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Histological Evaluation on the Biocompatibility and Degradation of Poly Lactic-co Glycolic Acid (PLGA) /Inorganic Filler Matrix in Surgically Created Intrabony 1- wall Defect in Beagle Dog.

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ABSTRACT

비글견 1벽성 골내낭에서 Poly Lactic-co Glycolic Acid (PLGA)/Inorganic Filler Matrix의 생체 친화성 및 흡수성에 대한 조직학적 연구

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치주 질환으로 인하여 소실된 치주조직을 재생시키려는 여러 술식이 많이 연구되고있다. 그 중 bioactive factor의 적용은 치주조직의 재생에 있어서 우수한 치료법으로 평가되고 있으며, 이를 수용부에 적절히 적용하기 위한 운반체로 생체친화적인 중합체가 이용되고 있다. 본 연구의 목적은 PLGA를 inorganic filler에 혼합시킨 재료를 성견의 일벽성 골내낭에 적용하여 이 재료의 생체 친화성과 생체 흡수도를 보고자 하는 것이다.

5마리의 비글견에서 제 3 소구치를 모두 발치한 뒤, 8주간의 치유기간이 지나고 제 2 소구치 원심면과 제 4 소구치 근심면에 5mm 깊이, 4mm폭의 일벽성 골내낭을 형성하였다. 좌측 defect에는 PLGA/inorganic filler matrix를 이식하였고 우측에는 아무것도 이식하지 않은 대조군으로 나누어 술 후 8주에 희생하여 치유 결과를 조직학적으로 비교 관찰하였다.

조직학적 분석 결과, 모든 결손부에서 염증의 소견이 관찰되지 않았으며 치근흡수와 유착은 발견되지 않았다. 백악질과 치조골, 치주인대를 포함한 치주조직의 재생에 있어서 대조군, 실험군 간에 조직학적으로 치유양상에 있어 차이를 많이 보이지 않았으며 PLGA/inorganic filler matrix는 8주 내에 완전히 흡수되어 결합조직이나 신생골 내에서 그 흔적을 발견할 수 없었다.

이러한 결과는 PLGA/inorganic filler matrix는 생체친화성 및 생체흡수성이 우수한 재료로서 치주 조직의 재생 치료에 있어서 신체활성인자의 scaffold로 사용될 수 있는 가능성을 보여주었다.

KEY WORDS(주요단어) : PLGA, 운반체, 치주재생, 일벽성 골내낭

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I. INTRODUCTION

Regeneration of tooth supporting tissues that caused by periodontal and peri-implant tissue destruction is a major goal of reconstructive therapy. One approach to induce periodontal regeneration, bioactive growth factors have been locally applied to the root surface in order to facilitate the cascade of wound-healing events. Bioactive growth factors require appropriate scaffolds to provide longer-term release of growth factors for wound healing. The scaffolds should be biocompatible and biodegradable. It also should be space making and facilitate blood clot stabilization.

Absorbable Collagen Sponge (ACS), Poly (Lactic-co-glycolic acid)(PLGA) and other bone substitutes were introduced by researchers as scaffolds of the growth factors. Collagen scaffolds has been regarded as one of the most useful scaffold for many years owing to its excellent biocompatibility and safety associated with its biological characteristics, such as biodegradability and weak antigenicity[23,24]. But the absorbable collagen sponge is easily collapsed and has short releasing time[24,25,26]. An appropriate carrier acting as a slow delivery vehicle for growth factor is required for maximal clinical effect[1]

PLGA is degraded by hydrolysis and enzymatic activity and have a range of mechanical and physical properties that can be engineered appropriately to suit a particular application[34].

