Characterization and eradication of the high concentration infection source of oral segment of Candida by infection examination diagnosis system

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Research Article

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Characterization and eradication of the high concentration infection source of oral segment of *Candida* by infection examination diagnosis system

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Abstract

Purpose: *Candida albicans* (CA) is a major pathogenic fungus that causes a critical infection and life-threatening disease. Currently, during the treatment of COVID-19 (in ICU), invasive Candidiasis and Candida colonization should be prevented to avoid the increase, in mortality, in SAPSII, and in length of stay. Our aim was to characterize and analyze the infection of CA for eradication.

Methods: We characterized and analyzed the infection source and infection field which are carrier field, diffusion field, and spread field, by using a new infection examination (diagnostic) system (IEDS) comprising a new dental formula (measurement analysis) medium (DFM) with time, space, and phase, and the simultaneous relative differential equations (C4RDE).

Results: This study showed that CA was not an endogenous microorganism, but a fungus that infected teeth causing dental caries that was successfully eradicated from all teeth of 353 Japanese patients.

Conclusion: Our methods help to eradicate the infection source, prevent critical infection, and reduce mortality. The IEDS is a powerful tool that can be applied for any infection easily. It is possible for the dentists to characterize CA by IEDS and eradicate CA from the oral cavity. IE(D)S will dramatically reduce the threat to the living body by pathogenic microorganisms.

Keyword: *Candida albicans*, IEDS, tooth caries, infection source, infection field, diffusion field, carrier field, spread field, infection chain, relative differentiation, ventilator, receptor field, resistance field, adhesive colony, fixed colony,

Abbreviation (see Supplementary **Abbreviation)**

Introduction

Candida albicans (CA) is a fungus that causes a life-threatening infection [1-3]. Furthermore, CA colonization on airway is associated with pneumonia, malignant tumors, failure of organ transplantation, ocular opacities, digestive disorders, and respiratory disorders [3-11]. Moreover, a recent study [12] suggested an adverse influence of COVID-19-associated pneumonia with CA, on the oral cavity, pharynx, heart, liver, spleen, central nervous system, urinary organs, eye, skin, vagina, and the endocrine system, any of which can be the cause of the infection [1, 2]. This leads to a synergistic biofilm formation which causes virulence [13]. Conventionally, as CA is considered an endogenous microorganism, there are few existing methods of disinfection of CA from a living body or the prevention of transmission to living body.

Materials and Methods

In our study, we have eradicated CA from all the teeth of 353 Japanese patients and showed that CA was not an endogenous microorganism (Tables 1, 2). The receptive property, resistance property, adhesion property (the condition equivalent to colonization [3-10],and fixing property (condition equivalent to pneumonia [3, 10]([4-9] may be included.) of the examination tissue and teeth are indicated by the infection examination system (IES) (Figs. 1, 2) which is the new dental formula (measurement analysis) medium (DFM) with time, space, and phase (Figs. 3–5) (Supplementary Notes S1–S27) and the simultaneous relative differential equations (C4RDE), calculated as the infection examination diagnosis system (IEDS) [14] especially the RDE (SOC). the observation object itself takes arithmetic circuit and display, one can observe phenomenon, directly [14](Fig. 1).

The medium is observed as the infection source (IS) (field), diffusion field (DF), spread field (SF), and carrier field (CF), which comprise the infection field (IF), and the continuum of the infection field (IF) is the infection chain (IC). (Figs. 1 and 2, and in Supplementary Notes S1–S10)

The fields and IS are expressed by the two equations in C4RDE [14], which describe the active property (active coefficient ε_a) and the decline property (decline coefficient ε_d) for the IF. In addition, the equation (C4RDE) provides the infection solution and the defense solution from the medium. The solutions are defined by infection function (infection coefficient γ_i) and defense function (defense coefficient γ_d). For each of the two or more factors (the number of factors is unrestricted), the active coefficient and decline coefficient are subtracted and added. This differentiation does not include rotation and vibration. It does not have an error with an infinitesimal real number. Therefore, The RDE is the only equation whose simultaneous is possible [14].

Each field and IC

In the diffusion field (DF), microorganisms diffuse from IS by potential energy. The spread field (SF) is defined as the spread, without a concentration gradient. The carrier field (CF) is defined as the field in which microorganisms are transferred by carrier (e.g., ventilator, toothbrush, floss, and

food). The infection field (IF) consists of the IS (in the segment) and the three fields (DF, SF, and CF). The continuum of the IF is the IC. In the IF, microorganisms encounter an organ. If the microorganism can adhere (adhesion stage colonization) to the substance that encountered the receptor field (RcF), it will colonize the substance. In suitable conditions, the proportions of microorganisms increase (fixing stage colonization), the IS will be generated, and the IF will progress. The IF begins from the IS and is infected in three ways (IFF is infection field function): DF, SF, and CF. In the IS, contaminated substances are generated by the IF.

The IEDS aims to stop the IC by eradication of IS, disinfection of CF, investigation of resistance field (RsF), and obstruction of RcF-ized for the substance.

Medium (DFM)

A sterilization swab (SS; trace) of the whole tooth without contact with the adjacent tooth (Fig. 3a) was performed. Then, the swab was applied to the area of dental formula (The trade mark and pat of dental formula, MicroDent Co., Ltd.) on corresponding medium (The CHROMagar™ Cnadida, BD BBL™, Becton, Dickinson and Company,) (**Supplementary Notes S11–S13**).

Medium (DFM): CHROMagar was used. Most of the fungi detected were *Candida albicans*. It can be sampled and cultivated at 37 °C for 48 h

The colony concentration (CC) on the medium in this sampling was calculated by the following equations. CAC refers to *Candida albicans* concentration.

CC of each dental formula area on the medium

∝ CAC of the tooth part (teeth parts) in contact with the SS (CWSS)

+ CAC in the saliva adhering to the teeth in CWSS

+ CAC in the saliva that is in the environment of CWSS

+ CAC of the (marginal) periodontal tissue in CWSS

+ CAC of the air in CWSS

This sampling includes swabs of the tooth, periodontal tissue, saliva, and air, and is the sum of the four parts. The CAC of tooth in contact with the SS includes CA and the IF from other teeth and organ(s).

1. The all-teeth examination (ATE) included the swab of all teeth (Fig. 3b).

2. The partial teeth examination (PTE) (tooth unit) included the swab of each tooth. The swab was applied to each area of the DFM (Fig. 3c). If a swab was negative after treatment and disinfection, this confirmed that the IS was tooth caries (Fig 4, 5).

Treatment procedure is provided in Supplement Fig S14.

C4RDE diagnosis equation is shown in Fig. 1 [14] (Supplementary SDE in C4RDE)

Results

According to C4RDE [14] and IFF (Fig. 1) corresponding to the model in Fig. 2, the infection is measured and analyzed, and it can be eliminated by treatment. The adhered (about γ_i=0) and fixed colonies (γ_i>0) can be detected, and disinfection can occur.

Especially, CA examination diagnostics (IEDS) by space, time, and phase analyses of teeth using DFM indicated that the biggest IF (including IS) of this fungus was tooth caries. (Fig. 4; Tables 3–10, Supplementary Table S23: space analysis) (Supplementary Notes S1–S28 and Supplementary Figs. S2–S5: time and phase analysis) (Supplementary Tables S1–S22). It is thought that the characteristics of IF and IC acquired from space, time, and phase analyses are useful for disinfection, prevention of transmission, and the control of microorganisms; however, they have also suggested the analysis of other microorganisms and viruses.

Treatment based on IEDS revealed that the CA elimination rate in the oral segment is approximately 100% as a result of elimination of dental caries and cleaning (Figs. 4, 5, Table 6, and Supplementary Fig S14). The combined treatment of dental caries and tooth cleaning by the antifungal agent [15] resulted in the eradication of CA by approximately 100% in the oral segment of the part of the IC.

CA and DMFT index (WHO) is decayed, missing, and filled tooth index.

Incidentally, in the first examination, the results were: CA (+) 40.5% (DMFT: 62.8%), CA (-) 56.4% (DMFT: 47.1%). The significant difference of DMFT was 15.7% ($P<0.01$). This showed that CA is not an endogenous microorganism. It suggests a relationship with dental caries (Table 1 and Supplementary Tables S1–S8).

CA in a dental-caries tooth

The IS is defined as the space that is colonized (adhesion stage or fixing stage) by microorganism (in the shape of microscopic image of caries tooth, multi formation images, such as fungi mycelium and spore, are seen (Supplementary Fig. 1)).

Fields

1. The main RcF is dental caries.

2. DF is about one tooth.

3. SF is not observed in CA; LB is observed.

4. CF is evaluated by the sampling by a swab; therefore, toothbrushes, ventilator, fine droplet nuclei, respiratory drop, ejected contaminated water drops by dental air turbine, and food are considered to be important CFs.

5. The tooth has a powerful RsF.

6. IF is eliminated in the oral segment.

7. IF is mathematically equivalent to any segment. It can be calculated for complicated systems using only two kinds of coefficient (active coefficient ε_a and decline coefficient ε_d).

8. The IS is detectable; therefore, it can be completely eradicated.

9. RcF is detectable, and hence, investigation of the IS is easy. Moreover, RcF, which is the risk field of infection, can be eliminated, and infection can be prevented.

