An Analysis of the Persistent Presence of Opportunistic Pathogens on Patient-Derived Dental Impressions and Gypsum Casts

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Purpose: This study aimed to assess the persistent presence of microorganisms on patient-derived dental impressions and gypsum casts, while highlighting important human pathogens such as *Candida,* methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa.* ***Materials and Methods:*** The practices and opinions regarding cross-infection control from 59 general dentists in Japan were obtained via a questionnaire. Alginate impressions were made from 56 patients. Using a brain heart infusion agar medium, impression and imprint cultures were carried out to visualize the microbial contamination on the surfaces of the impressions and gypsum casts, respectively. The colonies on the surfaces of the 30 impression cultures and 26 imprint cultures were collected by swabbing and then inoculated onto selective agar plates to detect streptococci, staphylococci, *Candida,* MRSA, and *P aeruginosa.* ***Results:*** The questionnaire showed that only 54% of general dentists had a cross-infection policy in their dental clinics, and only 30% to 40% were aware of the possible persistence of MRSA or *P aeruginosa* on impressions and gypsum casts. The impression/imprint cultures grew a large number of visible bacterial colonies on all of the impression/gypsum cast samples investigated. Selective agar cultures demonstrated the presence of streptococci (100, 100%), staphylococci (56.7, 65.4%), *Candida* (30, 46.2%), MRSA (26.7, 15.4%), and *P aeruginosa* (6.7, 7.7%) on the impressions and the gypsum casts, respectively. ***Conclusions:*** This investigation showed that patient-derived dental impressions and gypsum casts are contaminated with numerous microbes, including *Candida,* MRSA, and *P aeruginosa,* which are known pathogens responsible for nosocomial and/or life-threatening infection in the immunocompromised host. *Int J Prosthodont 2008;21:62–68.*

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Increased awareness of the importance of infectious diseases and recognition of the potential for transmission of numerous infectious microorganisms during dental procedures have led to an increased concern for, and attention to, infection control in dental practice.[1,2] In some countries, recommendations concerning the disinfection of items sent to dental laboratories have existed for several years.[3–5] These recommendations have, however, been followed only rarely.[6]

The need to block this potential route for the transmission of infectious disease via the disinfection of dental impressions and gypsum casts delivered to the dental laboratory is based primarily on theoretical considerations. Information on the microbial contamination of patient-derived (in vivo) impressions and gypsum casts is sparse. Some in vivo investigations

have shown the presence of bacteria on impressions,[7–10] although they did not attempt to identify specific pathogenic bacteria. Verification of the transfer of microorganisms to dental casts made from artificially contaminated (in vitro) typodonts and impressions has been documented.[11,12] However, there are few or no data in the literature on the actual carriage and persistence of oral flora on patient-derived gypsum casts. Therefore, evidence is limited regarding the risk of transmission of pathogenic microorganisms into the dental laboratory and to ancillary staff members from the handling of impressions or dental casts.

Opportunistic pathogens are bacteria that cause a disease in a compromised host that typically would not occur in a healthy (noncompromised) host. Flora normally found in and on the human body, such as *Staphylococcus aureus*, *Escherichia coli,* or *Candida albicans,* can cause an opportunistic infection, as can an organism such as *Pseudomonas aeruginosa* found in the environment. Methicillin-resistant *S aureus* (MRSA) is an important nosocomial pathogen that has recently been reported in patients without typical risk factors for nosocomial acquisition (community-associated MRSA).[13] Outbreaks of community-acquired MRSA infection in healthy children and adults have been described worldwide.[14] The most common site of MRSA colonization is the anterior nares,[15] but MRSA can also occasionally be isolated from the oral cavity,[16] the throat,[17] and saliva.[18,19] In these cases, it is colonizing as a part of the normal flora and is not causing any ill effects, but it may do so if transferred to other sites (eg, by breaking the skin) or if it is passed on to a susceptible person. Until now, there has been no information regarding whether these opportunistic pathogens really exist on patient-derived dental impressions and gypsum casts. To improve the basis for risk assessment and find a suitable strategy for reducing cross-contamination risks, there is a need for epidemiologic studies of the presence and persistence of microbial contamination of dental impressions and gypsum casts.

Therefore, the purpose of this study was to assess the persistence of opportunistic pathogens on patient-derived impressions and gypsum casts. Further, preliminary surveys of the practices and attitudes of 59 general dentists in Japan concerning cross-infection control and their awareness of the possibility of microbial contamination on dental impressions and gypsum casts were obtained and analyzed.

