Candida albicans growth on thermal cycled materials for maxillofacial prostheses *in vitro*.

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) fifteen commercial maxillofacial materials was investigated, by monitoring pH changes in growth media. The inhibitory effect of the tissue conditioners on fungal growth was analysed 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 materials (nonthermocycled and uncoated), significant antifungal effect was observed with two products. 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, $p<0.01$). When the interrelation between three parameters of fungal growth and the surface hydrophobicity of the materials were analysed, minimum pH of fungal growth on 10000-thermocycled materials correlated well with the contact angles of the materials (Student t-test, $p<0.01$), suggesting that thermocycling process reduced the unpolymerized components of the materials which showed the antifungal effects, resulted in that the cell growth depends on the surface hydrophobicity of the specimens.

These results, taken together, suggest that the ageing of the materials and the biological fluids of the host enhanced the fungal growth on maxillofacial materials.

Introduction

Resilient materials are frequently used for the fabrication of facial prostheses, and denture lining. Because of their biological inertness, technical simplicity, and viscoelastic properties, they act as shock absorbers and reduce and distribute the stress on the denture bearing tissues (Lytle, 1959; Udagama 1987). Their use for patient comfort and the treatment of the atrophic ridge, bone defects and undercuts has been known to be clinical beneficial (Boucher et al., 1975). Although these attributes are positive, there are also some physical and microbiological disadvantages to the use of these materials. Among these problems, one of the most serious has been colonization and infection of the material surface by *Candida albicans* and related *Candida* species (Udagama 1987, Pigno et al, 1994), resulting in being 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 particularly denture lining materials has been thought to be the first step (Rotrosen et al., 1986), followed by the growth of adherent cells or the coadhesion of floating cells to adherent ones. However only limited has been data available on the interactions between resilient liners and fungi. In addition, although the materials are known to show the age changes in their physical properties, little attention has been paid on the growth of *Candida* on aged materials.

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, the proteinaceous pellicle reduces the antifungal effects of tissue conditioners (Nikawa et al. 1997-b). Hence, the interactions between proteinaceous pellicles, (aged) materials frequently used for maxillofacial prostheses and *C. albicans* may be an important factor in regard to the in vivo fungal colonization and/or invasion of materials used long term. Thus the present study was concerned with investigating the growth of *C. albicans* on thermocycled and protein coated commercial maxillofacial materials used for facial prostheses.

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 maxillofacial materials

Samples of acrylics (Bio Resin, Shofu, Kyoto, Japan) and 15 commercial maxillofacial 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). The surface hydrophobicity of each material determined by contact angle measurements were shown in Table 2.

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 were taken after 0, 1000 and 10000 thermal cycling.

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× g for 15min at 4℃. Human serum was purchased from Sigma Chemical Co. (St Louis, MO, USA). Whole saliva and serum were stored at -25℃ before use (Nikawa et al. 1990, 1996).

Assay procedures

The growth assay was conducted using the procedure previously published(Nikawa et al. 1993, 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.

Results

Effect of maxillofacial materials 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 maxillofacial materials and times of thermal cycle on which *Candida* had grown, the reverse sigmoidal pH curves were observed with all samples, being similar to the results with tissue conditioners (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.

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 nonthermocycled and uncoated specimens, Astron Soft and Light Liner Soft were 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 3-a), followed by Ever Soft $\geqq$ Rebaron LC, Rebaron $\geqq$ Light Liner Hard, Astron LCH, and no significant effects were observed with the other materials, as compared with resin.

The highest rate of pH decline was observed with five silicone materials, *i.e.* Silkskin, CosmesilT001, Ideal, Episil, Cosmesil HC, and resin, decreased in the sequence, PER form$\geqq$ Lightdon-U$\geqq$Resiline$\geqq$ Astron LCH, Light Liner Hard, Rebaron, Rebaron LC and Ever Soft. Two products, *i.e.* Light Liner Soft and Astron Soft were the most effective to inhibit the fungal growth (ANOVA and multiple range test, $p<0.01$; Fig. 1-a, Tables 3-b).

Minimum pH also varied depending upon the samples on which *Candida* has grown, the case grown on resin, Lightdon-U, Resiline, PER form, Cosmesil HC or Rebaron, the lowest pH was observed (pH 2.60-2.81), increased in the order of Astron LCH $\leqq$Light Liner Hard and Silskin $\leqq$Ever Soft and Rebaron LC $\leqq$Ideal $\leqq$CosmesilT001 $\leqq$Episil. Light Liner Soft and Astron Soft showed the highest pH value (pH 4.03, and 4.07, respectively) (Fig. 1-a, Tables 3-c).

Effect of thermal cycles on antifungal effect of maxillofacial materials

As shown in Fig. 1-a through c, the antifungal effects of each materials appeared to be decreased with an increasing times of thermocycling. The time necessary to reach pH 5.5 was

not significantly affected by thermocycling, the rate of pH decline significantly promoted with Light Liner Soft and Astron Soft, and the minimum pH significantly subsided with Light Liner Soft and Astron Soft (Fig. 2-a.). However, reverse effects were observed with Ever Soft, Lightdon-U and resin (Fig. 2-a.).

Effect of proteinaceous pellicles on fungal growth

As shown in Figs. 1 and 2, saliva or serum pellicle tended to decrease the inhibitory effect of resilient liners on fungal growth. However, the time lag at the beginning of rapid decline was significantly elongated either by saliva-coat or by serum-coat, with Astron Soft and Light Liner Soft (ANOVA, $p<0.01$; Tables 4-a & b). As compared with uncoated samples, the significant effects on the rate of pH decline was not obserbed with most specimens (ANOVA, $p>0.05$; Tables 5-a & b). In contrast, the significant reduction in minimum pH was observed with saliva-coated or serum-coated Astron Soft and Light Liner Soft (Tables 6-a & b).

The effects of thermocycling on antifungal effect of saliva- or serum-coated resilient liners showed that the time necessary to reach pH 5.5 was significantly shortened, the rate of pH decline significantly promoted and the minimum pH significantly subsided with Astron Soft and Light Liner Soft (Fig. 2-a through c).

