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Persistence of ^{99m} Tc-Labelled Microorganisms on Surfaces of Impression Materials

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Abstract

Impression materials or prostheses can be contaminated with oral microflora and provide a significant source for cross-contamination. A study of such contamination was carried out using an approach different from that of infection control, which has often been investigated in previous studies.

The study focused on microorganisms known to cause local and systemic diseases and which are normally found in the oral flora. The persistence of *Streptococcus mutans (S. mutans), Escherichia coli (E. coli), Staphylococcus aureus* and *Candida albicans (C. albicans)* on zinc-oxide eugenol, silicone rubber, irreversible hydrocolloid and polyether-rubber was investigated using ^{99m} Tc-labelled microorganisms.

Ten specimens from each of the four impression materials were prepared as discs of 3 mm in height and 10 mm in diameter. After the specimens had been placed into a suspension of ^{99m} Tc-labelled microorganisms, remaining radioactivity was counted in a gamma counter.

According to own findings, *S. mutans* was the most, and *E. coli* the least persistent on the specimen surfaces. The number of microorganisms removed after washing was less than the amount remaining on the surfaces. *C. albicans* was removed most easily from all impression surfaces that bore persistent microorganisms after washing. Other microorganisms showed various degrees of persistence according to the impression material.

Introduction

In dentistry, there has been a considerable interest in infection control in recent years. Impression materials and prostheses can become contaminated with oral microflora and provide a significant source for cross-contamination, since spores, viruses and bacterial microorganisms can survive for prolonged periods of time away from their natural habitats[1-5]. Pathogenic microorganisms such as hepatitis B virus (HBV), human immunodeficiency virus (HIV), herpes virus (HSV), and *Mycobacterium tuberculosis* can cause infection in healthy humans, and furthermore microorganisms that are known to be avirulent can cause infection in immunocompromised patients by way of saliva, blood or direct contact[1,3,6-9]. Because of this, disinfection of impressions or patterns obtained from such patients has become of prime importance in recent years. In 1985, the American Dental Association published a guide for infection control including disinfection of impressions and prostheses in dental laboratories and clinics[10].

The effects of commonly used disinfectants and duration of disinfection on the dimensional accuracy of impression materials have been widely investigated[3,4,6,11-14]. However, reports in the literature on the persistence and carriage of oral flora on impression surfaces are limited[2,12].

The purpose of the present investigation was to test the adhesion of some microorganisms labelled with a radioisotope. *Staphylococcus aureus (S. aureus), E. coli, S. mutans and C. albicans* were labelled with ^{99m} Tc by the method of Ercan et al.[15] The impression materials tested were silicone rubber, irreversible hydrocolloid, polyether-rubber and zinc oxide eugenol paste.

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Materials and Methods

Ten specimens of each of irreversible hydrocolloid (CA 37, Cavex Holland B.V. Keur and Sneltjes Dental Mfg Co., Haarlem, Holland), silicone rubber (Thixoflex, Zhermack s. r. l., Via Bovazecchino, 100, 45021 Badia Polesine, Italy), zinc-oxide eugenol(Cavex Impression Paste, Cavex Holland B.V., Haarlem, Holland) and polyether-rubber (Espe Impregum F, Fabrik Pharmazeutischer praparate GMBH and Co., KG, D. 8031 Seefeld/Oberbay, Germany) impression materials were prepared in the form of discs 3 mm in height and 10 mm in diameter. Suspensions of each of the microorganisms were prepared at a concentration of 10^9 c.f.u./ml[16].

The microorganism suspensions were centrifuged at 3000 rpm for 10 min, and the supernatant was decanted. Ten milliliters of saline was added to the packed microorganisms, followed by mixing, washing and recentrifugation. The upper fluid layer was separated, then the following procedure was used to label the microorganisms with technetium -99m (^{99m}Tc)[15,17]:

1. L-D-Glucose 1-phosphate (Sigma Chemical,MO. Co., U. S. A.) (30mg) was dissolved in 2 ml distilled water. The pH of the solution was adjusted to 5-6 with 1 N HCl. Then 0.5 ml $SnCl_2 \cdot 2H_2O$ solution (1 mg/ml) was added and stirred for a few seconds. The mixture was passed through a 0.22-μm membrane filter [Millipore, MA,U.S.A.] directly into a sterile vial. Then 0.5 ml was taken from this mixture and added to the microorganism pellet, followed by stirring and left to react at room temperature (R. T.) for 20 min.[17]

2. ^{99m}Tc 555MBq was added to the microorganisms that reacted with glucose-phosphate and again the suspension was incubated at room temperature for 10 min.[15]