Matsumoto et al. previously reported that there was no signs of bone resorption or inflammation on the application of PLGA fixation screws to human patients18. Van Sliedregt et al. investigated four types of polylactides using cell cultures of rat epithelial cells in addition to human fibroblasts and osteosarcoma cells, and reported the biocompatibility of the PLGAs was satisfactory in general[33]. Piattelli reported limited residual amount of PLA/PGA(1%) at 6~8 months after implantation of PLA/PGA copolymer. Histomorphometric analysis revealed 43% of mineralized bone with complete absence of inflammatory response[18]. Consistently, PLGA composite 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 site[13]. Herberg et al. reported that the PLGA composite is a good candidate for scaffold of growth factor. The PLGA composite showed a highly porous space-providing structure and effectively induced coagulation exhibiting an intimate interaction with the fibrin clot. Additionally, the composite was conveniently injectable for ease of use[35].

The purpose of this study was to histologically evaluate the biocompatibility and adequate degradation of newly developed PLGA/inorganic filler matrix in the surgically created 1-wall

intrabony defects of beagle dog.

II. MATERIALS AND METHODS

A. Materials

1. Animals

Five 15-month-old male beagle dogs, each weighing approximately 12kg, were used in this study. The animals were all systemically healthy and had intact dentition with healthy periodontium. The Institutional Animals Care and Use Committee, Yonsei Medical Center, Seoul, Korea approved the selection of the animals, managements, surgical protocol, and the preparation routines. The animals were fed a soft diet throughout the study, in order to reduce the chance of mechanical interference with the healing process during food intake.

2. PLGA/inorganic filler matrix Fabrication

The novel composite constituted a bioresorbable poly (Lactic-co-glycolic acid) (PLGA) and various ingredient (SCil Technology, Martinsried, Germany).

PLGA composite was manufactured as follows: Poly (D,L-lactic-co-glycolic acid) and polyethylene glycol 1500 were solved in polyethylene glycol 300 by heat treatment. Calcium sulfate, D(-)-Mannitol, and cellulose ether, were dispersed in the polymeric solution.

B. Methods

1. Surgical Procedures

All surgical procedures were performed under general anesthesia induced by an intravenous injection of atropine (0.04 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd., Seoul, Korea) and an intramuscular injection of a combination of xylazine (Rompun, Bayer Korea Co., Seoul, Korea) and ketamin (Ketara, Yuhan Co., Seoul, Korea), followed by inhalation (Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea). Routine dental infiltration anesthesia (2% lidocaine hydrochloride with 1/80,000 epinephrine) was used at the surgical sites. The mandibular third premolars were extracted prior to the experimental surgery, and the extraction sites were allowed for socket healing.

After 8 weeks of healing period, the defect was performed as the procedure by Kim et al. In briefly, full thickness mucoperiosteal flaps were elevated and 1-wall "box-type" intrabony defects(5 mm depth, 4 mm width, 4 mm height)were surgically created at the distal aspect of the second premolars, and at the mesial aspect of the fourth premolar[28, 29]. Following root planning, a reference notches were made with a 1/4-round bur on the root surface at the base of the defects. Defects in the experimental sides were filled with PLGA and defects in the control sides were received a flap operation only.

Next, the mucoperiosteal flaps were repositioned and sutured with resorbable suture materials (Vicryl 5.0 Polyglactin 910, Ethicon. Johnson & Johnson, USA). Post-surgical management included intramuscular administration of antibiotics (Cefazoline Sodium 20mg/kg; Yuhan Corporation, Seoul,

Korea) for 3 days and daily topical dressing of 0.2% chlorhexidine solution (Hexamedin®, Bukwang Pharmaceutical Co., Seoul, Korea) for infection control for 7 days.

The remaining dentition received oral prophylaxis during the healing period. 8 weeks later, the animals were euthanized using an overdose of pentobarbital (90~120mg/kg; IV) and block sections including the surgical sites were removed for the histologic analysis.

2. Clinical Records

Clinical observation were done through the experimental time.

3. Histological Procedures

Block sections including defect sites and tooth, surrounding alveolar bone and soft tissues were collected. The block specimens were fixed in 10% buffered formalin for 10 days, decalcified in 5% formic acid for 14 days, dehydrated in ethanol and embedded in paraffin. The sections were stained with hematoxylin-eosin. Serial sections of 4㎛ thick thickness were cut in a mesial-distal direction at 80㎛ intervals. The three most central sections of each defect site were observed using incandescent and polarized light microscopy (Olympus Multi-view microscope BH2, Japan).