Materials and Methods

Questionnaire

The questionnaire consisted of 2 sections with a total of 12 questions. The first section solicited information regarding the practitioner's use of cross-infection policies and specific disinfection procedures, as well as the clinician's intention to implement a cross-contamination policy in the future. The second section concerned the clinician's awareness of contamination of dental impressions and gypsum casts with oral cavity-derived microorganisms, such as caries/periodontitis-associated bacteria, *Candida* fungus, hepatitis viruses, MRSA, and *P aeruginosa*. The questionnaire was distributed at an alumni meeting of the Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, to 65 general dentists in private practice in Japan. Replies were received from 59 practioners (91% response rate).

Subjects and Materials

The present study was conducted in accordance with a protocol approved by the Ethical Committee of the Osaka University Graduate School of Dentistry, and informed consent was obtained from all subjects. The subjects comprised 56 adults, randomly selected from the patients of the Department of Fixed Prosthodontics at Osaka University Dental Hospital, with the following inclusion criteria: *(1)* no complete denture on either arch; *(2)* more than 10 existing teeth in the maxilla; *(3)* over 20 years of age; and *(4)* had not been given oral hygiene/tooth brushing instructions. The alginate impression material (Aroma Fine DFII, GC), dental stone (New Plastone LE, GC), rubber bowl, spatulas, and boxing wax were sterilized with ethylene oxide.[20] As a negative control, a maxillary arch of a standard typodont with rubber-simulated soft tissue was also sterilized with ethylene oxide. Other instruments and materials (impression trays, water, etc) were sterilized by autoclave.

Assessment of Oral Microorganism Carriage on Dental Impressions

An alginate impression was made of the maxillary arch of 30 subjects (19 women and 11 men; mean age: 69.8 years; age range: 24 to 83 years) including 6 removable partial denture wearers. As a negative control, an alginate impression was also made of 5 sterilized typodonts. After setting for 2 minutes in the subject's mouth, the impression was removed. The carriage of oral flora on the impressions was evaluated using a modified impression culture technique.[21] Brain heart infusion (BHI) agar medium (5.2% BHI and 3.7% Bacto-Agar, Difco) was prepared at 50°C and poured into the impression. After 1 hour of cool down at 4°C, the hardened BHI agar was aseptically separated from the impression and incubated at 37°C aerobically for 48 hours. Photographs of the impression culture

Table 1 Responses to the Question "Do You Think These Microorganisms Persistently Exist on Dental Impressions or Gypsum Casts if Dental Impressions Are Not Disinfected?"

	Impressions (%)			Gypsum cast (%)		
	Yes	No	No idea	Yes	No	No idea
Caries/periodontitis-associated bacteria	80	2	19	54	15	31
Hepatitis viruses	76	2	22	51	3	46
Candida	68	2	31	46	10	44
MRSA	44	2	54	32	12	56
P aeruginosa	41	5	54	32	12	56

surface were taken, and the existence of any colonies was determined by visual observation.

Assessment of Oral Microorganism Carriage on Gypsum Casts

An alginate impression was made of the maxillary arch of 26 subjects (18 women and 8 men, mean age 55.7, 24 to 76 years of age), including 11 removable partial denture wearers. The impressions were poured with sterile dental stone, which was mixed with sterile water under sterile conditions in a sterile laminar-flow hood. After the dental stone had hardened, the casts were aseptically separated from the impression material and allowed to set further for 1 hour under the sterile hood for drying. The carriage of oral flora onto gypsum casts was evaluated using a modified imprint culture technique.[21] Paraffin wax was used to form a box around the gypsum casts. BHI agar medium at 50°C was poured onto the boxed gypsum casts and maintained at 4°C for 1 hour. The hardened BHI agar was separated from the casts and incubated at 37°C aerobically for 48 hours. As a negative control, 5 sterilized typodonts were prepared and an alginate impression was made, followed by the exact same procedure described earlier. Photographs of the imprint culture surface were taken and the existence of any colonies was determined by visual observation.

