Effect of Recombinant Human Growth/Differentiation Factor-5 Concentration with Injectable PLGA Composite Carrier on Periodontal Healing/Regeneration of 1 Wall Intrabony Defects in Dogs

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감사의 글

본 논문이 완성되기까지 학위 과정뿐 아니라 여러 면에서 부족한 저를 이끌어 주시고 조언해 주신 김종관 지도 교수님께 항상 존경과 깊은 감사를 드립니다. 부족한 부분을 항상 웃음으로 대해주시고 지도 해주신 채중규 병원장님 그리고 많은 관심 가져주시고 진행 과정을 꼼꼼히 챙겨 주신 최성호 과장님께도 진심으로 감사드립니다. 항상 따뜻한 격려과 조언을 해주시는 조규성, 김창성 교수님께도 감사드립니다. 바쁘신 일정에도 부족한 논문에 꼼꼼한 지도를 주신 이승종 교수님, 석사 과정뿐만 아니라 이번 학위과정에서도 저의 부족한 부분을 항상 너그럽게 이해해 주시고 지도해 주신 유윤정 교수님께도 깊은 감사를 드립니다.

연구 기간 내내 많은 도움을 주었던 정의원 임상교수님, 박정철, 김영택 선생님 그리고 모든 의국원 후배님들께 감사의 말을 전합니다.

논문 준비하느라 병원 업무에 다소 소홀함이 있었겠지만 옆에서 늘 격려해 주고 함께해 준 나의 파트너 조호진, 김영범 원장, 절친한 동기이자 이번 논문에 많은 도움을 준 주영이형 그리고 고운미소 식구들께 고마 움을 전합니다.

항상 사랑으로 지켜주시는 양가 부모님께 감사드리며 늘 건강하셨으면 좋겠습니다. 늘 걱정과 격려를 해주시는 대구 이모님을 비롯한 친척, 친지 여러분께도 감사를 드립니다.

마지막으로 사랑하는 나의 가족 아내 지연, 예쁜 경빈, 경원과 이 기쁨을 함께 하고 싶습니다.

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저자 씀

Table of Contents

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Effect of Recombinant Human Growth/Differentiation Factor-5 Concentration with Injectable PLGA Composite Carrier on Periodontal Healing/Regeneration of 1 Wall Intrabony Defects in Dogs

Recombinant human growth/differentiation factor-5 (rhGDF-5), a member of the transforming growth factor-β superfamily was reported as a suitable factor for enhancing healing in bone defect and ectopic bone formation. For clinical application, biodegradable and biocompatible polymers are useful as carriers for bone morphogenetic proteins(BMPs).

The aim of this study was to evaluate the effect of different concentrations of rhGDF-5 with injectable poly-lactide-co-glycolide-acid(PLGA) composite carrier on periodontal healing/regeneration of one-wall intrabony defects in dogs.

Bilateral 4 x 5 mm (width x depth) one-wall, critical-size, intrabony periodontal defects were surgically created at the mandibular second and fourth premolar teeth in 15 Beagle dogs.

The animals were allocated to 5 test groups of high, mid, low dose, control(only PLGA) and sham.

The dose delivered in the present study was 16 μg, 325 μg and 1625 μg rhGDF-5 in low, mid and high dose group.

Each 5 animals were as follows.

5 animals: P2/P4 intrabony defects were implanted on one side with rhGDF-5 High-dose and Sham surgery performed on the other side.

5 animals: P2/P4 intrabony defects were implanted on one side with rhGDF-5 Mid-dose and rhGDF-5

0-dose on the other side.

5 animals: P2/P4 intrabony defects were implanted with rhGDF-5 Low-dose on one side.

The animals were euthanized following an 8-week healing interval for clinical, radiographic, histological and histometric evaluation.

High dose of rhGDF-5/PLGA significantly increased bone height more than low, carrier, sham and induced more cementum regeneration than sham significantly. Cementum and alveolar bone regeneration was dependant on concentrations of rhGDF-5/PLGA but there was no statistically significant difference except in the high dose group. The injectable rhGDF-5/PLGA composite used in this study was

biocompatible and biodegradable because histologically, it didn't show inflammatory response and resorbed almost completely. It was well manipulable.

In conclusion, the high dose of injectable rhGDF-5/PLGA composite appears to be effective in regenerating cementum and alveolar bone in periodontal defects in dogs.

Key words : rhGDF-5, PLGA, periodontal regeneration, carrier, dog

Effect of Recombinant Human Growth/Differentiation Factor-5 Concentration with Injectable PLGA Composite Carrier on Periodontal Healing/Regeneration of 1 Wall Intrabony Defects in Dogs

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I. INTRODUCTION

The management of periodontal defects has been an ongoing challenge in clinical periodontics. This is mainly a result of the fact that the tissues which comprise the periodontium, the periodontal ligament and the cementum and alveolar bone, represent three unique tissues in their own right. Thus, reconstruction of the periodontium is not just a simple matter of regenerating one tissue but involves at least three quite diverse and unique tissues.

Currently, clinical and scientific research is focusing on a number of approaches

for periodontal regeneration.

Although utilization of various types of bone grafting materials for periodontal defects may result in some gain in clinical attachment levels and radiographic evidence of bone fill, careful histological assessment usually reveals that these materials have little osteoinductive capacity and generally become encased in a dense fibrous connective tissue (Garraway et al.1998).

In another approach to induce periodontal regeneration, polypeptide growth factors have been locally applied to the root surface in order to facilitate the cascade of wound-healing events that lead to the formation of new cementum and connective tissue.

One of promising group of polypeptide growth factors is the bone morphogenetic proteins (BMPs), which offer good potential for stimulating bone and cementum regeneration (Ripamonti et al.1994).

Studies have shown that BMP-2, -4, -5, -6 and -7 have osteoinductive properties (Yamaguchi et al.1991;King et al.1997;Chang et al.1994). However, these BMP showed complications like root resorption or ankylosis (Saito et al.2003; Wikesjo et al. 1999).

Recombinant human growth/differentiation factor-5 (rhGDF-5), a member of the transforming growth factor-β superfamily was reported as a suitable factor for enhancing healing in bone defect, ectopic bone formation and inducing bone

augmentation (Spiro et al. 2000; Yoshimoto et al. 2006; Kuniyasu et al. 2003).

