

Interactions between thermal cycled resilient denture lining materials, salivary and serum pellicles and *Candida albicans* in vitro. Part I. Effects on fungal growth.

Summary

In the present study, the growth of a single isolate of *C. albicans* on saliva-, serum-coated or protein free (uncoated), thermocycled (4°C-70°C for 1min, respectively; 0, 1000 and 10000 times) seven commercial resilient lining materials was investigated, by monitoring pH changes in growth media. The inhibitory effect of the tissue conditioners on fungal growth was observed using three parameters viz: i) delay in the onset of the rapid decline in pH, ii) reduction in the rate of pH change and iii) the pH minima reached. In the case of control soft liners (not thermocycled and uncoated), the antifungal effect appeared to depend upon the type of commercial soft liner used. Thus, an initial delay in pH decline, and very high pH minima were observed with fluoridated and heat cured silicone materials. High pH minima were also observed with cold cured acrylic soft liners, whereas cold cured silicone materials, did not significantly differ from heat cured acrylic resin ($p > 0.05$). However, the antifungal effect of the materials was significantly reduced both by thermal cycling (ANOVA; $p < 0.01$) and a layer of protein coating (saliva, $p < 0.05$; serum, $p < 0.01$).

These results, taken together, suggest that the ageing of the materials and the biological fluids of the host, particularly serum, promote yeast growth on soft lining materials.

Introduction

Resilient denture liners because of their viscoelastic properties, act as shock absorbers and reduce and distribute the stress on the denture bearing tissues (Lytle, 1959). Their use for patient comfort and the treatment of the atrophic ridge, bone undercuts, bruxism, xerostomia, and dentures opposing natural teeth has been known to be clinically beneficial (Boucher et al., 1975). Although these attributes are positive, there are also some physical and microbiological disadvantages to the use of these materials. One of the most serious has been colonization and infection of the material surface by *Candida albicans* and related *Candida* species, resulted in the source of infections such as, denture stomatitis, oral, gastrointestinal and pneumopulmonary candidosis (Budtz-Jorgensen, 1990; Nikawa et al, 1998).

In the successful colonization, subsequent plaque formation and development of pathogenesis, the adherence of *Candida* to solid surfaces such as acrylic resin or denture lining materials has been thought to be first step (Rotrosen et al., 1986), followed by the growth of adherent cells or the coadhesion of floating cells to adherent ones. Particularly, with *Candida* infection, the growth of it on these lining materials is thought to be one of the most important factors. However only limited data is available on the interactions between resilient liners and fungi. In addition, although the materials are known to show age changes in their physical properties, little attention has been paid on the growth of *Candida* when this has occurred.

In addition, we have demonstrated that denture pellicle comprising salivary or serum protein promotes film-like colonization (biofilm formation) of *C. albicans*, hyphal emergence and invasion into tissue conditioning materials (Nikawa et al. 1993). Further work showed that the proteinaceous pellicle reduced the antifungal effects of tissue conditioners (Nikawa et al. 1997a,b). Hence, the interactions between proteinaceous pellicles, (aged) lining materials and *C. albicans* should be the most important factors with regard to the in vivo fungal colonization and/or invasion of long term used soft lining materials. Thus in the present study, the growth of *C. albicans* on thermocycled and protein coated commercial soft lining materials was investigated by monitoring pH changes of growth media and other circumstances involved in resilient lining materials affecting fungal growth.

Materials and Methods

Microorganisms and growth condition

Candida albicans IFO 1385, purchased from the Institute for Fermentation, Osaka, was used and cultured as previously described (Nikawa et al., 1989; Nikawa & Hamada 1990). Briefly, the yeasts were grown at 37°C with reciprocal shaking (150 rev/min), in yeast nitrogen base medium (Difco, Detroit, USA) containing 250mM glucose. Batches of medium were inoculated with overnight cultures of the yeast, the yeast was harvested in the late exponential growth phase, washed twice with distilled water and suspended to final concentrations (10^7 cells/ml) (Nikawa et al., 1997a,b)

Acrylic resin and resilient lining materials

Samples of acrylic resins (Bio Resin, Shofu, Kyoto, Japan) and seven commercial resilient lining materials summarized in Table 1 were processed according to manufacturer's directions, and each of them was prepared to a uniform size (10mm × 10mm × 0.7mm thickness) with smooth surfaces by placing glass slides over them as previously described (Nikawa et al., 1994, 1995, 1996).

Specimens of each of the materials were thermocycled between 4°C and 70°C with an immersion time of 60 seconds in each bath, and the growth assay was taken after thermal cycling 0, 1000 and 10000 times.

Saliva and serum

Pooled unstimulated whole saliva was collected from five healthy candidates and clarified, according to the method of Cannon et al. (1995) with modification, by centrifugation at $12,000 \times g$ for 15min at 4°C. Human serum was purchased from Sigma Chemical Co. (St Louis, MO, USA). Whole saliva and serum were stored at -25°C before use (Nikawa & Hamada, 1990; Nikawa et al., 1996).

