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Effect of fibroblast growth factor on fibroblast differentiation from bone marrow stromal cell in dog

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지도교수 장 현 선

이 논문을 치의학 석사학위신청 논문으로 제출함.

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서 영 종

서영종의 석사학위 논문을 인준함.

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섬유모세포성장인자가 개의 골수기질세포로부터 섬유모세포 분화에 끼치는 효과

서 영 종

지도교수 : 장 현 선

조선대학교 대학원 치의학과

다양한 조직으로 분화가 가능한 줄기세포를 치주조직의 치유에 응용할 수 있다면 향후 심한 치주염 환자들의 조직 재생에 더 유용할 것이다. 그러나, 다양한 분화가 가능한 줄기세포에서 섬유모세포로의 분화에 관한 연구는 미비한 실정이다. 이에 본 연구는 줄기세포에 섬유모세포성장인자를 적용하여 섬유모세포로의 분화 가능성에 대하여 연구하고자 한다.

줄기세포는 Dog의 골수기질세포를 실험에 이용하였다. 줄기세포에 섬유모세포성장인자을 첨가한 것을 실험군으로 하였다. 실험군을 세분하였는데, 섬유모세포성장인자을 적용한 것을 실험1군, 치주인대섬유모세포-조건배지를 적용한 것을 실험 2군, 그리고 섬유모세포성장인자와 치주인대섬유모세포-조건배지를 함께 적용한 것을 실험 3군으로 세분하였다. 대조군은 아무런 처치를 하지 않고 단순히 골수기질세포만을 배양한 것으로 하였다. 치주인대섬유모세포 조건배지는 개의 치주인대섬유모세포를 배양하여 그 배양액을 조건배지로 이용하였다. 세포는 60 mm 배양접시에서 10% Fetal Bovine Serum(FBS, Gibco BRL)이 함유된 Dulbecco's Modified Eagles Medium(DMEM, Gibco BRL)을 이용하여 5% CO_2, 37℃, 100% 습도 조건에서 배양하였다. 섬유모세포성장인자는 3ng/ml로 적용하였고, 치주인대섬유모세포 조건배지는 치주인대섬유모세포만 배양하여 얻은 그 배양액을 줄기세포 배양액과 1:1 비율로 적용하였다. 배지는 이틀에 한번씩 교체하고 14일동안 배양한 후, 치주인대관련 유전자 발현을 평가하기 위하여 Trizol reagents (Gibco,

BRL, Rockville, USA)를 이용하여 각각의 세포에서 total RNA를 추출하였다. 줄기세포에서 섬유모세포로의 분화를 평가하기 위하여 UNCL, S100A2, S100A4, Nestin, nucleostemin, mRNA에 대한 의 RT-PCR를 시행하였다. 이 연구 결과, 섬유모세포 표지자로 이용되는 UNCL, S100A2, S100A4 mRNA의 발현이 줄기세포에 FGF를 적용한 실험군에서 두드러졌다. 줄기세포의 섬유모세포로의 분화에 FGF를 이용할 경우, 향후 치주염의 치료 및 치주조직 재생에 널리 응용할 수 있을 것으로 생각된다.

주요어: 줄기세포, 섬유모세포, 섬유모세포성장인자, 유전자

Introduction

The main purpose in periodontal therapy is the in regenerating periodontal tissue. The periodontal tissue is composed of cementum, bone, gingiva, and periodontal ligament. Periodontitis can be caused by infection, mechanical stress and aging. Once the periodontal tissue is impaired, the tissue has a limited capacity for regeneration[1]. Since cementoblasts, osteoblasts, and periodontal ligament fibroblasts are derived from periodontal ligament[2], regeneration of periodontal tissue can be limited in severe periodontitis patients with periodontal ligament loss. For this regeneration, human periodontal ligament fibroblast (hPDLF) is crucial. PDLF have a several potential, such as migration, proliferation, differentiation osteoblast-like, cementoblast-like, & periodontal ligament fibroblasts, achieve the promotion of tissue regeneration[3-6]. Melcher *et al.*[7] stated that the phenotypes of cells re-collected in the root surface (such as gingival epithelium, gingival lamina propria, periodontal ligament, cementum, and alveoloar bone) would determine the adhesion, regeneration characteristics, and quality.

