Study on Oral Health Care Aiming to Maintain and Promote General Health through the Management of Periodontal Disease

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Abstract

Periodontal disease is an infectious disease caused by oral bacteria. As the infection progresses, periodontal tissues become chronically inflamed, and the periodontal tissues that support the teeth (gingiva, alveolar bone) are destroyed, resulting in eventual tooth loss. New therapeutic agents are needed because periodontal disease is a chronic inflammatory state caused by bacterial infection and the effects of the few antibiotics available on the market are limited. Periodontal disease might negatively affect general health. An epidemiological survey in 1990 of American Pima Indians who congenitally develop type 2 diabetes revealed a link between diabetes and periodontal disease, and since then, several epidemiological surveys have been conducted, mostly in the USA and Europe. Periodontal disease might be involved in the progression of obesity, cardiovascular, chronic respiratory, and non-communicable diseases (NCDs) such as Alzheimer disease. However, little is understood about the causal relationship between periodontal and other diseases and the effects of treating periodontal disease on general health.

Here, I developed a novel, non-antibiotic agent with which to treat periodontal disease and analyzed the relationship between periodontal disease and general health among Japanese adults. I also clarified the feasibility of maintaining and promoting oral health care among the Japanese population. The causative agent of periodontal disease, *Porphyromonas gingivalis* (*P. gingivalis*), produces the cysteine proteases Arg-gingipain (Rgp) and Lys-gingipain (Kgp) that exacerbate periodontal disease through the degradation of proteins and plasma and destruction of the immune system. Therefore, inhibitors of these proteases should be an effective treatment for periodontal disease. Dual inhibitors derived from the Rgp and Kgp structures were synthesized and analyzed their biological potential. The activities of low concentrations of the inhibitors expressed as Ki were 40 and 0.27 nM for Rgp and Kgp, respectively. It was also confirmed that the organism exerts antibacterial

activities by eliminating the selectivity for proteases and the pathophysiological functions of *P. gingivalis*. I assessed the inhibition of vascular permeability in guinea pigs and the ability to suppress periodontal inflammation in Beagle models of canine periodontal disease. I developed the first dual inhibitor of the two pathogenic enzymes produced by *P. gingivalis* and confirmed its value as a new therapeutic agent against periodontal disease.

To verify the epidemiological relationship between periodontal disease and general health in Japanese adults, Hitachi Ltd. collaborated with the Faculty of Dentistry at Osaka University to conduct a cross-sectional study that introduced dental examinations into the annual health checks that Japanese companies arrange for all employees. Three parties designed the research protocol, and I assumed the lead in data acquisition and analysis. After obtaining approval from the Hitachi Institutional Review Board, the program proceeded at the Hitachi Health Management Center from 2014 to 2017 and included 1,022 Hitachi employees (male, n = 914; female, n = 108). Periodontal disease (periodontal pocket depth (PPD), occlusal force and obesity (BMI), diabetes (HbA1c), impaired glucose tolerance (IGT), fasting blood glucose (FBG), chronic obstructive pulmonary disease (% FEV_1), atherosclerotic heart disease, and associations with smoking were analyzed, and the odds ratios (ORs) for mutual risk was calculated. It was clarified that periodontal disease might significantly exacerbate obesity (OR: 1.42), impaired glucose tolerance (OR: 1.48), and chronic obstructive pulmonary disease (OR: 1.38). Among general health indicators, BMI (OR: 1.28), HbA1c (OR: 4.34), FBG (OR: 1.08), % FEV_1 (OR: 0.95), and smoking (OR: 2.32) were significantly associated with periodontal disease and the possibility of its exacerbation. Therefore, I clarified the relationship between periodontal and systemic diseases among Japanese adults, and that diabetes and hyperglycemic conditions might affect the bite force. I also added dental examinations, to corporate health checks and clarified the feasibility of oral health care that maintains and promotes general health through preventing and managing periodontal disease.

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Chapter 1. General introduction

Oral bacteria cause periodontal diseases that are accompanied by chronic inflammation that destroys the periodontal tissues supporting teeth and this can lead to tooth loss (1). Since tooth loss is directly linked to a decrease in the quality of life, prevention and treatment of periodontal disease is important for ensuring wellbeing at all ages. Severe periodontal disease was the sixth most prevalent condition worldwide in 2010 (2). The prevalence of severe periodontal disease enough to result in tooth loss, was 9.8% in 2017, and 796 million persons were infected among whom, 267 million (3.3%) lost teeth (3). The direct cost of treating oral disease was about $356 billion during 2015, and the loss of productivity from oral diseases was about $188 billion, of which 21%, or $38 billion, was due to severe periodontal disease (4). Thus, periodontal disease has significant social impact, and the importance of oral care centered around disease prevention and early treatment, is now globally recognized.

The Ministry of Health, Labor and Welfare conducts a dental disease fact-finding survey every five years to determine the prevalence of oral diseases in Japan. Figure 1 shows the transition of the survey results on periodontal pocket depth and bleeding between 2005 and 2016. The proportion of patients with periodontal pockets ≥ 4 mm deep increased with age. The 2016 survey found more periodontal pockets and higher bleeding across almost all age groups. In addition, the numbers teeth lost by individuals aged ≥ 70 years decreased annually, whereas the incidence of periodontal disease increased.

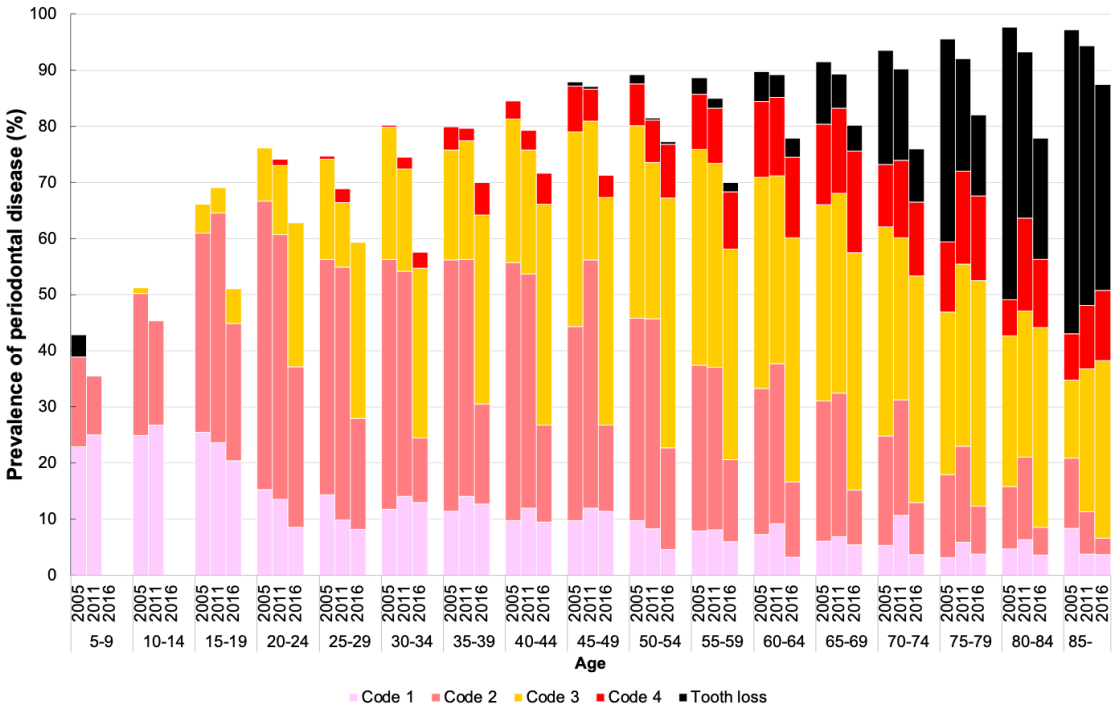

Fig. 1. Changes in periodontal disease prevalence in Japan.

This figure was created based on the "Survey of Dental Diseases" conducted by Ministry of Health, Labor and Welfare in Japan. Community Periodontal Index (CPI) criteria range from Codes 0 to 4 as follows: Code 0, healthy periodontal condition; Code 1, gingival bleeding on probing; Code 2, calculus and bleeding; Code 3: periodontal pocket 4–5 mm; Code 4, periodontal pocket ≥ 6 mm).

These findings indicate that reducing the prevalence of periodontal disease is an important issue in Japan. I consider that decreasing the prevalence of periodontal disease requires the development of new periodontal disease tests, prevention and treatment technologies and a change in consumer awareness of periodontal disease. In addition to increasing the numbers of teeth remaining among elderly persons as previously emphasized, measures to prevent the development of periodontal disease should also be implemented simultaneously. Periodontal disease might be involved in the progression of non-communicable diseases (NCDs) such as cancer, diabetes, cardiovascular, chronic respiratory, and Alzheimer diseases (5,6). Current views indicate that chronic inflammation caused by periodontal disease might cause bacteria to invade the circulatory system, which might

activate and exacerbate the inflammatory response. However, the ability of periodontal treatment to help improve cardiovascular outcomes has not been confirmed. Medical expenses for NCDs including dentistry are rising annually in developed countries (7). The same is true of Japan, where society is rapidly aging (8), and preventing soaring medical costs has become a global issue. If periodontal disease can be treated or better, avoided, and if general health deterioration can be prevented, medical expenses might be decreased by a behavioural paradigm shift towards daily oral care. A practical examination network of linked dental clinics and medical departments would be needed to universally realize that periodontal disease prevention and treatment might reduce risk of systemic diseases.

Objectives

Under these circumstances, I developed a therapeutic drug against periodontal disease with excellent safety and specificity, which could replace the conventional prevention and treatment drugs. That is, we synthesized dual inhibitors for the cysteine proteases, RGP and KGP that are specifically produced by *Porphyromonas gingivalis*, one of the pathogens of periodontal disease, and verified their applicability as therapeutic agents against periodontal disease.

I also devised and conducted a cross-sectional study in which periodontal examinations were added to the annual health checks for employees of Japanese companies. Based on evidence of a relationship between periodontal disease and general health in Japanese individuals, we considered the possibility of an oral health care model that maintains and promotes general health through the prevention and treatment of periodontal disease.

Chapter contents

Chapter 2 describes the development of a dual inhibitor of Arg- and Lys-gingipains that are virulence factors of *P. gingivalis* and examined its effects on gingival inflammation in a collaboration among the University of Kyushu, Taiho Pharmaceutical Corporation and LION Corporation.

Chapter 3 describes a cross-sectional study to clarify the relationship between periodontal disease and NCD-centered health diagnostic indicators among Japanese employees. I addressed the issues for realizing oral health care to maintain and/or promote systemic health by preventing and treating periodontal disease. Hitachi Limited, Hitachi Health Care Center, Osaka University and LION Corporation collaborated in that investigation.

Chapter 2. Development of a dual inhibitor of Arg-gingipains and Lys-gingipain produced from *Porphyromonas gingivalis*, pivotal periodontal pathogen.

2-1. Introduction

Gram-negative *Porphyromonas gingivalis* (*P. gingivalis*) comprises ~ 85% of the > 500 oral bacterial species that have been detected in the periodontal pockets of patients with periodontal diseases (9,10). *P. gingivalis* secretes various extracellular proteinases and on the cell surface to acquire heme and vitamin K that are needed for growth. These enzymes include a unique class of cysteine proteinases that are Arg-specific (Rgp) and Lys-specific (Kgp) gingipains that are encoded by the *rgpA* and *rgpB* genes (Rgp), and the *kgp* gene (Kgp) (11,12). Gingipains cause local tissue destruction and perturb host antimicrobial defenses and functions, resulting in the dysbiotic overgrowth of commensal bacteria within the oral cavity and gut as well as the dissemination of bacteria, including *P. gingivalis*, to other body sites. The overproduction of toxic metabolites caused by *P.gingivalis*-induced dysbiosis promotes metabolic disorders (13). Such interspecific interactions and disruption of host immune responses can contribute to the emergence and persistence of various bacterial communities and are therefore potential targets for therapeutic approaches to periodontal disease (14).

I developed a new active ingredient based on a new concept and found that it was safe, specific, and suppressed the progression of periodontal disease. That is, inhibitors of

RGP and KGP that are specifically produced by *P. gingivalis* were synthesized, and their usefulness as therapeutic agents against periodontal disease was verified.

2-2. Structure and function of cysteine proteinase produced from *P. gingivalis*.

Several periodontal pathogens have been identified in periodontal pockets of patients with periodontal disease, which include *P. gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans*. Among these pathogens, *P. gingivalis* is considered to be closely associated with major types and stages of periodontal disease, such as chronic adult periodontitis. Because *P. gingivalis* is an asaccharolytic organism, special attention has been given to its high proteolytic nature. Actually, this organism produces a wide variety of extracellular and/or cell-associated proteases, to acquire amino acids and peptides utilizable as carbon/energy sources for its growth and survival (15). Molecular genetic analysis revealed that Rgp was encoded by two genes, *rgp* A and *rgp* B, and KGP was encoded by a single gene, *kgp*. RGP and KGP have a common structure consisted of a proteolytic domain and adhesin domain (HGP, HbR) which encoded hemagglutinin gene (Fig. 2) (16).