For clinical application, biodegradable and biocompatible polymers are useful as carriers for BMP. Previous studies indicated that synthetic biodegradable polymers combined with BMP could induce new bone formation in animal models (Lucas et al.1990; Miyamoto et al. 1993).

Applications of poly-lactic-co-glycolic-acid (PLGA) combined with BMPs have been extensively studied (Kenley et al. 1994).

Isobe (Isobe et al. 1996) suggested that the PLGA capsules could be a promising carrier for recombinant BMPs to induce bone in clinics.

Although the recombinant BMPs are in themselves capable of inducing de novo bone formation, a stuiable delivery system typically enhances rhBMPs bioactivity.

Numerous biomaterials such as bone matrix, collagen, bioceramics, titanium and synthetic polymers have been investigated as carriers for both naturally occurring and recombinant BMPs. Desired characteristics of the carriers include biocompatibility, surface characteristics for cell attachment and differentiation and biodegradability (Lindholm et al. 1993; Winn et al. 1998). Additional criteria for an ideal carrier for BMPs are the retention of incorporated molecules and clinical accessibility. Longer retention ultimately resulted in higher osteoinductive activity (Uludag et al. 2000).

One common disadvantage with most of the carriers screened in animal and clinical studies is that an invasive procedure is always involved in placing the carrier

constituted with BMPs in vivo. The need for implantation of the current delivery systems undoubtedly restricts clinical access of BMPs to only a handful of applications. The therapeutic potential of BMPs will therefore be significantly increased if the delivery carrier can be extended to an injectable formulation (Gao et al. 2002).

The materials used in the present study consist of a paste-like composite in a pre-filled glass syringe and lyophilized rhGDF-5 in a special glass vial, which are mixed.

The aim of this study was to evaluate the effect of different concentrations of rhGDF-5 with injectable PLGA composite carrier on periodontal healing/regeneration of one-wall intrabony defects in dogs.

II. MATERIALS AND METHODS

A. Materials

1. Animals

15 male Beagle dogs, approximately 15 months old, weight 10-15 kg, bred exclusively for biomedical research purposes, were used. The animals exhibited an intact dentition with a healthy periodontium. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals had *ad libitum* access to water and a pelleted laboratory diet with the exception of one week postsurgery when they were fed on a canned soft dog food diet.

2. Injectable PLGA composite[§]

The novel composite consisted of a bioresorbable PLGA and various excipients, which were enhanced by rhGDF-5. To avoid incompatibilities between the active drug and the carrier, the two components were stored separately. The final pharmaceutical formulation consisted of the paste-like composite in a pre-filled glass

syringe and lyophilized rhGDF-5 in a special glass vial, which were mixed (Figure 1).

3. rhGDF-5[§]

rhGDF-5 Lyophilisate low dose was 16 μg rhGDF-5 per vial in powder 5.3mg

rhGDF-5 Lyophilisate mid dose was 325 μg rhGDF-5 per vial in powder 5.6mg

rhGDF-5 Lyophilisate high dose was 1625 μg rh GDF-5 per vial powder 6.9mg

Placebo Lyophilisate was 0 μg rhGDF-5 per vial

B. Methods

1. Experimental Design

The animals were allocated to 5 test groups of high, mid, low dose, control (only PLGA) and sham.

The dose delivered in the present study was 16 μg, 325 μg and 1625 μg rhGDF-5 in low, mid and high dose group.

Each 5 animals were as follows.

5 animals: P2/P4 intrabony defects were implanted on one side with rhGDF-5 High-dose and Sham surgery performed on the other side.

5 animals: P2/P4 intrabony defects were implanted on one side with rhGDF-5 Mid-

dose and rhGDF-5 and 0-dose on the other side.

5 animals: P2/P4 intrabony defects were implanted with rhGDF-5 Low-dose on one side.

2. Surgical Protocol

Surgical procedures were performed under general anesthesia induced by intravenous injection of atropin[†] and intramuscular injection of a combination of xylazine[‡] and ketamin[§§] followed by inhalation anesthesia[**]. Routine dental infiltration anesthesia was used at the surgical sites.

The mandibular first and third premolar teeth were extracted under general anesthesia prior to experimental surgeries and the extraction sites were allowed to heal for 8 weeks. Remaining teeth received oral prophylaxis in conjunction with the extractions.

The experimental surgery included elevation of buccal and lingual mucoperiosteal flaps to surgically create "box-type", 4 x 5 mm (width x depth), one-wall, intrabony defects at the distal aspect of the 2nd and the mesial aspect of the 4th mandibular premolar teeth (Figure 2) (Kim et al. 2004). Following root planing, a reference notch was made with a round bur on the root surface at the base of the defect. Both

intrabony defect sites received either rhGDF-5 high, medium, low, 0 dose (PLGA only,control)or served as sham-surgery (see above). Next, the mucogingival flaps were advanced, adapted, and sutured using a resorbable suture material[¶].

Radiographs of the defect sites were obtained pre-surgery, immediately post-surgery, and at the day of euthanasia.

3. Postoperative Management

The animals received an intramuscular administration of a broad-spectrum antibiotic[‖] and daily topical application of a 0.2% chlorhexidine solution[#] for infection control. The animals were fed on soft dog-food diet throughout the healing interval. Observations of experimental sites with regards to gingival health, maintenance of suture line closure, edema, tissue necrosis or infection were made daily until suture was removed, and at least twice weekly thereafter. Clinical recordings including photographs and radiographs were completed at surgery; immediately post-surgery; and at 8 weeks.

4. Clinical and Histological Procedures

Photographic and radiographic recordings are completed at surgery; immediately

post-surgery; and at 8 weeks post-surgery when the animals were euthanized using an overdose of pentobarbital sodium (90 -120 mg/kg; IV). Block sections including defect sites and surrounding alveolar bone and mucosal tissues were collected. The block specimens were rinsed in sterile saline and immersed in 10% neutral buffered formalin at a volume 10 times that of the block section for 10 days. After rinsing in sterile water, the sections were decalcified in 5% formic acid for 14 days, trimmed, dehydrated in a graded ethanol series, and embedded in paraffin. Step-serial sections, 5-µm thick, were cut in a mesial-distal vertical plane, at approximately 80-µm intervals. The sections were stained using hematoxylin/eosin and Masson's trichrome stains. The 4 most central sections of each defect site selected based on the width of the root canal were used for the histological and histometric analysis.

