increased gradually from 10 min to 60 min. In SLPI/PDLF, the expression of paxillin gradually increased up to 30 min and increased by 1.3 times at 60 min. When comparing paxillin expression between the two groups, SLPI/PDLF showed a significant increase from 10 min to 60 min compared to PDLF (Figs. 2A and B).

The distribution of F-actin protein observed using immunofluorescence showed a gradual increase in the formation of F-actin at 30 min, 60 min, and 3 h in PDLF as well as SLPI/PDLF. However, when comparing the two cell groups, the formation of F-actin in SLPI/PDLF was significantly increased after 30 min and 3 h compared to that in PDLF (Fig. 3).

Immunofluorescence analysis was performed using paxillin protein to confirm the increase in focal adhesion of PDLF by SLPI treatment. The expression pattern of paxillin protein was observed as the head of an arrow along the edge of the cell membrane. Both PDLF and SLPI/PDLF showed a time-dependent increase. In addition, the number of paxillin contact points of SLPI/PDLF significantly increased

from 30 min to 3 h compared to those of PDLF (Figs. 4A and B).

Cell proliferation of PDLF and SLPI/PDLF on the Cp-Ti surface gradually increased from 12 to 24 h. The proliferation of SLPI/PDLF increased by approximately 1.2 times than that of PDLF at all time points (Fig. 5). The expression patterns of Grb2, Ras, and pERK1/2 proteins was increased in a time-dependent manner from 10 to 60 min. Their expression was higher in SLPI/PDLF at all times compared to that in PDLF. The expression of Grb2 protein was 2.1, 1.2, and 1.2 times higher in SLPI/PDLF than that in PDLF at 10, 30, and 60 min, respectively. The expression of Ras protein was 1.9, 1.3, and 1.2 times higher in SLPI/PDLF than that in PDLF at 10, 30, and 60 min, respectively. ERK1/2 phosphorylation was 2.3, 1.2, and 1.6 times higher in SLPI/PDLF than that in PDLF at 10, 30, and 60 min, respectively (Figs. 6A and B).

Ⅲ-2. SLPI decreases the expression of pro-inflammatory cytokines, iNOS/COX-2, and the production of NO/PGE_2 in PDLF on the Ti surface

The expression of mRNA and protein corresponding to TNF-α and IL-1β significantly decreased at all time points in PDLF treated with LPS and SLPI (LPS/SLPI/PDLF) compared to that in PDLF treated with LPS only (LPS/PDLF). The mRNA expression of TNF-α decreased 2 times, 1.2 times, 1.4 times, 1.3 times, and 1.2 times in LPS/SLPI/PDLF from 2 h to 24 h, respectively, compared to that in LPS/PDLF. IL-1β mRNA expression decreased by 2.6 times, 2 times, 2.1 times, 2.2 times, and 1.8 times in LPS/SLPI/PDLF at 2, 4, 6, 12, and 24 h, respectively, compared to that in LPS/PDLF (Figs. 7A and B).

TNF-α protein expression in LPS/SLPI/PDLF decreased 1.3, 1.4, 1.5, 1.8, and 1.7 times at 2, 4, 6, 12, and 24 h, respectively, compared to that in LPS/PDLF. IL-1β protein expression in LPS/SLPI/PDLF decreased 2.2 times, 1.8 times, 1.4 times, 1.4 times, and 1.3 times at 2, 4, 6, 12, and 24 h, respectively, compared to that in LPS/PDLF (Figs. 8A and B).

In LPS/SLPI/PDLF, iNOS protein expression decreased 1.2 times, 1.5 times, 1.6 times, 1.5 times, and 1.4 times at 2, 4, 6, 12, and 24 h, respectively, compared to

that in LPS/PDLF. In LPS/SLPI/PDLF, COX-2 protein decreased by 1.5 times, 1.6 times, 1.3 times, 1.5 times, and 1.5 times compared to that in LPS/PDLF, respectively (Figs. 9A and B).

The amount of NO secreted was significantly decreased 1.1, 1.2, and 1.2 times in LPS/SLPI/PDLF compared to that in LPS/PDLF at 6, 12, and 24 h, respectively (Fig. 10A). The amount of PGE_2 secreted decreased 1.3 times and 1.4 times in LPS/SLPI/PDLF at 12 and 24 h, respectively, compared to that in LPS/PDLF (Fig. 10B).

Ⅳ. DISCUSSION

The periodontal ligament (PDL) contains fibers, which are secreted and maintained by cells such as fibroblasts, osteoblasts, osteoclasts, and cementoblasts as well as epithelial cells, undifferentiated mesenchymal cells, matrix, blood vessels, and nerves (Nuñez et al., 2012). Interestingly, among PDL cells, periodontal ligament fibroblasts (PDLF) express bone formation-related markers such as alkaline phosphatase (ALP), osteocalcin (OC), bone morphogenic protein (BMP), bone sialoprotein (BSP), and osteopontin (OPN) and which exhibit characteristics similar to osteoblasts such as calcified nodules formation in vitro (Ohshima et al., 1988; Arceo et al., 1991; Ivanovski et al., 2001). PDLFs not only play a role in synthesizing and secreting PDL fibers in the process of healing and regeneration of periodontal tissues but are also involved in the secretion of the matrix proteins of bone and proteins related to mineralization. Hence, they play an important role in replacing some of the main functions of osteoblasts and cementoblasts.

Dental implants are osseointegrated implants that directly form structural and functional bonding without intervening connective tissue between bones and implants. Titanium (Ti), which is the most commonly used dental implant, was initially used without surface treatment. However, since such implants require a long bone adhesion time, various surface treatment technologies have been developed to improve bone adhesion. Among these, the surface modification method, which physically and chemically controls the roughness of the surface of the implant, increases the contact area between the implant and alveolar bone, along with the affinity with the osteoblast. It induces rapid adhesion of cells and improves bone adhesion (Anders et al., 2010). However, such surface modification only exhibits features of osteoconduction, and the effect of osteoinduction cannot be expected. These osseointegrated implants lack the PDL of natural teeth. Hence, despite many advantages, they exhibit physical properties that are vulnerable to various biomechanical problems such as stress absorption and concentration (Minkle

et al., 2014). Therefore, in addition to Ti surface modification to increase osteoconduction and osteoinduction, the formation of periodontal tissue similar to that of natural teeth after implantation as well as fusion with bone tissue is expected to promote fusion with the surrounding tissues after implantation.

Recently, to compensate for the fundamental limitations of osseointegrated implants, various studies on periodontio-integrated implants examining the regeneration of periodontal tissue by implanting PDL around the implant in tissue engineering methods have been conducted (Gault et al., 2010; Lee et al., 2017; Garg and Deepa, 2018; Kaoru et al., 2018). The formation of periodontal tissues such as cementum and PDL was promoted after the transplantation of a collagen sponge scaffold cultured with canine PDL cells and a CaP polycaprolactone (CaP-PCL) coated scaffold with rat PDL cell sheets (Tsumanuma et al., 2011; Hongxia et al., 2014). In a study in which a ligaplant cultured on a PDL cell cultured on a Ti pin coated with hydroxyapatite (HA) was implanted in the alveolar bone of a canine, it was confirmed that a morphology similar to the PDL was formed around the implant. When it was implanted in humans, mechanical properties similar to those of natural teeth and regeneration of the alveolar bone and lamina dura were observed (Gault et al., 2010). These results demonstrate that the application of the periodontio-integrated implant along with the modification of the implant surface can lead to the development of implants that provide high mechanical stability in the long term by early osseointegration and the formation of periodontal tissue similar to natural teeth.

In recent years, the demand for implants and aesthetic treatments of the elderly and chronically ill persons is increasing; therefore, studies for the development of bio-implants with surface treated bioactive substances promoting bone formation, such as bone formation proteins or growth factors, are underway to shorten transplant time, promote early osseointegration, and increasing bones. Fibronectin increases the adhesion of osteoblasts and bone formation on the surface of the Ti. Transforming growth factor-beta 1 (TGF-β1) and bone morphogenic protein-7 (BMP-7) promote osteoblast differentiation and increase the activity of ALP (Zhang et al., 2003; Pugdee et al., 2007; Chen et al., 2013). In addition, a secretory

leukocyte protease inhibitor (SLPI) and thymosin β4 (Tβ4) are known to promote adhesion, proliferation, and differentiation of osteoblasts on the Ti surface (Choi et al., 2015; Jeong et al., 2015b; Choi et al., 2016; Jeong and Jeong, 2016). Among them, bone morphogenic protein-2 (BMP-2) is a physiologically active substance applied to implants to improve regeneration of bone tissue and surrounding bone matrix and has been widely used in clinical practice in recent years. However, BMP-2 is only used for implant placement using maxillary sinus elevation due to complications such as excessive bone growth and ectopic bone formation. Since it is easily decomposed by enzymes in the body and due to its short duration of action and high price, the need for the development of bioactive substances that can replace them is emerging (Rosa-María et al., 2015). In addition, fusion with surrounding tissues, including bones as well as inhibition of inflammation, is also an important factor for a successful implant procedure. Antibiotics used in the treatment of infectious diseases have many problems and side effects such as an increase in resistant bacteria due to long-term use, a decrease in the effectiveness of therapeutic drugs, multiple infections, and an increase in medical expenses.