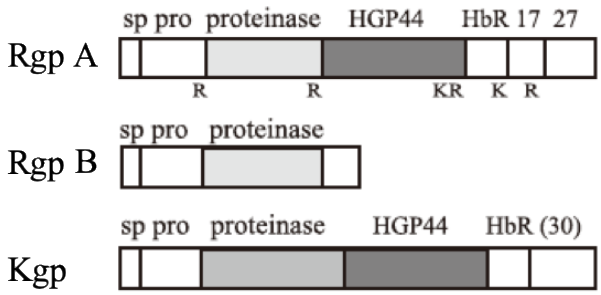

Fig. 2 Domain structures and homologies of gingipain adhesins RgpA, RgpB, Kgp.

RgpA and Kgp consist of signal peptide, propeptide, mature proteinase, and adhesin domain regions (HGP44, HbR, HGP17, and HGP27). RgpB has signal peptide, propeptide, and RgpB proteinase domain regions. R and K indicate cleavage sites for Rgp and Kgp, respectively (16).

These results indicated that RGP and KGP were the key enzymes for *P. gingivalis* to grow and survive in oral cavity. I thus envisioned an opportunity to develop a potent, selective dual inhibitor of Rgp and Kgp as a new therapeutic and preventive measure for periodontal disease. To date, however, no single molecules capable of inhibiting both Rgp and Kgp have been demonstrated in the literature. The lack of similarity in the overall structures in these enzymes may be a critical barrier to the design of the potent dual inhibitor. In addition, given that there is a fear of unfavorable side effects of such inhibitors on host proteases, it is very important to develop a dual inhibitor that is selective against these two gingipains. I report on the synthesis and biological evaluation of this dual inhibitor and its efficacy in attenuating the entire virulence of *P. gingivalis in vitro* and *in vivo*.

2-3. Materials and Methods

(1) Materials

KYT-1, the Rgp-specific inhibitor carbobenzoxy (Cbz)-Lys-Arg-CO-Lys-N(NH_3)$_2$, and KYT-36, the Kgp-specific inhibitor Cbz-Glu [NHN(CH_3) Ph]-Lys-CO-NHCH_2Ph, were synthesized at the Peptide Institute Inc. (Osaka, Japan).

(2) Synthesis of a dual inhibitor of RGP and KGP

Briefly, a dual inhibitor of RGP and KGP, KYT-41, was synthesized through 5 steps. Compound 1, which was derived from the hydrogenated methyl (3S)-3-benzyloxy-carbonylamino-7-tert-butoxycarbonylamino-2-hydroxy-heptanoate, was reacted with lithium hydroxide monohydrate to synthesized compound 2. Compound 3, which was synthesized via a condensation reaction from compound 2, was added to Dess-Martin reagent and oxidized to synthesized compound 4. Compound 4 was hydrolyzed, then final product (compound 5) was termed KYT-41.

(3) Bacterial strains and preparations of RGP and KGP

P. gingivalis ATCC33277 and W83 strains and *Actinomyces viscosus* (*A. viscosus*) NY-1 were grown under anaerobic conditions (10% CO_2, 10% H_2, 80% N_2) in enriched

brain– heart infusion broth. Rgp and Kgp were purified from the culture supernatant as described previously (17,18).

(4) Enzyme assays

For enzyme inhibition assay, all inhibitors were dissolved in dimethyl sulfoxide and used as a final concentration of 0.1%. The active concentration of cathepsin B, L, H, endogenous lysosomal cysteine proteinase, and trypsin, serine peptidase, were determined by titration against their specific inhibitors. The specificity of KYT inhibitors against Rgp and Kgp was determined by the same assay. All measurements were carried out at 40°C for 10 min with synthetic substrates for each enzyme.

(5) Degradation of human proteins

The degradation of various human proteins, including acid soluble type I collagen, fibronectin, α2-macrogloblin, γ-globulin, and fibrinogen (10 μg of protein each) by the *P. gingivalis* culture supernatant (0.6 g of protein) at pH 7.5 for 1 h was determined as described previously (19). After the reaction was complete, each sample was separated by SDS-PAGE in a 5 to 15% polyacrylamide gel. Gels were then stained with Coomassie Brilliant Blue R-250.

(6) Assessment of cell viability

Human gingival fibroblasts and human umbilical vein endothelial cells (HUVECs) were seeded into 96-well plates (10^4 cells/well) and incubated with *P. gingivalis* culture supernatant (83 g) in the absence and presence of various KYT inhibitors (0.1 mM for each) at 37°C for 6 and 3 h. Adhesion and viability were determined by the floating cells rate (%) and death cells rate (%) compared with calcein-AM labeled live cells.

(7) Hemagglutination and coaggregation assay

Effects of KYT inhibitors on coaggregation between *P. gingivalis* and *A. viscosus*. Each type of bacterium was harvested by centrifugation at 8000 g and adjusted to an optical density (OD) of 1.0 at 550 nm in the coaggregation buffer (10 mM Tris-HCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.02% NaN_3, and 0.15 M NaCl, pH 8.0) after washing twice

with the same buffer. Aliquots of 500 μL of each bacterium were mixed in a test tube and left to stand at room temperature for 30 min. Each inhibitor (0.1 mM) was added to the *P. gingivalis* suspension before 30 min of mixing with *A. viscosus*. After the coaggregation reaction, aggregated cells were removed by gentle centrifugation at 70 g for 30 min, and the OD of the supernatant was measured. Coaggregation values were expressed as (OD of mixture of 2 bacteria) × (sum of the OD of the controls for each bacterium) $^{-1}$ × 100.

Effects of KYT inhibitors on hemagglutination was determined by the following methods. The suspension of *P. gingivalis* in PBS at an OD at 550 nm of 0.4 was preincubated with or without KYT inhibitors at 37°C for 10 min. 100 μL aliquot of the suspension was mixed with an equal volume of sheep erythrocyte suspension (2.5% in PBS) and incubated in a round-bottomed microtiter plate at room temperature for 3 h.

(8) Periodontal disease model

Animal study procedure was approved by the Institutional Animal Care and Use Committee of Kyushu University. Experiments with beagle dogs were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of the animal research committees at Lion Corporation (Odawara, Japan). Beagle dogs were used for establishing a periodontal disease. Systemically healthy 4 beagle dogs (age; 4–5.6 years, 10.1–13.7 kg body weight) were received dental scaling and cleaning with daily tooth blushing for a month to establish gingival health (gingival index of 0–0.5). For this period, dogs were fed hard diet (DS-A; Oriental Yeast Co., Ltd., Tokyo, Japan). Then, dogs were fed a water-moistened soft diet (300 g DS-A dissolved in 300 mL hot water) once daily during 4 or 5 weeks to induce periodontal inflammation.

The level of periodontal inflammation was determined by measuring gingival clavicular fluid (GCF) volume. Dogs were sedated with a ketamine-droperidol cocktail (Sankyo Pharmaceutical Inc., Tokyo, Japan). GCF was collected from 6 mandibular premolars of each animal at 1 time a week with a filter paper strip, periopaper. Periopaper inserted to gingival sulcus and GCF was collected for 30 sec. Then, GCF volume was

determined with Periotron 8000 system (Ora Flow Inc., Smithtown, NY, USA) (Fig. 3). Four dogs were used for evaluating the ability of KYT-41 to suppress the gingival inflammation. One side of the mouth randomly allocated to the placebo site, while another side to the KYT-41-treated site. The applicant gel consisted of carrageenan and 3% propylene glycol, with or without 0.5 mM KYT-41. Topical application of KYT-41 or the placebo gel formulation was performed with syringes. During the 5 weeks experimental period, 1.0 mL of each of the formulations was applied around 6 mandibular premolars (the left and right 2nd, 3rd, and 4th lower premolars) of each side 2 times a day.

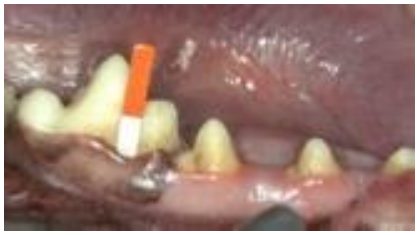

Fig. 3 Measurement of gingival crevicular fluid from gingival pocket of beagle dog.

Periopaper was inserted into the test site pocket under anesthesia. After 30 seconds, the amount of gingival crevicular fluid absorbed in the periopaper was measured with a Periotron 8000 system.

(9) Detection of black-pigmented anaerobic bacteria (BPAB) in the beagle dog gingival pockets

Three sterile paper points were inserted in the beagle dog gingival pockets. After 10 s, the paper points were removed, placed into Todd Hewitt broth (THB), and sonicated for 30 s. The THB fluid was diluted 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} and then applied to duplicate blood agar plates by using a spiral plater. Total colony forming units were determined after 5 d of growth under anaerobic conditions. Black-pigmented anaerobic bacteria (BPAB) were grown in THB anaerobically at 37°C for 48 h. The culture supernatants were separated by centrifugation and stored at -40°C until use for Western blot analysis.

(10) Statistical analysis

All results from in vitro experiments are expressed as the mean ± SD. The statistical significance of the difference between 2 groups was analyzed by a 2-tailed unpaired Student's t test. In animal models, ANOVA was performed to compare disease progression for all time points with the GCF volume levels between KYT-41- and placebo gel-treated sites. The Welch t test was also used to analyze for differences at each time point. In either case, differences were considered significant at $p < 0.05$.

2-4. Results

(1) Design and properties of KYT-41

My previous findings demonstrated that Rgp requires the presence of Lys residue in the P1' and P2 positions for efficient cleavage of human histatins, whereas Kgp needs the presence of basic amino acids in the P1' position and Glu residue in the P2 position for specific cleavage of the substrates (19). It has also been demonstrated that the Rgp- and Kgp-specific inhibitors need Lys-Arg-CO-Lys-$N(CH_3)_2$ and Glu-Lys-CO-Lys-$N(CH_3)_2$ molecules to inhibit the respective enzymes. In addition, there is a significant sequence similarity in the vicinity of the active site His and Cys residues between the two enzymes (20–22), suggesting that they might share part of the binding pattern of natural substrates. With this information at hand, I designed and synthesized the small molecule Cbz-Glu-Lys-CO-Arg-$CONH(CH_2)_2Ph$, termed KYT-41 as a novel, potent, dual inhibitor of both gingipains (Fig. 4A). In this compound, the functional Arg projected into the S1 position of Rgp was introduced to the position subsequent to the functional Glu-Lys residue for Kgp (Fig. 4B).

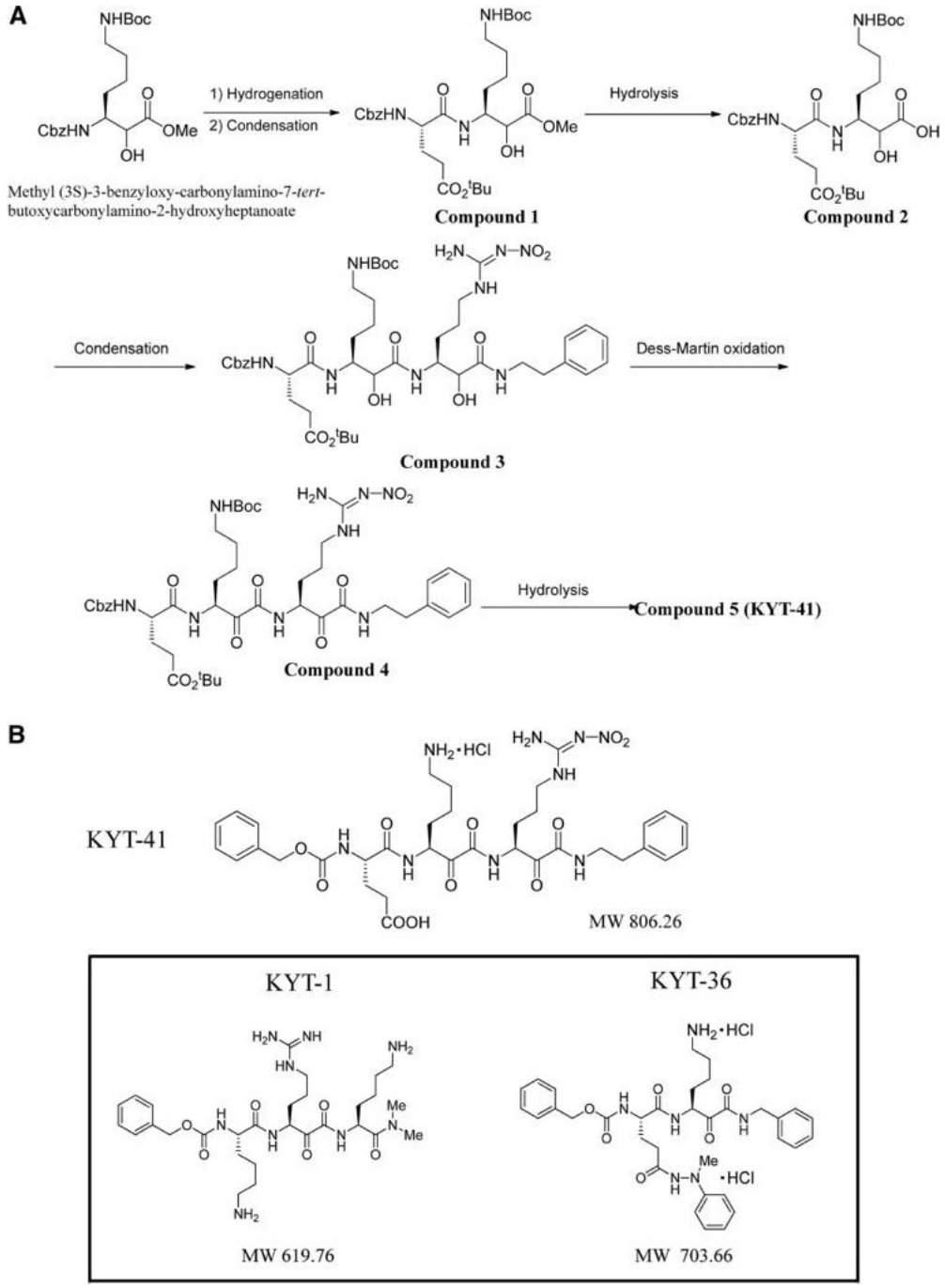

Fig. 4 Synthetic pathway for the dual inhibitor KYT-41.

