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Analysis of germ reduction methods for maxillofacial prostheses by physical methods

KEYWORDS: germ number reduction, maxillofacial prosthesis, physical methods, tumor care

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ABSTRACT *Maxillofacial prostheses are removable aids used to cover tumor-associated surface defects and are germ-contaminated. Chemical microbicides are useful for germ reduction when used with a proper dosage and exposure time. Chemical stress might be applied onto the contact area of prosthesis and the defect, so practical and easy-to-use physical treatment methods are to be examined in order to reduce germ populations.*

One example of the maxillofacial prostheses material groups - silicone, polymethyl methacrylate resin (PMMA) - was included. Test microorganisms were Candida albicans and Staphylococcus aureus. Using standardized conditions, physical methods for germ reduction were used: scrubbing, boiling, microwaves and ultrasound. Changes to material parameters mass, color and surface roughness were additionally analyzed. The used statistical method was the WILCOXON – test (α=0.05).

Scrubbing achieved an effective germ reduction and should be used weekly, especially for PMMA maxillofacial prostheses. Boiling showed long-term germ reduction. This method should only be used for silicone maxillofacial prostheses. Usage of microwaves or ultrasound is not recommended,

Introduction

Maxillofacial prostheses are removable means of treatment used to cover tumor associated surface defects. Commonly this means a partial or complete replacement of nose, ear or the orbital area [1,2]. The maxillofacial prostheses are made of PMMA (polymethyl methacrylate) resin and more and more frequently silicone[3]. Maxillofacial prostheses are cleaned and treated in order to reduce microbial load in distinct ways. Within the framework of another study, germ reduction by chemical microbicides and care habits were identified, material samples taken and temperatures measured, all concerning patients with nasal or orbital prostheses. All maxillofacial prostheses were inhabited by a mixed bacterial flora. Most commonly found were yeasts, *Staphylococcus aureus* and coagulase negative staphylococci as well as *Enterococcus faecalis*. The temperature at the contact area of the maxillofacial prostheses was 36 °C on average. The cleaning or "disinfection" intervals were reported to be between 1 and 3 weeks by the patients. The used materials encompassed : tap water, cologne (in order to improve the smell), dish-washing detergent, surgical spirit, cosmetic pads and soft cloths. This group of patients complained mostly about color changes (Figure 1), loss of accuracy of fit and development of bad smell [4]. The results were used in order to shape the conditions of the current study. With the help of chemical microbiocides, the germ count on maxillofacial prostheses can be reduced with varying effects. Exact dosage and exposure time are necessary for this. The risk of chemical stress onto the contact area between prosthesis and the edge of the tissue defect remains. To avoid these insecurities and possible dangers, methods for patients using physical, household-related methods to reduce microbial load are to be tested. The effect on the properties on commonly used maxillofacial prostheses materials is to be examined as well.

The following hypotheses were formulated:
–A significant reduction in microbial load will be achieved by using the physical methods of scrubbing, boiling, microwaving and ultrasound
– Device independent methods (scrubbing, boiling) will show the best results
– Material change will almost be imperceptible when using physical methods.

Materials and methods

Materials

One specimen of each of the most common maxillofacial prosthesis material groups was included into the study [3]. For polymethyl methacrylate the heat-curing Epicryl hot® (from Candulor, Wangen, Switzerland) in base color scheme C was used. More and more prostheses are made of silicone. The sample used for silicone was Episil E® (from Dreve, Unna, Germany) in base color scheme E 2.

Specimens

To work with identical specimen dimensions, a plate made of V2A steel was created in order to be used in the production of the specimens. This plate contained 30 “drilled holes” of identical dimensions which were created by an industrial laser to reach an optimal precision. Afterwards the transition into the aforementioned maxillofacial prosthesis materials took place. All materials were handled as specified by the manufacturer. For the production of the Epicryl specimens, the EWL 5509 heat-cure device (from KaVo, Biberach, Germany) was used. For the Episil specimens the polymer

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formation took place in a heating furnace of the Moulinex X93 model from Moulinex, Cormelle-le-Royale, France) for 60 minutes at 80 °C. The specimens presented a diameter of 7 mm and height of 2 mm. Until they were used, the specimens were stored in a lightproof container at room temperature. [5].

Test organisms

As a result of information gathered in prior studies, the examined test organisms were Staphylococcus aureus (ATCC 25923) and Candida *albicans* (ATCC 90028) [4].

