The change of periodontium after extraction of periodontally involved tooth in rats

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The change of periodontium after extraction of periodontally involved tooth in rats

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감사의 글

논문이 완성되도록 부족한 저를 이끌어주신 최성호 교수님께 우선 깊은 감사의 인사를 드립니다. 아울러 옆에서 조언과 격려로 이 논문의 부족함을 메꾸어 주시고 심사를 해주신 김창성 교수님, 정의원 교수님께도 특별히 감사의 인사를 드립니다. 또한 항상 인자한 미소를 머금으시며 옆에서 격려를 해주신 채중규 교수님, 조규성 교수님, 이승종 교수님으로부터도 많은 힘을 얻었습니다. 박정철 교수님과 이중석 교수님의 도움도 감사드립니다.

연구 내내 아낌없는 자문과 실질적으로 궂은 일을 맡아서 해주신 차재국 선생님, 황지완 선생님, 양혜주 선생님, 조아란 선생님을 비롯한 치주과 의국원들께도 감사의 말씀을 드립니다.

위에 언급한 분들의 도움에 보답하는 길은 이 논문을 시작으로 앞으로 더욱더 좋은 논문을 내는 것이라 생각하며, 학문에 더 열심히 정진하도록 노력하겠습니다.

마지막으로 항상 저를 응원하여 주시고 정신적인 지주가 되어주시는 부모님, 든든한 후배이자 동생인 김영주 선생에게도 감사의 인사를 드리며 모든 분들의 건강을 기원합니다.

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ABSTRACT

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Recent interest has focused on intentional replantation as an alternative choice to restore an original tooth instead of replacing it with prosthesis or an implant. Some studies dealing with immediate replantation has shown successful results with intentional replantation for periodontally involved teeth. For long-term success of replantation, a healthy periodontal status of recipient site is required so that delayed replantation is more suitable for periodontally involved teeth.

To reveal the ideal timing for delayed replantation of periodontally involved teeth, healing process of extraction sockets after extraction of periodontitis-induced teeth in rats were evaluated.

Twenty eight rats were randomly divided into two groups; control group (n=8) and test group (n=20). In the test group, periodontitis was induced by a ligature around the cervix of mandibular first molar of all rats. Two weeks later, the mandibular first molars were extracted in all animals of control and test groups. The animals were sacrificed on days 0, 3, 7 and 10 after extraction (n=2 in control group and n=5 in test group on each day) and mandibles were retrieved for histological and

immunohistochemical analysis. The change in amount of inflammatory cells and cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α were quantified.

In histological analysis of test group, inflammatory cell infiltrate was found abundantly in the remaining periodontium at the first 3 days after tooth extraction and decreased gradually at later time points. In immunohistochemical analysis of test group, both IL-6 and TNF-α were numerous in the furcation area at each post-extraction day. IL-6 was stained more heavily at between 3 and 7 days after extraction; at days 10 after extraction, little staining was observed. TNF-α staining was more intense at 3 days after extraction and gradually became weaken at later time points.

Within the limits of this study, it takes at least 10 days to resolve periodontal inflammation in rat extraction socket. Further studies are required to reveal the effect of delayed replantation in human model and compare between immediate and delayed replantation of periodontally involved teeth.

Key Words: tooth extraction, periodontitis, tooth socket, replantation, immunohistochemistry

The change of periodontium after extraction of periodontally involved tooth in rats

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I. INTRODUCTION

Intentional replantation has been defined as "The act of deliberately removing a tooth and following some procedures, returning it to its original socket." (Grossman, 1982) Recent interest has focused on intentional replantation as an alternative choice to restore an original tooth instead of replacing it with a prosthesis or implant. Some studies dealing with immediate replantation have shown successful results with intentional replantation for periodontally involved teeth. Lu (Lu, 1986) replanted a periodontally involved and endodontically mistreated mandibular first premolar and reported that replanted tooth remained functional and asymptomatic for 32 months.

Demiralp et al. (Demiralp et al., 2003) suggested that intentional replantation can be an alternative approach to extraction in cases where advanced periodontal destruction is present and no other treatments could be considered.

Although numerous studies (Demiralp et al., 2003; Lu, 1986) have shown successful results with intentional replantation, especially for periodontally involved teeth, a few of complications should be considered. Common complications are root resorption and ankylosis that leads to a gradual resorption of the dental hard tissues and their replacement by bone (Andreasen, 1980; Hammarstrom et al., 1989). To solve the complications, clinical and experimental studies showed that a healthy status of periodontium is of critical importance. With this understanding, two aspects could be considered. One is the extracted root surface and the other is the periodontium of extraction socket.

Most studies suggested not to touch the root surfaces of the tooth to be replanted if its periodontal ligament attachment is intact and sound (Dryden and Arens, 1994; Kratchman, 1997; Lu, 1986; Messkoub, 1991; Sharma and Duggal, 1994). But our concern was the replantation of periodontally involved teeth that had deeper periodontal pockets, advanced bone loss and the diseased periodontal ligament with necrotic cementum. They lead to above-mentioned complications. Lindskog et al. (Lindskog et al., 1985) reported root-surface resorption with or without accompanying ankylosis when the necrotic periodontal ligament was not removed prior to replantation of teeth. They suggested that denuding the root surface

chemically prior to replantation of the teeth without vital periodontal ligament would prevent resorption. Mahajan & Sidhu (Mahajan and Sidhu, 1982) also reported that the removal of the periodontal ligament raised the success rate of tooth replantation. This may be thought due to either the effective elimination of necrotic periodontal ligament and/or microorganisms prior to replantation.

Kratchman (Kratchman, 1997) suggested not to touch the socket walls and to rinse with sterile saline only if necessary, because touching the walls of socket might increase the risk of ankylosis. Furthermore, recent studies showed granulation tissues contained mesenchymal stem cells that were of help to healing of socket. On the other hand, most of the clinicians have performed curettage on the socket before replantation of periodontally involved teeth. It was generally agreed that the removal of granulation tissue raised the success rate of replantation (Madison, 1986; Sharma and Duggal, 1994). In an aspect of the absence of inflammation for replantation, “delayed replantation” also could be considered. Delayed replantation is to replant an extracted tooth after passing some treatment and time. This may allow decrease of complications by replantation after resolution of inflammation. The period between preparation of the recipient bed and replantation of the tooth also allows improvement of the nutrition and preservation of the vitality of the cells of the remaining periodontal ligament and the root cementum. Although Nethander et al. (Nethander et al., 2003) failed to show a difference between the immediate and delayed methods in terms of frequency of various types of root resortion, the teeth used in this study were not periodontally involved teeth. In case of periodontally involved teeth, delayed

replantation may be more suitable. However, there has been no study that reported the optimum replantation timing during the healing process of extraction socket for delayed replantation of periodontally involved teeth until recently.