A) Synthetic pathway via compounds 1–4. Cbz, carbobenzoxy group; Boc, tert-butoxycarbonyl group; tBu, tert-butyl group. B) Chemical structure of KYT-41 compared with those of KYT-1 and KYT-36.

This dual inhibitor exhibited potent inhibition of Rgp (IC_{50} =10^{-8}, Ki=4.0×10^{-8} M) and Kgp (IC_{50}=10^{-10} M, Ki=2.7×10^{-10} M) (Tables 1 and 2). IC_{50} values of cysteine cathepsins

and pancreatic trypsin by KYT-41 were 10^3 and 10^5 times less than those of Rgp and Kgp, respectively. KYT-41 also strongly inhibited the cell-associated Rgp and Kgp of the *P. gingivalis* ATCC33277 and W83 strains with 10^5 to 10^6 cfu of bacteria in 1 μL (ATCC33277: $IC_{50}<10^{-12}$ M for Rgp and $<10^{-14}$ M for Kgp; W83: $IC_{50}<10^{-8}$ M for Rgp and 10^{-10} M for Kgp).

Table 1 Inhibition activities of KYT-41 against various proteases.

Protease	IC_{50} (M)
Rgp	10^{-8}
Kgp	10^{-10}
Cathepsin B	10^{-5}
Cathepsin L	$>10^{-5}$
Cathepsin H	No inhibition
Trypsin	10^{-5}

Table 2 Inhibition of Rgp and Kgp by KYT-41 compared with KYT-1 and KYT-36.

Compound	K_i (M)	
	Rgp	Kgp
KYT-41	4.0×10^{-8}	2.7×10^{-10}
KYT-1	2.4×10^{-10}	2.6×10^{-6}
KYT-36	$>10^{-4}$	1.3×10^{-10}

(2) Effect of KYT-41 on degradation of various human proteins by *P. gingivalis* culture supernatant

There are several reports indicating that Rgp and Kgp can cooperatively degrade a wide variety of human proteins, including type I collagen, immunoglobulins, albumin, complement factors, coagulation factors, hemoglobin, cytokines, cell adhesion molecules,

and immune cell receptors (15,19,23–27). I thus examined whether or to what extent KYT-41 inhibits the degradation of various host proteins with *P. gingivalis* culture supernatant. The results indicate that the degradation of all proteins examined was strongly inhibited by KYT-41, which is comparable to that by a combination of KYT-1 and KYT-36 (Fig. 5).

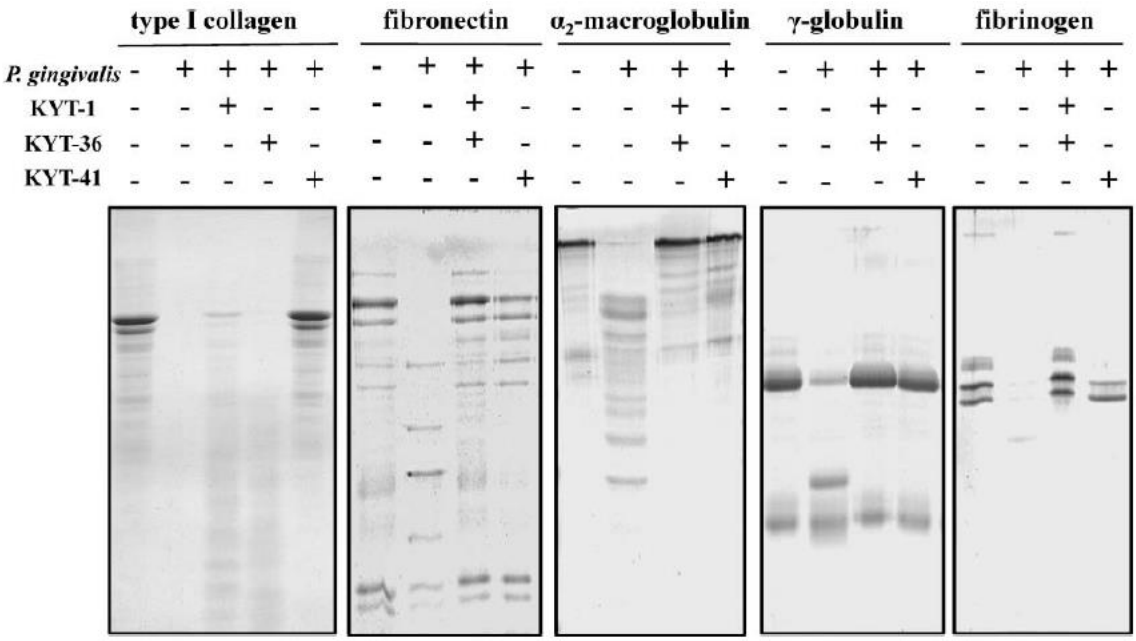

Fig. 5 Effect of KYT-41 as well as KYT-1 and KYT-36 on the degradation of various human proteins by *P. gingivalis* culture supernatant.

Each protein (10 g) was incubated with Pg culture supernatant (0.6 g of protein) at 37°C (in the case of type I collagen, at 25°C) for 1 h in the absence or presence of KYT-41 as well as KYT-1 and/or KYT-36 at a final concentration of 0.1 mM. After incubation, the reaction was terminated by the addition of an inhibitor cocktail containing leupeptin, tosyl-l-phenylalanine chloromethyl ketone, and tosyl-l-lysine chloromethyl ketone (a final concentration of 0.1 mM for each). The samples were then subjected to SDS-polyacrylamide gel electrophoresis in 5 to 15% polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue R-250.

(3) Effect of KYT-41 on the adhesion and viability of host cells by *P. gingivalis* culture supernatant

I determined the effect of KYT-41 on the adhesion and viability of human gingival fibroblasts and HUVECs. Loss of the adhesion and viability of these 2 cell types by *P. gingivalis* culture supernatant was strongly inhibited by a single administration of KYT-41 as well as KYT-1, but not KYT-36, at a final concentration of 0.1 mM (Fig. 6), indicating that the cytotoxic effects of *P. gingivalis* culture supernatant on the host cells is mainly due to Rgp.

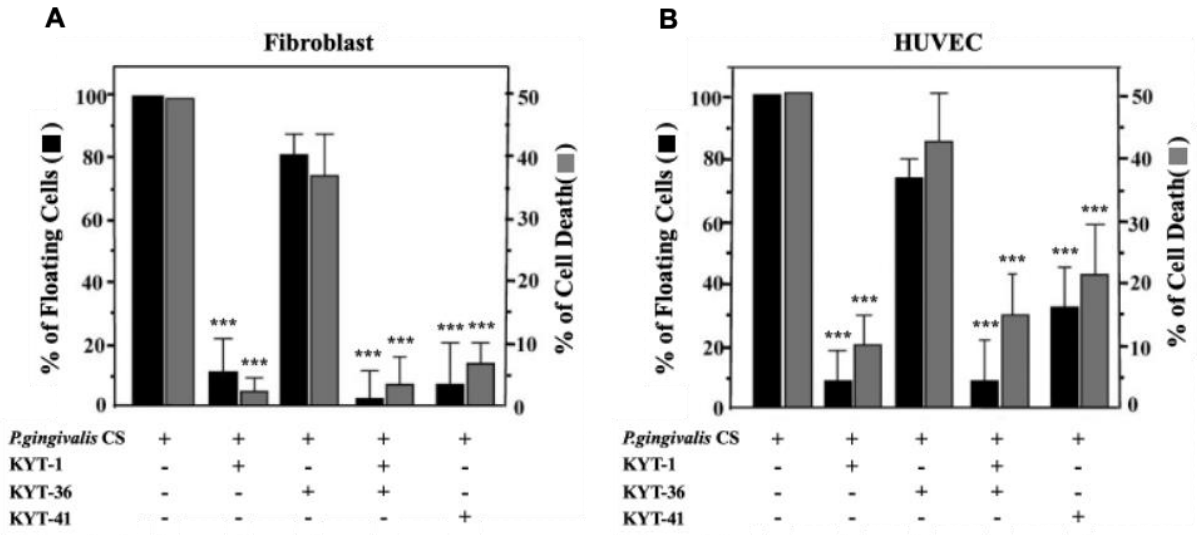

Fig. 6 Effects of KYT inhibitors on the loss of the adhesion and viability of human gingival fibroblasts and HUVECs induced by *P. gingivalis* culture supernatant.

Human gingival fibroblasts (A) and HUVECs (B) in culture were seeded into 96-well plates (10^4 cells/well) and incubated with *P. gingivalis* culture supernatant (83 g) in the absence and presence of various KYT inhibitors (0.1 mM for each) at 37°C for 6 and 3 h, respectively. Adhesion and viability were determined by staining of the attached cells with calcein-AM and by counting the viable cells with a cell-viability kit, respectively. *P < 0.001 vs. *P. gingivalis* treatment without inhibitors.

(4) Effect of KYT-41 on the various physiological processes of *P. gingivalis* necessary for its growth and survival

Coaggregation of *P. gingivalis* with various oral bacteria is recognized to be an early and critical step in its infectious processes and to play an important role in the formation and maturation of a biofilm that facilitates the adherence and colonization of the organism in periodontal regions. I thus assessed whether or to what extent the coaggregation activity could be inhibited by KYT-41. The results indicate that coaggregation between *P. gingivalis* and *A. viscosus* was strongly inhibited by a single use of KYT-41 (Fig. 7A). The extent of this inhibition was comparable to that with a combination of KYT-1 and KYT-36, confirming that the coaggregation activity is totally dependent on the proteolytic activities of both Rgp and Kgp. Hemagglutination is a distinctive characteristic of the organism that discriminates it from other asaccharolytic BPAB (28) and is a particularly important process for protoheme acquisition from hemoglobin for its survival. Previous studies from our and other laboratories revealed that the initial translational products of the *rgp*A, *kgp*, and *hag*A genes had hemagglutinin and hemoglobin-binding hemoglobin receptor (HbR) domains in their carboxy-terminal regions (Fig. 2) (17,29). Molecular genetic study with insertional gene inactivation also revealed that these genes are responsible for hemagglutination of *P. gingivalis* (30) and that HbR is generated by proteolytic processing by Rgp and Kgp (31). This hemagglutination was strongly inhibited by KYT-41 and by KYT-1, at a concentration of 10^{-6} M (Fig. 7B). However, KYT-36 showed little or no effect on it. Furthermore, the growth of the organism in α-KG/BSA-defined medium, which contains BSA as the sole energy/carbon source, was shown to be completely inhibited by a single use of KYT-41, which was comparable to a combination of KYT-1 and KYT-36 (Fig. 7C). These results strongly suggest that most of the pathological and physiological functions were abolished by the dual inhibitor of Rgp and Kgp.

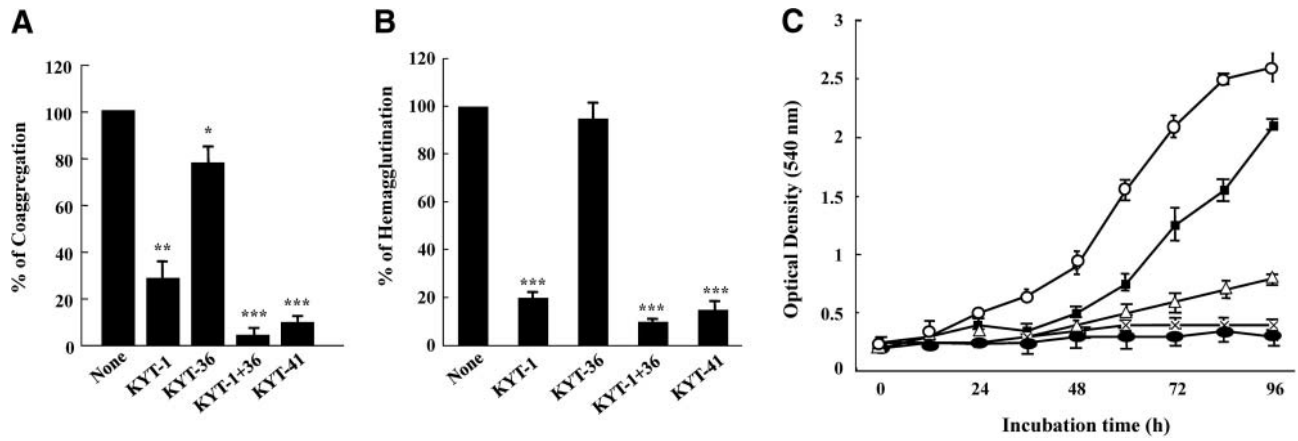

Fig. 7 Effect of KYT-41 as well as KYT-1 and/or KYT-36 on physiological functions of *P. gingivalis*.