III. RESULTS

1. Clinical observations

Surgical procedures were uneventful and without complication. Despite the extent and size of the surgically involved areas, wound closure was successfully maintained throughout the experiment for all defects. During this study, no sign of infection or clinical complication were found.

2. Histologic observations

All of the specimens were well tolerated and

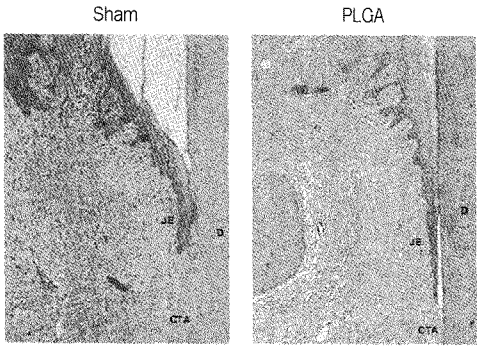

Fig 1. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 100). Apical extension of junctional epithelium (JE) varied along the root surface. Healing appeared similar within and among treatments.

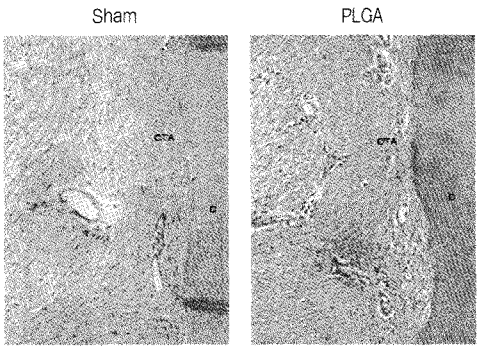

Fig 2. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 200). Connective tissue attachment(CTA) varied extension along the root surface. No PLGA could be detected and both groups didn't show undermining root resorption

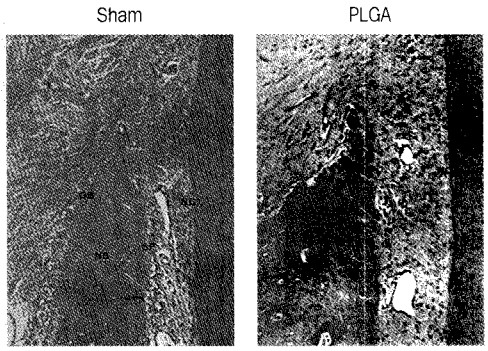

Fig 3. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 200). Regenerated alveolar bone varied extension along the root surface. No PLGA could be detected and both groups didn't show ankylosis.

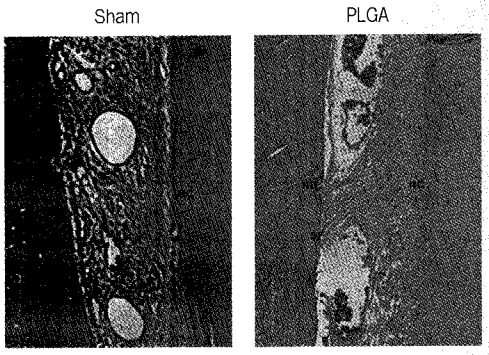

Fig 4. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 200). Obliquely or perpendicularly oriented new Sharpey's fibers (SF) inserted in the new bone (NB) and new cementum (NC). Blood vessels (V) and organized form of periodontal ligament was shown in the both groups. Healing appeared similar within and among the groups.

there were no histologically observable pathological tissue reactions at the time of necropsy. However, some cases seemed to cause an initial inflammation of the bone surrounding the intrabony 1-wall defects that were subsequently repaired along with the bone healing of the defects.