For that reason, the aims of the present study were to observe healing process of extraction socket of periodontally involved teeth and determine the point of resolution of inflammation of periodontium so that its results serve as reference of ideal timing for delayed replantation.

II. MATERIALS AND METHODS

Study design

Twenty eight of six-week-old Sprague-Dawley rats were used for the present study. The animal experiment was conducted in accordance with the guidelines approved by the Animal Ethics Committee of the Yonsei University College of Dentistry, Seoul, Korea. All animals were randomly divided into two groups; control group (n=8) and test group (n=20). In all rats of the test group, periodontitis was induced with ligature placement on the mandibular first left molars. Two weeks later, the mandibular first left molars of all rats in control and test groups were extracted. The rats in each group were sacrificed on day 0, 3, 7 and 10 after extraction sequentially; n=2 in control group and n=5 in test group on each day (Fig. 1). Mandibles were retrieved for histological and immunohistochemical analysis (Fig. 2). Neutral buffered formalin (10%) was required to fix the dissected block sections for 10 days.

Method

Male Sprague-Dawley rats (280 to 330 g) were obtained from a company. Food and water were provided ad libitum. The animals were maintained in a temperature-controlled room (22℃) on a 12-hour light-dark cycle. In test group, rats were anesthetized with a 1: 2 mixture of zoletin and ketamine(~1.5 $\mu\ell$ anestheic per gram

of body weight). A sterilized nylon-thread ligature was placed around the cervix of the mandiblular left first molar and knotted mesially. This induction of periodontitis was performed according to protocols as previously described (Duarte et al., 2010). In control group, rats were remained without any intervention. Two weeks later, the mandibular first left molars of all rats in control and test group were extracted. Body weights and food intake were measured daily during the experimental period.

Evaluation method

-Histological evaluation

After rinsing the block in sterile water, the sections were decalcified in 5% formic acid for 2 weeks, dehydrated in a graded ethanol series, and embedded in paraffin. Step-serial sections, 5-μm thick, were cut in the apico-coronal vertical plane. The most-central section was selected based on the position of the adjacent roots. Inflammatory infiltrates, new bone formation and epithelium proliferation in the sections were observed under a microscope after being stained with hematoxylin-eosin.

-Immunohistochemical evaluation

After deparaffinization and rehydration, the antigen was retrieved using a microwave-based technique in citrate buffer (pH 9.0) for 30 min. The endogenous peroxidase was blocked with 3% hydrogen peroxide for 5 min. The sections were incubated for 30 min at room temperature with primary antibodies: IL-6 (SC-1265,

Santa Cruz Biotechnology, CA, USA) diluted at 1:100, and TNF-a (ab6671, Abcam, Cambridge, UK) diluted at 1:100. After washing three times, immunodetection was performed using a commercially available kit (EnVisionTM Detection System, Dako REALTM, Kyoto, Japan) according to the manufacturer's instructions. Slides were then counterstained with hematoxylin.

III. RESULTS

Histological analysis

In control group, all extraction sockets have shown uneventful and general healing processes. At 0 day, blood clot from the remained periodontium and the gingival tissue filled extraction socket (Fig. 3). At 3 days, the contracted clot was replaced by granulation tissue composed of fibroblasts and endothelial cells. Epithelium began to proliferate to cover extraction socket. At 7 days, woven bone and trabeculae pattern started to appear at the apical half of the socket firstly. At 10 days, newly formed bone was found much more and matured. Epithelium fully covered extraction socket (Fig. 4). Figure 5 shows histologic views of the extraction sockets at each day after tooth extraction in control group.

In test group, extraction socket was also filled with blood clot at 0 day. The blood clot mainly consisted of erythrocytes and fibrin strands with various leukocytes. In a few of sokets, fractured root rests were remained at apical portion because first molars had multiple roots which were long and thin, and easy to be fractured during extraction (Fig. 6). At coronal portion of socket, infiltration of inflammatory cells was found in the remained periodontium and observed until 3 days abundantly. At 7 days, the clot was replaced by granulation tissue and osteoblastic activity was observed along alveolar wall at the first. From 7 days, infiltration of inflammatory cells decreased gradually and was found a little in the coronal half of the socket at 10 days.

At 10 days, newly formed bone including the capillary-like vessels became more obvious in the apical of the socket histologically and epithelium had proliferated from the sides of the wound to cover the wound surface (Fig. 7). Figure 8 shows histologic views of the extraction sockets at each day after tooth extraction in test group.

Immunohistochemical analysis

Immunoreactivities of cytokines examined were consistently localized in the cytoplasm of the cells, showing a dark brown-colored reaction (Fig. 9a).

Figure 9, 10, 11 and 12 show respectively the immunohistochemical reactivities and table 1 and 2 show subjectively determined staining intensities of the tested cytokines in control and test groups. For comparison of immunoreactivities of the individual cytokines, the labeling intensity of cells was scored on the following scale of 0 to ++++ using subjective criteria, similar to that described previously (Ackermann et al., 1994; Chilosi et al., 1990; Pulford et al., 1990) : 0, no staining; +, weak staining (minimally detectable); ++, moderate staining; +++, strong staining; and ++++, very strong staining (dark brown, almost black).

In control group, IL-6 was little observed at 0 day and stained more strongly at between 3 and 7 days after extraction (Fig. 9b, 9c); at days 10 after extraction, little staining was observed like 0 day (Fig. 9d). Although it was somewhat weaker at 0 and 10 days, similar weak staining intensities had shown throughout healing period in case of TNF-α (Fig. 10).

In test group, IL-6 was stained very strongly at between 3 and 7 days after extraction (Fig. 11b, 11c); at days 10 after extraction, little staining was observed (Fig. 11d). TNF-α staining was more intense at 3 days after extraction and gradually became weaken at later time points (Fig. 12).