10. As RsF is detectable, the prevention of transmission is possible.

Space analysis by dental formula (Supplementary Notes S18–S24)

The local IS indicated that teeth caries included the fixing colonization part of the CA (Fig. 4, Tables 3–10, and Supplementary Table S21). CA was detected in 243 (68.3%) teeth caries (C1 to C3 in 356 teeth). In adjacent healthy teeth (true healthy tooth [Adj-THT] + restored healthy teeth [Adj-RHT]) and non-adjacent healthy teeth {true healthy tooth [nAdj-THT] + restored healthy teeth [nAdj-RHT]), the values were 46 (12.9%) and 57 (16.0%), respectively.

When CA was present in many teeth, in the oral segment, it appeared to be an endogenous microorganism (Fig. 3e and 5) [22]. In contrast, when CA infections was present in a few teeth, it was fixed to the tooth and caused teeth caries (Fig. 3d and 4). A CA(+) caries tooth and a CA(-) caries tooth existed in the same mouth. (Supplementary Table S18). These indicate existence of a RsF, because, infection cannot be realized with the influence of CF because of tooth brushing.

Phase analysis by dental formula

1. The phase of time is observed as Fig4 and 5. It is useful for the prediction of infection.

2 The phase of space is observed as Fig4 and 5. It finds one IS buried in multiplex IS.

3. The phase of microorganisms (Especially, Only phase analysis, It define as comparison of multiple microorganisms.) It detects RcF or RsF. An interesting relationship between RsF and *Lactobacillus* (LB) is indicated (Supplementary Fig. 5).

Re-infection

Moreover, light adhesion (adhesive colony) was observed in some cases which could lead to re-infection; however, these cases were easily removed.

Discussion

There are many examples that omit the IEDS based on specific space, time, and phase of the IF, and these are confusing. There are studies describing the involvement of dental caries [15-18]; in contrast, there are studies not related to dental caries [19, 20]. It may be the feature of CA that discussion in [3-12] similar to VAP has generated. In these studies, there was no analysis of the infection caused by CA, the application of the antifungal agent was stopped, and there was no plan against re-infection; moreover, studies have been reported regarding the use of antifungal agents for infection in root canal (*Pseudomonas* [9] is treated as an important pathogenic bacteria of apical periodontitis), but there is a lack of studies on the propriety of application of the antifungal agent after disinfection, and the technique and extent of maintenance to achieve permanent prevention of transmission and full disinfection. Therefore, these methods have not resulted in the eradication of infection.

In the space analysis of the CA(+) non caries tooth we found that in 32 of 46 adjacent healthy teeth with CA(+), CA was removed by professional cleaning or brushing (after dental caries operation); (Table 5a). In 36 of 57 non-adjacent healthy teeth with CA(+), CA was removed by professional cleaning or brushing; (after dental caries operation) (Table 5b).

These are considered to be light CA infections, which depends on the internal transmission by IF (from the caries teeth), or on the external transmission (food, dust splash, hospital infection, etc.) by IF, and adhesion. Internal transmission was considered for DF that was less than one tooth away (Fig. 2, Supplementary Figs. 11–13).

Four-level classification

In our study, the infection of CA was classified into one of the following four levels:

1. Encountering tissue (There is no invasion)

2. Adhesive colonization (surface colonization) (Supplementary Note S14) (colony equivalent to colonization [3-10]). This level is removable by cleaning.

3. Fixing colonization (invasive colonization) (Supplementary Note S15) (In the lung, the fixing colony will cause pneumonia.) [3, 11], It causes a infection.

4. Outbreak (of colonization) in organs (tissue, substance) (Supplementary Note S16)

The first-and second-level CA (from all true healthy teeth and the restored healthy teeth in Table 7) can be mostly disinfected by cleaning; however, at the third level, disinfection cannot be accomplished without treatment. The fourth level is a colony concentration (CC) of 100 or more, and a specific exponential increase was also observed on the chart (Table 7).

5. Four colony level (see Supplementary Detail (contents) of colony level)

Colony **level 1**: adhesion level (1–4) (The number of colony)

Colony **level 2:** transient level, increased growth (5-14)

Colony **level 3**: fixing level, increased growth (15-99)

Colony **level 4**: outbreak (100–)

Adhesion

The result of CA adhesion in healthy tooth is given in (Table 5). CA was detected on teeth, except for the caries tooth. It was not a fixed CA, but an adherent CA. We assumed the possibility of internal transmission from the 243 CA(+) caries tooth to the others. We found that in 32 teeth, adhesive colonization was removed by tooth cleaning. Among the non-adjacent healthy teeth, in which there was a strong possibility of internal transmission and/or external transmission, 36 CA (+) teeth had removed adhesive colonization by tooth cleaning, which accounted to a total of 311 teeth (87.4%; 311/356). Furthermore, the dummy is the result of the external and/or internal transmission; therefore, 10 teeth can be added to the total which resulted in 90.2% (321/356) of the teeth. If the 35 unclear teeth are removed, this becomes 100% (321/321). Therefore, it is thought that caries tooth occurs from a fixed CA. The IS is a caries tooth, which was treated

with an antifungal agent, resulting in disinfection (Supplementary Note S24). If CC in each dental formula area is 0, it must also be 0 in saliva, in periodontal tissue, and in the air.

The oral segment is the entrance for the living body.

For the oral segment, which is the entrance for the living body, to become CA-free, the infection chain (IC) must be broken in the connected segment that could be infected from the oral segment. There is a strong possibility that the teeth constitute the first habitat of CA; thus, CA disinfection of the teeth was very effective (Supplementary Notes S26–S27). Moreover, resin filling, artificial tooth for the final prosthetics, denture and healthy teeth, which are clear on DFM, also occasionally have adherent CA. The information from IEDS can examine the IR (IR subtracts IS from IC), but in many cases, it is possible to maintain a patient's oral segment CA-free continuously. In the oral segment, which is the entrance to the living body, ref. 2 (P87) suggests food as the source of IF (Japan is an exception [23]), IES shows the specific dental caries are because of infection from a source other than food. The treatment, based on detailed measurement analysis of the IF, shows that the IC can be eliminated, including internal and external infections from the natural environment.

No endogenous microorganism

In the oral segment, one or more CA-infected teeth may exist. In the case of the latter, two or more IF(s) (and accordingly, DF, SF, and CF) exists in the restricted space of the oral segment; thus, the IF(s) suggest that CA is the causative endogenous microorganism. Importantly, there is a possibility that RsF is powerful in the oral segment. Moreover, it is thought that the reason for the negative value of ATE was the small amount of external transmission.

This may indicate that the prevalence of CA(s) in Japan, such as in food, soil, and water, was very low (Table 1). Furthermore, the Food Sanitation Law of Japan [23] does not recognize the contamination of pathogenic microorganisms. The same is true of the Water Supply Law [24].

The characterizations of CA

CA must consider the phase of the space-time of <u>infection source</u>, <u>infection field</u>, <u>receptor field</u>, <u>resistance field</u>, <u>adhesive colony</u>, <u>fixed colony</u> and infection chain. Moreover, CA must consider the phase of microorganisms. If they are neglected, in the statistical research or the medical treatment etc., the study cannot obtain the correct result.

Conclusion

In the treatment and prevention of infection, specification of the characteristics of IS and IF of the microorganisms is the highest priority. The IEDS, including the DFM, indicates the exact DF, SF, CF, RsF, RcF, dental caries time, IC, and other properties of microorganisms such as CA, and can simultaneously show the infection route and infection medium (Fig. 1, 2) (Supplementary Note S28). The surveillance of CA disinfection and the information from IEDS of specific teeth are very effective in the prevention of infection by CA.

It is necessary to perform dental formula examination of the microorganisms contained in dental caries and group it according to three phases (the infrared analysis method is important for *Streptococcus mutans*). Accordingly, information from IEDS can prevent infection, while preventing various diseases in other internal organs. Additionally, it is able to perform high-precision dental caries treatment (including operation), and prevent tooth loss. It is necessary to classify and verify the internal transmission from the untreated fixing teeth and active internal transmission by dental operation.

The outer CA infection routes (IR) include soil, food, pets, and care, through which progression can occur. Inner infections can transfer from the oral segment to respiratory organs or digestive organs, nasal cavity, and paranasal sinus. Moreover, disinfection of the ventilator used for treatment is important.

As mentioned above, the field of CA is mainly observed as CF.

The use of the ventilator

Although the use of the ventilator through the oral cavity in which dental-caries tooth exists is not recommended, it is inevitable in cases of infection. Therefore, in cases of infection, medical treatment is recommended. IEDS is very effective in prevention of hospital infection.

Solutions

$_1N$, is the other microorganisms and antibacterial factors, $_2N$ is the number of CA. In the treatment, the active factor of CA is only the growth factor (due to the influence of RcF and RsF). The $\gamma_i = 0.4–0.5$[25]. Correspondingly, the control of γ_i and/or γ_d is important for treatment and prevention. The doctor should be treated as $\gamma_d>0$. (**Supplementary POE S1**)

It is important that doctors increase the number $_1N$ of inhibitory factors. In addition, IE(D)S shows the interaction of two or more kinds of microorganisms, such as CA and LB.

Summary

Eradication of the infection source (IS) of the oral segment(s) and breaking the infection chain (IC) by IE(D)S will dramatically reduce the threat to the living body by pathogenic microorganisms.

Declarations

Funding

'Not applicable'

Conflicts of Interest

The author owns the patents, trademark, and copy right of DFM.