Assay procedures

The growth assay was conducted as follows (Nikawa et al. 1993, 1994). The specimens of acrylic and resilient liners were coated with saliva or serum by placing them in wells of

Multiwell tissue culture plates (Nunclon[®] Delta, Nunc, Kamstrup, Denmark), into which were dispensed 500 μ l of the protein solution per well, and incubating for 1 hour at 37°C. Saliva or serum was substituted with an equal volume of sterile distilled water in the control wells. After incubation the protein solution was aspirated, 50 microliters of yeast suspension (1×10^7 cells/ml) was inoculated on the surface of each acrylic or resilient liner specimen and the whole assembly was incubated at 37°C for 2 hrs to promote yeast adherence and colonization. Subsequently, 2.0 ml of Sabouraud broth was carefully dispensed into each well, incubated at 37°C for upto 120hrs and pH changes in these media during this period were measured using a pH meter (pH meter 245, Corning, New York, USA) as a parameter of fungal growth (Nikawa et al., 1994).

The assays were carried out on two independent occasions, with quadruplicated samples on each occasion and the values obtained were averaged to give the final data with standard deviations. All the numerical data obtained were analyzed by analysis of variance (ANOVA) and Tukey's multiple range test at 5% level.

Inhibitory assay

Inhibitory effect on *C. albicans* of the components of resilient lining materials were investigated as follows. Sterilized filterpaper (5.0 mm in diameter) was immersed in liquid of each lining materials for 10 minutes. The paper discs of each material were placed on yeast-inoculated agar plates, incubated at 37°C for 24 hours, and the inhibitory zone recorded.

Results

Effect of resilient liners on fungal acid production and/or growth

As shown in Fig. 1-a through c, although the pH changes in media were varied depending upon resilient lining materials on which *Candida* had grown and times of thermal cycle, the reverse sigmoidal pH curves were observed with all samples, being similar to our previous study (Nikawa et al. 1994). Initially, the pH of the media with all samples decreased slightly, and a remarkable, rapid and linear decline in pH from ca. 5.5 was observed. After 10-50 hrs of incubation, the rate of pH change levelled off in each case.

In the case of non-thermocycled and uncoated resilient liners, the retardation of fungal growth appeared to depend upon the type of liners, i.e. initial delay in decline and high minimum pH were observed with fluoroc and heat cured silicone materials (KD and MB). High minimum pH was observed with cold curing acrylic resilient liners (SF and SR), and, as compared with acrylic resin, no remarkable change was observed with cold cured silicone materials (EV, TSR and MO).

As in the previous study (Nikawa et al, 1994), the inhibitory effects of tissue conditioners on fungal growth were observed as following three types; delay of beginning of rapid decline in pH, decreases in the rate of pH change and increases in minimum pH. We further analysed the effect of resilient liners and protein-coats on yeast growth with regard to these three parameters. As to non-thermocycled and uncoated specimens, the beginning of rapid decline was observed with SR, SF and acrylic resin samples at about 10-hours incubation, followed by $MO \leq EV \leq TSR$, and KD and MB was the most effective to delay the beginning of *Candida* acid production or growth (ANOVA and multiple range test, $p < 0.01$; Fig. 1-a, Tables 2-a). The highest rate of pH change was observed with the case yeasts grown on TSR, MO and EV, decreased in the sequence $resin \geq SF$, and SR MB and KD showed the most inhibitory effect (ANOVA and multiple range test, $p < 0.01$; Fig. 1-a, Tables 2-b). Minimum pH also varied depending upon the samples on which *Candida* has grown, the case grown on MO showed the lowest (pH 2.88), increased in the order of EV, resin and $TSR < SR$, and SF, MB and KD showed the highest pH value (pH 4.09, 4.12 and 4.20, respectively) (Fig. 1-a, Tables 2-c).

Effect of thermal cycles on antifungal effect of resilient liners

As shown in Fig. 1-b and c, the antifungal effects of each material appeared to decrease with increasing times of thermocycling. In case of 1000 thermocycling, the period necessary to reach pH 5.5 significantly shortened with MB (Table 2-a), the rate of pH decline significantly promoted with MB, SR, resin and TSR (Table 2-b), and the minimum pH significantly subsided with MB and SF (Table 2-c). As to the 10000 thermocycled specimens, the antifungal effects of each material were more weakened to reduce the differences between the materials (Fig.1-c). The time necessary to reach pH 5.5 significantly shortened with MB and KD (Table 2-a), the rate of pH decline significantly promoted with MB, KD, resin, SR and TSR (Table 2-b), and the minimum pH significantly subsided with MB, SF, SR and KD (Table 2-c).

