Mycotic growth and soft denture lining materials

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Mycotic flora was studied from the dentures and denture bearing mucosae of 39 persons who wore soft-lined (Molloplast B) mandibular dentures and heat-cured acrylic resin maxillary dentures.
Fungal growth was detected in 85% of the mandibular dentures and in 44% of the maxillary dentures ($p < 0.001$). On the mandibular mucosa fungal growth was revealed in 74% and on the mucosa of the maxilla in 69%. In connection with inflamed mucosae fungal growth was always detected on the mandibular denture and on the mandibular mucosa in 93% as well as on the maxillary denture in 50% and on the maxillary mucosa in 75%. Considering the healthy mandibular mucosa fungus was found in 75% on the mandibular dentures and in 62% on the mucous membranes. In connection with healthy maxillary mucosae the corresponding figures were 42% and 68%. The specimens revealed 7 different yeasts and 2 moulds. The most common fungi were *Candida albicans* (86%), *Torulopsis glabrata* (31%), and *C. tropicalis* (14%). The uncured Molloplast material caused a definite inhibition of candida growth *in vitro*, while the cured material indicated no growth inhibition.

Key-words: Fungi; moniliasis; prosthodontics; stomatitis, denture

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Mycotic infection is considered the most important etiological factor in denture stomatitis. Clinically it has been found that mycotic flora easily adheres to the soft lining materials causing irritation in the mucous membranes of the mouth *Masella, Dolan & Laney,* 1975). *In vitro* some soft liners have been found to sustain the growth of Candida species (*Gruber, Lucatorto & Molnar,* 1966; *Engelhardt,* 1974) and some to inhibit it (*Williamson,* 1968). Others, have been found to be without any effect on the growth of *Candida albicans* (*Williamson,* 1968; *Masella et al.* 1975).

Molloplast B is one of the materials that have been mentioned to have an inhibitory effect (*Williamson,* 1968). One of the authors (E. M.) has used the silicone material mentioned in an attempt to soothe the soreness beneath mandibular dentures and

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has often noticed mycotic foci in the soft material (*Mäkilä*, 1976a). No fungal growth visible to the eye could be observed in the acrylic resin maxillary dentures of the same persons. Thus it is apparent that in the oral environment Molloplast B is not able to inhibit fungal growth.

In the following the mycotic flora has been studied from the dentures and mucous membranes of persons who wear soft-lined complete dentures in the mandible and heat-cured acrylic resin complete dentures in the maxilla. Also the ability of the cured and non-cured Molloplast B materials to inhibit the growth of *C. albicans* has been studied in fungus cultures on agar plates.

MATERIAL AND METHODS

Those patients to whom one of the workers (E.M.) had made soft-lined mandibular complete dentures in 1970 – 1975 as well as those to whom at the Institute of Dentistry of the University of Turku a corresponding mandibular denture had been made in 1974, were invited into an examination in June and September 1975. Forty-two persons were invited and 39 of them (9 men, 30 women) arrived (93%).

The average age of the persons examined was 64 years (29–89 years). The mandibular dentures were lined with the Molloplast B® silicone compound (Köstner & Co., Oberursel, West Germany). All the persons examined wore heat-cured polymethyl methacrylate resin complete dentures in the maxilla. The dentures had been worn from 2 weeks to 5 ½ years with an average of 1 year and 5 months.

Prior to taking a scraping, the dentures were kept under cold running water for 20 seconds and they were dried with air. The scraping was taken from the basal surface of the denture always from the same place directly onto an incubation dish, in the mandibular denture from an area corresponding to the location of the first right molar and in the maxillary denture from an area corresponding to the right glandular area of the palate. From the corresponding areas swabs were taken from the mucous membranes. The yeasts were cultured on *Kimmig's* test agar and maltose agar at 37 °C. Identification of the yeasts was carried out as described by *Lodder* (1970). The inhibitory effect of the Molloplast material on candida growth *in vitro* was studied on test agar plates. The agar surface was seeded with *C. albicans* for even growth and about 5 x 10 mm pieces of Molloplast, cured and non-cured, were immediately placed against the agar surface. The plates were kept at the + 37 °C temperature for 3 days to allow the candida to grow. The size of the area lacking candida growth is a rough estimate of the inhibitory effect due to Molloplast.