5. Histological and Histometric Analysis

The 4 most central sections from each defect site were observed using incandescent and polarized light microscopes[##]. Histometric analysis was performed by one masked experienced examiner using an image-analysis software[¶¶]. The following parameters were recorded (Figure 3):

- Defect height: distance from the apical extension of the root surface notch to the cemento-enamel junction (CEJ)

- Epithelial attachment: distance from the CEJ to the apical extension of an epithelial attachment on the root surface. This parameter included any gingival recession.
- Cementum regeneration: distance from the apical extension of the root surface notch to the coronal extension of newly formed cementum or a cementum-like substance on the root surface.
- Bone regeneration (height): distance from the apical extension of the root surface notch to the coronal extension of newly formed bone along the root surface;
- Root resorption
- Ankylosis

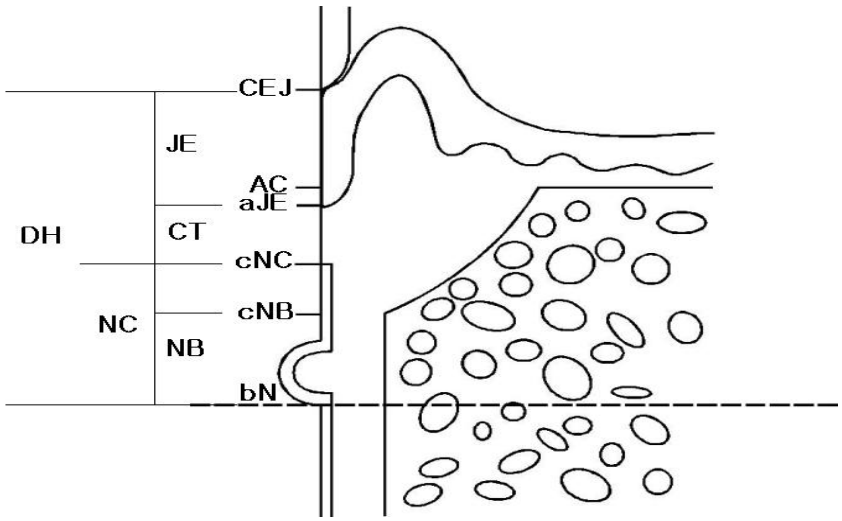

Figure 3. A schematic diagram depicting the experimental design, the landmarks and the parameters used in histometric analysis.

CEJ: cementoenamel junction DH: defect height
JE: junctional epithelium migration CT: connective tissue adhesion
NC: new cementum regeneration NB: new bone regeneration
bN: base of notch a: apical c: coronal

6. Statistical Analysis

Statistical differences were determined using ANOVA with Statistics software. A p value < 0.05 was considered significant.

§ PLGA, rh-GDF5, Scil Technology GmbH, Martinsried, Germany

† 0.04 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd., Seoul, Korea)

‡ Rompun, Bayer Korea Co., Seoul, Korea

§§ Ketara, Yuhan Co., Seoul, Korea

** Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea

¶ Vicryl 5.0 Polyglactin 910, Ethicon, Johnson & Johnson, New Jersey, USA

ǁ Cefazoline Sodium 20 mg/kg, Yuhan Co., Seoul, Korea

Hexamedin®, Bukwang Pharmaceutical Co., Seoul, Korea

Olympus Multi-view BH2, Tokyo, Japan

¶¶ Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA

III. RESULTS

1. Clinical observations

All sites healed uneventfully with minimal signs of inflammation and some gingival recession.

2. Radiographic observation

Radiographic observations made from Pre-surgery, immediately post-surgery and at 8 weeks are shown in Figure 4. New bone formation varied among and within the experimental groups, however groups receiving high dose generally showed more bone formation than that observed in the others.

3. Histological Observations

Photomicrographs of experimental sites are shown in Figure 5-8. The healing observations appeared similar within and among treatments. Inflammatory cell infiltration was minimal in all sites of defect and PLGA appeared almost completely

resorbed, with minimal residual PLGA observed.

All experimental sites showed new bone and cementum formation along the planed root surface, and the amount of those varied in each group. Both Cellular cementum and thin acellular cementum were present. Differential deposition of cellular and acellular cementum was not found in all groups (Figures 5-8).

The newly formed bone consisted mostly of woven bone with primary osteons and minimal lamellar bone. It was hypercellular and high in density, so that it appeared noticeably different from normal alveolar bone (Figure 5-8).

New periodontal ligament space appeared to have formed in regenerated area of experimental defects.

Intrinsic fibers and extrinsic fibers were intermingled in the periodontal ligament. Ankylosis and root resorption were not observed in any of the intrabony periodontal defects.

4. Histometric Analysis

The results from the histometric analysis are shown in Table 1. Induced defect height averaged (± SD) 4.88 ± 0.59, 4.67 ± 0.46, 4.55 ± 0.31, 4.41 ± 0.21 and 4.79 ± 0.65 mm for defect sites receiving high, mid, low, control and sham, respectively, with no significant differences between the groups. The corresponding observations

for alveolar bone regeneration (height) averaged 2.36 ± 0.60, 1.74 ± 0.72, 1.23 ± 0.62, 1.38 ± 0.51 and 1.25±0.27 mm; sites receiving high dose showed significantly greater bone formation than the low, control and sham groups ($p < 0.05$).

Cementum regeneration averaged 2.85 ± 0.78, 2.74 ± 1.00, 2.18 ± 0.88, 1.92 ± 0.52 and 1.83±0.42mm for defect sites receiving high, mid, low, control and sham .The high dose group was significantly different from sham group ($p < 0.05$).

Connective tissue adhesion amounted 0.49 ± 0.37, 0.50 ± 0.36, 0.30 ± 0.13, 1.07 ± 0.33 and 0.98 ± 0.77 mm .

The low dose group was significantly different from the control and sham groups ($p < 0.05$).