Microorganisms were centrifuged at 3,000 rpm for 10 min. The radioactivity in the microorganisms and the supernatent was determined using a dose calibrator. Five milliliters of saline was added to the microorganism layer, mixed well and centrifuged. After the second washing, the radio activities of microorganism and supernatent layers were counted separately. Thus, the ^{99m}Tc not attached to the microorganisms was removed. This procedure was repeated until a constant radioactivity reading was obtained in the bacterial layer. Percentage labelling of microorganisms was found to be 44.1 % for *S. aureus*, 80.1 % for *C. albicans*, 1.8 % for *E. coli* and 26.0 % for *S. mutans*.

To the labelled microorganisms, 82 ml saline solution was added and mixed well. Then 2 ml was taken from this suspension and diluted to 100 ml. Aliquots of 1 ml were taken from this as 2/100 standard. Two-milliliter samples of the suspension were placed in 40 vials of equal volume.The specimens were placed in these vials, and covered completely with the microorganism suspension on both surfaces. After 15 min, the specimens were removed and placed on blotting paper. To dry them, both of the surfaces of each specimen were placed in full contact with the paper. They were then placed separately in counting tubes and the radioactivity counted in a gamma counter (BF Gammaszint 5300, Labor Prof. Dr. Berthold Wildbad, D-7547, Wildbad, Germany) for 1 min against a standard prepared using 2/100 dilution of the microorganism suspensions described above. After counting, the specimens were rinsed with distilled water for 15 s, washed, dried and counted again in the same gamma counter as for the first series. From the count rates, the uptake of different microorganisms on the impression surfaces as a percentage of the total radioactivity of the suspension the disc was immersed in was calculated:

$$\% \text{ Uptake} = \frac{\text{Count rate on the disc}}{\text{Count rate of the standard}} .$$

The persistence of microorganisms as a percentage of radioactivity adhering to the surfaces was obtained from the first count. The amount of radioactivity removed by washing and the amount remaining on the impression surfaces were obtained as percentages from the first and second counts. Percentages of

microorganisms remaining on the impression surfaces after washing were determined according to the formula:

$$\% \text{ Persistence} = \frac{\text{Count rate after washing}}{\text{Count rate before washing}} \times 100 .$$

The results were subjected to statistical analysis. To establish differences between groups, Kruskal-Wallis one-way analysis of variance, Wilcoxon and Mann-Whitney U tests were applied[18].

Results

The uptakes of microorganisms by the various impression surfaces are summarized in Fig. 1. The persistence of microorganisms on the impression surfaces after washing is given in Table 1. The uptake values were low (<3 %), but persistence was high (>39.5 %). According to the results of Kruskal-Wallis one-way analysis of variance, the difference between groups was significant (p=0.00). Groups were compared two by two by Wilcoxon and Mann-Whitney U tests. The differences between the groups were obtained.

1. *Comparison of uptake values before and after washing (Fig.1)*
   It was found that the uptake values for *S. mutans* were higher than the uptake values for the other microorganisms on all the impression materials before washing. The next highest was *S. aureus*, followed by *C. albicans* and *E. coli*.
   There was a significant decrease (p=0.01) when the uptake values for all microorganisms were read after washing. *S. mutans* had the highest count rate, followed in order by *S. aureus, C. albicans* and *E. coli*.
2. *Comparison of the percentages of microorganisms remaining on impression materials after washing (Tables 1,2)*
   It was found that *C. albicans* had the least persistence on all the impression materials. The other three microorganisms had a higher percentage of persistence than *C. albicans* on zinc-oxide eugenol and polyether-rubber. The difference between the percentage of persistence was not significant ($p > 0.05$).

Also on silicone rubber surfaces, it was shown that *S. mutans, S. aureus and E. coli* had significantly higher persistence than *C. albicans* ($p<0.05$). The difference in percentages between *S. aureus* and *E. coli,* and *S. aureus* and *S. mutans* was insignificant ($p>0.05$). *S. mutans* had higher persistence than *E. coli* ($p<0.05$).

E. coli had the highest and *C. albicans* the lowest persistence on irreversible hydrocolloid. The difference between the percentage persistence of *S. aureus* and *S. mutans* was not significant ($p>0.05$).

C. albicans had the least percentage persistence on all impression materials compared with the other microorganisms. It showed most persistence on polyether rubber (75.20 %).

