Keywords
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Testing of disinfection procedures for dental prosthetic materials

Summary

Study design: The objective of this study was to evaluate a disinfectant especially designed for immersion disinfection of dental impressions ; the disinfection product being primarily manufactured for the application on dental impression materials is supposed to simplify disinfection procedures in every day practise.

Method: Test objects of polymethacrylate were produced in a standardized manner with a rough and a smooth surface in order to simulate practical conditions of real dental prostheses. Testing of the disinfecting efficiency was done by applying an artificial contamination using *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, all done according to the guidelines set up by the German commission for disinfection testing. Furthermore an analysis of colour stability before and after disinfection of the test objects was done using spectrophotometry.

Results: As a result it can be stated, that a sufficient disinfection effect was determined for all test germs and test objects. Spectrophotometric data did not show any significant changes in colour after disinfection.

Conclusion: Other parameters necessary for acceptance of a disinfecting product have been tested already for dental impressions, therefore the application can be recommended under practical conditions for dental prostheses as well.

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Introduction

The procedures carried out in dental practices provide ample scope for transmission of pathogenic or facultatively pathogenic microorganisms. The oral cavity plays host to a plethora of microbes. It is well known that apart from the resident flora, transient flora is also often present depending on the patient's clinical antecedents, nutritional habits and lifestyle, immune status or individual physiology [1, 2, 3]. Dental workpieces such as impressions and prostheses can come into contact with these microbes, thus serving as potential vehicles for the spread of these, possibly pathogenic, microorganisms [4].

Disinfection of dental workpieces, e.g. of removable prostheses, impressions or occlusal overlay tracks, has in recent times been the focus of scientific discussions. These gave rise to the formulation of requirements set out in various guidelines in the field of dental hygiene [5, 6, 7, 8]. While these give recommendations for disinfection of dental workpieces in dental treatment situations, such measures have not yet been implemented in daily practice to the extent desired [9, 10].

However, disinfection of dental workpieces must be viewed as an indispensable component of a global hygiene concept in order to interrupt infection chains between the patient, dentist, dental assistants and technicians.

The present study was carried out in order to help develop practice-oriented procedures and thus have uniform recommendations, so as to avoid a scenario whereby different disinfection procedures are used for various types of prosthetic workpieces. In principle, it cannot be presupposed that a disinfectant procedure will be endowed with comparable efficacy and material compatibility in respect of various types of materials since, inter alia, the surface roughness and hence microbial adhesion characteristics determine different baseline situations [11, 12, 13, 14].

The aim of this study was to use a disinfectant procedure already approved as a surface disinfectant for immersion disinfection of basic dental prosthetic materials [15, 16]. The test product has already been subjected to in-depth testing in earlier tests carried out by the Work Group to determine its suitability for use on impressions [17, 18, 19]. Expansion of the application spectrum to basic prosthetic materi-

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als was based on the following criteria:

1. Adequate microbial reduction on selected basic prosthetic materials as per the definition of disinfection pursuant to the provisions of the German Society of Hygiene and Microbiology (DGHM) [20, 21] and of the German Association of Applied Hygiene (VAH) [22] in the modified practice-oriented model test [23].
2. There should be no sign of any colour changes that would exceed the usual limit values in the synthetic materials to be disinfected

The results were intended – incorporated into a hygienic concept – as a contribution to increasing acceptance of disinfectant measures where dental workpieces were concerned.

Materials and Methods

Within the framework of these tests, both microbiology and material-related methods were used. Details of the methodology can be consulted in the publication by Höhme [24].

The material used to manufacture the process challenge devices (PCDs) (n = 378), which were used as standardised models for prostheses, including prosthetic workpieces, was the basic prosthetic material Paladon® 65, which is often used in dentistry (Heraeus Kulzer GmbH & Co. KG, Wehrheim) as a hot polymerisate, and PalaXpress® (Heraeus Kulzer GmbH & Co. KG, Wehrheim), which was selected in the colours pink and clear as a cold polymerisate. Based on the manufacturer's instructions plastic plates were manufactured, sawn manually into 20 × 20 × 3 mm PCDs and fitted with a smooth and a defined rough side (instrument: TG 200, Wirtz-Buehler, Düsseldorf, SiC-paper, granulation 320) so as to simulate the situation of a prosthesis or other workpiece as encountered in practice.