Detection of Pathogenic Microbes

Colonies on the BHI impression and/or the imprint culture surface were collected by swabbing with a sterile cotton swab and then suspended in 1 mL of sterile phosphate-buffered saline. The colony suspension was plated on 5 selective agar medium plates: Mitis-Salivarius Agar (Becton Dickinson), *Candida* GE Agar (Nissui), Mannitol Salt Agar (Becton Dickinson), OPAII *Staphylococcus* Agar (Becton Dickinson), and *Pseudomonas Aeruginosa* Selective Agar (PASA) medium (Becton Dickinson) to detect the presence of *Streptococcus mutans* and other streptococci, *Candida*, staphylococci, MRSA, and *P aeruginosa*, respectively. After 48 hours of incubation under aerobic conditions at 37°C, the existence of positive colonies for each selective medium was visually determined according to the manufacturer's instructions (Figs 1a to 1e).

Results

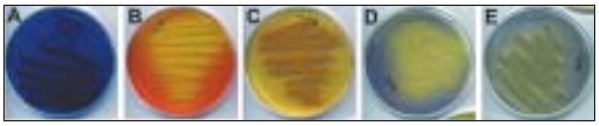
Questionnaire

In response to the question on the implementation of a cross-infection policy, only 32 of 59 respondents (54%) had a cross-infection policy in their dental clinics, and of those dental clinics with no existing policy, 8% intended to implement one in the future. While detailed information about the type of disinfectant and the exact disinfection procedure used at each clinic was not obtained from the questionnaires, the clinicians reported using glutaraldehyde, sodium hypochlorite, electrolyzed oxidizing water, and electrolyzed strong acid water as disinfectants. As a cross-infection policy, 3 practitioners reported that they only rinsed the impressions thoroughly under running water without using any disinfectants.

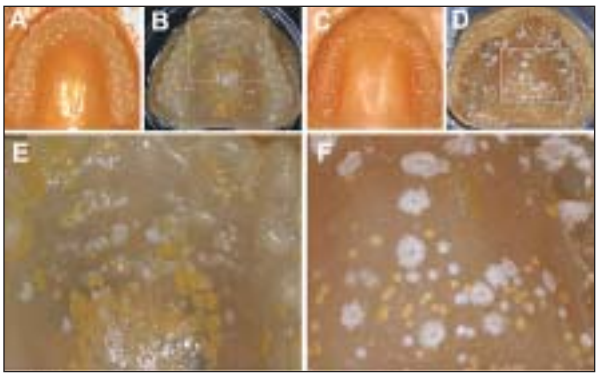
The responses to the questions concerning the dentists' awareness of the persistent presence of microorganisms on dental impressions and gypsum casts are given in Table 1. More than 68% of the respondents believe that caries/periodontitis-associated bacteria, hepatitis viruses, and *Candida* persistently exist on the dental impressions after removal from the patient's mouth. Approximately 40% of the respondents were aware of the persistent presence of MRSA and *P aeruginosa* bacteriae on impressions. As with the gypsum casts, approximately 50% of the surveyed clinicians were aware of contamination with caries/periodontitis-associated bacteria, hepatitis viruses, and *Candida*. Only 30% of the surveyed clinicians believe that MRSA and *P aeruginosa* bacteriae exist on the gypsum casts.

Visualization of Microbial Contamination on Impressions and Gypsum Casts

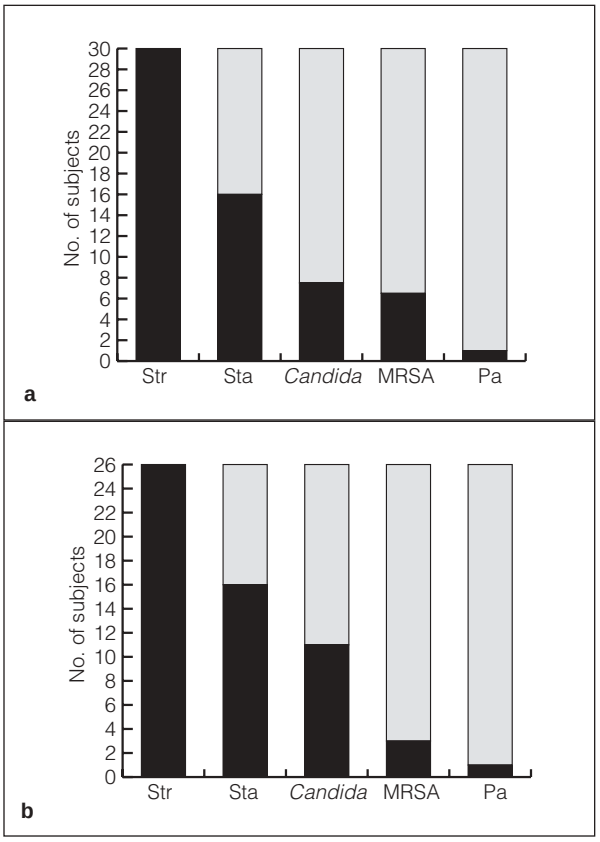
The use of BHI impression/imprint culture detection methods allowed a large number of obvious colonies, grown on all of the samples of the alginate impressions and on the gypsum casts, to be investigated (Figs 2a to 2f). These colonies were predominantly distributed over the areas of the palate and dental arch, and they varied in color, size, and form (Figs 2e and 2f). In contrast, no live colonies were observed in 5 of the BHI impression/imprint cultures of negative controls from sterilized typodonts (Figs 2a and 2c), thus indicating the adequacy of the sterilization procedures.