Test design

After the specimens were manufactured, their initial parameters including mass, color and surface roughness were measured. The initial sterilization of the samples occurred via low temperature plasma (model: STERRAD 100; from Johnson &Johnson Medical, Norderstedt, Germany). The colonization of the specimens was accomplished by attachment of Columbia blood agar (Oxoid article # PB 500 8A), each inoculated with the test organism in question, for 28 days at 36°C. The usual cleaning and disinfection intervals of the prostheses described by patients were considered. The maxillofacial prosthesis material was examined as follows:

Series 1 - three weeks of incubation, a single procedure of germ reduction was carried out

Series 2 - during the three week incubation period the germ reduction occurred in a weekly rhythm

Series 3 - after the three week incubation period a single germ reduction procedure was carried out. The specimens was then stored in a sterile nutrient broth for 10 days. This technique was used to examine the possibility of recontamination from the inside of the material and is subsequently described as "long-term effect" [6].

Each series included 16 specimens. The randomly chosen specimens 1 to 8 were used to determine the starting germ population count. The specimens 9 to16, which were created under identical circumstances, were each treated with a single method for germ reduction (scrubbing, boiling, microwaves or ultrasound). Afterwards the final germ count was measured. The recovery of the test organisms was accomplished via orbital shaker (model: Vortex 2; from IKA, Staufen, Germany; frequency: 400/ min., 15 minutes) in sterile physiological saline solution and using dilution series and surface cultures on nutrient agar. After an incubation period of 24 hours *(Staphylococcus aureus)* or 48 hours *(Candida albicans)* the colony-forming units (CFU) were counted. During the long-term test series (series 3) the samples were incubated in sterile nutrient broth for 10 days after a single germ reduction procedure. After 10 days there was examination if any clouding occurred in the broth. Clouding was graded as a microbial colonization. If clouding was positive, streaking onto nutrition agar and examination determining morphology and count of the colonies was carried out. "Bioburden" was determined according to ISO 11737-1.

After completion of the tests determining germ reduction, the samples were cleaned, disinfected and plasma-sterilized. Afterwards the final parameters for mass, color and surface roughness were measured.

Tools / devices used for germ reduction

Four different methods for germ reduction were used - scrubbing, boiling, microwaves and ultrasound. For scrubbing, plasma sterilized, commonly used flat head toothbrushes of the type P-35 - soft from Oral-B, Kronberg, Germany) were used. Scrubbing was done with one minute of flowing water per specimen. For every specimen a new brush was used. Boiling occurred for 10 minutes at 100°C in a common household cooking pot. Regular tap water was used (pH=7.5) The temperature profile was measured via temperature logger (type EBI 125 A from Ebro Electronic, Ingolstadt, Germany). The microwave device HMT 703 C (from Bosch, Salzgitter, Germany) was a common household device. It was used without adding fluid. It was equipped with a rotary plate which was used to move the specimen during the test in order to avoid so-called "cold spots". The microwave oven was used for 10 minutes at 600 Watt. For the use of ultrasound a device of the type Sonorex RM 16 U (from Bandelin, Berlin, Germany), was used due to the fact that dental ultrasound devices would be too small for the maxillofacial prostheses. This device is equipped with a hanging basket with a lifting device. It was used to move the specimens in order to avoid a placement in so-called "dead zones" (areas without ultrasound effect). The device operated at a frequency of 40 kHz. The power of the device amounted to 23 Watt per liter distilled water (22°C, pH=6.4) or 0.34 Watt/cm^2. The temperature profile was also measured via the aforementioned temperature logger.

Tools / Devices for examining material properties

The determination of the mass of the specimens was done via a Sartorius BA 110 S precision scale model (from Sartorius, Göttingen, Germany), measurement accuracy was 0.1 mg. To measure color position changes the colorimeter model GRETAG SPM 100 (from Gretag, Regensdorf / Zürich, Switzerland) was used. Measurements were done with norm light D 65, observation angle of 2° and absolute white cover in a dimmed cabin. To determine surface roughness a Hommel – Tester T 6000 device model (from Hommelwerke, Villingen / Schwenningen, Germany) was used in "roughness mode". The length of the measured route totaled 4.8 mm and the Gauss – filter was used with λ_c 0.8 mm [ISO 4287]. The hard material Epicryl hot® was measured using the TKE 100 scanner and the soft material Episil E® was measured using the TKC 300 scanner.