An epithelial attachment of variable length was observed in all sites irrespective of treatment with no clear distinction between the groups (Fig 1). Connective tissue attachment was in varing dimension along the root surface. Findings of PLGA was not detectable with minimal inflammatory cell infiltration in

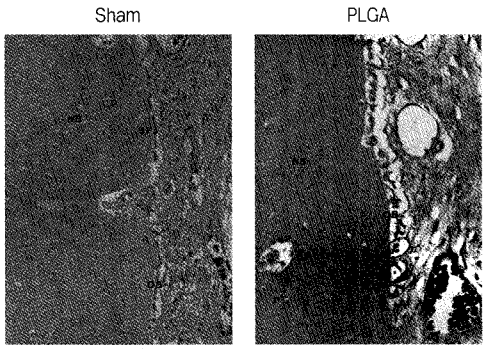

Fig 5. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 400). Osteoblasts (OB) were arranged along the new bone (NB) surface and Sharpey's fibers (SF) inserted in the new bone in the both groups. Healing appeared similar within and among the groups.

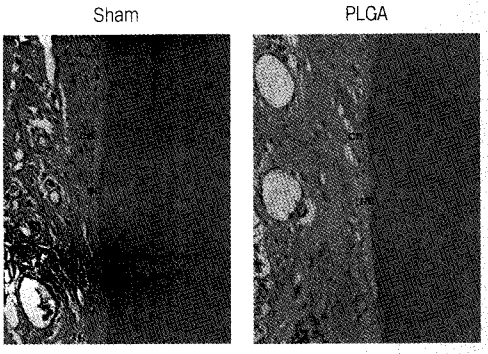

Fig 6. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 400). In the intrabony cementum layer, it was observed that the cementoblasts (CB) were arranged along the new cementum. Healing appeared similar in the both groups.

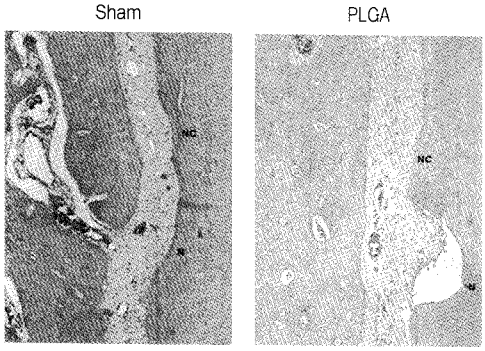

Fig 7. Representative photomicrographs of Sham and PLGA group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 100). New cementum (NC) formation above the notch (N) could be seen. No inflammation and ankylosis could be seen in the both groups.

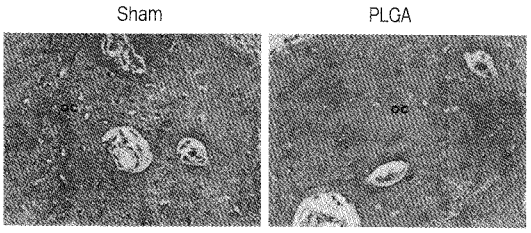

Fig 8. Representative photomicrographs of control group and test group at 8 weeks postsurgery; hematoxylin and eosin (original magnification x 400). Woven bone nature of the new bone showing more or less cellularity and bone in process of maturation. Notice the osteocyet(OC) within the newly formed bone. No PLGA could be detected and healing appeared similar in the both groups.

all the groups (Fig 2). Regenerated alveolar bone was more or less formed above the notch area (Fig 3).

Both groups maintained good periodontal ligament space, and showed no evidence of ankylosis and resorption. Periodontal ligament in both groups were observed to have a regular pattern and a dense fiber arrangement. Dense fibers showing periodontal regeneration were embedded into the newly formed bone and new cementum obliquely or perpendicularly (Fig 4,5,6).

All experimental sites showed new bone and cementum formation along the planed root surface (Fig 7). The newly formed cementum was compared of cellular and acellular cementum in both groups. In the cementum layer below the bone level, cementoblasts were arranged closely and the fibers were embedded perpendicularly (Fig 6). In addition, a thin layer of cementum was also observed above the newly formed bone level. In this cementum layer, the cementoblasts were rarely observed and the fibers showed a parallel orientation.