A) Effects of KYT inhibitors on coaggregation between *P. gingivalis* and *A. viscosus*. B) Effects of KYT inhibitors on hemagglutination of *P. gingivalis*. C) Effects of KYT inhibitors on the growth of *P. gingivalis* in α-KG/BSA medium. An overnight culture of *P. gingivalis* in enriched brain– heart infusion broth was diluted 10-fold with α-KG/BSA medium, with or without inhibitors (0.1 mM), and incubated anaerobically at 37°C. Growth was monitored by measuring the OD at 540 nm. ○, no inhibitor; ■, KYT-1; △, KYT-36; X, KYT-41; ●, combination of KYT-1 and KYT-36.

(5) Effect of KYT-41 on the progression of gingival inflammation in dog periodontal disease models

Periodontal disease is one of the most common infectious diseases affecting adult dogs and is more likely to mimic the pathological and physiological mechanisms of human periodontal disease (32). I thus used beagle dogs as a periodontal disease model for evaluating the effectiveness of KYT-41 in vivo. I first tried to establish equally healthy gingiva in all the beagle dogs during the pretreatment phase. For this purpose, all the dogs received a daily tooth brushing until a gingival index of 0 to 0.5 was reached. All the dogs had healthy, nonpigmented, pink gingiva with a smooth and regular texture and

a knife-edged gingival margin (Fig. 8A). Then, the dogs were placed on a water-moistened soft diet during the entire experimental period. At 5 weeks after the dogs began consuming the soft diet, the gingiva was swollen and bleeding on probing and displayed altered color, significant erythema, and edema accompanying subgingival plaque formation on tooth surfaces (Fig. 8A). Plaque samples were obtained from each of the teeth at this stage by using paper points and then incubated on blood agar plates. BPAB were isolated from the plates and grown in THB for 48 h at 37°C. The culture supernatants were separated by centrifugation and subjected to Western blot analysis with polyclonal antibodies against the proteinase domain of Rgp. The results showed that the culture supernatants of BPAB collected from each site were mostly positive for the antibodies (Fig. 8B), indicating the existence of Rgp-generating BPAB in dog periodontal disease models.

By using this model, we examined the efficacy of locally delivered KYT-41 in reducing the gingival inflammation. GCF volume was measured from 6 mandibular premolars to investigate inflammation level. One side of dog mandibular randomly selected in 4 dogs was used as the placebo site, and the other side served as the KYT-41-treated site. One milliliter of gel solution, with or without KYT-41 (0.5 mM), was applied to each site of the mandibular premolars 2 times/day for 4 weeks. At baseline (0 week), there was no significant difference in the mean GCF volumes between 3 mandibular premolars in the vehicle- and the KYT-41-treated sites. From 1 to 4 weeks after the treatment, however, the mean values of the GCF volume in the 3 mandibular premolars at the placebo site increased significantly, whereas that at the KYT-41-treated site decreased significantly ($p<0.01$, ANOVA; Fig. 8C). A significant difference was also observed in the GCF volume of 3 mandibular premolars between the vehicle- and KYT-41-treated sites at week 4 ($p<0.01$).

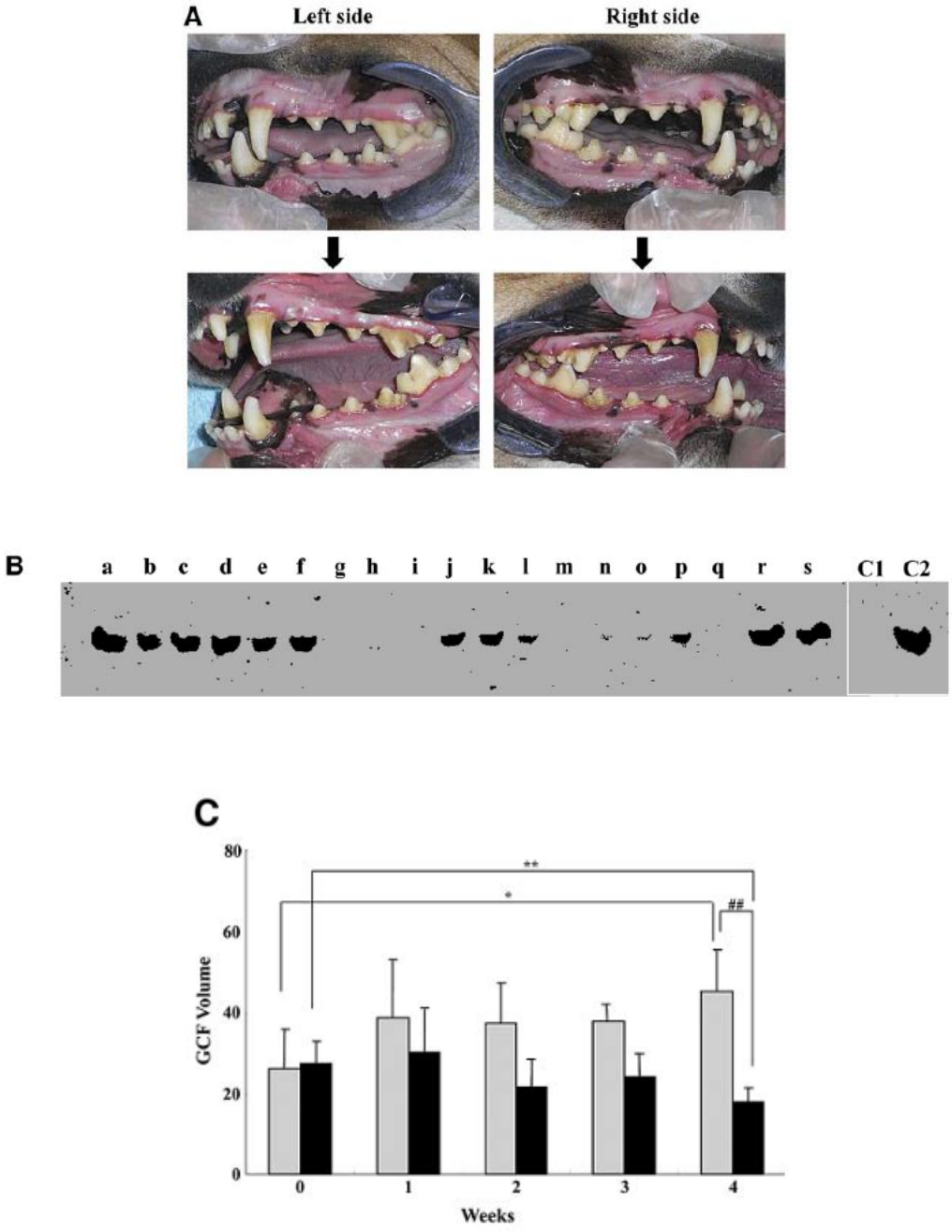

Fig. 8 Effect of KYT-41 on the progression of gingival inflammation in dog PD models.

A) Gross appearance of gingival tissues of the left and right maxillary and mandibular arcades of beagle doges before and after a hard diet was replaced with a soft diet. B) BPAB derived from gingival pockets of the PD models were grown THB anaerobically at 37°C for 48 h. The culture supernatants were separated by centrifugation and subjected to SDS-PAGE. The separated proteins were transferred to polyvinylidene difluoride membranes followed by immunostaining with polyclonal antibodies against the catalytic domains of Rgp. a–s) BPAB

obtained from various gingival pockets using sterile paper points, C1; *A. viscosus* T14V, C2; *P. gingivalis* ATCC33277. C) Topical application of KYT-41 (0.5 mM; black) or placebo gel (1.0 mL; gray) formulations to each PD dog 2/d during the 5 weeks experimental period.

2-5. Discussion

Tetracycline antibiotics are conventionally used to treat periodontal disease and bactericidal agents serve to prevent periodontal disease. Tetracyclines such as doxycycline and minocycline not only have antibacterial activity but inhibit tissue matrix metalloproteinases (MMP) that contribute to the hydrolysis of connective tissue by chelation (33). Among disinfectants, triclosan (2,4,4'-trichloro-2'hydrox-ydiphenylether) has been the most popular disinfectant in oral care products and cosmetics worldwide. However, triclosan released into environmental wastewater decomposes into methyl-triclosan and chlorinated phenol that are more toxic than triclosan, and aquatic microorganisms might acquire drug resistance (34). Triclosan has also been detected in breast milk, urine and plasma, and blood triclosan levels correlate with consumer antibiotic use (35). The US Food and Drug Administration (FDA) excluded triclosan from consumer products in September 2016 due to these safety concerns (36). Because the applications of fungicides require care in terms of human and environmental safety, I aimed to develop a safe, effective, and *P. gingivalis*-specific agent. *Porphyromonas gingivalis* is a benign bacterium that generates and converts biofilms to pathogenicity together with other bacteria that causes periodontal disease with destruction of periodontal tissue (37). I developed an inhibitor of the proteases that specifically suppresses the survival of *P. gingivalis* and verified its usefulness as a therapeutic agent against periodontal disease.

The developed dual inhibitor KYT-41 is specific for Rgp (Ki = 10^{-8} M) and Kgp (Ki = 10^{-10} M). We also individually synthesized the Rgp and Kgp inhibitors KYT-1 and KYT-36, and compared and verified their inhibitory activities. The inhibitory activity of KYT-41 against Rgp and Kgp was 160-fold lower than that of KYT-1 and similar to that of

KYT-36, respectively. I then assessed the specificity of the inhibitors using the cysteine proteases cathepsin and trypsin. IC_{50} values of cysteine cathepsins and pancreatic trypsin by KYT-41 were 10^3 and 10^5 times less than those of Rgp and Kgp, respectively. KYT-41 also inhibited Rgp and Kgp bound to *P. gingivalis* cells, suggesting that it is a direct antibacterial agent. KYT-41 also abolished various pathological functions of *P. gingivalis*.KYT-41 inhibited the degradation of various host proteins in *P. gingivalis* culture supernatant, whereas KYT-1 and KYT-36 alone did not.

Gingipain cleaves MMP propeptides, including MMP-1, -2, and -9 to produce mature enzymes *via* Rgp activity in human gingival fibroblasts (38,39), and stimulates MMP-1 expression (40). Fibroblasts, keratinocytes, and monocyte macrophages continuously secrete the MMP family of proteases is as latent precursors and they are activated by enzymes such as gingipain. Considering that the MMP family is also activated by enzymes derived from various bacteria, we considered that KYT-41 should suppress the progression of periodontal disease accompanied by tissue destruction.

I investigated the effects of KYT-41 using the Beagle dog model in which periodontitis spontaneously occurs with a pathology similar to that of human periodontitis (41,42). Beagle dogs harbor the human *P. gingivalis*-like gram-negative bacteria *P. salivosa*, *P. cangingivalis*, *P. canoris*, and *P. gulae* (43,44).

Firstly, I assessed bacterial cell samples sampled from periodontal pockets by western blotting using an anti-Rgp antibody to confirm that Rgp-like enzymes reside in the periodontal pockets of these dogs. The ability of a drug to suppress inflammation can be quantified using this model. Since the amount of inflammation reflects that of gingival crevicular fluid secreted into periodontal pockets that correlate with inflammatory exacerbation, an inflammation-free state is essential at the start of the test. Therefore, I removed tartar from the teeth including the test site for 2 weeks and removed plaque daily by brushing the teeth before starting the test.

The teeth were not brushed from the first day of the test, and the dogs were fed once a day with a soft diet. Since the onset of inflammation induced might differ among dogs, a placebo and the test agent were respectively assigned to the left and right jaws of each dog

for 4 weeks. I found that KYT-41 significantly suppressed periodontal inflammation compared with the placebo, indicating its usefulness as a therapeutic agent for periodontal disease. The Peptide Institute Inc. (https://www.peptide.co.jp) markets KYT-41, for which we have filed a patent application as an agent for treating periodontal disease (JP 4006598, WO 2003/042237).

Chapter 3. A cross-sectional study of relationships between periodontal disease and general health: The Hitachi Oral Healthcare Survey

3-1. Introduction

P. gingivalis has been identified in the coronary artery (45), placenta (46) and lever (47), and in patients with diabetes and severe periodontal disease. The relationship between periodontal disease and general health has attracted interest not only from dentists but also from investigators in various disciplines, and a two-way relationship has been emphasized. Nelson *et al*. investigated the relationship between diabetes and periodontal disease in an epidemiological study of Pima Indians with a high frequency of type 2 diabetes and found that the incidence of periodontal disease is increased 2.6-fold among persons with diabetes (48). Since then, many epidemiological studies in Europe and the USA that have investigated the relationship between periodontal and systemic diseases, have suggested that periodontal diseases are involved in the progression of non-communicable diseases (NCDs) including obesity, cardiovascular, chronic respiratory, and Alzheimer diseases (5,6).

The WHO proposed a “Global Strategy for Oral Health” in 2021 based on the notion that oral health is essential for general health and announced an action policy on dental examinations and treatment worldwide (49). The target for regular dental examinations in Japan was set at 65% in the Dental and Oral Health Law that came into effect during 2011. A need for increased reliability of evidence for a relationship between oral health and general health, and lifelong dental examination was established in the WHO “Basic Policy for Economic and Financial Management and Reform 2019”. Improved consultation rates of dental examinations and promoted medical-dental cooperation when systemic diseases might be identified and treated were emphasized as strengthening efforts for the early detection of diseases in the “Action Plan of the Growth Strategy” 2021. As a result of these policies, the rates of regular dental examinations increased to 34.1% in 2009, 47.8% in 2012, and 52.9% in 2016, but they are still far from the target value (50).