Effect of proteinaceous pellicles on fungal growth

As shown in Figs. 2 and 3, saliva or serum pellicle essentially decreased the inhibitory effect of resilient liners on fungal growth. The effects were, however, varied depending upon both protein-coats and materials used (ANOVA, $p < 0.01$). The time lag at the beginning of rapid decline was significantly shortened by a saliva-coat with MB, and by a serum-coat with MB, whereas the elongated time lag at the beginning of rapid decline was observed with saliva-coated SF, serum-coated SF, SR, EV, MO, resin and TSR. (ANOVA, $p < 0.01$; Tables 3-a & 4-a). In contrast, as compared with uncoated samples, the significant increase in the rate of pH decline was observed with most of all specimens, other than saliva-coated KD, serum-coated KD, MB and SF (ANOVA, $p < 0.01$; Tables 3-b & 4-b). The significant reduction in minimum pH was observed with saliva-coated EV, SF, serum-coated EV and SF. (Tables 3-c & 4-c).

When the effects of thermal cycles on antifungal effect of saliva-coated resilient liners were analysed, in the case of 1000 thermocycling, the time necessary to reach pH 5.5 significantly shortened with MB (Table 3-a), the rate of pH decline significantly promoted with SR, SF, MB and KD (Table 3-b), and the minimum pH significantly subsided with MB, SF, KD and SR (Table 3-c). As to the saliva-coated, 10000 thermocycled specimens, the antifungal effects of each material were more weakened to reduce the differences between the

materials (Fig.2-c). The time necessary to reach pH 5.5 significantly shortened with MB and KD (Table 3-a), the rate of pH decline significantly promoted with KD, SR, SF and MB (Table 3-b), and the minimum pH significantly subsided with all specimens, other than EV (Table 3-c).

When the effects of thermal cycles on the antifungal effect of serum-coated resilient liners were analysed, in case of 1000 thermocycling, no samples exhibited elongated lag time to reach pH 5.5 (Table 4-a), however, the rate of pH decline significantly promoted with MB, KD and SF (Table 4-b), and the minimum pH significantly subsided with MB (Table 4-c). As to the serum-coated, 10000 thermocycled specimens, the antifungal effects of each material were similarly more weakened to reduce the differences between the materials (Fig.3-c). The time necessary to reach pH 5.5 significantly shortened with EV, MB and KD (Table 4-a), the rate of pH decline significantly promoted with KD, SF, SR, MB and MO (Table 4-b), and the minimum pH significantly subsided with MB, SF, TSR, SR and KD (Table 4-c).

Inhibitory effects of the components of the soft lining materials

As shown in Table 5, liquid components of resin, the paste of MB and KD showed an inhibitory effect on *C. albicans*. The monomer of resin showed an inhibitory zone of 6.0 mm in diameter, the paste of KD showed 7.0mm inhibitory zone and the paste of MB showed the greatest inhibitory zone (11.0mm). As references, 25ug/ml and 5mg/ml of Amphotericin B exhibited 9.0 and 15.0mm inhibitory zones in diameter, respectively.

Discussion

Denture stomatitis is an erythematous pathogenic condition of the denture bearing mucosa and caused mainly by microbial factors, especially by *C. albicans*. It has shown that the main reservoir of *C. albicans* and related *Candida* species is the fitting surface of upper denture (Davenport 1970) and that soft lining materials, including tissue conditioners and resilient liners, are easily colonized and deeply infected by these organisms (Allison & Douglas 1973; Douglas 1979;). In the pathogenesis of denture stomatitis, growth of large numbers of *Candida* on the fitting surface of denture and the following acid production by grown yeasts (Odds 1988) which shows direct cytotoxicity, activates acid proteinase and

phospholipase produced by these yeasts and promotes *Candida* adherence are known as among the most important factors (Samaranayake & MacFarlane 1985, 1990; Samaranayake et al., 1986). In addition, it has been recently pointed out that the continuous swallowing or aspiration of microorganisms from denture plaque exposes patients, particularly the immunocompromised host or medicated elderly people to be vulnerable to further infection.

Since the growth of *C. albicans* on the resilient lining materials, particularly on the aged materials, is of importance in clinical terms, in the present study, fungal growth on the commercial resilient liners was investigated by monitoring the pH change of growth medium. (Nikawa et al., 1994).

In the previous study, we employed two different concentrations of yeast suspension to inoculate onto the lining materials ($50 \mu\text{l}$ of 10^5 cells/ml and $50 \mu\text{l}$ of 10^7 cells/ml; which corresponded to the final concentrations of 2.83×10^3 cells/cm² and 2.83×10^5 cells/cm², respectively), and revealed that the latent antifungal activity of these lining materials was overpowered by the later concentration (Nikawa et al., 1994). Further, Budtz-Jorgensen, Theilade & Theilade (1983) reported that the diagnostic criteria for *Candida*-associated denture stomatitis is at the concentration of 10^5 cells/cm². Thus, in the present study, we employed the latter concentration to examine the antifungal activity of test specimens under the condition representative of the oral states of *Candida*-associated denture stomatitis patients.