Data analysis and statistics

The initial and end microbial population was compared. The germ reduction was measured - as is common in microbiological analysis - in log level reduction (logarithmic display). The mass of each specimen was measured thrice and the arithmetic mean was used. The color position determination used the Lab-System. The used spectrophotometer displayed the values of L, a and b in its display. Using the CIELAB-formula changes in color position ΔE_{ab} were calculated. To specify changes in surface roughness, the significant parameter Rz – so-called averaged surface roughness - was used [9]. The applied statistical method was the WILCOXON – Test ($\alpha = 0.05$).

Results

Germ reduction via physical methods.

Both materials were able to be colonized with the test organisms. The initial population of Staphylococcus aureus was between 10^5 and 10^7 CFU / ml. *Candida albicans* had an initial population around 10^7 CFU / ml. All four physical methods resulted in a noticeable germ reduction, which varied when examined more closely. The initial and final germ populations for Epicryl hot® are comparatively shown in Figure 2. All four methods lead to a germ reduction when used on the PMMA material Epicryl hot®. The massive germ reduction connected to all test series using boiling was noticeable. Figure 3 shows all results of germ reduction for Episil E®. For all test series using boiling on the maxillofacial prosthesis silicone, a complete germ reduction could be observed. The comprehensive results for both materials, both test organisms and all germ reduction methods are displayed in Table 1. In the end, boiling resulted in the highest germ reduction. A high germ reduction was also observed for scrubbing both materials. Usage of the microwave oven only resulted in complete germ reduction for *Candida albicans* . The test series using *Staphylococcus aureus* only showed minor germ reduction using the microwave oven. The usage of ultrasound on both materials resulted in minor germ reduction for both test organisms. (Table 1). Comparing the initial and final test organism populations, all displayed significant differences (p – values between 0.012 and 0.025). The long-term effect (series 3) correlated to a recontamination from the interior of the

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material. Post-boiling, no recontamination was examined on these samples as well. The use of ultrasound had no long-term effect. Varied results were recorded for the microwave oven and scrubbing. The microwave was ineffective respective to Staphylococcus aureus, the same result was observed with scrubbing and *Candida albicans* (Table 2).

Changes to the material

Mass

Only small changes to mass of the PMMA material was observed. The mass difference before and after germ reduction measures amounted to a value between 0.0002 and 0.0005 g. Relative to the initial mass, this only amounted to 0.1 to 0.5 %. The highest loss in mass was observed when using ultrasound.

Color position differences

Higher color position differences were observed for all series using boiling as germ reduction measure. The highest difference occurred in the series using Epicryl hot®. The color position difference ΔE_{ab} amounted to values between 2.7 and 1.6. For all other series, color spot differences were between 0.4 to 0.8.

Surface roughness

The determined differences between initial and final averaged surface roughness R_z were always higher for the maxillofacial silicone material Episil E® directly compared to the identical, conditioned series including the PMMA resin material. The highest value was calculated for Episil E® after using ultrasound (between 3.1 and 2.4 µm). All other data gathered for Episil showed values ranging between 1.6 and 1.0 µm. The PMMA material presented Rz differences between 0.5 and 0.1 µm.

Comparative evaluation

The complex display of short- and long-term effects of the chosen germ reduction method, additionally regarding the effects on the material of the samples, is shown in Table 3. A long-term effect was not observed in the series using scrubbing. The effects on the silicone material were distinctive. The effective germ reduction when using boiling was in contrast to heavy changes to the material, especially concerning PMMA material. The use of microwaves showed no effective germ reduction, but distinctive changes to the material. Use of ultrasound displayed no effective germ reduction either, but resulted in the most comprehensive changes to the material.

Discussion

General aspects

There is validated knowledge that select microorganisms, which grow inside the oral cavity when it is undergoing pathologic change, can cause or support systemic illness in patients [10 - 14]. There are several studies that support a connection between germ populations in the palate oriented side of removable prostheses made from PMMA resin and occurrence of Stomatitis prothetica [15 - 17]. Microbial colonization of prosthesis adhesives may affect the contact area pathologically [18].

