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Evaluation of the Clinical Efficacy of Copine 7-Derived Peptides for Naturally Occurring Periodontitis in Dogs

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ABSTRACT

Aim: To assess the efficacy of copine7-derived peptide (CPNE7-DP) applied in conjunction with non-surgical dental treatments in improving naturally occurring periodontitis in dogs.

Materials and Methods: This study included 24 client-owned dogs with mild to moderate periodontitis, randomly assigned to three groups: CPNE7-DP; a placebo control group receiving carrier material; and a negative control group left untreated. Experimental agents were applied after professional dental cleaning. Clinical assessments were conducted at Weeks 0, 4 and 8, which involved measurements of gingival index (GI), periodontal pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BoP). Alveolar bone loss (ABL) was evaluated using intraoral radiographs.

Results: Application of CPNE7-DP resulted in a significant improvement in GI, PPD, CAL and BoP compared with both placebo and negative control groups. ABL was significantly reduced in the CPNE7-DP group. These clinical outcomes indicated the potential regenerative effects of CPNE7-DP on periodontal tissues.

Conclusions: Topical application of CPNE7-DP in conjunction with non-surgical dental treatments was effective in reducing gingival inflammation, PPD, CAL and ABL in dogs with naturally occurring periodontitis. These findings suggest the potential of CPNE7-DP as an effective adjunctive therapeutic agent for periodontal treatment.

Trial Registration: Approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-220520-5-1)

1 | Introduction

Periodontitis, increasingly prevalent with age, is a common oral disease in dogs and humans and has been well studied as a model for human periodontitis (Albuquerque et al. 2012; Genco and Borgnakke 2013; Lund et al. 1999; Marshall et al. 2014). The disease begins with plaque formation, progressing to tissue destruction due to chronic inflammation triggered by host immune responses (Harvey 2005; Listgarten 1986). Extensive research on periodontitis and regenerative therapies has established a comprehensive understanding of canine disease pathogenesis and highlighted the canine model's value for evaluating advanced regenerative treatments (Kantarci et al. 2015).

Periodic mechanical removal of supra- and sub-gingival calculi and plaques is used to slow the disease progression and is the most basic periodontal treatment. Traditionally, various periodontal treatments such as perioceutics, pocket

reduction or open flap debridement have been applied depending on the severity of periodontal disease, with the expectation of some degree of periodontal tissue repair (Lobprise and Stepaniuk 2019; Stepaniuk 2019). Perioceutics refers to the use of pharmacological agents, such as antimicrobial and host modulatory therapies, specifically developed to manage periodontitis (Honibald et al. 2012). These treatments aim to modulate the excessive host inflammatory response and improve the oral microbiota and are a valuable adjunct to mechanical debridement in periodontal disease (Gulati et al. 2014). Subantimicrobial doses of doxycycline are commonly used systemically in both humans and dogs as part of these perioceutics, with local treatment formulations available for direct application to the periodontal pocket (Caton et al. 2001; Cleland Jr. 2000; Drisko 1998; Kim et al. 2016; Preshaw et al. 2004). Studies on treatment efficacy have focused on preventing further destruction of periodontal tissue through the modulation of inflammation and the consequent improvement in clinical parameters.

Ideally, the goal of periodontal treatment is to regenerate periodontal tissues such as the periodontal ligament (PDL), cementum, alveolar bone and gingiva that have been destroyed as a result of inflammation and to improve the adhesion of the regenerated PDL fibres to the cementum to restore the periodontal tissue to the original functional state (Wang et al. 2005). While the regeneration and attachment of periodontal tissue are required along with repair, the gingiva and alveolar bone do not provide the cells necessary to attach the PDL and cementum (Ramseier et al. 2012). Therefore, various tissue engineering approaches are being developed to restore and regenerate the periodontium to its original functional state (Ward 2022).