Junctional epithelium amounted to 1.52 ± 0.96, 1.43 ± 0.53, 2.04 ± 0.75, 1.41 ± 0.53 and 1.96 ± 0.47mm for high, mid, low, control and sham groups.

There were no significant difference from each group ($p < 0.05$).

Table 1. Results of the histometric analysis (group means ± SD in mm)

	CEJ-notch	bone height	cementum	epi.	C-T
High	4.88±0.59	2.36±0.60*	2.85±0.78¶	1.52±0.96	0.49±0.37
Medium	4.67±0.46	1.74±0.72	2.74±1.00	1.43±0.53	0.50±0.36
Low	4.55±0.31	1.23±0.62	2.18±0.88	2.04±0.75	0.30±0.13†
Carrier	4.41±0.21	1.38±0.51	1.92±0.52	1.41±0.53	1.07±0.33
Sham	4.79±0.65	1.25±0.27	1.83±0.42	1.96±0.47	0.98±0.77

* : stastically significant difference between High and Low,Carrier, Sham ($p<0.05$)

¶ : stastically significant difference between High and Sham ($p<0.05$)

†: stastically significant difference between Low and control, Sham ($P<0.05$)

IV. DISCUSSION

The aim of this study was to evaluate regeneration of alveolar bone and periodontal attachment, and to evaluate carrier material resorption following surgical implantation of rhGDF-5/PLGA with a different concentration in a preclinical canine model.

RhGDF-5 is one of the bone morphogenetic protein family of the TGF-β superfamily.

It was reported as having abilities of chondrogenesis, osteogenesis, odontogenesis and angiogenesis (Nishitoh et al. 1996; Akiyama et al. 2000; Baur et al. 2000; Aoki et al. 2001; Coleman et al. 2003).

In rat calvarial defect, ß-TCP coated with rhGDF-5 demonstrated to have superior bone regeneration compared to conventional bone materials (Pöhling et al. 2006).

Also Kuniyasu et al (Kuniyasu et al. 2003) reported that successful bone augmentation was induced by 1, 10 and 100 μg rhGDF-5-collagen composite on the rat calvaria.

Although the bone forming potential of rhGDF-5 was lower than rhBMP-2(Kang et al.2004), previous studies reported that rhGDF-5 has enough ability to induce bone augmentation and ectopic bone formation (Kuniyasu et al. 2003; Yoshimoto et al. 2006; Spiro et al. 2000; Hötten et al. 1996).

The numerous studies on BMPs in periodontal tissue regeneration have been done (Wikesjo et al. 2004; Choi et al. 2002; Selvig et al. 2002; Nakamura et al. 2003).

The potential of rhBMP-2 in an absorbable collagen sponge (ACS) carrier (rhBMP-2 at 0.2mg/ml) to induce periodontal regeneration was first shown in a pilot study using the supra-alveolar periodontal defect model (Sigurdsson et al. 1996)

Some previous researches reported that rh-BMP-2 had a favorable effect on bone formation and periodontal regeneration (Saito et al.2003; Wikesjo et al. 2004; King et al.1999). However, ankylosis was observed in supra-alveolar periodontal defects receiving rhBMP-2/ACS (rhBMP-2 at 0.05, 0.1, and 0.2mg/ml) without apparent correlation to rhBMP-2 concentration or dose(Wikesjo et al. 1999). Other studies also using rhBMP-2 with various carriers provided evidence of ankylosis in large experimental periodontal defects in rodent, canine, and non-human primate models(Sigurdsson et al. 1995; King et al. 1997; Kinoshita et al. 1997).

The Study on rhGDF-5 showed it induced angiogenesis which was not observed in rh-BMP-2 (Yamashita et al. 1997).

The rhGDF-5 is, therefore considered to be a candidate for the BMP to facilitate periodontal regeneration.

In this study, high dose rhGDF-5/PLGA increased bone height significantly but didn’t cause ankylosis and root resorption.

An appropriate carrier is essential for these effects of rh-GDF-5. It should be

biocompatible, biodegradable and have prerequisites including porosity, space provision and blood clot stabilization to support periodontal regeneration. Takaoka et al (Takaoka et al. 1988) reported that collagen was a suitable carrier for promoting osteoinduction when used in combination of BMP and collagen. But in the previous studies the absorbable collagen sponge has a disadvantage of collapsing in the space formation and maintenance and short releasing time and β-TCP has a delayed resorption rate and poor handling properties (McPherson. 1992; Uludag et al. 2001).

Historically, PLA/PGA polymers have been used to make biodegradable screw and fixation pins, rods, plates, suture anchors and sutures. With the advent of tissue engineering in recent years PLGA was also rapidly gaining recognition in the scaffolds or carriers of cells, extracellular matrix components, and bioactive agents (Athanasiou et al.1998). PLGA was considered to be suitable matrices for bone and soft connective tissue (Laurencin et al. 1999). In the calf muscles of Wistar rats it was suggested that the rhBMP-2 (10, 50 μg) was released in an active form at the implant site during the degradation of the copolymer (10mg PLGA), resulting in the induction of new bone formation within 3 weeks after implantation (Bessho et al.2002). More recently, it was demonstrated that transplantation of cloned cementoblasts into PLGA carrier leads to the repair of large periodontal alveolar bone defects in rodents (Zhao et al. 2004).

In this study, PLGA was easily managed because it was of the injectable putty type

so that it adapted well to the bone defect and began to set within seconds of contact with blood, which eased flap management. PLGA used in this study facilitated space formation and maintenance at aspect of easy-to-use.

This study presented increased bone regeneration in sites with high dose rhGDF-5/PLGA compared to sites with low dose rhGDF-5/PLGA and only PLGA.

Bone density of sites with rhGDF-5/PLGA was higher with smaller marrow spaces, and many osteocytes were present in the newly formed bone. These results would support the recent in vitro studies, suggesting that rhGDF-5 promotes an osteogenic differentiation in stromal cells (Shen et al. 2006; Zeng et al. 2006). In addition to direct effects on differentiation and proliferation at cellular level, rhGDF-5 induced angiogenesis in vitro and in vivo (Yamashita et al. 1997; Zeng et al. 2006). Angiogenesis and vascular invasion into bone matrix are important steps in the sequential cascade of the bone formation process (Reddi.1992). In the present study, bone formation might be caused by effects of both differentiation at cellular level and angiogenesis at tissue level. However, the woven nature of the new bone and its hypercellularity appeared different from normal alveolar bone. So the longer-term experiment will be required to determine whether this woven bone remodels to become the normal trabecular bone.