The test product was a formulation based on glutaral (pentane-1,5-dial) and glyoxal (ethandial) (Impresept®, Art. Nr. 25125, 3M Espe AG, Seefeld), which had already been certified as a surface disinfectant (see VAH List, as of 16 March 2007 [22]).

Microbiology Tests

The experimental design was based on the procedures recommended by DGHM [20, 21] and VAH [22] for testing surface disinfectants as well as on the proposals put forward by Borneff et al. [23] for modification of the test so as to render it suitable as an experimental design for dental impressions. In the context of this practice-oriented model test, the PCDs were contaminated with a suspension of different test organisms that could be encountered when wearing dental prostheses:

1. *Candida (C.) albicans* ATCC 10231 (DSM strain 1386, mean baseline microbial count 4.05×10^8 cfu/ml)
2. *Pseudomonas (P.) aeruginosa* ATCC 15442 (DSM strain, 939, mean microbial count 6.46×10^8 cfu/ml)
3. *Staphylococcus (S.) aureus* ATCC 6538 (DSM strain 799, mean baseline microbial count 1.37×10^8 cfu/ml)

Aliquots of 20 µl suspension of the test organisms were used to contaminate the PCDs; 30 PCDs and 30 controls were inoculated for each type of PCD and test organism. The test suspension was spread evenly on the PCDs using a Drigalski spatula and after leaving to dry for 10 min the PCDs were subjected to immersion disinfection with Impresept® for 10 min as per the manufacturer's instructions. The control PCDs were immersed for the same period of time in water of standardised hardness (17 °dH). The neutraliser used was based on the specifications by Sonntag [15].

Using decadic dilution series, aliquots of 100 µl were plated in triplicate batches onto trypticase soybean agar (Art. No. TV 5002 E, Oxoid GmbH, Wesel). The plates were then incubated at 36±1 °C for 48 h for the bacteria and for 72 h for fungi. The same procedure was used for the controls. Surviving microbes were counted manually with a colony counter (Colony Counter Type 603, IUL-Instruments GmbH, Königswinter) and the reduction factor (DGHM [20, 21]) was calculated.

Material-Related Tests

To investigate changes to materials the PCDs were subjected to colorimetric examination using remission spectrometry [25a, b, 26]. To that effect, the PCD was exposed to light radiation and the reflected component was measured. Measurements were conducted with a spectrophotometer (CM-2002, Minolta) before and after immersing once for 10 minutes in the disinfectant. The size of the area measured on the PCDs was 10 mm in diameter. Having calibrated the spectrophotometer, the PCDs were positioned with the rough side facing the spectrophotometer. Above this was placed a large reference disc (L* = 91.59, a* = -2.98, b* = 7.62), used by way of a defined background.

For each PCD type, an area with a diameter of 10 mm was measured four times for each of the 36 PCDs, with a 90° rotation being interposed between every two measurements. The coordinates were determined in the laboratory colour room for the average value obtained for each of the four measurements. The distances between the measuring points in Laboratory Colour Room1 before and after disinfection yielded delta E values (ΔE) for the individual PCDs.

Statistical Methods

A box plot (also called box-whisker plot) was used (SPSS Version 12.0, Windows) for graphic illustration of the results of the microbiology and material-related tests. It featured various measurements showing the central trend, scattering and skewed values in a single diagram. The median (2nd quartile), 1st and 3rd quartiles, 5 % and 95 % percentile and outliers are shown in each case.

For comparison of the microbiology results, both paired comparison, with the t-test as well as – since more than two medians had to be compared for each type of PCD – multiple comparison with Tukey testing between the medians of the control microbial counts were carried out (SPSS Version 12.0, Windows; n = 30; $\alpha = 0.05$).

Results

Microbiology Tests

Examination of all disinfected PCDs on completion of the experiment showed that no test organism survived disinfection.