Copine is a Ca^2-dependent phospholipid binding protein, and copine 7 (CPNE7), a member of the copine family, has been identified in pre-ameloblast-conditioned medium (PA-CM) and plays an important role as a signalling molecule in odontoblast differentiation and dentin formation during tooth development (Choung et al. 2016; Creutz et al. 1998; Oh et al. 2015). CPNE7 consists of 633 amino acids and induces PDL regeneration by regulating the rearrangement and adhesion of PDL cells (Choung et al. 2019). The CPNE7-derived peptide (CPNE7-DP) is a water-soluble, cell-penetrating peptide consisting of 10 amino acids with a function similar to that of the recombinant CPNE7 protein (Lee et al. 2020). In vitro, CPNE7-DP applied to human PDL cells (hPDLCs) has been found to enhance adhesion activity by directly regulating the cementum attachment protein (CAP). In an in vivo study, CPNE7-DP application to a three-wall-defect canine model was shown to significantly improve PDL attachment histologically (Bai et al. 2022). However, this canine periodontal defect model consisted of 4-year-old beagles and was applied to an artificially created three-wall defect, rather than naturally occurring periodontitis. Therefore, it is necessary to confirm whether similar results could be obtained when applied to clinically occurring chronic periodontal disease.

This study aimed to determine the efficacy of CPNE7-DP in improving naturally occurring periodontitis in dogs. As surgical periodontal treatment might obscure the effectiveness of this agent owing to the benefits of invasive surgery itself, a clinical trial was designed to determine the effects of CPNE7-DP on periodontitis when used in conjunction with non-surgical dental treatments.

2 | Materials and Methods

2.1 | Chemical Preparation

CPNE7-DP was manufactured by Anygen (Lot No. KH001-21-001, Gwangju, Republic of Korea). Propylene glycol alginate (PGA; Protanal ester SD 8440) was purchased from ALGINATOS (Lot No. 1G57235, Chile S.A). A 6% PGA solution was dissolved in sterilised water. The completely dissolved 6% PGA was pasteurised at 60°C for 30 min. The peptide solution (2 mg/mL) was prepared by dissolving CPNE7-DP in water and then sterilised using a 0.2-μm syringe filter. The gel-type peptide formulation was prepared by mixing 2 mg/mL peptide solution and 6% PGA solutions in 1:1 ratio. The final gel-type formulation was made to 3% PGA with 1 mg/mL peptide and dispensed into a sterilised syringe. The test materials were refrigerated at 2°C–8°C and used as provided by the manufacturer, with a 27-gauge cannula secured to the syringe outlet just before injection.

2.2 | Recruitment of Canine Patients

This was a prospective study of client-owned dogs that visited the Seoul National University Veterinary Medical Teaching Hospital between August 2022 and August 2023. The protocol for this clinical study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-220520-5-1). The recruited patients were dogs weighing less than 15 kg, were at least 1 year old, lived at home with their owners, in whom it was possible to apply a toothbrush and who had mild to moderate periodontitis with clinical attachment loss (CAL). Only dogs that allowed reliable buccal probing while conscious and whose owners provided written informed consent for the regimen, including daily tooth brushing, were included. Animals that were administered antibiotics, anti-inflammatory drugs or immunosuppressants, or those with hormonal or immune system disorders, were excluded from the study. Breed was not a selection criterion; dogs were enrolled consecutively based on periodontal disease suitability and the inclusion criteria. Group allocation was not disclosed to the investigator responsible for performing clinical procedures and assessments.

2.3 | Clinical Experimental Protocol

All examinations were conducted by an experienced veterinary dental clinician (S.E.K.) in a double-blinded fashion, using standardised methods to minimise intra-examiner variability. Twenty-four dogs were included and randomly assigned to three groups. Before anaesthesia, complete blood cell count and serum chemistry were performed for each dog to screen for general health. For evaluation of periodontal status and dental professional cleaning at Week 0, general anaesthesia was induced using alfaxalone (Alfaxan multidose, Jurox) and maintained with isoflurane (Ifran solution, Hana Pharmaceuticals) inhalation at 1.3%–1.7% of Et Iso. The clinical periodontal parameters of all teeth were recorded, including gingival index (GI),

