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ORIGINAL ARTICLE

Disinfection effect of dental impression tray adhesives

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Abstract
Objectives Iatrogenic infections are serious problems in dental offices. Impression tray adhesives are delivered in glass containers with a fixed brush attached inside the cap. Using the brush for application of the impression tray adhesive on a contaminated impression tray or prostheses, pathogen transmission by replacing the cap with the brush is possible.
Materials and methods Bacterial strains (patient strains and in vitro strains) were supervaccinated on Columbia agar. The bacterial solution was diluted with TSB and aerobically grown, and starting concentration was 1×10^7 cfu/ml. The stock solution was placed on Columbia agar. Alginate, polyether, and silicon impression tray adhesives were applied to the center of the particular blood agar plates and incubated for 48 h. The expansion of the inhibition zone assays were measured using a microscope.
Results Twenty-one different bacterial strains were selected in the saliva samples of 20 patients. The growth inhibition for alginate impression tray adhesive was 1.1 % (±0.3) of the patient strains. The overgrowth of polyether impression tray adhesive was 30.6 % (±9.3) and for silicon impression tray adhesive 11.8 % (±5.0). In in vitro strains, alginate impression tray adhesive performed an inhibition of 0.7 % (±0.3). The overgrowth of polyether impression tray adhesive was 7.0 % (±1.6) and for silicon impression tray adhesive was 6.5 % (±1.3).
Conclusions Using the fixed brush for application of the impression tray adhesive on multiple patients, a cross-contamination cannot be ruled out.
Clinical relevance An application of the impression tray adhesive with a pipette and a single-use brush would eliminate the contamination.

Keywords Impression tray adhesive · Cross-contamination · Antimicrobial properties · Microbiological reduction

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Introduction

In spring 2011, an epidemic of the verotoxin-producing *Escherichia coli* showed difficulty to find an indirect chain of infection [1]. For several weeks, the epidemic unsettled the population of Germany and parts of Europe. However, pathogen transmission is not only possible by consummation of infected food but also by medical treatment. This is a risk factor which should be known in every medical institution [2].

For the safety of patients and medical health care providers, infection control is a major concern also in dentistry [3]. For patients as well as for dental staff and technicians, multiple ways of infection are possible in dentistry. On one

hand, pathogens can be transmitted directly by contact of infected saliva or blood during invasive treatment. On the other hand, an indirect risk exists because of the multiple use of instruments and materials [4–6]. To reduce the potential risk of infections, the German Centre for Disease Control (Robert Koch Institute, RKI) has published guidelines for infection control in dental offices and laboratories.

Nevertheless, there are some routine treatment sequences in dentistry where an indirect infection cannot be ruled out. Especially during prosthodontics treatment, a lot of different materials and technical equipment is needed, and not all materials can be sterilized. Therefore, a transmission of pathogens, particularly when treating older and multimorbid patients is very dangerous, and the transmission of pathogens may also involve life-limiting risks for treated patients [7]. It is crucial to check complex treatment sequences for their hygiene level and, if necessary, to eliminate possible pathogen transmission especially for the quality management of dental practices [8].

In 1987, White and Jordan showed the possibility of cross-contamination when using impression tray adhesives [9]. The delivery in glass containers with a plastic cap that has a fixed brush inside for multiple uses was criticized. Generally, the risk of a cross-contamination increases by multiple usage instead of the use of a unit dose of that material. Also, the use of a sterile brush is possible which avoids reusable implements such as cotton swabs [10]. Using the fixed multiple use brush to apply impression tray adhesives on an impression tray which was not disinfected after a patient trial fitting or a prostheses for a relining impression, the adhesive will perpetuate cross-contamination. The contaminated impression tray adhesive is likely to get in indirect contact with one of the next patient transmitting pathogens.

There is no information about these problems listed in the materials safety data sheets of the manufacturer. However, the flavor of the impression tray adhesives is similar to that of liquid disinfectants and, therefore, simulates a disinfectant effect. Herman verified a disinfection effect of impression tray adhesives in his investigation [11]. However, this effect is not sufficient to be valid as a liquid disinfectant. In addition, Herman observed the survival of pathogens in the impression tray adhesives. Corresponding to current hygienic guidelines, the study design has to be changed. Based on the risk management of Spaulding, impression tray adhesives are categorized in group semi-critical [12]. Impression tray adhesives are in direct contact with the mucosa; therefore, they should be sterile [13, 14]. Moreover, not only the liquid itself but also the bottleneck is a crucial area for a contamination. The brush will contact the bottleneck when replacing the cap during the use of the adhesive. Because of evaporation of antimicrobial ingredients like isopropyl alcohol or acetone, the dried adhesive on the bottleneck of the glass container shows less antimicrobial effect [11]. The purpose of this study was to test three common impression tray adhesives for the risk of a cross-contamination, referring to the critical hygienic regulation of today's environment in dental clinics.