For periodontal regeneration, hPDLF cells having the potential to divide into various cells are important. Because conventional periodontal regeneration methods remain insufficient to obtain a complete regeneration in periodontitis patients, the concept of periodontal tissue engineering has resently been needed to improve the healing of periodontal tissues[8]. Generally, S100A4-, S100A2-, Periostin-mRNA could be used as a useful marker for distinguishing cultured gingival fibroblasts and periodontal ligament cells. Park et al.[9] reported that in RT-PCR and Northern analysis, the expression of S100A4 and periostin mRNA in GF was slightly detectable, and that the expression of S100A4 and periostin mRNA in PDLF was much higher than that in GF. On the other hand, S100A2 mRNA was highly expressed in both GF and PDLF.

The most abundant cell in periodontal connective tissue is the gingival fibroblast. PDLF and gigival fibroblasts (GF) display distinct functional activities in the regeneration and repair of the periodontal tissues as well as during inflammatory periodontal diseases[9-13]. Generally, severe periodontitis patients expected tooth extration have no PDLF. Han et al[14] reported that PDLF and GF appear to display different gene expression patterns that may

reflect intrinsic functional differences of the two cell populations and may well coordinate with their tissue-specific activities. However, it will be worth that human GF can be used as a hPDLF for the periodontal tissue engineering.

Attention is drawn to periodontal disease seen mainly in adults with aging population. Rather than treating periodontal disease once it develops, prevention is better by maintaining a healthy periodontium.[15)]

Stem cells are pluripotent cells with unlimited proliferation potential and differentiation capacity to all types of somatic cells. Periodontal tissue engineering based on in vitro expanded cells holds the promise to overcome the limitations associated with contemporary regenerative techniques. Stem cells may become a cell source with unlimited supply for periodontal tissue engineering applications[16)]. Lee *et al.*[17)] reported that fibroblastic differentiation from stem cells is of widespread significance in the engineering of virtually all tissues including tendons, ligaments, periodontal ligament, cranical sutures and as interstitial filler of all organs. Ogiso *et al.*[18)] reported that co-culture of human periodontal ligament fibroblast or rat skin fibroblast with bone marrow stromal cells (BMSCs) resulted in a large reduction of bone nodule formation, and that fibroblasts may inhibit osteoblast differentiation. Lee *et al.*[17)] observed that connective tissue growth factor (CTGF)-treated hMSCs failed to show osteogenic or chondrogenic differentiation, and that CTGF is an effective induction factor for fibroblastic differentiation of human human mesenchymal stem cells. Hermann *et al.*[19)] observed that their data provide an impetus for differentiating human bone marrow stromal cells in vitro into mature neuroectodermal cells. MSCs generally are positive for nestin, which is an early marker of neuronal progenitors[20)]. Suzuki *et al.*[20)] reported that BMSCls can be expanded rapidly in vitro and have the potential to be differentiated into neuronal cell types.

MSCs can become a valuable cell source as an autograft for clinical application involving tissue regeneration[21)]. Recent studies have shown that mesenchymal stem cells obtained from periodontal ligament (PDL-MSCs) are multipotent cells that have similar features of the bone marrow and dental pulp MSCs and are capable of proliferating and producing different types of tissue such as bone and tooth associated-tissues[21)].

Lu *et al.*[22)] reported the therapeutic potential of adult bone marrow stromal

cells (BMSCs). BMSCs expressed neural markers nestin. Nestin and GFAP double immunofluorescence showed differentiation of BMSCs into neural stem cells in vitro. Differentiated cells express neural specific genes(e.g. 68-kDa neurofilament, β-tubulin III and nestin) detected by RT-PCR method.

MOREAU *et al.*[23] reported that in vitro BMSCs growth may be enhanced through culture medium supplementation, mimicking the biochemical environment in which cells optically proliferate and differentiate. They suggested significant in vitro ligament development after only 14 days of culture when using a sequential growth factor approach. Kafienah et al.[24] reported that Nucleostemin is a marker of proliferating stromal stem cells in adult human bone marrow and that it is involved in the regulation of proliferation of these cells. Duarte *et al.*[25] reported the expression of S100A2 and S100A4 calcium-binding protein in periodontium using in situ hybridization and important role maintaning width of periodontal ligament without calcification.

The aim of this study was to investigate the possibility of fibroblast differentiation from BMSCs under the influence of fibroblast growth factor using the RT-PCR.