periodontal pocket depth (PPD), CAL and bleeding on probing (BoP). Periodontal parameters were measured at three points per tooth (mesial, buccal and distal) on the buccal side. Mobile teeth were excluded from the experimental group. Dental radiography was performed using a computed radiography system (CR7 Vet, IM3), and radiographic alveolar bone loss (RABL) was determined by calculating the ratio of the distance from the cemento-enamel junction to the alveolar bone margin to the distance from the cemento-enamel junction to the apex, using a software (ImageJ, National Institutes of Health). Teeth classified as having mild or moderate periodontitis between the second premolars and first molars of the maxilla and mandible were selected based on periodontitis staging criteria of the American Animal Hospital Association Dental Care Guidelines for Dogs and Cats (Bellows et al. 2019). A G*Power analysis was conducted to determine the sample size required for ANOVA, and 20 teeth were included in each group. All dogs underwent scaling and polishing of all teeth using an ultrasonic scaler, followed by closed root planing and subgingival curettage with a Colombia universal curette (CUC13–14, Osung). After cleaning periodontal pockets and achieving complete haemostasis, the pockets were thoroughly dried using a three-way syringe. Subsequently, in the CPNE7-DP group, periodontal pockets were injected with a mixture of CPNE7-DP and a carrier material; the placebo control (PC) group received an injection of the carrier material only. The negative control (NC) group was treated identically in terms of scaling, polishing and root planing, but no material was injected into the periodontal pockets. Table 1 presents the injected materials and ingredients according to groups. Materials were injected until the pockets were filled and leaked slightly (0.05–0.1 mL per site); the substance that leaked out of the periodontal pocket was wiped using a dry gauze after 1 min. After the non-surgical procedure was completed and the dogs recovered from anaesthesia, the owners were instructed to brush their dogs' teeth at least once daily using the provided toothbrush (CS Smart 7600, Curaprox) throughout the study period. Oral hygiene compliance was assessed through biweekly telephone check-ins and reconfirmed at each follow-up visit. All the owners were instructed to feed a dry pellet diet to the dogs included in this study during the experimental period.

During follow-up at Weeks 4 and 8, the general health and periodontal status of the dogs were examined. Dental examinations, including measurements of GI, PPD, CAL and BoP, were performed under conscious conditions at Week 4 and under general anaesthesia at Week 8 to additionally allow intraoral radiographic imaging for further RABL assessment.

2.4 | Statistical Analysis

All experimental data were statistically analysed using a software program (SPSS statistics version 26, IBM). In the same group, the clinical periodontal parameters and RABL before and after the administration of the test material were analysed using one-way analysis of variance (ANOVA) for each group. The dental parameters and RABL between the experimental groups were analysed using one-way ANOVA. The Tukey method was used for post hoc analysis. The significance level was set at $p = 0.05$.

3 | Results

3.1 | General Conditions and Local Reactions

The recruited animals included 9 spayed females, 13 castrated males, and 1 intact female and male each. The breeds included were Miniature Poodle ($n = 7$), Beagle ($n = 4$), Bichon frisé ($n = 3$), Pomeranian ($n = 3$), Coton de Tulear ($n = 2$), Miniature Schnauzer ($n = 1$), Dachshund ($n = 1$), Yorkshire terrier ($n = 1$), Maltese ($n = 1$) and mixed breeds ($n = 1$). Table 1 depicts the breed distribution and mean ± standard deviation of age and body weight for each group. No significant differences in age or weight were observed between the groups ($p > 0.05$).

The general physical examination of all dogs was normal during the experimental periods. No local or systemic adverse events, such as haemorrhage, inflammation, oedema, anorexia, vomiting or diarrhoea, were observed following treatment. Pain on palpation or percussion was assessed while the dogs were

TABLE 1 | Group allocation, breed distribution, age and body weight for each group.

	CPNE7-DP	PC	NC
Injected agent	$C_{62}H_{105}N_{21}O_{15}$ acetate in 3% PGA	3% PGA	None
Number of animals	8	8	8
Number of teeth	20	20	20
Age	8.25 ± 3.11	9.25 ± 2.25	9.63 ± 2.33
Body weight	6.59 ± 4.36	6.53 ± 2.33	5.15 ± 1.34
Breed distribution	Poodle ($n = 4$) Beagle ($n = 3$) Bichon frisé ($n = 1$)	Pomeranian ($n = 3$) Poodle ($n = 1$) Schnauzer ($n = 1$) Dachshunds ($n = 1$) Beagle ($n = 1$) Bichon frisé ($n = 1$)	Coton de Tulear ($n = 2$) Poodle ($n = 2$) Bichon frisé ($n = 1$) Maltese ($n = 1$) Yorkshire terrier ($n = 1$) Mixed breed ($n = 1$)

Note: Age and body weight data are presented as mean ± standard deviation.
Abbreviations: CPNE7-DP, copine7-derived peptides; NC, negative control; PC, placebo control (3% propylene glycol alginate).

conscious—during the visit at Week 4 and before anaesthesia at Week 8—and no dog showed any pain responses.