Materials and methods

Patients

Twenty patients (eight female and 12 male, mean age 58 years (± 10)) of the clinic of the Department of Prosthodontics (University of Halle, Germany) were included in this study. All patients were required to produce a minimum of 4 ml of saliva in 10 min. To avoid cross-contamination, the saliva was placed in tubes (Falcon Tubes (PP) sterile 50 ml, Becton Dickinson, Sparks, MD, USA). Exclusion criteria were hepatitis B, hepatitis C and HIV − infections of the patients.

All patients gave their written informed consent. This study was carried out in accordance with the Helsinki Declaration and approved by the local ethics committees.

Bacterial identification and quantification

In every saliva sample, facultative anaerobic-metabolizing bacteria were identified and quantified. For identification purposes, bacteria from saliva samples were placed on agar plates using inoculating loops (Greiner Bio-One, Frickenhausen, Germany). Columbia agar, McConkey agar, and cetrimide agar (all Oxoid, Basingstoke, United Kingdom) were used as bacterial growth substrate. Bacterial identification was performed using the Vitek biochemical identification system (senior model 120, bioMérieux, Marcy-l'Etoile, France) and the BBL Crystal identification system (Becton Dickinson, Sparks, MD, USA) for facultative anaerobes. The bacteria were quantified in triplicate by plating tenfold serial dilution on Columbia agar (Oxoid, Basingstoke, United Kingdom).

Impression tray adhesives

Three different impression tray adhesives were used in this study to investigate the antimicrobial effects of these adhesives: a product for alginate impressions (Fix, DENTSPLY DeTrey GmbH, Konstanz, Germany) was compared with a product for polyether impressions (Polyether Adhesive, Espe 3 M GmbH, Seefeld, Germany) and for silicone impression materials (Universal Adhesiv, Heraeus Kulzer GmbH, Hanau, Germany).

In vitro strains

Master solutions of in vitro strains were produced of the following microorganisms: *Pseudomonas aeruginosa* (ATCC 27853, ATCC 15692, ATCC 15442, ATCC 10145) *E. coli* (ATCC 35218), *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (ATCC 35556), and *S. aureus* (MRSA patient strain). Producing the master solution of the in vitro strains, a colony forming unit of each single strain was added to 4-ml nutrient-rich growth substrate (Tryptone Soy Broth (TSB), Oxoid, Basingstoke, United Kingdom). This mixture was grown overnight in an aerobic environment and diluted with TSB to a starting concentration of 1×10^7 cfu/ml.

Inhibition zone assays

Inhibition zone assays were performed to investigate the effects of the used impression tray adhesives of the identified microorganism. In this study, a modified Kirby-Bauer zone of the inhibition test system was used. The Kirby-Bauer zone of the inhibition test method is designed to qualitatively test the ability of antimicrobial agents to inhibit the growth of microorganisms over a serious of contacts. Known quantities of bacteria are grown on agar plates in the presence of a defined amount of the verified dental impression adhesives. If the bacteria are susceptible to the relevant agent, an area of clearing surrounds the areas of the adhesives where bacteria are not capable of growing (called a zone of inhibition).

Therefore, bacterial strains (patient strains and in vitro strains) were supervaccinated on Columbia agar. After 24 h of incubation, colony forming units were aerobically grown overnight in TSB at 37 °C and 400 rpm (HTMR-133, MS-Laborgeräte Schröder OHG, Wiesloch, Germany). The bacterial solution was diluted with TSB and incubated aerobically for 24 h. At the start, the concentration of the bacterial solution was adjusted at 1×10^7 cfu/ml and verified photometrically using a spectrophotometer (Biomate III, Novodirect, Kehl, Germany) and quantified microbiologically. These microbiological detection experiments of the test bacteria were repeated three times to confirm the results.

One hundred microliters of the respective stock solution was placed on Columbia agar. Following 10 μl of impression, tray adhesives were applied to the center of the selected blood agar plates using a sterile pipette (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). Afterwards, the blood agar plates were incubated aerobically for 48 h (Heraeus B6420, Kendro Laboratory Products, Hanau, Germany). Expansion of the inhibition zone assays were measured microscopically (Axiolab, Carl Zeiss Jena GmbH, Jena, Germany).