1) 2003–381874, Dental diagnostic system, November 12, 2003, in Japan

2) 2003–421304, Dental formula system, December 18, 2003, in Japan,

3) Japanese Pat No.5337353, 2007–138463, Dental formula Apparatus, May 24, 2007, in Japan

4) 2008–209406, Infection inflammation immune response measuring diagnostic apparatus, August 18, 2008, in Japan,

5) 2020–41422 Arithmetic unit, March 10, 2020, in Japan, especially regarding details of C4RDE,

6) Japanese Pat No6555573, 2019.8.7, Computer arithmetic unit, Riken Co., Ltd.,

7) Japanese Pat No6555574, 2019.8.7, Computer arithmetic unit, Riken Co., Ltd.,

8) Japanese Pat No6796295, 2020.11.18, Str Arithmetic unit, Riken Co., Ltd.,

9) Japanese Pat No6800460, 2020.11.27, Arithmetic unit, Riken Co., Ltd.,

10) Trade Mark of Dental Formula (Measurement Analysis) Medium (DFM), MicroDent Co., Ltd., No5341950

11) Copyright of dental formula (measurement analysis) medium (DFM), MicroDent Co., Ltd.,

Ethics approval

See Supp. Figure 0 no ethics problems,

Consent to participate

The clinic has always disclosed that treatment data without personal data will be used for research (See Supp. Figure 0)**,** and no participant (patients) has ever objected to this.

Consent for publication

The clinic has always disclosed that treatment data will be used for research (**See Supp. Figure 0**), and no participant has ever objected to this.

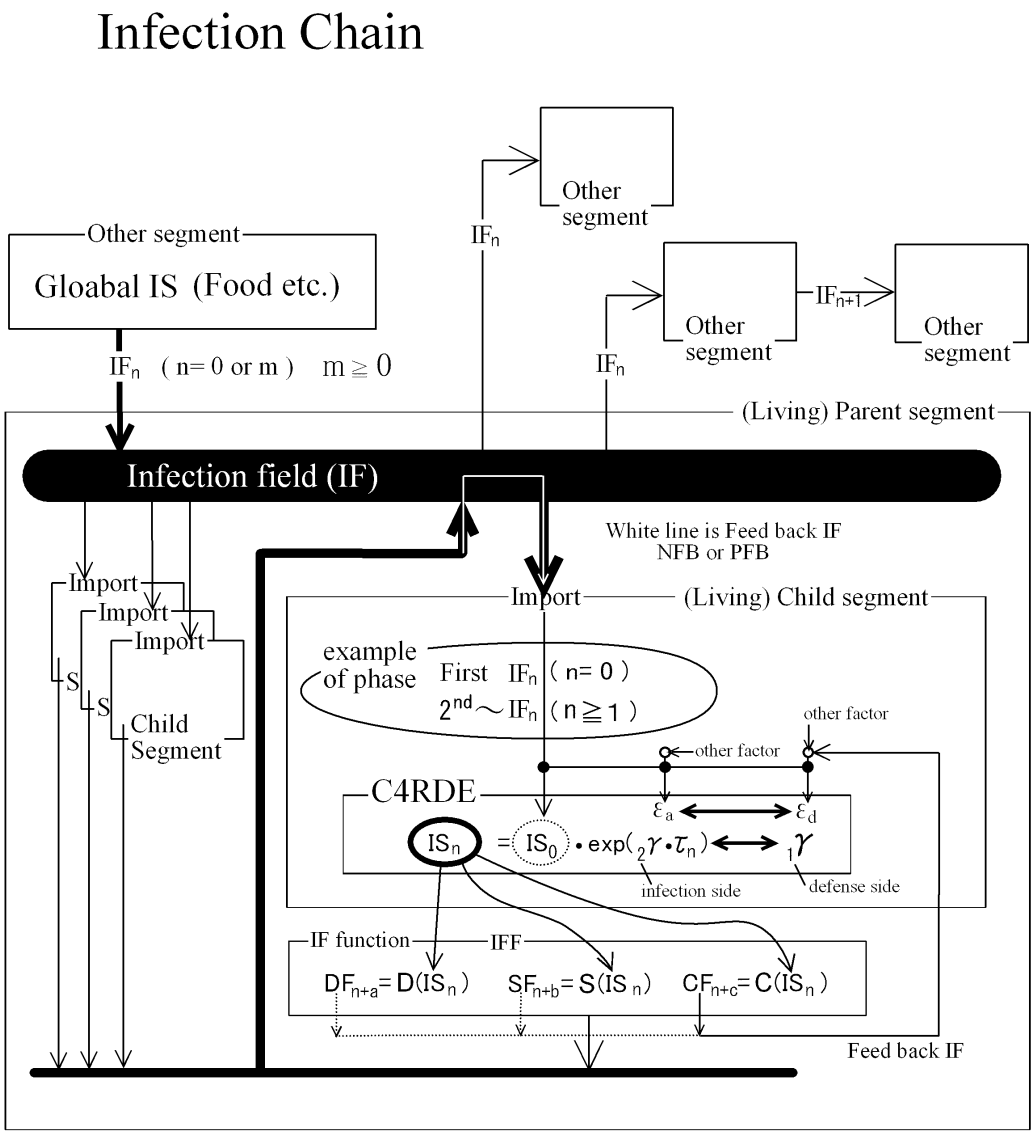
Availability of data and material

See Supplementary information,

Code availability

'Not applicable'

Figure Legends



IF : Infection field
DF : Diffusion field
SF : Spread field
CF : Carrier field
IS : Infection source

RcF : Receptor field
RsF : Resistant field
SOI : Solution of infedction
SOD : Solution of defens
ε_a active coefficient

m>0 : integer
n>0 : integer
a, b, c : integer
ε_d decline coefficient

Fig. 1 Definition of infection chain (IC) and various segment(s)

The infection source (IS) is defined as the space colonized by microorganisms. In the diffusion field (DF), microorganisms diffuse from the IS by potential energy. The spread field (SF) is defined as the spread that is not dependent on the concentration gradient. The carrier field (CF) is a field in which microorganisms are transferred by a carrier (e.g., toothbrush, floss, food). The infection field (IF) consists of the IS (in the segment) and three fields (DF, SF, and CF). The continuum of the IF is the IC (Fig. 3 and Supplementary Note S1-S10). They were measured in dental formula medium by C4RDE (C4SDE), which analyzes IF (including IS).

ε_d: Inhibition term (treatment, prevention, medicine, defense, decline term), as the relative decrease in factor and antigen: Decline coefficient ε_d

$$\varepsilon_d = \varepsilon_{\mathrm{d\_RsF}} + \varepsilon_{a\_\overline{\mathrm{DF}}} + \varepsilon_{a\_\overline{\mathrm{SF}}} + \varepsilon_{a\_\overline{\mathrm{CF}}} + \varepsilon_{\mathrm{d\_trt}}$$

$$\varepsilon_d = \varepsilon_{\mathrm{d\_RsF}} + \varepsilon_{\mathrm{d\_trt}} \text{ (without anti field.)}$$

ε_a: Infection term (microorganism term, CA term) as the relative increase in factor and antigen: Active coefficient ε_a

$$\varepsilon_a = \varepsilon_{a\_\mathrm{RcF}} + \varepsilon_{a\_\mathrm{DF}} + \varepsilon_{a\_\mathrm{SF}} + \varepsilon_{a\_\mathrm{CF}} + \varepsilon_{a\_\mathrm{growth}}$$

C 4 RDE with ε_d and ε_a

$$\frac{\frac{K}{n}\,|\,{}_1\mathrm{N}_\tau}{K\,|\,\tau_n} = \frac{{}_r|\,{}_1\mathrm{N}_{\tau_n}}{|\tau_n} = \frac{{}_r\mathrm{d}\,{}_1\mathrm{N}_{\tau_n}}{{}_r\mathrm{d}\tau_n} = \frac{\triangle_n\;{}_2N_\tau \cdot \varepsilon_d}{\triangle_n\tau} + \frac{\triangle_n\;{}_1N_\tau}{\triangle_n\tau}$$

$$\frac{\frac{K}{n}\,|\,{}_2\mathrm{N}_\tau}{K\,|\,\tau_n} = \frac{{}_r|\,{}_2\mathrm{N}_{\tau_n}}{{}_r|\tau_n} = \frac{{}_r\mathrm{d}\,{}_2\mathrm{N}_{\tau_n}}{{}_r\mathrm{d}\tau_n} = \frac{\triangle_n\;{}_2N_\tau}{\triangle_n\tau} + \frac{\triangle_n\;{}_1N_\tau \cdot \varepsilon_a}{\triangle_n\tau}$$

$${}_1\gamma_{\tau_n} = \frac{\varepsilon_d \cdot {}_{n-1}\triangle_n\;{}_2N_\tau + {}_n\triangle_{n+1}\;{}_1N_\tau}{\left|\varepsilon_d \cdot {}_{n-1}\triangle_n\;{}_2N_\tau\right| + \left|{}_n\triangle_{n+1}\;{}_1N_\tau\right|} \cdot \frac{2}{\tau}$$

$${}_2\gamma_{\tau_n} = \frac{{}_{n-1}\triangle_n\;{}_2N_\tau + \varepsilon_a \cdot {}_n\triangle_{n+1}\;{}_1N_\tau}{\left|{}_{n-1}\triangle_n\;{}_2N_\tau\right| + \left|\varepsilon_a \cdot {}_n\triangle_{n+1}\;{}_1N_\tau\right|} \cdot \frac{2}{\tau}$$

$${}_1\mathrm{N}_{\tau_n} = {}_1\mathrm{N}_0 \cdot \exp({}_1\gamma_{\tau_n} \cdot \tau_n)$$

$${}_2\mathrm{N}_{\tau_n} = {}_2\mathrm{N}_0 \cdot \exp({}_2\gamma_{\tau_n} \cdot \tau_n)$$

$$ {}_1N_{\tau_n} = {}_1N_0 \cdot \exp({}_1\gamma_{\tau_n} \cdot {}^{\tau_n}/_{\tau_r}) $$