Materials and Methods

Cell Culture

The bone marrow stromal cells (BMSCs) and periodontal ligament fibroblasts (PDLF) of dog were used for our study. Fresh media obtained from culturing human periodontal ligament fibroblasts of dog were used for conditioned media. 8 passage BMSC and 6 passage PDLF were used for this study. The BMSC and PDLF were cultured in Dulbecco′s Modified Eagles Medium (DMEM, Gibco BRL, USA) containing 10% fetal bovine serum (FBS) at 5% CO2, 37℃, and 100% humidity.

Conditioned medium experiments

The cells were cultured at 5% CO_2, 37℃, and 100% humidity in a 60 ㎜ Petri dish until 14 days. The media change was done in a humid incubator in a 2-day interval by exchanging DMEM medium containing 10% FBS. The control group was BMSC without treatment. The experimant groups were BMSC treated with FGF, FGF plus fresh media of hPDLF, fresh media of hPDLF, fresh media of dPDLF, FGF plus fresh media of dPDLF, respectively. The 3 ng/㎖ fibroblast growth factor (FGF) was applied in a 2-day interval when the experimental cell culturing media was changed. The FGF applied media was named by FGF-treated media.

Conditioned media obtained from culturing periodontal ligament fibroblasts of dog was applied in a 2-day interval when the experimental cell culturing media was changed. It is named by PDLF-conditioned media. The PDLF-conditioned media was obtained in a 2-day interval when the PDLFs culturing media was changed. The PDLF-conditioned media was made by mixture of 1:1 ratio with fresh media obtained from PDLF and culture media.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The total RNA was extracted according to the manufacturer′s instruction using Trizol Reagent (Invitrogen, USA). The first-strand cDNA was synthesized using 25U of oligo-d(T) primer and premix (Bioneer, Korea) per 1

㎍ of total RNA from cultured gingival fibroblast. The sense and anti-sense oligonucleotide primers for the base sequences of UNCL, S100A2, S100A4, nestin, nucleostemin and GAPDH were ordered (Table 1). Using the template of cDNA processed from the reverse transcription (RT) process, 20 ㎕ of PCR mixture was prepared by adding 20 pmols of primer, 5 ㎕ of cDNA, AccuPower Premix (Bioneer, Korea), and distilled water.

PCR was done by 30-35 cycles of denaturation, annealing, and extension (Table 2) using PTC-200 (MJ Research Inc., Watertown, MA, U.S.A). PCR products were analyzed by eletrophoresing in 1.5% agarose gel to confirm gene expressions.

Table 1. Nucleotide sequences of the primers used for RT-PCR

	Primer	Sequences 5′→3′	Predicted size (base pairs)
GAPDH	Sense	CCATGGAGAAGGCTGGG	200
	Anti-sense	CAAAGTTGTCATGGATGACC	
UNCL	Sense	ACCAGTGGGCCAGAGATGACC	493
	Anti-sense	GAAAGCCCGTAGAGCAGAATCAGA	
S100A2	Sense	AAGAGGGCGACAAGTTCAAGC	278
	Anti-sense	GAATGTTGCAGGAAACAGCCA	
S100A4	Sense	CTCAGCGCTTCTTCTTTCTT	295
	Anti-sense	ATCATGGCGATGCAGGACA	
Nestin	Sense	GCCCTGACCACTCCAGTTTA	98
	Anti-sense	GGAGTCCTGGATTTCCTTCC	
Nucleostemin	Sense	GGG AAG ATA ACC AAG AGT GTG	220
	Anti-sense	CCT CCA AGA AGT TTC CAA AGG	

Table 2. Conditions for RT-PCR

	Temperature (℃)			Time (min.)
	S100A2, S100A4, Nestin, GAPDH	Nucleostemin	UNCL	
Predenaturation	94	95	94	5
denaturation	94	95	94	1
Annealing	55	58	45	1
Polymerization	72	72	72	*

*Polymerization time: GAPDH, UNCL (1.5min), S100A2, S100A4(2min), nestin(0.5), neleostemin(0.25), min.: minute