IV. DISCUSSION

Periodontal disease is mainly caused by interactions between bacteria of the dental plaque and components of the cellular and humoral host immune response, including cytokines and biological mediators released by activated immunocompetent cells (Kornman et al., 1997; Page et al., 1997). Each cell type in the inflammatory infiltrates (neutrophils, T- and B-lymphocytes, plasma cells, and macrophages) plays a specific role in the progression of periodontal disease (Page et al., 1997). On the contrary, it is suggested that the periodontal disease will be resolved if plaque, inflammatory infiltrates and cytokines disappear. Therefore, the present experiment was designed to analyze healing of sockets after extraction of periodontally involved teeth in rats histologically and immunohistochemically and to determine optimum timing of resolution of inflammation for delayed replantation. In all of the rats, healing was generally uneventful.

In histological analysis, extraction sockets of test group were filled with blood clot and periodontium of coronal portion demonstrated large numbers of inflammatory infiltrates on day 0. Until the first 3 days after tooth extraction, inflammatory infiltrates were found abundantly in the remaining periodontium and decreased at later time points. At 7 days, the proliferative granulation tissue was found in the extraction socket. Granulation tissue originates from the fibroblasts and the endothelial cells in the remained periodontium (Johansen, 1970; Todo, 1968).

Moreover, the fibroblasts in the granulation tissue are suggested to differentiate into osteoblasts, thus forming new bone during socket healing (Lin et al., 1994). The extraction socket was observed to undergo healing process and bone formation in the socket became obvious histologically from 7 days after extraction (Fig. 8). When comparing with control group and previous study (Sato and Takeda, 2007) which demonstrated the socket healing process after sound tooth extraction, we found that socket healing after periodontally involved tooth extraction showed the delay of 2-4 days. In fact, previous study (Sato and Takeda, 2007) found first new bone formation had begun from 5 days when we didn't observe in present study. It was assumed that inflammation might cause delayed socket healing.

In immunohistochemical analysis, we quantified interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) among cytokines at furcation area of extraction socket because IL-6 and TNF-α play an important role in inflammation.

In response to *P. gingivalis* oral gavage, mice with genetically deleted IL-6 had decreased bone loss compared to wild-type mice, suggesting that the production of IL-6, which is proinflammatory, contributed to bone resorption (Baker et al., 1999). Borch et al. (Borch et al., 2010) also suggested that an exaggerated production of IL-6 occurs in generalized aggressive periodontitis (GAgP). However, according to several studies, IL-6 didn't seem to disappear instantly as the source of inflammation disappeared. A recent randomized-controlled clinical trial confirmed that intensive periodontal treatment resulted in a temporary increase of serum levels of IL-6 while 6

months after the treatment (Tonetti et al., 2007). Lopez Carriches et al. (Lopez Carriches et al., 2006) have found that levels of IL-6 were higher after surgical extraction of lower third molars and remained high until the seventh day after. Miyawaki et al. (Miyawaki et al., 1996) have proved that the level of IL-6 in plasma increases after different operations (cystectomy, benign tumor extirpation, etc.). Cruickshank et al. (Cruickshank et al., 1990) studied the response of IL-6 in patients who had undergone different types of operations (minor surgery, cholecystectomy, hip surgery, colorectal surgery and major vascular surgery), finding that levels of IL-6 were related to the duration of surgery. The authors concluded that IL-6 is a sensitive and early marker of tissue damage. Results of the present experiment also showed a similar correspondence with theirs. In both control and test group, IL-6 was stained strongly at between 3 and 7 days after extraction; at 10 days after extraction, was decreased significantly. This finding suggests that IL-6 increased temporarily until 7 days through tissue damage of extraction and decreased at 10 days as inflammation was resolved. But in overall impression, IL-6 in test group showed stronger staining than IL-6 in control group due to induced inflammation.

TNF-α also has a significant role in the bone loss. Several studies showed TNF-α was increased with host response stimulated by dental plaque and bacterial products, raised osteoclastic activity and consequently accelerated bone resorption and periodontal breakdown (Garlet et al., 2007; Gaspersic et al., 2003; Graves et al., 2001). In other words, the decrease in TNF-α seemes to reduce the host response, thereby reducing expression of the cytokines that stimulate bone resorption, which

resulted in less net bone loss. In the study by Liu et al. (Liu et al., 2006) that examined the potential impact of TNF-α, bone resorption was induced following the placement of ligatures around rat molars for 7 days. At 4 and 9 days following removal of the ligatures, new bone formation occurred in normal mice with bone resorption's ceasing. In the present study, TNF-α of control group showed similar weak staining from 0 to 7 days. On the other hand, TNF-α of test group showed the peak at 3 days and was decreased gradually at later time points. At 10 days after extraction, TNF-α almost faded out and it implied the resolution of inflammation.

In fact, this study using rats can't be applied to human model directly, as there are differences between rats and human in healing process and time after extraction of periodontally involved teeth. The design of this study was to serve as reference of ideal timing for delayed replantation in various studies using rats. Thus, further studies are required to reveal the effect of delayed replantation in human model and compare between immediate and delayed replantation of periodontally involved teeth.

V. Conclusion

Within the limits of this study, it takes at least 10 days to resolve periodontal inflammation in the rat extraction socket. It can be a baseline information for delayed replantation following periodontally involved tooth extraction.

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REFERENCES

Ackermann MR, DeBey BM, Stabel TJ, Gold JH, Register KB, Meehan JT: Distribution of anti-CD68 (EBM11) immunoreactivity in formalin-fixed, paraffin-embedded bovine tissues. Vet Pathol 31: 340-348, 1994.

Andreasen JO: Analysis of pathogenesis and topography of replacement root resorption (ankylosis) after replantation of mature permanent incisors in monkeys. Swed Dent J 4: 231-240, 1980.

Baker PJ, Dixon M, Evans RT, Dufour L, Johnson E, Roopenian DC: CD4(+) T cells and the proinflammatory cytokines gamma interferon and interleukin-6 contribute to alveolar bone loss in mice. Infect Immun 67: 2804-2809, 1999.

Borch TS, Holmstrup P, Bendtzen K, Nielsen CH: In vitro cytokine responses to periodontal pathogens: generalized aggressive periodontitis is associated with increased IL-6 response to Porphyromonas gingivalis. Scand J Immunol 71: 440-446, 2010.

Chilosi M, Mombello A, Montagna L, Benedetti A, Lestani M, Semenzato G, et al.: Multimarker immunohistochemical staining of calgranulins, chloroacetate esterase, and S100 for simultaneous demonstration of inflammatory cells on paraffin sections. J Histochem Cytochem 38: 1669-1675, 1990.

Cruickshank AM, Fraser WD, Burns HJ, Van Damme J, Shenkin A: Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. Clin Sci (Lond) 79: 161-165, 1990.