Statistics

The statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Bacterial growth of the three impression tray adhesives was compared with a single factor analysis of variance and Bonferroni correction. Level of significance was set to 5 % ($p<0.05$).

Results

Bacterial spectrum of saliva samples

Results are given as means ± standard deviation. In 21 saliva samples of the 20 patients, nine different facultative anaerobic-metabolizing bacterial genera were identified. The nine bacterial genera could be subclassified in 21 independent strains (Fig. 1). The mainly identified genera were *streptococci* ($4.56\times10^7\pm3.64\times10^7$ cfu/ml), *staphylococci* ($3.64\times10^7\pm2.89\times10^7$ cfu/ml) and *corynebacteria* ($1.23\times10^7\pm1.14\times10^7$ cfu/ml).

Inhibition zone assays for patient strains

Bacterial growth inhibition for alginate impression tray adhesive of the identified patient strains was very limited 1.1 % (± 0.3) (Fig. 2), but it was significantly higher than the results for polyether and silicon impression tray adhesives ($p<0.05$, data not shown). This result has been intensified for polyether and silicon impression tray adhesives. Both groups of impression tray adhesives were overgrown by our patient strains. The overgrowth of polyether impression tray adhesive was 30.6 % (± 9.3) and for silicon impression tray adhesive 11.8 % (± 5.0) (Fig. 2).

Inhibition zone assays for laboratory strains

The used laboratory strains performed similar results of the inhibition zone assays for alginate impression tray adhesive compared to the patient strains. Percentages of bacterial growth reduction of alginate impression tray adhesive were negligible: *S. mutans* (ATCC 25175) 0.2 % (± 0.15), *P. aeruginosa* (ATCC 15692) 1.6 % (± 0.7), *P. aeruginosa* (ATCC 27853) 2.2 % (± 0.8), *P. aeruginosa* (ATCC 15442) 0.8 % (± 0.3), *S. aureus* (ATCC 35556) 0.7 % (± 0.1), *S. aureus* (MRSA patient strain) 0.6 % (± 0.1), *E. coli* (ATCC 35218) 1.0 % (± 0.3), and *P. aeruginosa* (ATCC 10145) 0.0 % (± 0.0) (Fig. 3). The specimens of polyether impression tray adhesive were generally overgrown by the laboratory strains: *S. mutans* (ATCC 25175) 9.3 % (± 1.2), *P. aeruginosa* (ATCC 15692) 3.3 % (± 0.6), *P. aeruginosa*

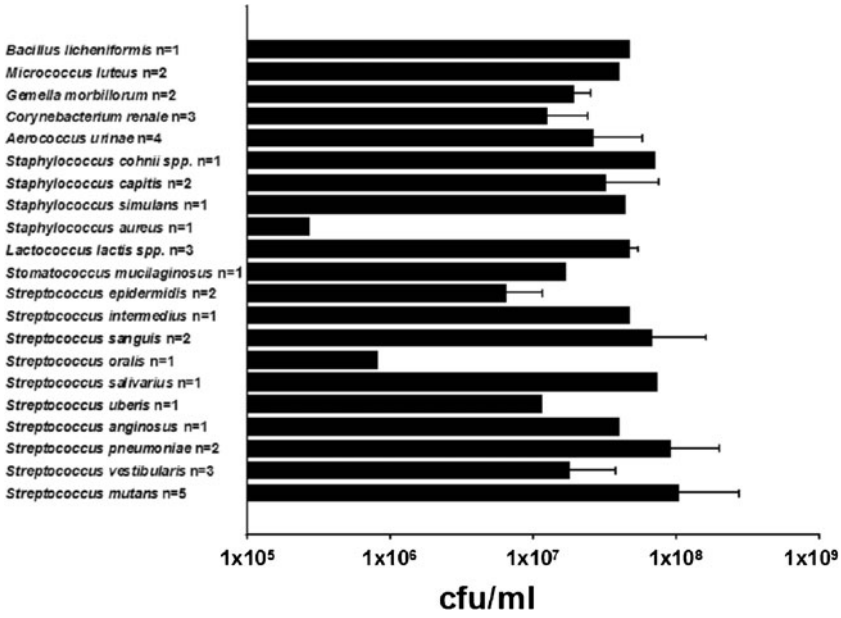

Fig. 1 Facultative anaerobic bacteria detected in the saliva of the investigated patients (shown in colony forming unit per milliliter saliva, mean and standard deviation)

(ATCC 27853) 3.3 % (± 1.2), *P. aeruginosa* (ATCC 10145) 10.3 % (± 1.5), *P. aeruginosa* (ATCC 15442) 6.0 % (± 1.0), *S. aureus* (ATCC 35556) 4.7 % (± 0.9), *S. aureus* (MRSA patient strain) 11.7 % (± 6.2), and *E. coli* (ATCC 35218) 7.7 % (± 0.5) (Fig. 3).

