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[UCI]I804:24011-200000237597

2009년 2월
박사학위논문

Immunohistochemical study of vascular endothelial cell differentiation from dog bone marrow stromal cell cultured with VEGF on scaffold

조선대학교 대학원

치 의 학 과

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비계상에 *VEGF*와 함께 배양된 성견의 골수기질세포의
혈관내피세포로의 분화에 관한 면역조직화학적 연구

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지도교수 장 현 선

이 논문을 치의학 박사학위신청 논문으로 제출함.

2008년 10월 일

조선대학교 대학원

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2008 년 11월 일

조선대학교 대학원

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국문초록

비계상에 VEGF와 함께 배양된 성견의 골수기질세포의
혈관내피세포로의 분화에 관한 면역조직화학적 연구

조 일 준
지도교수 : 장 현 선
조선대학교 대학원 치의학과

치주치료의 궁극적인 목적은 치주조직의 재생이라 할 수 있다. 치주조직 재생에 필수적인 치주인대의 회복은 심한 치주염으로 파괴된 치주염 환자에서 추구해야할 목표라고 할 수 있다. 또한 최근에는 무치악 부위에 임플란트 치료가 일반화되었는데 치주염으로 흡수된 부위의 골조직의 증대는 선결조건이 되었다. 최근에는 조직재생의 한계를 극복하기 위하여 치,의학계에서 조직공학적 시도가 이루어지고 있는데, 심한 치주염으로 인해 조직 재생의 한계를 나타내는 치주염 환자들에서 세포, 비계, 성장인자를 실험실상에서 치주조직으로 형성하여 적용할 경우 임상에 유용할 것으로 생각된다. 또한 조직공학적으로 치주조직(연조직, 경조직)을 형성할 경우 치유에 필수적인 혈관 신생을 동시에 형성하여 치주조직 재생에 적용할 경우 치유의 효과 및 재생 시간을 단축시킬 수 있을 것으로 생각된다. 그러나 치주조직공학적으로 세포, 비계, 성장인자를 이용한 혈관신생에 관한 연구는 미비한 실정이다. 이 연구의 목적은 임상에서 흔히 이용되고 있는 흡수성교원질막에 골수줄기세포를 배양한 후 혈관내피세포성장인자를 적용하여 줄기세포에서 혈관세포로의 분화 가능성을 연구하고자 하였다.

줄기세포는 성견의 골수기질세포를 실험에 이용하였다. 골수기질세포를 흡수성교원질막에서 배양하면서 혈관내피세포성장인자를 적용하였다. 흡수성교원질막에서 골수기질세포만 배양한 것을 대조군으로 하였고, 흡수성교원질막에서 골수기질세포를 배양하면서 혈관내피세포성장인자(50 ng/㎖)를 적용한 것을 실험군으로 하였다. 대조군, 실험군 모두 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. 골수기질세포에서 혈관세포로의 분화 가능성을 조직학적으로 평가하기 위하여 1주, 2주 대조군과 실험군 조직을 10% 중성 포르말린에 고정하고 통상적인 방법으로 hematoxylin-eosin 염색과 면역조직화학적 염색을 시행한 후 조직학적으로 관찰하였다.

대조군보다 실험군에서 세포증식이 더 뚜렷하게 관찰되었고, 실험군에서 CD31 양성 세포들이 대조군에 비하여 더 뚜렷하게 관찰되었다. 이 연구 결과 흡수성교원질막에 골수기질세포를 배양하면서 혈관내피세포성장인자를 적용할 경우 혈관내피세포로의 분화 가능성을 시

사해주고있다.

주요어: 골수기질세포, 혈관내피세포성장인자, 흡수성교원질막, CD31

Introduction

The triad of tissue engineering is composed of cell, scaffold, and signaling molecule. Tissue engineering has developed in response to an inadequately of organs and tissues for patients requiring organ/tissue replacement[1). Periodontitis can be caused by infection, mechanical stress and aging. The main purpose in periodontal therapy is the in regenerating periodontal tissue. Once the periodontal tissue is impaired, the tissue has a limited capacity for regeneration[2). Since cementoblasts, osteoblasts, and periodontal ligament fibroblasts are derived from periodontal ligament[3), 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[4-7). Melcher *et al.*[8) 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. Generally, severe periodontitis patients expected tooth extration have no PDLF. 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 regenerate periodontal tissues[9).