RESULTS

Occurrence of fungi

Fungus occurred in the mouth of 35 persons, (90%), either on the dentures or on the mucous membranes, and the specimens of only 4 persons proved negative. Of the women fungal growth was discovered in 28 (93 %) and of the men in 7 (78 %). Table I and Fig. 1 show that mycotic growth was present in about 85% of the soft-lined mandibular dentures. Regarding the maxillary dentures without soft lining the corresponding figure was approximately 44%. The difference is statistically significant ($\chi^2 = 14.26$; $p < 0.001$). No significant difference was observed between the mandibular and maxillary mucous membranes with respect to mycotic growth. From the mucous membranes under the soft material fungal growth was detected in 74% and from the maxilla bearing hard dentures in 69% (Table I, Fig. 1). Regarding the maxilla, the scrapings taken from the denture contained fungus more rarely than swabs taken from the mucous membranes ($\chi^2 = 5.21$; $p < 0.05$) (Table I). Also when

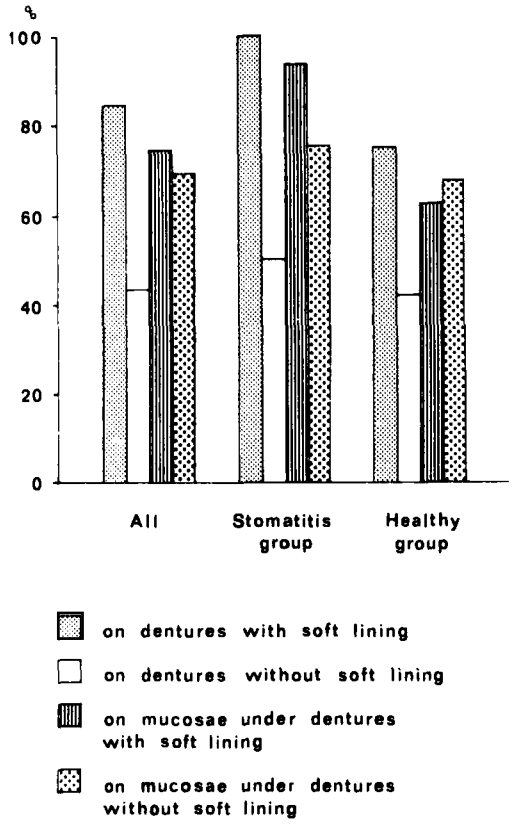

Fig. 1. Positive fungal growth on denture and mucosae expressed as per cent.

Table I. *Fungal growth in each jaw. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Fungal growth Denture/Mucosa	Mandible No.	Maxilla No.
+/+	29	16
+/−	4	1
−/+	0	11
−/−	6	11
Total	39	39

observing the occurrence of mycotic flora on the denture and on the mucosa of the same jaw it was found that fungus grew on the mucosa but not on the denture of the maxilla in 11 cases (28%). With respect to the lower jaw no such case was found. Opposite cases in which the scraping taken from the denture contained fungal growth but the swab taken from the mucous membrane did not, were found 4 (10%) in regard to the lower jaw and 1 (3%) in regard to the upper jaw (Table I).

In comparing the existence of fungi in connection with denture stomatitis (localized or generalized) and healthy mucous membranes it was found that mycotic flora occurred on the mandibular denture in all cases of mandibular stomatitis. Mycotic flora was found on the maxillary denture (Table II, Fig. 1) in 50% of the cases of maxillary stomatitis. In connection with healthy mucous membranes of the mandible fungal growth could be detected in 75% on the mandibular denture, in the maxilla the corresponding figure was 42% ($\chi^2 = 6.01$; $p < 0.05$) (Fig. 1). In the group with stomatitis fungal growth was revealed on the soft-lined denture bearing mucosa of the mandible in 93% and under the maxillary denture without soft lining in 75%. In the group with healthy mucous membranes the corresponding figures were 62% and 68% (Table II, Fig. 1). In Table III the same has been viewed in relation to individuals. When denture stomatitis appeared in the mouth fungus was also found at least in one of the four areas examined. When the mucous membranes of the mouth were healthy, mycotic growth occurred in the specimens of 17 persons (81%). It has to be noticed, however, that every time when the fungus finding was negative the mucous membranes were healthy (Table III).