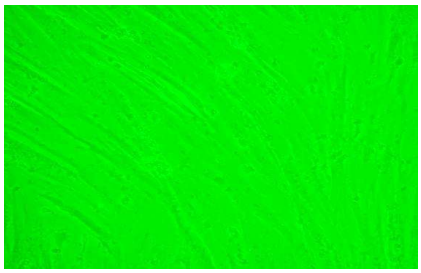
Results

1. Morphology of the cells

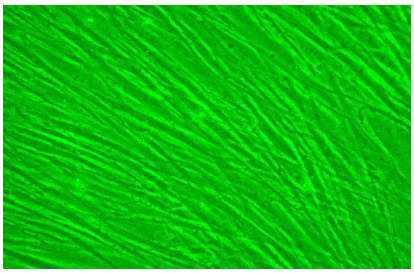
Morphological changes according to the FGF-treated media and/or PDLF-conditioned media of BMSC was shown.

Morphological changes of the cells were observed under a phase contrast microscope. At 14 days, more proliferation was observed under the FGF treated, PDLF-conditioned, FGF plus PDLF-conditioned groups than the control group.

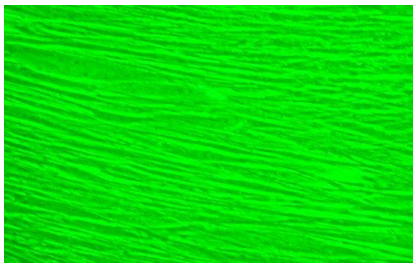
A. Control



B. FGF-tx



C. PDLF conditioned media-tx



D. FGF-tx/PDLF conditioned media-tx

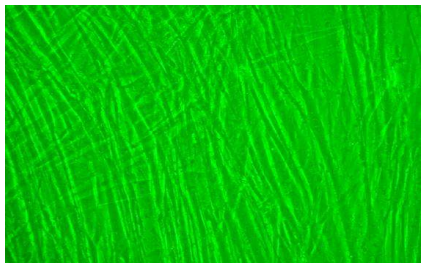


Figure 1. Effect of FGF treated and/or PDLF-conditioned media on bone marrow stromal cell. At 14 days, cell proliferation was more observed in FGF and/or PDLF-conditioned groups than the control group. A. Untreated BMSC cultures, B. FGF-treated BMSC, C, PDLF-conditioned medium, D. FGF-treated plus PDLF-conditioned medium.

2. Pattern of gene expression using RT-PCR

The S100A4 mRNA and S100A2 mRNA were expressed similarly in between the experimental groups (FGF treated, FGF plus PDLF-conditioned media and the PDLF-conditioned media group) and the control group at one day. The expression of UNCL mRNA were expressed weakly at one day.
The nestin mRNA showed strongly in the control group. The nucleostemin mRNA was expressed strongly in FGF treated group.

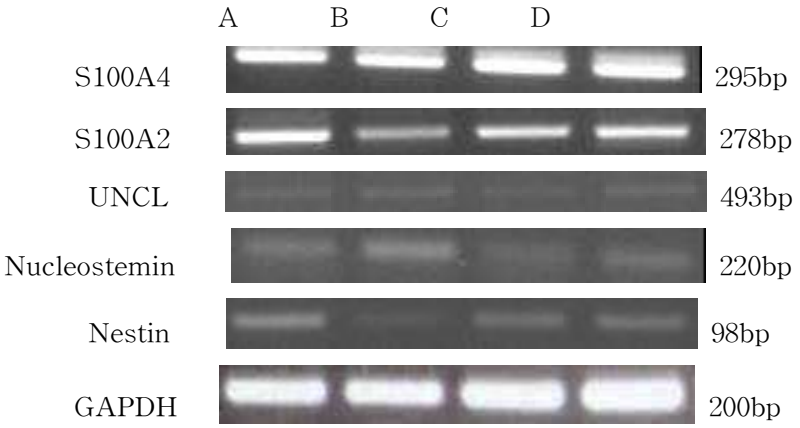


Figure 2. Gene (S100A4, S100A2, UNCL, Nestin, Nucleostemin-mRNA) expression pattern according FGF-treated and/or PDLF-conditioned media. At 1 day. A. Untreated BMSC cultures, B. FGF-treated BMSC, C, PDLF-conditioned medium, D. FGF-treated plus PDLF-conditioned medium.