Surface hydrophobicity of the materials and fungal growth

When the interrelation between three parameters of fungal growth and the surface hydrophobicity of the materials were analysed, minimum pH of fungal growth on 10000-thermocycled materials correlated well with the contact angles of the materials, and the result gave a level of significance (Fig. 3, $r=0.691$; $p<0.01$)

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) shows direct cytotoxicity, acid proteinase and phospholipase produced by these yeasts is activated and promotes *Candida* adherence (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, to the risks of unexpected infections (Nikawa et al. 1998).

Similarly to our previous results (Nikawa et al. 1994), as compared with acrylics, resilient lining materials showed inhibitory effects to a more or less extent on the growth of *C. albicans* in the following three ways, i.e. delay of beginning of rapid decline in pH, decreases in the rate of pH change and increases in minimum pH (Figs. 1 &2, Tables 3 through 6).

As to non-thermocycled and uncoated specimens, Asron Soft and Light Liner Soft were 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 3-a). The result suggested that the delay of beginning of rapid decline in pH caused by Asron Soft and Light Liner Soft, , which are the soft acrylic materials comprised of VLC type monomer and polymer, should be attributed to their fungicidal effects agaist initially inoculated fungi. However, such effects of these two materials were significantly reduced by thermocycling (ANOVA, $p<0.01$; Fig. 2-b). In contrast, all of the five silicone materials and VLC materials comprising either single paste or single gel showed the minimal effects on the fungal growth, as compared with the heat-curing acrylic resin. Thus, these results, taken together, imply that the fungicidal effects of the materials against inocula should be derived from their unpolymerized component.

In the present study, two products, *i.e.* Light Liner Soft and Astron Soft significantly reduced the fungal growth rate. As the pH values correlates with the number of total fungal cells in the well at any incubation periods (Nikawa et al., 1994), the decrease in the rate of pH reduction accompanied by fungal growth, could be explained to be caused by either the continuous contact of the fungal cells with the antifungal components of products or the leaching out of the some inhibitory components from materials to the growth medium.

As shown in Fig. 1-a and Tables 3-c, minimum pH also varied depending upon the samples on which *Candida* has grown, and Light Liner Soft and Astron Soft showed the highest pH value (pH 4.03, and 4.07, respectively). As minimum pH could be considered to correspond to the maximum number of fungal cells on each products (Nikawa et al, 1994), which should be the results of both the delay in initial growth and the suppression of growth rate. Thermocycling significantly reduced the minimum pH (promoted fungal growth) with Light Liner Soft and Astron Soft (ANOVA, $p<0.01$; Fig. 2-a), which showed the highest

inhibitory effects in the case of nonthermocycled.

When the interrelation between three parameters of fungal growth and the surface hydrophobicity of the materials were analysed, minimum pH of fungal growth on 10000-thermocycled materials correlated with the contact angles of the materials, and the result gave a level of significance (Fig. 3, $r=0.691$; $p<0.01$). In our previous studies, although the fungal growth was related to the hydrophobicity or adherence capacity of the substrate (Nikawa et al. 1994), the components or the composition of the materials affects, to greater extent, on the fungal growth (Nikawa et al. 1995). Thus thermocycling process should reduce the effects of fungal growth resulted in being drawn out the latent effects of surface hydrophobicity of maxillofacial materials on fungal growth.

As shown in Figs. 1 through 3 and Tables 2 through 5, saliva or serum pellicle essentially decreased, the inhibitory effect of maxillofacial materials and facillitated fungal growth on the materials as compared with uncoated samples, 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 the significant antifungal effects. This 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). 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.

Lastly, our findings, that some of commercial maxillofacial materials exhibit the antifungal activity, but that this acivity is significantly reduced either by thermocycling or proteinaceous pellicles, taken together, comfirms that appropriate control of denture plaque is essential to the long-term clinical usage of maxillofacial materials.

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Legends to Figures

Fig. 1 The pH curves of medium in which *Candida albicans* grown on uncoated(a), saliva-coated(b), and serum-coated(c), thermocycled (0 times, 1000 times, 10000 times) acrylic and maxillofacial materials.

Fig. 2 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 of the medium in which *Candida albicans* grown on uncoated(a), saliva-coated(b), and serum-coated(c) thermocycled (0 times, 1000 times, 10000 times) acrylic and maxillofacial materials. Materials with the significant reduction in antifungal effects were indicated with asterisk (*), and that with the reverse effects were indicated with double asterisks (**) (ANOVA, $p<0.01$).

Fig. 3 Correlation between minimum pH accompanied with the fungal growth on 10000-times thermocycled maxillofacial materials, and the surface hydrophobicity of the materials.

Table 1 Resilient Materials

	manufacturer	type
Astron LC Hard [a]	Astron Dental Corp, USA	VLC*
Astron LC Soft [a]	Astron Dental Corp, USA	VLC
COSMESIL HC[b]	Principality Medical Ltd, UK	condensation curing
COSMESIL T001[b]	Principality Medical Ltd, UK	addition curing
EPISIL[b]	Dreve-Dentamid GmbH, Germany	addition curing
Ever Soft [c]	Myerson Austenal Ltd, UK	cold curing
IDEAL[b]	Orthomax, UK	addition curing
Resiline[a]	Dentsply/York Div, UK	VLC
Lihgt Liner Hard[a]	H.J. Bosworth, USA	VLC
Light Liner Soft[a]	H.J. Bosworth, USA	VLC
Lightdon-U	Dreve-Dentamid GmbH, Germany	VLC
PER form[a]	Whaledent Dentalprodukte GmbH, Germany	VLC
Rebaron LC [a]	GC Inc. Japan	VLC
Rebaron [a]	GC Inc. Japan	cold curing
SILSKIN 2000[c]	DePuy Healthcare, UK	addition curing
Bio Resin (resin)[b]	Shofu Inc. Japan	heat curing

*VLC: visible light curing

[a]VLC acrylic materials cured in the TRIAD 2000curing unit.

[b]Silicone materials cured at room temperature 23±2oC and 50±5 % relative humidity.

[c]Acrylic materials cured at room temperature 23±2oC and 50±5 % relative humidity.