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[10). Lee *et al.*[11) 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.*[12] 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.*[11] observed that connective tissue growth factor (CTGF)-treated human mesenchymal stem cells (hMSCs) failed to show osteogenic or chondrogenic differentiation, and that CTGF is an effective induction factor for fibroblastic differentiation of hMSCs.

The rapid and transient growth of new capillaries, the process called angiogenesis, is central to most human life processes including tissue divelopment, regeneration, and repair[13].

MSCs can become a valuable cell source as an autograft for clinical application involving tissue regeneration[14]. 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[14].

Moreau *et al.*[15] reported that in vitro BMSCs growth might 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.

Among these factors, vascular endothelial growth factor (VEGF) shows promise as a molecule that may enhance the vascularization of engineered tissues, as it has been shown to act most specifically on endothelial cells (ECs)[16]. CD31 identifies platelet endothelial cell adhesion molecule (PECAM) present on endothelial cells and in non-mesoderm-derived cells[17].

The aim of this study was to investigate the possibility of endothelial cell differentiation from dog bone marrow stromal cell cultured with VEGF on scaffold immunohistologically.

Materials and Methods

Cell Culture

The dog bone marrow stromal cells (BMSCs) were used for our study. 2 passage BMSCs were used for this study. The BMSCs 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 7 days and 14 days, respectively. The 50 ng/㎖ vascular endothelial growth factor (VEGF) was applied every 2 days until 7 days. The control group was BMSC cultured in the resorbable collagen membrane (Bio-Gide) without VEGF treatment. The experimenta group was BMSC cultued in the resorbable collagen membrane (Bio-Gide) with VEGF treatment. The experimant one group was cultured at 5% CO_2, 37℃, and 100% humidity in a 60 ㎜ Petri dish until 7 days and the 50 ng/㎖ VEGF was applied in a 2-day interval until 7 days. The experimant two group was cultured at 5% CO_2, 3 7℃, and 100% humidity in a 60 ㎜ Petri dish until 14 days and the 50 ng/㎖ VEGF was applied in a 2-day interval until 7 days.

Histologic study and Immunohistochemical study

The specimen was immediately fixed in a solution containing 10% neutral formalin. They were processed routinely and were embedded in paraffin, The paraffin blocks were sectioned in 6 ㎛ thin slices and the sections were stained with hematoxylin-eosin for general histological observations. The sections were evaluated under a light microscope for detection of endothelical-like cell.

For the immunohistochemistry, monoclonal mouse anti-human antibodies were purchased from Dako Cytomation Inc. (Glostrup, Denmark). The primary Abs used were CD31. Sections were stained by the biotin-streptavidin-peroxidase method (Vectastain® ABC-AP kit, Burlingame, CA, USA.). The paraffin sections were pre-incubated with 1.5 % blocking normal serum diluted in 1X PBS for 20 min, and incubated for overnight with primary antibody (anti-CD31) diluted (1:200) in the diluted normal serum. After washing in 1X PBS, the sections were incubated for 30 min at room temperature with diluted biotinylated universal sencondary antibody. The sections were then rinsed briefly with 1X PBS, reacted with Vectastain® ABC-AP Reagent for 30 min. After color development with 0.05% DAB (diaminobenzidine tetrahydrochloride), the sections were washed and counterstained with hematoxylin. The positively stained cells in the experimental and control group were compared with each other.

Results

Morphological changes of BMSC cultured in the resorbable collagen membrane was shown according to the VEGF. Morphological changes of the cells were observed under a light microscope.

In microscopic analysis, the cell prolifeation was shown in experimental group than control group (Figure. 1-6). We have evaluated $CD31^{+}$ endothelial cells by

using immunohistochemistry in BMSC cultured in the resorbable collagen membrane (Bio-Gide) with VEGF. This study showed that there were more $CD31^+$ endothelial cells in the experimental group than the control group (Figure. 7). Moreover, the CD31 positive cells were associated with a significant increase in numbers of endothelial cells in the experimental group.