3.2 | Clinical Periodontal Parameters

Table 2 presents the mean ± standard error values of periodontal parameters for each group at Weeks 0, 4 and 8 following injection of the test material. All groups showed a reduction in GI at Weeks 4 and 8 compared with baseline ($p < 0.05$). Compared to that at Week 4, GI at Week 8 was slightly increased on average in all groups; this difference was significant in the CPNE7-DP group ($p < 0.05$). Significant improvements in PPD and CAL were observed exclusively in the CPNE7-DP group at Weeks 4 and 8 ($p < 0.05$), indicating periodontal pocket reduction and clinical attachment gain. No significant changes were found in the PC or NC groups during the same periods. The incidence of BoP was significantly decreased in the CPNE7-DP and PC groups at Weeks 4 and 8 compared with that at Week 0 ($p < 0.05$), but there was no significant difference between Weeks 4 and 8. No significant differences in the BoP were observed in the NC group during the experimental periods.

Figure 1 illustrates the between-group comparisons of each clinical dental parameter at each time point. Clinical parameters in all groups were not significantly different at Week 0. The GI and BoP of the CPNE7-DP and PC groups revealed significant difference at Weeks 4 and 8 compared with the NC group ($p < 0.01$). PPD of all groups at Week 4 was not significantly different but showed a significant difference only between the CPNE7-DP and NC groups at Week 8 ($p < 0.05$). CAL in the CPNE7-DP group was significantly lower than in the PC and NC groups at both Weeks 4 and 8 ($p < 0.001$), demonstrating clinical attachment gain compared to the controls.

3.3 | RABL

Figure 2 depicts the representative clinical images and dental radiographs of all experimental groups at Weeks 0 and 8. At Week 0, the RABL of all experimental groups was not significantly different. At Week 8, only the CPNE7-DP group showed a significant decrease in RABL ($p < 0.05$) (Table 3), indicating alveolar bone gain. No significant changes in RABL were observed in the PC or NC groups. Between-group comparison at week 8 revealed significantly lower RABL in the CPNE7-DP group compared to both control groups ($p < 0.01$; Figure 3), suggesting that significant alveolar bone gain was limited to the CPNE7-DP group.

4 | Discussion

In this study, we found CPNE7-DP application with non-surgical therapy improved the periodontal parameters in dogs. A notable improvement in GI was observed across all groups, likely attributable to scaling, root planing and curettage performed prior to drug application. Nevertheless, all groups showed a subsequent increase in GI at Week 8, likely due to long-term plaque deposition. The significant increase in the CPNE7-DP group was probably influenced by population-specific environmental differences such as brushing. However, this result was unlikely to reflect the efficacy of CPNE7-DP, as no significant differences were found when compared with the NC group.

The normal PPD in medium-sized dogs is typically no more than 2–3 mm, while in small dogs a PPD of ≤2 mm is considered normal (Bellows et al. 2019). At Week 0, the mean PPD of all groups was ≥3 mm, indicating periodontal disease. By Weeks 4 and 8, the mean PPD in all groups decreased to <3 mm; however, a significant decrease was observed only in the CPNE7-DP group, reaching

TABLE 2 | Values of periodontal parameters for each group.

Groups	Parameters	Week 0	Week 4	Week 8
CPNE7-DP	GI	2.55 ± 0.14	1.10 ± 0.12*	1.55 ± 0.11*,†
	PPD	3.20 ± 0.21	1.93 ± 0.21*	1.83 ± 0.20*
	CAL	2.65 ± 0.24	1.18 ± 0.24*	1.13 ± 0.24*
	BoP	1.00 ± 0.00	0.30 ± 0.11*	0.20 ± 0.09*
PC	GI	2.35 ± 0.17	1.10 ± 0.16*	1.30 ± 0.18*
	PPD	3.00 ± 0.27	2.30 ± 0.24	2.20 ± 0.22
	CAL	2.88 ± 0.33	2.48 ± 0.31	2.53 ± 0.33
	BoP	1.00 ± 0.00	0.55 ± 0.11*	0.45 ± 0.11*
NC	GI	2.55 ± 0.11	1.75 ± 0.12*	2.00 ± 0.00*
	PPD	3.10 ± 0.18	2.55 ± 0.20	2.55 ± 0.20
	CAL	3.00 ± 0.21	2.70 ± 0.21	2.70 ± 0.21
	BoP	0.90 ± 0.07	0.95 ± 0.05	0.95 ± 0.05

Note: Data are presented as mean ± standard error.
Abbreviations: BoP, bleeding on probing; CAL, clinical attachment loss; CPNE7-DP, copine7-derived peptides; GI, gingival index; NC, negative control; PC, placebo control (3% propylene glycol alginate); PPD, periodontal pocket depth.
*Significantly different compared to baseline (Week 0, $p < 0.05$).
†Significantly different compared to Week 4 ($p < 0.05$).