Table 2 Contact angles of resilient materials

	contact angle*
Astron LCH	70.42 ±4.76
Astron Soft	86.07 ±2.07
COSMESIL HC	113.08 ±0.91
COSMEIL T001	109.43 ±1.81
EPISIL	105.12±1.38
Ever Soft	99.58±2.29
IDEAL	110.93±2.03
Resiline	60.02 ±3.63
Lihgt Liner Hard	87.68 ±5.18
Light Liner Soft	79.07 ±4.69
Lightdon-U	86.68±2.98
PER form	99.82±2.06
Rebaron LC	84.70 ±3.85
Rebaron	81.70 ±0.88
SILSKIN 2000	108.10 ±5.37
Bio Resin (resin)	58.80 ±1.20

*Mean contact angle ±SD calculated from six specimens by the sessile drop method using distilled water as sensing liquid.

Table 3-a Time to reach pH 5.5
uncoated control sample

cont 0	cont 1000	cont 10000
Silskin	Resiline	Silskin
CosmesT001	Episil	CosmesT001
Episil	Astron LCH	Ideal
Ideal	Silskin	CosmesilHC
Lightdon-U	PER form	Astron LCH
resin	Light Liner Hard	Episil
PER form	CosmesT001	Light Liner Hard
Resiline	Lightdon-U	PER form
CosmesilHC	Rebaron LC	Resiline
Astron LCH	Rebaron	Rebaron LC
Light Liner Hard	Ideal	Rebaron
Rebaron	CosmesilHC	Lightdon-U
Rebaron LC	*resin*	*resin*
Ever Soft	Ever Soft	Light Liner Soft
Light Liner Soft	Astron Soft	Ever Soft
Astron Soft	Light Liner Soft	Astron Soft

* No significant differences were observed with the sample connected by bars.

Table 3 b Rate of pH reduction

uncoated control samples

cont 0		cont 1000	cont 10000
Astron Soft	*	Light Liner Soft	Light Liner Soft
Light Liner Soft		Astron Soft	Astron Soft
Ever Soft		Ever Soft	Ever Soft
Rebaron LC		CosmesilHC	Episil
Rebaron		Ideal	Rebaron LC
Light Liner Hard		*resin*	CosmesT001
Astron LCH		Rebaron	CosmesilHC
Resiline		Rebaron LC	Ideal
Lightdon-U		CosmesT001	*resin*
PER form		Lightdon-U	Silskin
resin		Light Liner Hard	Light Liner Hard
CosmesilHC		Episil	Astron LCH
Episil		Silskin	Rebaron
Ideal		Astron LCH	Lightdon-U
CosmesT001		Resiline	PER form
Silskin		PER form	Resiline

* No significant differences were observed with the sample connected by bars.

Table 3-c Minimum pH
uncoated control samples

cont 0		cont 1000	cont 10000
resin	*	Resiline	Resiline
Lightdon-U		PER form	PER form
Resiline		Rebaron	Lightdon-U
PER form		Astron LCH	Light Liner Hard
CosmesilHC		*resin*	Rebaron
Rebaron		Rebaron LC	Rebaron LC
Astron LCH		Lightdon-U	*resin*
Light Liner Hard		Light Liner Hard	Astron LCH
Silskin		Ever Soft	Ever Soft
Ever Soft		CosmesT001	Astron Soft
Rebaron LC		Episil	Light Liner Soft
Ideal		Silskin	Episil
CosmesT001		Ideal	Silskin
Episil		CosmesilHC	CosmesilHC
Light Liner Soft		Light Liner Soft	Ideal
Astron Soft		Astron Soft	CosmesT001

* No significant differences were observed with the sample connected by bars.

Table 4-a Time to reach pH 5.5
saliva-coated sample

sal 0	sal 1000	sal 10000
CosmesT001	CosmesT001	CosmesT001
CosmesilHC	Silskin	Silskin
Ideal	Ideal	Ideal
Episil	Episil	Episil
Silskin	CosmesilHC	CosmesilHC
PER form	Lightdon-U	Resiline
resin	*resin*	Lightdon-U
Light Liner Hard	Resiline	Astron LCH
Rebaron LC	Ever Soft	Ever Soft
Lightdon-U	PER form	*resin*
Resiline	Astron LCH	Rebaron
Astron LCH	Rebaron	PER form
Rebaron	Rebaron LC	Rebaron LC
Ever Soft	Light Liner Hard	Light Liner Hard
Light Liner Soft	Light Liner Soft	Astron Soft
Astron Soft	Astron Soft	Light Liner Soft

* No significant differences were observed with the sample connected by bars.

Table 4-b Time to reach pH 5.5
serum-coated sample

ser 0	ser 1000	ser 10000
CosmesilHC *	Episil	Ideal
CosmesT001	CosmesT001	CosmesT001
Episil	Silskin	Episil
Silskin	CosmesilHC	Silskin
Ideal	Ideal	CosmesilHC
resin	Lightdon-U	Resiline
Lightdon-U	Rebaron	PER form
PER form	*resin*	Lightdon-U
Ever Soft	PER form	Astron LCH
Resiline	Astron LCH	Light Liner Hard
Rebaron	Light Liner Hard	*resin*
Astron LCH	Rebaron LC	Rebaron LC
Light Liner Hard	Ever Soft	Rebaron
Rebaron LC	Resiline	Ever Soft
Light Liner Soft	Astron Soft	Light Liner Soft
Astron Soft	Light Liner Soft	Astron Soft

* No significant differences were observed with the sample connected by bars.

Table 5-a Rate of pH reduction
saliva-coated samples

sal 0	sal 1000	sal 10000
Astron Soft *	Astron Soft	Light Liner Soft
Light Liner Soft	Light Liner Soft	Astron Soft
Astron LCH	Light Liner Hard	Episil
Rebaron	Ever Soft	Light Liner Hard
Light Liner Hard	Rebaron LC	Rebaron LC
Rebaron LC	Astron LCH	Ideal
Ever Soft	Rebaron	Ever Soft
Lightdon-U	Resiline	Silskin
PER form	PER form	Astron LCH
Resiline	*resin*	*resin*
resin	Lightdon-U	Rebaron
Silskin	Episil	CosmesT001
Episil	CosmesilHC	Lightdon-U
Ideal	Silskin	PER form
CosmesilHC	Ideal	CosmesilHC
CosmesT001	CosmesT001	Resiline

* No significant differences were observed with the sample connected by bars.