Demiralp B, Nohutcu RM, Tepe DI, Eratalay K: Intentional replantation for periodontally involved hopeless teeth. Dent Traumatol 19: 45-51, 2003.

Dryden JA, Arens DE: Intentional replantation. A viable alternative for selected cases. Dent Clin North Am 38: 325-353, 1994.

Duarte PM, Tezolin KR, Figueiredo LC, Feres M, Bastos MF: Microbial profile of ligature-induced periodontitis in rats. Arch Oral Biol 55: 142-147, 2010.

Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR, Avila-Campos MJ, Cunha FQ, et al.: The dual role of p55 tumour necrosis factor-alpha receptor in

Actinobacillus actinomycetemcomitans-induced experimental periodontitis: host protection and tissue destruction. Clin Exp Immunol 147: 128-138, 2007.

Gaspersic R, Stiblar-Martincic D, Osredkar J, Skaleric U: Influence of subcutaneous administration of recombinant TNF-alpha on ligature-induced periodontitis in rats. J Periodontal Res 38: 198-203, 2003.

Graves DT, Oskoui M, Volejnikova S, Naguib G, Cai S, Desta T, et al.: Tumor necrosis factor modulates fibroblast apoptosis, PMN recruitment, and osteoclast formation in response to P. gingivalis infection. J Dent Res 80: 1875-1879, 2001.

Grossman LI: Intentional replantation of teeth: a clinical evaluation. J Am Dent Assoc 104: 633-639, 1982.

Hammarstrom L, Blomlof L, Lindskog S: Dynamics of dentoalveolar ankylosis and associated root resorption. Endod Dent Traumatol 5: 163-175, 1989.

Johansen JR: Repair of the post-extraction alveolus in the Wistar rat. A histologic and autoradiographic study. Acta Odontol Scand 28: 441-461, 1970.

Kornman KS, Page RC, Tonetti MS: The host response to the microbial challenge in periodontitis: assembling the players. Periodontol 2000 14: 33-53, 1997.

Kratchman S: Intentional replantation. Dent Clin North Am 41: 603-617, 1997.

Lin WL, McCulloch CA, Cho MI: Differentiation of periodontal ligament fibroblasts into osteoblasts during socket healing after tooth extraction in the rat. Anat Rec 240: 492-506, 1994.

Lindskog S, Pierce AM, Blomlof L, Hammarstrom L: The role of the necrotic periodontal membrane in cementum resorption and ankylosis. Endod Dent Traumatol 1: 96-101, 1985.

Liu R, Bal HS, Desta T, Krothapalli N, Alyassi M, Luan Q, et al.: Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. J Dent Res 85: 510-514, 2006.

Lopez Carriches C, Martinez Gonzalez JM, Donado Rodriguez M: Variations of interleukin-6 after surgical removal of lower third molars. Med Oral Patol Oral Cir Bucal 11: E520-526, 2006.

Lu DP: Intentional replantation of periodontally involved and endodontically

mistreated tooth. Oral Surg Oral Med Oral Pathol 61: 508-513, 1986.

Madison S: Intentional replantation. Oral Surg Oral Med Oral Pathol 62: 707-709, 1986.

Mahajan SK, Sidhu SS: Periodontal ligament, extra-oral period and use of fluorides in replantation of teeth. Indian J Med Res 75: 441-445, 1982.

Messkoub M: Intentional replantation: a successful alternative for hopeless teeth. Oral Surg Oral Med Oral Pathol 71: 743-747, 1991.

Miyawaki T, Maeda S, Shimada M: Elevation of plasma interleukin-6 level in patients undergoing oral and maxillofacial surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 81: 15-20, 1996.

Nethander G, Skoglund A, Kahnberg KE: Experimental autogenous tooth transplantation in the dog: a comparison between one- and two-stage surgical techniques. Acta Odontol Scand 61: 223-229, 2003.

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 2000 14: 216-248, 1997.

Pulford KA, Sipos A, Cordell JL, Stross WP, Mason DY: Distribution of the CD68 macrophage/myeloid associated antigen. Int Immunol 2: 973-980, 1990.

Sato H, Takeda Y: Proliferative activity, apoptosis, and histogenesis in the early stages of rat tooth extraction wound healing. Cells Tissues Organs 186: 104-111, 2007.

Sharma NK, Duggal MS: Replantation in general dental practice. Br Dent J 176: 147-151, 1994.

Todo H: Healing mechanism of tooth extraction wounds in rats. I. Initial cellular response to tooth extraction in rats studied with 3H-thymidine. Arch Oral Biol 13: 1421-1427, 1968.

Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al.: Treatment of periodontitis and endothelial function. N Engl J Med 356: 911-920, 2007.

LEGENDS

Figure 1. Sequence of the experiment in the test group.

Figure 2. Mandible retrieved for histological and immunohistochemical analysis. The arrow is the extraction site of the mandibular first left molar.

Figure 3. Histologic view of the extraction sockets at 0 day after tooth extraction in control group. Blood Coagulum (BC), Remained Periodontium (RP). (x 40, Hematoxylin and Eosin stain, scale bar= 1mm)

Figure 4. Histologic view of the extraction sockets at 10 days after tooth extraction in control group. Note proliferated epithelium (PE) covers extraction socket fully and new bone (NB) is formed. (x 40, Hematoxylin and Eosin stain, scale bar= 1mm)

Figure 5. Histologic view of the extraction sockets at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction in control group. (a) erythrocytes (black arrows), (c) woven bone (black arrows), (d) matured bone (black arrows), blood vessel (white arrows). (x 400, Hematoxylin and Eosin stain, scale bar= 100μm)

Figure 6. Histologic view of the extraction sockets at 0 day after tooth extraction in test group. Inflammatory Infiltrates (II), Blood Coagulum (BC), Root Rest (RR). (x 40, Hematoxylin and Eosin stain, scale bar= 1mm)

Figure 7. Histologic view of the extraction sockets at 10 days after tooth extraction in test group. Note proliferated epithelium (PE) from the sides of the wound to cover the wound surface except in the central region and the newly formed bone (NB) regenerated next to the alveolar wall within the socket. (x 40, Hematoxylin and Eosin stain, scale bar= 1mm)

Figure 8. Histologic view of the extraction sockets at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction in test group. (a) erythrocytes (black arrows), (b) osteoclasts (black arrows), plasma cell (white arrow), (c) woven bone (black arrow), osteocyte(white arrow), (d) osteoblast (black arrow), blood vessel (white arrows). (x 400, Hematoxylin and Eosin stain, scale bar= 100μm)