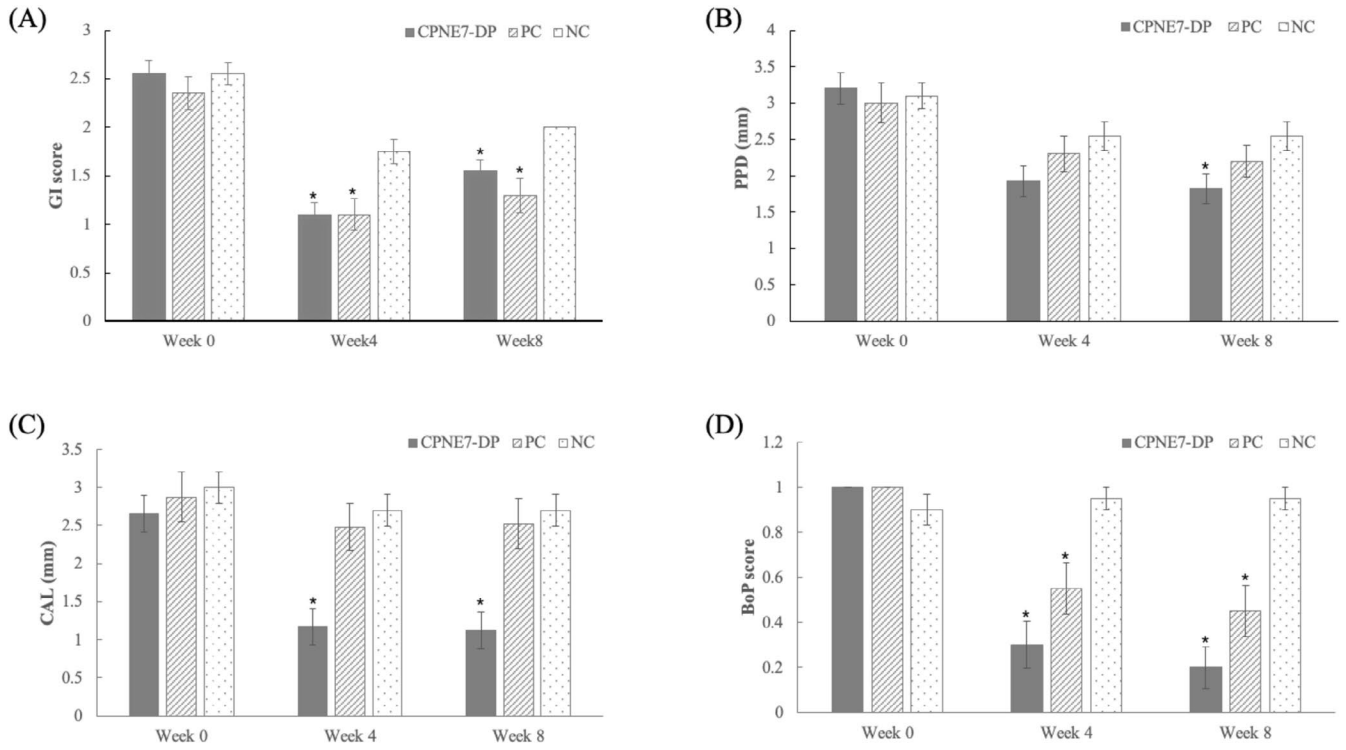

FIGURE 1 | Comparisons of clinical parameters in the same week. Inter-group comparisons were conducted using data from Table 2. (A) Gingival index, (B) Periodontal pocket depth, (C) Clinical attachment loss, (D) Bleeding on probing. One-way ANOVA with Tukey's post hoc test; $n = 20$ teeth per group. *Significant difference compared to the NC group at the same week; $p < 0.05$.