However, this did not provide for calculation of a reduction factor (RF) as per the formula:

$$RF = \log_{10} N_0 - \log_{10} N$$

(N_0: cfu of test organisms before disinfection; N: cfu of test organisms after disinfection)

since the decadic logarithm of "0" had not been mathematically defined. To calculate

the reduction factor, the number of surviving test organisms after disinfection could be assumed to be "1". This would permit calculation of the reduction factor once again (DGHM [20, 21]), however, then all fluctuations in the reduction factors calculated derived exclusively from fluctuations in the cfus in the test organisms before disinfection.

For that reason the medians of the test organisms' concentration of the positive controls of all types of PCDs and test organisms were calculated, so as to identify the main focus of the respective concentration of test organisms (Figure 1). The median for *C. albicans* was 10^4 for all PCD types. The highest level of *C. albicans* adhesion was found on the cold polymerisate (clear), while the lowest rate of adhesion was to the hot polymerisate PCD type. Scattering for *C. albicans* was lowest for the cold polymerisate (pink) PCD type.

The median for *P. aeruginosa* was > 10^5 for all PCD types. The highest level of *P. aeruginosa* adhesion was found on the cold polymerisate (clear) PCD type, while the lowest rate of adhesion was to the hot polymerisate (pink) PCD type.

The median for *S. aureus* was also > 10^5 for all PCD types. The highest level of *S. aureus* adhesion was found on the cold polymerisate (pink) PCD type, while the lowest rate of adhesion was to the cold polymerisate (clear) PCD type.

No growth in test organisms was seen for three PCDs of the cold polymerisate (pink) type with the test organism *S. aureus*. It was not possible to elucidate the reason for this. (Nonetheless, the values were included in the calculations).

Comparison of statistical median of the number of test organisms for the controls produced the following picture: for *C. albicans* no significant difference was seen between the PCD types, however, for *P. aeruginosa* a significant difference was discerned between the cold polymerisate (pink) and cold polymerisate (clear) PCD types. Comparison of the other PCDs did not show any significant differences. For

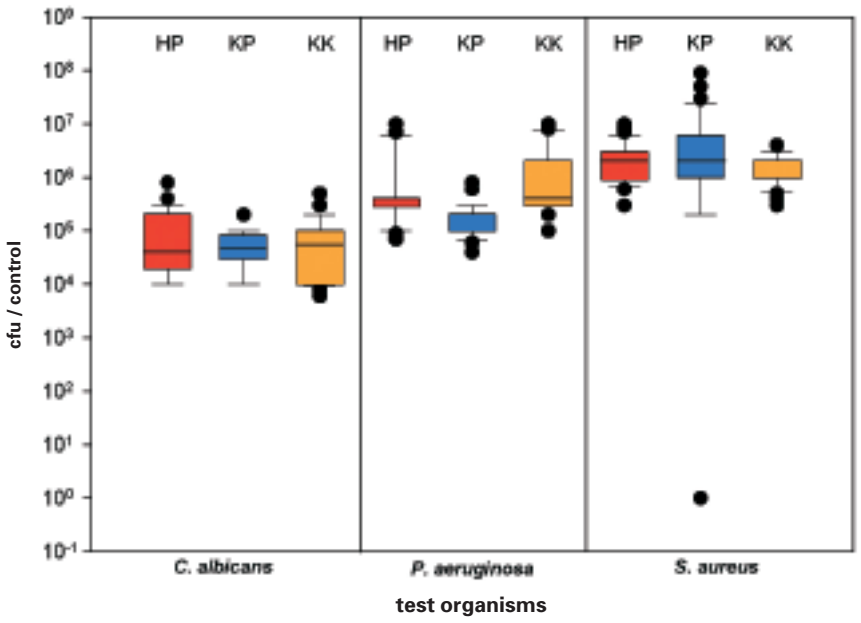

Figure 1: Medians of microbial counts (colony forming units (cfu/PCD) of positive controls of C. albicans, P. aeruginosa and S. aureus shown as box plot for the hot polymerisate (HP), cold polymerisate pink (KP) and cold polymerisate clear (KK) PCD types (n = 30 per PCD type).