Figure 9. Interleukin-6 in the extraction sockets of control group at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction. (a) cytoplasms showing a dark brown-colored reaction (arrows) (x 400, Immunohistochemical stain, Scale bar= 100μm)

Figure 10. Tumor necrosis factor-α in the extraction sockets of control group at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction. (x 400, Immunohistochemical stain, Scale bar= 100μm)

Figure 11. Interleukin-6 in the extraction sockets of test group at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction. (x 400, Immunohistochemical stain, Scale bar= 100μm)

Figure 12. Tumor necrosis factor-α in the extraction sockets of test group at days (a) 0, (b) 3, (c) 7 and (d) 10 days after tooth extraction. (x 400, Immunohistochemical stain, Scale bar= 100μm)

TABLES

Table 1. Reactivities and staining intensities of tested cytokines in the extraction socket of control group. (a Subjective scale: 0, no staining; +, weak staining; ++, moderate staining; +++, strong staining; ++++, very strong staining.)

Cytokines	0 day	3 day	7 day	10 day
IL-6	+	++	++	0~+
TNF-α	0~+	+	+	0~+

(IL-6 : Interleukin-6, TNF-α : Tumor Necrosis Factor- α)

Table 2. Reactivities and staining intensities of tested cytokines in the extraction socket of test group. (a Subjective scale: 0, no staining; +, weak staining; ++, moderate staining; +++, strong staining; ++++, very strong staining.)

Cytokines	0 day	3 day	7 day	10 day
IL-6	++	+++	++	+
TNF-α	++	+++	++	+

(IL-6 : Interleukin-6, TNF-α : Tumor Necrosis Factor- α)

FIGURES

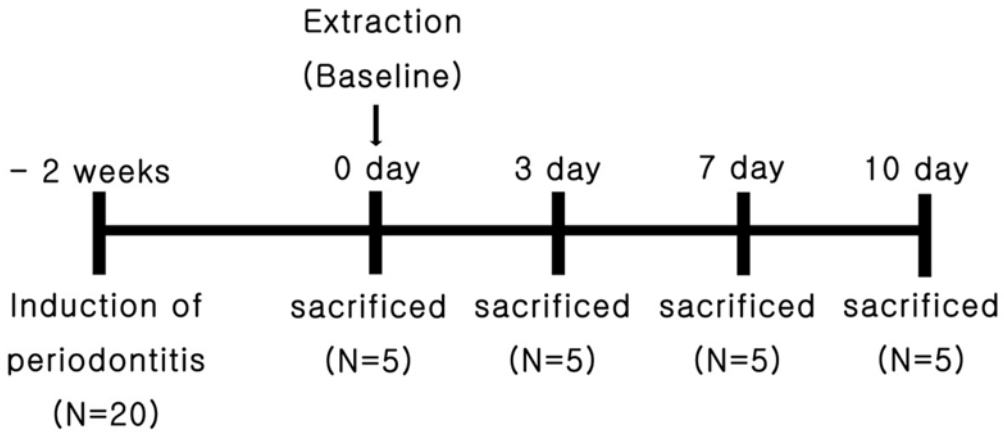


Figure 1

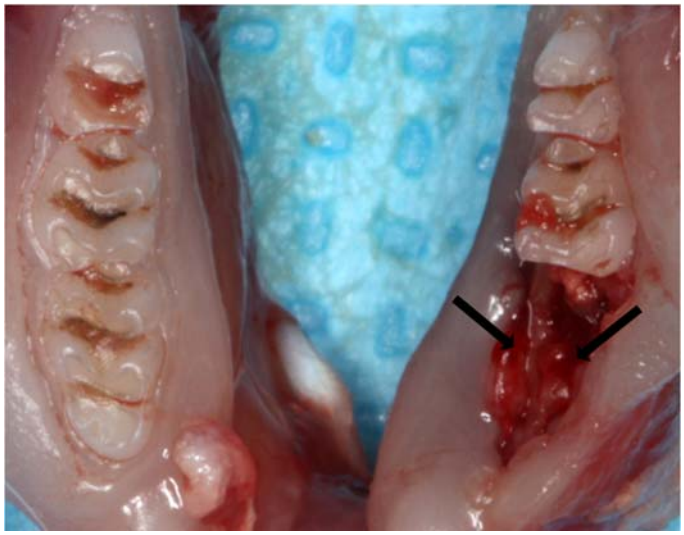
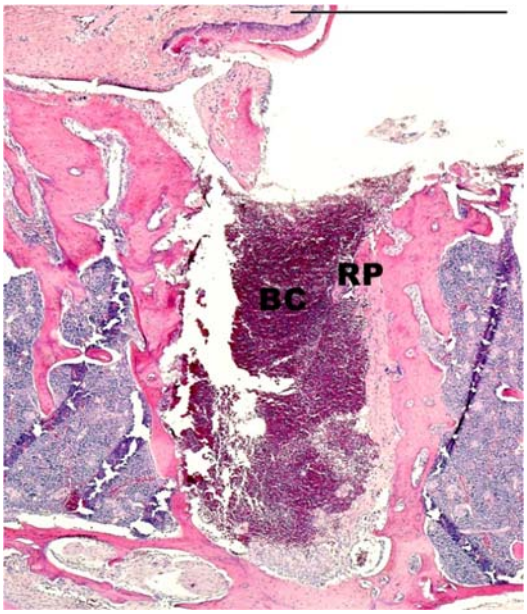


Figure 2



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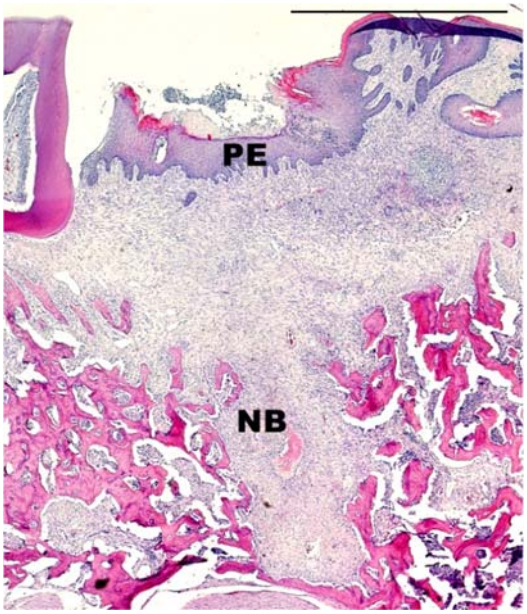