Cementum regeneration observed in this study included both cellular and acellular

cementum. Newly formed cellular cementum had the same structure as it is observed at natural cementum, whereas acellular cementum appeared similar only in density to natural cementum on H-E staining histology. Regenerated cementum in each test group was consisted of mainly cellular type. Previous studies described uniformly thin and acellular mineralized tissue on the denuded dentin surface after periodontal therapy (Miyaji et al. 2006; Lindskog & Blomlöf. 1992). Formation of this mineralized tissue was known to be dependent on initial resorption of root dentin surface, and BMP promoted these resorption and formation by facilitating osteoclastic effects (Miyaji et al. 2006; Lindskog & Blomlöf 1992). In this study, sites received high dose rhGDF-5/PLGA showed the most cementum regeneration (including cellular and acellular types). The high dose group was significantly different from the sham group ($P<0.05$).

Newly formed periodontal ligament was observed between the new bone and cementum along the involved root surface, and could be considered to be in an immature state, which was not as dense as PDL in uninvolved portions of the same tooth. Intrinsic fibers and extrinsic fibers are intermingled in the periodontal ligament. Extrinsic fibers observed in this space were embedded perpendicularly or obliquely to the cementum. These results demonstrate that rhGDF-5 might increase the regeneration of periodontal attachment apparatus including cementum, alveolar bone and periodontal ligament.

All sites which received rhGDF-5/PLGA and only PLGA showed no residual materials. Neither of ankylosis and root resorption were observed in any of the intrabony periodontal defects.

These result showed no difference was between rhGDF-5 dose and resorption rate of materials at 8weeks.

However further studies are needed to determine effect of rhGDF-5 dose on resorption rate of PLGA material.

Matsumoto et al (Matsumoto et al. 2005) reported that there was no signs of bone resorption or inflammation on the application of PLGA fixation screws to human patients. PLGA sponge implanted in post-extraction sites resulted in the formation of matured, mineralized and well-structured bone after 6 months of healing. Particles of grafted material could not be identified in any of the biopsied sites (Serino et al. 2003).

Through these results, rhGDF-5 seemed to play a role on the periodontal tissue regeneration and it showed prominent bone formation ability and PLGA was demonstrated to be a good carrier, satisfying the aforementioned other requirements.

V. CONCLUSIONS

High dose of rhGDF-5/PLGA significantly increased bone height more than low dose, carrier and sham groups and induced more cementum regeneration than the sham group significantly. Cementum and alveolar bone regeneration was dependant on concentrations of rhGDF-5/PLGA but there was no statistically significant difference except in the high dose group. The injectable rhGDF-5/PLGA composite used in this study was biocompatible and biodegradable because histologically, it didn't show inflammatory response and resorbed almost completely. It was well manipulable.

In conclusion, the high dose of injectable rhGDF-5/PLGA composite appears to be effective in regenerating cementum and alveolar bone in periodontal defects in dogs.

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LEGENDS

Table1. Results of the histometric analysis (group means ± SD in mm)

Figure 1. The final pharmaceutical formulation (C)-injectable composite consisted of the paste-like composite in a pre-filled glass syringeand lyophilized rhGDF-5 in a special glass vial(A), which were mixed(B)

Figure 2. Surgically created, critical-size, one-wall, intrabony periodontal defect at the distal aspect of the mandibular 2nd and mesial aspect of the mandibular 4th premolar teeth (A). Application of rhGDF-5/PLGA (B). Mucoperiosteal flaps adapted and sutured for primary intention healing (C). Healing at 8 weeks post-surgery (D).

Figure 3. A schematic diagram depicting the experimental design, the landmarks and the parameters used in histometric analysis.

Figure 4. Representative radiographs showing defect sites receiving high dose rhGDF-5/PLGA (A), only PLGA(Carrier) (B), immediately post-surgery (1) and at 8 weeks post-surgery (2).

Figure 5. Photomicrographs from defect sites receiving high dose rhGDF-5/PLGA displaying the coronal extension of the newly formed bone and cementum and the apical extension of an epithelial attachment (hematoxylin/eosin, original magnification X10 & X40).

Figure 6. Photomicrographs from defect sites receiving mid dose rhGDF-5/PLGA displaying the coronal extension of the newly formed bone and cementum and the apical extension of an epithelial attachment (hematoxylin/eosin, original magnification X10 & X40).

Figure 7. Photomicrographs from defect sites receiving low dose rhGDF-5/PLGA displaying the coronal extension of the newly formed bone and cementum and the apical extension of an epithelial attachment (hematoxylin/eosin, original magnification X10 & X40).

Figure 8. Photomicrographs from defect sites receiving only PLGA carrier displaying the coronal extension of the newly formed bone and cementum and the apical extension of an epithelial attachment (hematoxylin/eosin, original magnification X10 & X40).

Figure 9. Main results from the histometric analysis (mm)