S. aureus, as in the case of *P. aeruginosa*, a significant difference was seen between cold polymerisate (pink) and cold polymerisate (clear) PCD types. Comparison of the other PCDs did not show any significant differences.

In summary it must be stated that adhesion, or the concentration of test organisms found on the various PCD types (controls) differed greatly but no uniform trend could be discerned. Different scattering patterns and fluctuations were seen depending on how well the test organisms could be rinsed off from the PCDs. Independently of that, the reduction obtained of > 10^4 for fungi (*C. albicans*) and > 10^5 for bacteria (*S. aureus*, *P. aeruginosa*) met the required value attesting to adequate disinfection in respect of fungi and bacteria (DGHM [20, 21]).

Results of the Material-Related Tests

The disinfection procedure used did not cause any changes in colour that could be subjectively perceived on inspection. Only in three cases did remission spectrometry measurements show, in the case of hot polymerisate, as per the definition, "a colour difference that could be optically perceived in the laboratory colour room" before and after disinfection.

For the cold polymerisate (pink) and cold polymerisate (clear) PCDs the value for all PCDs measured (n = 36) was less than the specified limit value ($\Delta E \leqslant 1$); for the hot polymerisate (n = 36) the ΔE value, apart from 3 PCDs, were within the specified range of $\Delta E \leqslant 1$ (Figure 2).

The standard deviation (Figure 3) pattern differed, with the hot polymerisate and cold polymerisate (pink) being somewhat identical. The cold polymerisate (clear) showed a markedly lower standard deviation, as can be explained by the lack of colour pigments in the plastic material.

In summary it can be stated that in terms of material-related aspects, the disinfection procedure used did not result in any colour differences that could be optically perceived. With the exception of three cases (hot polymerisate: PCD 24 (ΔE = 1.05) 29 (ΔE = 1.11) and 30 (ΔE = 1.01), probably due to inhomogeneous materials, but which could not be subjectively perceived, the delta E values were within the specified range of $\leqslant 1$.

[1] A method commonly used for precise definition of a colour is to depict it in a three-dimensional colour space. Each colour is clearly defined by means of three values L, a and b. The colour space features principle three axes, with L representing the brightness value of the test object. The a-value defines the red-green component of the test object. The yellow-blue component of a colour is determined by the b-value. If two colours are to be compared with each other in this colour space, this is done by comparing two points in the three-dimensional axis system. Using Pythagoras' theorem ($c^2 = a^2 + b^2$) the following formula is obtained with respect to the lab axis system:

$\Delta E = \sqrt{dL^2 + da^2 + db^2}$

ΔE = difference between two colours, d = difference in each case between the three axes L, a and b

Discussion

Disinfection of dental and technical/dental materials and instruments has been in the spotlight once again not least in relation to the recommendation for "Infection prevention in dentistry – hygiene requirements" compiled by the Robert Koch Institute (RKI) [6] It explicitly states that "Dental / technical workpieces, impressions, bite-corrective devices, etc. must be viewed as being microbially contaminated …" and that "…infection of patients, personnel in the dental laboratory or third parties during transport …" must be ruled out by taking appropriate hygiene measures. In view of the manifold nature of the materials to be disinfected, it is recommended that the respective manufacturer be consulted about the procedure to be used [6]. This statement, which should be construed as being a "foregone expert opinion", should also be borne in mind in a forensic setting [27, 28].

Evaluation of a pilot project carried out in Frankfurt am Main on hygiene standards in dental practices [10] highlighted the fact that, despite intensive new legal regulations (Protection against Infection Act - IfSG [29]) and expansion of guidelines (RKI [6]) , there continued to be shortcomings in compliance with, and implementation of, a standard infection control policy. It was also discussed whether an improvement in the structural quality of hygiene behavioural patterns could be achieved through an improvement in the economic aspects. In that respect, a uniform procedure, inter alia, for dental impressions and prostheses would be beneficial.