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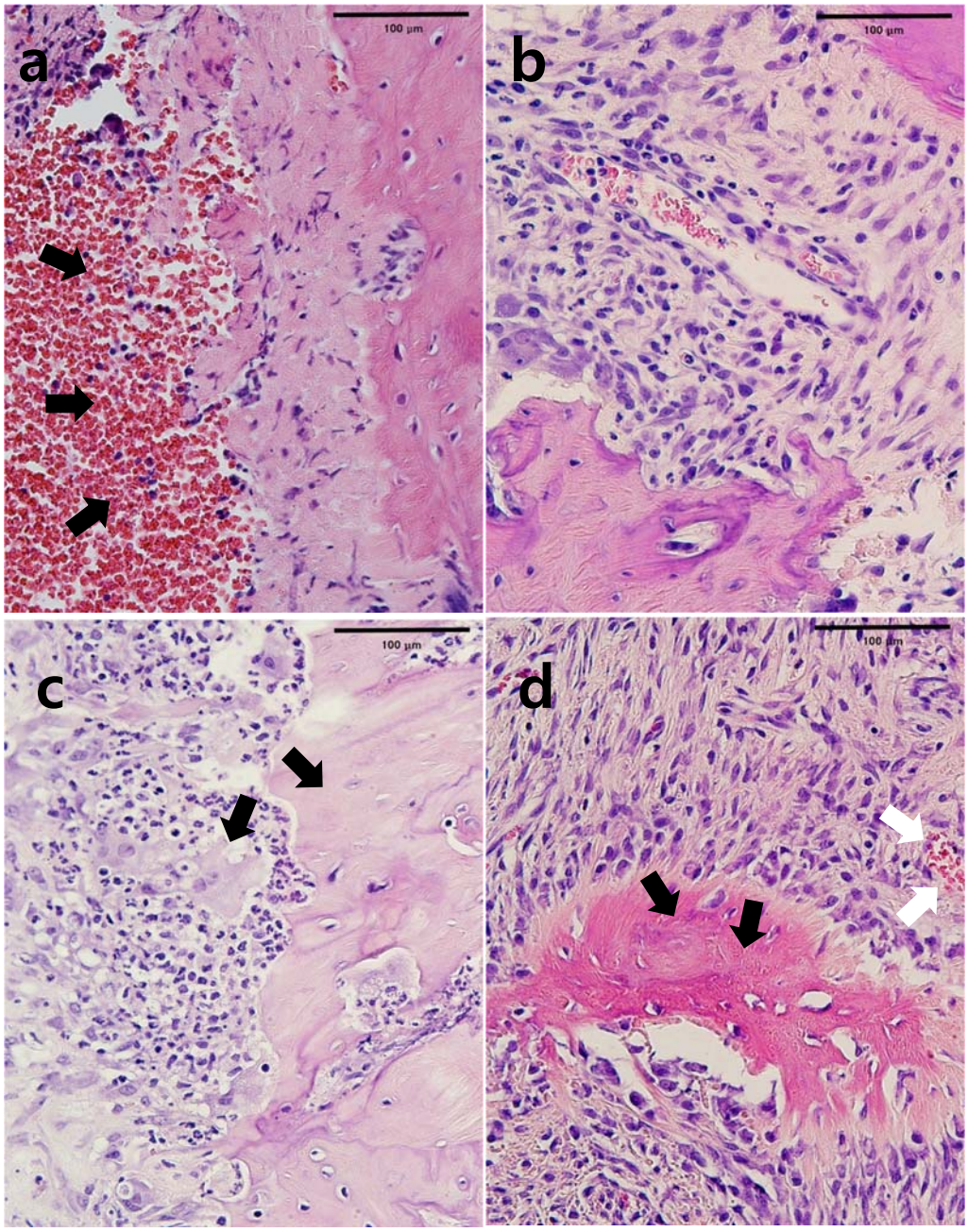

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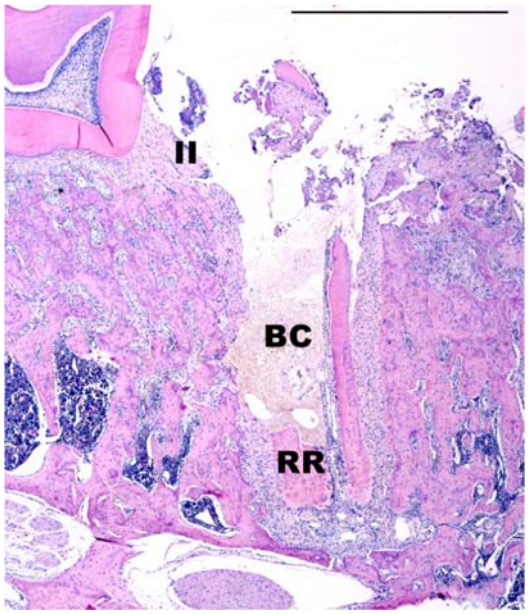

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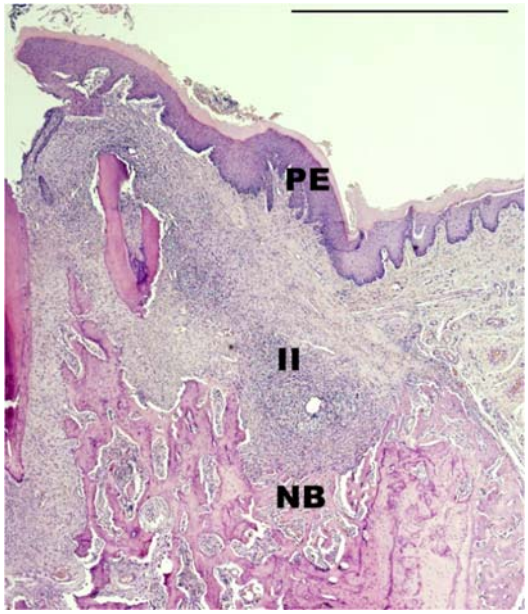