Table 3 b Rate of pH reduction

serum-coated samples

ser 0		ser 1000	ser 10000
Astron Soft	*	**Light Liner Soft**	**Astron Soft**
Light Liner Soft		**Astron Soft**	**Light Liner Soft**
Rebaron LC		**Ever Soft**	**Episil**
Ever Soft		**Resiline**	**Silskin**
Light Liner Hard		**Rebaron LC**	**Ever Soft**
Astron LCH		**Light Liner Hard**	**CosmesilHC**
Resiline		**Astron LCH**	**CosmesT001**
Rebaron		**CosmesilHC**	**Ideal**
Lightdon-U		**Ideal**	**Rebaron LC**
PER form		**Silskin**	***resin***
Ideal		***resin***	**Astron LCH**
Silskin		**CosmesT001**	**Lightdon-U**
Episil		**Lightdon-U**	**Rebaron**
resin		**Episil**	**Light Liner Hard**
CosmesT001		**PER form**	**Resiline**
CosmesilHC		**Rebaron**	**PER form**

* No significant differences were observed with the sample connected by bars.

Table 6-a Minimum pH
saliva-coated samples

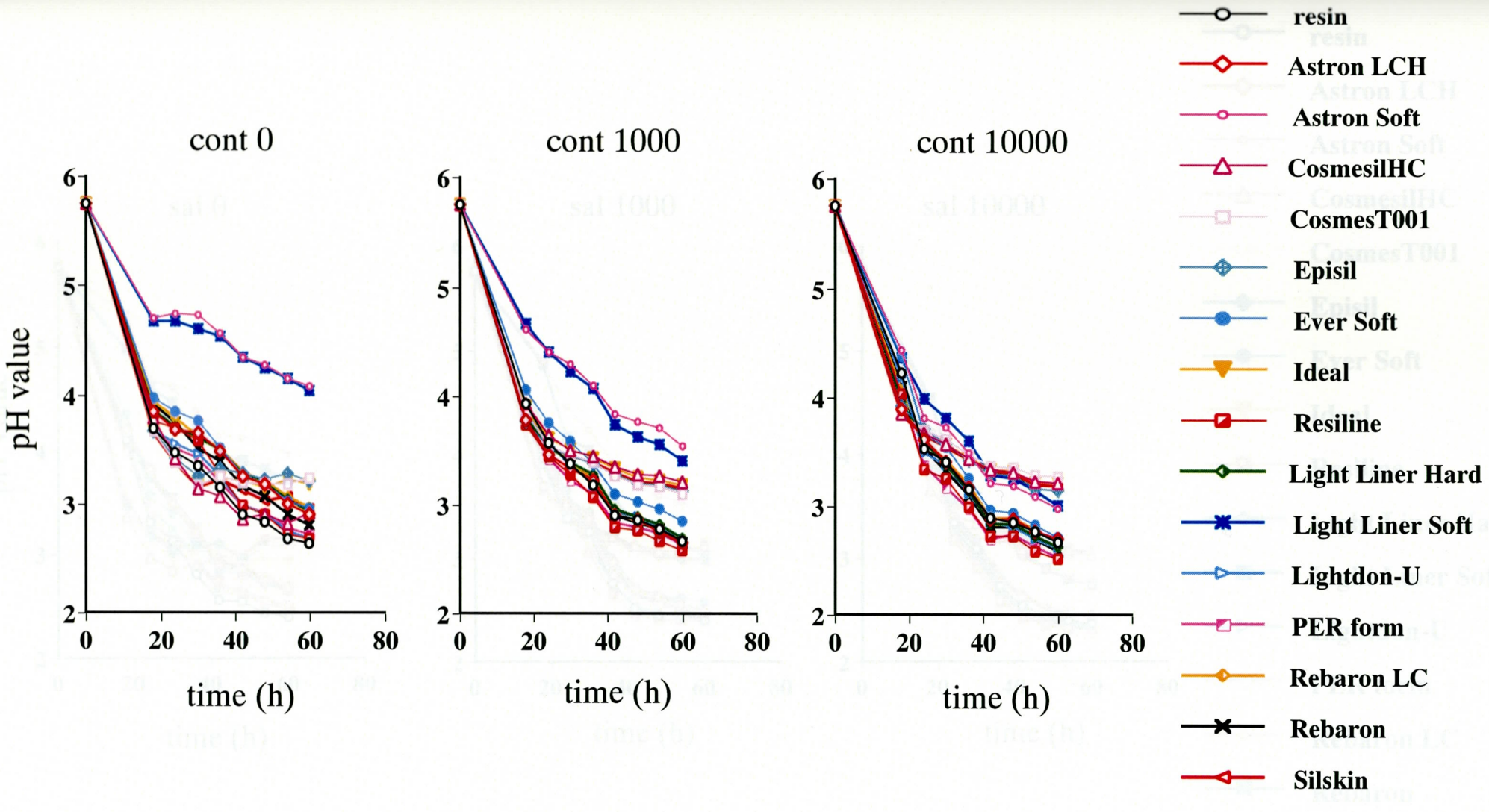
sal 0		sal 1000	sal 10000
resin	*	*resin*	Resiline
PER form		Resiline	PER form
Resiline		Lightdon-U	Rebaron
Lightdon-U		Rebaron	Lightdon-U
Ever Soft		PER form	*resin*
Light Liner Hard		Astron LCH	Ever Soft
Astron LCH		Light Liner Hard	Light Liner Hard
CosmesilHC		Rebaron LC	Rebaron LC
Rebaron		Ever Soft	Astron LCH
Rebaron LC		Silskin	Light Liner Soft
CosmesT001		Episil	Astron Soft
Ideal		Ideal	CosmesT001
Episil		CosmesT001	CosmesilHC
Silskin		Light Liner Soft	Ideal
Light Liner Soft		CosmesilHC	Silskin
Astron Soft		Astron Soft	Episil