Overgrowth of silicon impression tray adhesive assays was related to the polyether results: *S. mutans* (ATCC 25175) 2.5 % (± 0.0), *P. aeruginosa* (ATCC 15692) 8.3 (± 2.9 %), *P. aeruginosa* (ATCC 27853) 0.0 % (± 0.0), *P. aeruginosa* (ATCC 10145) 4.2 % (± 1.4), *P. aeruginosa* (ATCC 15442) 18.3 % (± 2.9), *S. aureus* (ATCC 35556) 5.7 % (± 1.5) *S. aureus* (MRSA patient strain) 8.3 % (± 0.9), and *E. coli* (ATCC 35218) 4.7 % (± 0.9) (Fig. 3). The differences of bacterial growth inhibition between the three used impression tray adhesives were significant ($p<0.05$, data not shown). Only alginate impression tray adhesive showed small antibacterial characteristics which are not able to avoid any microbiological cross-contamination sufficiently.

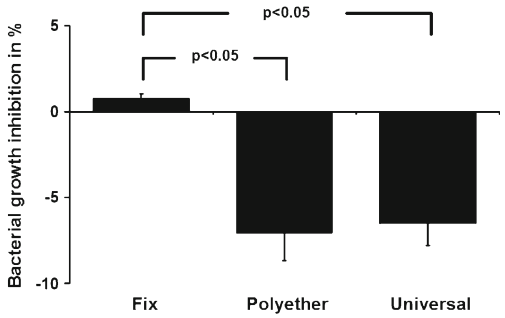

Fig. 2 Inhibition of bacterial growth of patient strains in alginate (*Fix*) impression tray adhesive, polyether (*Polyether*) impression tray adhesive, and silicon (*Universal*) impression tray adhesive in percentage. The bacterial growth inhibition differed significantly ($p<0.05$)

Discussion

Impression tray adhesives were delivered in glass containers with a plastic cap that has a fixed brush inside. Using the brush on an undisinfected impression tray after a patient trial fitting or a removable prostheses for a relining impression can contaminate the adhesive because of remaining saliva or biofilm. Multiple use of the brush can transmit pathogens to further patients. A general risk of potential cross-infection is given through the massive bacterial contamination of the investigated saliva (up to 1×10^8 cfu/ml; Fig. 1).

In this study, the impression tray adhesives for polyether and silicone were overgrown in the zone of inhibition assays

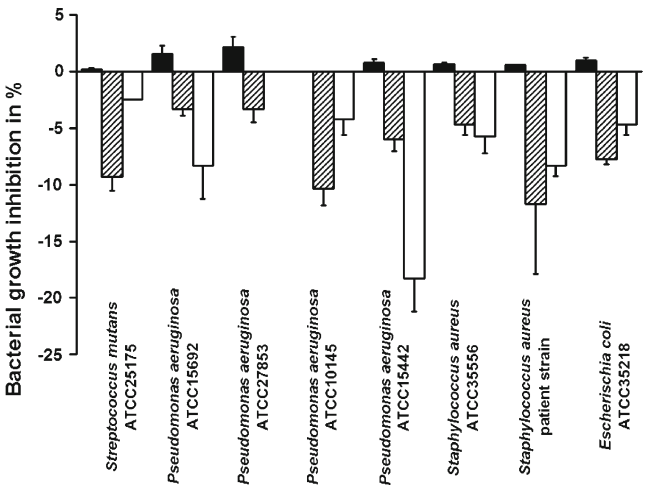

Fig. 3 Mean and standard deviation in percentage of the inhibition of bacterial growth in alginate (*black*) impression tray adhesive, polyether (*diagonal lines*) impression tray adhesive, and silicon (*white*) impression tray adhesive of the laboratory strains

from the patient strains and from laboratory strains (*S. mutans*, *P. aeruginosa*, *E. coli*, and *S. aureus*). Only the alginate impression tray adhesive showed marginal growth inhibition (Figs. 2, 3) and is not inhibiting any bacterial growth sufficiently. This is the case for the patient strains as well as for the laboratory strains. The small differences shown by the results of the inhibition of the impression tray adhesives may adhere in the disparities of the concentration of the antimicrobiological agents included in the impression tray adhesives. However, these results indicate that the alginate impression tray adhesive has neither the ability to avoid any bacterial cross-infection sufficiently.

