Pharmacokinetic and pharmacodynamic evaluation of local minocycline agents in treating peri-implant mucositis and peri-implantitis: An experimental *in vivo* study

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Pharmacokinetic and pharmacodynamic evaluation of local minocycline agents in treating peri-implant mucositis and peri-implantitis: An experimental *in vivo* study

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감사의 글

치주학을 배울 수 있다는 기대감을 안고 치주과에 입사한 지 어느덧 3년이란 세월이 지났습니다. 전공의 기간 동안 임상에서의 다양한 술식들의 매력은 물론, 연구영역에서의 치주과의 진정한 매력을 느낄 수 있었으며 후학들이 이렇게 안락한 환경에서 마음껏 연구를 진행할 수 있도록 발판을 마련해 주신 치주과 교수님들께 진심으로 감사드립니다.

가장 먼저, 누구보다도 논문 작성에 세심하고 꼼꼼한 손길로 큰 도움을 주신 정의원 교수님께 큰 감사를 드리며 항상 풍부한 경험과 아낌없는 조언을 해주신 조규성, 최성호 교수님께도 감사드립니다. 또한 임상 및 연구에 대해 항상 큰 가르침을 주신 김창성, 이중석, 백정원 교수님, 본 연구 및 논문 작성에 있어 가장 가까이에서 함께 고민하고 많은 도움을 주신 차재국, 송영우 교수님께도 진심으로 감사드립니다.

본 논문을 작성하는데 있어 길라잡이를 해주신 Mariano Sanz, Ignacio Sanz Martin 교수님께도 깊이 감사드립니다. 이 외에도 실험 및 논문에 도움을 주신 구태환, 김명지, 팽경원 선생님 그리고 제 옆에서 항상 힘이 되어준 저의 동기들께도 감사의 마음을 전합니다.

마지막으로, 항상 저를 믿어주고 지지와 격려를 보내주시는 부모님과 누나에게도 사랑과 감사의 마음을 전합니다.

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Abstract

Pharmacokinetic and pharmacodynamic evaluation of local minocycline agents in treating peri-implant mucositis and peri-implantitis: An experimental *in vivo* study

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Purpose: The objective of this preclinical investigation was to evaluate the differential pharmacokinetic and pharmacodynamic properties of two local minocycline microsphere carriers: chitosan-coated alginate (CA) and poly(meth)acrylate-glycerin (PG).

Materials and methods: Four dental implants were placed bilaterally in the edentulous mandible of six beagle dogs. Each implant was randomly assigned to receive one of the following four treatments: (i) CA (CA-based minocycline), (ii) Placebo (CA substrate without minocycline), (iii) PG (PG-based minocycline) and (iv) Control (mechanical debridement only). After inducing peri-implant mucositis (mucositis) and

peri-implantitis at each side of mandible, the randomly assigned treatments were administered into the gingival sulcus twice at a 4-week interval using a plastic-tipped syringe. Pharmacokinetic (carrier and bacteriostatic effect sustainability) and pharmacodynamic (clinical, radiographical and cell-marker intensity) evaluations were performed after each administration.

Results: The CA microspheres remained longer around the healing abutment compared to PG microspheres in both experimentally induced peri-implant mucositis and peri-implantitis environment. Longer bacteriostatic effect was observed during the second administration in CA [7.0 ± 5.7 days (mucositis); 9.3 ± 5.7 days (peri-implantitis)] compared to PG [1.2 ± 2.6 days (mucositis); 1.2 ± 2.9 days (peri-implantitis)] in both mucositis and peri-implantitis group ($p < 0.05$). The efficacy of the applied therapies based on clinical, radiographical and histological analyses were comparable across all treatment groups.

Conclusions: CA microspheres showed longer carrier and bacteriostatic effect sustainability when compared to PG microspheres, however, longer drug sustainability did not lead to improved treatment outcomes.

Key words: pharmacokinetic, pharmacodynamic, chitosan-alginate microspheres, peri-implant mucositis, peri-implantitis, local minocycline agent

Pharmacokinetic and pharmacodynamic evaluation of local minocycline agents in treating peri-implant mucositis and peri-implantitis: An experimental *in vivo* study

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I. INTRODUCTION

Dental implants have demonstrated a high degree of predictability to rehabilitate absent teeth or tooth loss, with long-term (≥10 years) survival rates exceeding 94% [1]. Despite these survival rates, the prevalence of peri-implant diseases remains a major health problem. Peri-implant mucositis (mucositis) — defined by the presence of peri-implant tissue inflammation, identified by bleeding on probing (BOP) and by an increase in probing depth due to tissue swelling or a decrease in probing resistance, but without the evidence of peri-implant bone loss [2] — occurs in about half of the population with dental implants [3-5]. Peri-implantitis, an irreversible peri-implant inflammatory disease presenting all of the clinical symptoms of mucositis with the evidence of suppuration and peri-implant bone loss, is present in 20% of the population with dental implants [3, 4]. Therefore, an effective

modality to treat inflammatory peri-implant disease is necessary owing to the fact that the number of implants is ever-increasing.

Among several implicated predisposing factors, bacterial infection has been recognized as a major risk factor for inflammatory peri-implant disease [6]. To control the balance of oral microflora, mechanical debridement of implant fixture is prerequisite [7]. However, mechanical debridement alone is not sufficient to remove deeply infiltrated bacterial species [8, 9]. Therefore, adjunctive therapies involving the local delivery of antimicrobial agents have been advocated [10]. Local delivery has the advantage of applying the antimicrobial agents at high concentrations at the targeted site with a relatively low systemic concentration [11]. However, the constant flow of the gingival crevicular fluid (GCF) and the cleansing activity of saliva may restrict their efficacy, which demands studies that evaluate the pharmacokinetic and pharmacodynamic properties of any proposed local antimicrobial agent for oral use [12].

Minocycline, a second generation tetracycline, due to its broad spectrum antimicrobial activity and long-term sustainability, has been formulated as minocycline-loaded microsphere agents and demonstrated improved clinical results when used as an adjunct to mechanical debridement therapy in the treatment of peri-implantitis [13]. Chitosan-coated alginate (CA) carriers have also been reported to have prolonged biodegradable sustainability for the controlled release of minocycline [14, 15]. The use of this biopolymer has been advocated due to its biodegradability, improved wound healing, low toxicity and antimicrobial activity [16]. Other types of biodegradable polymers have included poly(meth)acrylate-glycerin (PG) microspheres with faster release of the contained chemical via diffusion control, thus rapidly creating a highly concentrated antimicrobial environment shortly after the delivery [17]. The aim of this experimental *in vivo* investigation was to evaluate pharmacokinetic and pharmacodynamic aspects of CA minocycline microspheres, when compared to PG microspheres, in an experimentally induced mucositis and peri-implantitis model in beagle dogs.

II. MATERIALS AND METHODS

1. Experimental Animals, Housing and Husbandry

This preclinical *in vivo* study was approved by the International Animal Care and Use Committee, Yonsei Medical Research Center, Seoul, South Korea (Permission no.2017-31-0547) and followed the ARRIVE guidelines [18]. This experimental *in vivo* investigation was carried out in six male beagle dogs (12–15 months old) with a mean weight of 15 kg. All animals were sheltered under the supervision of professional veterinarians at Avison Biomedical Research Center of Yonsei University, Seoul, South Korea at a room temperature of 15–20 °C and humidity of ≥30%. The animals were quarantined for 2 weeks, and then were approved for use in the experiments after performing a careful health examination.

General anaesthesia was induced by inhalation anaesthesia with isoflurane followed by the intramuscular injection of xylazine (Rompun, BayerKorea, Seoul, South Korea) and the intravenous injection of atropine (Kwangmyung Pharmaceutical, Seoul, South Korea). Lidocaine HCl (2% with epinephrine 1:80,000; Kwangmyung Pharmaceutical) was injected at the surgical site for local anaesthesia. Intramuscular antibiotic injection (cefazolin sodium, Yuhan Pharmaceutical, Seoul, South Korea) was administered after the surgery.

2. Experimental procedures

The overall study design is outlined in Figure 1.

2.1 Tooth extraction and Implant Installation

The second to fourth premolars and the first molar were extracted from both sides of the mandible of each dog. After an 8-week healing period, four implants were installed 7 mm apart at the level of the bone crest in each side of the mandible (Figure 2a, i). The

implants were all of internal connection design with a sandblasted, large-grit, acid-etched surface and a size of 3.6 × 8 mm (Implantium; Dentium, Suwon, South Korea). Healing abutments (4.0 × 2.0 mm; Dentium) were then connected to the implant fixture and the wounds healed by primary closure. A 4-week healing period was applied during which plaque control was performed three or four times weekly using a toothbrush and chlorhexidine gluconate solution (Hexamedine, Bukwang Pharmaceutical, Seoul, South Korea).

2.2 Experimental peri-implant mucositis and peri-implantitis

Four weeks after implant installation, hemi-mandibles of the dogs were randomly allocated to mucositis or peri-implantitis group. To develop mucositis, the oral hygiene measures were discontinued and animals were fed a soft diet for 6 weeks to induce plaque accumulation [19]. To develop peri-implantitis, silk ligatures (2-0 Gingi-pak®, Camarillo, USA) were placed into the gingival sulcus around healing abutments for 6 weeks to induce peri-implantitis [20]. New healing abutments (4.0 × 2.0 mm) were replaced after inducing peri-implant disease.