Figs 1a to 1e Representative photographs of positive colonies on **(a)** Mitis-Salivarius Agar (streptococci), **(b)** Mannitol Salt Agar (staphylococci), **(c)** *Candida* GE Agar *(Candida),* **(d)** OPAII *Staphylococcus* Agar (MRSA), and **(e)** PASA medium *(P aeruginosa)* detected from patient-derived impression samples.



Figs 2a to 2f Representative photographs of the impression cultures **(a and b)** and imprint cultures **(c and d)** using BHI agar to visualize microbial contamination on the surface of alginate impressions and gypsum casts. As negative controls, the impression cultures **(a)** and imprint cultures **(c)** were carried out using sterilized typodonts instead of human subjects. **(e and f)** Magnified images of the microbial colonies on the impression culture **(e)** and imprint culture **(f)**.



Figs 3a and 3b The detection of streptococci (Str), staphylococci (Sta), *Candida,* MRSA, and *P aeruginosa* (Pa) from 30 impression samples **(a)** and 26 gypsum cast samples **(b).** Positive *(black bars)* and negative *(gray bars)* subjects for each of the microbes are indicated.

Potential Persistence of Pathogenic Microbes on Impressions and Gypsum Casts

Selective agar culture demonstrated that streptococci colonies were detected on both the impressions (Fig 3a) and gypsum casts (Fig 3b) from all subjects. Of the 30 impression samples investigated, staphylococci, *Candida*, MRSA, and *P aeruginosa* were detected in 17 (56.7%), 9 (30%), 8 (26.7%), and 2 (6.7%) samples, respectively (Fig 3a). It is significant that these pathogens were also detected on the gypsum casts. Of the 26 gypsum cast samples investigated, 17 (65.4%), 12 (46.2%), 4 (15.4%), and 2 (7.7%) yielded growth of colonies of staphylococci, *Candida*, MRSA, and *P aeruginosa*, respectively (Fig 3b). No positive colony for these microorganisms was detected on these selective agar culture plates for negative control samples of 5 impressions and 5 gypsum casts. This indicated that "background" contamination with these pathogenic microbes was therefore absent in the stone, impression material, and working environment.

Discussion

Considerable variation has been reported regarding the implementation of disinfection procedures for impressions in dental schools and laboratories. Various surveys report that 37.5%[6,22] to 90%[23] of impressions are disinfected in routine cases. The present study's finding that only 54% of the 59 clinicians surveyed had a cross-infection policy in their dental clinics was disappointing in light of the increased societal awareness of the need for caution in handling potentially hazardous biologic materials. Moreover, some respondents (5%) reported that they only rinse impressions thoroughly under water as a disinfection procedure. The rinsing of impressions under water without the use of a disinfectant is not a sufficient procedure for infection control, and thus it should not be recommended.[3] The use of an inadequate disinfection procedure in the handling of dental materials not only places the unwary staff at risk but also results in a high level of avoidable cross-contamination.

The questionnaire revealed a high degree of recognition among the surveyed clinicians that the pathogenic microbes that are causative of the well-known oral diseases, such as dental caries, periodontal diseases, and candidosis, as well as hepatitis viruses, which are of greater risk to dental personnel,[6,24] are frequently retained on dental impressions. It is noted that only 32% to 44% of the surveyed practitioners were aware that the important nosocomial pathogens MRSA and *P aeruginosa* are also retained on impressions and/or gypsum casts. As expected, the surveyed practitioners recognized that gypsum casts tend to be less contaminated with pathogenic microbes compared to dental impressions. Only 54% of the respondents reported an awareness of the possible bacterial contamination of gypsum casts. It is theorized that the low appreciation of the microbial persistence on gypsum casts results from an absence of concrete in vivo data on the carriage of oral microorganisms on gypsum casts. These alarming replies to the questionnaire prompted the investigation of microbial contamination in patient-derived dental impressions and gypsum casts.