The lowest persistence was observed on silicone rubber (39.5 %). The percentages of persistence on zinc-oxide eugenol and irreversible hydrocolloid were significantly higher than on silicone rubber, and significantly lower than on polyether-rubber ($p < 0.05$). The difference between the other impression materials was not significant ($p >0.05$).

Discussion

Bacteria, viruses and resistant spores can easily contaminate dental equipment and impression materials. Because of this, dentists and dental staff must protect both themselves and patients[1].

Immunocompromised patients may have an altered flora, and normally harmless microorganisms can cause infection in these patients[1,7,9]. Microorganisms known to cause local and systemic diseases and normally found in the oral flora were used in this study[7,14,19]. They can infect the skin or mucosa, or may cause serious infections such as endocarditis, encephalitis and meningitis[16].

^{99m}Tc-labelling of microorganisms was used in this study. This method had been used previously in our

laboratory to determine the adhesive properties of various dental materials[20-22]. It was modified from the red blood cell labelling method of Ercan and Bernay[15] and applied for the first time for studying microorganism persistence in dentistry. A similar method was used before by Pfister et al.[23] in persistence studies of ^{113m}In-labelled *S. mutans* on the surfaces of different restorative materials. Later, ^{99m}Tc was substituted for ^{113m}In, as reported previously [24],because of the more favorable physical characteristics of ^{99m}Tc (T1/2 = 6h, Eγ =140 keV), and its easy availability. It allows precise determination of microorganism adhesion to surfaces by radioactivity measurements. The experiments can be carried out *in vitro* with a minimum amount of radioactivity so that the radiation dose to which personnel are exposed is low. It is also a simple and inexpensive procedure.

Our results revealed that microorganisms cannot be removed completely from surfaces just by washing. Most of the original radioactivity remained on the surface, whatever the type of microorganism used and the type of surface. Thus, washing alone is not sufficient to prevent cross-contamination. Different disinfectants can be tested to this effect in future work. We found that *E. coli* had the least persistence on all impression materials before and after washing, whereas *S. mutans* had the most.

When the count rates obtained after washing were compared with those obtained before washing, it was observed that *C. albicans* was removed more efficiently after washing. This shows that it attaches only weakly to all impression materials compared with the other microorganisms. *C. albicans* had the lowest percentage persistence on silicone rubber. That is, compared with other microorganisms, it could be removed easily by washing. The percentage persistence of *S. mutans* and *S. aureus* on silicone rubber was not low, but was lowest on irreversible hydrocolloid. The difference in the persistence of various microorganisms on different materials may have been due to the different surface characteristics of the microorganisms, such as electric charge, shape, polarity and molecular units attached to their surfaces. Consequently, it was revealed that all microorganisms could not be removed equally easily from impression materials by washing.

In the study by Samaranayake et al.[14] of the persistence of microorganisms on elastomeric impression materials and irreversible hydrocolloid surfaces, higher persistence was observed on the latter material. This result was attributed to the roughness of the irreversible hydrocolloid surface. However, our findings indicated that other mechanisms might play a role in addition to the surface roughness of impression materials. However, our results cannot be compared directly with their findings, because they used a different method and determined the survival of microorganisms on impression surfaces.

More studies are needed in order to reveal the parameters that affect the persistence of microorganisms on impression surfaces and to what extent. ^{99m}Tc-labelling of microorganisms for such studies is a simple and inexpensive approach. In future studies this can be used to test the efficiencies of various disinfectants for removing the bacteria adhering to impression surfaces.

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Table 1 Arithmetic means and standard deviations of persistence of microorganisms on various impression materials after washing

Microorganisms	Impression materials			
	Zinc-oxide eugenol	Polyether-rubber	Silicone rubber	Irreversible hydrocolloid
S. aureus	84.6±8.7	86.2±6.7	83.2±7.0	72.7±9.7
C. albicans	60.7±13.4	75.2±6.3	39.5±6.9	59.3±15.7
E. coli	86.5±4.4	86.9±9.7	79.7±6.8	89.4±5.3
S. mutans	83.4±14.2	86.0±5.3	86.0±2.5	66.7±6.5

Table 2 Statistical comparisons of groups according to Mann-Whitney U test

Microorganisms	Impression Materials			
	Zinc-oxide eugenol	Polyether-rubber	Silicone rubber	Irreversible hydrocolloid
S. aureus	U=93.5	U=83	U=100	U=81
C. albicans	p<0.05	p<0.05	p<0.05	p<0.05
S. aureus	U=53	U=56	U=69	U=83
E. coli	p>0.05*	p>0.05*	p>0.05*	p<0.05
S. aureus	U=54	U=57	U=56	U=70
S. mutans	p>0.05*	p>0.05*	p>0.05*	p>0.05*
C. albicans	U=93	U=83	U=89	U=96
E. coli	p<0.05	p<0.05	p<0.05	p<0.05
C. albicans	U=89	U=81	U=93	U=75
S. mutans	p<0.05	p<0.05	p<0.05	p<0.05
E. coli	U=66	U=51	U=76	U=83
S. mutans	p>0.05*	p>0.05*	p<0.05	p<0.05