2.3 Antimicrobial Interventions

After professionally administered plaque control with an ultrasonic scaler, local delivery agents (LDA) were placed into the gingival sulcus by means of a disposable injection syringe twice at a 4-week interval.

Each implant in both mucositis and peri-implantitis group was randomly assigned to receive one of the following four treatments (Figure 2c, k):

1. 0.5 g of minocycline hydrochloride with CA microsphere carrier (CA; Minocline®, Dongkook Pharmaceutical, Seoul, South Korea),
2. CA microsphere carrier without the antimicrobial agent (placebo; prepared by the Dongkook Pharmaceutical company),

3. 0.5 g of minocycline hydrochloride with PG microsphere carrier (PG; Periocline®, Sunstar, Osaka, Japan),
4. mechanical debridement only (negative control).

3. Pharmacokinetic evaluation

The sustainability of LDA was evaluated at macroscopical and microbiological level. To evaluate the sustainability of the LDA carriers, healing abutments were retired from the implants of CA, placebo and PG groups, and circular inspection of the subgingival area proceeded macroscopically in plain sight for the presence of the residual antimicrobial agent (Figure 3) at 14 and 28 days after the first drug administration and at 1, 3, 7, 14 and 28 days after the second drug administration. The length of carrier sustainability was recorded as the time between the day of drug administration to the day when LDA were no longer visible from the subgingival part of the healing abutments. The mean length of carrier sustainability was calculated as an average of all six implants from each of the mucositis and peri-implantitis CA, placebo and PG groups.

To assess the sustainability of the bacteriostatic effect, GCF and residual agent (if present) samples of the CA, PG and control groups were collected from the mesial, distal, buccal and lingual gingival sulcus around the healing abutments with a single stroke using a Gracey curette (Osung MND, Seoul, South Korea) at 1, 3, 7, 14 and 28 days after each drug administration. These samples were immediately placed in centrifuge plastic tubes (Eppendorf Tube®, Hamburg, Germany) containing 200 μL of distilled water and then stored in a deep freezer at −80 °C.

A single-blinded broth dilution assay was carried out by centrifuging the GCF sample solutions and serial dilution using phosphate-buffered saline (LPS solution, Daejeon, South Korea) [21]. A total amount of 100 μL of each diluted sample solution was added to a 96-well plate, and 5 μL of *Staphylococcus aureus* solution (2.5×10^7 CFU) in LB broth (95 μL of 2× LB broth; BD Diagnostics, Sparks, MD, USA) was subsequently added to each well.

After incubating the solutions for 24–48 h at 37 °C, bacterial cell growth was evaluated at 600 nm using a microplate reader (SpectraMax M2, Molecular Devices, San Jose, CA, USA).

4. Pharmacodynamic evaluation

4.1 Clinical and Radiographical Outcomes

Clinical measurements were recorded in all the implants by a single examiner (M.J.K.) at the following time points: (a) baseline (T1), (b) 4 weeks after the first drug administration (T2) and (c) 4 weeks after the second drug administration (T3). BOP, plaque index (PLI) and gingival index (GI) [22] were measured to evaluate the severity of inflammation. Probing pocket depths (PPD) were measured using a periodontal probe with 1-mm marking (Qulix CP-15 UNC SE, Hu-Friedy, IL, USA).

Peri-apical radiographs of the implants were obtained using a customized film holder (XCP, Dentsply Rinn, Konstanz, Germany) with a portable X-ray device (DIOX-602, Digimed, Seoul, South Korea) (Figure 2e-h, m-p). The radiographs were obtained immediately after the fixture installation (Surgery, S0) and at 1, 2 and 6 weeks after plaque inducement and ligature placement to ensure that the marginal bone levels were maintained. Peri-apical radiographs were then obtained at T1, T2 and T3 to examine the bone level changes around the implant fixtures. The healing abutment–fixture junction (A/F) was marked as a reference point, and the average marginal bone level changes were recorded [23].

4.2 Histological Preparation and Outcomes

The dogs were euthanized 30 days after the second administration with an overdose of sodium pentobarbital. The mandibles of the dogs were retrieved and the healing abutments were carefully removed. Tissue blocks prepared using a diamond saw were fixed in 10% buffered neutral formalin (Sigma Aldrich, Yong-in, South Korea) for 2 weeks and then were decalcified using 10% EDTA (Chelatocal, National Diagnostics, Atlanta, GA, USA). Before the blocks were fully decalcified, the fracture technique was performed to

obtain two units from each block by slicing the centre of the buccal and lingual aspects parallel to the implant axis [24]. The decalcified blocks were then dehydrated with a graded series of ethanol concentrations. The units were embedded in paraffin and then sectioned at a thickness of 3 μm using an automated rotary microtome (Leica RM2255, Leica Biosystems, Nussloch, Germany). Each tissue section was then processed with immunohistochemistry (IHC) staining.

The preparation for IHC included de-paraffinization with xylene prior to the staining. Sodium citrate buffer solutions (pH 6.0) were used for antigen retrieval. The sections were gently washed with Tris buffer saline (TBS) 0.025% Triton X-100 and then drained with tissue paper. Primary antibodies were diluted in TBS with 1% bovine serum albumin and were applied to the sections via a pipette. The sections were incubated overnight at 4 °C and then rinsed with TBS 0.025% Triton X-100 the following day. Horseradish peroxidase was used to conjugate with primary antibodies, and positive cells were detected using DAB (3,3'-diaminobenzidine) staining. The primary antibodies used in the IHC cell marker analysis were CD3 (ab828, Abcam, Cambridge, UK), CD20 (PA5-16701, Thermo Fisher scientific, Seoul, South Korea) and IgG (PAA544Ca01, Cloud-Clone corp., Katy, TX, USA). The specificity and dilution rates of the primary antibodies used in the IHC staining are listed in Table 1 [23].

Digital images of the specimens were obtained (Panoramic 250 Flash III, 3D, Case Viewer 2.0, 3D Histech, Budapest, Hungary) and the intensities of CD3, CD20 and IgG antibody staining were evaluated using the IHC profiler in Image J image-processing software [25]. Areas of 0.5 × 0.5 mm above and below the A/F were selected as regions of interest (ROIs) (Figure 4).

5. Statistical Analysis

Statistical analysis was performed using standard software (SPSS version 25, IBM, Armonk, NY, USA). The mean values of the carrier sustainability length and bacteriostatic duration were calculated for each group and the mean values of measured variables were

calculated for each implant and group in clinical, radiographical and histological evaluations. The null hypotheses of this study were as follows: 1. CA and PG will exhibit similar carrier degradability and bacteriostatic duration; 2. All treatment groups will present comparable treatment outcomes. Due to the small sample size, a non-parametric Kruskal—Wallis test was performed to compare the carrier sustainability length and bacteriostatic duration after each drug administration and to compare IHC cell marker intensity after sacrifice. If the results were significant ($p < 0.05$), a Mann—Whitney U test was performed as a post-hoc test with the significance criterion adjusted according to Bonferroni's method (i.e., $p < 0.0083$). A Kruskal—Wallis test ($p < 0.05$) was also used to compare the clinical and radiographical outcomes at each time point (T1, T2 and T3) between the groups. To compare pre- and post-treatment differences within each group, a Wilcoxon signed-rank test ($p < 0.05$) was applied to assess intragroup treatment outcomes.

III. RESULTS

The healing processes after tooth extraction and implant surgeries were uneventful at all surgical sites. Measures from all six dogs were included in the results.

1. Pharmacokinetic evaluation

Carrier sustainability

Macroscopic evaluation of the healing abutments revealed longer carrier sustainability with CA-based carriers (CA and placebo) compared to PG carrier in both mucositis and peri-implantitis groups. In mucositis, drug agent residues were detected from three implants (out of six) in CA (7.0 ± 7.0 days; $p > 0.05$, vs. PG) and four implants in the placebo (11.7 ± 9.6 days; $p < 0.01$, vs. PG) group 14 days after the administration, while no drug agent remnants were found from the PG group implants at day 14 (0.0 ± 0.0 days) in the first administration (Figure 5a). In the second administration, drug agent residues were found in three implants from both CA (12.2 ± 8.1 days) and placebo (15.2 ± 9.4 days) groups at day 14, and two implants from the CA and one implant from the placebo group remained up to 28 days, while drug agent remnants were found in only one implant from the PG (0.2 ± 0.4 days; $p = 0.002$, vs. CA and placebo) group at day 1 and were all dissolved by day 3 (Figure 5b).

In peri-implantitis, five implants (out of six) from CA (16.3 ± 10.5 days, $p = 0.015$, vs. PG) and placebo (18.7 ± 11.4 days, $p = 0.015$, vs. PG) were remained at day 14 while no LDA retention was observed from PG at day 14 (0.00 ± 0.00 days) in the first administration (Figure 5c). After the second administration, five implants from CA (22.2 ± 9.30 days, $p = 0.002$, vs. PG) and three implants from placebo (12.8 ± 8.18 d $p = 0.002$, vs. PG) groups were remained at day 14 while PG (0.67 ± 1.21 days) showed significantly low LDA retention rate when compared to CA carriers (Figure 5d).