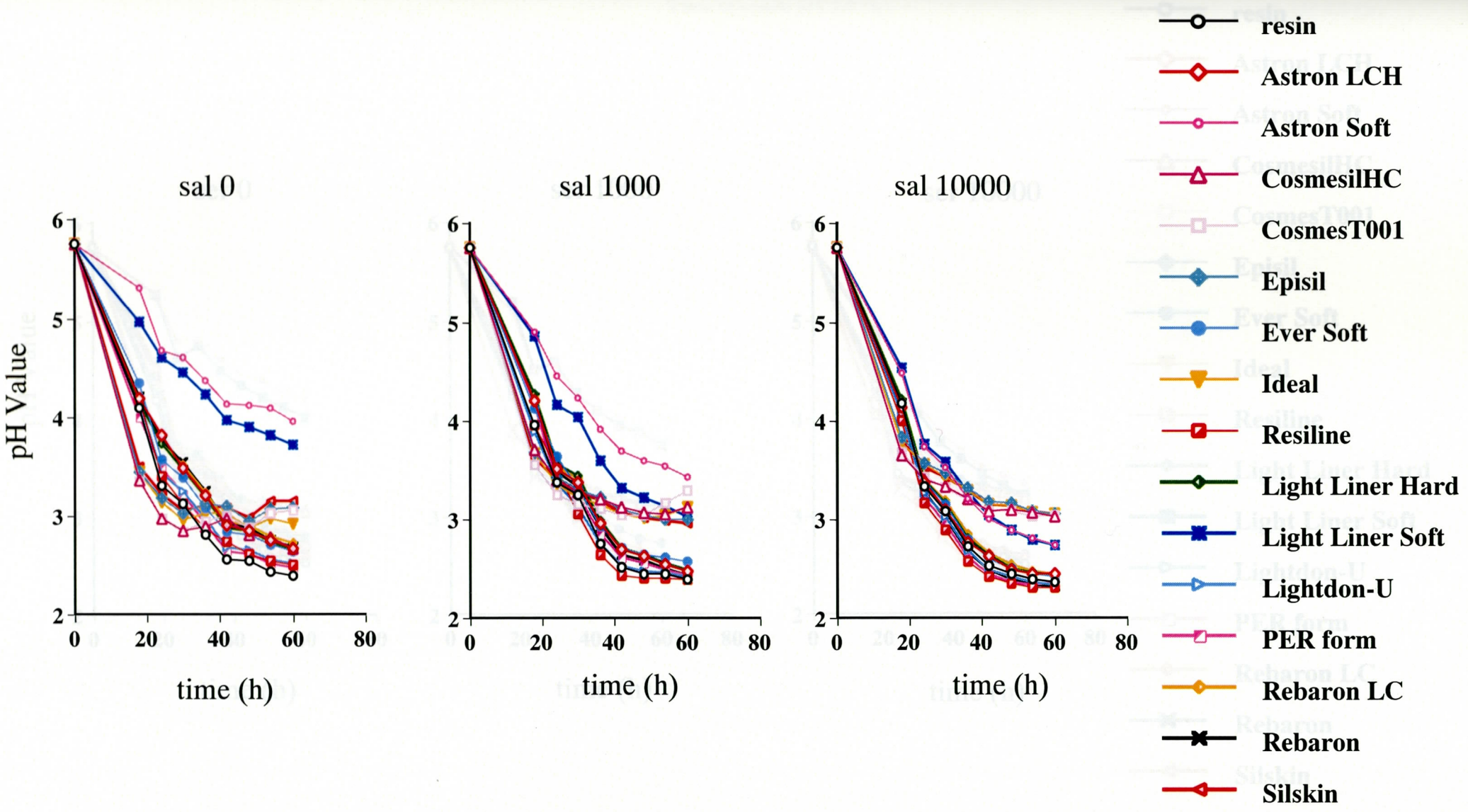
* No significant differences were observed with the sample connected by bars.

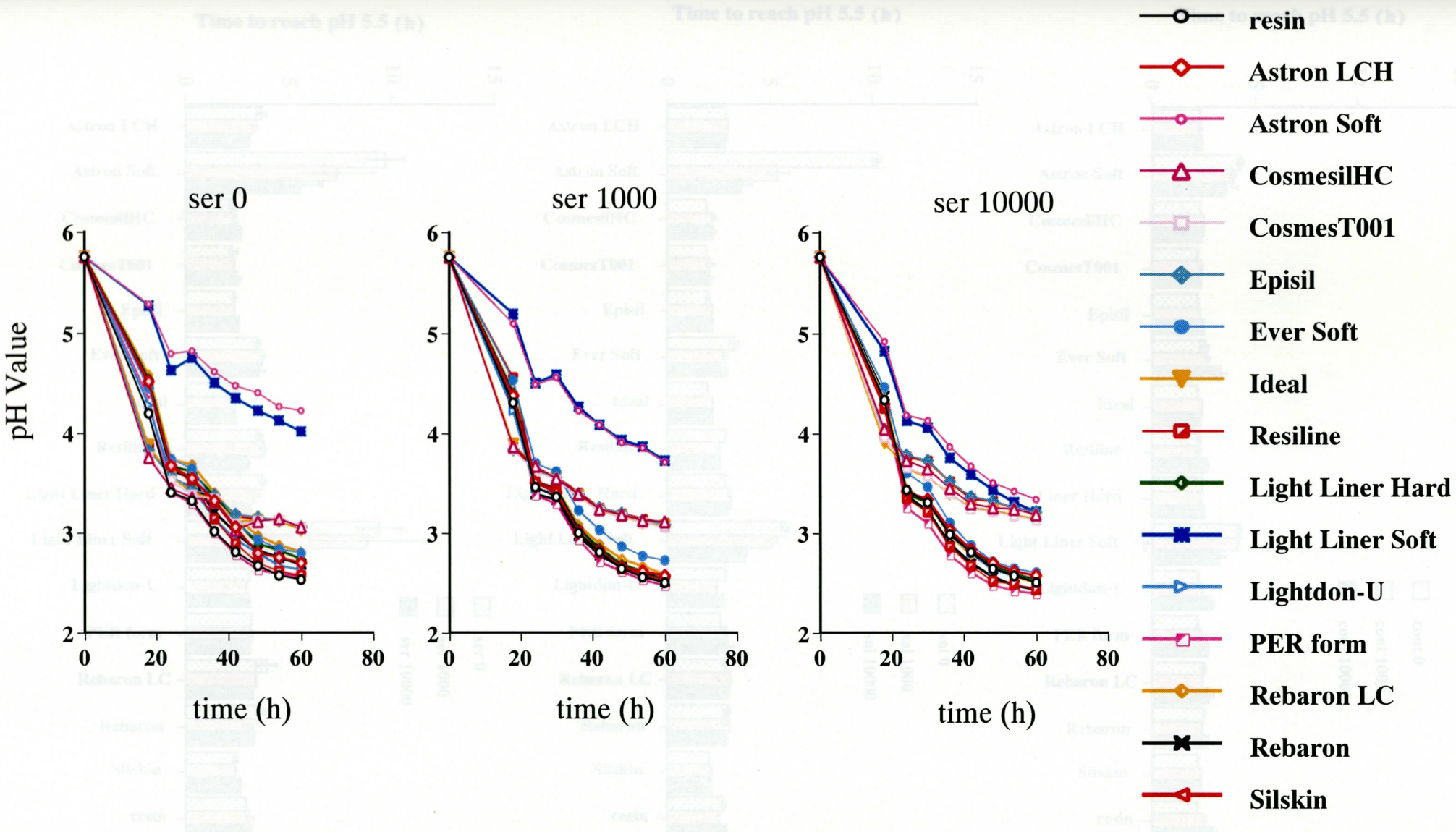
Table 6-b Minimum pH
serum-coated samples