${}_1N_{\tau n}$ is the value of the inhibitor, and ${}_2N_{\tau n}$ is the value of the pathogenic object (in the case of CA) (Supplementary **POE S1**). The τ ratio (τ_r) is used if the reaction time of existence 1 (${}_1N$) and existence 2 (${}_2N$) is different. ${}_1N_0$ is the initial value of the inhibitor, and ${}_2N_0$ is the initial value of the pathogenic object (in the case of CA). The τ ratio (τ_r) is used if the reaction time of existence 1 (${}_1N$) and existence 2 (${}_2N$) is different [14]

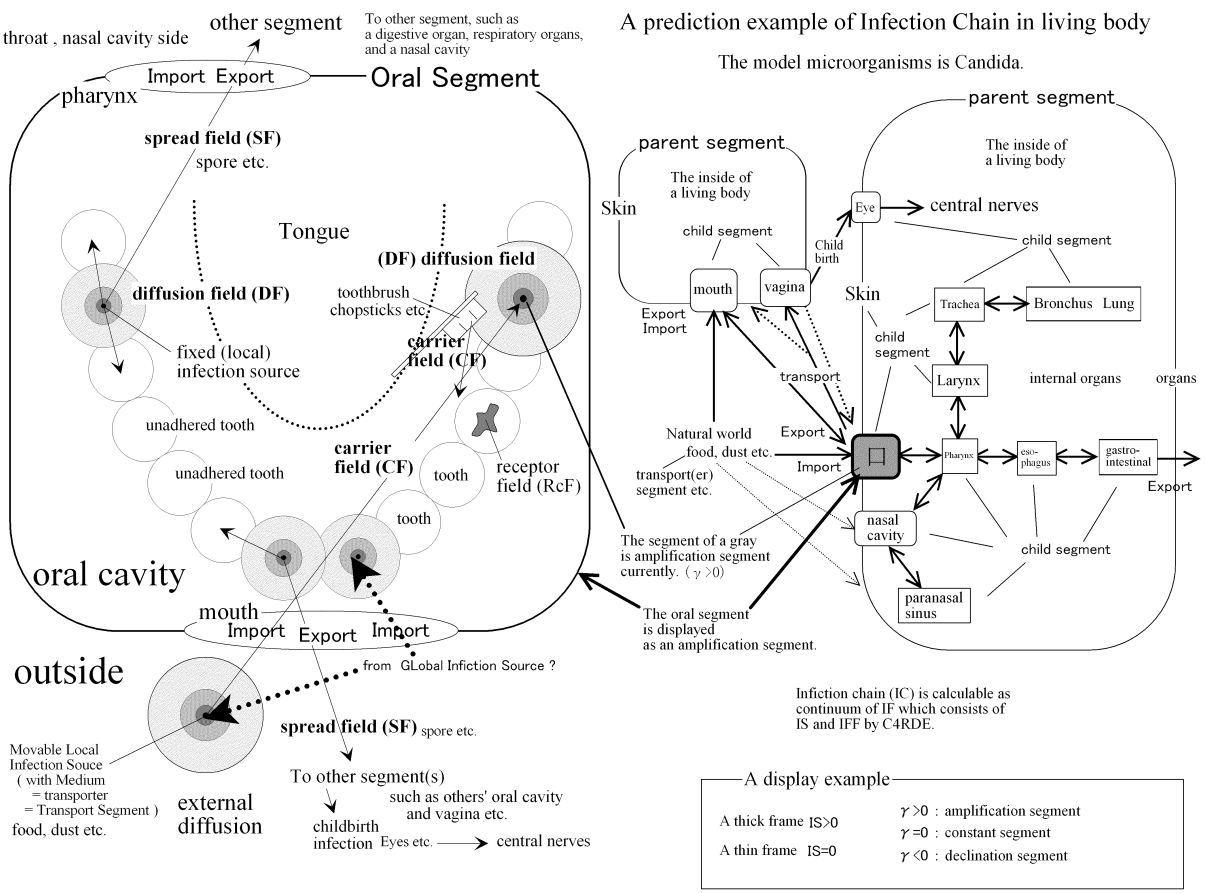


Fig. 2 Each field in the oral segment and an example of the infection chain in living body. (In the case of *Candida*).

The amplification segment is an example of an oral segment. Thus, the IS and infection coefficient γ is displayed, showing cooperation with other regions. The transmission of infection can occur by the oral segment to the lung (including the airway), oral segment to the vagina at the time of childbirth.

It is thought that this relatively easy examination, analysis, and diagnostics are the most fundamental and important examinations in the prevention of infection by CA in the living body, including for dental caries tooth treatment

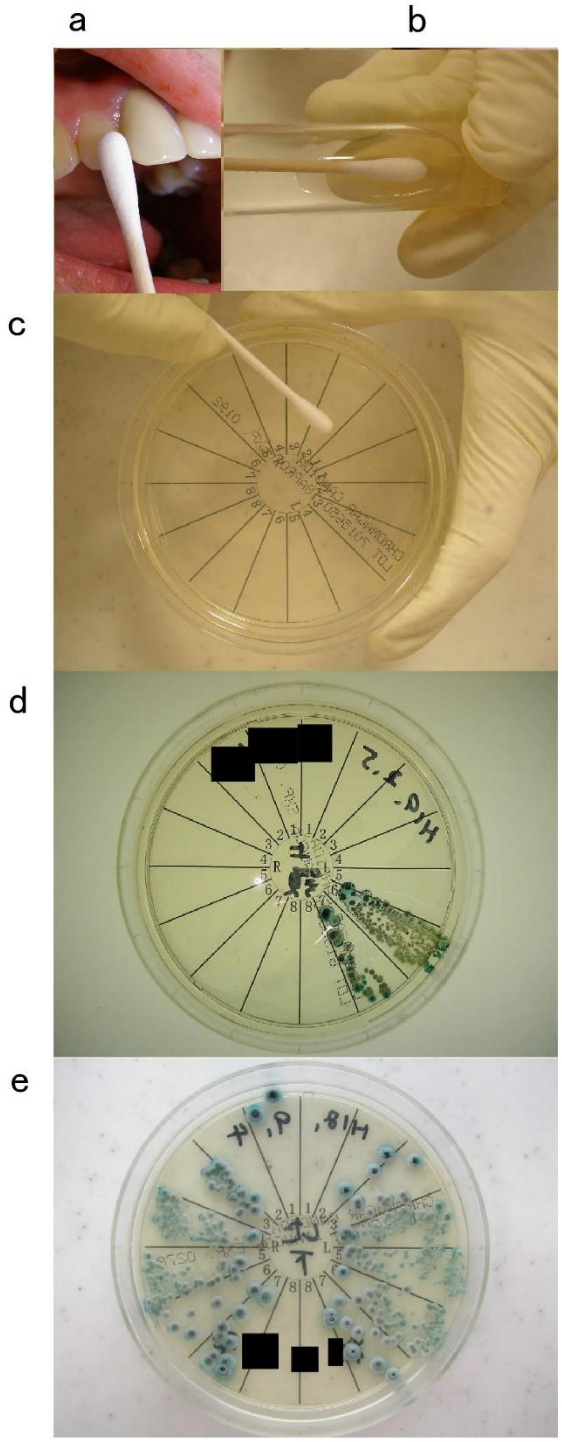


Fig. 3 The sampling method of a sample by dental formula medium (DFM),

a, A sterile swab of the teeth (crown). (Additionally, tracing the teeth cervix traces the entrance of

the periodontal pocket, and samples soft tissues, such as the marginal gingiva and interdental papilla.) **b,** All teeth swabs were applied to medium (oral cavity unit examination). **T**he all teeth examination (ATE), **c,** The sterile swab applied to the tooth that corresponded to each dental formula and the swab was applied to the area of dental formula on corresponding medium. The partial teeth examination (PTE) (tooth unit), **d,** As one or more CA-positive caries were observed, the infection source (IS) was tooth decay. **e,** As many CA-positive caries were observed, growth similar to that of endogenous microorganisms occurred. **c-e,** Dental formula medium (DFM) analyzes the sample according to the dental formula. The dental formula information on this DFM permitted space, time, and phase analyses (Table 3). The results in DFM clarified the infection by CA (Fig. 3d and e).

1. Space analysis shows the above DF, SF, CF, and the character of RcF {CA(+) dental caries} where it adheres specifically.