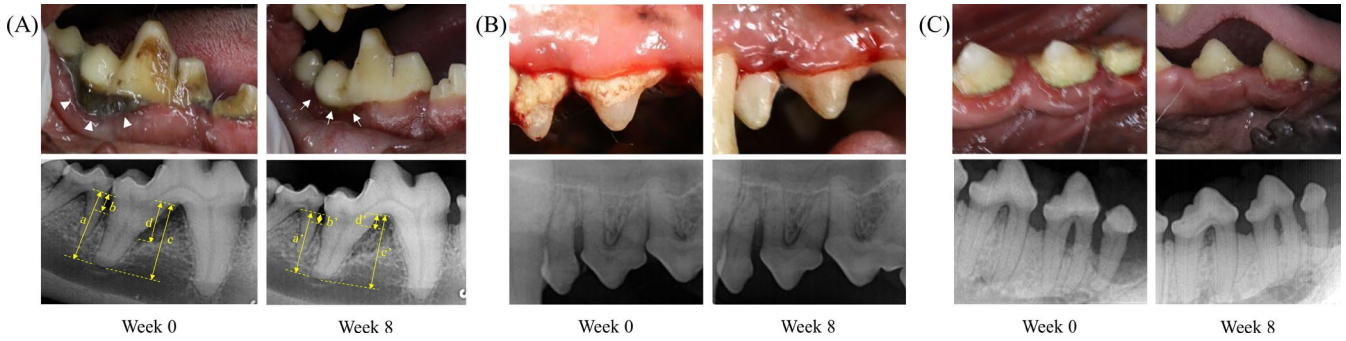

FIGURE 2 | Representative clinical images and dental radiographs at Weeks 0 and 8. (A) CPNE7-DP group. The clinical image shows marked root exposure due to gingival recession at Week 0 (white arrowheads), with soft tissue recovery at Week 8 (white arrows). Intraoral radiographs show alveolar bone gain at Week 8 compared to Week 0. Radiographic alveolar bone loss was calculated as the ratio of the distance from the cemento-enamel junction (CEJ) to the alveolar bone margin (b and d, b′ and d′) to the distance from the CEJ to the apex (a and c, a′ and c′), respectively. When mesial and distal values differed, their mean was used. (B) Placebo control group and (C) negative control group. No notable changes in alveolar bone level were observed between Weeks 0 and 8.

TABLE 3 | Values of radiographic alveolar bone loss (%).

Groups	Week 0	Week 8
CPNE7-DP	26.94 ± 2.74	16.92 ± 2.65*
PC	32.63 ± 3.80	31.29 ± 3.73
NC	32.29 ± 2.71	31.49 ± 2.92

Note: Data are presented as mean ± standard error.
Abbreviations: CPNE7-DP, copine7-derived peptides; NC, negative control; PC, placebo control (3% propylene glycol alginate).
*Significantly different compared to baseline (Week 0, $p < 0.05$).

the normal PPD range for small dogs (≤ 2 mm). The insignificant and modest PPD reduction seen in the PC and NC groups was likely due to gingival recession following calculus removal.

CAL is an indicator of the attachment level of the periodontal tissue, and a decrease in CAL signifies the regeneration of periodontal tissue following the actual removal of calculus and plaque, which causes the disease. Here, only the CPNE7-DP group showed a trend of significant CAL reduction post administration compared with pre-administration levels. Additionally, there was no significant difference in CAL before administration