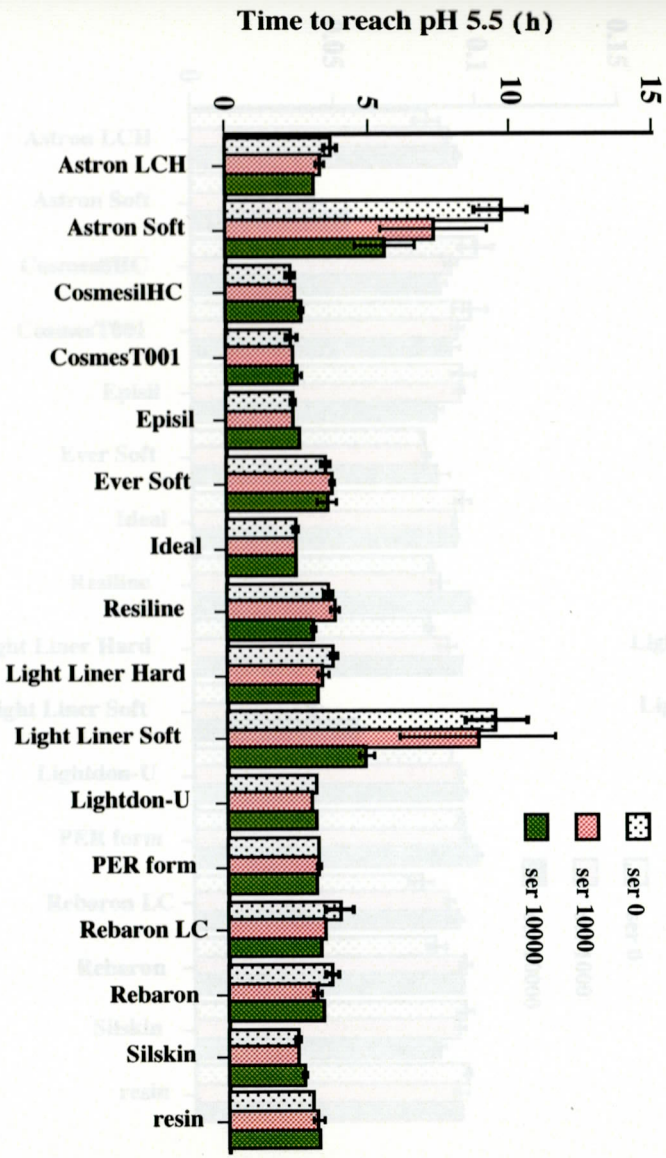
ser 0	*	ser 1000		ser 10000
Resiline		**PER form**		**PER form**
Rebaron		***resin***		**Rebaron**
PER form		**Rebaron**		**Resiline**
Lightdon-U		**Lightdon-U**		**Rebaron LC**
resin		**Light Liner Hard**		***resin***
Ever Soft		**Resiline**		**Lightdon-U**
Light Liner Hard		**Astron LCH**		**Light Liner Hard**
Rebaron LC		**Rebaron LC**		**Astron LCH**
Astron LCH		**Ever Soft**		**Ever Soft**
Light Liner Soft		**CosmesT001**		**CosmesT001**
Astron Soft		**Ideal**		**Ideal**
CosmesilHC		**Episil**		**CosmesilHC**
CosmesT001		**Silskin**		**Silskin**
Ideal		**CosmesilHC**		**Light Liner Soft**
Episil		**Astron Soft**		**Episil**
Silskin		**Light Liner Soft**		**Astron Soft**