Bacteriostatic Effect Sustainability

Longevity of LDA bacteriostatic effects were evaluated using the broth dilution assay. In mucositis group, all six implants from the CA group presented the same bacteriostatic effect up to day 3 in the first drug administration. Five out of six implants from the PG group showed a bacteriostatic effect up to day 3 in the first drug administration (CA: 3.0 ± 0.0 days, PG: 2.5 ± 1.2 days; $p > 0.05$) (Figure 6a). In the second drug administration, two out of six implants from the CA group presented a bacteriostatic effect up to day 14 and two other implants were effective up to day 7 for CA group (7.0 ± 5.7 days), while only one implant from the PG group lasted up to day 7 (1.2 ± 2.6 days, $p = 0.132$) (Figure 6b).

In peri-implantitis group, all six implants from CA and PG showed bacteriostatic effect at day 3 and one implant from CA has shown bacteriostatic effect up to day 7 [CA (3.67 ± 1.63 days), PG (3.0 ± 0.0 days); $p > 0.05$] (Figure 6c) in the first delivery. During the second delivery, prolonged bacteriostatic effect was observed between CA (9.33 ± 5.72 days) and PG (1.17 ± 2.86 days; $p < 0.05$) (Figure 6d). Five implants from CA has shown bacteriostatic effect at day 7 and three implants were effective up to day 14. On the other hand, only one implant from PG has shown bacteriostatic effect up to day 7.

2. Pharmacodynamic evaluation

Clinical findings

Clinical parameters (mean PPD, BOP, GI and PLI values) at different time points are depicted in Figure 7, Table 2, and 3. In mucositis, none of the clinical parameters differed significantly between the groups at T1, T2 and T3. Most of the interval changes were minimal, but all groups showed significantly reduced PLI between T1 and T2 ($p = 0.026$, 0.027, 0.028 and 0.027 in the CA, placebo, PG and control groups, respectively). Furthermore, mean PPD was reduced in the CA group (−0.39 ± 0.49 mm), while other groups showed slight interval changes (−0.11 ± 0.18 mm (placebo) to 0.09 ± 0.40 mm (PG)) after the second drug administration.

In peri-implantitis, mean PPD was significantly reduced within all the groups at T3 compared to T1 and T2 (p = 0.027 for all groups). CA was the only group that showed significant reduction of mean PPD between T1 and T2 ($p < 0.05$). Mean PLI was also significantly reduced within CA, placebo and PG groups at T3 compared to T1 and T2 [T1-T3: (CA: p = 0.028, placebo and PG: p = 0.027); T2-T3: (CA and placebo: p = 0.027, PG: p = 0.026)] while the control group showed significant reduction of PLI between T1 and T3 (p = 0.027). PG and Control group showed significantly reduced BOP (%) at T2 and T3 compared to T1 ($p < 0.05$) and PG was the only group that presented significantly improved GI at T2 and T3 compared to T1 ($p < 0.05$).

Radiographical findings

Radiographical measurements from implant sites taken at S0, T1, T2 and T3 are presented in Figure 8. In mucositis, mean bone level changes between S0 and T1 were −0.38 ± 0.26, −0.47 ± 0.30, −0.39 ± 0.38 and −0.44 ± 0.30 mm in the CA, placebo, PG and control groups, respectively, with no significant differences between any of the groups ($p > 0.05$) (Figure 8a). Negligible bone loss was observed in all treatment groups between T1 and T3 (−0.37 ± 0.26, −0.34 ± 0.23, −0.25 ± 0.23 and −0.44 ± 0.29 mm in the CA, placebo, PG and control groups, respectively ($p > 0.05$).

In peri-implantitis, -0.9 mm (PG) to -1.6mm (placebo) of mean marginal bone loss occurred after inducing peri-implantitis with a ligature (Figure 8b). Minimal mean marginal bone gain occurred from CA (0.17 ± 0.28 mm) and placebo (0.12 ± 0.29 mm) while PG (-0.15 ± 0.24 mm) presented negligible mean marginal bone loss after the first delivery. Minimal mean marginal bone level change was also observed after the second delivery ranging from -0.06 ± 0.29 mm (placebo) to -0.024 ± 0.25 mm (CA). All results were comparable between the groups.

IHC cell marker analysis

The cell marker intensities in the upper ROI, lower ROI and total mean (upper and lower ROI combined) are summarized in Table 4. In mucositis, the IHC cell marker intensities from all three antibodies (CD3, CD20 and IgG) were comparable between the groups. Moreover, there were no significant differences between the upper and lower ROIs within each group.

In peri-implantitis, CD3, T-cell antibody, has a total mean positive score in range of 6.34 (Control) to 9.29 % (placebo). CD20, B-cell antibody, has a total mean positive score in range of 7.85 (placebo) to 8.55 % (Control). IgG, B-cell and Plasma cell antibody, has a total mean positive score in range of 6.59 (CA) to 11.33 % (Control). All groups showed comparable positive score at upper and lower ROI. The positive score of upper and lower ROI within the group were also comparable.

IV. Discussion

This study evaluated pharmacokinetic and pharmacodynamic parameters when using two different carriers for local minocycline delivery in an experimentally induced mucositis and peri-implantitis environment. More implants from CA microspheres presented prolonged sustainability of the carrier (CA and placebo) and a bacteriostatic effect (CA) compared to PG microspheres. Clinical, radiographical and IHC cell marker analysis presented comparable results between the groups.

The controlled release rate of minocycline in CA microspheres is primarily attributable to the stability of chitosan in aqueous solution. In CA microspheres, chitosan is coated around alginate microspheres via ionic coacervation using a cross-linking agent such as calcium chloride [14]. Chitosan is degraded inside the body by lysozyme, and so CA microspheres will dissolve and degrade in proportion to the amount of lysozyme present in the surrounding environment [26]. From a previous study which evaluated the biodegradability of a minocycline-loaded CA microsphere in relation to the concentration of chitosan in microsphere fabrication, a slower release rate of minocycline was observed [14]. Furthermore, this previous study reported a 50% microsphere weight decrease in two weeks in an *in vitro* setting of GCF with HEPES buffer and lysozyme (20 μg/mL) and suggested that a CA microsphere can maintain drug concentration for a week [14, 27]. Among 12 CA microsphere-applied implants (CA and placebo) in mucositis group, the local antibiotic agent remained at 14 days in 7 and 6 implants in the first and second trials, respectively. In peri-implantitis group, among the 12 CA microsphere-applied implants (CA and placebo), 6 and 9 implants were remained at day 14. With their slowly degradable property, a longer bacteriostatic effect was also observed in the broth dilution assay in both mucositis and peri-implantitis CA group (up to 14 days post-delivery) when compared to PG microspheres. A previous clinical trial which evaluated the effects of CA minocycline microspheres observed significant reduction in both aerobic and anaerobic bacterial counts

even after 6 weeks and that BOP (%) and mean PPD were significantly reduced compared to the conventional supragingival scaling group [15].

In addition to the slow degradation of chitosan, CA microspheres also have the advantages of the anti-inflammatory effect of chitosan and the bio-adhesion ability of alginate to mucosa [28, 29]. When minocycline-containing alginate microspheres are released to the gingival sulcus, alginate will adhere to the mucosa so as to prevent it from washing out due to the GCF and salivary flow. Furthermore, even when minocycline is completely released into the surrounding environment, chitosan can act as an anti-inflammatory material itself. Thaya et al. [29] reported antibacterial and antibiofilm activities of CA microspheres against Gram-positive and -negative species in *in vitro* tests. These observations indicate that CA microspheres are a highly effective carrier for the local delivery of antibacterial agents.

On the other hand, PG carrier controls the drug release rate via diffusion. The microsphere is hydrophilic and comprises poly(meth)acrylate, glycerin and hydroxyethyl cellulose [30]. Therefore, when microspheres are locally injected into the gingival sulcus, they will instantaneously dissolve to release minocycline. A previous study of a locally injected PG minocycline microsphere reported that the minocycline concentration is immediately increased to 1300 μg/mL and then decreased to 90 μg/mL within 7 hours after being delivered [17]. Another *in vivo* study which evaluated the drug retention rate of minocycline-loaded methoxy-poly(ethylene glycol) poly(lactic acid) nanoparticles with a PG minocycline microsphere determined the concentration of minocycline in GCF using high-performance liquid chromatography [31]. The study revealed that the minocycline concentration of the PG carrier fell under 3 μg/mL after 2 days post-delivery and the action of the drug (>1 μg/mL) lasted up to 8 days. Comparable to the present study, most locally injected agents were dissolved and the residual agent was detected in only one (mucositis) and two (peri-implantitis) implants at day 1 from the PG group, and the bacteriostatic effect lasted up to day 7 during the second trial in both mucositis and peri-implantitis. However, care is needed before assuming that one agent is superior to the other, because the

cumulative amount of minocycline released in the two types of microspheres may be equivalent since both the CA and PG groups contained 10 mg of minocycline hydrochloride.

From the clinical results of mucositis group, the CA group showed a slight reduction of the mean PPD (0.4 mm) after the second administration, while the PG group showed a minimal increase (0.1 mm) over the same interval (Table 2). There was also a slight reduction of the mean BOP relative to the CA group (5.6%) after the second administration, while the PG group presented a slight increase (2.7%). However, this apparent difference between these two groups was not statistically significant.

Clinical assessment of peri-implantitis group showed significantly reduced mean PPD at T3 compared to baseline in all of treatment groups ($p < 0.05$). Since progression of peri-implantitis can be measured by increased probing depth [2], this result implies that the disease has not been progressed during the treatment period. In PPD, CA was the only group that showed significant reduction in PPD after each delivery. All of treatment groups showed significantly reduced mean PLI at T3 compared to baseline ($p > 0.05$) while PG was the only group that showed significant reduction from both BOP and GI scores. However, despite these intragroup changes, all clinical outcomes were also comparable between the groups.