Therefore, I and my team devised and conducted a cross-sectional study that added periodontal examinations into the annual health checks for employees of Japanese companies. Considering the evidence supporting a relationship between periodontal disease and general health among Japanese persons, I considered the possibility of establishing an oral health care model that maintains and promotes general public health by preventing and treating periodontal diseases.

3-2. Materials and Methods

(1) Study design and population

A cross-sectional study was performed from 2014 to 2017 at the Hitachi Health Care Center (HHCC, Hitachi, Ibaraki, Japan), and data were collected for one week in December of each year. Written informed consent was obtained from all employees before participation in this study. The study was called the Hitachi Oral Healthcare (HTC-OHC) Survey. The Ethics Review Board at Hitachi Ltd. approved this study (approval No. 2014-58), which was conducted in accordance with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (51). Dental examinations were introduced as part of the regular annual Hitachi Ltd. employee health checks (about 80 employees/day) at HHCC.

(2) Periodontal examination

Dental examinations were conducted by dental hygienists from The LION Foundation for Dental Health (LDH). The dental hygienist performed a calibration of periodontal disease measurements prior to the start of the study to improve reliability and reduce bias in dental index measurements. Probes for periodontal pocket measurement (CP-12, Hu-Friedy Mfg. Co. LLC, Chicago, IL, USA) were used to train the dental hygienists to achieve an average probing pressure of 35 g across 16 individuals. Thereafter, the probing pocket depth (PPD) and the presence or absence of bleeding were measured and recorded from six locations per tooth (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual, and mesio-lingual) for all the teeth of five Lion Corporation employees who

volunteered to help with calibration. These series of calibrations were performed twice a year prior to the start of the study to improve and homogenize the accuracy of investigator measurements. Calculation of kappa statistics resulted in a range of good (0.7) to excellent (0.8) values.

(3) Data collection

Health data were obtained from annual health checks conducted at the HHCC. The 47 endpoints determined at this facility included height, weight, abdominal circumference, hearing, vision, blood pressure, blood flow, electrocardiography, and other blood tests as part of the routine physical examinations. Indices for obesity, diabetes, impaired glucose tolerance (IGT), chronic obstructive pulmonary disease (COPD), atherosclerotic cardiovascular disease (ASCVD) were calculated from test results anonymized for use as a general health index. Periodontal disease indicators (number of teeth, PPD, BOP sites, periodontal epithelial surface area (PESA), periodontal inflamed surface area (PISA), and occlusal force were also measured during dental examinations. Detailed descriptions of measurements and calculations for each indicator are described below.

(4) Classification of pathological conditions

Periodontal disease index and pathophysiological classification

Periodontitis was classified based on the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (52). We did not measure clinical attachment level (CAL), but classified periodontitis stages from maximal PPD values. I compared employees without PPD ≥ 4 mm and haemoglobin A1C (HbA1c) level < 5.7 (healthy) with those who had mild (stages I and II: maximum PPD ≥ 4 mm, but < 6 mm) or moderate/severe (stages III and IV: maximum PPD ≥ 6 mm) periodontitis. I calculated the PESA and PISA to quantify pocket epithelium surface areas and periodontal inflammation loads (53) using Microsoft Excel (54).

Obesity index and pathological classification

Obesity is defined as BMI ≥ 25 kg/m^2 according to the obesity classifications of the Japan Society for the Study of Obesity (55). Participants were classified into two groups: healthy (18.5-24.9 kg/m^2) and obese (≥ 25 kg/m^2). A stratified analysis revealed that among participants considered as healthy based on BMI, those with HbA1c level < 5.7% were considered healthy in analyses.

Diabetes indicators and pathological classification

Diabetes was defined by HbA1c levels according to the American Diabetes Association (56). HbA1c was measured using high-performance liquid chromatography (HLC723-G11, TOSOH Co. Ltd., Tokyo, Japan). Individuals with HbA1c level ≥ 6.5%, 5.7%–6.4%, or < 5.7% were classified as having confirmed, suspected (borderline), or no diabetes mellitus (healthy), respectively.

Blood glucose index and pathophysiological classification

Plasma glucose (mg/dL) was measured enzymatically (GA09 II, A&T Co. Ltd., Tokyo, Japan). Levels of fasting blood glucose (FBG) < 100, 100–125, and ≥ 126 mg/dL were classified based on the guidelines of the American Diabetes Association (57) as being normal, impaired fasting glucose (IFG), or impaired glucose tolerance (IGT), respectively.

Chronic obstructive pulmonary disease index and pathophysiological classification

Lung function was assessed as postbronchodilator forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) by using a spirometer (SP-770COPD, Fukuda Denshi Co. Ltd., Tokyo, Japan). The FEV_1/FVC ratio (%FEV_1) was also calculated for classification analyses (58). Levels of COPD taken as %FEV_1 according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (58) were determined as mild (≥ 80%), moderate (50%–79%), severe (30%–49%), or very severe (< 30%). Participants with HbA1c level < 5.7% classified as mild by %FEV_1 served as the healthy group during stratification analyses.

Atherosclerotic index and pathophysiological classification

The cardio-ankle vascular index (CAVI) was measured to determine arterial stiffness that reflects the elastic properties of arterial walls (59). CAVI was measured using the VaSera VS-3000 (Fukuda Denshi Co. Ltd., Tokyo, Japan). Values of < 8.0, 8.0–8.9, and ≥ 9.0 were defined as healthy, borderline, and suspected atherosclerosis, respectively. Participants with CAVI < 8.0 and HbA1c level < 5.7% were defined as healthy in the stratified analysis.

Classification of smoking status

Smoking status was categorized as having never smoked (never), previously smoked (former), and currently smoked (current) based on questionnaire responses.

(5) Statistical analysis

Pathophysiology was stratified based on the indicators of periodontal disease, obesity, diabetes, COPD, ASCVD. Differences between dental hygiene and health diagnostic indices were analysed using one-way ANOVA with post hoc Dunnet's tests, chi-squared tests, Wilcoxon rank-sum tests, or Kruskal-Wallis tests with post hoc Bonferroni correction for multiple comparison. Differences in indices between disease and healthy groups were analysed using Mann-Whitney U tests. Associations between smoking status and disease states were also analysed using Pearson's chi-squared tests (categorical variables).

The risk of developing obesity, diabetes, IGT, COPD, and ASCVD with moderate/severe periodontitis, was estimated through multiple logistic regression analyses that included dental indices (tooth count and PPD, PESA, and PISA) and health diagnostic indices (BMI, HbA1c, FBG, $\%FEV_1$, and CAVI). Model 1 (M1) is the reference model, which is based on healthy participants with HbA1c level < 5.7. Model 2 is M1 adjusted for age. Model 3 is M1 adjusted for age and smoking status. The odds ratios (ORs) and 95% confidence intervals (CI) between disease and control groups were then analysed. Associations between periodontal disease indices, general health indices, and occlusal force were clarified using multiple linear regression analyses. All data were

statistically analysed using SPSS Statistics Version 27.0 (IBM Corp., Armonk, NY, USA). Values with $p < 0.05$ were considered statistically significant.

3-3. Results

All participants were aged ≥ 20 years who confirmed their intention to participate in the study through writing. Those who are taking medication or undergoing treatment for systemic disease and those with full dentures were excluded upon participant recruitment. Upon applying the exclusion criteria, we targeted a total of 1,183 people: 248 in 2014, 294 in 2015, 322 in 2016, and 319 in 2017. However, there was an overlap in the participants because 161 participants had already been examined; as such, the duplicate data were deleted. The final number of participants was 1,022 (Fig. 9). Table 3 shows baseline characteristics of participants in this study. Median age was 50 (28-77) years, consisting of 914 male (89.4%) and 108 female (10.6%), among whom 30% smoked and 40% did not. The median of number of teeth, levels of BMI, and HbA1c were 28, 23.7 kg/m^2, and 5.6 %, respectively.

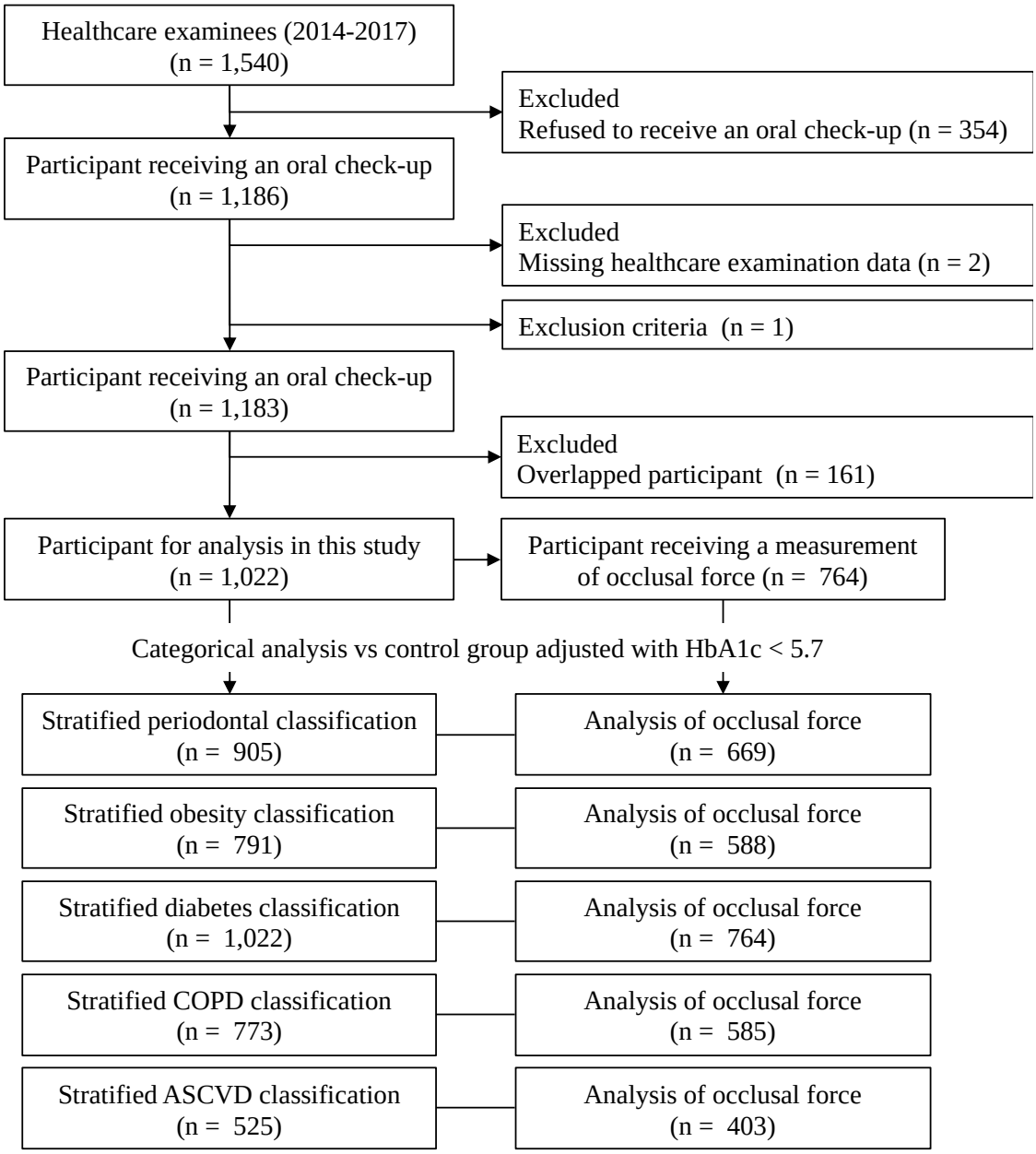

Fig. 9 Flowchart of inclusion and exclusion of participants in the Hitachi Oral Health Care Survey.

Table 3 Characteristics of the study participants.

n [Male / Female]	1022 [914 / 108]
Age [years]	50 (14)
Number of teeth	28 (4)
BOP [sites / mouth]	11 (22)
Biting force [N]	368.2 (329.7)
Hight [cm]	170.0 (9.6)
Body weight [kg]	68.7 (15.1)
BMI [kg/m^2]	23.7 (4)
HbA1c [%]	5.6 (0.5)
FBG [mg/dL]	104 (15)
% FEV_1	80 (8)
CAVI	7.80 (1.29)
Smoking [n, [%]]	
Never	406 [40]
Former	309 [30]
Current	307 [30]

Data are presented as the median (interquartile range) or number or percentage.

(1) Classification based on periodontal disease index

Table 4 shows that 62 (6.9%) of the employees had good oral health, whereas 487 (54.2%), and 349 (38.9%) had mild and moderate/severe periodontitis, respectively. The PPD, BOP sites, PESA, and PISA were significantly increased in those with mild or moderate/severe periodontitis compared with healthy employees. In addition, mild or moderate/severe periodontitis was associated with a significant increase in BMI, FBG and HbA1c levels. The groups with

moderate/severe periodontitis had significantly fewer teeth, lower high-density lipoprotein (HDL), and % FEV_1 levels than the healthy group.