The endothelial cell differentiation from bone marrow stromal cell cultured with VEGF on scaffold was marked by a significant increase in numbers of $CD31^+$ endothelial cells at 2 weeks (Figure. 8). At 2 weeks, the endothelial cells were predominantly present in experimental group compared with control group. At one week, there was a slight increase in the numbers of $CD31^+$-labeled cells in the experimental group. In the experimental group, the expression of CD31 positive cells were expressed weakly at one week compared with two weeks.

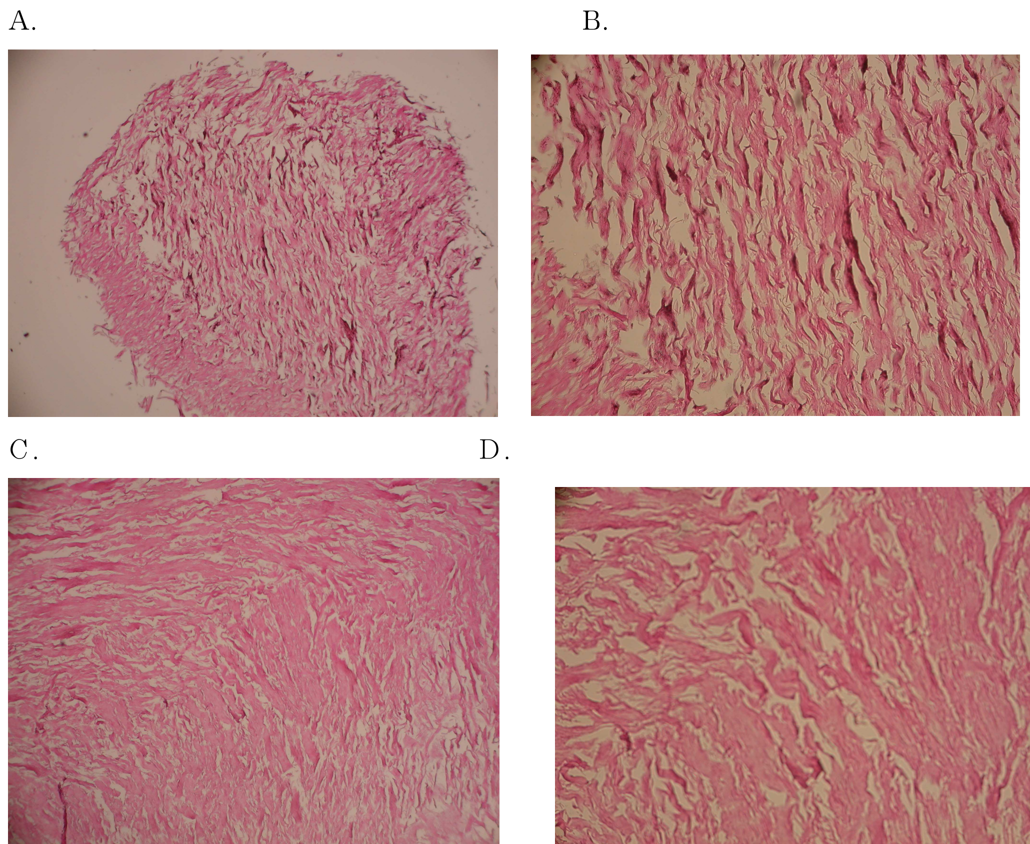
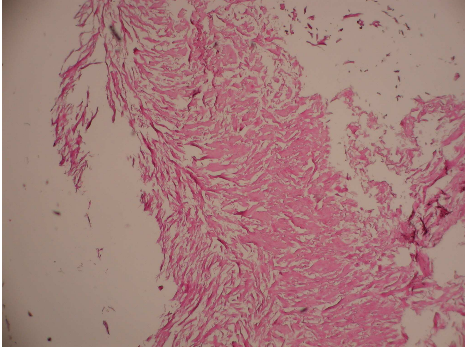


Figure 1. Histologic features of endothelial cell differentiation from bone marrow stromal cell cultured with VEGF on scaffold. Cells in experimental group was proliferated more than control group. At 1 week. Hematoxylin-easin staining. magnification, ×40(A,C), ×100(B,D). A,B. VEGF-treated (Experimental group), C,D. VEGF-untreated (control group)

A.