The process of osseointegration, which is one of the important factors for clinical success based on the biological stability of the implant and surrounding tissues after implant placement, involves bone affinity phase, bone conduction phase, and bone adaptation phase. The bone affinity phase involves a period of approximately 1 month from implant placement until a woven bone is formed. During this period, blood coagulation reaction by platelets and deposition of osteoid by osteoblasts occurs on the implant surface. The bone conduction phase involves a period when a woven bone is replaced with lamellar bone and parallel-fibered bone, and a larger amount of bone is deposited on the implant surface. The process takes approximately 2-3 months. The bone adaptation phase involves the entire life from 3 or 4 months after implantation and involves bone remodeling via replacement by a mature bone (Parithimarkalaignan and Padmanabhan, 2013). Among these osseointegration processes, anti-inflammatory reactions and cell adhesion are the most important parts that must precede the deposition of bone on the implant surface during the bone affinity phase. Subsequently, in the bone conduction and

the bone adaptation phase, it is necessary to increase the bone matrix formation by cell division. Therefore, the purpose of this study was to find an effective new molecule for increasing PDL formation and fusion with alveolar bones after implantation before application of periodontio-integrated implants to the animal model. Thus, the function of SLPI, a molecule known to play an important role in the anti-inflammatory reaction and bone formation process, was investigated in PDLF on the Ti surface.

SLPI is a non-glycosylated protein with a molecular weight of 11.7 kDa and is found in the saliva, seminal plasma as well the mucus of the cervix and bronchi (Thompson & Ohlsson, 1986; Moreau et al., 2008). As a protease inhibitor, the function of SLPI is to inhibit the activity of serine proteases such as elastase and cathepsin G of neutrophil, trypsin and chymotrypsin of pancreatic acinar cells, and chymase of mast cells (Fritz, 1988; Walter et al., 1996; Wright et al., 1999). In addition, SLPI is involved in cell proliferation, migration, inhibition of inflammatory response, and acceleration of matrix remodeling during the wound healing process in the skin and oral mucosa (Ashcroft et al., 2000; Angelov et al., 2004). SLPI not only acts as an anti-inflammatory factor in the inflammatory response, but also demonstrates anti-bacterial and anti-fungal effects and the inhibition of HIV infection (McNeely et al., 1995; Shugars & Wahl, 1998; Sallenave, 2002). SLPI reduces the expression of TNF-α by inhibiting the activation of nuclear factor-kappaB (NF-κB) in LPS-treated monocytes and also suppresses the early inflammatory response of odontoblasts induced by LPS stimulation via the inhibition of the activation of NF-κB (Taggart et al., 2005; Choi et al., 2009). In addition, SLPI directly inhibits NF-κB activation, which induces the expression of tumor necrosis factor-α (TNF-α) and interleukin-1beta (IL-1β) after irradiation with 660nm red LED in LPS-stimulated macrophages (Jeong et al., 2015a). SLPI inhibited the inflammatory response and destruction of the surrounding cartilage and bone by inhibiting the production of TNF-α and nitric oxide (NO) in rat knee arthritis (Song et al., 1999). It also inhibited the resorption of the alveolar bone and the expression of inflammatory cytokines such as TNF-α and IL-1β in rat periodontitis (Lee et al., 2016). In addition, in human PDL cells injected with SLPI

siRNA, SLPI decreased the expression of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and toll-like receptor 2, 4 (TLR2, 4), which were increased upon LPS stimulation, and inhibited NF-κB activation (Svensson et al., 2017). SLPI is involved in the differentiation of odontoblasts and mineralization of dentin during dentinogenesis by increasing the expression of BSP, OC, osteonectin (ON), and collagen type I (Col I) (Jeong et al., 2015c). It also promotes the adhesion and differentiation of osteoblasts and mineralization of the bone matrix on the Ti surface (Jeong et al., 2015b; Choi et al., 2016).

Cell adhesion is essential for the regulation of various cellular processes, including cell proliferation, migration, differentiation, and survival. In this cell adhesion process, focal adhesion (FA) is a mechanical structure that mediates the binding between the cell and extracellular matrix via integrin and plays an important role in physiological regulation such as development, wound healing, inflammatory response, migration, and differentiation of cells (Giancotti and Ruoslahti, 1999). In the signaling process for FA formation, focal adhesion kinase (FAK), a signaling molecule, and paxillin, a cytoskeletal adapter protein, are involved in regulating the integration and processing of signals related to adhesion and growth factors. Tβ4 increased the adhesion of osteoblasts to the Ti surface by increasing the expression of paxillin mediated by FAK (Choi et al., 2015). In epithelial and mesenchymal tumor cells overexpressing FAKs such as colon cancer, breast cancer, and sarcoma in humans, the invasion was promoted by metastasis (Owens et al., 1995). Paxillin expression was decreased in osteoblasts of FAK knockout mice (Castillo et al., 2012). The expression of paxillin was observed in melanoma cells by immunofluorescence staining after treatment with genistein, a major component of soybean. The number of paxillin observed as the shape of arrowheads around the cell membrane was decreased. In the case of F-actin, the distribution of G-actin was increased in melanoma cells treated with genistein (Cui et al., 2017). In the Ti surface treated with SLA/NaOH, F-actin of MG-63 cells was increased, and more cells were attached compared to the control (Park et al., 2014). Plasminogen activation inhibitor-1 (PAI-1) promoted the migration of human smooth muscle cells and the formation of the actin cytoskeleton (Degryse et al.,

2004), and SLPI promoted the adhesion of osteoblasts on the Ti surface (Jeong et al., 2015b).

The results of this study demonstrated that SLPI significantly attached PDLF to the Ti surface in a time-dependent manner and increased the expression of phosphorylated FAK (pFAK) and paxillin proteins compared to that observed in the control group. In addition, immunofluorescence staining analysis was performed to confirm the morphological expression of F-actin and paxillin protein. The formation of F-actin and the number of paxillin contacts were increased in the SLPI-treated cell group compared to that in the control group. Therefore, SLPI increased initial cell adhesion and F-actin via the FAK/paxillin signaling pathway related to FA, which effectively attached the PDLF to the Ti surface, suggesting an effective molecule involved in the promotion of cell proliferation and osseointegration.

Stable adhesion of cells to the extracellular matrix or the surface can induce survival-related signaling pathways, such as increased proliferation and decreased apoptosis. pFAK directly interacts with the SH2 domain of growth factor receptor-bound protein 2 (Grb2) during cytoskeleton formation and cell adhesion, which activates Ras protein, resulting in increased phosphorylation of extracellular regulated kinase 1 and 2 (ERK1/2) related to cell proliferation (Koch et al., 1991; Giancotti and Ruoslahti, 1999). FAK overexpression in human fetal pancreatic islet cells (islets of Langerhans) increased the survival of islet cells compared to the control group by increasing the phosphorylation of ERK1/2 (Saleem et al., 2009). In addition, tissue-type plasminogen activator (tPA) promotes mitosis of renal stromal fibroblasts by activating FAK/ERK1/2 signaling (Hao et al., 2010). Tβ4 reduced apoptosis induced by UV or TNF via FAK/paxillin signaling in fibroblasts (Niu and Nachmias, 2000), and increased viability and proliferation of endothelial progenitor cells (EPCs) via increased phosphorylation of ERK (Zhao et al., 2011). T β4 promoted the proliferation of osteoblasts on the Ti surface by activating FAK/Grb2/Ras/pERK1/2 signaling (Choi et al., 2015). SLPI promoted the proliferation of endometrial epithelial cells by activating the Ras/MAPK signaling pathway (Rasool et al., 2010). In addition, SLPI increased cell proliferation and migration during the wound healing process of oral mucosa and keratinized cells

(Wang et al., 2011) and promoted proliferation of osteoblasts on the Ti surface via the FAK/Grb2/Ras/pERK1/2 pathway (Jeong et al., 2015b).

In this study, SLPI significantly increased the proliferation of PDLF on the Ti surface in a time-dependent manner. In addition, the expression of Grb2 and Ras proteins and phosphorylation of ERK1/2 was significantly increased in the PDLF treated with SLPI compared to that in the control group. Therefore, SLPI increased the proliferation of PDLF via the Grb2/Ras/ERK1/2 signaling transduction process related to mitosis on the Ti surface, suggesting that it induces an increase in the secretion and mineralization of collagen by increasing the division of PDLF.

Peri-implantitis caused by a bacterial infection in the oral cavity and toxicity of the implant material, one of the causes of failure in osseointegrated implant procedures, unlike natural teeth, can occur commonly because there is no PDL around the implant, including a vascular network. Therefore, for the development of implants without peri-implantitis involving the suppression of inflammation and reduction of bacterial adhesion, many studies are underway for the development of novel strategies for the improvements in implant, such as the antimicrobial coating on the implant surface (Brohede et al., 2009), TiN or F coating on the Ti surface, and anodic oxidation of the Ti surface using sodium chloride electrolyte (Yoshinari et al., 2001; Shibata et al., 2004). However, there is no reported study examining the inhibition of peri-implantitis, which can also occur in periodontio-integrated implants.