Table 4 Intergroup comparisons of participants stratified by periodontal status (*n* = 898)

Variables	Healthy *n* = 62 (6.9%)	Mild periodontitis (Stage I and II) *n* = 487 (54.2%)		Moderate/Severe periodontitis (Stage III and IV) *n* = 349 (38.9%)		*P* value
Periodontal parameters						
Number of teeth	27.3 (3.0)	27.1 (3.6)		25.1 (5.6)	***	< 0.001†
PPD (mm)	2.5 (0.2)	2.7 (0.3)	***	3.2 (0.7)	***	< 0.001†
BOP sites / mouth	0.0 (0.0)	17.1 (19.4)	***	28.9 (26.9)	***	< 0.001†
PESA (mm^2)	1277.4 (188.2)	1363.9 (221.7)	***	1568.2 (444.0)	***	< 0.001†
PISA (mm^2)	0.0 (0.0)	169.3 (196.1)	***	393.4 (424.5)	***	< 0.001†
Health examination						
Age	49.8 (9.2)	49.9 (9.7)		52.9 (9.9)	*	< 0.001¶
Weight (kg)	62.8 (10.1)	69.6 (12.1)	***	70.6 (12.4)	***	< 0.001¶
BMI (kg/m^2)	22.2 (2.5)	24.1 (3.5)	***	24.5 (3.7)	***	< 0.001†
Triglyceride (mg/dL)	103.8 (80.5)	125.8 (90.0)		132.8 (98.4)		0.070†
LDL (mg/dL)	123.7 (26.2)	122.8 (29.5)		119.5 (30.9)		0.239¶
HDL (mg/dL)	63.3 (15.4)	58.2 (15.1)	*	58.6 (15.4)	*	0.045¶
FBG (mg/dL)	98.7 (8.0)	107.7 (18.6)	***	110.9 (19.5)	***	< 0.001†
HbA1c (%)	5.44 (0.21)	5.72 (0.73)	***	5.80 (0.74)	***	< 0.001†
% FEV_1	81.1 (6.9)	80.2 (5.8)		78.3 (7.0)	*	0.023†
CAVI	8.0 (1.0)	7.8 (0.9)		8.0 (0.9)		< 0.044†
FIB4 score	1.13 (0.48)	1.08 (0.47)		1.18 (0.49)		0.012¶

Smoking (n, (%))				< 0.001§
Never	38 (61.3)	207 (42.5)	104 (29.8)	
Former	13 (21.0)	143 (29.4)	118 (33.8)	
Current	11 (17.7)	137 (28.1)	127 (36.4)	
Occlusal function (Total *n* = 669)				
n	33	352	284	
Biting force (N)	453.1 (279.7)	411.1 (244.7)	372.9 (237.4)	0.058¶

Data are shown as means ± SD. P value was calculated from one way analysis of variance (ANOVA) ¶ or Kruskal-Wallis tests for continuous variables or Pearson's chi-squared tests§ for categorical variables. *p < 0.05, ***p < 0.001 vs. healthy group.

(2) Health status stratified by obesity index

Table 5 shows that 421 (53.2%), 22 (2.8%) and 384 (44.0%) employees were healthy, underweight and overweight according to obesity pathophysiology. The underweight group did not differ significantly from the healthy group in terms of periodontal disease indicators. The obese group had significantly deeper PPD, higher BOP, PESA, and PISA, and fewer teeth than the healthy group. The obese group had also significantly higher %FEV_1 and triglyceride, LDL, FBG, and HbA1c levels, with lower HDL values.

Table 5 Intergroup comparisons of participants stratified by Body Mass Index (*n* = 791)

Variables	**Healthy**	**Underweight**	**Obesity**		***P* value**
	n = 421 (53.2%)	*n* = 22 (2.8%)	*n* = 348 (44.0%)		
Periodontal parameters					
Number of teeth	27.0 (4.2)	26.4 (3.7)	25.4 (5.8)	***	< 0.001†
PPD (mm)	2.7 (0.5)	2.7 (0.5)	2.9 (0.7)	***	< 0.001†

BOP sites / mouth	17.1 (20.8)	15.5 (17.3)		22.1 (25.3)	***	0.012†
PESA (mm^2)	1392.7 (308.5)	1325.4 (267.2)		1429.8 (413.6)	*	0.166†
PISA (mm^2)	192.0 (284.1)	162.3 (212.6)		268.3 (350.5)	***	0.004†
Health examination						
Age	48.7 (9.7)	49.1 (8.0)		51.0 (9.2)	**	0.003¶
Weight (kg)	64.4 (8.0)	47.6 (5.4)	***	80.7 (12.0)	***	< 0.001†
BMI (kg/m^2)	22.2 (1.7)	17.5 (0.9)	***	28.0 (3.1)	***	< 0.001†
Triglyceride (mg/dL)	109.8 (78.2)	69.3 (29.2)	**	150.5 (98.9)	***	< 0.001†
LDL (mg/dL)	118.7 (28.0)	107.1 (37.3)		127.1 (30.1)	***	< 0.001†
HDL (mg/dL)	63.0 (15.8)	81.8 (21.8)	***	51.7 (11.1)	***	< 0.001†
FBG (mg/dL)	100.9 (8.3)	97.6 (10.1)		115.5 (24.6)	***	< 0.001†
HbA1c (%)	5.37 (0.20)	5.59 (0.29)	***	5.99 (0.92)	***	< 0.001†
% FEV_1	79.0 (6.9)	82.9 (8.3)	*	80.6 (5.4)	**	0.001†
CAVI	7.8 (0.9)	8.1 (0.8)		7.8 (0.9)		0.426¶
FIB4 score	1.09 (0.46)	1.11 (0.34)		1.08 (0.46)		0.916¶
Smoking (*n*, (%))						<0.001§
Never	200 (47.5)	10 (45.5)		117 (33.6)		
Former	120 (28.5)	2 (9.1)		104 (29.9)		
Current	101 (24.0)	10 (45.5)		127 (36.5)		
Occlusal function (Total *n* = 588)						
n	316	12		260		
Bite force (N)	410.5 (243.9)	426.1 (307.1)		395.5 (240.5)		0.727¶

Data are shown as means ± SD. P value was calculated from one way analysis of variance (ANOVA) ¶ or Kruskal-Wallis tests for continuous variables or Pearson's chi-squared tests§ for categorical variables. *p < 0.05, **p < 0.01, ***p < 0.001 vs. healthy group.

(3) Health status stratified by diabetes index

Table 6 shows 582 (56.9%), 353 (34.5%), and 87 (8.5%) employees were healthy, borderline diabetic, and diabetic according to the diabetes pathophysiology classification index. The diabetes group had fewer teeth and deeper PPD than the healthy group. They were also heavier and had a higher BMI and higher triglyceride, FBG, and CAVI levels, but lower HDL levels and occlusal force.

Table 6 Intergroup comparisons of participants stratified by HbA1c (*n* = 1,022).

Variables	**Healthy**	**Borderline**		**Diabetes**		***P* value**
	n = 582 (56.9%)	*n* = 353 (34.5%)		*n* = 87 (8.5%)		
Periodontal parameters						
Number of teeth	26.7 (4.6)	25.8 (5.2)	***	23.7 (6.5)	***	< 0.001†
PPD (mm)	2.8 (0.6)	2.8 (0.6)		3.0 (0.6)	***	0.005¶
BOP sites / mouth	18.0 (21.0)	19.9 (24.8)		19.9 (24.2)		0.415†
PESA (mm^2)	1402.7 (327.5)	1405.0 (396.8)		1347.2 (410.2)		0.465†
PISA (mm^2)	206.0 (283.2)	239.9 (348.4)		251.5 (351.1)		0.202†
Health examination						
Age	49.0 (9.7)	53.2 (9.4)	***	56.0 (9.2)	***	< 0.001¶
Weight (kg)	67.8 (11.0)	70.5 (13.6)	***	76.8 (15.3)	***	< 0.001†
BMI (kg/m^2)	23.4 (3.1)	24.6 (3.8)	***	26.7 (4.5)	***	< 0.001†
Triglyceride (mg/dL)	121.6 (89.4)	126.6 (80.0)		154.2 (113.5)	***	0.037†
LDL (mg/dL)	121.1 (29.2)	123.1 (29.9)		119.2 (29.2)		0.429¶
HDL (mg/dL)	60.7 (16.0)	58.0 (14.7)	*	52.3 (13.5)	***	< 0.001†
FBG (mg/dL)	101.4 (8.6)	109.7 (12.9)	***	154.3 (28.8)	***	< 0.001†
HbA1c (%)	5.39 (0.19)	5.89 (0.19)	***	7.70 (1.15)	***	< 0.001†
% FEV_1	79.5 (6.7)	79.6 (6.4)		79.5 (5.9)		0.962¶
CAVI	7.8 (0.9)	8.0 (0.9)	***	8.4 (1.0)	***	< 0.001¶

FIB4 score	1.08 (0.45)	1.15 (0.52)	1.24 (0.45)	***	0.003†
Smoking (*n*, (%))					0.004§
Never	260 (44.6)	122 (34.6)	24 (27.6)		
Former	165 (28.4)	112 (36.3)	32 (36.8)		
Current	157 (27.0)	119 (33.7)	31 (35.6)		
Occlusal function (Total *n* = 764)					
n	431	264	69		
Bite force (N)	416.5 (243.7)	384.1 (249.8)	334.3 (287.5)	*	0.002¶

Data are shown as means ± SD. P value was calculated from one way analysis of variance (ANOVA) ¶ or Kruskal-Wallis tests for continuous variables or Pearson's chi-squared tests§ for categorical variables. *p < 0.05, ***p < 0.001 vs. healthy group.

(4) Health status stratified by chronic obstructive pulmonary disease index

Table 7 shows that 309 (40.0%) and 464 (60.0%) employees, respectively, had mild and moderate COPD according to the pathophysiology classification index. Fewer teeth, higher PPD, FBG, HbA1c, and CAVI, and significantly decreased occlusal force were associated with moderate COPD than with mild COPD.

Table 7 Intergroup comparisons of participants stratified by %FEV_1 (*n* = 773).

Variables	**Mild**	**Moderate**	***P* value**
	n = 309 (40.0%)	*n* = 464 (60.0%)	
Periodontal parameters			
Number of teeth	27.3 (3.8)	25.3 (5.8)	< 0.001†
PPD (mm)	2.7 (0.5)	2.9 (0.6)	< 0.001†
BOP sites / mouth	18.5 (21.0)	18.4 (22.1)	0.931†
PESA (mm^2)	1412.7 (293.1)	1393.2 (412.5)	0.618†

PISA (mm^2)	203.7 (260.9)	230.5 (345.4)	0.569†
Health examination			
Age	46.7 (8.9)	54.1 (9.4)	< 0.001¶
Weight (kg)	68.1 (12.0)	68.8 (11.0)	0.408†
BMI (kg/m^2)	23.6 (3.5)	23.8 (3.0)	0.280¶
Triglyceride (mg/dL)	123.3 (89.0)	128.5 (100.4)	0.450¶
LDL (mg/dL)	120.5 (28.5)	120.9 (30.4)	0.893¶
HDL (mg/dL)	60.7 (16.3)	59.6 (15.4)	0.333¶
FBG (mg/dL)	100.9 (8.8)	109.6 (19.7)	< 0.001†
HbA1c (%)	5.38 (0.18)	5.78 (0.73)	< 0.001†
% FEV_1	84.1 (3.4)	74.0 (4.8)	< 0.001†
CAVI	7.6 (0.9)	8.1 (0.9)	< 0.001¶
FIB4 score	1.00 (0.40)	1.21 (0.51)	< 0.001†
Smoking (*n*, (%))			< 0.001§
Never	160 (46.5)	180 (32.8)	
Former	93 (27.0)	202 (36.9)	
Current	91 (26.5)	166 (30.3)	
Occlusal function (Total *n* = 585)			
n	218	367	
Bite force (N)	432.3 (241.2)	373.8 (238.1)	0.001¶

Data are shown as means ± SD. †Mann Whitney U tests. Smoking data are shown as *n* (%). §Pearson's chi-squared tests.

(5) Health status stratified by atherosclerotic cardiovascular disease index

Table 8 shows that 245 (46.7%), 187 (35.6%) and 93 (17.7%) employees were respectively healthy, borderline, and had suspected ASCVD according to the atherosclerotic pathophysiology classification index. Those with ASCVD had fewer teeth,

increased FBG, and HbA1c levels, and decreased %FEV_1 and occlusal force. However, BMI did not significantly differ among groups.

Table 8 Intergroup comparisons of participants stratified by CAVI (*n* = 525).