The crest of the newly formed bone was mostly woven bone, and the newly formed bone appeared moderate cellularity and density in both control and PLGA groups (Fig 8).

Sites receiving the PLGA composite showed no apparent effect on bone formation, indicating that this biomaterial did not appear to obstruct or otherwise compromise bone formation.

IV. DISCUSSION

The major challenges in contemporary periodontal therapy are to re-establish soft tissue attachment to newly formed cementum on the root surface and to restore lost bone. This requires regeneration of gingival connective tissues destroyed by inflammation, formation of new cementum

and restoration of bone loss, and, most importantly, new attachment of connective tissue fibers to previously diseased root surfaces[30,31]. More recently, the application to the root surfaces of a variety of growth and differentiation factors to stimulate cell repopulation of periodontal defects and subsequently induce regeneration has been investigated[27]. Bioactive growth factors require appropriate scaffolds to provide long-term release of growth factors to heal wounds[19].

Poly (lactic acid), poly (glycolic acid), and their copolymers have been approved by the Food and Drug Administration for use as sutures, vascular grafts, drug carriers, and scaffolds for tissue engineering. PLGA copolymers degrade over time by releasing of lactic acid monomers that are oxidized to pyruvic acid[12] and its framework makes the space maintained for enough time. Because the mechanical properties of these polymers are dependent on the molecular weight of the component polymers, high-molecular-weight poly (DL-lactide-co-glycolide) (PLGA) copolymers are used as scaffolds for tissue engineering[9,10]. The use of low-molecular weight PLGA copolymers as a delivery vehicle for bioactive factors around dental and orthopedic implants has yet to be investigated[5,6].

With the advent of tissue engineering in recent years PLGA is also rapidly gaining recognition in the scaffolds or carriers of cells, extracellular matrix components, and bioactive agents[34]. Polylactide and polyglycolide acids are considered to be suitable matrices for bone and soft connective tissue[14]. It is apparent that the rhBMP-2 was released in an active form at the implant site during the degradation of the copolymer, resulting in the induction of new bone formation within 3 weeks after implantation[6]. More recently, it has been demonstrated that transplantation of cloned cementoblasts into PLGA carrier leads to the repair of large periodontal alveolar bone defects in rodents[17].

Minenna et al. evaluated the clinical outcome of re-constructive surgery in deep intra-osseous defects by means of open flap curettage in conjunction with a PLA/PGA copolymer implant as compared with open flap curettage procedure alone. The results demonstrated that the use of PLA/PGA did not provide an additional benefit in terms of CAL gain and PD reduction compared with control procedure[22].

The results of this study indicate that regeneration of the periodontal tissues following was similar in the intrabony defects where PLGA was inserted compared to what was observed in the intrabony defect of natural healing. The histological observation indicated that, there was more or less cellularity and bone formation in process of maturation in both two groups. In addition, dense and well organized form of periodontal ligaments was shown. Herberg et al. previously reported that the PLGA composite seems to be effective in periodontal

regeneration[35]. These results support that PLGA can be a good candidate for bioactive growth factor carrier.

The PLGA composite showed a highly porous space providing structure. It effectively induce coagulation exhibiting an intimate interaction with the fibrin clot. 8 weeks following operation, no particles of the grafted materials could be identified in the specimens without complications such as ankylosis or root resorption. Absence of inflammatory and foreign-body giant cells in the biopsies indicate a complete biodegradation of the materials during the period of observation.

In the interpretation of these results, a PLGA composite has great potential for the use in delivery of cells/factors to the periodontal tissue regeneration without any complications. This finding is in agreement with the study of others with no signs of bone resorption or inflammation on the application of PLGA[18, 33].

V. CONCLUSION

In conclusion, the application of PLGA/inorganic filler matrix in the 1-wall intrabony defects showed to be biocompatible and biodegradable. These results support that PLGA/inorganic filler matrix in conjunction with periodontal regeneration materials could be a good candidate for scaffold of bioactive growth factors in promoting periodontal tissue regeneration.

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