FIGURES

Figure 1. Injectable rhGDF-5/PLGA composite

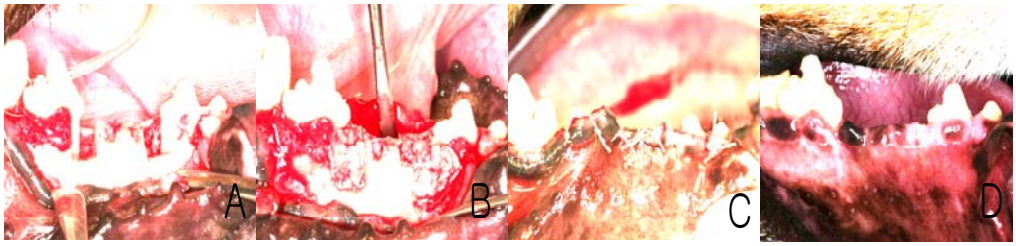

Figure 2. Surgical Protocol

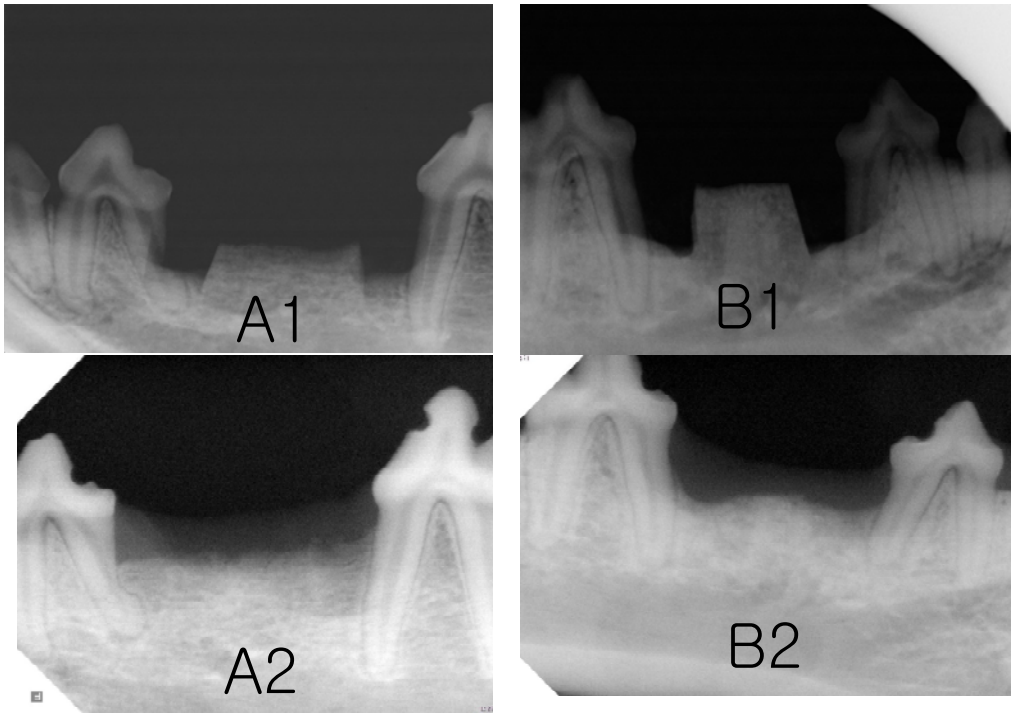

Figure 4. High dose rhGDF-5/PLGA (A), only PLGA(Carrier) (B), immediately post-surgery (1) and at 8 weeks post-surgery (2).

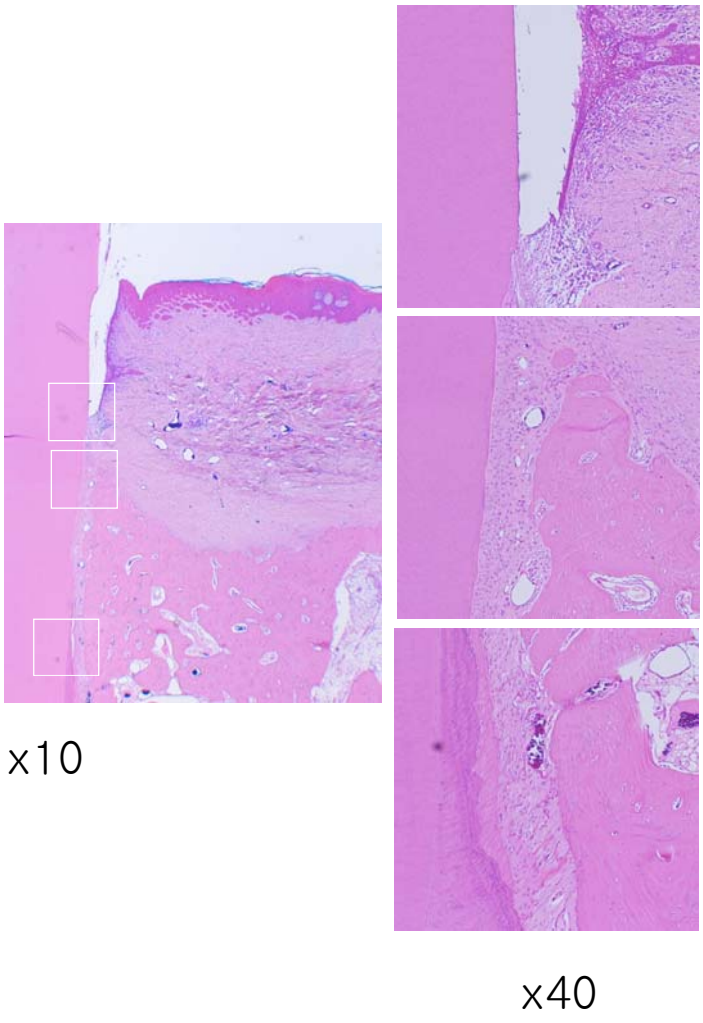

Figure 5.High dose rhGDF-5/PLGA (X10 & X40).