Variables	Healthy	Borderline		ASCVD		*P* value
	n = 245 (46.7%)	*n* = 187 (35.6%)		*n* = 93 (17.7%)		
Periodontal parameters						
Number of teeth	27.4 (6.9)	25.6 (4.8)	***	23.2 (6.9)	***	< 0.001†
PPD (mm)	2.7 (0.5)	2.8 (0.6)		2.9 (0.7)		0.016†
BOP sites / mouth	18.4 (22.0)	17.7 (21.0)		13.5 (16.0)		0.059†
PESA (mm^2)	1399.0 (274.6)	1385.6 (346.5)		1247.8 (359.6)	***	0.001†
PISA (mm^2)	201.5 (262.2)	210.9 (297.7)		157.4 (195.9)		0.141†
Health examination						
Age	45.9 (7.7)	56.6 (8.5)	***	64.2 (6.4)	***	< 0.001†
Weight (kg)	69.1 (11.9)	66.6 (10.5)	*	65.5 (9.8)	*	0.009†
BMI (kg/m^2)	23.6 (3.4)	23.9 (3.1)		23.3 (2.7)		0.335†
Triglyceride (mg/dL)	116.7 (73.6)	130.5 (108.7)		127.6 (87.1)		0.251†
LDL (mg/dL)	123.3 (27.9)	119.7 (28.7)		118.0 (27.0)		0.200¶
HDL (mg/dL)	60.4 (14.7)	58.5 (15.1)		60.7 (16.6)		0.375¶
FBG (mg/dL)	100.5 (8.4)	110.7 (21.2)	***	118.1 (20.4)	***	< 0.001†
HbA1c (%)	5.38 (0.20)	5.84 (0.76)	***	5.95 (0.68)	***	< 0.001†
% FEV_1	80.2 (6.3)	79.0 (6.2)	*	76.7 (7.6)	***	< 0.001†
CAVI	7.2 (0.5)	8.4 (0.3)	***	9.5 (0.5)	***	< 0.001†
FIB4 score	1.00 (0.35)	1.24 (0.49)	***	1.65 (0.61)	***	< 0.001†
Smoking (*n*, (%))						0.001§
Never	110 (44.9)	70 (37.4)		36 (38.7)		
Former	65 (26.5)	69 (36.9)		45 (48.4)		

Current	70 (28.6)	48 (25.7)		12 (12.9)		
Occlusal function (Total *n* = 403)						
n	186	140		77		
Bite force (N)	456.3 (256.6)	357.8 (199.2)	***	316.0 (200.0)	***	< 0.001[†]

Data are shown as means ± SD. P value was calculated from one way analysis of variance (ANOVA) [¶] or Kruskal-Wallis tests for continuous variables or Pearson's chi-squared tests[§] for categorical variables. [*]p < 0.05, [***]p < 0.001 vs. healthy group.

(6) Factor analysis of oral and systemic health status interdependence

Regarding Model 3 (representing reference Model 1, adjusted for both age and smoking status), multiple logistic analyses were carried out to examine the bidirectional effects on oral and systemic health. Analysis of the effect of higher tooth number on systemic disease revealed a significantly decreased risk of obesity (OR, 0.95; 95% CI, 0.92–0.99; p = 0.006), and diabetes mellitus (OR, 0.96; 95% CI, 0.92–1.00; p = 0.040). However, risk of the following: IGT (OR, 0.96; 95% CI, 0.91–1.01; p = 0.077), moderate COPD (OR, 0.98; 95% CI, 0.94–1.02; p = 0.249), and ASCVD (OR, 1.02; 95% CI, 0.96–1.09; p = 0.456) was not decreased. A deeper PPD also significantly correlated with increased risk of obesity (OR, 1.42; 95% CI, 1.09–1.84; p = 0.009), IGT (OR, 1.48; 95% CI, 1.00–2.20; p = 0.049), and moderate COPD (OR, 1.38; 95% CI, 1.02–1.88; p = 0.038). However, the risks of diabetes (OR, 1.36; 95% CI, 0.96–1.91, p = 0.083) and ASCVD (OR, 1.61; 95% CI, 0.91–2.87; p = 0.105) were not increased (Fig. 10).

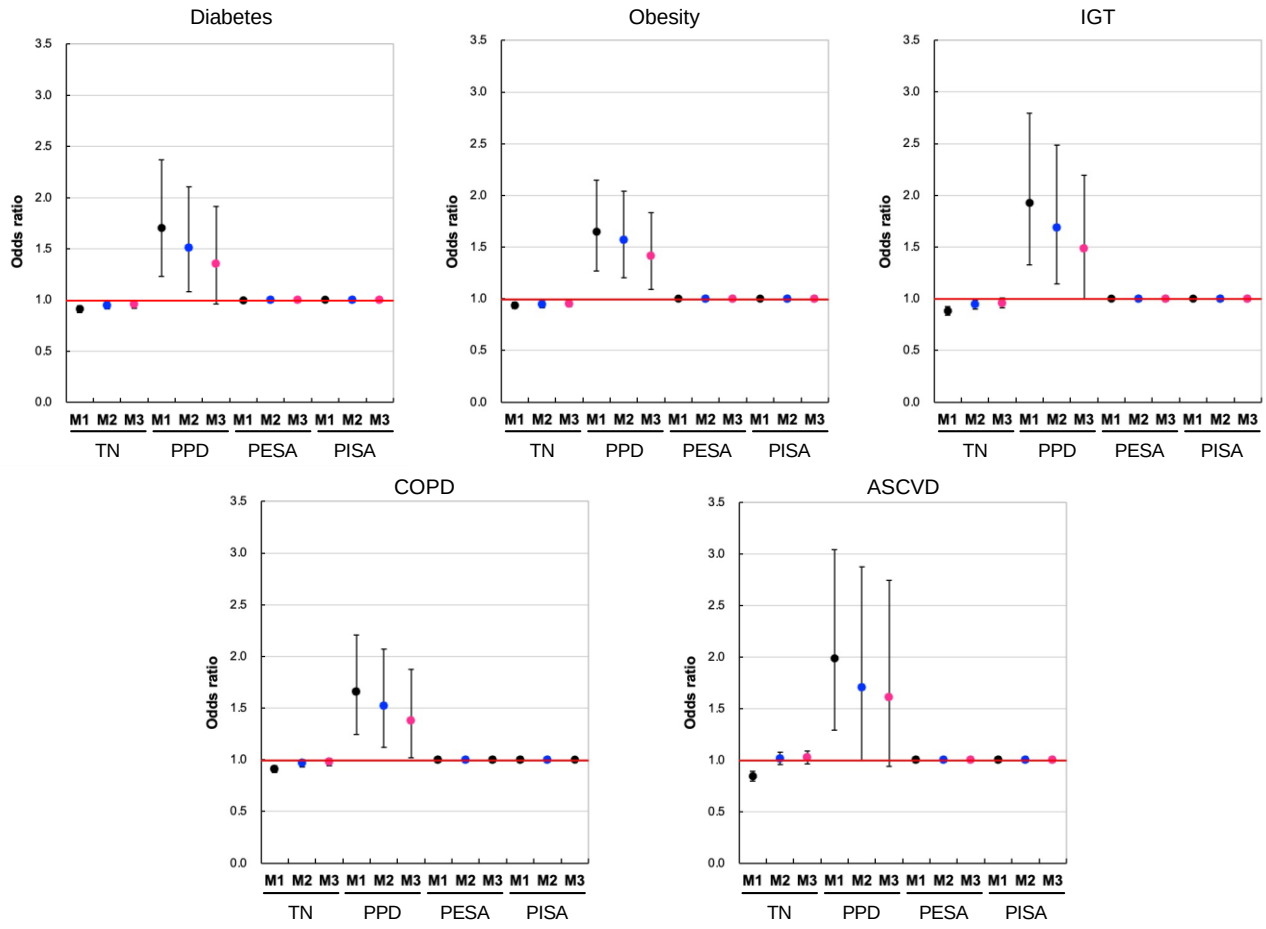

Fig. 10 Effects of periodontal disease indicators on risk of diabetes, obesity, IGT, COPD, and ASCVD.

Model 1 (M1) represents the reference model, created based on healthy subjects with an HbA1c value below 5.7. Model 2 (M2) represents M1 adjusted for age. Model 3 (M3) represents M1 adjusted for age and smoking status. Markers indicate the estimated odds ratio (OR). Vertical ranges indicate the 95% confidence interval (CI).

Meanwhile the effect of systemic disease on periodontal disease status revealed that a high BMI (OR, 1.28; 95% CI, 1.15–1.42; $p < 0.001$), HbA1c (OR, 4.34, 95% CI, 1.89–9.98, $p < 0.001$), FBG (OR, 1.08; 95% CI, 1.04–1.11; $p < 0.001$), and smoking (OR, 2.32; 95% CI, 1.62–3.33; $p < 0.001$; Model 2) significantly increased with worse periodontal disease. A low $\%FEV_1$ (OR, 0.95; 95% CI, 0.91–1.00; $p = 0.031$) significantly increased

with worse periodontal disease. However, CAVI (OR, 0.90, 95% CI, 0.52–1.43; p = 0.569) did not affect risk of developing periodontal disease (Fig. 11).

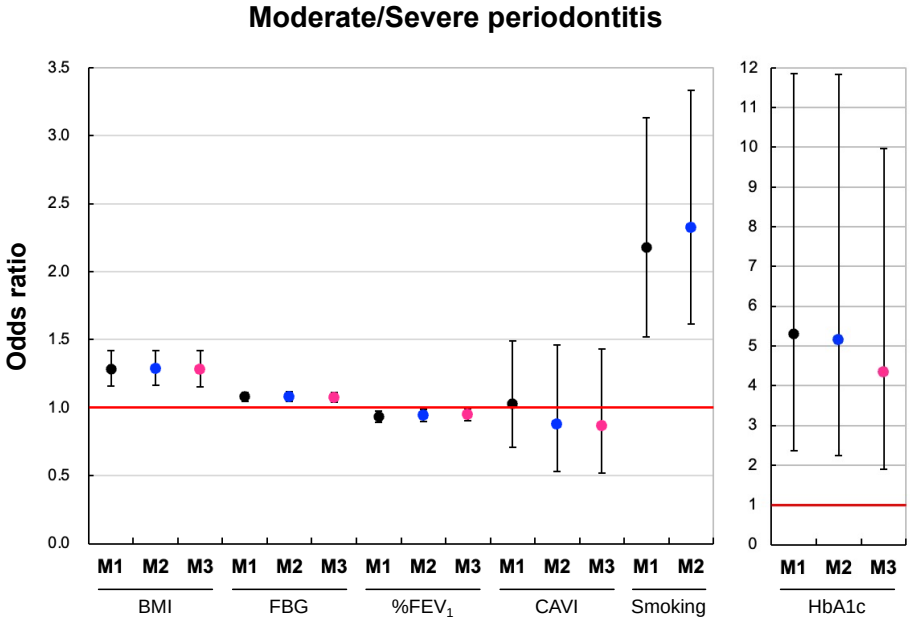

Fig. 11 Effects of BMI, FBG, % FEV_1, CAVI, smoking, and HbA1c on severe periodontal disease.

Model 1 (M1) represents the reference model, created based on healthy subjects with an HbA1c value below 5.7. Model 2 (M2) represents M1 adjusted for age. Model 3 (M3) represents M1 adjusted for age and smoking status. Markers indicate the estimated odds ratio (OR). Vertical ranges indicate the 95% confidence interval (CI).

3-4. Discussion

(1) Study on the relationship between periodontal disease and general health

Evidence about a relationship between periodontal disease and general health has accumulated, but the causal relationship and the effect of treating periodontal disease on improving general health remains obscure.

This epidemiological study aimed to maintain and improve systemic health by preventing and managing periodontal disease more effectively in the future, as well as relevant research.

Considering such a model, a new oral health workplace model that allows consumers to participate in semi-enforced health examinations at the lowest possible cost is needed rather than leaving the choice of whether or not to undergo such checks up to individuals. Thus, I considered that the most efficient way to achieve this would be to add periodontal disease tests to the annual medical examinations for employees, which is obligatory for all Japanese companies.

(2) Issues of cross-sectional study on periodontal disease and general health

I examined the likelihood of an epidemiological association between periodontal and systemic diseases centered on NCDs in Japanese adults. This required the random selection of a statistically relevant number of participants of various ages, continuous accurate and reproducible quantitation, introduction of periodontal examinations, and appropriate data management. The Hitachi Health Care Center (HHCC) of Hitachi, Ltd., which conducted the clinical part of this study uses information technology to prevent the onset and aggravation of lifestyle-related diseases such as diabetes among employees and to determine the quality of medical care using medical data. The HHCC actively promotes enhancements in the overall medical examination process with the aim of improving care and reducing costs. The HHCC conducts medical examinations for ~80 employees/day and 12,000–18,000 employees annually. A periodontal examination was introduced to their medical examinations and a research protocol was established through collaboration with a dental hygienist from Lion Dental Hygienist Institute and a periodontal disease specialist from the Department of Periodontal Disease Treatment, Faculty of Dentistry, Osaka University. Thus, I and my team designed the research protocol, and conducted data acquisition and I analyzed the data. The entire research plan was formulated by three parties, and I took the lead in data acquisition and analysis.

The criteria for determining periodontally and systemically healthy were required. Although many epidemiological studies have investigated the relationship between periodontal disease and general health, differences among the criteria applied to diagnose periodontal disease have hampered reproducibility (60,61). Periodontal disease severity is determined by measuring clinical attachment loss (CAL), which is the condition under which the gingiva adheres to and supports teeth. However, this requires precise measurements of numerous participants which is inappropriate for large-scale epidemiological studies, and several methods can be applied. For example, examining representative teeth using the Community Periodontal Index (CPI), developed by the WHO, is often adopted in large-scale cohort studies, but some problems are associated. It is useful as a surveillance index of periodontal status in populations but is unsuitable as an outcome of analytical epidemiological studies (62), and periodontal status might be underestimated. Furthermore, sex is a factor that can affect periodontal status (63,64). Furthermore, the periodontal disease index in all teeth should be determined to improve the accuracy of the results (65). Therefore, we measured periodontal pocket depth and bleeding in six sites per tooth. The consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions reclassified periodontitis and added stages (52). I compared healthy employees without periodontal pocket depth PPD ≥ 4 mm and HbA1c < 5.7 with those who had stages I and II (maximum PPD ≥ 4 to < 6 mm) or stages III and IV (maximum PPD ≥ 6 mm) periodontitis.