Similar to our previous studies (Nikawa et al. 1994), as compared with acrylic resin, resilient lining materials showed inhibitory effects on the growth of *C. albicans* to a more or less extent in following three manners, i.e. delay of beginning of rapid decline in pH, decrease in the rate of pH change and increase in minimum pH (Figs. 1 through 3, Tables 2 through 4).

As to the nonthermocycled and uncoated samples, the effect on the delay of the start of rapid decline in pH, KD and MB were the most effective, and the paste of these materials showed the direct inhibitory effects (Table. 5). The results suggested that the delay of the start of rapid decline in pH caused by KD and MB should be attributed to their fungicidal effects against initially inoculated fungi. However, such effects of these materials are significantly reduced by thermocycling (ANOVA, $p < 0.01$; Table 2), implying that the fungicidal effect of the materials is derived from their unpolymerized components. The lowest rate of pH change

was observed with the case yeasts grown on SR, MB and KD and increased in the sequence SF \geq resin, and EV, MO and TSR showed the highest (ANOVA and multiple range test, $p < 0.01$; Fig. 1-a, Tables 2-b). As the pH values correlates with the number of total fungal cells in the well at each incubation period (Nikawa et al., 1994), the decrease in the rate of pH reduction accompanied by fungal growth, could be explained as caused by either the continuous contact of the fungal cells with the antifungal components of resilient liners, such as KD and MB, or the leaching out of the some inhibitory components from resilient liners to the growth medium. Since the acrylic resilient liner is reported to tend to show the relatively higher solubility than MB (Braden and Wright, 1983; Kawano et al, 1994), we speculate that the inhibitory effects of acrylic resilient liner, i.e. SR or possibly SF, against fungal growth rate should be attributed to the leaching out of the some inhibitory components to the growth medium. This resulting in the elongated fungal growth rate. In fact, thermocycling significantly reduced the such effect of SR (ANOVA, $p < 0.01$; Table 2). However, to date, we do not have accurate data available on the solubility of the materials examined, further study will be needed. Minimum pH could be considered to correspond to the maximum number of fungal cells on each soft liner (Nikawa et al, 1994), which could be the results of both the delay in initial growth and the suppression of growth rate, and also varied depending upon the samples on which *Candida* has grown. The case grown on MO showed the lowest (pH 2.88), increased in the order of EV, resin and TSR < SR, and SF, MB and KD showed the highest pH value (pH 4.09, 4.12 and 4.20, respectively) (Fig. 1-a, Tables 2-c). Thermocycling significantly reduced the minimum pH (promoted fungal growth) with MB, SF, SR and KD (ANOVA, $p < 0.01$; Table 2), which showed the relatively high inhibitory effects in the case of unthermocycled.

As shown in Figs. 1 through 3 and Tables 2 through 4, saliva or serum pellicle essentially decreased, to a more or less extent, the inhibitory effect of resilient liners and facilitated fungal growth on the materials as compared with uncoated samples, this being consistent with the results of our previous findings (Nikawa et al., 1997b). However, as to the delay in beginning of pH decline, salivary pellicle showed no effects and serum pellicle showed significant antifungal effects. These phenomena are not surprising, because it is known that some salivas suppress candidal growth while others do not (Samaranayake, Hughes & MacFarlane, 1984) and hence it is possible our observation that saliva does not

affect fungal growth on acrylic specimens could either be due to the quality of the saliva used and/or its high dilution in the incubation medium. A similar explanation could be offered for its growth on serum-coated acrylics as the serum is known to affect candidal cell kinetics in a variety of ways (Odds, 1988). In contrast, the results observed in the present study, that saliva or serum pellicle essentially facilitated fungal growth on the resilient lining materials, particularly significant promotion of the rate of pH decline and subsided minimum pH, should be partly attributed to the prevention of direct contact of yeast cells with lining materials caused by proteinaceous pellicles. This is similar to our previous results with tissue conditioners (Nikawa et al., 1997b). In addition, it is pointed out that the nutrient-rich environment of the oral cavity might overpower the inhibitory effect of these materials (Graham et al., 1991; Okita et al., 1991). Hence the phenomena may be additionally attributed to the local nutrient factors derived from pellicles. Further studies, however, are required to substantiate the current observations and to clarify the interactions between these oral fluids and candidal growth on denture materials.

Our findings, that some of commercial resilient lining materials exhibit antifungal activity, but that this activity is significantly reduced either by thermocycling or proteinaceous pellicles, taken together, suggests that appropriate control for denture plaque is essential to the long-term clinical use of resilient lining materials.

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Legends to Figures

Fig. 1 The pH curves of medium in which *Candida albicans* grown on (a: 0 times, b: 1000 times and c: 10000 times) thermocycled acrylic and resilient lining materials.