Lipopolysaccharide (LPS) is a major component of the cell wall of gram-negative bacteria that induces peri-implantitis, LPS of *P. gingivalis* and *A. actinomycetemcomitans* stimulates the production of pro-inflammatory cytokines such as TNF-α, IL-6, and interleukin-8 (IL-8) from monocytes or macrophages (Cazalis et al., 2008; Holden et al., 2014). In addition, LPS of *E. coli,* a gram-negative anaerobic bacterium, increases the production of TNF-α, IL-1β, and IL-6 in THP-1 cells, a human monocyte cell line (Zhang et al., 2008). It also increases the expression of TLR4, NF-κB, and pro-inflammatory cytokines such as colony-stimulating factor 2 (CSF2), IL-2, and IL-6 in human PDL cells (Sun et al., 2008). The expression of TNF-α and IL-1β was increased in rat periodontitis

tissue and supernatant of cultured whole blood induced by a combination of ligation and topical application of LPS (Jonarta et al., 2010). In human PDLF, LPS increased the expression of TNF-α, IL-1β, and receptor activator of nuclear factor kappa-B ligand (RANKL), and in the periodontal tissues of canine, it increased the expression of IL-6 and IL-8 and caused the destruction of periodontal tissues and resorption of the alveolar bone (He et al., 2018). In addition, LPS increased the expression of TNF-α and IL-1β in osteoblasts (Die et al., 2012) and promoted osteoclast activity by increasing the expression of RANKL and decreasing the expression of osteoprotegerin (OPG) (Yamano et al., 2010).

TNF-α and IL-1β are pro-inflammatory cytokines that induce acute inflammation. They are mainly secreted from macrophages; however, they might also be secreted from PDL cells, gingival fibroblasts, and osteoblasts. TNF-α and IL-1β accelerate the proliferation and differentiation of osteoclasts by stimulating the formation of prostagrandin E_2 (PGE_2) and RANKL in the fibroblasts of periodontal tissue (Popova and Mlachkova, 2010). Moreover, TNF-α and IL-1β induce bone resorption and destruction of the tissue via the degradation of the extracellular matrix by increasing the expression of collagenolytic enzymes and matrix metalloproteinases (MMPs) (Page et al., 2000; Graves and Cochran, 2003). Expression of TNF-α and IL-1β increased in saliva and gingival crevicular fluid (GCF) of periodontitis patients, which caused more serious inflammation in periodontitis lesions. It was higher in the sera of patients with chronic periodontitis, cardiovascular disease, and diabetes (Masada et al., 1990; Martínez-Aguilar et al., 2019). The antioxidant pyrrolidine dithiocarbamate (PDTC) decreased the expression of IL-1, IL-6, IL-8, and TNF-α in peri-implantitis periodontal tissues of canine and PDLF stimulated with LPS. It also inhibited NF-κB, p65, and TRL4 (He et al., 2018). Curcumin repressed the inflammatory response and reduced the loss of the alveolar bone by decreasing the expression of TNF-α, IL-6, and RANKL in rat periodontitis (Zhou et al., 2013). Bovine lactoferrin (bLF) reduced the expression of TNF-α in LPS-treated gingival tissue and PDL of rats and repressed the destruction of alveolar bone related to periodontitis by reducing the number of osteoclasts on the surface of alveolar bone (Yamano et al., 2010).

In this study, SLPI significantly reduced the expression of TNF-α and IL-1β, which was increased in LPS-treated PDLF on the surface of the Ti. With respect to the relationship between previously reported functions of SLPI and LPS, TNF-α, and IL-1β, the inhibitory effect of pro-inflammatory cytokines of SLPI on the Ti surface was suggested to alleviate peri-implantitis by reducing inflammation of PDLF attached to the surface of the Ti. Therefore, to identify the inflammation inhibitory mechanism of SLPI, here, we investigated the expression of inducible nitric oxide synthase (iNOS)-NO and cyclooxygenase-2 (COX-2)-prostaglandin E_2 (PGE_2), which are signal-transducing molecules related to TNF-α and IL-1β that are the pro-inflammatory cytokines repressed by SLPI.

NO is a free radical produced from l-arginine by nitric oxide synthase (NOS). NO is an important cell signal-transducing molecule that participates in various physiological functions of mammals, including vasodilatation, relaxation of smooth muscles, neural transmission, and immune reactions. NOS is produced in three subtypes: endothelial NOS (eNOS), neuronal NOS, (nNOS), and inducible NOS (iNOS). NO produced by each enzyme differs in expression, patterns of control, and tissue distribution. eNOS and nNOS are involved in intracellular signaling transduction, protein activity, or inhibition by continuously producing low concentrations of NO in normal cells. In contrast, iNOS acts as a cytotoxic substance by producing a large amount of NO in neutrophils, mast cells, monocytes, and macrophages via the stimulation of proinflammatory cytokines or LPS. Overexpression of iNOS or excessive production of NO aggravates the inflammatory response, causing tissue damage, genetic mutation, and nerve damage (Beckman and Koppenol, 1996; Kendall et al., 2001).

Cyclooxygenase (COX) is the main enzyme that accelerates the reaction by reducing arachidonic acid to prostaglandin (PG). There are two subtypes of COX. COX-1 is constantly expressed in most tissues and is responsible for physiological functions, including platelet aggregation, gastric mucosal protection, and control of kidney function (Queiroz-Junior et al., 2009). COX-2 is involved in the inflammatory response and cell proliferation mediated by pro-inflammatory cytokines or growth factors, respectively (Holzhausen et al., 2005). PGE_2 synthesis

by COX-2 leads to inflammation, angiectasia, edema, fever, and pain, and is involved in tissue damage by inducing the production of MMPs in inflammatory diseases such as periodontitis and rheumarthritis (Shimizu et al., 2000). Moreover, PGE_2 secreted from macrophages induces bone resorption by activating osteoclasts during the process of osseointegration (Landgraeber et al., 2014).

TNF-α and IL-1β increased the expression of iNOS in human corneal cells, pulmonary epithelial cells (Donnelly and Barnes, 2002; Kim et al., 2015), and LPS and interferon-gamma (IFN-ɣ) enhanced the expression of iNOS in human gingival fibroblasts (Daghigh et al., 2002). Scutellaria baicalensis (SB) extract inhibited the expression of TNF-α, IL-6, and NF-κB activation increased by LPS in PDL cells. It significantly repressed the production of NO and PGE_2 by reducing the expression of iNOS and COX-2 (Ming et al., 2018). Expression of iNOS and COX-2 was increased in macrophages, neutrophils, and lymphocytes of periodontitis tissue compared to that in the normal tissue of humans and mice (Jagadish and Mehta, 2014). Moreover, Tβ4 decreased inflammatory response during hypoxia by repressing synthesis of NO and PGE_2 by controlling the expression of iNOS and COX-2 in H_2O_2 stimulated osteoblasts on the Ti surface (Choi et al., 2018). The phenolic compound hydroxytyrosol (HT) reduced the expression of iNOS and COX-2 increased by H_2O_2 in chondrocytes of osteoarthritis (Facchini et al., 2014) and flavonoid of persimmon leaves reduced the expression of iNOS and COX-2 by inhibiting the expression of NF-κB/p65 in H_2O_2 stimulated osteoblasts (Sun et al., 2014). NO induced apoptosis of macrophages and osteoblasts stimulated with LPS and inhibition of NO synthesis in the periapical lesions of rats reduced inflammation and inhibited bone resorption (Lin et al., 2007). Aminothiazoles reduced the production of PGE_2 in LPS-stimulated PDL cells along with the development of monocytes into osteoclasts (Kats et al., 2019).

Similar to other studies, SLPI significantly reduced the protein expression of iNOS and COX-2 and synthesis of NO and PGE_2, which is increased upon LPS stimulation in PDLF on Ti surface compared to that in the control group. SLPI inhibits signaling pathways via iNOS/NO and COX-2/PGE_2, indicating that it can suppress peri-implantitis by reducing bacterial inflammation and oxidative stress

caused by hypoxia in PDLF on the Ti surface.

In summary, SLPI can effectively enhance initial osseointegration by promoting collagen synthesis and mineralization afterward via adhesion of PDLF and inhibition of inflammation. To verify this, further studies on the genes and proteins of collagen and mineralization-related genes in inflamed PDLF and on the SLPI function for inflamed osteoblast differentiation and activation factor of osteoclasts should be conducted (Fig. 11).

V. REFERENCES

Ana Carolina de Faria Morandini, Carla Renata Sipert, Erivan Schnaider Ramos-Junior, Daniel Thomas Brozoski, Carlos Ferreira Santos : Periodontal ligament and gingival fibroblasts participate in the production of TGF-β, interleukin (IL)-8 and IL-10. Braz Oral Res 25: 157-162, 2011.

Anders Palmquist, Omar M Omar, Marco Esposito, Jukka Lausmaa, Peter Thomsen : Titanium oral implants: surface characteristics, interface biology and clinical outcome. J R Soc Interface 7: S515-527, 2010.

Angelov N, Moutsopoulos N, Jeong MJ, Nares S, Ashcroft G, Wahl SM : Aberrant mucosal wound repair in the absence of secretory leukocyte protease inhibitor. Thromb Haemost 92: 288-297, 2004.

Arceo N, Sauk JJ, Moehring J, Foster RA, Somerman MJ : Human periodontal cells initiate mineral-like nodules in vitro. J Periodontol 62: 499-503, 1991.

Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song XY, Wahl SM : Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat Med 6: 1147-1153, 2000.

Avizienyte E, Wyke AW, Jones RJ, McLean GW, Westhoff MA, Brunton VG, Frame MC : Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. Nat Cell Biol 4: 632-638, 2002.