2. Time analysis shows the infection tendency in real-time and the growth trends in dental caries over time.

3. Phase analysis shows the phase difference of Lactobacillus (LB), SF, and RsF (Supplementary Fig. 5).

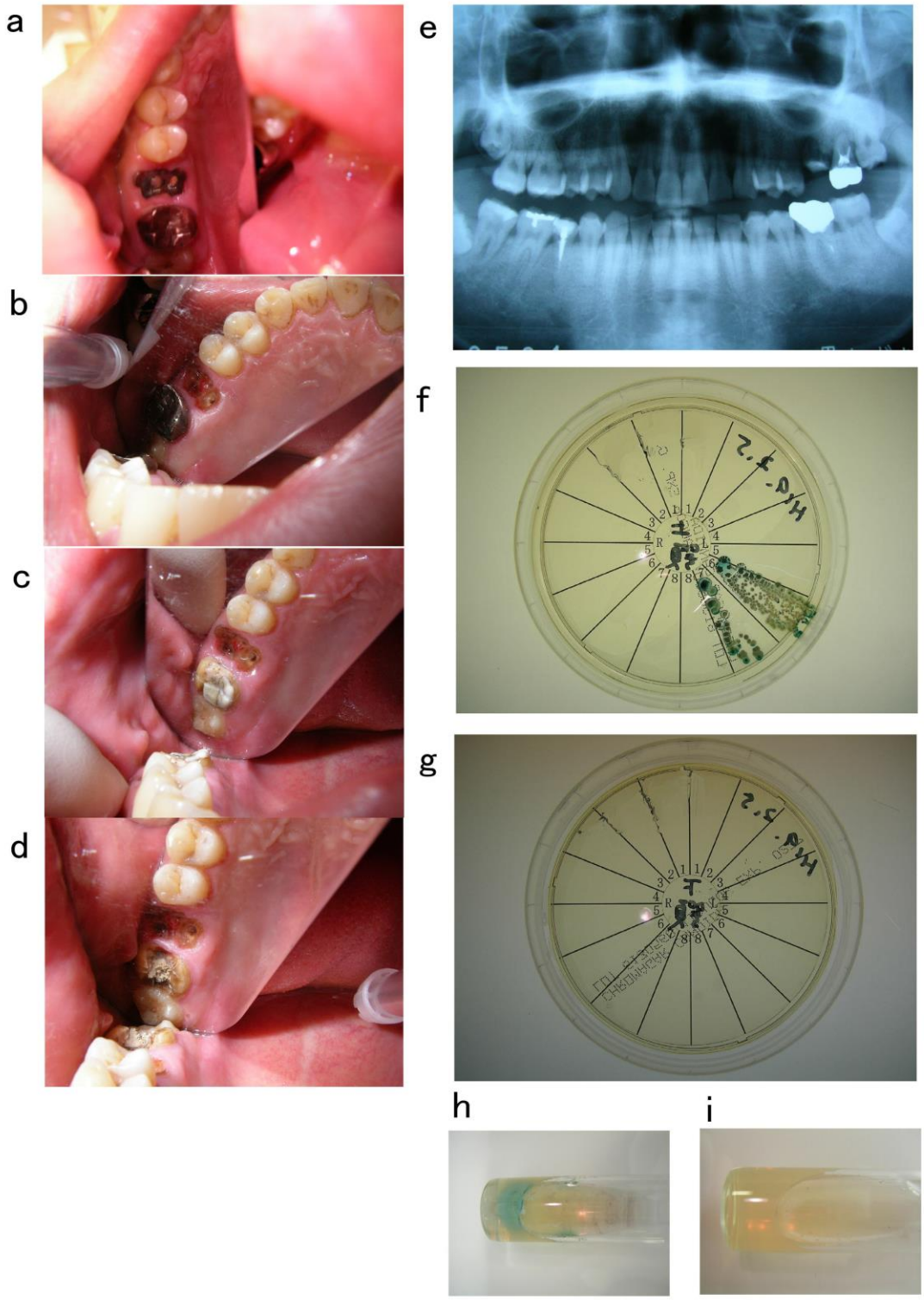
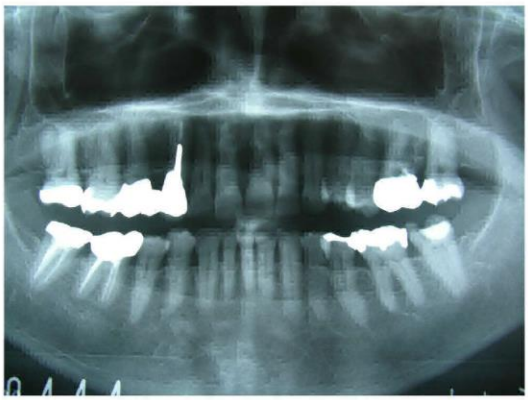


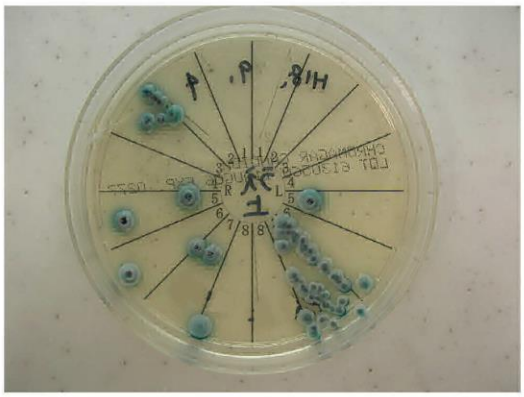
Fig. 4 Disinfection case, a few positivity tooth,

a, Before the operation, the upper left teeth No. 6 and 7 show dental-caries. X-ray findings showed caries in No 6 and 7. **b,** The caries in the upper left No. 6 tooth were removed. **c,** The upper left No. 7 crown was removed. **d,** The upper left No. 7 core was removed**. e,** Orthopantomography at the first dental examination. **f,** Dental formula medium upper jaw is CA colony level 4 (100- colony counts, the level of outbreak) in No. 6 tooth and level 3 (15-99 colony counts, fixing level with increased growth) in No. 7 tooth. **g,** Dental-formula medium lower jaw is clear. **h,** Before treatment, all teeth were CA-positive. **i,** After treatment, all teeth were CA-negative

a



b



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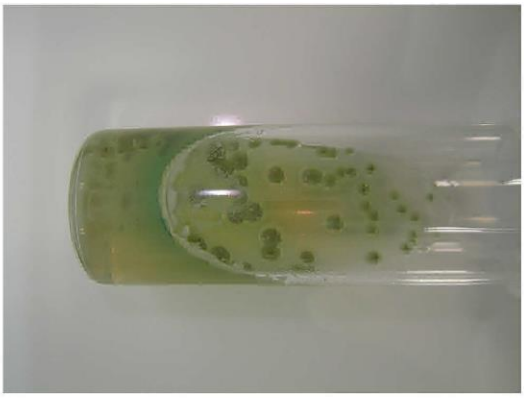

e

Fig. 5 Disinfection case, many positive teeth,

a, Orthopantomography at the first dental examination, **b,** Dental-formula medium upper jaw **c,** Dental-formula medium lower jaw. In this case, many teeth were CA infected. CA in the oral segment appears to be an endogenous microorganism. **d,** Before treatment, all teeth were CA-positive. **e,** After treatment, all teeth were CA-negative

Tables:

Table 1: First examination of 353 patients (using CHROMagar medium)

first examination		number(of persons)	rate (%)	average DMFT index	±	SD
before caries treatment	all the patients	353	100.0	53.7	±	26.2
	CA(−)patients	199	56.4	47.1	±	24.2
	CA(+)patients	143	40.5	62.8	±	26.4
	CA(±)patients	11	3.1	53.4	±	22.7

Table 2: *Candida albicans* (CA) examination and DMFT in the whole (teeth, periodontal tissue) oral cavity

first examination		number(of persons)	rate (%)	average DMFT index	±	SD
before caries treatment	CA(−)patients	199		47.1	±	24.2
	CA(+)patients	143	100.0	62.8	±	26.4
	CA(+1)patients	45	31.5	55.5	±	22.6
	CA(+2)patients	43	30.1	67.0	±	24.0
	CA(+3)patients	50	35.0	63.0	±	30.0
	CA(+4)patients	5	3.5	91.2	±	15.9

Table 3 Space analysis operator

Karte No	Name	DMFT																
Date																		Total
	Colony count		3	1	2		7							1	1	48		
	Colony stage		1	1	1		2							1	1	3		
	Treatment																	
	Dental caries stage		C2	C2	C2		C3頬								C3per	C2		
		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1	UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8	
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1	LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8	
	Dental caries stage		C3per	C3per	C2	C2								C2	C2	C2		
	Treatment																	
	Colony stage		2	2	3	3	3		1		1	2	3	3	3	2		
	Colony count		10	7	約40	約30	29		2		3	10	53	61	42	11		

Karte No	Name	DMFT																
Date																		Total
	Colony count														70	33		
	Colony stage														3	3		
	Treatment																	
	Dental caries stage														C3per	C3per		
		UR8	UR7	UR6	UR5	UR4	UR3	UR2	UR1	UL1	UL2	UL3	UL4	UL5	UL6	UL7	UL8	
		LR8	LR7	LR6	LR5	LR4	LR3	LR2	LR1	LL1	LL2	LL3	LL4	LL5	LL6	LL7	LL8	
	Dental caries stage																	
	Treatment																	
	Colony stage																	
	Colony count																	

Table 4. CA (CHROMagar) examination of 49 patients, 19.8 remaining teeth

total of each condition	The number of CA colony detection tooth	356	100.0
	C1	2	0.6
	C2	124	34.8
	C3pul	10	2.8
caries tooth	C3per	65	18.3
243	C3 restored tooth	42	11.8
adjacent teeth	adjacent true healthy tooth	27	7.6
46	adjacent restored healthy tooth	19	5.3
non-adjacent teeth	non-adjacent true healthy tooth	23	6.5
57	non-adjacent restored healthy tooth	34	9.6
10	Dumy(Po)	10	2.8

Table 5 Whole circumstances of colony detection for teeth, except for teeth with caries in Table 4

Table 5a

contents	Number (tooth)
negative	32
attending the hospital is impossible	10
During dental treatment	0
During professional cleaning	4
Tooth extraction required	0
Total of result of the adjacent tooth with the CA colony	46

Table 5b

contents	Number (tooth)
negative	36
attending the hospital is impossible	16
During dental treatment	1
During professional cleaning	4
Tooth extraction required	0
Total of result of the non-adjacent tooth with the CA colony	57

Table 6 Disinfection results

	Number of people	Rate
Before dental treatment CA(+)	143	100.0
Dental treatment impossible patient	7	4.9
The progress unknown during dental treatment etc.	67	46.9
1st treatment, the end of dental treatment	69	48.3
After the end of 1st dental treatment, CA(-)	68	
After the end of 1stdental treatment, CA(+)	1	0.7
After the end of 1st dental treatment, Decreasing	1	
After the end of 1st dental treatment, disinfection	68	98.6
2nd treatment, the end of dental treatment	1	
After the end of 2nd dental treatment, CA(-)	1	100.0
After the end of 2nd dental treatment, CA(+)	0	0.0
After the end of 2nd dental treatment, disinfection		100.0

Table 7 Stage of dental caries and colony count using CHROMagar.