The colonization is an additional burden for patients already suffering from multiple problems due to their tumor therapy. Clinical observation of local inflammation in the contact area between maxillofacial prostheses and tumor associated defects supports this. Orbita, nose and ear prostheses are worn up to 16 hours a day. The maxillofacial prostheses are removed only during night time [19]. On the other hand, the use of conventional cleaning and disinfection methods is not optimal [4, 20]. Chemical microbicides require exact dosage and exposure time. This is a challenge for the affected patients, since the life-impairing effects of tumor extraction, radio- and chemotherapy often dominate their life. Incorrect dosing can cause irritation of the contact area of the maxillofacial prosthesis and the adjacent tissue (which is often subject to surgery or radiotherapy) and thus can be detrimental for the patient. For this reason the analysis of practical physical methods of germ reduction that can be used in everyday life proves to be a useful study. The test conditions were derived from patient experience and simulate clinical conditions [4].

Germ reductions

Maxillofacial prostheses are to be considered as colonized with microorganisms [20]. Permanent soft materials – such as silicone - are more heavily colonized [21]. Most prostheses in the facial area are increasingly being produced from silicone. [3]. The necessity of germ reduction for prosthesis material that has permanent contact to mucous membranes has to be emphasized. [22]. Antifungal therapy for prostheses is also required [23]. The microbial colonization of voice prostheses and tracheal cannulas made of silicone is displayed as well [24, 25].

Microbiologically, displaying initial and final microbial count using log levels is customary. Regarding disinfection a reduction of germ count by 5 log levels is considered to be effective. [26]. A comparison of this study with other tests is not effectively possible since the methodology differs significantly.

The benefit of the highly effective germ reduction using boiling has to be evaluated when other prosthesis parts such as artificial eyes or eyelashes are integrated into the prosthesis. The use of microwaves in order to reduce microbial population has been mentioned by different authors [27 - 29]. An antifungal effect of admixtures into the silicone material could not be observed [23].

Changes to the material

Additionally the effects of all germ reduction methods on the commonly used maxillofacial prosthesis materials were tested. The changes to the mass of the samples were only minimal. Although significant differences between the different methods were observed regarding this parameter, all changes were actually of such small magnitude that they result in no practical consequences. To evaluate the changes in color spot difference Δ Eab, the actual changes noticeable by the naked eye are important. The following table is to give an overview to compare objectively measured color spot differences and the actual, subjective perception [30] :

- 0 to 1.14 – imperceptible
>1.14 to 1.72 – slightly perceptible
>1.72 to 4.58 – moderately perceptible
> 4.58 – considerably perceptible.

Actually perceivable color changes only occurred for the method of boiling. This is a big disadvantage for the prosthesis patients. Subjectively color changes to a prosthesis were considered to be a major flaw [4]. For the PMMA material Epicryl hot® changes were even more distinct compared to Episil E®. The exposition to natural sun or UV light was eliminated in order to exclude this issue as a factor and produce comparable results [31]. An increase in surface roughness was observed excluding the use of ultrasound. To increase germ reduction effectiveness and long-term effect, chemical and physical methods were combined. [32 - 34]. The long term chemical influence in combination with toothbrushes as mechanical interaction caused an increase in surface tension as well as the combination of microwaves and toothbrushes [35, 36]. Such changes also occurred when microwaves were combined with chemical disinfection [37]. The sole use of chemical disinfection was also considered to increase surface roughness as well [38]. Rougher surfaces carry the disadvantage that they offer more suitable circumstances for the formation and adhesion of a new biofilm. [39].

Regarding the hypothesis it is to be noted:

Significant germ reduction occurred using all four methods. This hypothesis is to be accepted. Device-independent methods proved to result in the highest germ reduction. This hypothesis is only to be

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partially accepted, since the use of microwaves resulted in a comparable reduction regarding Candida albicans.

The changes to the material were only minimal. This hypothesis is only to be partially accepted, since changes to the color position were perceivable when using boiling as germ reduction method. Scrubbing resulted in effective germ reduction. It should be used once per week and should be the preferred method for PMMA maxillofacial prostheses. The germ reduction when using boiling showed a long term effect. Since changes to the material proved to be detrimental, this method should be used for silicone maxillofacial prostheses. The use of microwaves or ultrasound is not to be recommended.

Table 1 - Mean germ count reduction, taking series 1 and 2 into account, as a function of the kinds of test germs and all the methods used in direct comparison. The results are given in log levels (logarithmic portrayal). The marking (*) means that a complete reduction of germs was observed.