Fig. 2 The pH curves of medium in which *Candida albicans* grown on saliva-coated and (a: 0 times, b: 1000 times and c: 10000 times) thermocycled, acrylic and resilient lining materials.

Fig. 3 The pH curves of medium in which *Candida albicans* grown on serum-coated and (a: 0 times, b: 1000 times and c: 10000 times) thermocycled, acrylic and resilient lining materials.

Table 1 Materials

Type of Materials	Type of curing	Products	Abbr.	Manufacturers
Acrylic Resin	heat curing	Bio Resin	resin	SHOFU Inc. Kyoto, Japan
Resilient Lining Materials				
Acrylic	cold curing	Soften Nissin Soft Reverse	SF SR	Kamemizu Chem.Ind. Co., Ltd. Osaka, Japan Nissin Dental Products Inc. Kyoto, Japan
Silicone	cold curing	Mollosil Evatouch Tokuyama Soft Relining	MO EV TSR	DETAX. Karl Huber. GmbH & Co. KG, Ettlingen, Germany Neo Dental Chemical Products Co., LTD. Tokuyama Corp. Tokyo, Japan
	heat curing	Molloplast B	MB	DETAX. Karl Huber. GmbH & Co. KG, Ettlingen, Germany
Fluoric	heat curing	Kurepeet Dough	KD	Kreha Chemical Industry Co. Japan

Table 2-a Time necessary to reach pH 5.5 (h)
uncoated control samples

cont 0	cont 1000	cont 10000
SR 8.29 ±0.54	SR 10.73 ±0.46	MB* 12.28 ±0.40
SF 11.24 ±0.69	resin 11.50 ±1.19	EV 16.00 ±3.39
resin 11.68 ±0.72	SF 17.33 ±0.42	SF 17.82 ±0.68
MO 15.37 ±1.34	MO 18.36 ±0.24	resin 17.98 ±0.32
EV 17.93 ±1.38	TSR 19.21 ±1.72	SR 18.42 ±0.23
TSR 19.36 ±1.56	EV 19.37 ±0.57	MO 18.60 ±0.20
KD 45.70 ±4.49	MB* 31.68 ±7.17	TSR 18.89 ±0.88
MB 46.85 ±5.76	KD 47.75 ±6.70	KD* 27.35 ±4.69

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

* Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

Table 2-b Rate of decrease in pH (unit/h)
uncoated control samples

cont 0	cont1000	cont10000
KD -0.0264 ±0.0027	KD -0.0302 ±0.0013	SF -0.0477 ±0.0219
MB -0.0264 ±0.0025	SF -0.0392 ±0.0121	MB* -0.0515 ±0.0069
SR -0.0306 ±0.0017	EV -0.0515 ±0.0126	EV -0.0550 ±0.0076
SF -0.0337 ±0.0067	MB* -0.0553 ±0.0036	KD* -0.0594 ±0.0119
resin -0.0492 ±0.0052	SR* -0.0610 ±0.0031	resin* -0.0679 ±0.0042
EV -0.0498 ±0.0034	resin* -0.0662 ±0.0059	SR* -0.0748 ±0.0041
MO -0.0507 ±0.0040	MO -0.0714 ±0.0061	TSR* -0.0816 ±0.0025
TSR -0.0539 ±0.0152	TSR* -0.0726 ±0.0116	MO -0.0840 ±0.0029

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

*Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

Table 2-c Minimum pH of growth meidium
uncoated control samples

cont 0	cont 1000	cont 10000
MO 2.88 ± 0.037	EV 3.18 ± 0.130	EV 3.13 ± 0.077
EV 3.16 ± 0.046	MO 3.26 ± 0.070	MO 3.18 ± 0.020
resin 3.17 ± 0.079	TSR 3.36 ± 0.080	MB* 3.34 ± 0.018
TSR 3.25 ± 0.019	resin 3.49 ± 0.084	TSR 3.34 ± 0.042
SR 3.91 ± 0.080	MB* 3.82 ± 0.029	SF* 3.41 ± 0.083
SF 4.09 ± 0.100	SF* 3.87 ± 0.077	SR* 3.48 ± 0.045
MB 4.12 ± 0.040	SR 3.92 ± 0.062	resin 3.60 ± 0.025
KD 4.20 ± 0.260	KD 4.08 ± 0.075	KD* 3.70 ± 0.069