Number	C1	C2	C3pul	C3per	C3restored	nAdj-THT	nAdj-RHT	Adj-THT	Adj-RHT	Dumy(Po)	
1-4	2	65	7	19	24	18	24	21	13	6	
5-9	0	14	0	12	4	0	5	1	3	1	
10-14	0	14	0	3	2	4	1	0	0	0	
15-19	0	5	0	2	2	0	1	1	2	0	
20-24	0	4	0	1	2	1	0	0	0	0	
25-29	0	1	1	3	4	0	0	2	0	0	
30-34	0	4	1	4	0	0	1	0	0	1	
35-39	0	1	1	2	0	0	0	1	0	0	
40-44	0	4	0	2	0	0	1	0	0	0	
45-49	0	1	0	3	0	0	0	0	1	0	
50-54	0	1	0	1	2	0	1	1	0	1	
55-59	0	1	0	0	0	0	0	0	0	0	
60-64	0	2	0	1	2	0	0	0	0	1	
65-69	0	0	0	2	0	0	0	0	0	0	
70-74	0	2	0	1	0	0	0	0	0	0	
75-79	0	0	0	1	0	0	0	0	0	0	
80-84	0	0	0	2	0	0	0	0	0	0	
85-89	0	1	0	0	0	0	0	0	0	0	
90-94	0	0	0	0	0	0	0	0	0	0	
95-99	0	0	0	0	0	0	0	0	0	0	
100-	0	4	0	6	0	0	0	0	0	0	
	2	124	10	65	42	23	34	27	19	10	356

nAdj-THT, nAdj-RHT, Adj-THT, and Adj-RHT: **(see Supp. Abbreviation)**

Table 8**a** A CA-positive single tooth with dental caries in a single jaw (Using CHROMagar (a and b))

Number	C1	C2	C3pul	C3per	C3rest	
1-4	2	26	4	5	6	
5-9	0	4	0	0	2	
10-14	0	3	0	1	1	
15-19	0	2	0	0	0	
20-24	0	0	0	0	1	
25-29	0	0	1	0	0	
30-34	0	1	0	1	0	
35-39	0	0	0	1	0	
40-44	0	0	0	0	0	
45-49	0	0	0	1	0	
50-54	0	0	0	0	0	
55-59	0	0	0	0	0	
60-64	0	0	0	0	1	
65-69	0	0	0	0	0	
70-74	0	0	0	0	0	
75-79	0	0	0	1	0	
80-84	0	0	0	1	0	
85-89	0	0	0	0	0	
90-94	0	0	0	0	0	
95-99	0	0	0	0	0	
100-	0	0	0	1	0	
	2	36	5	12	11	66

Table 8**b** A CA-positive single tooth with dental caries in the whole jaw

Number	C1	C2	C3pul	C3per	C3rest	
1-4	2	17	4	4	4	
5-9	0	4	0	0	1	
10-14	0	3	0	1	1	
15-19	0	2	0	0	0	
20-24	0	0	0	0	1	
25-29	0	0	1	0	0	
30-34	0	1	0	0	0	
35-39	0	0	0	1	0	
40-44	0	0	0	0	0	
45-49	0	0	0	1	0	
50-54	0	0	0	0	0	
55-59	0	0	0	0	0	
60-64	0	0	0	0	0	
65-69	0	0	0	0	0	
70-74	0	0	0	0	0	
75-79	0	0	0	1	0	
80-84	0	0	0	0	0	
85-89	0	0	0	0	0	
90-94	0	0	0	0	0	
95-99	0	0	0	0	0	
100-	0	0	0	0	0	
	2	27	5	8	7	49

Table 9 The stage of dental caries and colony count immediately after the shift to positive from negative in the first examination (Using CHROMagar)

Number	C1	C2	C3pul	C3per	C3rest	nAdj-TIIT	nAdj-RIIT	Adj-TIIT	Adj-RIIT	Dumy(Po)	
1-4	0	1	0	0	3	11	29	1	1	4	
5-9	0	0	0	0	1	2	5	0	0	3	
10-14	0	0	0	0	0	0	1	0	0	0	
15-19	0	0	0	0	0	0	1	0	0	0	
20-24	0	0	0	0	0	0	3	0	0	0	
25-29	0	0	0	0	0	0	0	0	1	0	
30-34	0	0	0	0	0	0	1	0	0	1	
35-39	0	0	0	0	0	0	0	0	0	0	
40-44	0	0	0	0	0	0	1	0	0	0	
45-49	0	0	0	0	0	0	0	0	0	0	
50-54	0	0	0	1	0	0	1	0	0	0	
55-59	0	0	0	1	0	0	0	0	0	0	
60-64	0	0	0	0	0	0	0	0	0	0	
65-69	0	0	0	0	0	0	0	0	0	0	
70-74	0	0	0	1	0	0	0	0	0	0	
75-79	0	0	0	0	0	0	0	0	0	0	
80-84	0	0	0	0	0	0	0	0	0	0	
85-89	0	0	0	0	0	0	0	0	0	0	
90-94	0	0	0	0	0	0	0	0	0	0	
95-99	0	0	0	0	0	0	0	0	0	0	
100-	0	0	0	0	0	0	0	0	0	0	
	0	1	0	3	4	13	42	1	2	8	74

Table 10**a** CA-positive single healthy tooth in a single jaw (Using CHROMagar)

Number	adjacent restored healthy tooth	non-adjacent restored healthy tooth	adjacent true healthy tooth	non-adjacent true healthy tooth	
1 - 4	3	15	3	12	
5 - 9	0	2	0	0	
10 - 14	0	0	0	0	
15 - 19	0	0	0	0	
20 - 24	0	0	0	0	
25 - 29	0	0	0	0	
30 - 34	0	0	0	0	
35 - 39	0	0	0	0	
40 - 44	0	0	0	0	
45 - 49	0	0	0	0	
50 - 54	0	0	0	0	
55 - 59	0	0	0	0	
60 - 64	0	0	0	0	
65 - 69	0	0	0	0	
70 - 74	0	0	0	0	
75 - 79	0	0	0	0	
80 - 84	0	0	0	0	
85 - 89	0	0	0	0	
90 - 94	0	0	0	0	
95 - 99	0	0	0	0	
100-	0	0	0	0	
	3	17	3	12	35

Table 10**b** CA-positive single healthy tooth in the whole jaw (Using CHROMagar)

Number	adjacent restored healthy tooth	non-adjacent restored healthy tooth	adjacent true healthy tooth	non-adjacent true healthy tooth	
1 - 4	0	15	0	12	
5 - 9	0	2	0	0	
10 - 14	0	0	0	0	
15 - 19	0	0	0	0	
20 - 24	0	0	0	0	
25 - 29	0	0	0	0	
30 - 34	0	0	0	0	
35 - 39	0	0	0	0	
40 - 44	0	0	0	0	
45 - 49	0	0	0	0	
50 - 54	0	0	0	0	
55 - 59	0	0	0	0	
60 - 64	0	0	0	0	
65 - 69	0	0	0	0	
70 - 74	0	0	0	0	
75 - 79	0	0	0	0	
80 - 84	0	0	0	0	
85 - 89	0	0	0	0	
90 - 94	0	0	0	0	
95 - 99	0	0	0	0	
100-	0	0	0	0	
	0	17	0	12	29