The expression of UNCL mRNA were expressed strongly at 11 days than one day. Expecially, The UNCL mRNA were expressed stronly in FGF treated and control group. The S100A4 mRNA was expressed strongly in FGF treated at 11 day. The expression of nucleostemin mRNA were expressed strongly at 11 days than one day. Expecially, the nucleostemin mRNA were expressed stronly in FGF treated and control group. The S100A2 mRNA was expressed similarly between experimental groups and the control group at 11 day. At 11 day, the expression of the nestin mRNA was weak.

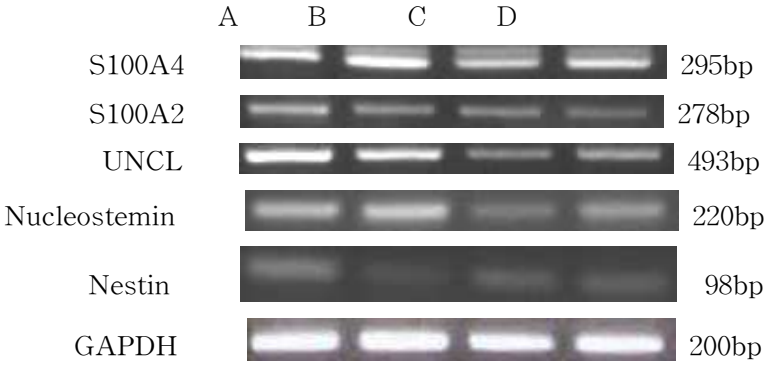


Figure 3. Gene (S100A4, S100A2, UNCL, Nestin, Nucleostemin-mRNA) expression pattern according FGF-treated and/or PDLF-conditioned media. At 11 days. A. Untreated BMSC cultures, B. FGF-treated BMSC, C, PDLF-conditioned medium, D. FGF-treated plus PDLF-conditioned medium. at 7 days.

The expression of UNCL mRNA were expressed strongly in FGF treated and FGF plus PDLF-conditioned media groups at 14 days. The S100A4 mRNA was expressed strongly in FGF treated at 14 day. The S100A2 mRNA was expressed stronly in all experimental groups (FGF treated, FGF plus PDLF-conditioned media and the PDLF-conditioned media group). The nucleostemin mRNA were expressed stronly in FGF treated at 14 days.

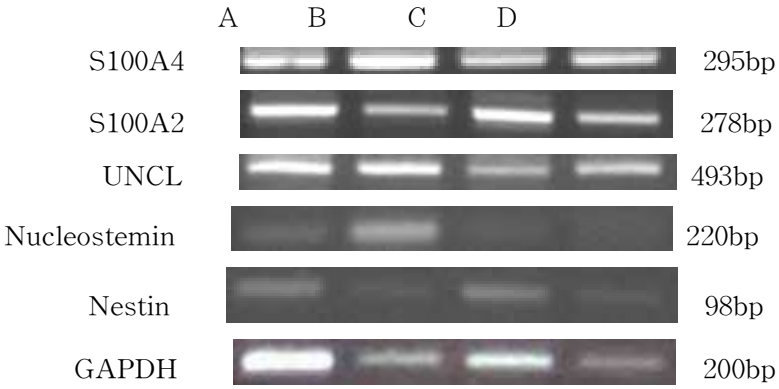


Figure 4. Gene (S100A4, S100A2, UNCL, Nestin, Nucleostemin-mRNA) expression pattern according FGF-treated and/or PDLF-conditioned media. At 14 days. A. Untreated BMSC cultures, B. FGF-treated BMSC, C, PDLF-conditioned medium, D. FGF-treated plus PDLF-conditioned medium. at 7 days.

Discussion

The ultimate purpose of clinical periodontal therapy in periodontal defects is regeneration of all lost structures including the alveoloar bone, cementum, and periodontal ligament. Attempts at regeneration of complicate periodontal defects by guided tissue regeneration have not always yielded predictable results. Recently, attempts at engineering the defects using various materials have shown promising results. Chong et al.[26] reported human periodontal fibroblast response to enamel matrix derivative, amelogenin, and platelet-derived growth factor-BB. They reported that the combination of EMD and PDGF-BB produces greater proliferative and wound-fill effects on PDLS cells than each by themselves, and that amelogenin alone may not trigger the regenerative potential of periodontal tissues and that it requires a combined interaction with other enamel matrix components of EMD to direct the regenerative process.