Progression of marginal bone loss is a distinguishable clinical characteristic of peri-implantitis from mucositis. In the radiographical analysis of peri-implantitis group, all treatment groups showed minimal marginal bone level changes during the treatment period ranging from -0.07 (CA) to -0.25 mm (PG). Taking into an account that the mucositis group showed mean marginal bone loss occurred at a range of -0.25 (PG) to -0.44 (Control) mm, minimal bone level changes occurred in both mucositis and peri-implantitis groups indicate that all treatment groups have impeded the disease progression. Furthermore, a previous *in vitro* study asserted on the possible negative impingement of CA microspheres in the healing process after treatment due to its long sustainability remained in gingival sulcus [30]. The radiographical outcome of this study showed that longer sustained microsphere does not interfere with the healing process or create a further bone loss.

An immune response begins with cell-mediated immunity involving phagocytes and T-cells, and then initiates humoral immunity as helper T-cells stimulate B cells to differentiate to plasma and memory cells after undergoing mitosis. This means that B cells and plasma cells are mostly involved in the latter inflammatory stage. Concurrently, most (about 60%) of the inflammatory cells in periodontal disease are largely comprised of B cells and plasma cells [32]. The IHC cell marker analysis examined three different types of antibodies: CD3, CD20 and IgG. In the IgG cell marker analysis using an antibody specific to B cells and plasma cells, the CA group presented the lower tendency of cell marker intensity in both the upper and lower ROIs of the A/F among all other test groups in both mucositis and peri-implantitis group. In the histological slide samples, infiltrated connective tissue (ICT), an area of which inflammatory cells are distributed, is present throughout the coronal and apical portion of the pocket along the implant fixture (Figure 4). Carcuac et. al. [23] reported that histological evaluation of ICT in experimentally induced peri-implantitis revealed larger ICT and longer ulcerated pocket epithelium compared to experimentally induced periodontitis due to lack of ability to encapsulate the lesion. A closer examination of the histological samples of this current study also revealed that pocket epitheliums were stretched to the bone crest with no epithelial barrier in the most apical part of the ICT.

The efficacy of an adjunctive local antibacterial therapy in periodontal or peri-implant disease is still controversial. A review article reported that the BOP tendency and probing depths can be reduced compared to those in non-surgical therapy by applying adjunctive local antibacterial delivery [33]. Other controlled clinical studies reported that the use of adjunctive local minocycline delivery after non-surgical therapy showed improved BOP, PPD and bacterial counts after 12 months of treatment [34, 35]. However, in an *in vivo* study which evaluated the antiseptic effects of chlorhexidine in experimentally induced mucositis in nine cynomolgus monkeys, comparable mean PLI and GI scores and mean PPD reductions were observed between the mechanical treatment group and the adjunctively treated chlorohexidine group [36]. While significant clinical improvements

were observed from the treatment groups when compared to the no treatment group, mechanical treatment alone was not sufficient to achieve clinical resolutions of mucositis lesions. The clinical results from this study also demonstrated that the treatment outcome of mechanical debridement alone is comparable.

A recent randomized placebo-controlled clinical trial emphasized the importance of the repeated administration of local antibiotic agents after treatment [13]. In this clinical study, a local minocycline agent was delivered into the gingival sulcus of the patients with peri-implantitis at the surgical treatment, stitch-out, 1 month and 3 months post-operation. Improved clinical and radiographical results were observed in the minocycline ointment-treated group when compared to the placebo ointment-treated group. The present study also repeatedly injected the local antibiotic agent at 1-month interval. Although a reduced mean PPD was observed from the all of treatment groups in an experimentally induced peri-implantitis environment ($p < 0.05$) after the second administration within each group, no statistical differences were observed in clinical and radiographical measures between the groups. The present authors carefully interpreted that the effect of a single delivery at 1-month interval may have been an overly distant period to properly evaluate the effect of the repeated application of a local antibiotic agent since healing in dogs is believed to occur two to three times faster than in humans [37].

The discrepant treatment results from this *in vivo* study with the studies of those which presented an improved outcome when using adjunctive local antibacterial therapy may have been associated with some of the limitations of this study. The present study investigated the sustainability of the agents. Therefore, care was taken to ensure that the sulcular environment did not interfere with oral hygiene care after each delivery. For this reason, only the supragingival parts of the healing abutments were cleansed using a hydrogen peroxide-soaked gauze during the investigation period. Since oral hygiene control is known to be a critical factor in relieving inflammation, the authors carefully speculate that the effects of the restricted oral hygiene care may have surpassed the effects of local minocycline. Furthermore, the treatment responses varied between the included

animals. The author evaluations of the periodontal status of the studied animals revealed that two dogs presented less-favourable soft-tissue healing patterns, showing more gingival swelling and remaining redness than the others. Due to the small size of the sample, such variations among individuals produced relatively large standard deviations that influenced the statistical power of the study.

This study evaluated the pharmacokinetic and pharmacodynamic parameters of local minocycline with different constituents on a monthly basis for two months. Following the ARRIVE guidelines, the authors only evaluated short-term clinical and radiographical outcomes. Moreover, histological data were collected only after a sacrifice, and so interval changes between before and after the treatment were not determined. Thus, further studies are needed to evaluate the long-term effects with a sufficient delivery interval and microbiological evaluations that can present interval changes.

V. Conclusion

Within the limitations of this study, pharmacokinetic evaluations from this study presented prolonged carrier sustainability and a bacteriostatic effect of CA minocycline-containing microspheres when compared to diffusion-controlled PG minocycline microspheres. However, prolonged sustainability of minocycline did not lead to improved treatment results.

References

1. Moraschini, V., et al., *Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review.* Int J Oral Maxillofac Surg, 2015. **44**(3): p. 377-88.
2. Berglundh, T., et al., *Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.* Journal of periodontology, 2018. **89**: p. S313-S318.
3. Renvert, S., C. Lindahl, and G.R. Persson, *Occurrence of cases with peri-implant mucositis or peri-implantitis in a 21-26 years follow-up study.* J Clin Periodontol, 2018. **45**(2): p. 233-240.
4. Renvert, S., et al., *Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations.* Journal of clinical periodontology, 2018. **45**: p. S278-S285.
5. Sanz, M., I.L. Chapple, and W.G.o.t.V.E.W.o. Periodontology*, *Clinical research on peri-implant diseases: consensus report of W orking G roup 4.* Journal of clinical periodontology, 2012. **39**: p. 202-206.
6. Heitz-Mayfield, L.J., *Peri-implant diseases: diagnosis and risk indicators.* J Clin Periodontol, 2008. **35**(8 Suppl): p. 292-304.
7. Rams, T.E. and J.J.P. Slots, *Local delivery of antimicrobial agents in the periodontal pocket.* 1996. **10**(1): p. 139-159.
8. Mombelli, A., et al., *Actinobacillus actinomycetemcomitans in adult periodontitis. I. Topographic distribution before and after treatment.* 1994. **65**(9): p. 820-826.
9. Renvert, S., et al., *Effect of root debridement on the elimination of Actinobacillus actinomycetemcomitans and Bacteroides gingivalis from periodontal pockets.* 1990. **17**(6): p. 345-350.
10. Herrera, D., et al., *A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients.* Journal of Clinical Periodontology, 2002. **29**: p. 136-159.

11. van Winkelhoff, A.J., T.E. Rams, and J. Slots, *Systemic antibiotic therapy in periodontics.* Periodontol 2000, 1996. **10**(1): p. 45-78.
12. Oosterwaal, P.J., F.H. Mikx, and H.H. Renggli, *Clearance of a topically applied fluorescein gel from periodontal pockets.* J Clin Periodontol, 1990. **17**(9): p. 613-5.
13. Cha, J.K., J.S. Lee, and C.S. Kim, *Surgical Therapy of Peri-Implantitis with Local Minocycline: A 6-Month Randomized Controlled Clinical Trial.* J Dent Res, 2019. **98**(3): p. 288-295.
14. Park, Y.J., et al., *Injectable polysaccharide microcapsules for prolonged release of minocycline for the treatment of periodontitis.* Biotechnol Lett, 2005. **27**(22): p. 1761-6.
15. Yeom, H.R., et al., *Clinical and microbiological effects of minocycline-loaded microcapsules in adult periodontitis.* J Periodontol, 1997. **68**(11): p. 1102-9.
16. Lee, D.W., et al., *Strong adhesion and cohesion of chitosan in aqueous solutions.* Langmuir, 2013. **29**(46): p. 14222-9.
17. Vanderkerckhove, B.N.A., M. Quirynen, and D. Van Steenberghe, *The use of locally-delivered minocycline in the treatment of chronic periodontitis. A review of the literature.* Journal of clinical periodontology, 1998. **25**(11): p. 964-968.
18. Kilkenny, C., et al., *Animal research: reporting in vivo experiments: the ARRIVE guidelines.* British journal of pharmacology, 2010. **160**(7): p. 1577-1579.
19. Berglundh, T., et al., *Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog.* Clin Oral Implants Res, 1992. **3**(1): p. 1-8.
20. Fickl, S., et al., *Experimental Peri-Implantitis around Different Types of Implants - A Clinical and Radiographic Study in Dogs.* Clin Implant Dent Relat Res, 2015. **17 Suppl 2**: p. e661-9.
21. Balouiri, M., M. Sadiki, and S.K. Ibnsouda, *Methods for in vitro evaluating antimicrobial activity: A review.* J Pharm Anal, 2016. **6**(2): p. 71-79.
22. Loe, H. and J. Silness, *Periodontal Disease in Pregnancy. I. Prevalence and Severity.* Acta Odontol Scand, 1963. **21**(6): p. 533-51.
23. Carcuac, O., et al., *Experimental periodontitis and peri-implantitis in dogs.*

Clinical Oral Implants Research, 2013. **24**(4): p. 363-371.