Microbial contamination of dental impressions derived from patients has been documented by Samaranayake et al[8] and Sofou et al.[10] They demonstrated contamination by detecting bacterial colonies from a piece of alginate impression sample on tryptic soy agar medium. The present study used impression/imprint culture techniques with BHI agar to determine the microbial contamination on the surface of the impressions/gypsum casts. BHI agar is an enriched nonselective medium used for the isolation and cultivation of a wide variety of bacteria, yeast, and molds.[25] These techniques provide visualization of the bacterial contamination and the distribution on the sample surface. The results demonstrated that all impression and gypsum cast samples were covered with a large number of obvious colonies. These colonies varied in color, size, and form, thus indicating the presence of different types of microbes on the surface. These results indicate that a large number of microbes are retained on impression materials and are viably transferred onto the surface of stone casts. This visual evidence of microbial contamination of impressions and gypsum casts should provide motivation for dental practitioners and their ancillary staff members to take precautions to prevent cross-contamination.

At present, there have only been a few in vivo studies documenting the presence of pathogenic microorganisms on dental materials. One study showed that 12% of modeling compound impressions taken from patients known to have tuberculosis harbored *Mycobacterium tuberculosis*.[26] Powell et al[27] reported that the isolated bacteria on materials transmitted to dental laboratories were predominately alpha-hemolytic streptococci, staphylococci, and different species of Enterobacteriaceae. They also examined the submitted material for presence of viruses but found no positive samples. The present study showed extensive contamination of alginate impressions with oral streptococci and staphylococci. In addition, this study is the first report of the presence of *Candida*, MRSA, and *P aeruginosa* species in the impression samples.

In vitro transfer of microorganisms to dental casts made from impressions of contaminated typodonts has been documented.[11] A study involving the artificial bacterial contamination of dental stone demonstrated no reduction in the level of contamination 4 hours following inoculation.[28] Microorganisms that have artificially contaminated the surface of an impression can be recovered readily from gypsum casts 24 hours following the pouring of the impression.[12] In this study, it was demonstrated that not only oral streptococci and staphylococci were present on the surface of the gypsum casts, but also *Candida*, MRSA, and *P aeruginosa* species. To the authors' knowledge, this report is the first in vivo study documenting the carriage and persistence of pathogens on the surface of patient-derived gypsum casts.

These detected organisms are basically opportunistic pathogens, which are transiently found in the oral cavity. They are important human pathogens that cause a broad spectrum of infections, from the trivial to the life threatening. *Candida* causes a common opportunistic infection known as oral candidosis, which is seen in compromised patients. The oral carriage rate for *Candida* in healthy individuals is approximately 17% when samples are collected on swabs, and the oral carriage rate increases to 47% when the more accurate imprint culture methods are used.[29] *P aeruginosa* is a common nosocomial contaminant, and epidemics have been traced to many items in the hospital environment.[30] MRSA has traditionally been considered a health care–associated pathogen in patients with established risk factors. However, it has emerged in patients without established risk factors and is a serious infection control concern.[13] The most common site of colonization is the anterior nares,[15] but MRSA can also be isolated in other areas, including the oral cavity[16] and throat.[17] Healthy people will generally exhibit no signs or symptoms of infection resulting from the incidental colonization of MRSA. Salam et al[18] investigated the isolation frequencies of opportunistic pathogens in the saliva of elderly Japanese subjects. The isolation frequencies of *P aeruginosa*, MRSA, and *Candida* in the saliva samples were reported to be 1%, 6%, and 17%, respectively. Smith et al[16] reported isolating MRSA from the oral cavity of 37 (6%) of 615 subjects studied. Honma et al[19] reported that the