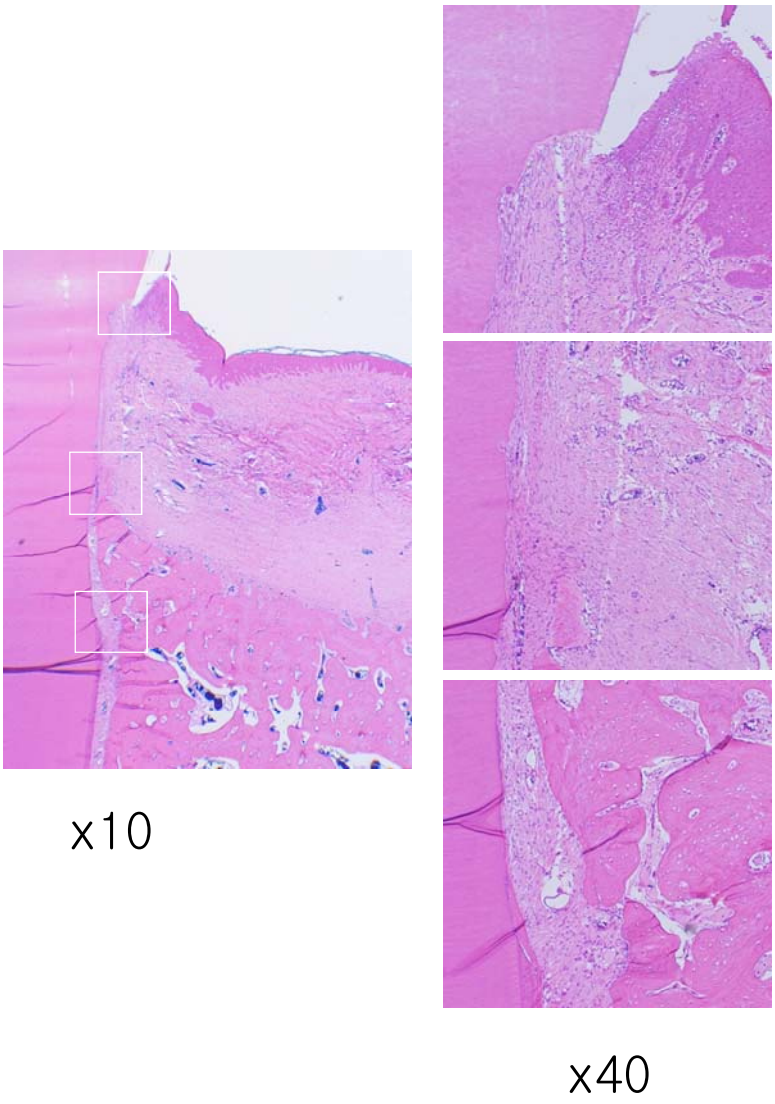

Figure 6. Mid dose rhGDF-5/PLGA (X10 & X40).

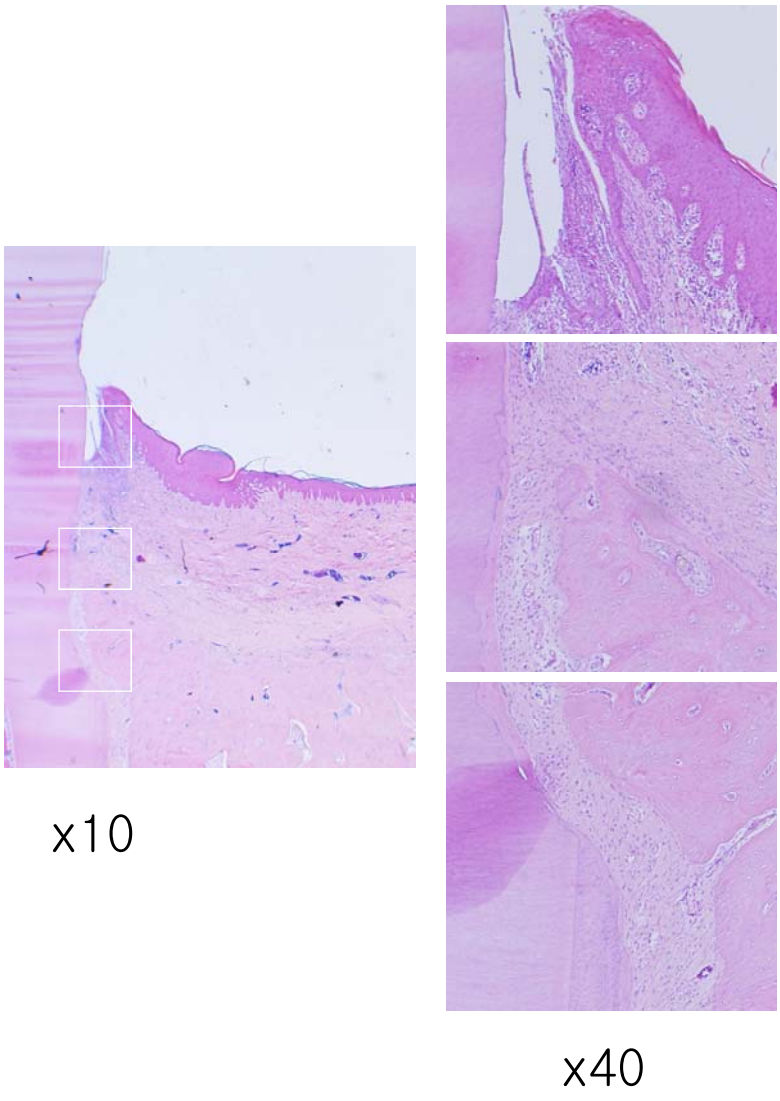

Figure 7. Low dose rhGDF-5/PLGA (X10 & X40).