24. Berglundh, T., et al., *The topography of the vascular systems in the periodontal and peri-implant tissues in the dog.* J Clin Periodontol, 1994. **21**(3): p. 189-93.
25. Sanz-Martín, I., et al., *Structural and histological differences between connective tissue grafts harvested from the lateral palatal mucosa or from the tuberosity area.* Clinical oral investigations, 2019. **23**(2): p. 957-964.
26. Guarino, V., et al., *Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications.* Aims Materials Science, 2015. **2**(4): p. 497-502.
27. Alvarez, A.L., F.O. Espinar, and J.B. Mendez, *The application of microencapsulation techniques in the treatment of endodontic and periodontal diseases.* Pharmaceutics, 2011. **3**(3): p. 538-71.
28. Miyazaki, S., et al., *Drug release from oral mucosal adhesive tablets of chitosan and sodium alginate.* International journal of pharmaceutics, 1995. **118**(2): p. 257-263.
29. Thaya, R., et al., *Synthesis of chitosan-alginate microspheres with high antimicrobial and antibiofilm activity against multi-drug resistant microbial pathogens.* Microbial pathogenesis, 2018. **114**: p. 17-24.
30. Lee, J.-B., et al., *Characteristics of Local Delivery Agents for Treating Peri-implantitis on Dental Implant Surfaces: A Preclinical Study.* Journal of Oral Implantology, 2019. **45**(2): p. 116-126.
31. Yao, W., et al., *Local delivery of minocycline-loaded PEG-PLA nanoparticles for the enhanced treatment of periodontitis in dogs.* Int J Nanomedicine, 2014. **9**: p. 3963-70.
32. Berglundh, T., N.U. Zitzmann, and M. Donati, *Are peri-implantitis lesions different from periodontitis lesions?* J Clin Periodontol, 2011. **38 Suppl 11**: p. 188-202.
33. Renvert, S., A.M. Roos-Jansåker, and N. Claffey, *Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review.* Journal of clinical periodontology, 2008. **35**: p. 305-315.
34. Bassetti, M., et al., *Anti-infective therapy of peri-implantitis with adjunctive local drug delivery or photodynamic therapy: 12-month outcomes of a randomized*

controlled clinical trial. Clin Oral Implants Res, 2014. **25**(3): p. 279-287.

35. Renvert, S., et al., *Mechanical and repeated antimicrobial therapy using a local drug delivery system in the treatment of peri-implantitis: a randomized clinical trial.* J Periodontol, 2008. **79**(5): p. 836-44.
36. Trejo, P.M., et al., *Effect of mechanical and antiseptic therapy on peri-implant mucositis: an experimental study in monkeys.* Clinical oral implants research, 2006. **17**(3): p. 294-304.
37. Schenk, R.K., et al., *Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible.* Int J Oral Maxillofac Implants, 1994. **9**(1): p. 13-29.

Figure legend

Figure 1. Study outline

Figure 2. Clinical photographs and radiographs**.** Clinical photographs of mucositis (a – d) and peri-implantitis (i – l) at different time points. Peri-apical radiographs of mucositis (e – h) and peri-implantitis (m – p) at different time points.

Figure 3. Macroscopic evaluation of carrier sustainability

a) No residue and b) residual agent visible at the subgingival part of healing abutment.

Figure 4. Immunohistochemical (IHC) stained histological slides of mucositis and peri-implantitis with different types of primary antibody – CD 3, CD 20, IgG antibody. A/F – abutment-fixture function; upper (blue) and lower (maroon) region of interest (ROI) selected for cell marker intensity analysis.

Figure 5. Macroscopic carrier sustainability evaluation

Figure 6. Longevity of bacteriostatic effect from broth dilution assay

Figure 7. Clinical interval changes

Figure 8. Marginal bone level change

Tables

Table 1. Types of monoclonal antibodies used for IHC staining

Antibody	Specificity	Dilutions	Source
CD3	**T cells**	**1:200**	**Abcam**
CD20	**B cells**	**1:800**	**Thermofisher scientific**
IgG	**Plasma / B Cells**	**1:800**	**Cloud-Clone corp.**

Table 2. Clinical parameters (mean±S.D.) measured at different time points of experimentally induced mucositis group

Clinical parameter	Group	Baseline(T1)	4 weeks (T2)	8 weeks (T3)
Mean PPD (mm)	CA	2.56 ± 0.46	2.64 ± 0.49	2.25 ± 0.27
	Placebo	2.39 ± 0.31	2.44 ± 0.54	2.33 ± 0.36
	PG	2.41 ± 0.22	2.47 ± 0.20	2.56 ± 0.60
	Control	2.47 ± 0.30	2.50 ± 0.17	2.50 ± 0.44
p-value		0.975	0.847	0.585
Mean GI	CA	1.44 ± 0.23	1.53 ± 0.11	1.56 ± 0.33
	Placebo	1.56 ± 0.25	1.58 ± 0.13	1.64 ± 0.15
	PG	1.44 ± 0.21	1.44 ± 0.18	1.47 ± 0.31
	Control	1.42 ± 0.08	1.44 ± 0.08	1.44 ± 0.23
p-value		0.680	0.267	0.501
Mean BOP (%)	CA	44.3 ± 22.9	52.8 ± 11.7	47.2 ± 26.2
	Placebo	55.5 ± 24.7	58.5 ± 13.0	63.8 ± 14.8
	PG	47.3 ± 22.5	44.5 ± 18.5	47.2 ± 31.1
	Control	41.5 ± 8.5	44.3 ± 8.0	44.3 ± 22.9
p-value		0.646	0.267	0.449
Mean PLI	CA	2.75 ± 0.23	2.67 ± 0.22	1.47 ± 0.40‡
	Placebo	2.64 ± 0.22	2.78 ± 0.18	1.45 ± 0.41‡
	PG	2.78 ± 0.25	2.69 ± 0.32	1.42 ± 0.37‡
	Control	2.72 ± 0.25	2.70 ± 0.20	1.47 ± 0.37‡
p-value		0.760	0.811	0.972

‡ - significantly different from T2 within each group
p-value – Clinical parameters compared (Kruskal–Wallis test) between the groups at each time point

Table 3. Clinical parameters (mean±S.D.) measured at different time points of experimentally induced peri-implantitis group

Clinical parameter	Group	Baseline(T1)	4 weeks (T2)	8 weeks (T3)
Mean PPD (mm)	CA	3.81 ± 0.99	3.06 ± 0.59‡	2.00 ± 0.62‡,*
	Placebo	3.81 ± 0.91	3.67 ± 0.83	2.17 ± 0.58‡,*
	PG	3.44 ± 0.30	3.19 ± 0.35	2.42 ± 0.60‡,*
	Control	3.47 ± 0.41	3.11 ± 0.62	2.17 ± 0.65‡,*
p-value		0.980	0.506	0.782
Mean GI	CA	1.92 ± 0.13	1.78 ± 0.12	1.78 ± 0.18
	Placebo	1.92 ± 0.13	1.72 ± 0.16	1.53 ± 0.35
	PG	1.89 ± 0.12	1.69 ± 0.18‡	1.44 ± 0.34‡
	Control	1.81 ± 0.22	1.58 ± 0.08	1.61 ± 0.31
p-value		0.720	0.154	0.491
Mean BOP (%)	CA	91.7 ± 12.7	77.8 ± 12.2	77.8 ± 18.3
	Placebo	91.7 ± 12.7	69.7 ± 14.9	69.5 ± 29.4
	PG	88.8 ± 12.4	69.5 ± 17.7‡	44.5 ± 34.1‡
	Control	91.5 ± 8.5	64.0 ± 11.4‡	50.2 ± 33.3‡
p-value		0.955	0.433	0.414
Mean PLI	CA	2.70 ± 0.34	2.33 ± 0.62	1.45 ± 0.29‡,*
	Placebo	2.75 ± 0.25	2.39 ± 0.57	1.31 ± 0.35‡,*
	PG	2.75 ± 0.25	2.50 ± 0.53	1.25 ± 0.48‡,*
	Control	2.67 ± 0.26	1.91 ± 0.98	1.17 ± 0.37‡
p-value		0.890	0.383	0.585

‡-significantly different from the baseline.
*-significantly different from T2
p-value – Clinical parameters compared (Kruskal–Wallis test) between the groups at each time