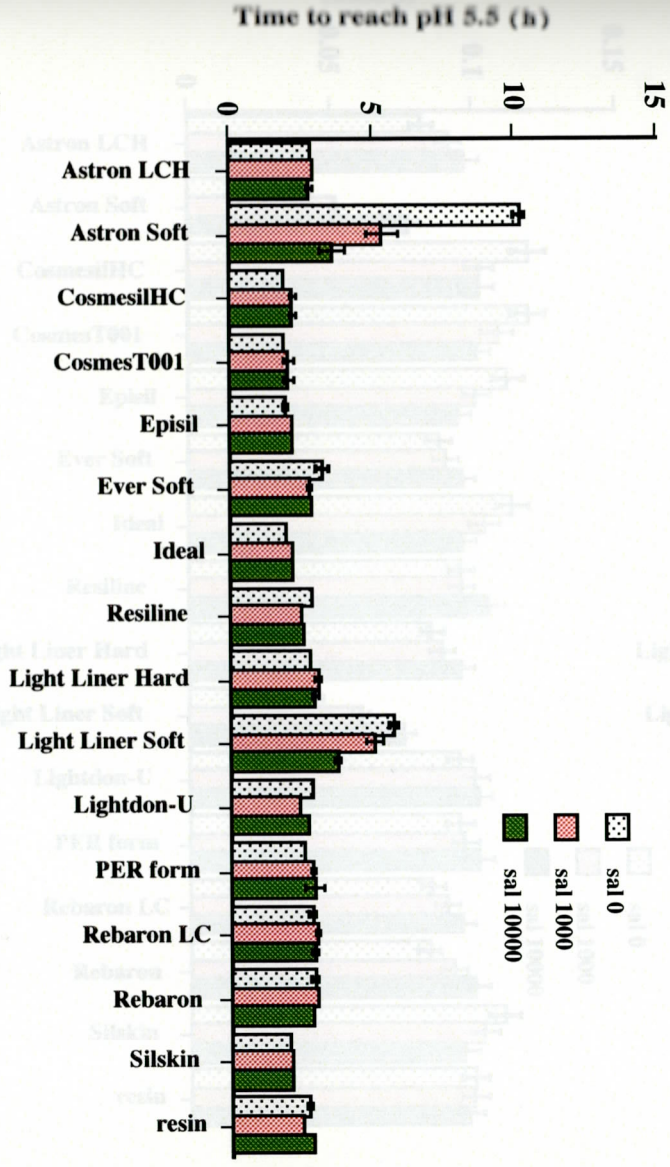
* No significant differences were observed with the sample connected by bars.

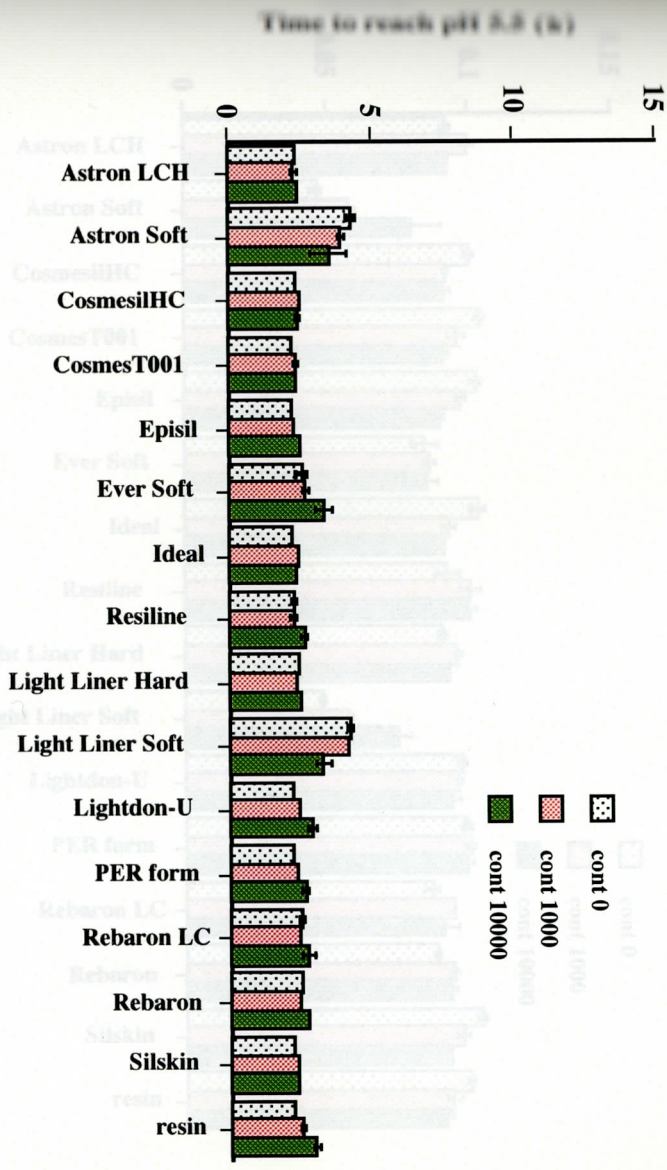

cont 0
cont 1000
cont 10000
pH value
time (h)
time (h)
time (h)
6
5
4
3
2
0
20
40
60
80
resin
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin


sal 0
sal 1000
sal 10000
pH Value
time (h)
time (h)
time (h)
resin
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin


ser 0
ser 1000
ser 10000
pH Value
time (h)
time (h)
time (h)
6
5
4
3
2
0
20
40
60
80
resin
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin


Time to reach pH 5.5 (h)
0
5
10
15
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin
ser 10000
ser 1000
ser 0