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Figures

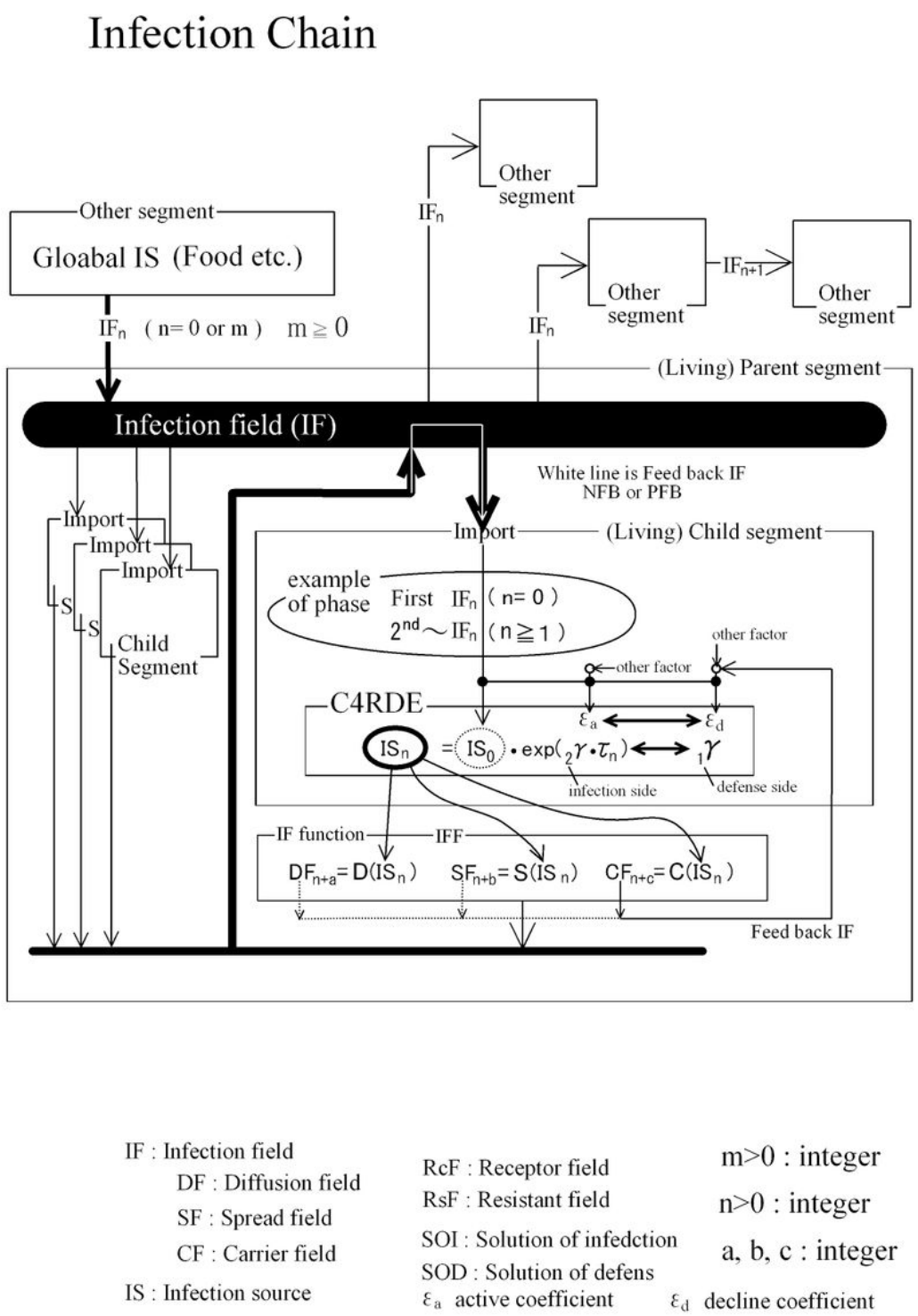

Figure 1

Definition of infection chain (IC) and various segment(s)

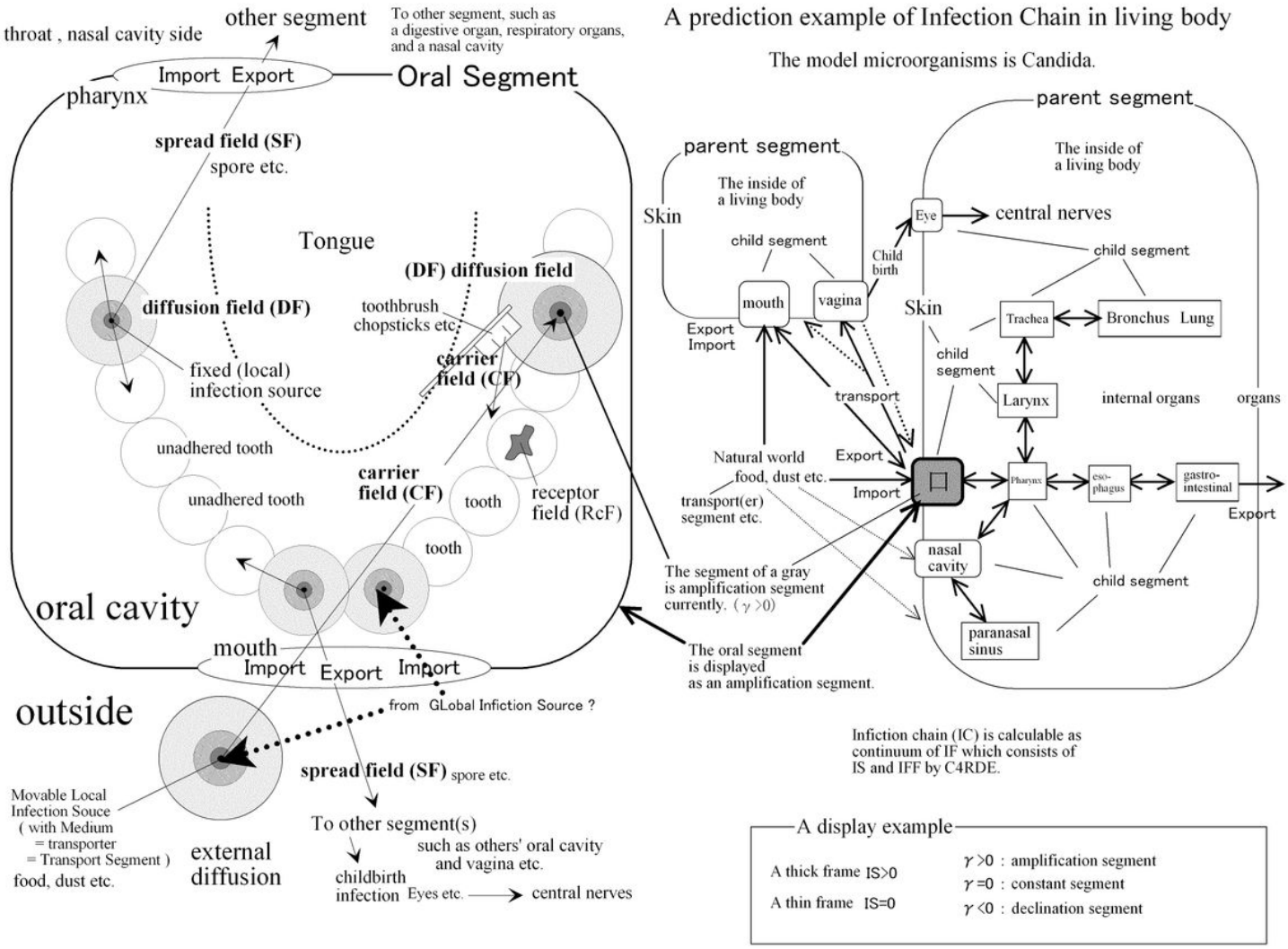

Figure 2

Each field in the oral segment and an example of the infection chain in living body. (In the case of Candida).

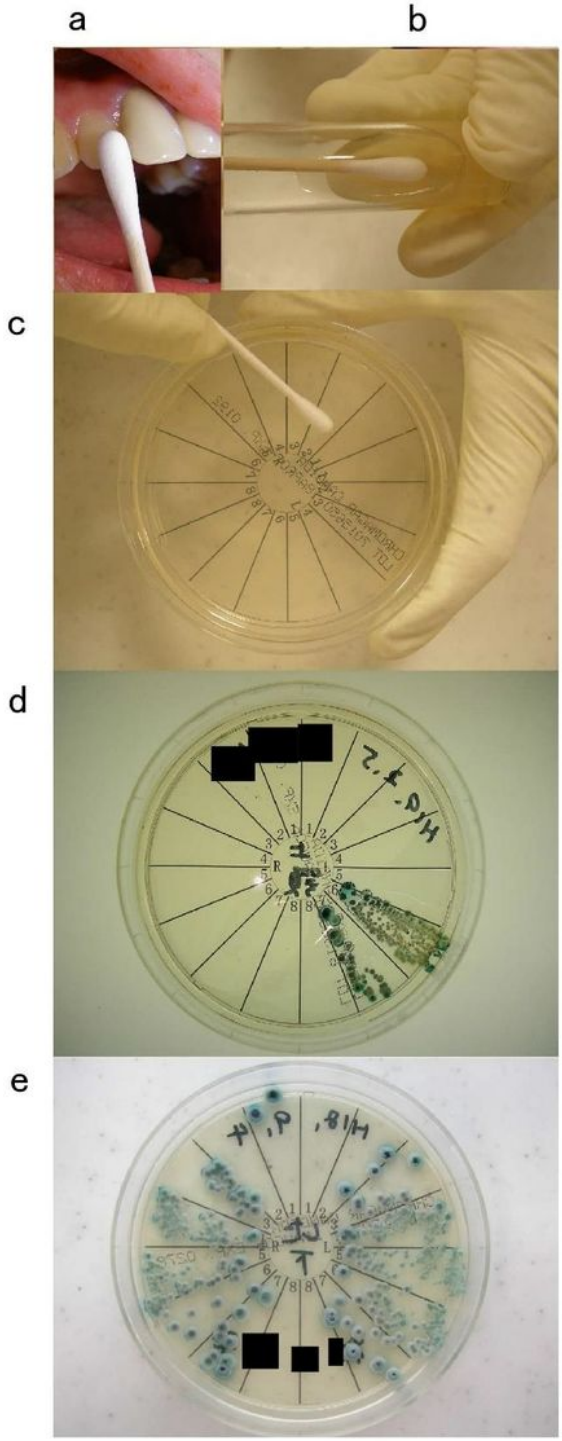

Figure 3

The sampling method of a sample by dental formula medium (DFM), a, A sterile swab of the teeth (crown). (Additionally, tracing the teeth cervix traces the entrance of the periodontal pocket, and samples soft tissues, such as the marginal gingiva and interdental papilla.) b, All teeth swabs were applied to medium (oral cavity unit examination). The all teeth examination (ATE), c, The sterile swab applied to the tooth that corresponded to each dental formula and the swab was applied to the area of dental formula

on corresponding medium. The partial teeth examination (PTE) (tooth unit), d, As one or more CA-positive caries were observed, the infection source (IS) was tooth decay. e, As many CA-positive caries were observed, growth similar to that of endogenous microorganisms occurred. c-e, Dental formula medium (DFM) analyzes the sample according to the dental formula. The dental formula information on this DFM permitted space, time, and phase analyses (Table 3). The results in DFM clarified the infection by CA (Fig. 3d and e).