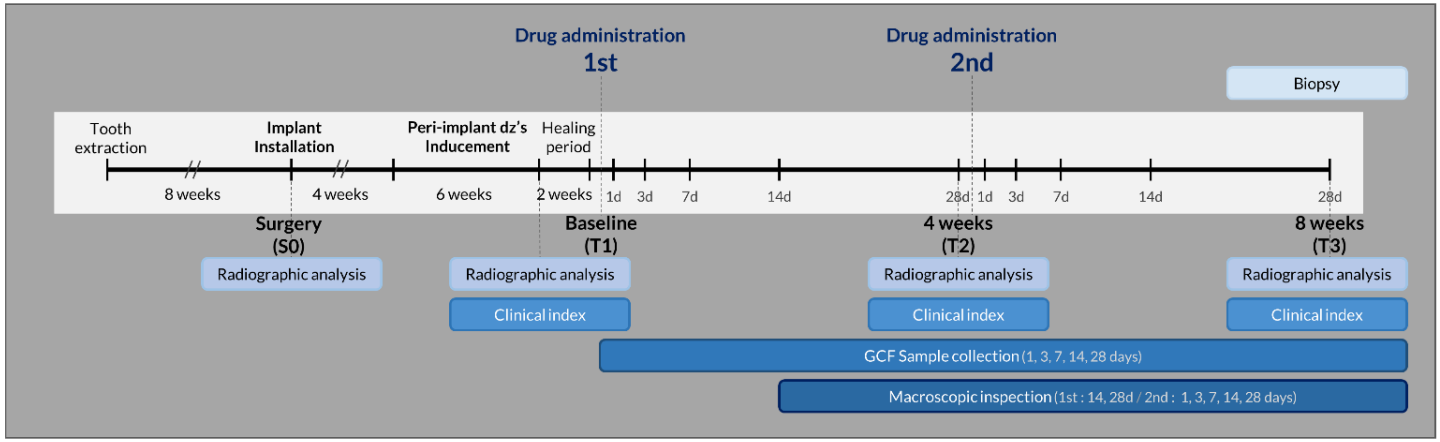
Table 4. IHC cell marker analysis

			CA	Placebo	PG	Control	*p*-value
Mucositis	**CD3 (%)**	**Upper**	8.60±4.27	8.66±4.05	8.70±5.35	6.60±4.41	**0.615**
		Lower	8.30±3.90	7.49±4.50	7.59±4.57	5.94±5.43	**0.340**
		Total mean	8.60±4.27	8.02±4.34	8.26±5.08	6.31±4.90	
	CD20 (%)	**Upper**	6.35±3.38	6.70±3.89	4.34±2.51	6.26±3.54	**0.572**
		Lower	6.76±7.71	7.57±5.16	5.46±2.20	7.36±4.01	**0.539**
		Total mean	6.55±5.86	7.10±4.54	4.90±2.43	6.84±3.83	
	IgG (%)	**Upper**	6.33±3.52	15.18±10.95	8.34±7.16	11.20±9.33	**0.226**
		Lower	6.14±5.49	10.16±7.95	8.82±6.56	6.14±3.73	**0.369**
		Total mean	6.23±4.61	12.78±9.95	8.61±6.83	8.67±7.54	
Peri-implantitis	**CD3 (%)**	**Upper**	7.14±2.84	9.24±6.09	9.49±6.84	5.99±3.65	**0.516**
		Lower	8.20±3.78	9.34±5.89	7.69±6.28	6.73±4.34	**0.824**
		Total mean	7.67±3.39	9.29±6.00	8.59±6.63	6.34±4.01	
	CD20 (%)	**Upper**	9.21±4.67	8.28±4.58	8.76±9.07	9.93±4.67	**0.388**
		Lower	8.02±4.14	7.48±4.50	8.06±4.15	7.17±5.32	**0.887**
		Total mean	8.48±4.39	7.85±4.56	8.43±7.19	8.55±5.19	
	IgG (%)	**Upper**	8.41±4.99	9.75±6.29	11.72±10.99	13.10±9.90	**0.898**
		Lower	4.97±3.25	7.93±8.23	8.45±5.26	9.57±8.80	**0.467**
		Total mean	6.59±4.50	8.84±7.38	10.00±8.63	11.33±9.53	

** The numbers represent mean precentage of cell marker intensity distribution of upper ROI, lower ROI and mean score of the two ROIs combined (Total mean). They are summated scores of high positive and positive values from IHC profiler, Image J.

p-value – IHC cell marker distribution compared (Kruskal–Wallis test) between the groups at top and bottom ROI.

FIGURES



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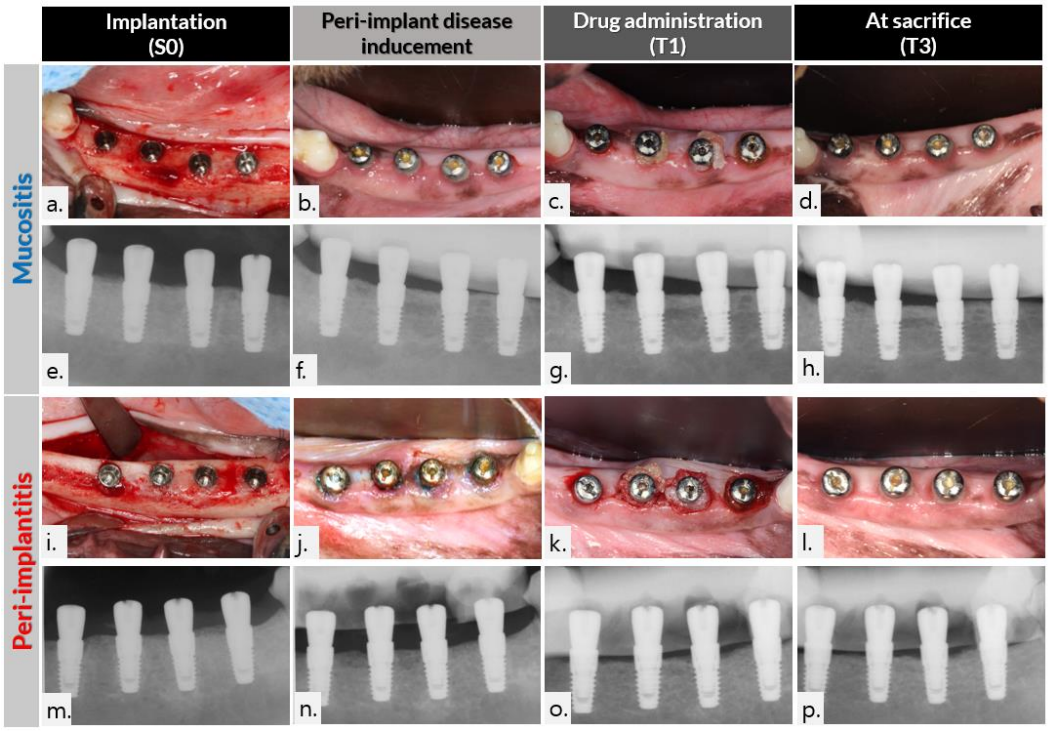

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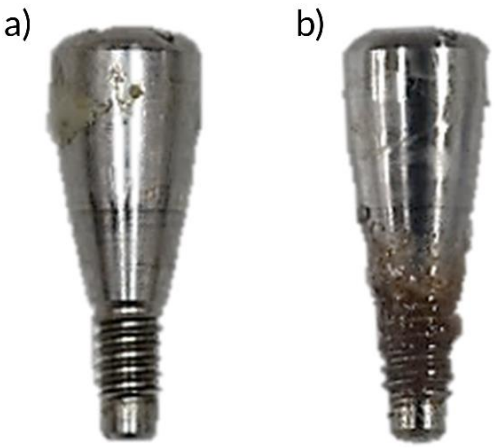

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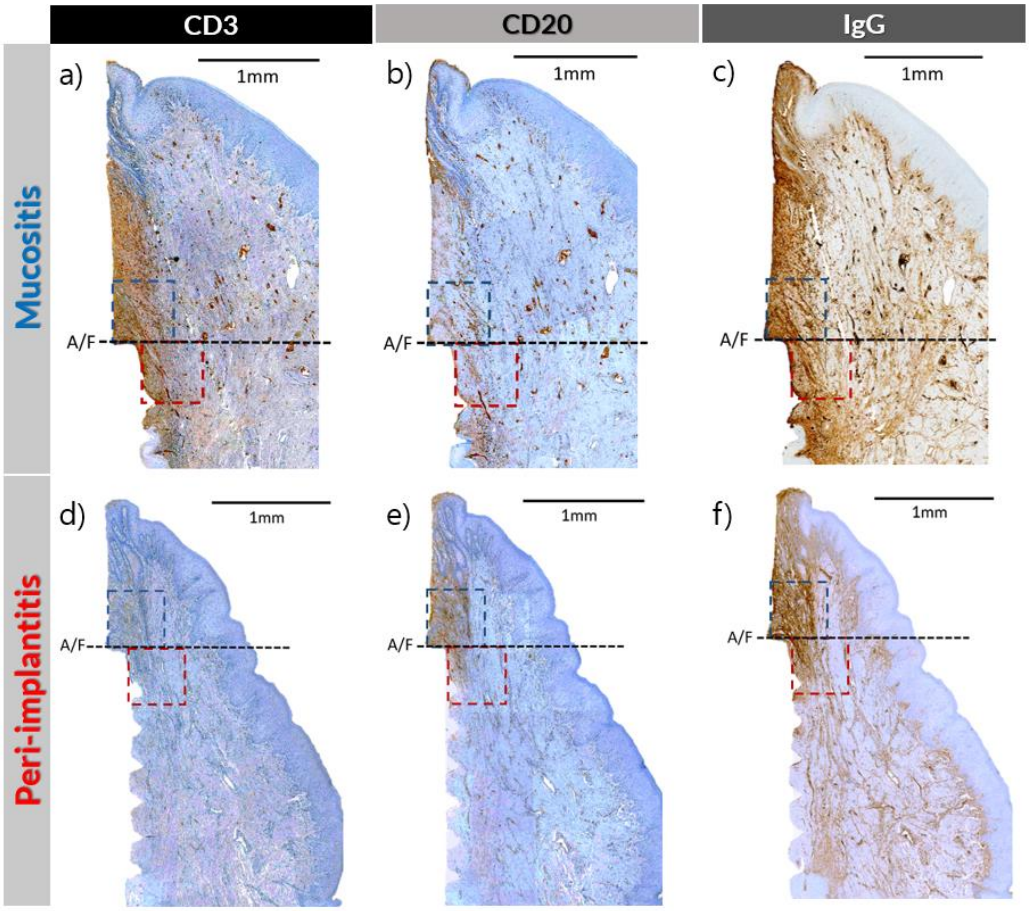