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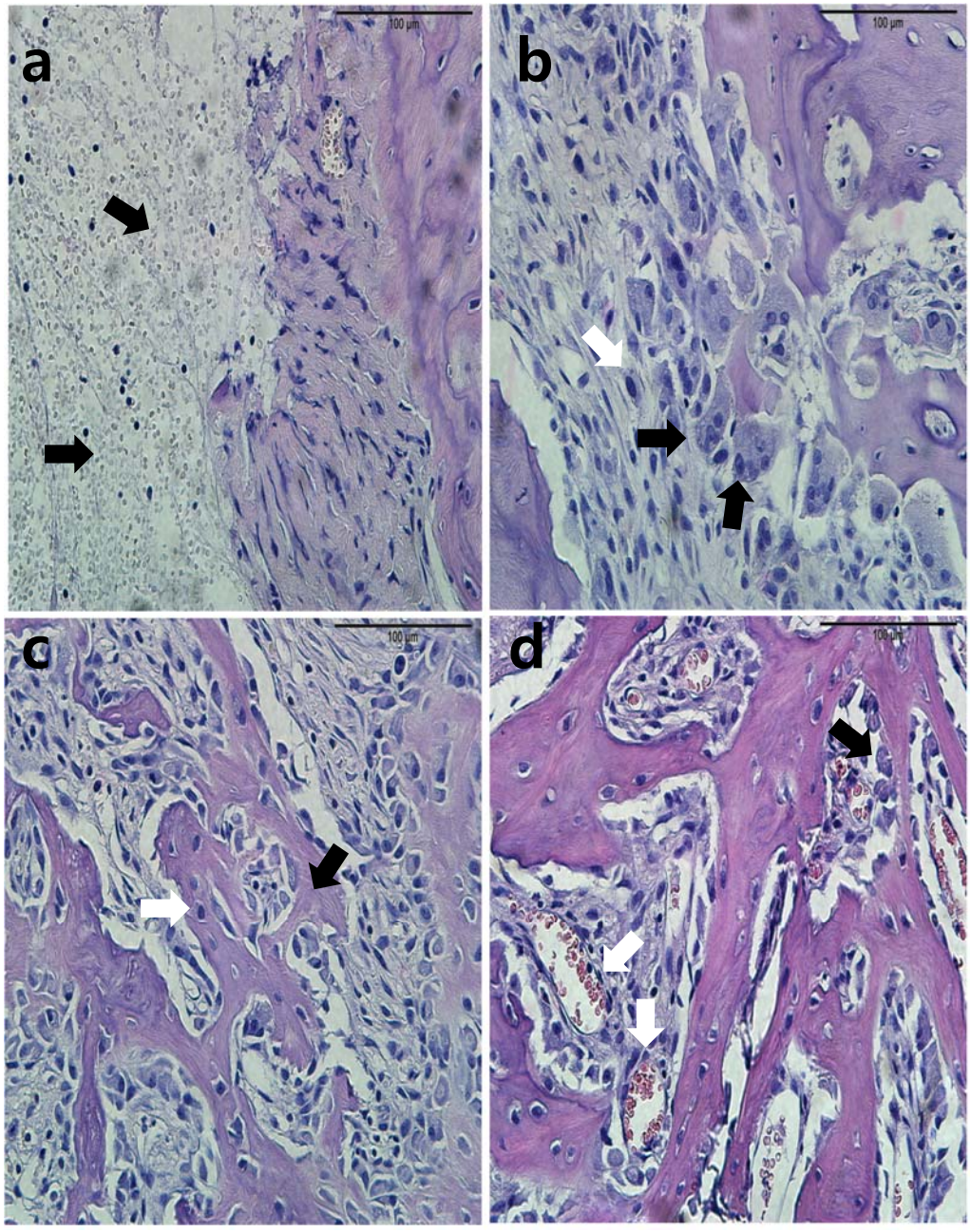

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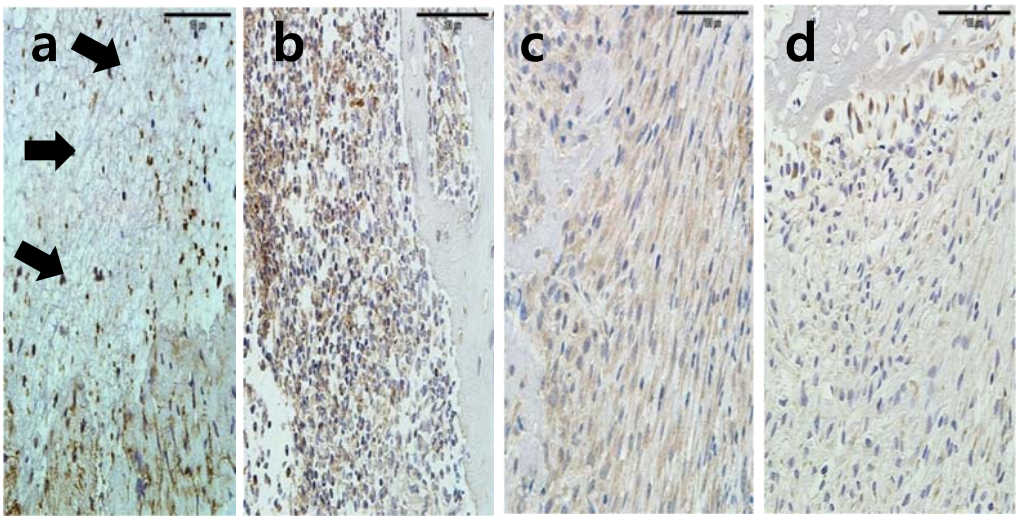

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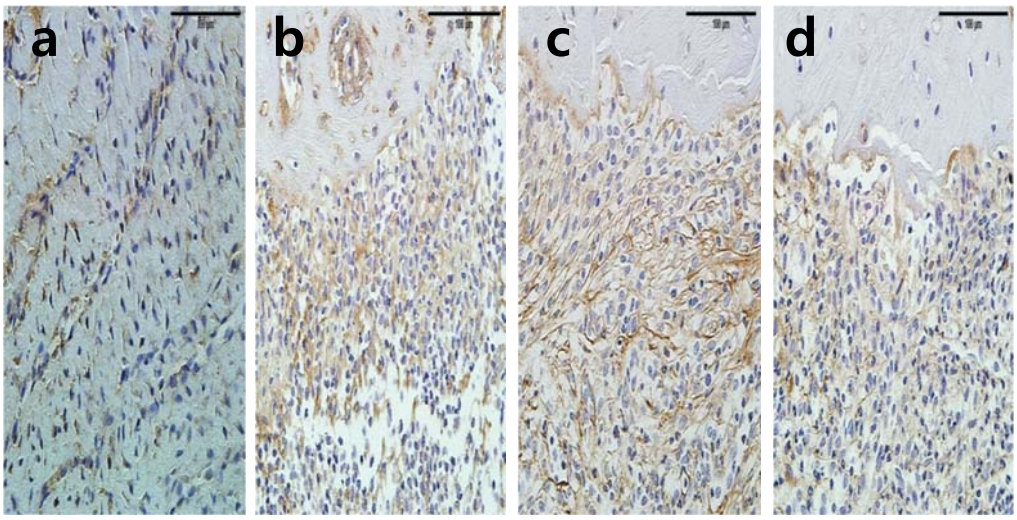