Presence of different species of fungi

Seven different yeasts and two moulds were discovered; their distribution in relation to the sexes is given in Table IV. The most common fungus was *C. albicans* (about 86%) the next

Table II. *Fungal growth in connection with inflamed and healthy mucous membranes in each jaw. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Fungal growth Denture/Mucosa	Denture stomatitis		Healthy mucosa	
	mandible	maxilla	mandible	maxilla
	No.	No.	No.	No.
+/+	14	3	15	13
+/−	1	1	3	0
−/+	0	3	0	8
−/−	0	1	6	10
Total	15	8	24	31

Table III. *Fungal growth in connection with inflamed and healthy mucous membranes in the mouth. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Fungal growth	Denture stomatitis in mandible and/or maxilla	Healthy mucosa in mandible and maxilla	Total
	No.	No.	No.
Positive	18	17	35
Negative	0	4	4

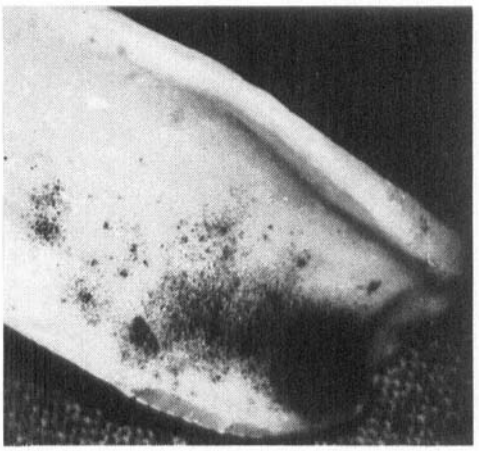

Fig. 2. Black mould (*Ochroconis constrictum*) on soft lined mandibular denture.

were *Torulopsis glabrata* (31%), and *C. tropicalis* (14%). *C. albicans* was found in 77% of the entire material, in women more often (83%) than in men (56%) ($\chi^2 = 3.00$; $p < 0.10$); *T. glabrata* in 28%, slightly more often in women (30%) than in men (22%) ($\chi^2 = 0.21$), and *C. tropicalis* in approximately 13%, in men twice as often as in women ($\chi^2 = 0.92$). The differences between the sexes were not statistically significant.

C. albicans was the most common fungus on the dentures as well as on the mucous membranes. On the dentures the next in prevalence was *C. tropicalis* and on the mucous membranes *T. glabrata* (Table V). It is seen in the same table that in the scrapings taken from the maxillary dentures without soft lining only three different fungi grew while on the mandibular dentures with soft lining seven different fungi appeared. Moulds grew only on the mandibular dentures but not on the mucous membranes (Fig. 2). *C. parapsilosis* and *Saccharomyces cerevisiae* occurred only on the mucous membranes, not on either of the dentures (Table V).

In viewing the occurrence of *C. albicans* in the whole material it was noticed to exist more often (67%) on the soft surface of the mandibular denture than on the acrylic resin surface of the maxillary denture (36%) ($\chi^2 = 7.39$; $p < 0.01$) (Fig. 3) and also more often on the mandibular denture (67%) than on the mandibular mucosa (49%). In the maxilla *C. albicans* occurred more often on the mucous membrane (49%) than on the denture (36%). The differences between the dentures and the

Table IV. *Occurrence of different fungi in denture wearers*

	Men No.	Women No.	Total No.
Yeasts: *Candida albicans*	5	25	30
Candida tropicalis	2	3	5
Candida parapsilosis	0	1	1
Candida rugosa	1	0	1
Candida krusei	0	1	1
Torulopsis glabrata	2	9	11
Saccharomyces cerevisiae	1	0	1
Moulds: Aspergillus sp.	0	1	1
Ochroconis constrictum	0	1	1
	11 [1]	41 [2]	52

[1]) Four men with two fungi
[2]) Nine women with two fungi and two women with three fungi

Table V. *Occurrence of different fungi on dentures and mucous membranes. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

	Dentures		Mucosae	
	mandibular No.	maxillary No.	mandibular No.	maxillary No.
Yeasts: *Candida albicans*	26	14	19	19
Candida tropicalis	4	3	4	4
Candida parapsilosis	0	0	1	1
Candida rugosa	1	1	1	–
Candida krusei	1	0	1	1
Torulopsis glabrata	2	0	7	9
Saccharomyces cerevisiae	0	0	1	1
Moulds: Aspergillus sp.	1	0	0	0
Ochroconis constrictum	1	0	0	0

mucous membranes were not statistically significant. On the mucous membranes of the upper and lower jaws *C. albicans* were detected equally, in 19 persons (49%) (Table V, Fig. 3).

In connection with healthy mucous membranes *C. albicans* was found in 54% of the soft-lined dentures and in 32% of the maxillary dentures without soft material. In the group with stomatitis the corresponding figures were 87 % and 50% (Table VI, Fig. 3).