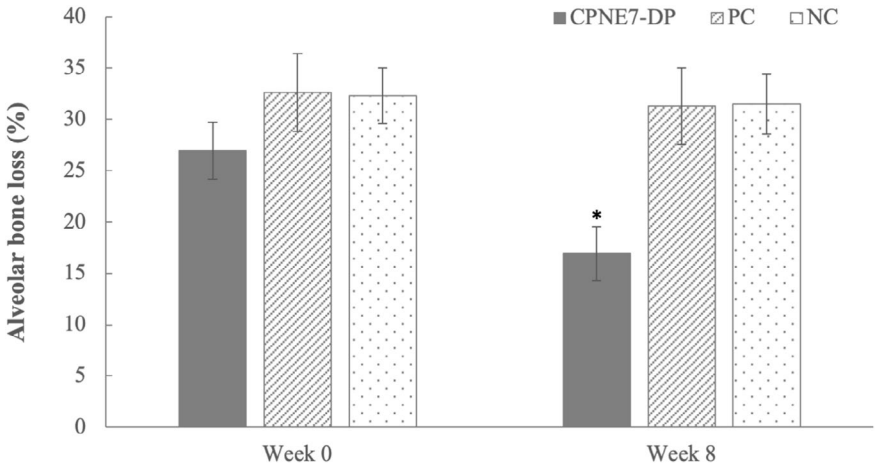

FIGURE 3 | Comparisons of radiographic alveolar bone loss (%) in the same week. Inter-group comparisons were conducted using data from Table 3. One-way ANOVA with Tukey's post hoc test; $n = 20$ teeth per group. *Significant difference compared to the NC group at the same week; $p < 0.05$.

in any groups, but the CPNE7-DP group showed a significant improvement in CAL levels compared with the other groups after Week 4. These findings highlight the potential of CPNE7-DP to enhance periodontal attachment through mechanisms promoting PDL regeneration.

BoP is used alongside GI as an indicator of the degree of inflammation in the gingiva. At Week 0, most teeth (all teeth in CPNE7-DP and PC groups) showed BoP. After injection of the experimental substance according to the assigned groups, a significant decrease in BoP and GI was observed in the CPNE7-DP and PC groups, while the NC group showed no significant change throughout the experimental period. PGAs employed as physical carriers for CPNE7-DP application in the periodontal pocket have shown antibacterial effects (Sculean et al. 2001). Hence, here the 3% PGA treatment group (PC group) was included as a control, considering the potential for improvement in the effect of PGA. The between-group comparison analysis also indicated the effectiveness of CPNE7-DP and PGA in improving GI and BoP, possibly because of the effects of 3% PGA used as the base for the CPNE7-DP and PC groups rather than the effects of the mixed experimental drugs.

PDL is a connective tissue located between the cementum and alveolar bone, which not only attaches the tooth to the alveolar bone but also acts as a cushion against mechanical forces. Orientation and good alignment of the fibres are important for the functioning of the PDL. PDL cells are polarised and their long axes are aligned parallel to the main bundle of the PDL (Cho and Garant 2000). PDL contains stem cells necessary for regeneration; however, a new normal cementum must be created for the PDLs to attach to the root surface (Chen and Shi 2014; Narayanan and Bartold 1996). Currently, tissue engineering methods for periodontal regeneration include the application of scaffolds, signalling molecules, stem cells and gene therapy. Periodontal tissue regeneration occurs to some extent when these treatments are applied alone or in combination to experimental dogs with periodontal disease, but the outcome is still unpredictable (Ward 2022). Guided tissue regeneration has been the gold standard method for periodontal regeneration; however, it is a technique-sensitive procedure and has limitations in its application areas, making treatment results unpredictable (Tonetti et al. 1998; Villar and Cochran 2010). Enamel matrix derivative (EMD) is a widely studied and commercially available bioactive material, but its regenerative efficacy in the periodontal apparatus has been still controversial (Sculean et al. 2005). Here we evaluated ABL using dental radiography, providing a two-dimensional assessment of the three-dimensional bone structure. The CPNE7-DP group exhibited a significant reduction in RABL compared with pre-treatment levels, underscoring its potential role in alveolar bone regeneration. Owing to inherent limitations of radiographic analysis, ABL measurements here were based solely on height changes, without considering the number of bony walls in defect sites. Additionally, radiographic evaluation was not performed at Week 4 because intraoral radiography for assessing alveolar bone levels in animals requires general anaesthesia. Moreover, as confirmed during probing, most ABL sites here consisted of one- or two-wall defects, which are less responsive to traditional guided-tissue regeneration. Despite these limitations, RABL was significantly reduced in the group in which CPNE7-DP was applied to these pathological sites, highlighting its potential as a therapeutic agent for treating complex periodontal defects.

Previous studies have suggested that the microtubule-associated protein tau (TAU), which regulates axon elongation, may play a role in the alignment and rearrangement of PDLCs by cytoskeletal reorganisation (Miyazaki et al. 2015). In vitro, TAU was strongly expressed at the leading edge of hPDLCs treated with CPNE7, leading to enhanced alignment. In a canine model with experimentally induced periodontal defects, application of CPNE7-DP, which exerts similar effects to recombinant CPNE7, resulted in a four-fold increase in perpendicularly aligned PDL fibres at the attachment site and the formation of a cementum-like layer on the exposed dentin (Bai et al. 2022). Collectively, these findings suggest that CPNE7-DP may contribute to functional periodontal regeneration by promoting cytoskeletal reorganisation of PDL cells and cementum formation.