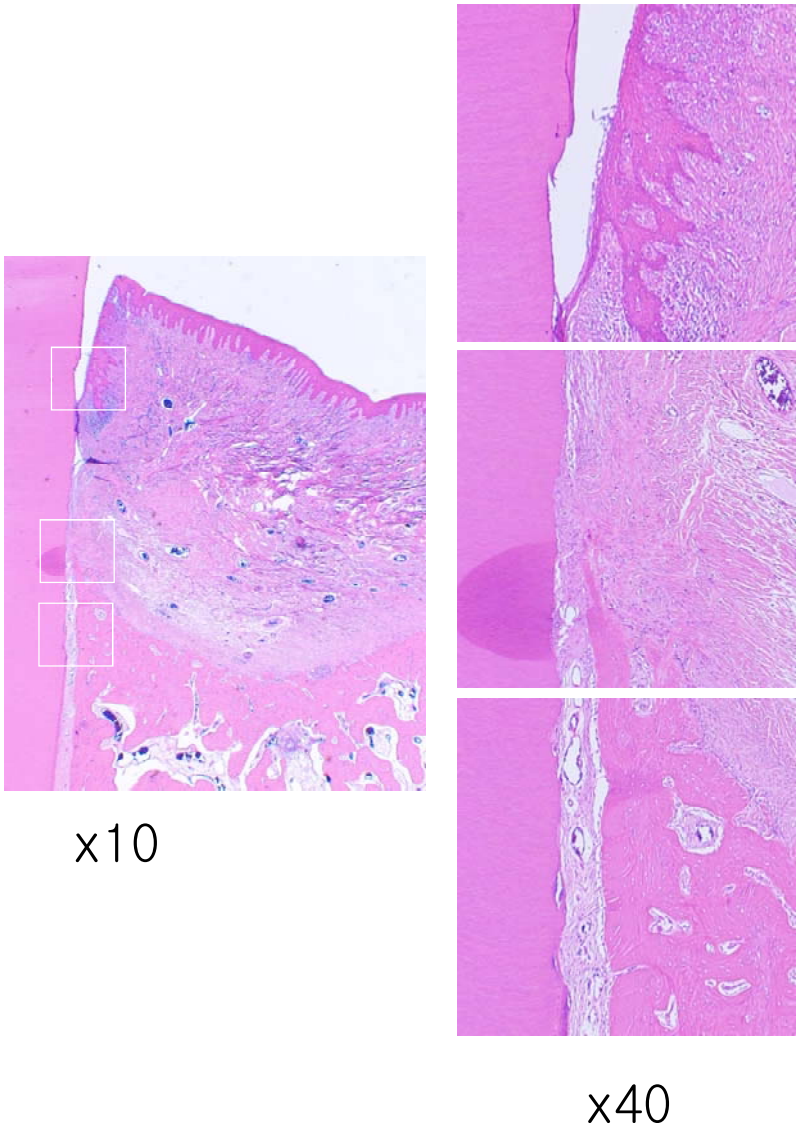

Figure 8. only PLGA carrier (X10 & X40).

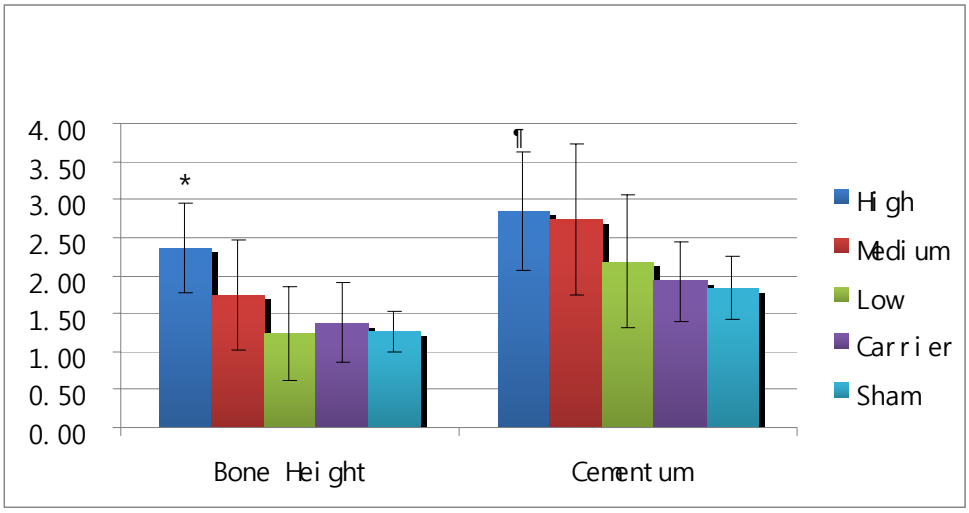

Figure 9. Graph of the histometric analysis (mm)

* $p < 0.05$ compared to Low,Carrier, Sham .

¶ $p < 0.05$ compared to Sham .

-국문요약-

성견 일벽성 골내낭에서 Injectable PLGA Composite 운반체로 사용시 Recombinant Human Growth/Differentiation Factor-5의 농도별 치주조직 치유와 재생 효과

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민 천 기

골결손부에서의 치유와 골형성에서 recombinant human Growth/ Differentiation Factor-5 (rhGDF-5)는 적합한 요소의 하나로 보고 되어지고 있고 임상에서 BMP의 운반체로 생체친화적인 중합체가 이용되고 있다. 이 실험의 목적은 서로 다른 농도의 주사형 rhGDF-5/PLGA를 비글견의 일벽성 골내낭에 적용하여 치조골, 백악질 형성과 같은 치주조직의 치유와 재생의 정도를 비교하고자 하는 것이다.

15마리의 비글견에서 제 3소구치를 모두 발치한 뒤, 8주간의 치유기간이 지나고 제 2소구치 원심면과 제 4소구치 근심면에 백악법랑경계에서 5mm 깊이, 협설측으로 4mm 폭의 일벽성 골내낭을 형성했다. 실험군은 고농도, 중농도, 저농도의 rhGDF-5를 적용한 것과 운반체만 적용한 경우 수술만 한 경우의 5군으로 하며, 5마리씩 3그룹으로 나누어 1그룹 5마리는 좌우측에 한쪽은 고농도 (1625μg) rhGDF-5를 적용하고, 반대쪽에는 수술만을, 2그룹 5마리는 좌우측에 한쪽에는 중농도 (325μg) rhGDF-5와 반대쪽에는 운반체 (PLGA)만을, 3그룹 5마리는 우측에만 저농도 (16μg)의 rhGDF-5를 적용했다 (각 군당 10부위). 8주간의 치유기간이 지난 뒤, 조직학적 분석과 조직계측학적 분석을 위하여 희생하였다.

조직학적, 조직계측학적 분석 결과 고농도의 rhGDF-5를 적용한 군이 골형성 높이에 있어서 저농도, 운반체만 적용한 군 그리고 수술만 한 군에 비해 유의성 있는 증가를 보였고 백악질형성에 있어서 고농도의 rhGDF-

5를 적용한 군이 수술만 한 군에 비해 유의성 있는 증가를 보였다. 치주조직 재생에 있어서는 서로 다른 농도의 rhGDF-5를 적용시 군 간에 농도에 따른 차이가 발견되었으나 고농도외에는 유의성 있는 차이를 보이지 않았다. rhGDF-5/PLGA를 적용한 모든 군에 있어서 생체친화성, 생체흡수성을 보였으며 재료적용시 다루기가 편리했고, 치근흡수와 유착은 나타나지 않았다.

이러한 결과는 고농도의 주사형 rhGDF-5/PLGA가 치주조직의 재생에 있어서 효과적으로 사용될 수 있음을 보여준다.

Key Words : rhGDF-5, PLGA, 치주재생, 운반체, 개