In principle, it is possible to use both sterilisation and disinfection processes. The most reliable method for eradication of all viable microbes from the surface or from deeper levels of a dental prosthesis is sterilisation, e.g. in an autoclave (saturated steam under pressure at 120–134 °C and 1–2 bar). But such processes cannot be used because materials are heat sensitive and would cause major changes in dimensions [30]. Sterilisation with a low-temperature process, e.g. ethylene oxide (EO) could be contemplated in principle; but this would not lend itself to everyday practices in view of sterilizer-specific safety precautions and the prolonged degassing times needed [31, 32].

While the possibility of using Á rays for sterilisation, as investigated by Setz and Geis-Gerstorfer [33], can be viewed as optimal in terms of maximum microbial reduction (up to 13 log levels for spores), for economic reasons it is not feasible for daily practice. Likewise, the plasma sterilisation process (generation of reactive hydroxyl radicals at 45 °C, 5 % relative humidity) does not appear to be suitable for treatment of dental prosthetic materials due to the high financial investment needed in the dentistry setting.

Since sterilisation of workpieces is not possible, or only after major investment in time and financial resources, preference is given to disinfection procedures because at least there is a general consensus that these materials need not be sterile since they are not implanted into a sterile body cavity, but rather are fitted in, and withdrawn from, the oral cavity (see. RKI [6]). As such, a clear distinction must be made between these and those materials designed for intracorporeal use, e.g. catheters, synthetic heart valves and endoprosthetic devices.

Under everyday use conditions, it is mainly chemical disinfection processes, possibly with the aid of ultrasound [2, 34], which are suitable. In that respect, Grün and Engelhardt investigated the use of sodium hypochlorite solutions backed up by ultrasound for ≤30 min as "…still tolerable in everyday practice" [2]. On investigating alkaline peroxide products, Abelson was able to achieve an additional 40–45 % microbial reduction when using this product backed up by ultrasound as opposed to immersion in the disinfectant solution alone [34].

The latest literature also draws attention to the use of sodium hypochlorite solution as a 10-minute immersion disinfection [35, 36]. Their investigations into the extent to which the deeper synthetic layers, too, lent themselves to disinfection demonstrated that the best results were obtained for 5 % sodium hypochlorite solution in respect of a dental prosthesis that had been worn. There is much controversy about the colour changes induced in synthetic materials by such a form of disinfection. The disinfectant performance of sodium hypochlorite also proved to be inadequate in the presence of organic loads [37].

As far as compliance with the valid requirements is concerned, the disinfectant performance of the disinfection procedure Impresept® investigated can be deemed