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

*Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

Table 3-a Time necessary to reach pH 5.5 (h)
saliva-coated samples

	sal0	sal1000	sal10000
	SR 10.90 ± 0.81	resin 10.39 ± 3.70	resin 10.08 ± 2.00
	resin 13.94 ± 4.20	SR 10.42 ± 2.39	SR 12.00 ± 1.96
	MO 18.01 ± 1.44	MO 15.72 ± 3.36	TSR 14.02 ± 2.57
	TSR 18.02 ± 1.45	TSR 16.08 ± 3.39	MO 15.20 ± 3.93
▲	SF 18.93 ± 0.95	SF 19.62 ± 0.32	MB* 19.19 ± 0.62
	EV 19.95 ± 1.38	EV 21.12 ± 0.65	SF 19.89 ± 0.51
▼	MB 31.29 ± 4.97	MB* 26.44 ± 2.46	EV 20.59 ± 0.81
	KD 40.74 ± 6.84	KD 37.48 ± 3.72	KD* 25.89 ± 2.05

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

*Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 3-b Rate of decrease in pH (unit/h)
saliva-coated samples

sal0	sal1000	sal10000
KD	-0.0295 ± 0.0101	KD* -0.0442 ± 0.0038
▲ SR	-0.0378 ± 0.0031	TSR -0.0591 ± 0.0080
▲ SF	-0.0454 ± 0.0045	resin -0.0605 ± 0.0030
▲ MB	-0.0475 ± 0.0078	SR* -0.0617 ± 0.0061
▲ resin	-0.0615 ± 0.0027	MO -0.0668 ± 0.0111
▲ MO	-0.0675 ± 0.0032	SF* -0.0686 ± 0.0018
▲ EV	-0.0677 ± 0.0010	EV -0.0727 ± 0.0036
▲ TSR	-0.0694 ± 0.0018	MB* -0.0788 ± 0.0022

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

*Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 3-c Minimum pH of growth meidium
saliva-coated samples

	sal10	sal1000	sal10000
▼ EV	2.87 ± 0.046	MO 2.91 ± 0.022	MO* 2.47 ± 0.050
MO	2.93 ± 0.037	EV 2.92 ± 0.032	resin* 2.65 ± 0.007
TSR	3.07 ± 0.013	TSR 3.13 ± 0.014	TSR* 2.77 ± 0.014
resin	3.21 ± 0.031	resin 3.19 ± 0.007	SR* 2.78 ± 0.110
▼ SF	3.83 ± 0.170	MB* 3.57 ± 0.029	EV 2.90 ± 0.032
MB	4.02 ± 0.040	SF* 3.60 ± 0.120	MB* 3.06 ± 0.029
KD	4.06 ± 0.033	KD* 3.78 ± 0.062	SF* 3.23 ± 0.120
SR	4.08 ± 0.087	SR* 3.84 ± 0.110	KD* 3.55 ± 0.062

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

*Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 4-a Time necessary to reach pH 5.5 (h)
serum-coated samples

	ser0	ser1000	ser10000
▲ SF	18.74 ± 4.04	SR 24.53 ± 1.52	EV* 20.55 ± 0.63
▲ SR	22.88 ± 2.24	resin 24.90 ± 3.17	SF 21.37 ± 0.85
▲ EV	26.18 ± 3.82	EV 25.90 ± 3.68	MB* 21.83 ± 2.03
▲ MO	26.94 ± 3.40	MB 28.42 ± 3.93	KD* 25.90 ± 3.38
▼ MB	27.48 ± 4.58	SF 28.56 ± 4.01	SR 32.55 ± 5.13
▲ resin	34.95 ± 4.03	MO 33.47 ± 4.30	MO 34.78 ± 1.70
KD	35.14 ± 2.22	KD 35.04 ± 6.35	resin 37.46 ± 14.15
▲ TSR	42.73 ± 15.00	TSR 53.41 ± 12.00	TSR 37.77 ± 13.00

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

* Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 4-b Rate of decrease in pH (unit/h)
serum-coated samples

	ser0	ser1000	ser10000
	KD -0.0290 ±0.0022	MO -0.0424 ±0.0080	KD* -0.0431 ±0.0068
	MB -0.0298 ±0.0024	SR -0.0552 ±0.0126	TSR -0.0563 ±0.0110
	SF -0.0301 ±0.0030	MB* -0.0558 ±0.0039	SF* -0.0584 ±0.0030
▲	SR -0.0506 ±0.0016	TSR -0.0595 ±0.0023	SR* -0.0620 ±0.0101
▲	MO -0.0544 ±0.0136	KD* -0.0598 ±0.0056	EV -0.0652 ±0.0014
▲	TSR -0.0632 ±0.0023	SF* -0.0604 ±0.0053	resin -0.0684 ±0.0087
▲	EV -0.0683 ±0.0024	EV -0.0652 ±0.0071	MB* -0.0685 ±0.0069
▲	resin -0.0684 ±0.0077	resin -0.0658 ±0.0053	MO* -0.0727 ±0.0088