*insignificant

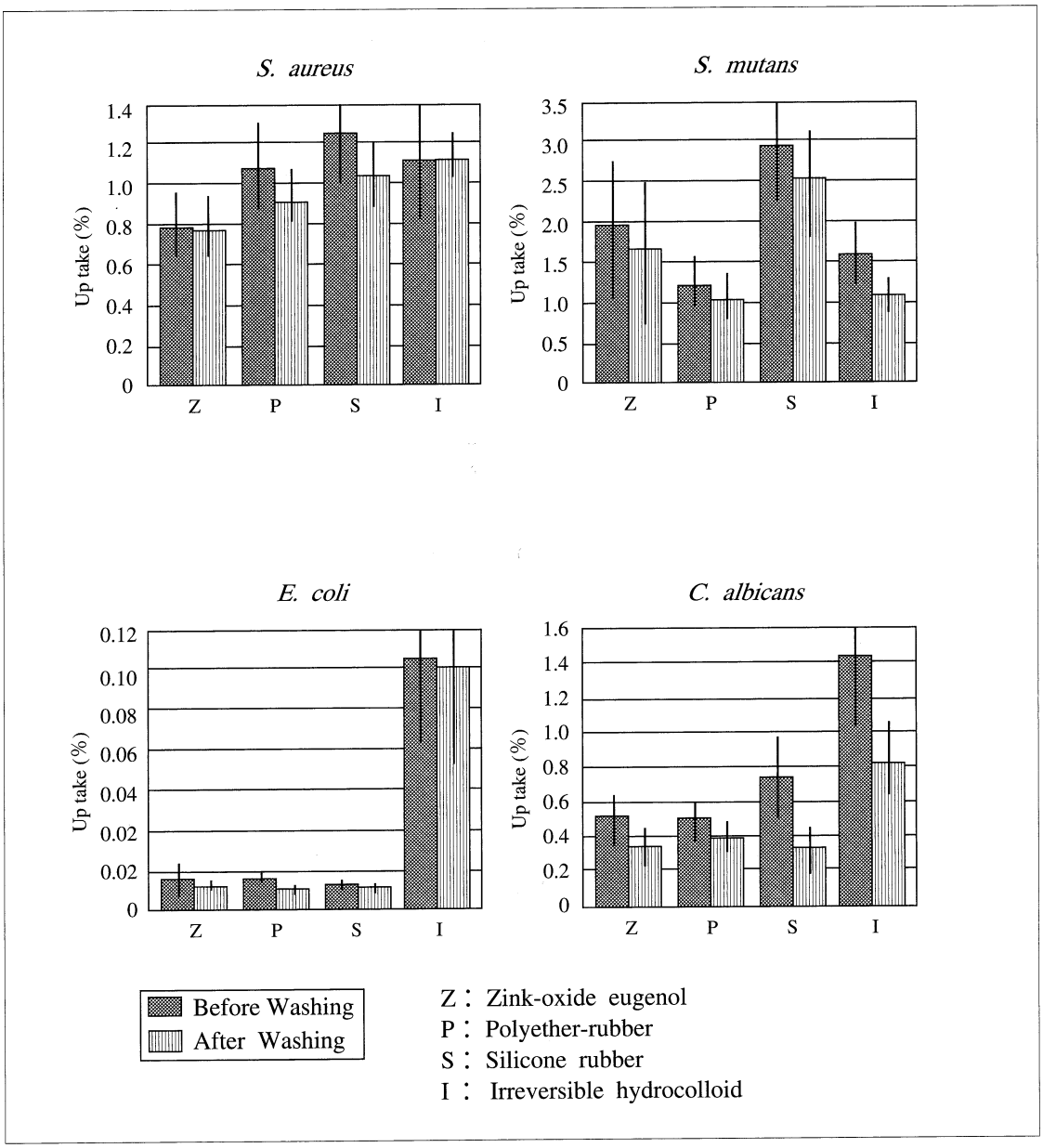

Fig. 1 The uptake(%) of ^{99m}Tc-labelled microorganisms by various impression materials immersed in a suspension of labelled microorganisms (mean ± SD)