Most periodontitis experiments in animals have been performed using artificially induced periodontitis in laboratory animals. Periodontitis and peri-implantitis experiments in medium-sized

animals are widely recognised as good models for evaluating treatment efficacy and safety (Kantarci et al. 2015). Experiments using diseases induced in laboratory animals have the advantage of a controlled environment; however, in the case of chronic diseases, the onset age or disease mechanism may not be exactly the same as those in laboratory animals. Additionally, because research requires a large number of animals and causes animal suffering, there has been a global trend to reduce the use of laboratory animals, such as the 3R (Reduction, Refinement, Replacement) programme. Among alternative methodologies, the use of natural animal disease models, which respond in a similar way from a pathological and therapeutic point of view, is recommended as a promising tool (Capela et al. 2022). Here we conducted experiments to assess the degree of clinical improvement in periodontitis using a naturally induced canine periodontitis model, which revealed significant differences in treatment effectiveness. The naturally occurring canine periodontitis model used in this study may serve as a promising tool for future periodontal disease research, with potential for evaluating the significance of treatment effects in humans with comparable pathological and recovery mechanisms.

No dogs in this study had systemic diseases that could affect tissue healing, and they were fed a diet of similar composition, with no significant differences in weight or age between the groups. However, this study had some limitations. The experimental teeth were randomised between the premolars and first molars, and the animal breed, age, sex and size were inconsistent, as dogs with naturally occurring periodontitis were recruited. Additionally, because all dogs were client-owned, invasive procedures such as histological analysis were not feasible. Furthermore, clinical periodontal measurements were limited to the buccal aspect, which may not fully represent the overall periodontal status, particularly in lingual sites. Despite efforts to maintain consistency in home care through owner education, variations in periodontal management may have influenced the outcomes. These limitations may be overcome by applying the CPNE7-DP to more animals and comparing results of retrospective studies. The other limitation of this study is that all clinical and radiographic assessments were performed by a single blinded examiner. While this approach ensured procedural consistency, it may have introduced measurement bias due to the lack of inter-examiner validation. This limitation may be addressed in larger scale future studies involving multiple independent examiners.

In conclusion, this study demonstrated that topical CPNE7-DP application following closed root planing significantly improved multiple clinical periodontal indices, including GI, PPD, CAL, BoP and RABL, in canine patients with naturally occurring periodontitis. Thus, CPNE7-DP may serve as an effective adjunctive therapeutic agent for improving periodontal outcomes with naturally occurring periodontitis. However, further large-scale studies are necessary to verify its long-term efficacy and clinical applicability.

Author Contributions

Conceptualisation: Se Eun Kim, Dong-Seol Lee, Han Woong Kim and Joo-Cheol Park. Data curation: Se Eun Kim, Hyeonu Sung, Sehan Shin, Jinhee Bae and Giyeon Kim. Formal analysis: Se Eun Kim, Jin-Seok Seo, Song Yi Roh and Joo-Cheol Park. Investigation: Hyeonu Sung, Sehan Shin, Jinhee Bae and Giyeon Kim. Methodology: Se Eun Kim, Dong-Seol Lee, Han Woong Kim, Joo-Cheol Park and Chul Son. Project administration: Han Woong Kim, Jin-Seok Seo, Song Yi Roh, Hyeonu Sung, Sehan Shin, Jinhee Bae and Giyeon Kim. Software: Se Eun Kim. Supervision: Joo-Cheol Park. Visualization: Se Eun Kim. Writing – original draft: Se Eun Kim. Writing – review and editing: Jin-Seok Seo, Song Yi Roh, Su-Jin Park, C Son, Song Yi Roh, Jeongmin Park and Joo-Cheol Park.

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Ethics Statement

The protocol for this clinical study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-220520-5-1).

Conflicts of Interest

Dong-Seol Lee was employed by HysensBio Co. Ltd. Dong-Seol Lee is an inventor on the Korean patent grant 10-2500280 and the PCT patent application (PCT/KR2021/006812) filed by HysensBio Co. Ltd. These patents are based on this work.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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