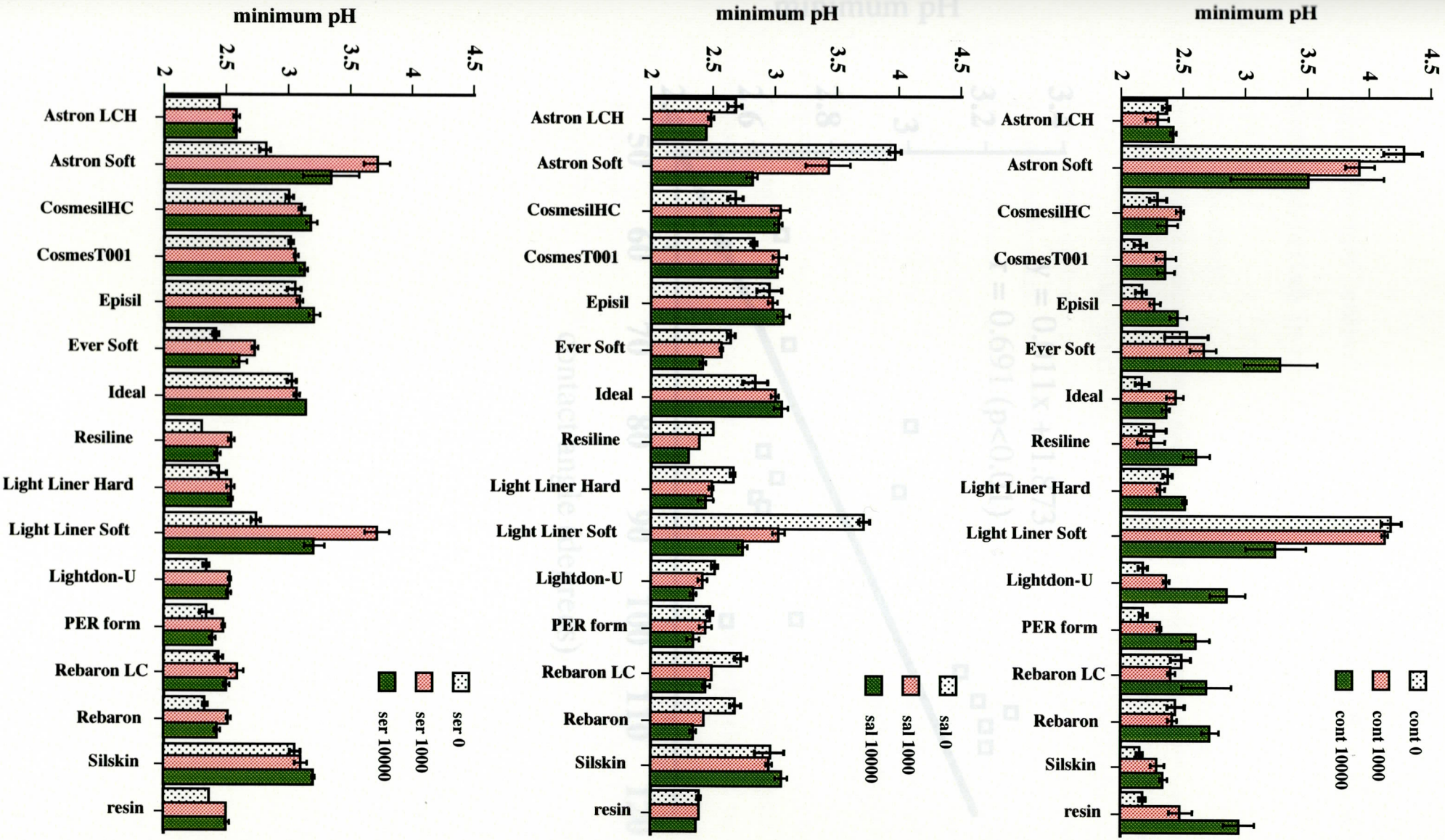

Time to reach pH 5.5 (h)
0
5
10
15
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin
sal 10000
sal 1000
sal 0

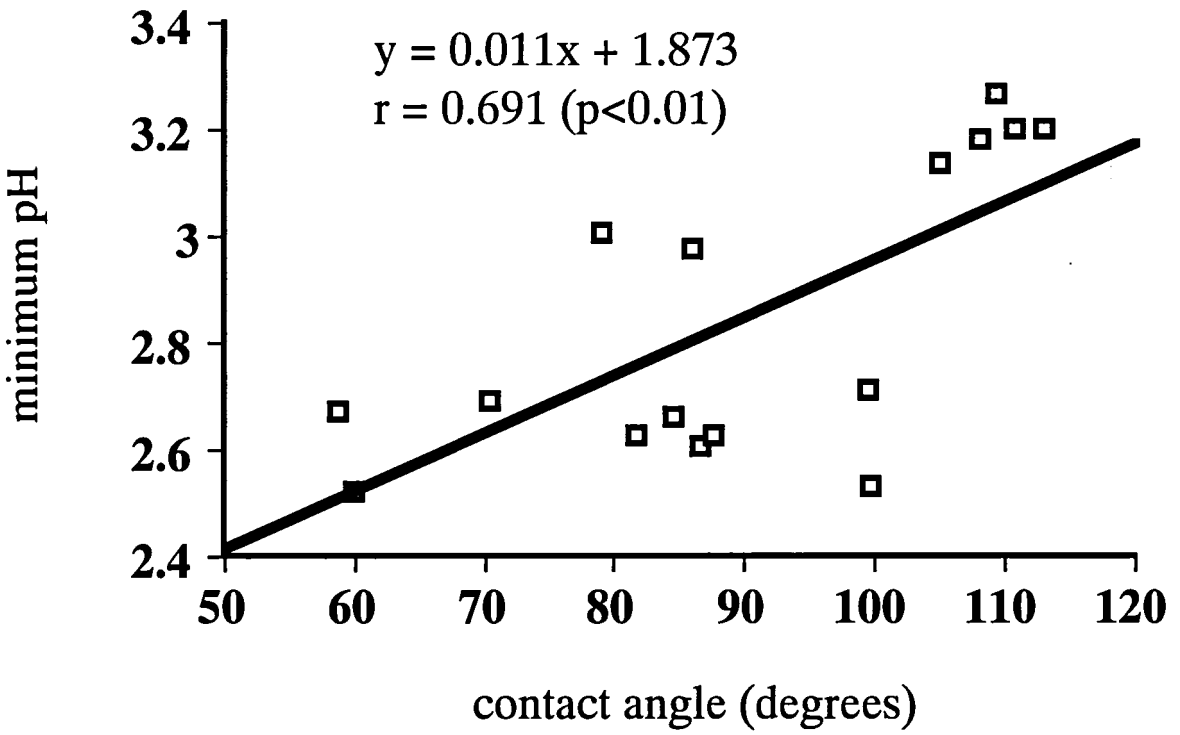

Time to reach pH 5.5 (h)
0
5
10
15
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin
cont 10000
cont 1000
cont 0

cont 0
cont 1000
cont 10000
0.15
0.1
0.05
0
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin

sal 0
sal 1000
sal 10000
0.15
0.1
0.05
0
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin

ser 0
ser 1000
ser 10000
0.15
0.1
0.05
0
Rate of decline in pH (unit/h)
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin


minimum pH
2
2.5
3
3.5
4
4.5
Astron LCH
Astron Soft
CosmesilHC
CosmesT001
Episil
Ever Soft
Ideal
Resiline
Light Liner Hard
Light Liner Soft
Lightdon-U
PER form
Rebaron LC
Rebaron
Silskin
resin
ser 0
ser 1000
ser 10000
sal 0
sal 1000
sal 10000
cont 0
cont 1000
cont 10000


y = 0.011x + 1.873
r = 0.691 (p<0.01)
minimum pH
3.4
3.2
3
2.8
2.6
2.4
50
60
70
80
90
100
110
120
contact angle (degrees)