From the healthy mandibular mucous membranes bearing soft-lined dentures *C. albicans* was discovered in 37% of the cases and from the maxillary mucous membranes bearing dentures without soft lining in 45%. Considering the inflamed mucous membranes the corresponding figures were 67% and 62% (Table VI, Fig. 3).

When denture stomatitis was present, *C. albicans* was regulary found except for one case in which the specimens contained *C. tropicalis* and *C. rugosa*. Thus, *C. albicans* was present in 94% in the group with denture

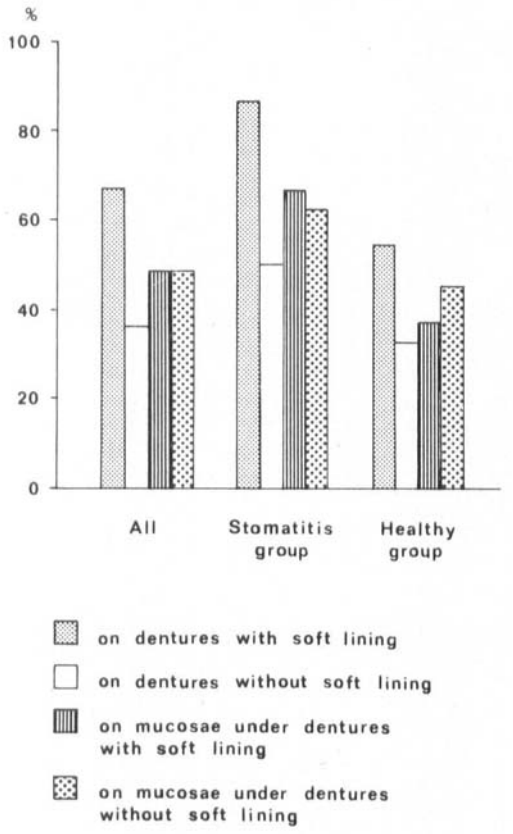

Fig. 3. Presence of *Candida albicans* on dentures and mucosae, per cent.

stomatitis. In those persons whose both mandibular and maxillary membranes were healthy growth of *C. albicans* was found in 62%. In 8 persons of 9 in whom *C. albicans* proved negative the mucous membranes were healthy (Table VII).

Table VIII shows all the fungus combinations observed in connection with both inflamed and healthy mucous membranes.

Effect of Molloplast on candida growth in vitro

The effect of Molloplast on the growth of *C. albicans in vitro* is seen in Fig. 4. The uncured material of Molloplast caused a definite

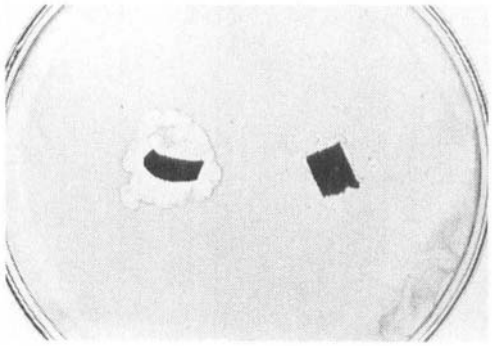

Fig. 4. The effect of Molloplast on the growth of *Candida albicans in vitro*. On the left an inhibitory ring is seen around the uncured Molloplast. On the right there is no clear inhibition of Candida growth around the cured Molloplast. 2-day-old culture.

inhibition of candida growth as indicated by a clear ring around the material. The cured material, on the other hand, exhibited no growth inhibition.

DISCUSSION

The percentage of oral candida in subjects with clinically normal mouths has been estimated as between 30–60% (*Bartels & Blechman*, 1962; *Heber, Reinel & Vogel*, 1975). *Williamson* (1972) found a diurnal variation with an early morning peak in the number of candida organisms in dentulous subjects; wearing dentures increased this candida count. The finding of such a high frequency of candida in the present study is thus not unexpected. The species of yeasts isolated in this study from oral mucosa and dentures are those which are most commonly found in the mouth (*Kahanpää*, 1972; *Olsen*, 1974; *Budtz-Jørgensen, Stenderup & Grabowski*, 1975). The most common fungi in the mouth were *C. albicans*, *T. glabrata*, and *C. tropicalis*. The individual findings of Aspergillus sp. and *Ochroconis constrictum* are to be regarded as incidental (*Mäkilä*, 1976b).