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

* Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 4-c Minimum pH of growth medium
serum-coated samples

	ser0	ser1000	ser10000
▼ EV	3.01 ± 0.025	3.05 ± 0.033	2.83 ± 0.023
MO	3.04 ± 0.060	3.08 ± 0.050	2.96 ± 0.058
resin	3.17 ± 0.034	3.23 ± 0.027	3.11 ± 0.051
TSR	3.56 ± 0.250	3.45 ± 0.210	3.17 ± 0.060
▼ SF	3.77 ± 0.150	3.62 ± 0.110	3.24 ± 0.063
MB	4.01 ± 0.037	3.64 ± 0.170	3.31 ± 0.180
KD	4.05 ± 0.037	3.92 ± 0.094	3.35 ± 0.037
SR	4.11 ± 0.096	4.01 ± 0.170	3.77 ± 0.050

No significant differences were observed between the values connected by bars. (ANOVA, p>0.01)

* Antifungal effects were significantly reduced by thermal cycling. (ANOVA, p<0.01)

An arrow indicates the significant change caused by protein coats.

Table 5 Inhibitory effects on *Candida albicans*
of components of materials examined

Diameter of inhibitory zone (mm)		
resin	liquid	6.0
	powder	-
SF	liquid	-
	powder	-
SR	liquid	-
	powder	-
MO	base paste	-
	catalyst liquid	-
EV	base paste	-
	catalyst paste	-
TSR	base paste	-
	catalyst paste	-
MB	Dough	7.0
KD	Dough	11.0
Amphotericin B	5mg/ml	15.0
	25ug/ml	9.0

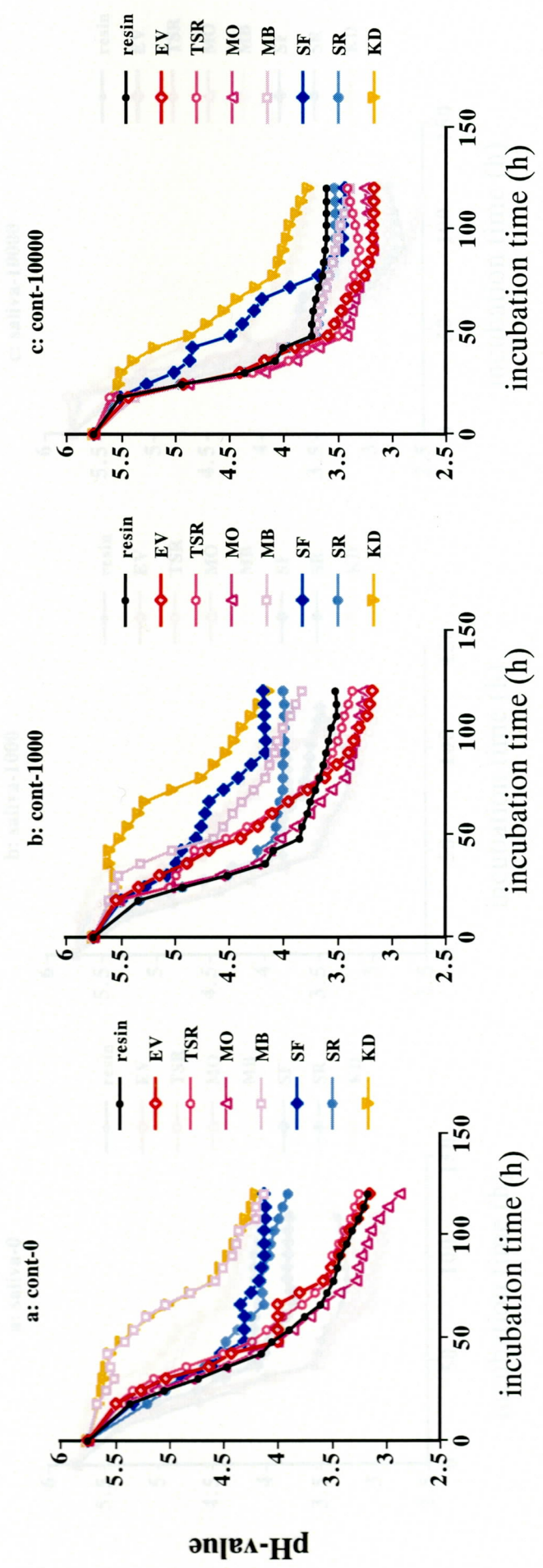


Fig. 1 Nikawa et al.