Method	*Staphylococcus aureus*		*Candida albicans*	
	Epicryl hot (PMMA)	Episil E (Silicone)	Epicryl hot (PMMA)	Episil E (Silicone)
Scrubbing	5.5 to 6.5	5.5 to 6	5 to 6	6.5 to 7 *
Boiling	5.5 to 7 *	5 to 5.5 *	7 *	7 *
Microwave	1	1 to 4	7 *	7 *
Ultrasound	5	4.5 to 6	4.5	4.5 to 5.5

Table 2 - Detection of test germs on the series 3 specimens (long-term storage) following decontamination (examinations with germ detection/total number of examinations). In the marked series of examinations (#) the total number was n = 7 because one sample could not be evaluated.

	Staphylococcus aureus		*Candida albicans*	
	Epicryl hot (PMMA)	Episil E (Silicone)	Epicryl hot (PMMA)	Episil E (Silicone)
Scrubbing	7 / 8	3 / 8	8 / 8	8 / 8
Boiling	0 / 8	0 / 8	0 / 8	0 / 7 #
Microwave	8 / 8	8 / 8	2 / 8	5 / 7 #
Ultrasound	8 / 8	8 / 8	8 / 8	8 / 8

Table 3 - Comparative portrayal of the tested physical methods with a view to germ reduction and the effects on the materials. In the lines and columns in which no different influencing of the kinds of germs or the material reactions took place, the assessments have only been stated once.

Effects of germ reduction: +++ very good; ++ good; + satisfactory; - slight; - - very slight; - - - non-existent.
Effects on the materials: m – minimum; d – distinct; e – extensive.

Method	Effects of the germ count reduction		Effects on the materials	Proposal for application
	Short-term effect	Long-term effect		
Scrubbing	+ +	for Staph. aureus -	for PMMA - m	1 x a week possible only for PMMA maxillofacial prostheses
		for Cand. albicans - - -	for Silicone – d	
Boiling	+ + +	+ + +	for PMMA – e	1 x a week possible only for silicone maxillofacial prostheses
			for Silicone - d	
Use of microwave	for Staph, aureus - -	for Staph. aureus - - -	d	not to be recommend
	for Cand. albicans + + +	for Cand. albicans -		
Use of ultrasound	+	- - -	e	not to be recommended

Legends of Illustrations

Figure 1 An orbita maxillofacial prosthesis fitted to spectacles has been portrayed. It was worn for 2½ years. The deposits and discolorations can be seen above all on the contact areas of maxillofacial prosthesis and defect.

Figure 2 Comparative portrayal of the initial and final germ counts with the PMMA synthetic Epicryl hot® for *Staphylococcus aureus* (top) and for *Candida albicans* (bottom). The initial germ count has been portrayed in red and the final germ count in green columns. Each series of tests entailed n =8. The mean values and the standard deviations have been shown.

Series 1 –single germ reduction; Series 2 – weekly germ reduction. Germ reduction method: A – scrubbing; B – boiling; C – microwave; D – ultrasound.

Figure 3 Comparative portrayal of the initial and final germ counts with the Episil E® silicone for *Staphylococcus aureus* (top) and for *Candida albicans* (bottom). The initial germ count has been portrayed in red and the final germ count in green columns. Each series of tests entailed n =8. The mean values and the standard deviations have been shown. Series 1 –single germ reduction; Series 2 – weekly germ reduction.

Germ reduction method: A – scrubbing; B – boiling; C – microwave; D – ultrasound.

Fig. 1

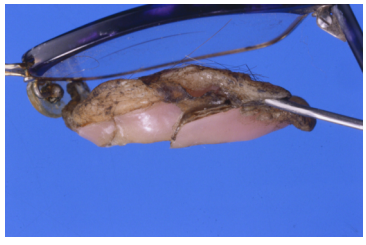


Fig. 2

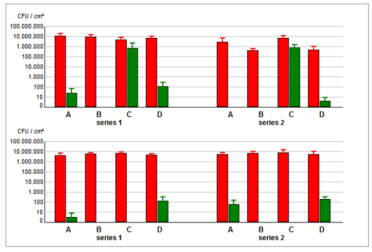
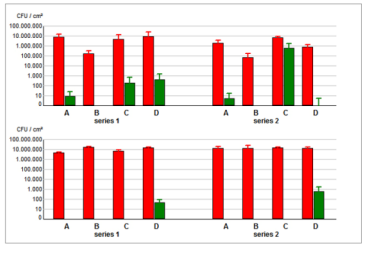


Fig. 3



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