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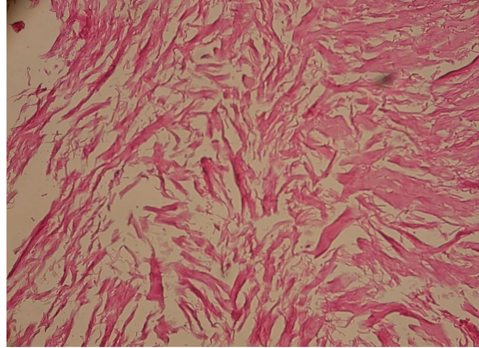

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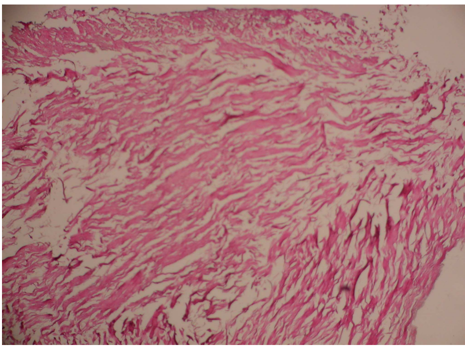

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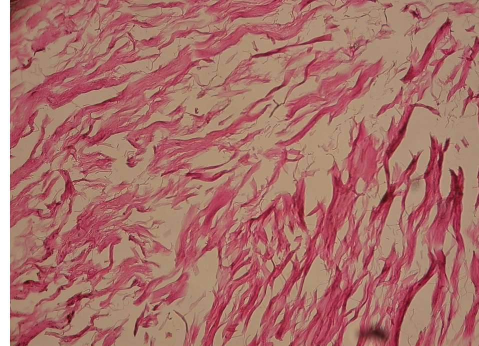
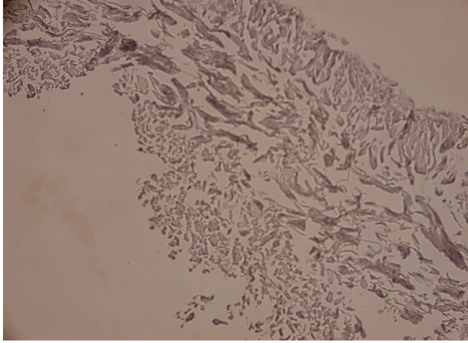
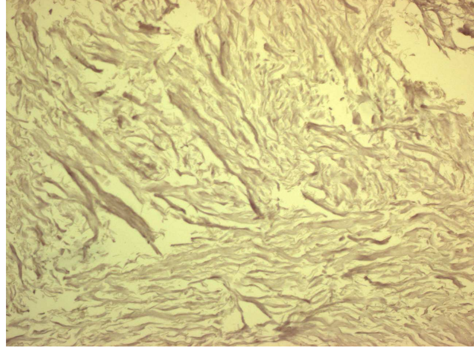


Figure 2. Histologic features of endothelial cell differentiation from bone marrow stromal cell cultured with VEGF on scaffold. The vascular endothelial-like cells in experimental group was show more than control group. At 2 week. Hematoxylin-easin staining. magnification, ×40(A,C), ×100(B,D). A,B. VEGF-treated (Experimental group), C,D. VEGF-untreated (control group)

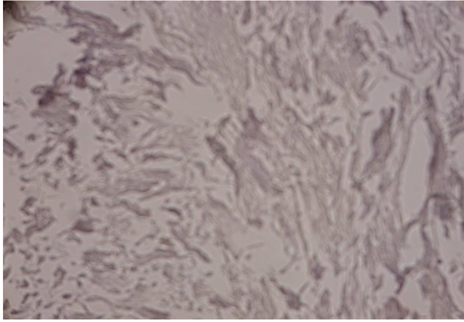
A.



B.



C.



D.

Figure 3. Histologic features of endothelial cell differentiation from bone marrow stromal cell cultured with VEGF on scaffold. CD 31 positive cells in experimental group was shown more than control group. The expression of CD31 positive cells were expressed weakly at one week compared with two weeks. 1 week (A,C) and 2 week (B,D). Immunohistochemical staining. magnification, ×100.

Discussion

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.

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.[18] 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.