Tissue engineering is applied to overcome limited tissue regeneration using the factors that would stimulate the regeneration of alveoloar bone and periodontal attachment. Human periodontal ligament fibroblast (hPDLF) can be differentiated and proliferated into osteoblast-like cell and cementoblast-like cell, playing a central role in periodontal regeneration. People need their teeth longer as the life expectancy increased and want to prepare for healthy older years by maintaining healthy periodontal tissue in shape-wise and from esthetic point of view.

This study was performed to investigate the possibility of fibroblast differentiation from bone marrow stromal cells (BMSC) using the fibroblast growth factor (FGF) and to explore their potential use for periodontal ligament engineering. RT-PCR was used to examine expression of nestin, nucleostemin, UNCL, S100A4 and S100A2-mRNA in the fibroblast growth factor and/or periodontal ligament fibroblast conditioned media-treated BMSCs at 1, 11, 14 days, respectively.

The causes of periodontitis is known aging, infection, and mechanical stress. Chronic periodontitis is a common in adult people. Generally, severe periodontitis patients have a inflammatory PDL or PDL loss. For the prevention and regeneration in periodontitis patients, inhibition of aging in periodontal cells is essential, and it will be worth using the hGF as a hPDLF.

Bone marrow stromal cells (BMSCs) reside in bone marrow and provide a lifelong source of new cells for various tissues[27]. Jeong *et al.*[27] reported that a BMSC-specific genetic catalog may facilitate future studies on molecular mechanisms governing core properties of these cells. The dental follicle is an ectomesenchymal tissue surrounding the developing tooth germ. It is believed that this tissue contains stem cells and lineage committed progenitor cells or precursor cells (PCs) for cementoblasts, periodontal ligament cells, and osteoblasts. Morsczeck *et al.*[28] reported the isolation of PCs derived from dental follicle of human third molar teeth and that demonstrated that cultured PCS are unique undifferentiated lineage committed cells residing in the periodontium prior or during tooth eruption. They compared gene expressions of PCs, human mesenchymal stem cells (hMSCs), periodontal ligament cells (PDL-cells) and osteoblasts (MG63) for delimination of PCS. These fibroblast-like, colony forming and plastic adherent cells expressed putative stem cell markers Notch-1 and nestin.

In this study, periodontal ligament fibroblast-conditioned medium was obtained from cultures of dog periodontal ligament fibroblasts that were untreated (plain) or treated with the indicated concentrations (3 ng/ml) of fibroblast growth factor for the 1-, 11- and 14 days culture period. Stem cell marker gene (nestin mRNA) expression pattern was increased in the treated group with the indicated concentrations (3 ng/ml) of fibroblast growth factor (FGF). PDL fibroblast marker genes (UNCL-, S100A2-mRNA) expression pattern was increased in the periodontal ligament fibroblast-conditioned medium group and the combined group than the control.

Bone marrow stromal cells (BMSCs) are pluripotent stem cells with self-renewal property and potential to differentiate into a variety of cell types[29, 30]. Yang *et al.*[31] founded that rat bone marrow stromal cells expressed NSE and nestin mRNA, but only weak expression of NF1 mRNA was detected. After Salvia miltiorrhiza induction for 24 hours, NSE and nestin expression maintained the same level, but NF1 expression increased apparently compared to the control. rBMSC could also differentiate into neuron-like cells and express neuron phenotype as previously described.

Cells of the periodontal attachment (cementoblast, osteoblasts, and periodontal ligament fibroblasts) are descended from a common progenitor (the cranial

neural crest). Lallier TE.[32] suggested that differential expression of semaphorins and plexins may be involved in regulating cell-sorting in the formation and regeneration of the periodontal attachment structure. Ogiso *et al.*[33] reported that fibroblasts secrete prostaglandins which can inhibit bone formation, and that this may be one mechanism whereby fibroblasts can modulate osteogenesis at the interfaces of soft and mineralizing connective tissues. Fibroblast-conditioned medium was obtained from cultures of human periodontal ligament fibroblasts that were untreated (plain) or treated with the indicated concentrations of indomethacin for the entire culture period[33].