Figure 10

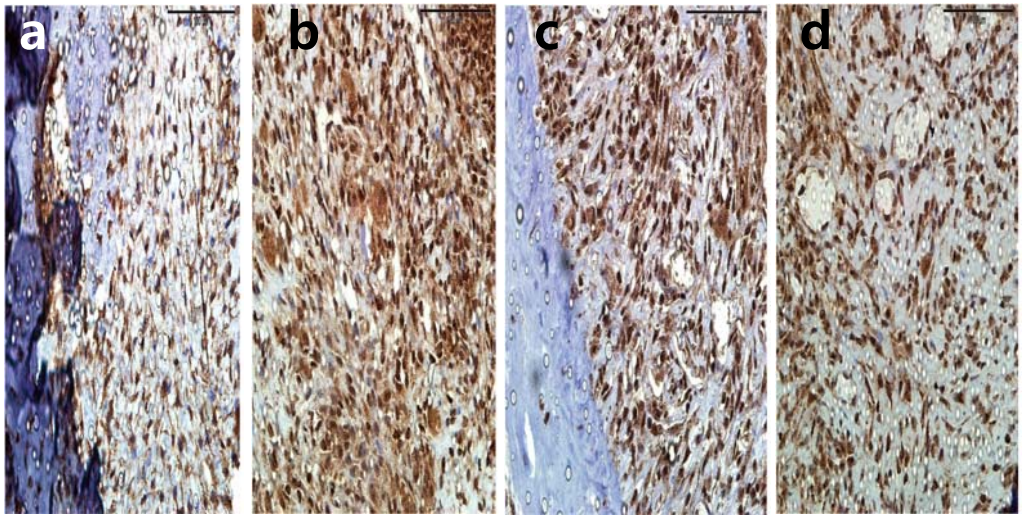

Figure 11

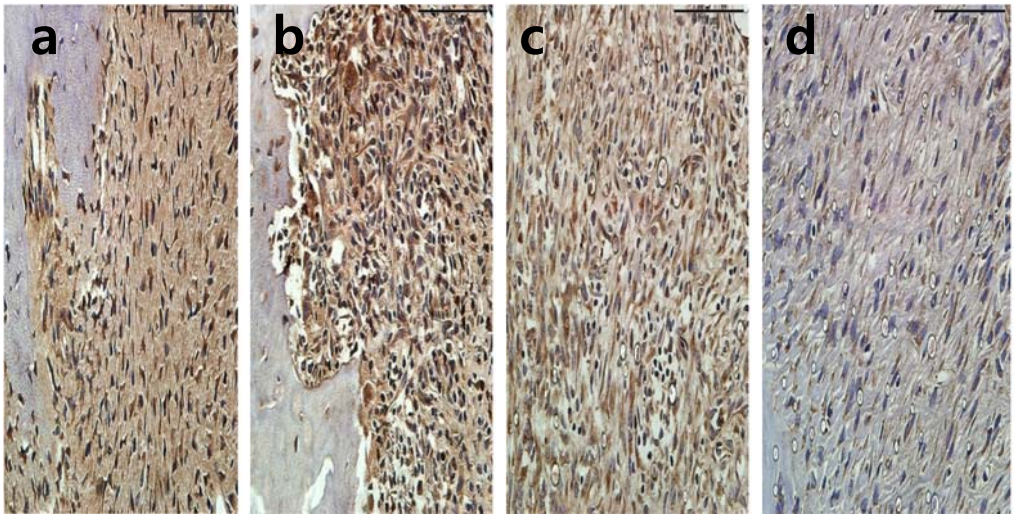

Figure 12

국문요약

쥐에서 치주 질환에 이환된 치아의 발치 후 치주조직의 변화

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최근 치아를 수복하는데 있어서 보철이나 임플란트의 대안으로 의도적 재식술(intentional replantation)이 관심을 받고 있다. 여러 연구에서 치주 질환에 이환된 치아의 즉시 재식술(immediate replantation)은 높은 성공률을 보여왔다. 그러나 장기적인 성공을 위해서는 발치와의 건강한 치주조직 상태가 무엇보다도 중요하며, 그런 의미에서 치주 질환에 이환된 치아에서는 지연 재식술(delayed replantation)이 더 적당하다고 할 수 있다.

이번 연구는 쥐의 치아에 유도 치주염을 일으킨 다음 발치한 후 치유 과정을 관찰함으로써 적당한 지연 재식술의 시기를 판단하기 위해 시행되었다.

28마리의 쥐를 임의로 실험군(n=20)과 대조군(n=8)으로 나눈 다음 실험군의 쥐들에서는 발치 2주전에 하악 제1대구치에 견사를 결찰하여 치주

염을 유도하였다. 2주 후 모든 실험군과 대조군 쥐들의 하악 제1대구치를 발치한 다음 0, 3, 7, 10일째 날에 희생하여 조직학적, 면역조직화학적 방법으로 분석하였다. 면역조직화학적 분석에는 interleukin-6 (IL-6)와 tumor necrosis factor-α (TNF-α)가 이용되었다.

조직학적 분석에서 실험군의 발치와에서는 발치 3일째까지 많은 염증세포의 침윤을 볼 수 있었으나 그 후 점점 감소하였다. 면역조직화학적 분석에서 IL-6는 발치 후 3일과 7일째 사이에 좀 더 많은 염색량을 보였으며, 10일째에는 많이 감소한 모습을 보였다. TNF-α는 3일째 더 많은 염색량을 보였으며, 그 후 차차 감소하는 양상을 보였다.

결론적으로 치주 질환에 이환된 쥐의 치아는 발치 후 10일째 날에 많은 염증의 감소를 보이기 때문에 이 시기에 지연 재식술이 시행되는 것이 적당하다고 할 수 있다.

핵심되는 말 : 치아 발치, 치주염, 발치와, 재식술, 면역조직화학