Recently, four factors (cell, matrix, growth factor, angiogenesis) is essential for periodontal tissue engineeing. A variety of growth factors that promote the formation of a new microvasculature have been identified[19,20], and they could potentially be utilized to accelate the ingrowth of blood vessels in developing tissue[21].

This study was performed to investigate the possibility of endothelial cell differentiation from bone marrow stromal cells (BMSC) cultured with VEGF on scaffold and to explore their potential use for periodontal tissue engineering.

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. Bone marrow stromal cells (BMSCs) reside in bone marrow and provide a lifelong source of new cells for various tissues[22]. Bone marrow stromal cells (BMSCs) are pluripotent stem cells with self-renewal property and potential to differentiate into a variety of cell types[23, 24].

Moreau *et al.*[15] 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 5 days, 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.*[15] 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.*[25] 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[15].

One of the fundamental principles that underlies tissue-engineering strategies using cell transplantation is that a newly formed tissue must acquire and maintain sufficient vascularization in order to support its growth. Enhacing angiogenesis through delivery of growth factors is one approach to establishing a vascular network to these tissues[26]. Kaigler et al.[26] reported that the growth and differentiation of cultured ECs were enhanced in response to exposure to BMSC conditioned medium (CM) that human BMSCs secrete sufficient quantities of VEGF to enhance survival and differentiation of endothelial cells in vitro. Brody et al.[27] reported about characterizing nanoscale topography of the aortic heart valve basement membrane for tissue engineering heart valve scaffold design. Yu et al.[28] reported about a preparation and endothelialization of decelluarised vscular scaffold for tissue-engineered blood vessel.

Bone marrow stromal cells (BMSCs) play a central role in the repair and regeneration of mesenchymal tissues. Hankemeier *et al.*[29] reported modulation of proliferation and differentiation of human bone marrow stromal cells by fibroblast growth factor 2 for potential implications for tissue engineering for tendons and ligaments. In our study, cells in experimental group was proliferated more than control group. In H & E staining, the vascular endothelial-like cells in experimental group was show more than control group at 2 week. In immunohistochemical study, CD 31 positive cells in experimental group was shown more than control group. The expression of CD31 positive cells were expressed weakly at one week compared with two weeks.

Conclusion

We suggest that the VEGF can be used for vascular endothelial cell differentiation from bone marrow stromal cell cultured on scaffold and that a engineering vascular tissue can be made in vitro.

Reference

1. Langer R. Vacanti JP. Tissue engineering. Science 260:920, 1993.
2. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontology 2000. 40:164-172, 2006.
3. Melcher AH. Cells of periodontium: their role in the healing of wounds. Ann R Coll Surg Engl 67:130-131, 1985
4. Polson AM, Caton J. Factors influencing periodontal repair and regeneration. J Periodontol 1982;53(10):617-625.
5. Kawanami M, Sugaya T, Gama H, et al. Periodontal healing after replantation of intentially rotated teeth with healthy and denuded root surfaces. Dental Traumatology 2001;17(3):127-133.
6. Shimono M, Ishikawa T, Ishikawa H, et al. Regulatory mechanisms of periodontal regeneration. Microsc Res Tech 2003;60(5):491-502.
7. Silva TA, Rosa AL, Lara VS. Dentin matrix proteins and soluble factors: intrinsic regulatory signals for healing and resoption of dental and periodontal tissues. Oral Diseases 2004;10(2):63-74.
8. Melcher AH. On the repair potential of periodontal tissues. JP. 1976;47(5):256-260.
9. Benatti BB, Silverio KG, Casati MZ, Sallum EA, Nociti FH Jr. Physiological features of periodontal regeneration and approches for periodontal tissue engineering utilizing periodontal ligament cells. J of Bioscience and Bioengineering 103(1):1-6, 2007.
10. Inanc B, Elcin AE, Unsal E, Balos K, Parlar A, Elcin YM. Differentiation of human embryonic stem cells on periodontal ligament fibroblasts in vitro. Artif Organs 32(2):100-109, 2008
11. Lee CH, Moioli EK, Mao JJ. Fibroblastic differentiation of human mesenchymal stem cells using connective tissue growth factor. Conf Proc IEEE Eng Med Biol Soc. 1:775-778, 2006.
12. Ogiso B, Hughes FJ, Melcher AH, Mcculloch CAG. Fibroblasts inhibit mineralized bone nodule formation by rat bone marrow stromal cells in vitro.
13. Folkman J, Cotran R. Relation of vascular proliferation to tumor growth. Int. Rev Exp Pathol 1:207, 1976.