Basdra EK, Komposch G : Osteoblast-like properties of human periodontal ligament cells: an in vitro analysis. Eur J Orthod 19: 615-621, 1997.

Beckman JS, Koppenol WH : Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and the ugly. Am J Physiol 271: 1424-1437, 1996.

Brohede U, Forsgren J, Roos S, Mihranyan A, Engqvist H, Strømme M : Multifunctional implant coatings providing possibilities for fast antibiotics loading with subsequent slow release. J Mater Sci Mater Med 20: 1859-1867, 2009.

Castillo AB, Blundo JT, Chen JC, Lee KL, Yereddi NR, Jang E, Kumar S, Tang WJ, Zarrin S, Kim JB, Jacobs CR : Focal adhesion kinase plays a role in osteoblast mechanotransduction in vitro but does not affect load-induced bone formation in vivo. PLoS One 7: e43291, 2012.

Catledge SA, Fries MD, Vohra YK, Lacefield WR, Lemons JE, Woodard S, Venugopalan R : Nanostructured ceramics for biomedical implants. J Nanosci Nanotechnol 2: 293-312, 2002.

Cazalis J, Bodet C, Gagnon G, Grenier D : Doxycycline reduces lipopolysaccharide-induced inflammatory mediator secretion in macrophage and ex vivo human whole blood models. J Periodontol 79: 1762-1768, 2008.

Chen F, Terada K, Handa K : Anchorage effect of various shape palatal osseointegrated implants: a finite element study. Angle Orthod 75: 378-385, 2005.

Chen S, Yang J, Wang H, Chao Y, Zhang C, Shen J, Zhang P : Adenovirus encoding BMP-7 immobilized on titanium surface exhibits local delivery ability and regulates osteoblast differentiation in vitro. Arch Oral Biol 58: 1225-1231, 2013.

Choi BD, Jeong SJ, Wang G, Kim HJ, Kim BO, Hwang HK, Lim DS, Kim SH,

Jeong MJ : Temporal induction of secretory leukocyte protease inhibitor (SLPI) in odontoblasts by lipopolysaccharide and wound infection. J Endod 35: 997-1002, 2009.

Choi BD, Jeong SJ, Lee HY, Lim DS, Lee BH, Bae CS, Jeong MJ : The effect of thymosin β4 for osteoblast adhesion on titanium surface. J Nanosci Nanotechnol 15: 5663-5667, 2015.

Choi BD, Lee SY, Jeong SJ, Lim DS, Cha HJ, Chung WG, Jeong MJ : Secretory leukocyte protease inhibitor promotes differentiation and mineralization of MC3T3-E1 preosteoblasts on a titanium surface. Mol Med Rep 14: 1241-1246, 2016.

Choi BD, Lim DS, Lee SY, Nho TH, Jeong SJ, Ko YM, Gang SN, Kim YJ, Piao XH, Jeong MJ : Thymosin β4 reduces H_2O_2 induced oxidative stress in MC3T3-E1 cells on titanium surface. J Nanosci Nanotechnol 18: 893-897, 2018.

Cui S, Wang J, Wu Q, Qian J, Yang C, Bo P : Genistein inhibits the growth and regulates the migration and invasion abilities of melanoma cells via the FAK/paxillin and MAPK pathways. Oncotarget 8: 21674-21691, 2017.

Daghigh F, Borghaei RC, Thornton RD, Bee JH : Human gingival fibroblasts produce nitric oxide in response to proinflammatory cytokines. J Periodontol 73: 392-400, 2002.

Degryse B, Neels JG, Czekay RP, Aertgeerts K, Kamikubo Y, Loskutoff DJ : The low density lipoprotein receptor-related protein is a motogenic receptor for plasminogen activator inhibitor-1. J Biol Chem 279: 22595-22604, 2004.

Die L, Yan P, Jun JZ, Min HT, Cai W, Xing L : Glycogen synthase kinase-3 beta

inhibitor suppresses Porphyromonas gingivalis lipopolysaccharide-induced CD40 expression by inhibiting nuclear factor-kappa B activation in mouse osteoblasts. Mol Immunol 52 :38 - 49, 2012.

Donnelly LE, Barnes PJ : Expression and regulation of inducible nitric oxide synthase from human primary airway epithelial cells. Am J Respir Cell Mol Biol 26: 144-51, 2002.

Facchini A, Cetrullo S, D'Adamo S, Guidotti S, Minguzzi M, Facchini A, Borzì RM, Flamigni F : Hydroxytyrosol prevents increase of osteoarthritis markers in human chondrocytes treated with hydrogen peroxide or growth-related oncogene α. PLoS One 9: e109724, 2014.

Fritz H : Human mucus proteinase inhibitor (human MPI). Human seminal inhibitor I (HUSI-I), antileukoprotease (ALP), secretory leukocyte protease inhibitor (SLPI). Biol Chem Hoppe Seyler 369: 79 - 82, 1988.

Gault P, Black A, Romette J-L, Fuente F, Schroeder K, Thillou F, Brune T, Berdal A, Wurtz T : Tissue-engineered ligament: implant constructs for tooth replacement. J Clin Periodontol 37: 750 - 758, 2010.

Garg H, Deepa D : Bioengineered periodontal ligament: Ligaplants, a new dimension in the field of implant dentistry - Mini review. J Oral Res Rev 10: 92-95, 2018.

Giancotti FG, Ruoslahti E : Integrin signaling. Science 285: 1028-1032, 1999.

Giubellino A, Burke TR Jr, Bottaro DP : Grb2 signaling in cell motility and cancer. Expert Opin Ther Targets 12: 1021-1033, 2008.

Graves DT, Cochran D : The contribution of interleukin-1 and tumor necrosis

factor to periodontal tissue destruction. J Periodontol 74: 391-401, 2003.

Hao S, Shen H, Hou Y, Mars WM, Liu Y : tPA is a potent mitogen for renal interstitial fibroblasts role of β1 integrin/focal adhesion kinase signaling. Am J Pathol 177: 1164 - 1175, 2010.

He CY, Jiang LP, Wang CY, Zhang Y : Inhibition of NF-κB by pyrrolidine dithiocarbamate prevents the inflammatory response in a ligature induced peri-implantitis model: A canine study. Cell Physiol Biochem 49: 610-625, 2018.

Hedia HS : Effect of coating thickness and its material on the stress distribution for dental implants. J Med Eng Technol 31: 280-287, 2007.

Holden JA, Attard TJ, Laughton KM, Mansell A, O'Brien-Simpsonb NM, Reynolds EC : Porphyromonas gingivalis lipopolysaccharide weakly activates M1 and M2 polarized mouse macrophages but induces inflammatory cytokines. Infect Immun 82: 4190 - 4203, 2014.

Holzhausen M, Spolidorio DM, Muscara MN, Hebling J, Spolidorio LC : Protective effects of etoricoxib, a selective inhibitor of cyclooxygenase-2, in experimental periodontitis in rats. J Periodont Res 40: 208-211, 2005.

Hongxia Dan, Cédryck Vaquette, Anthony G Fisher, Stephen M Hamlet, Yin Xiao, Dietmar W Hutmacher, Saso Ivanovski : The influence of cellular source on periodontal regeneration using calcium phosphate coated polycaprolactone scaffold supported cell sheets. Biomaterials 35: 113-122, 2014.

Ivanovski S, Li H, Haase HR, Bartold PM : Expression of bone associated macromolecules by gingival and periodontal ligament fibroblasts. J Periodontal Res 36: 131-141, 2001.

Jagadish R, Mehta DS : Comparative evaluation of the efficacy of the cyclooxygenase pathway inhibitor and nitric oxide synthase inhibitor in the reduction of alveolar bone loss in ligature induced periodontitis in rats: An experimental study. J Indian Soc Periodontol 18: 59-64, 2014.

Jeong SJ, Choi BD, Lee HY, Hwang YH, Kim BH, Cho YI, Yun JJ, Lee BH, Jeong MJ : 660nm red LED induces secretory leukocyte protease inhibitor (SLPI) in lipopolysaccharidestimulated RAW264.7 cell. J Nanosci Nanotechnol 15:5610 - 5616, 2015a.

Jeong SJ, Wang G, Choi BD, Hwang YH, Kim BH, Ko YM, Jeong MJ : Secretory leukocyte protease inhibitor (SLPI) increases focal adhesion in MC3T3 osteoblast on titanium surface. J Nanosci. Nanotechnol 15: 200-204, 2015b.

Jeong JO, Wang G, Jeong SJ, Choi BD, Lee HY, Jeong MJ : Function of secretory leukocyte protease inhibitor (SLPI) in odontoblast during mouse tooth development. J Nanosci Nanotechnol 15: 120 - 124, 2015c.

Jeong SJ, Jeong MJ : Effect of thymosin beta4 on the differentiation and mineralization of MC3T3-E1 Cell on a titanium surface. J Nanosci Nanotechnol 16: 1979-1983, 2016.

Jonarta AL, Asmara W, Astuti I, Tandelilin RTC : Systemic IL-1β and TNF-α productions of E. coli lipopolysaccharide-induced periodontitis model on rats. The Indonesian J Dent Res 1: 49-54, 2010.