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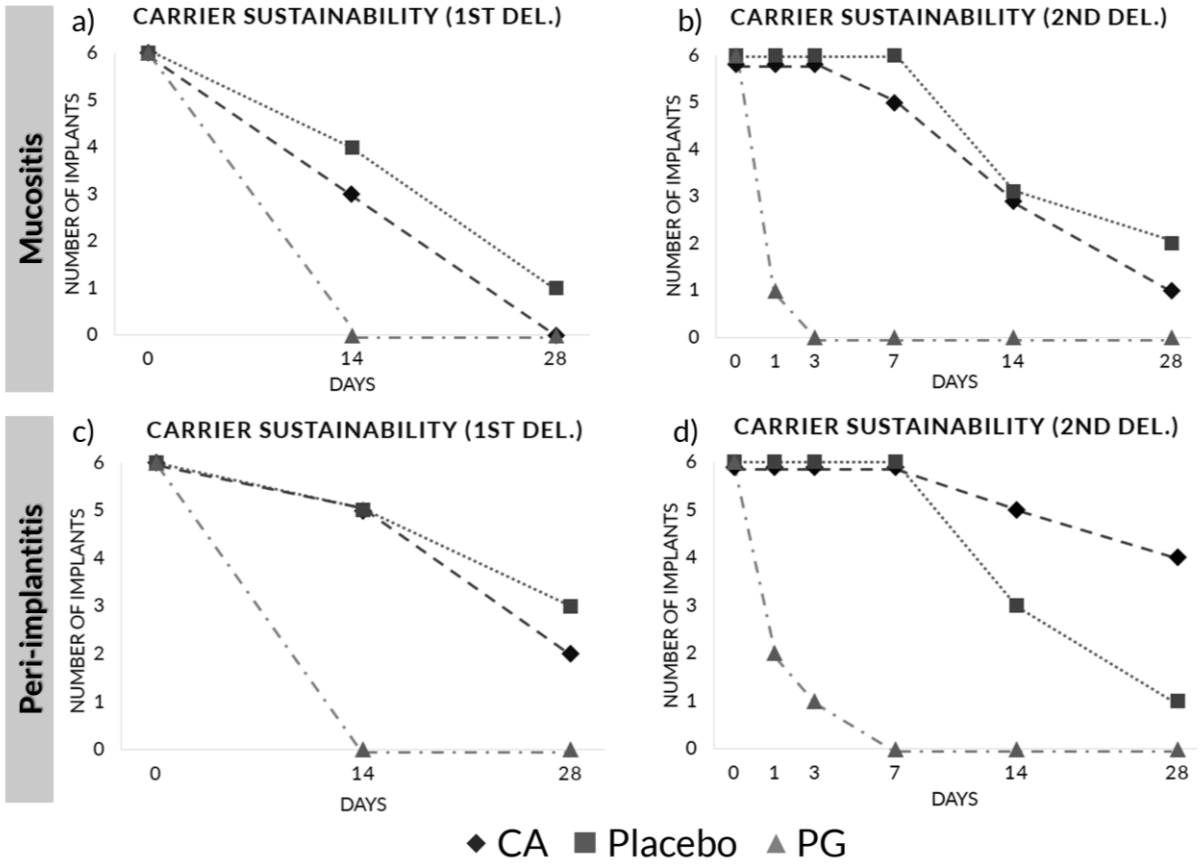

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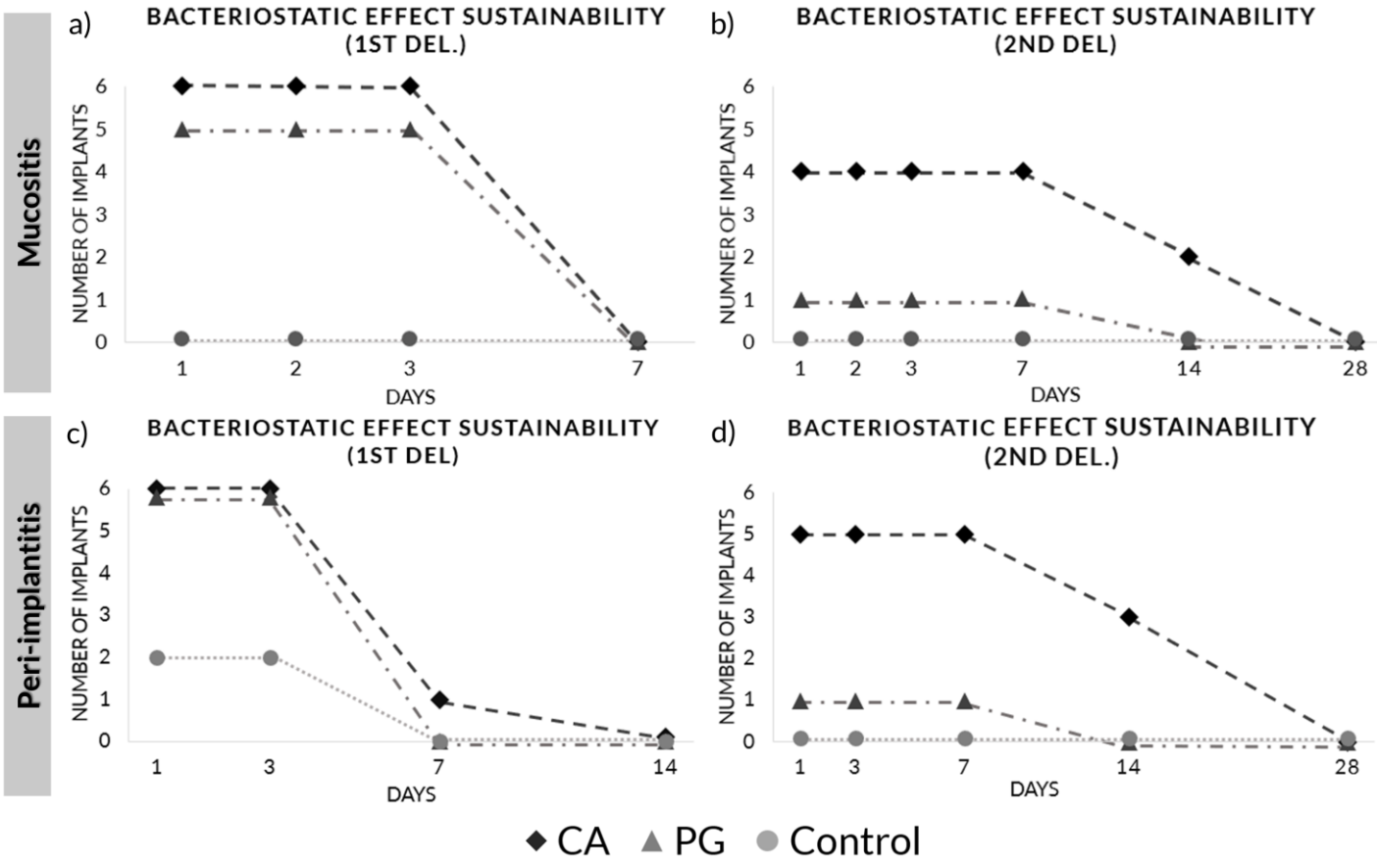