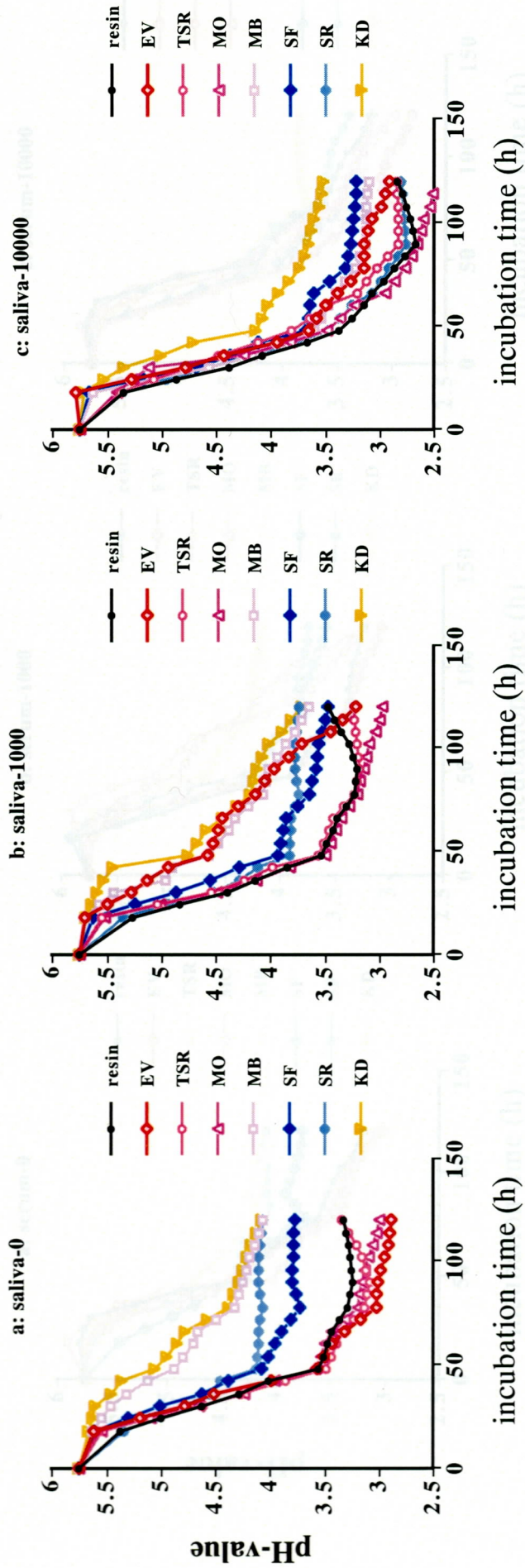


Fig. 2 Nikawa et al.

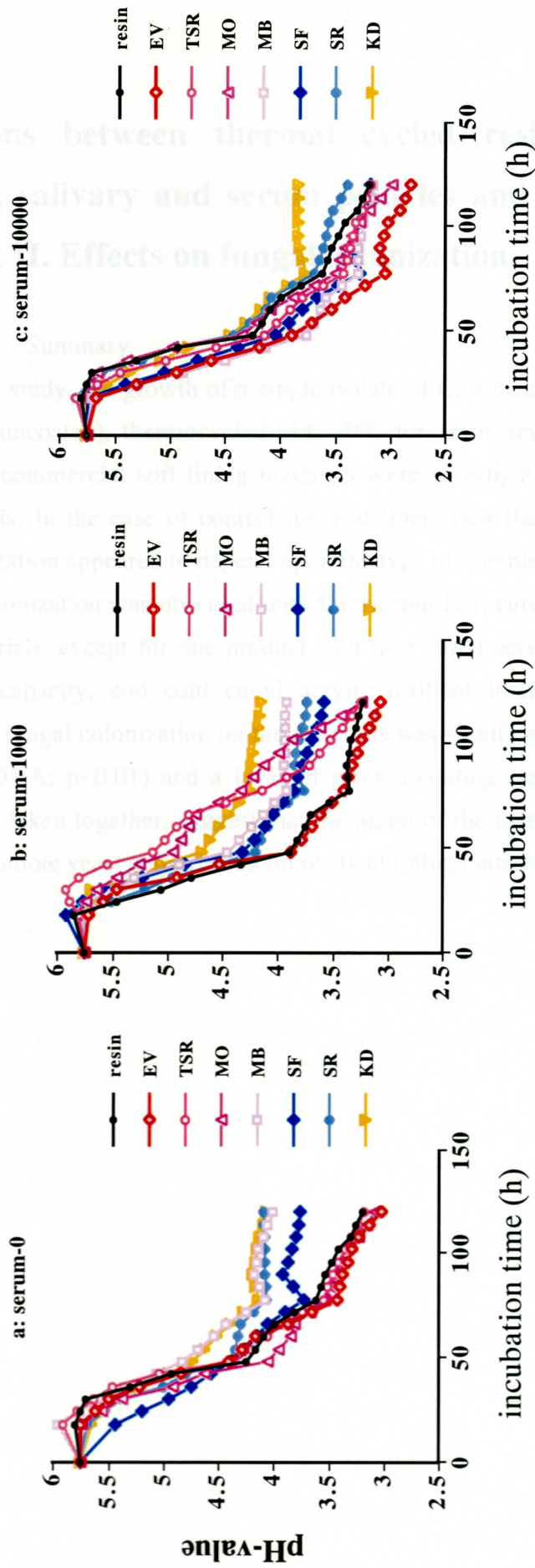


Fig. 3 Nikawa et al.