Kaoru Washio, Yusuke Tsutsumi, Yuka Tsumanuma, Kosei Yano, Supreda Suphanantachat Srithanyarat, Ryo Takagi, Shizuko Ichinose, Walter Meinzer, Masayuki Yamato, Teruo Okano, Takao Hanawa, Isao Ishikawa : In Vivo Periodontium formation around titanium implants using periodontal ligament cell sheet. Tissue Eng Part A 24: 1273-1282, 2018.

Kats A, Gerasimcik N, Näreoja T, Nederberg J, Grenlöv S, Lagnöhed E, Desai S, Andersson G, Yucel-Lindberg T : Aminothiazoles inhibit osteoclastogenesis and PGE 2 production in LPS-stimulated co-cultures of periodontal ligament and RAW 264.7 cells, and RANKL-mediated osteoclastogenesis and bone resorption in PBMCs. J Cell Mol Med 23: 1152-1163, 2019.

Kendall HK, Marshall RI, Bartold PM : Nitric oxide and tissue destruction. Oral Dis 7: 2-10, 2001.

Kim MY, Lim YY, Kim HM, Park YM, Kang H, Kim BJ : Synergistic inhibition of tumor necrosis factor-alpha-stimulated pro-inflammatory cytokine expression in HaCaT cells by a combination of rapamycin and mycophenolic acid. Ann Dermatol 27: 32 - 39, 2015.

Koch CA, Anderson D, Moran MF, Ellis C, Pawson T : SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* 252: 668-674, 1991.

Kokubo T, Pattanayak DK, Yamaguchi S, Takadama H, Matsushita T, Kawai T, Takemoto M, Fujibayashi S, Nakamura T : Positively charged bioactive Ti metal prepared by simple chemical and heat treatments. J R Soc Interface 7: S503-513, 2010.

Landgraeber S, Jäger M, Jacobs JJ, Hallab NJames : The pathology of orthopedic implant failure is mediated by innate immune system cytokines. Mediators Inflamm 2014: 185150, 2014.

Lee DJ, Lee JM, Kim EJ, Takata T, Abiko Y, Okano T, David W. Green, Shimono M, Jung HS : Bio-implant as a novel restoration for tooth loss. Sci Rep 7: 7414, 2017.

Lee JH, Frias V, Lee KW, Wright RF : Effect of implant size and shape on implant success rates: a literature review. J Prosthet Dent 94: 377-381, 2005.

Lee SY, Nho TH, Choi BD, Jeong SJ, Lim DS, Jeong MJ : Secretory leukocyte protease inhibitor reduces inflammation and alveolar bone resorption in LPS-induced periodontitis in rats and in MC3T3-E1 preosteoblasts. Animal Cells and Systems 20: 344-352, 2016.

Lin SK, Kok SH, Lin LD, Wang CC, Kuo MYP, Lin CT, Hsiao M, Hong CY : Nitric oxide promotes the progression of periapical lesion via inducing macrophage and osteoblast apoptosis. Oral Microbiol Immunol 22: 24-29, 2007.

Lin Y, Gallucci GO, Buser D, Bosshardt D, Belser UC, Yelick PC : Bioengineered periodontal tissue formed on titanium dental implants. J Dent Res 90: 251-256, 2011.

Martínez-Aguilar VM, Carrillo-Ávila BA, Sauri-Esquivel EA, Guzmán-Marín E, Jiménez-Coello M, Escobar-García DM, Pozos-Guillén A : Quantification of TNF-α in patients with periodontitis and type 2 diabetes. Biomed Res Int 2019: 7984891, 2019.

Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC : Measurement of interleukin-1 alpha and -1 beta in gin gival crevicular fluid: implications for the pathogenesis of periodontal disease. J Periodontal Res 25: 156-163, 1990.

McCulloch CA : Basic considerations in periodontal wound healing to achive regeneration. Periodontology 2000 1: 16-25, 1993.

McNeely TB, Dealy M, Dripps DJ, Orenstein JM, Eisenberg SP, Wahl SM : Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J Clin Inves 96: 456-464, 1995.

Ming J, Zhuoneng L, Guangxun Z : Protective role of flavonoid baicalin from Scutellaria baicalensis in periodontal disease pathogenesis: A literature review. Complement Ther Med 38: 11-18, 2018.

Minkle Gulati, Vishal Anand, Vivek Govila, Nikil Jain, Pavitra Rastogi, Rohit Bahuguna, Bhargavi Anand : Periodontio-integrated implants: A revolutionary concept. Dent Res J (Isfahan) 11: 154-162, 2014.

Mitra SK, Hanson DA, Schlaepfer DD : Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol 6: 56-68, 2005.

Moreau T, Baranger K, Dadé S, Dallet-Choisy S, Guyot N, Zani ML : Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family. Biochimie 90: 284-295, 2008.

Niu M, Nachmias VT : Increased resistance to apoptosis in cells overexpressing thymosin beta four: A role for focal adhesion kinase pp125FAK. Cell Adhes Commun 7: 311-320, 2000.

Nuñez J, Sanz-Blasco S, Vignoletti F, Nuñez F, Arzate H, Villalobos C, Nuñez L, Caffesse RG, Sanz M : Periodontal regeneration following implantation of cementum and periodontal ligament-derived cells. J Periodont Res 47: 33-44, 2012.

Oates TW, Valderrama P, Bischof M, Nedir R, Jones A, Simpson J, Toutenburg H,

Cochran DL : Enhanced implant stability with a chemically modified SLA surface: a randomized pilot study. Int J Oral Maxillofac Implants 22: 755-760, 2007.

Ohshima M, Kuwata F, Otsuka K, Saito R, Sato K, Shioji S, Suzuki K : Alkaline phosphatase activities of cultured human periodontal ligament cells. J Nihon Univ Sch Dent 30: 208-217, 1988.

Okada N, Kobayashi M, Mugikura K, Okamatsu Y, Hanazawa S, Kitano S, Hasegawa K : Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid. J Periodontal Res 32: 559-569, 1997.

Owens LV, Xu L, Craven RJ, Dent GA., Weiner TM., Kornberg L, Liu ET, Cance WG : Overexpression of the focal adhesion kinase ($p125^{FAK}$) in invasive human tumors. Cancer Res 55: 2752-2755, 1995.

Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS : Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 14: 216 - 248, 2000.

Parithimarkalaignan S and Padmanabhan TV : Osseointegration: An Update. J Indian Prosthodont Soc 13: 2 - 6, 2013.

Park JC, Kim JH, Kang ES, Ryu JJ, Huh JB : Effects of SLA surface treated with NaOH on surface characteristics and response of osteoblast-like cell. J Korean Acad Prosthodont 52: 211-221, 2014.

Park JM, Koak JY, Jang JH, Han CH, Kim SK, Heo SJ : Osseointegration of anodized titanium implants coated with fibroblast growth factor-fibronectin (FGF-FN) fusion protein. Int J Oral Maxillofac Implants 21: 859-866, 2006.

Pivodova V, Frankova J, Ulrichova J : Osteoblast and gingival fibroblast markers in dental implant studies. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 155: 109-116, 2011.

Popova Chr, Mlachkova A : Gingival tissue IL-1β and PGE2 levels in patients with chronic periodontitis after additional therapy with non-steroidal anti-inflammatory drugs. Journal of IMAB - Annual Proceeding (Scientific Papers) 16: 27-30, 2010.

Pugdee K, Shibata Y, Yamamichi N, Tsutsumi H, Yoshinari M, Abiko Y : Gene expression of MC3T3-E1 cells on fibronectin-immobilized titanium using tresyl chloride activation technique. Dent Mater J 26: 647-655, 2007.

Rasool N, LaRochelle W, Zhong H, Ara G, Cohen J, Kohn EC : Secretory leukocyte protease inhibitor, SLPI, antagonizes paclitaxel in ovarian cancer cells. Clin Cancer Res 16: 600 - 609, 2010.

Queiroz-Junior CM, Pacheco CM, Maltos KL, Caliari MV, Duarte ID, Francischi JN : Role of systemic and local administration of selective inhibitors of cyclo-oxygenase 1 and 2 in an experimental model of periodontal disease in rats. J Periodont Res 44: 153-160, 2009.

Rosa-María Díaz-Sánchez, Rosa-María Yáñez-Vico, Ana Fern ndez- Olavarría, Regina Mosquera-Pérez, Alejandro Iglesias-Linares, Daniel Torres-Lagares : Current approaches of bone morphogenetic proteins in dentistry. J Oral Implantol 41: 337-342, 2015.

Saleem S, Li J, Yee SP, Fellows GF, Goodyer CG, Wang R : beta1 integrin/FAK/ERK signalling pathway is essential for human fetal islet cell differentiation and survival. J Pathol 219: 182-192, 2009.

Sallenave JM : Antimicrobial activity of antiproteinases. Biochem Soc Trans 30: 111-115, 2002.

Schou S, Holmstrup P, Stoltze K, Hjørting-Hansen E, Kornman KS : Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth. Clin Oral Implants Res 4: 12-22, 1993.

Shibata Y, Kawai H, Yamamoto H, Igarashi T, Miyazakiet T : Antibacterial titanium plate anodized by being discharging in NaCl solution exhibits cell compatibility. J Dent Res 83: 115-119, 2004.

Shimizu N, Ogura N, Yamaguchi M, Goseki T, Shibata Y, Abiko Y, Iwasawa T, Takiguchi H : Stimulation by interleukin-1 of interleukin-6 production by human periodontal ligament cells. Arch Oral Biol 37: 743-748, 1992.