14. Trubiani O, Orsini G, Zini N, Di Iorio D, Piccirilli M, Piattelli A, Caputi S. Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: A morphological report. J Biomed Mater Res A. 2008.
15. Moreau JE, Chen J, Horan RL, Kaplan DL, Altman GH. Sequential growth factor application in bone marrow stromal cell ligament engineering. Tissue Engineering. 11(11-12):1887-1897, 2005.
16. Ferrara N. Vascular endothelial growth factor. Eur. J. Cancer 32A:2413, 1996.
17. Rafii S. Circulation endothelial precursors: mystery, reality, and promise. J Clin Invest. 105:17-19, 2000.
18. Chong CH, Carnes D, Moritz A, Oates T, Ryu OH, Simmer J, Cochran D. Human Periodontal Fibroblast Response to Enamel Matrix Derivative, Amelogenin, and Platelet-Derived growth Factor-BB. JP 77;1242-1252, 2006.
19. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev. Physiol 53:217, 1991
20. Polverini PJ. The pathophysiology of angiogenesis. Crit. Rev Oral Biol Med 6:230, 1995.
21. Kaigler D, Krebsbach PH, Polverini PJ, Mooney DJ. Role of vascular endothelial growth factor in bone marrow stromal cell modulation of endothelial cells. Tissue engineering 9(1):95-103, 2003.
22. Yaghoobi MM, Mowla SJ, Tiraihi T. Nucleostemin a coordinator of self-renewal, is expressed in rat marrow stromal cells and turns off after induction of neural differentiation. Neuroscience Letters 390:81-86, 2005.
23. Lallier TE. Semaphorin profiling of periodontal fibroblasts and osteoblasts. J Dent Res 83(9):677-682, 2004.
24. Ogiso B, Hughes FJ, Davies JE, Mcculloch CAG. Fibroblastic regulation of osteoblast function by prostaglandins. Cellular signaling 4(6):627-639, 1992.
25. Farhadi J, Jaquiery C, Barbero A, Jakob M, Schaeren S, Pierer G, Heberer M, Martin I. Differentiation-dependent up-regulation of BMP-2, TGF-β1, and VEGF expression by FGF-2 in human bone marrow stromal cells. Plast. Reconstr. Surg. 116:1379-1386, 2005.
26. Kaigler D, Krebsbach PH, Polverini PJ, Mooney DJ. Role of vascular endothelial growth factor in bone marrow stromal cell modulation of

endothelial cells. Tissue engineering 9(1):95-103, 2003.

27. Brody S, Anilkumar T, Liliensi다 S, Last JA, Murphy C, Pandit A. Characterizing nanoscale topography of the aortic heart valve basement membrane for tissue engineering heart valve scaffold design. Tissue engineering. 12(2):413-421, 2006)
28. Yu XX, Wan CX, Chen HQ. Preparation and endothelialization of decelluarised vscular scaffold for tissue-engineered blood vessel. J Mater Sci:Mater Med 19:319-326, 2008.
29. Hankemeier S, Keus M, Zeichen J, Jagodjinski M, Barkhausen T, Bosch U, Krettek C, Grisensven M. Modulation of Proliferation and Dirrerentiation of Human Bone Marrow Stromal Cells by Fibroblast GROWTH Fator 2: Potential Implications for Tissue Engineering of Tendons and Ligaments. 11(1-2):41-49, 2005.

저작물 이용 허락서

학 과	치의학과	학 번		과 정	박 사
성 명	한글 : 조 일 준 　　한문 : 　　　영문 :				
주 소					
연락처	016-652-2804				
논문제목	한글 : 비계상에 VEGF와 함께 배양된 성견의 골수기질세포의 혈관내피세포로의 분화에 관한 면역조직화학적 연구 영문 : Immunohistochemical study of vascular endothelial cell differentiation from dog bone marrow stromal cell cultured with VEGF on scaffold				

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 : 조 일 준 (서명 또는 인)

조선대학교 총장 귀하