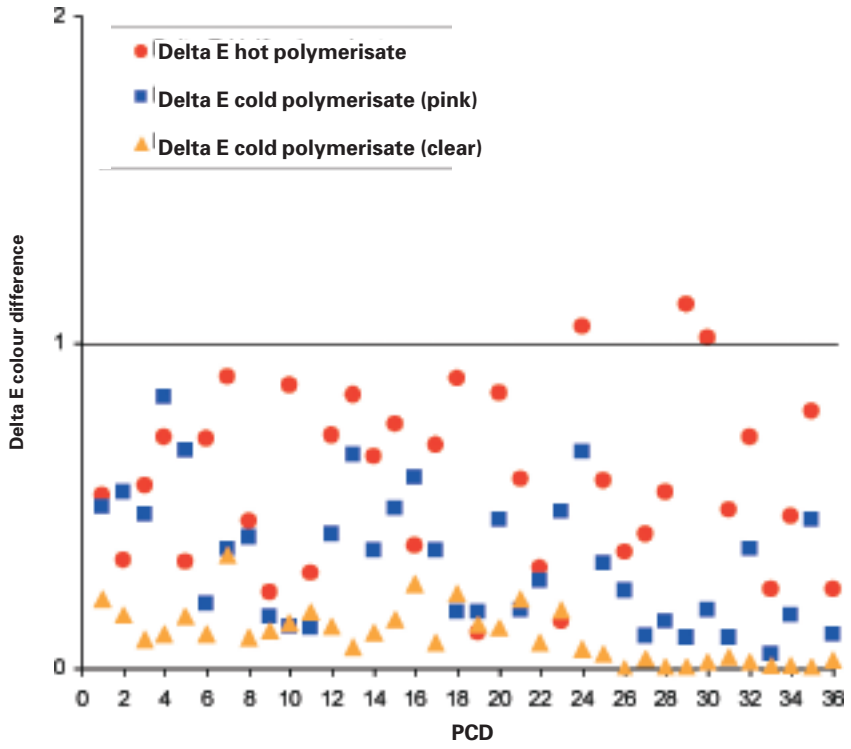

Figure 2: Delta E values of L a* b* colour space based on spectrophotometry, of individual PCD types (n = 36 per PCD type with 4 measurements per PCD).*

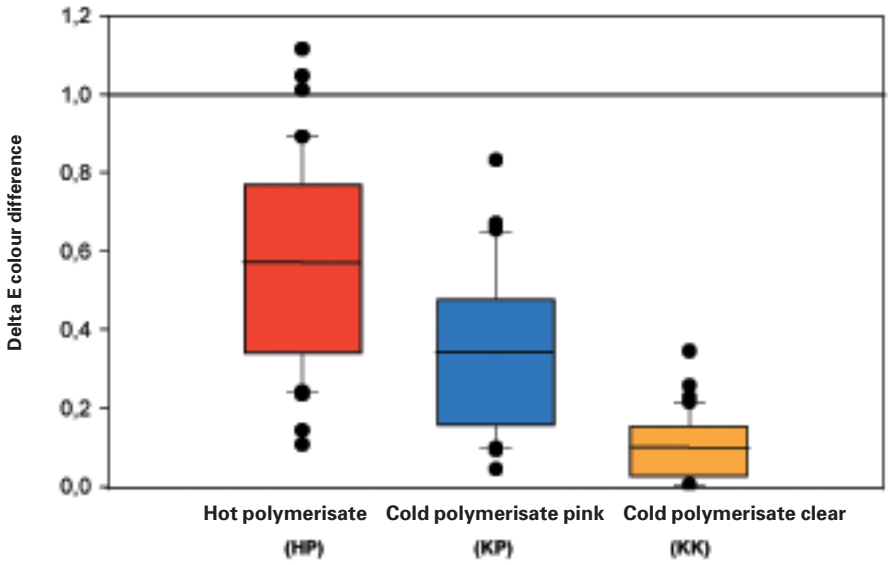

Figure 3: Delta E values (n = 36 per PCD type with 4 measurements per PCD) of individual PCD types shown as box plot (line at "1" corresponds to limit value for ΔE).

to be endowed with adequate efficacy on the basis of the microbiology test series conducted. Differences were seen between the various PCDs which, however, were negligible in terms of the prescribed performances.

The material-related test series using spectrophotometry-based colorimetry demonstrated that no changes in colour could be optically perceived following the disinfection procedure used. The delta E values, with three exceptions relating to the hot polymerisate PCD type, were within the specified range of

$\Delta E \leqslant 1$ and as such do not give rise to any change in colour that can be seen with the human eye. Further experimental model test series should investigate any disinfection-induced change in the dimensions of synthetic materials so as to identify accurate fit in the patient's mouth.

Pursuant to the instructions specified in the corresponding safety sheet, the workpiece should be thoroughly rinsed under running water before fitting it in the patient's mouth in view of the fact that the disinfectant used is a glutardialdehyde-based disinfectant (see Safety Sheet 3M Espe [38]). Because of the aldehyde component, sensitisation is possible following inhalation or skin contact with the product. Furthermore, glyoxal has been assigned to Carcinogenicity Category 3 [38]. Hence appropriate precautions must be taken when handling this product.

Under everyday use conditions, the test procedure confers the special advantage of being suitable for multiple disinfection of dental impressions and prostheses as well as orthodontic materials, such as removable dental prosthetic devices or occlusal overlay tracks. As such, it meets the needs of dental practices and hospitals for standardisation of the working practices.

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Conflict of Interest

None declared.

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