Shimizu N, Yamaguchi M, Uesu K, Goseki T, Abiko Y : Stimulation of prostaglandin E2 and interleukin-1beta production from old rat periodontal ligament cells subjected to mechanical stress. J Gerontol A Biol Sci Med Sci 55: B489-495, 2000.

Shugars DC, Wahl SM : The role of the oral environment in HIV-1 transmission. J Am Dent Assoc 129: 851 - 858, 1998.

Song Xy, Zeng L, Jin W, Thompson J, Mizel DE, Lei Kj, Billinghurst RC, Poole AR, Wahl SM : Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. J Exp Med 190: 535-542, 1999.

Sun L, Zhang J, Fang K, Ding Y, Zhanga L, Zhang Y : Flavonoids from persimmon (Diospyros kaki) leaves (FPL) attenuate H2O2-induced apoptosis in MC3T3-E1 cells via the NF-κB pathway. Food Funct 5: 471-479, 2014.

Sun Y, Shu R, Zhang MZ, Wu AP : Toll-like receptor 4 signaling plays a role in triggering periodontal infection. FEMS Immunol Med Microbiol 52: 362-369, 2008.

Svensson D, Aidoukovitch A, Anders E, Jönsson D, Nebel D, Nilsson BO : Secretory leukocyte protease inhibitor regulates human periodontal ligament cell production of pro-inflammatory cytokines. Inflamm Res 66: 823 - 831, 2017.

Taggart CC, Cryan SA, Weldon S, Gibbons A, Greene CM, Kelly E, Low TB, O'Neill SJ, McElvaney NG : Secretory leucoprotease inhibitor binds to NF-κB binding sites in monocytes and inhibits p65 binding. J Exp Med 202: 1659 - 1668, 2005.

Thompson RC, Ohlsson K : Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. Proc Natl Acad Sci USA 83: 6692-6696, 1986.

Tsumanuma Y, Iwata T, Washio K, Yoshida T, Yamada A, Takagi R, Ohno T, Lin K, Yamato M, Ishikawa I, Okano T, Izumi Y : Comparison of different tissue derived stem cell sheets for periodontal regeneration in a canine 1 wall defect model. Biomaterials 32: 5819, 2011.

Walter M, Plotnick M, Schechter NM : Inhibition of human mast cell chymase by secretory leukocyte proteinase inhibitor: enhancement of the interaction by heparin. Arch Biochem Biophys 327: 81-88, 1996.

Wang G, Lim DS, Choi BD, Park JJ, Jeong SJ, Kim JS, Kim JD, Park JS, Kim EK, Kim BH, Ham JH, Jeong MJ : Effect of secretory leukocyte protease inhibitor on migration and invasion of human KB oral carcinoma cells. Anim Cells Syst 15: 139-146, 2011.

Wang XX, Xie L, Wang R : Biological fabrication of nacreous coating on titanium dental implant. Biomaterials 26: 6229-6232, 2005.

Wright CD, Kennedy JA, Zitnik RJ, Kashem MA : Inhibition of murine neutrophil serine proteinases by human and murine secretory leukocyte protease inhibitor. Biochem Biophys Res Commun 254: 614-617, 1999.

Yamano E, Miyauchi M, Furusyo H, Kawazoe A, Ishikado A, Makino T, Tanne K, Tanaka E, Takata T : Inhibitory effects of orally administrated liposomal bovine lactoferrin on the LPS-induced osteoclastogenesis. Lab Invest 90: 1236 - 1246, 2010.

Yoshinari M, Oda Y, Kato T, Okuda K : Influence of surface modifications to titanium on antibacterial activity in vitro. Biomaterials 22: 2043-2048, 2001.

Zhang D, Chen L, Li S, Gu Z, Yan J : Lipopolysaccharide (LPS) of Porphyromonas gingivalis induces IL-1beta, TNF-alpha and IL-6 production by THP-1 cells in a way different from that of Escherichia coli LPS. Innate Immun 14: 99-107, 2008.

Zhang H, Ahmad M and Gronowicz G: Effects of transforming growth factor-beta 1 (TGF-beta1) on in vitro mineralization of human osteoblasts on implant materials. Biomaterials 24: 2013-2020, 2003.

Zhao Y, Qiu F, Xu S, Yu L, Fu G : Thymosin β4 activates integrin linked kinase and decreases endothelial progenitor cells apoptosis under serum deprivation. J Cell Physiol 226: 2798-2806, 2011.

Zhou T, Chen D, Li Q, Sun X, Song Y, Wang C : Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats. Acta Odontologica Scandinavica 71: 349 - 356, 2013.

Ⅵ. FIGURE LEGENDS

Figure 1. Adhesion of SLPI-treated PDLF on Ti discs.
SLPI significantly increased the adhesion of PDLF compared to that of untreated PDLF. $*p < 0.05$.

Figure 2. Protein expression of the FA related signaling molecules in PDLF on Ti discs with SLPI treatment.
(A) The expression of pFAK and paxillin was increased significantly in PDLF on Ti discs after SLPI treatment compared to that in the untreated PDLF. (B) Quantitative analysis of the expressed band density between the SLPI-treated and untreated PDLF on Ti discs. $*p < 0.05$.

Figure 3. The formation of F-actin in PDLF on Ti discs with SLPI treatment.
The formation of F-actin in the SLPI-treated PDLF on Ti discs increased with time compared to that in the untreated PDLF. All scale bars are 5 ㎛.

Figure 4. The paxillin contacts in PDLF on Ti discs with SLPI treatment.
Paxillin (arrowhead) in PDLF on Ti discs was increased upon SLPI treatment in a time-dependent manner compared to that in untreated PDLF. All scale bars are 5 ㎛. $*p < 0.05$.

Figure 5. Proliferation in PDLF on Ti discs with SLPI treatment.
SLPI significantly increased the proliferation of PDLF compared to that in the untreated cells. $*p < 0.05$.

Figure 6. Protein expression of the mitosis-related signaling molecules in PDLF on Ti discs with SLPI treatment.

(A) The expression of Grb2, Ras, pERK1/2 was increased significantly in PDLF on Ti discs after an SLPI treatment compared to that in the untreated cells. (B) Quantitative analysis of the expressed band density between the SLPI-treated and untreated PDLF on Ti discs. $*p < 0.05$.

Figure 7. The mRNA expression of TNF-α and IL-1β in LPS- or SLPI/LPS-treated PDLF on Ti discs.

(A) The expression of TNF-α and IL-1β mRNA was decreased in SLPI/LPS/PDLF compared to that in LPS/PDLF. (B) Quantitative analysis of the expressed band density between the SLPI-treated and untreated PDLF on Ti discs. M, marker; C, control. $*p < 0.05$.

Figure 8. The protein expression of TNF-α and IL-1β in LPS- or SLPI/LPS-treated PDLF on Ti discs.

(A) The expression of TNF-α and IL-1β protein was decreased in SLPI/LPS/PDLF compared to that in LPS/PDLF. (B) Quantitative analysis of the expressed band density between the SLPI-treated and untreated PDLF on Ti discs. C, control. $*p < 0.05$.

Figure 9. The protein expression of iNOS and COX-2 in LPS- or SLPI/LPS-treated PDLF on Ti discs.

(A) The expression of iNOS and COX-2 protein was decreased in SLPI/LPS/PDLF compared to that in LPS/PDLF. (B) Quantitative analysis of the expressed band density between the SLPI-treated and untreated PDLF on Ti discs. C, control. $*p < 0.05$.

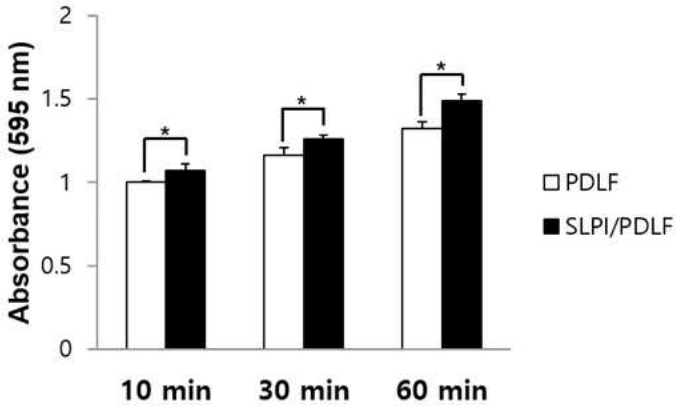
Figure 10. Nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in LPS- or SLPI/LPS-treated PDLF on Ti discs.

The production of (A) NO and (B) PGE_2 was decreased in SLPI/LPS/PDLF compared to that in LPS/PDLF. C, control. $*p < 0.05$.

Figure 11. Diagram of SLPI function in PDLF and osteoblasts on the titanium surface.

Ⅶ. FIGURES

Figure 1.