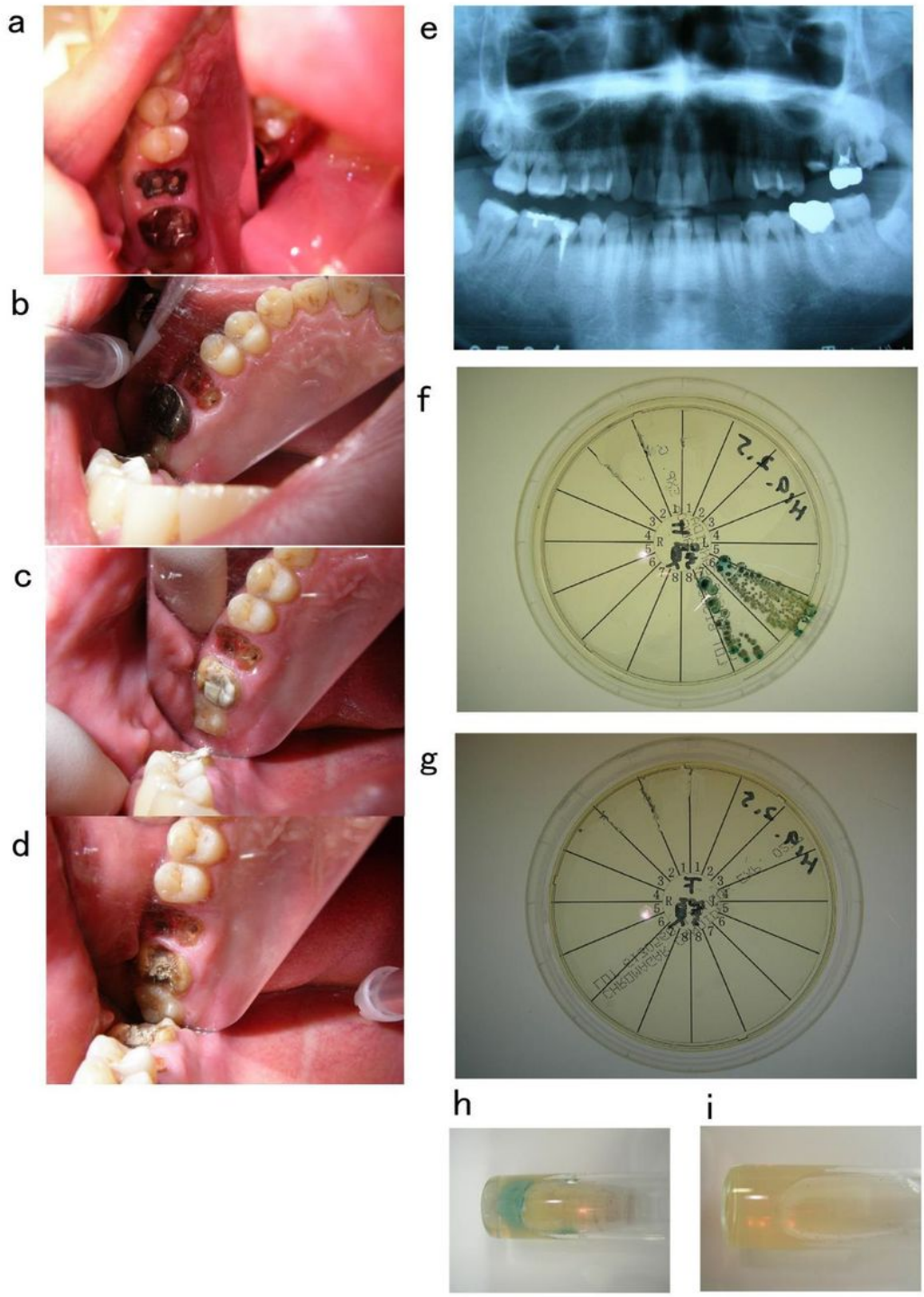

Figure 4

Disinfection case, a few positivity tooth, a, Before the operation, the upper left teeth No. 6 and 7 show dental-caries. X-ray findings showed caries in No 6 and 7. b, The caries in the upper left No. 6 tooth were removed. c, The upper left No. 7 crown was removed. d, The upper left No. 7 core was removed. e, Orthopantomography at the first dental examination. f, Dental formula medium upper jaw is CA colony level 4 (100- colony counts, the level of outbreak) in No. 6 tooth and level 3 (15-99 colony counts, fixing level with increased growth) in No. 7 tooth. g, Dental-formula medium lower jaw is clear. h, Before treatment, all teeth were CA-positive. i, After treatment, all teeth were CA-negative

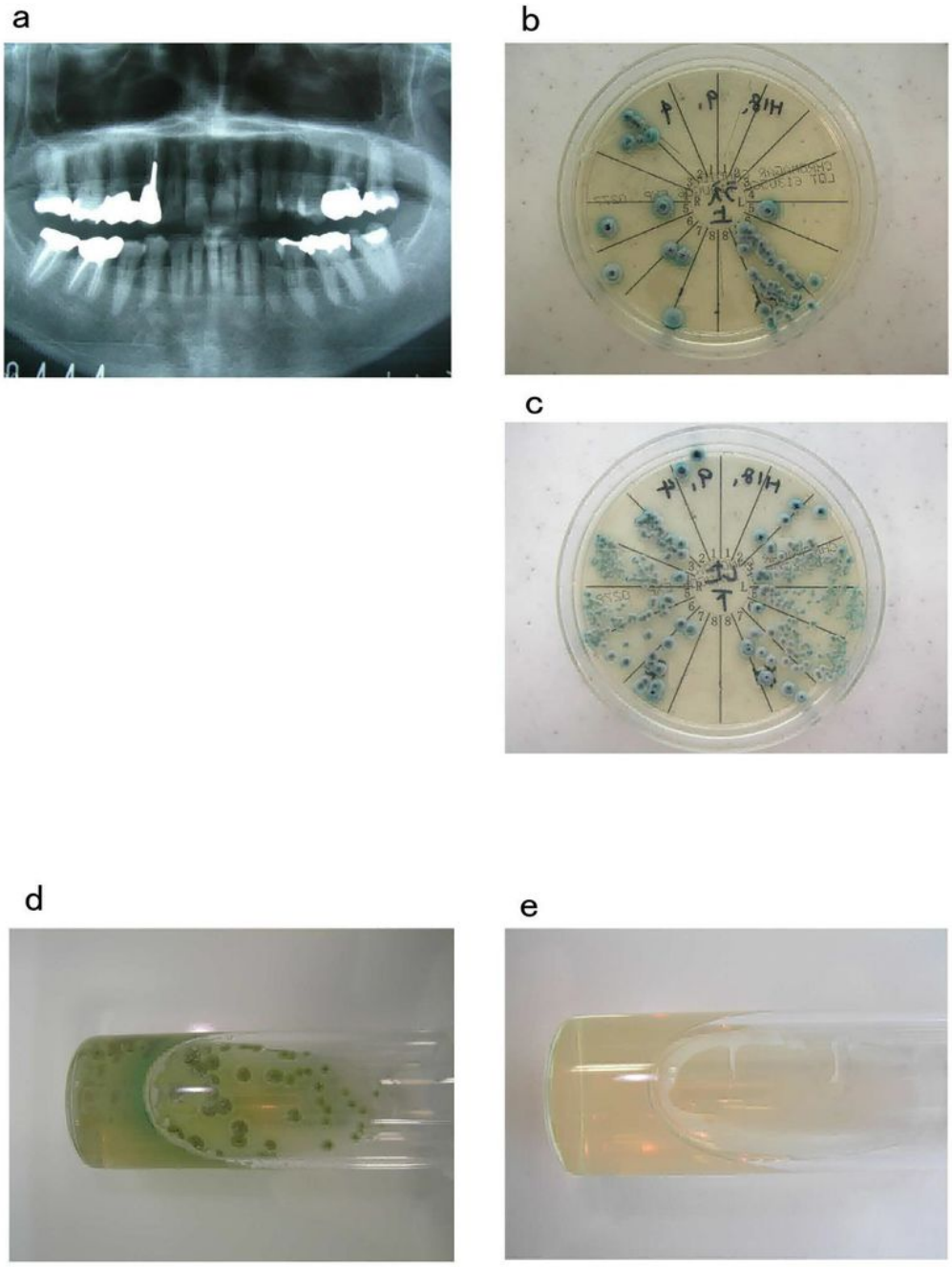

Figure 5

Disinfection case, many positive teeth, a, Orthopantomography at the first dental examination, b, Dental-formula medium upper jaw c, Dental-formula medium lower jaw. In this case, many teeth were CA infected. CA in the oral segment appears to be an endogenous microorganism. d, Before treatment, all teeth were CA-positive. e, After treatment, all teeth were CA-negative

Supplementary Files

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- SupplementaryInformation1828E7.pdf