In 1987, White and Jordan criticized the use of the fixed brush in the cap of the container. Therefore, they postulated instructions for hygienic use of impression tray adhesives [9]. White and Jordan recommended using single-use applicators instead of the fixed brush [10]. To avoid a pathogen contamination of the impression tray adhesive, the brush should not be replaced in the glass container after its use on a nonsterile impression tray or prostheses. Therefore, it is recommended that the impression tray adhesive is filled into a separate container for single use. The remaining adhesive must be discarded and not added to the original glass container. To avoid the single use applicators, the application of the impression tray adhesive could be undertaken before the fitting trial of the impression tray on the sterile tray. However, some ingredients of the impression tray adhesive can irritate the mucosa by extensive contact. On the other hand, the study of Chai showed that the contamination of the impression tray adhesive with saliva reduces significantly its adhesive power [15]. The first scientific study to investigate the antimicrobial character of impression tray adhesives was done by Herman [11]. He showed that impression tray adhesives have antimicrobial effects. However, the minor microbial reduction of pathogens was minor and did not avoid any contamination of the adhesives. The results did not warrant the discontinuance of proper infection control procedures but rather demonstrated an additional benefit of the antimicrobial character of the impression tray adhesives. Herman investigated the effect of the adhesive fluid only with laboratory strains in low concentration.

A problem of current hygienic requirements is the survival of pathogens in the fluid and in critical areas, for example, on the bottleneck of the glass container. Bacterial contamination of the bottleneck by replacing the fixed brush may lead to a pathogen settling on the dried impression tray adhesive because antimicrobial ingredients, such as isopropyl alcohol or acetone, usually evaporate immediately.

The purpose of this study was to complete the study from Herman in critical areas. To simulate a situation on a daily basis such as in dentist clinics, not only laboratory strains but also patient strains were used. Patient strains were cultivated out of patient saliva and the pathogens identified, quantified and used for the inhibition zone assay.

Generally, the intraoral environment provides ideal conditions for bacteria [16]. The most facultative anaerobic bacteria in saliva are *Streptococci*, *Staphylococci*, and *Corynebacteria* [17–21]. Especially, these bacteria were found in the saliva of the examined patients. Some of these bacteria belong to the category of opportunistic pathogens [22]. The amount of bacteria in some of the patient's saliva samples was relatively high. The majority of the patients of the test group showed a noticeable xerostomia due to a previous head and neck radiation. Based on literature data [11] and the results of this study, bacteria are able to survive in impression tray adhesives in a quantity that allows duplication. Furthermore, a contamination with pathogens of infections typically spread in hospitals such as *P. aeruginosa* and multiresistant *S. aureus* may be possible. Even the transmission of other microorganisms, i.e., verotoxin-producing *E. coli*, cannot be eliminated with impression tray adhesives.

Primarily, the ingredients of the impression tray adhesives have a high suction capacity to ensure that the impression materials stay attached to the impression tray or prostheses when removing the impression from the mouth. Therefore, the impression tray adhesives are not bactericide and only limited bacteriostatic. Regarding the hygienic guidelines of centers for disease control, a multiple use of the fixed brush is not recommended. Therefore, the manufacturer should eliminate the brush in future. Disinfecting the impression tray after fitting the size of the tray in the oral cavity might be an alternative procedure avoiding cross-infection in dental clinics. The disinfecting procedure must be performed prior to the application of the impression tray adhesive. When these instructions are followed, the use of the brush can be considered. The disinfection has to be added to the material data sheets of the impression tray adhesives. Some manufacturers offer impression tray adhesives in the form of a spray. Hereby, the problem of pathogen transmission would be eliminated. However problems occur, concerning the work protection. The usage of an aerosol is the major concern. The material data sheets of these spray impression tray adhesives described the likelihood of an irritation of the mucosa by inhalation of the aerosol. Hence, to protect dental health care providers from this aerosol, a disposable pipette would be the simplest solution. The application of the impression tray adhesive on the impression tray or the prostheses could be undertaken with a single use brush.

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Conflict of interest The authors declare that they have no conflict of interest.

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