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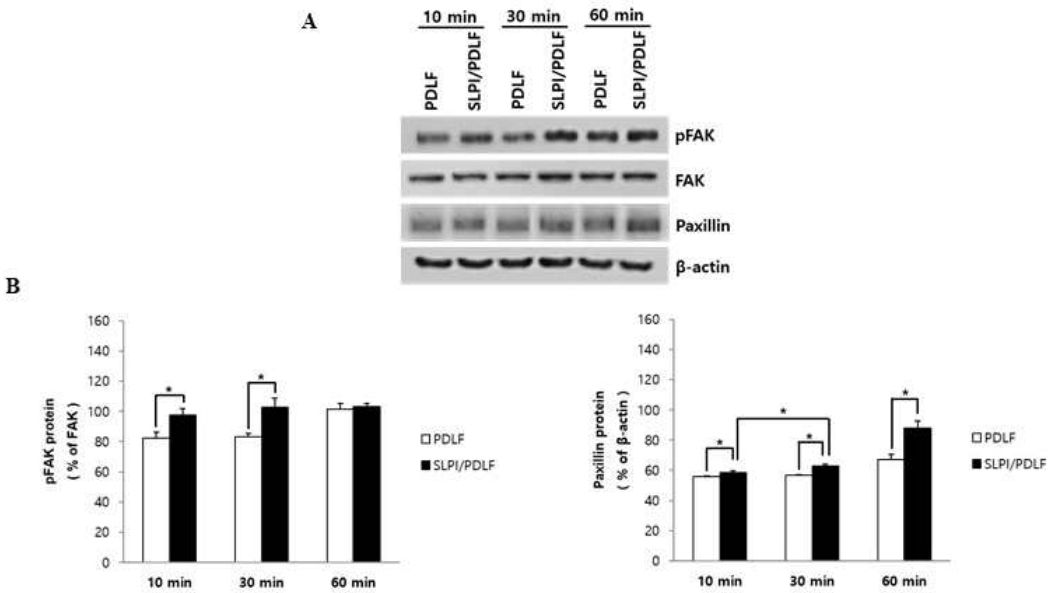

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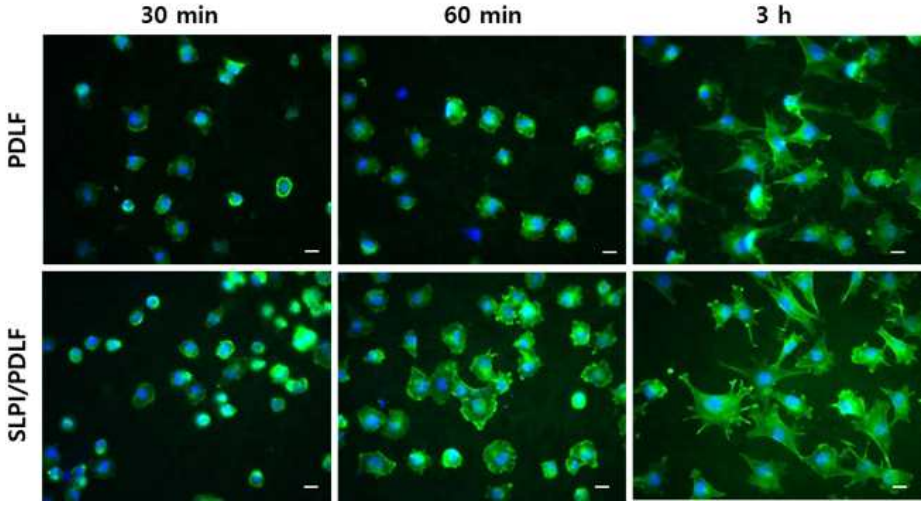

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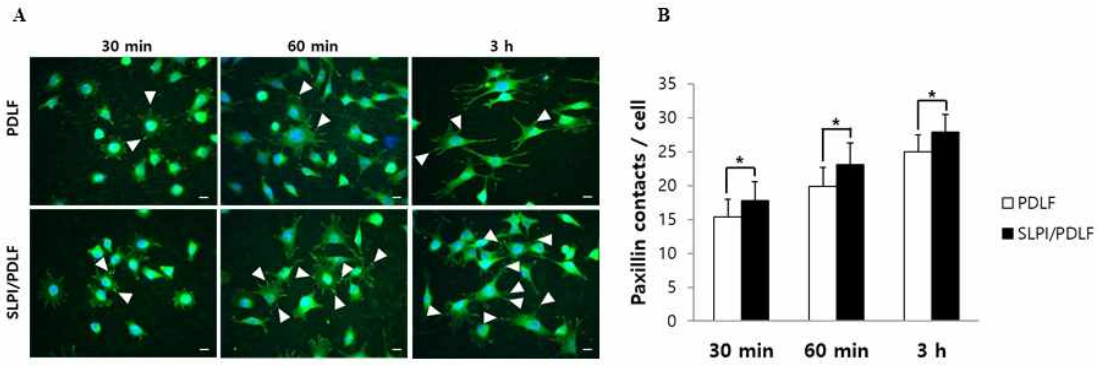

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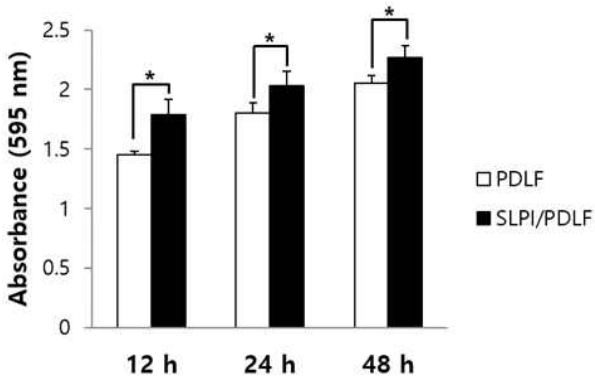

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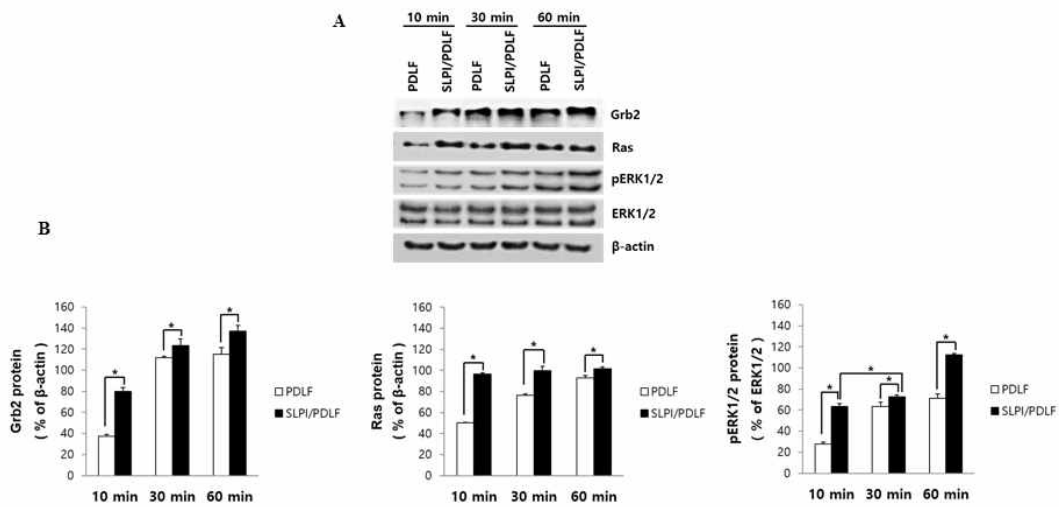

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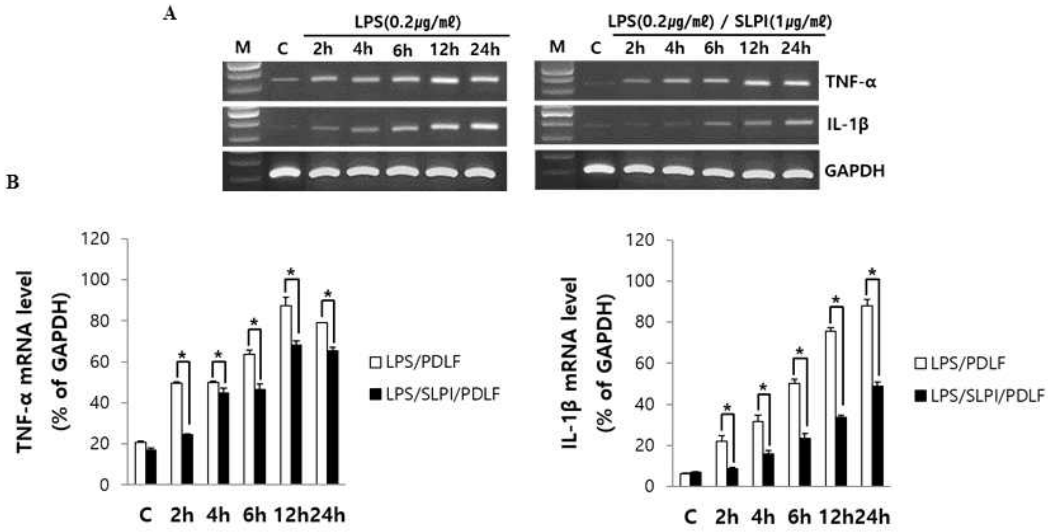