The criteria for determining diseases differ among races. Although many epidemiological studies have investigated the link between diabetes and obesity and periodontal disease in the USA and Europe, the results do not necessarily provide a comprehensive perspective in other populations (66,67). For example, the proportion of obese Asians with a body mass index (BMI) ≥ 30 kg/m^2, is lower than in Western Europe (67), but obesity-related complications increase. The Japan Society for the Study of Obesity defines obesity as BMI ≥ 25 kg/m^2 (55). Thus, considering differences among races, epidemiological studies in Japan were required to clarify the relationship between periodontal disease and the general health of Japanese citizens.

The criteria for healthy controls required some adjustment. Stratified analyses of data from the healthy group and the disease group classified by disease indexes are problematic. Initially, I stratified participants as healthy, or having mild/moderate, or severe periodontal disease according to the periodontal disease classification criteria and analyzed the general health indicators of each group. The mean diabetes index (HbA1c) and FBG of the healthy group were 5.71% ± 0.76% and 105.7 ± 19.1 mg/dL, respectively. The HbA1c and the FBG of healthy participants defined < 5.7% and < 100 mg/dL according to the diabetes diagnostic criteria and the fasting blood glucose standard, respectively. Therefore, when healthy participants were stratified only by the periodontal disease classification, a relationship with general health status could not be accurately verified. Then I introduced the condition HbA1c < 5.7% into the classification of "healthy", excluded irrelevant data, and repeated the stratification analysis. This reduced the number of healthy participants (n = 62), but the average HbA1c in the healthy group according to the periodontal disease classification was 5.44% ± 0.21%, and the FBG was 98.7 ± 8.0 mg/dL, which improved the accuracy of the results. Therefore, HbA1c < 5.7% was included as a criterion for health in subsequent analyses.

(3) Relationship between periodontal disease and diabetes

Diabetes is a metabolic disorder characterized by chronic hyperglycemia (56), which causes abnormalities in various organs and complications such as retinopathy, nephropathy, neuropathy, and cardiovascular disease (68) and increases the risk of periodontal disease (69). Here, I analyzed the effects of the periodontal disease indices, *periodontal* probing depth (PPD), bleeding on probing (BOP), periodontal surface area (PESA), periodontal inflamed surface area (PISA) and tooth counts on diabetes. The PPD results showed that periodontal disease increased the risk of diabetes 1.4-fold and the HbA1c results showed that diabetes increased the risk of periodontal disease 4.3-fold, regardless of age, sex, or smoking habit. Kocher *et al*. (70) summarized the findings of typical epidemiological studies of periodontal disease and diabetes and found that the OR of periodontal disease risk associated with diabetes was increased 1.17- to 3.84-fold,

and a study of Pima Indians found an 11.4-fold increase. he OR in the present study was higher than that previously reported. This might have been due to the improved stratification of healthy controls with respect to periodontal disease. Our findings confirmed a bidirectional relationship between periodontal disease and diabetes, but this will require further verification in a larger cohort.

(4) Relationship between periodontal disease and obesity

Obesity is a chronic inflammatory disease characterized by excessive fat accumulation in adipose tissues (71). Increased oxidative stress can lead to periodontal disease as well as local and systemic vascular endothelial damage (72). I found that employees with mild and moderate/severe periodontal disease had a significantly higher BMI than healthy employees with HbA1c < 5.7%. The results of multiple logistic regression analysis showed that high BMI increased the risk of moderate/severe periodontal disease 1.3–fold. Conversely, having more teeth was associated with a reduced risk of obesity, and an increase in PPD increased risk of obesity 1.4–fold. Blood HDL values were significantly reduced and increased in employees classified as obese and underweight ($p < 0.001$ for each). High-density lipoprotein exerts anti-inflammatory effects (73). Decreased HDL levels cause bacterial toxin-induced inflammation and blood coagulation responses that increase susceptibility to inflammatory stimuli (74). *P. gingivalis* induces HDL oxidation and induces inflammation through interaction with monocytes/macrophages (75), and derived LPS suppresses adiponectin secretion from adipocytes (76). The present findings clearly support a relationship between periodontal disease and obesity.

(5) Relationship between periodontal disease and chronic obstructive pulmonary disease

Periodontal disease and COPD are related because they are chronic inflammatory diseases accompanied by the destruction of connective tissues and have many common factors such as advanced age and smoking (77). The $\%FEV_1$ was significantly lower among employees with severe periodontal disease than those who were classified as healthy. The results of multiple logistic analysis adjusted for age and smoking also showed that COPD increased the risk of periodontal disease. In contrast, having more

teeth reduced the risk of COPD, and an increase in PPD could increase the risk of COPD 1.4-fold. However, the BOP and PISA indicators that are associated with inflammation did not significantly differ. A meta-analysis of an investigation into the association between periodontal disease and COPD found increased PPD and attachment loss in patients with COPD, but bleeding and inflammation differed, thus the results were unclear (78). I did not find an association between COPD and BMI or the dental inflammation indices BOP and PISA. This might have because none of employees were classified as having Type III or IV COPD in the Chronic Obstructive Pulmonary Disease Classification according to the Gold Guidelines, so further verification is needed. A comparison of dentate employees diagnosed with COPD and those with healthy periodontal tissue (teeth, gingival condition) showed that more dentate patients were hospitalized, indicating a high risk of death and that teeth affect the pathophysiology of COPD (79). These findings indicated that risk for COPD might be reduced by early and appropriate treatment of periodontal disease.

(6) Relationship between periodontal disease and atherosclerotic cardiovascular disease

The link between periodontal and atherosclerotic heart diseases is a topic of interest, given the overlap with chronic inflammatory diseases with common risk factors such as connective tissue destruction, advanced age and smoking (80). The cardio-ankle vascular index (CAVI) of atherosclerosis was significantly increased in employees with moderate/severe periodontal disease. The results of multiple logistic regression analyses adjusted for age and smoking suggested that a higher CAVI increases risk for moderate/severe periodontal disease. Conversely, PPD did not differ, but employees classified as having atherosclerotic heart disease had significantly fewer teeth and a smaller PESA compared with the healthy group. An association between periodontal and cardiovascular diseases has been established and characterized, but the cause(s) remains unclear (81).

(7) Necessity of introducing dental medical examination to corporate medical examination

Considering current dental practice, major reforms in the following key areas would be required to achieve the fundamental goals of preventing periodontal disease: universally available, essential oral health-care services that meet the most prevalent population needs, innovative oral health workforce models and training, an health system governance context that would enable a flexible continuum of patient-centered support, with appropriate quality of services, integrated surveillance, program monitoring, and implementation research to ensure appropriate health outcomes, and a shift in intervention focus to upstream population-wide policies (82). Poudel *et al.* (83) and Sanchez *et al.* (84) have introduced mechanisms to improve the oral health of patients, and they have medical personnel educate patients about the increased risk of oral complications and provide advice regarding regular dental examinations. Income and education are important factors that affect general health, and the existence of health inequalities has become a major problem (85). To help resolve this, I encourage people to change their awareness of and attitudes to maintaining and promoting general health through evidence-based education and information dissemination regarding the relationship between care of the oral cavity and general health, and socio-economic factors. I aimed to build an oral health care system that allows anyone to participate in medical examinations regardless of their socioeconomic status.

The corporate medical examination system in Japan is unique. he Industrial Safety and Health Act of Japan requires employers to provide their employees with annual health checks, but dental checks are not a requirement (86). This health care system allows all employees to participate in annual medical examinations without discrimination by income; thus, the accumulated annual data can monitor individual health status before the onset of a disease and the status of a disease over time. A future predictive model of health status might be generated from analyzing such data. This system that essentially forces employees to undergo medical and periodontal examinations as well as disease monitoring is less likely to cause economic disparity than other medical examinations. Mass periodontal disease examinations would lead to identifying the risk of general

health deterioration without incurring high examination costs, and the importance of oral health care could be disseminated.

This cross-sectional study proceeded at HHCC, which collects health information about its employees from the moment they are employed until they retire. Thus, the company has implemented the early detection of systemic diseases and other health care measures for employees to prevent extant diseases worsening. I revealed that maintaining employee health was associated with better productivity throughout the company. Moreover, reduced medical expenses lead to a more sustainable health care system. However, Japanese companies do not implement dental checks with the same vigilance as regular health checks. We introduced our first dental check for one week in 2014 at no additional cost. The participation rate in the dental checks at that time was 63.6% (2014) and few employees were aware of their importance. However, repeating the same dental checks annually thereafter resulted in the participation rates of 81.8% (2015), 89.7% (2016), and 85.1% (2017). Visits to dental clinics in Japan are often limited to the appearance of subjective symptoms, and dentistry remains outside the scope of regular health maintenance or checks. However, this trend is not unique to Japan. Economic status and lack of knowledge about oral healthcare are also barriers to including dentistry in regular health checks in other countries (84). The association between periodontal disease and general health was communicated to the employees *via* the internal public relations department, which improved collective understanding of the importance of dental health examinations, at least in that workplace. Moreover, I and my team provided these examinations as part of the usual health checks without incurring additional cost, which increased participation rates. When a need for dental treatment was detected during the examination, we provided referral letters to encourage more thorough investigation. We also established a medical test feedback system to evaluate the extent to which dental treatment contributes to better health, and its effects on health insurance expenditure. Japan has the highest life expectancy in the world, but healthcare costs are increasing due to the expansion of advanced medical care and the aging population. I consider that

expanding systems such oral healthcare will help to damp continuously increasing medical expenses.

The cross-sectional study added dental checks to the regular annual health checks of employees at Hitachi Ltd. I identified an association between periodontal disease and occlusal status with NCD-centered health indicators among Japanese employees. I found that changes in occlusal force varied by age, and that future health issues could be more accurately predicted from results of routine dental checks. The introduction of dental checks into compulsory annual corporate health checks facilitated the early detection of periodontal disease as well as therapeutic intervention. Such medical-dental collaborations reflect the importance and potential of building a comprehensive regional health care system that maintains and promotes good general health.

Chapter 4. General discussion and conclusion

Oral health is a key indicator of general health and includes many important functions such as breathing, eating, talking, smiling and socializing. These are essential elements of a good quality of life QOL, and they should be maintained and improved throughout life. Considering the prevalence of periodontal disease in Japan and overseas and its relationship with NCDs, the development of new therapeutic agents and an oral healthcare system that can monitor the health status of the oral cavity as well as the rest of the body will contribute to a reduction in medical expenses. I developed KYT-41, a dual inhibitor that can suppress most of the pathogenic effects of *P. gingivalis*, the causative agent of periodontal disease. KYT-41 is safe, specific, stabile, and is thus considered useful as a therapeutic agent. Obtaining approval as a new treatment for periodontal disease requires time and development costs. A US research group has announced the potential of anti-gingipain antibodies to control the progression of Alzheimer disease (6) and is applying to the FDA for clinical trials. I also consider promoting technological innovation to control and treat systemic and periodontal diseases in Japan. I and my team devised and implemented a model that utilized annual corporate health checks to secure evidence of a relationship between

periodontal disease and general health among Japanese employees and to promote periodontal disease prevention and treatment. I secured mutual risk data between disease and general health and showed the usefulness of the system. Evidence-based information dissemination and early detection and treatment intervention for periodontal disease utilizing such a system should become the standard model for future healthcare systems not only in Japan but also elsewhere. The Japanese Cabinet Office has announced that it will introduce a national directive that will require all citizens to undergo an annual dental examination. This directive will be published in the Basic Policy on Economic and Fiscal Management and Reform 2022 that will be compiled in June 2022. Regular dental checks will reduce the effects on periodontal disease and general health, leading to a reduction in medical expenses. I consider that the relationship between periodontal disease and general health among Japanese workers identified herein and the oral health care model will greatly contribute to national policies and help ease the burden of skyrocketing healthcare costs.

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Original publications

1. A novel, potent dual inhibitor of Arg-gingipains and Lys-gingipain as a promising agent for periodontal disease therapy.

   Shinsuke Kataoka, Atsuyo Baba, Yoshimitsu Suda, Ryosuke Takii, Munetaka Hashimoto, Tomoyo Kawakubo, Tetsuji Asao, Tomoko Kadowaki, and Kenji Yamamoto.

   The FASEB Journal, 2014, 28(8): 3564-3578.

   https://doi.org/10.1096/fj.14-252130

2. A cross-sectional study of relationships between periodontal disease and general health: The Hitachi Oral Healthcare Survey.

   Shinsuke Kataoka, Mitsuo Kimura, Tsuguno Yamaguchi, Kenji Egashira, Yu Yamamoto, Yasushi Koike, Yuki Ogawa, Chika Fujiharu, Toshiko Namai, Kanako Taguchi, Momoko Takahashi, Asami Kameda, Tomoka Kasen, Asami Higashi, Komomi Kubota, Masayuki Sato, Hiroaki Yamaga, Kaori Nohara, Mikiko Shirasawa, Chika Sekine, Maki Fukuda, Arisa Aoki, Yurina Takeuchi, Misaki Mugiyama, Kenta Mori, Keigo Sawada, Yoichiro Kashiwagi, Masahiro Kitamura, Takeshi Hayashi, Tohru Nakagawa, Shinya Murakami.

   BMC Oral Health, 2021 Dec 15;21(1): 644-660.

   https://doi: 10.1186/s12903-021-01990-6.