Denture stomatitis is more often found in women than in men (*Nyqvist*, 1952; *Turrell*,

Table VI. *Growth of* Candida albicans *in connection with inflamed and healthy mucous membranes. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Growth of *Candida albicans* Denture/Mucosa	Denture stomatitis		Healthy mucosa	
	mandible	maxilla	mandible	maxilla
	No.	No.	No.	No.
+/+	10	3	9	7
+/−	3	1	4	3
−/+	0	2	0	7
−/−	2	2	11	14
Total	15	8	24	31

Table VII. *Growth of* Candida albicans *in connection with inflamed and healthy mucous membranes in the mouth. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Growth of *Candida albicans* on dentures and/or on mucous membranes	Denture stomatitis in mandible and/or maxilla	Healthy mucosa in mandible and maxilla	Total
	No.	No.	No.
Positive	17	13	30
Negative	1	8	9

1966; *Budtz-Jörgensen*, 1972). Also fungi appeared more often to be present in the mouth of women than of men.

It seems clear that mycotic flora easily adheres to the soft lining material used in this study. According to *Davenport* (1970) in the cases of stomatitis candida is more often revealed on the denture than on the mucous membrane. The same observation was made in this material in connection with soft-lined dentures. But in connection with maxillary dentures without soft lining the reverse was true. This was apparently, due to the fact that prior to taking the scraping the denture was rinsed in running tap water. From the soft surface the mycotic flora adhered to it was not rinsed away while from the hard acrylic resin surface the fungi could loosen. In connection with the healthy mucosae the same was true: fungi were more often found on the soft-lined dentures than on the mucosae bearing them and on the mucosae bearing dentures without soft lining more often than on the dentures. From the specimens of denture wearers with clinically healthy mucosae yeast-like fungi have been cultured in 25–42% and in connection with denture stomatitis in 68–96% (*Lilienthal*, 1950; *Turrell*, 1966; *Ritchie et al.*, 1969; *Budtz-Jörgensen*, 1972).

In this study where specimens were taken from four places, at least one specimen proved positive in all patients if stomatitis occurred in the mouth. Although the oral mucosae were clinically healthy in 82% of the patients at least one fungus culture was positive. Such a high frequency of fungi is apparently due to the fact that all 39 patients wore soft-lined dentures and that fungus grew on 33 of them, i.e. it is difficult to get the soft material clean. On some dentures a covering layer could be seen and its removal required extremely strong brushing. The therapeutic result of the soft linings is good in relieving tissue soreness (*Mäkilä*, 1976a). Nevertheless difficult in maintaining good hygiene is an obvious disadvantage in their use.

The present findings show that the uncured Molloplast has an inhibitory effect on candida growth. It contains some inhibitory substance which is readily diffusible into the agar gel. The cured material, on the other hand, did not

Table VIII. *Growth of different fungal species in connection with inflamed and healthy mucous membranes in each jaw. In the mandible a soft-lined denture, in the maxilla a denture without soft lining*

Fungal growth Denture/Mucosa		Denture stomatitis		Healthy mucosa	
		mandible	maxilla	mandible	maxilla
		No.	No.	No.	No.
C. albicans	/ *C. albicans*	8	2	7	4
C. albicans	/ *C. albicans* + *T. glabrata*	1	1	2	3
C. albicans + *O. constrictum*	/ *C. albicans*	1	0	0	0
C. albicans	/ *T. glabrata*	1	0	0	0
C. albicans + *T. glabrata*	/ *T. glabrata*	0	0	1	0
C. albicans	/ *C. parapsilosis*	0	0	1	1
C. albicans	/ *S. cerevisiae*	1	0	0	1
C. albicans + *C. tropicalis*	/ *C. tropicalis* + *T. glabrata*	0	0	0	1
C. albicans	/ negative	1	1	2	0
negative	/ *C. albicans*	0	2	0	5
negative	/ *C. albicans* + *T. glabrata*	0	0	0	2
negative	/ *T. glabrata*	0	1	0	0
C. tropicalis	/ *C. tropicalis* + *T. glabrata*	0	0	1	1
C. tropicalis	/ *C. tropicalis*	1	0	2	1
C. rugosa	/ *C. tropicalis*	0	0	0	1
C. rugosa	/ *C. rugosa*	1	0	0	0
C. krusei	/ *C. krusei*	0	0	1	0
negative	/ *C. krusei*	0	0	0	1
Aspergillus sp.	/ negative	0	0	1	0
negative	/ negative	0	1	6	10
		15	8	24	31

inhibit candida growth. Either the inhibitory substance disappeared during cooking or it was after that in nondiffusible form in the material.

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