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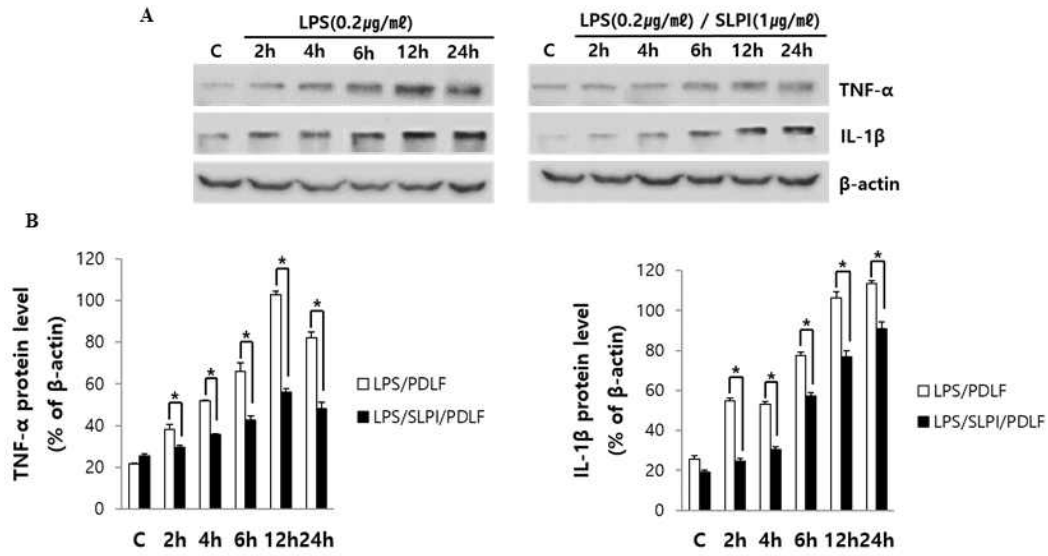

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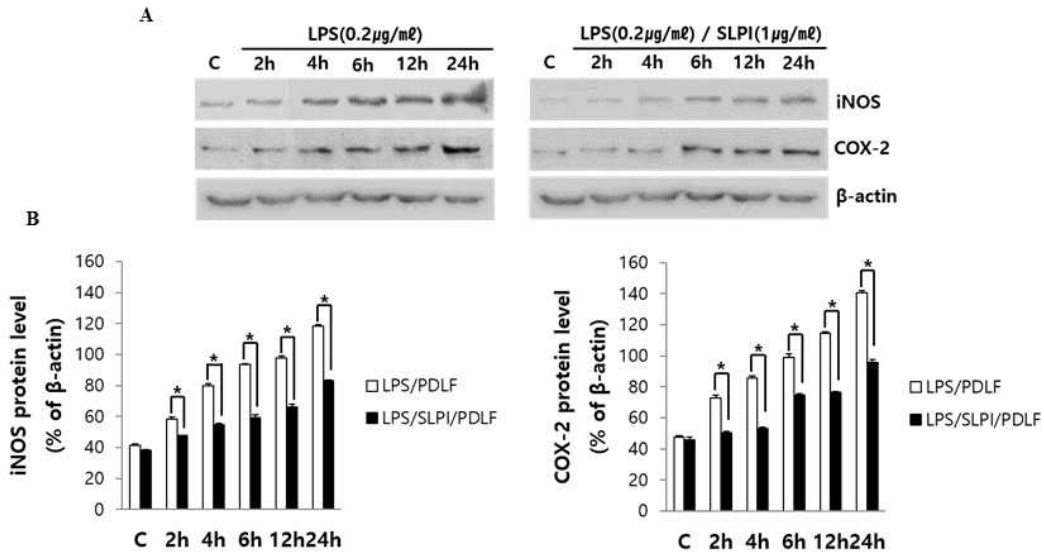

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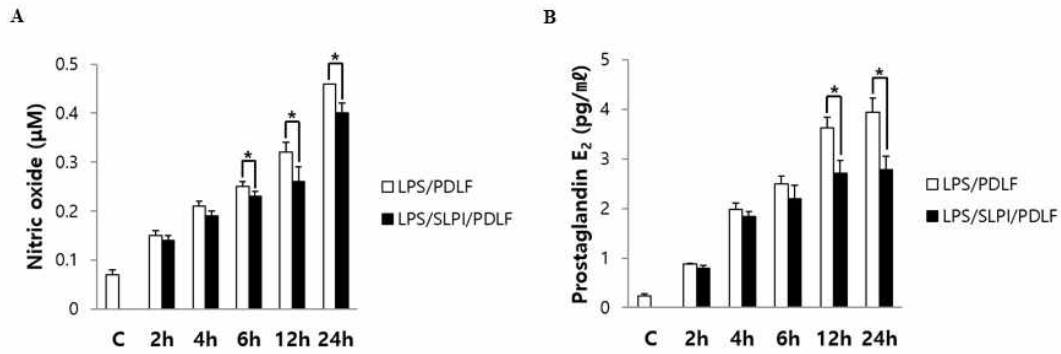
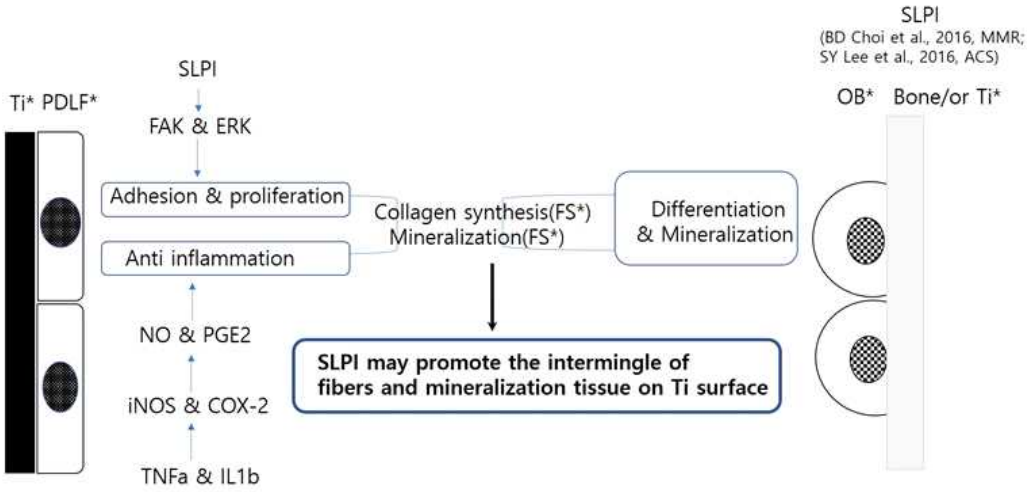


Figure 11.



*) PDLF, periodontal ligament fibroblast; OB, osteoblast; Ti, Titanium; FS, Further Study

ABSTRACT

Effects of SLPI on the attachment and inflammatory response of periodontal ligament fibroblasts cultured on titanium surface for development of periodontio-integrated implant

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The periodontal ligament (PDL) is involved not only in tooth support but also in the repair and regeneration of the alveolar bone and cementum. Titanium (Ti) is a widely used material as an implant fixture and an orthodontic mini-screw because of its high success rate of osseointegration demonstrating excellent mechanical strength and biocompatibility. Because of the removed PDL, this osseointegrated implant has a lower buffering effect on the occlusal force and lower immunity to external inflammation compared to the natural teeth. Recently, various studies have been conducted examining periodontio-integrated implants for the regeneration of periodontal tissue around implants.

Secretory leukocyte protease inhibitor (SLPI) promotes wound healing and proliferation. In addition, it reduces alveolar bone resorption in periodontitis and suppresses the expression of proinflammatory cytokines in osteoblasts. SLPI also increases the adhesion, differentiation, and mineralization of osteoblasts on the Ti surface. To date, even though many studies have been reported on the relationship between Ti and periodontal ligament fibroblasts (PDLF), only a few studies have

investigated the molecules that promote adhesion and activity in PDLF. Furthermore, no study has been published examining the function of SLPI in PDLF cultured on Ti. Therefore, this study aimed to examine the function of SLPI in the adhesion of PDLF and inhibition of the inflammatory response in PDLF on the Ti surface, for the modeling of periodontio-integrated implants.

After treatment of cultured PDLF on the Ti surface with SLPI, MTT assay, immunofluorescence staining, and western blotting were performed to confirm the function of SLPI in the adhesion and proliferation of PDLF. After SLPI and LPS treatment of the PDLF on the Ti surface, the inhibitory effect of SLPI on inflammation was analyzed by PCR, western blotting, and by analyzing the expression of nitric oxide (NO) and prostaglandin E_2 (PGE_2). The adhesion, viability, and proliferation of SLPI-treated PDLF were higher than those observed in the control cells. The formation of actin stress fibers, paxillin expression, and FAK phosphorylation was increased in the SLPI-treated PDLF compared to that in the control. The expression of Grb2 and Ras proteins and phosphorylation of ERK1/2 was increased in the SLPI-treated PDLF compared to that in the control. The mRNA and protein expression corresponding to TNF-α and IL-1β were lower in the SLPI-treated PDLF after LPS exposure than those observed in the LPS-treated PDLF. In addition, SLPI decreased the protein expression of iNOS and COX-2 and inhibited secretion of NO and PGE_2 in LPS-treated PDLF on the Ti surface.

Therefore, the results of this study demonstrated that SLPI promoted the adhesion and proliferation of PDLF on the surface of Ti, and reduced the secretion of inflammatory cytokines and mediators, suggesting an effective molecule that can increase the clinical success rate after implantation by providing biological and mechanical stability during the osseointeration process. Further studies should be performed to determine the effect of SLPI on collagen expression and mineralization between PDLF and osteoblasts on Ti.