Moreau *et al.*[34] reported that the sequential administration of growth factors to first proliferate and then differentiate BMSCs cultured on silk fiber matrices will support the enhanced development of ligament tissue in vitro. Confluent second passage (P2) BMSCs obtained from purified bone marrow aspirates were seeded on RGD-modified silk matrices. Seeded matrices were divided into three groups for 5 days of static culture, with medium supplement of basic fibroblast growth factor (B) (1 ng/ml), epidermal growth factor (E; 1ng/ml) or growth factor-free control (C). After day 5, medium supplement was changed to transforming growth gactor-β1 (T; 5 ng/ml) or C for an additional 9 days of culture. Sequential growth factor application promoted significant increases in collagen type I transcript expression from day 5 of culture to day 14, for five of six groups tested. Moreau *et al.*[34] reported that the findings indicates significant in vitro ligament development after only 14 days of culture when using a sequential growth factor approach.

Farhadi *et al.*[35] reported that human bone marrow stromal cells from six donors were expanded for two passages (expansion phase) and subsequently cultivated in osteogenic medium containing ascorbic acid, β-glycerophosphate, and dexamethasone (differentiation phase). After each phase, cells were transferred into serum-free medium with or without FGF-2 at different concentrations and for different times, and the expression of BMP-2, TGF-β1, and VEGF was quantified at the mRNA level. In response to 5 ng/ml FGF-2 for 24 hours, the mRNA expression of VEGF increased at both culture phases (up to 6.1 fold), whereas that of BMP-2 and TGF-β1 significantly increased only after the expansion (3.1-fold) or differentiation phase (2.1-fold), respectively. FGF-2 up-regulates the expression of BMP-2, TGF-β1, and

VEGF in human bone marrow stromal cells, in a pattern dependent on the cell-differentiation stage. These findings prompt for in vivo investigations on the delivery of FGF-2 for the temporally/functionally regulated enhancement of bone marrow stromal cell-based bone induction[35].

In our study, the expression of UNCL mRNA were expressed strongly at 11 days than one day. Expecially, The UNCL mRNA were expressed stronly in FGF treated and control group. The S100A4 mRNA was expressed strongly in FGF treated at 11 day. The expression of nucleostemin mRNA were expressed strongly at 11 days than one day. Expecially, the nucleostemin mRNA were expressed stronly in FGF treated and control group. The S100A2 mRNA was expressed similarly between experimental groups and the control group at 11 day. At 11 day, the expression of the nestin mRNA was weak.

Bone marrow stromal cells (BMSCs) play a central role in the repair and regeneration of mesenchymal tissues. Hankemeier *et al.*[36] reported modulation of proliferation and differentiation of human bone marrow stromal cells by fibroblast growth factor 2 for potential implications for tissue engineering fo tendons and ligaments. They analyzed the effect of low-dose (3 ng/ml) fibroblast growth factor 2 (FGF-2) and high-dose FGF-2 (30 ng/ml) on proliferation, differentiation (transcription of collagen I, collagen III, fibronectin, elastin, α-smooth muscle actin, and vimentin, reverse transcription-polymerase chain reaction) of human BMSC, and compared the results with those of a control group without FGF-2. They reported that microscopic investigation of the cell cultures with low-dose FGF-2 showed more homogeneous, dense, fibroblast-like, spindle-shaped cells with long cell processes compared with cultures with high-dose, or no FGF-2, and showed that low-dose FGF-2 may be useful for tissue engineering of ligaments and tendons by increasing BMSC proliferation and stimulating mRNA expression of specific extracellular matrix proteins and cytoskeletal elements.

In this study, the expression of UNCL mRNA were expressed strongly in FGF treated and FGF plus PDLF-conditioned media groups at 14 days. The S100A4 mRNA was expressed strongly in FGF treated at 14 day. The S100A2 mRNA was expressed stronly in all experimental groups (FGF treated, FGF plus PDLF-conditioned media and the PDLF-conditioned media group). The nucleostemin mRNA were expressed stronly in FGF treated at 14 days.

Conclusion

In this study, UNCL-, S100A2-, S100A4-mRNA was expressed strongly in FGF experimental groups treated in BMSCs. These findings suggest that FGF can be associated for engineering periodontal ligament by providing the initial evidence of a reproducible protocol for fibroblastic differentiation of bone marrow stromal cells.

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논문제목	한글 : 섬유모세포성장인자가 개의 골수기질세포로부터 섬유모세포 분화에 끼치는 효과 영문 : Effect of fibroblast growth factor on fibroblast differentiation from bone marrow stromal cell in dog				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

2008 년 10 월 일

저작자 : 서 영 종 (서명 또는 인)

조선대학교 총장 귀하