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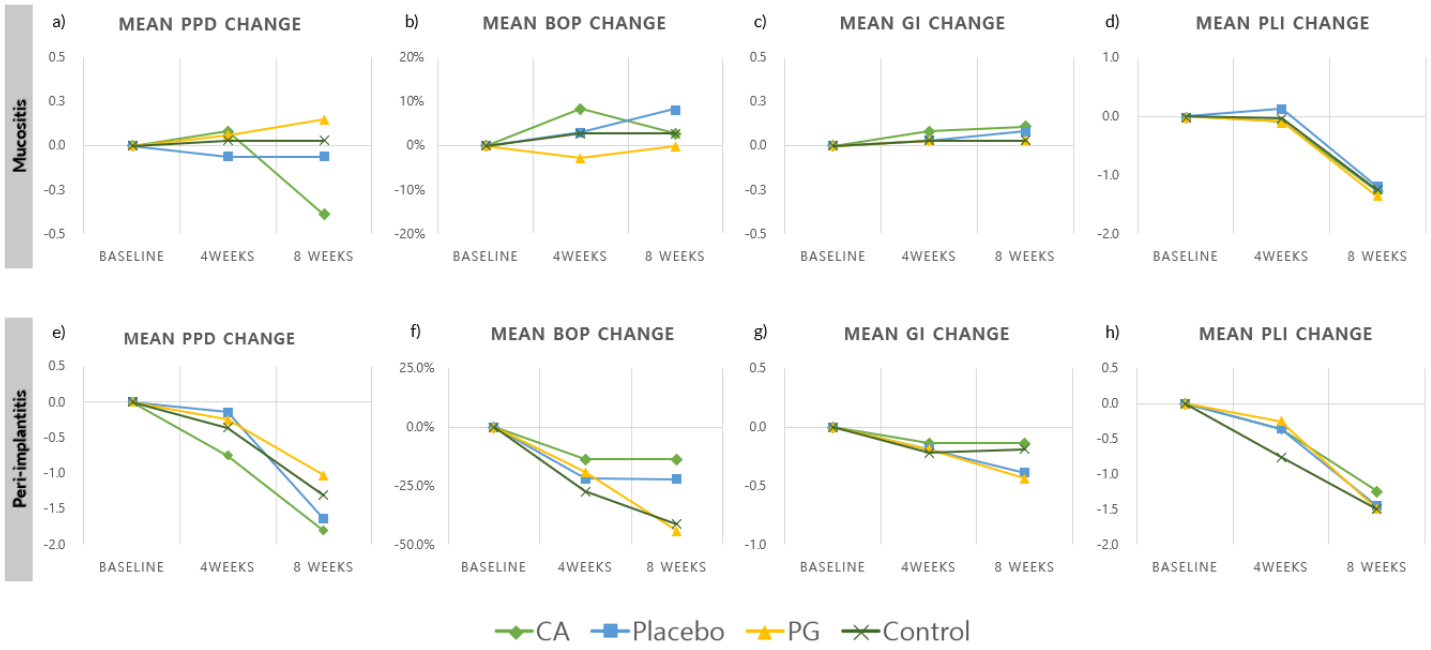

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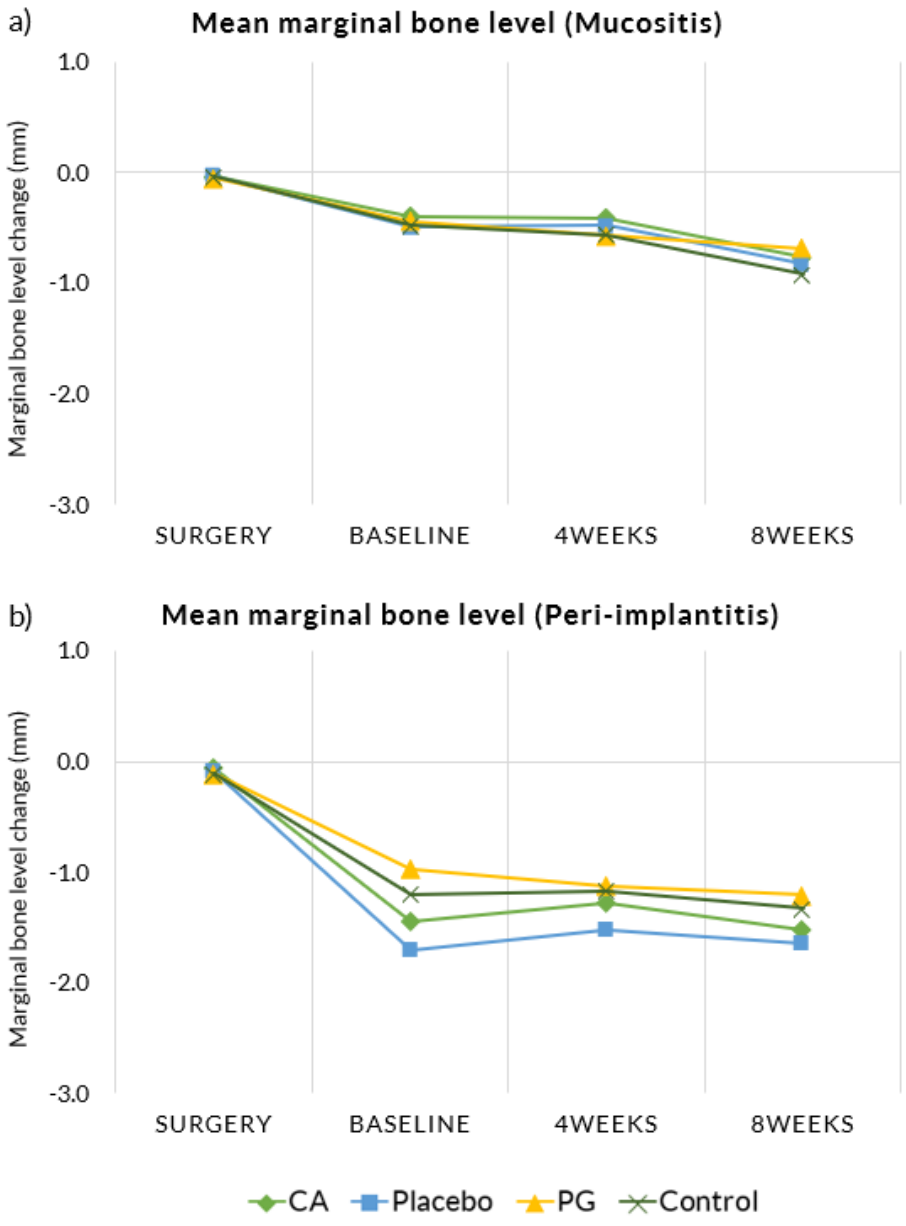

Figure 8

국문요약

임플란트 주위 점막염 및 임플란트 주위염 치료를 위한 국소 미노사이클린 송달제제의 약역학 및 약동학적 분석

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염증성 임플란트 주위 질환은 임플란트 주위 점막염(이하 점막염)과 임플란트 주위염으로 나뉜다. 임플란트를 식립한 환자들 중 점막염은 약 50%, 임플란트 주위염은 약 20%의 높은 발병률이 관찰된다. 임플란트의 식립이 지속적으로 증가함에 따라 임플란트 주위 질환에 대한 효과적인 치료방법이 필요하다. 여러 관련 병인 중 세균 감염이 염증성 임플란트 주위 질환의 주요 위험요인으로 보고되었으며 구강내 미생물장의 균형을 맞추기 위해선 임플란트 주위의 기계적인 제거술이 전제되어야 하지만 기계적인 제거술 만으로는 임플란트 및 주위 병소의 해부학적 요인들에 의해 깊이 침투된 세균을 제거하기가 부족하다. 따라서 치료의 효율성을 높이기 위해 부가적인 국소 항생제 송달제

제들이 연구되어 왔다. 그러나 지속적인 치은열구액의 지속적인 흐름과 타액의 정화 활동은 약물의 효능을 제한할 수 있으며, 이는 경구 사용을 위해 제안된 국소 항생제의 약동학 및 약역학적 특성을 평가하는 연구가 필요하다. 본 연구의 목적은 비글 견에서 실험적으로 유도된 점막염과 임플란트 주위염에서 두 종류의 미소구체[Chitosan-coated alginate (CA) microsphere; Poly(meth)acrylate-glycerin (PG) microsphere]를 사용하여 제작된 국소 미노사이클린 송달제제의 약동학 및 약역학적 측면을 평가하는 것이다.

총 6 마리의 비글 견의 무치악 하악에 4개의 임플란트를 양측으로 식립했다. 각 임플란트는 다음 4 가지 치료 중 하나를 받도록 무작위로 배정되었다: (i) CA 군(미노사이클린을 포함한 CA 미소구체 국소 송달제제), (ii) 위약군(미노사이클린을 포함하지 않은 CA 미소구체 국소 송달제제), (iii) PG 군(미노사이클린을 포함한 PG 미소구체 국소 송달제제), (iv) 대조군(기계적 제거술). 하악 양쪽에서 점막염과 임플란트 주위염을 유도한 후, 플라스틱 팁 주사기를 사용하여 4 주 간격으로 2 회에 걸쳐 치주낭에 무작위로 배정된 치료제를 투여함. 각 투여 후 약동학(운반체 및 정균 효과 지속성) 및 약역학(임상, 방사선 사진 및 조직학적 분석) 평가를 시행하였다.

연구 결과 실험적으로 유도된 점막염과 임플란트 주위염 환경 모두에서 CA 군 미소구체가 PG 군 미소구체에 비해 임시치유 지대주 주변에 더 오래 남아 있었다. 두 번째 투여 후 점막염과 임플란트 주위염 그룹 모두에서 정균 효과

가 CA 군에서 [7.0 ± 5.7 일(점막염); 9.3 ± 5.7 일(임플란트 주위염)] PG 군에 비해 [1.2 ± 2.6 일(점막염); 1.2 ± 2.9 일(임플란트 주위염)] 더 길게 관찰되었다($p < 0.05$). 점막염과 임플란트 주위염에서 모든 치료군의 효능은 임상, 방사선 사진 및 조직학적 분석에서 모두 비슷하게 관찰되었다.

결과적으로 CA 군 미소구체는 PG 군 미소구체에 비해 더 긴 운반체 및 정균 효과 지속성이 관찰되었지만, 더 긴 약물 지속성은 개선된 치료 결과로 이어지진 않았다.

핵심되는 말: 약역학, 약동학, 키토산-알지네이트 미소구체, 임플란트 주위 점막염, 임플란트 주위염, 국소 미노사이클린 송달제제