detected percentage of MRSA from the saliva samples of 166 dental students was 2.3%. Compared to these published studies, the present study shows a higher rate of detection. This discrepancy is likely the result of differences in the methods used for the isolation of the pathogens. In the other studies, the researchers inoculated saliva or swabbed samples directly on the selective agar plates, while in this study, the operators first amplified the number of microbes in the original samples by culturing the organisms in BHI agar culture before inoculating the selective agar plates. As a preliminary study, bacterial samples were collected by swabbing 10 impressions and directly inoculating the swabs on the selective agar media; however, no colonies of MRSA and *P aeruginosa* grew (data not shown). This may suggest that the original number of these pathogens on the impressions is very low and that they are difficult to detect by the usual methods of sampling. Nonetheless, the presence of these potentially persisting pathogens on impressions and gypsum casts creates the risk of transmission to the dental staff and to any other contacts. The possibility exists that further colonization may occur and may result in a serious infection. Further in vivo research is needed to establish an appropriate procedure for the disinfection of impressions and gypsum casts to prevent carriage and reduce the potential for the transmission of pathogenic organisms.

Conclusions

The present study demonstrated the potential persistence of opportunistic pathogens on patient-derived dental impressions and gypsum casts. The isolated pathogens include *Candida*, MRSA, and *P aeruginosa*, all of which can produce nosocomial and/or life-threatening infection in the immunocompromised host. Approximately half of the dental clinics surveyed in Japan did not use an appropriate disinfection procedure for the handling of impressions. Infection control is a dynamic and ever-changing aspect of medical and dental practice. It is imperative that all dental staff be made aware of the most recent information and that procedures be in place to prevent the transmission of infection. The entire dental staff should understand the importance of these infection control procedures and appreciate their necessity. This study confirms the need for dental clinics to use adequate infection control procedures and to prevent the possibility of cross-contamination, resulting in infection by opportunistic pathogens, among patients and dental office and laboratory personnel.

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Literature Abstract

Clinical evaluation of the supraosseous gingivae before and after crown lengthening

The purpose of this study was 3-fold: *(1)* to determine whether transsulcular probing (TSP) accurately and reproducibly defines the supraosseous gingival (SOG) dimension compared to direct bone-level (DBL) measurement at surgery; *(2)* to compare the SOG dimension 6 months after crown lengthening surgery (CLS) to that observed preoperatively; and *(3)* to determine whether the preoperative SOG for a particular tooth can be used to predict the post-crown-lengthening dimension. Nineteen patients (19 to 67 years of age; mean age: 35 ± 13.1 years) underwent CLS with the surgical tooth acting as both the control and the test site. The SOG dimension was measured by TSP before and 6 months after surgery. Stents were used as fixed reference points. DBL, after flap reflection and before and after bone removal was also measured from the stent reference. All measurements were made at 6 sites (midfacial, midlingual/palatal, mesial-facial, mesial-lingual/palatal, distal-facial, distal-lingual/palatal) using a CP-15 UNC SE periodontal probe (with marking differences < 0.2 mm). Intraclass correlations were calculated to test for the reliability of TSP measurements versus DBL measurements. A Wilcoxon signed-rank test was used to compare the mean difference between SOG dimensions at baseline and 6 months after surgery. Intraclass correlation coefficients for TSP measures of SOG to DBL measures of SOG ranged from 83.4% to 91.9% agreement at different sites, with all correlations being highly significant ($P < .001$), indicating a high degree of agreement between TSP and DBL. The differences in SOG dimensions 6 months after surgery compared to baseline were as follows: mean buccal, 0.51 mm; mean lingual/palatal, 0.61 mm; overall mean, 0.56 mm. These differences were significant for all 3 comparisons ($P < .001$, $P < .004$, and $P < .001$, respectively). The change in SOG dimensions interproximally was found to be similar to that at the midfacial sites. The authors concluded that TSP is an accurate alternative to DBL in clinically determining SOG dimensions. Also, there is a statistically significant overall reduction in SOG dimension, ranging from 0.51 to 0.61 mm, 6 months postsurgically compared to the presurgical measurements. The authors also concluded that when subjected to CLS, the postoperative SOG dimension of a particular tooth can be estimated using knowledge of its preoperative measurement, although any tests to prove this statement were not published.

Perez JR, Smukler H, Nunn ME. *J Periodontol* 2007;78:1023–1030. **References:** 29. **Reprints:** Dr José R. Perez, Health Profession Division, College of Dental Medicine, Department of Periodontology, Nova Southeastern University, 3200 S. University Dr, Fort Lauderdale, FL 33328-2018. E-mail: jrperezperio@aol.com—*Tapan N. Koticha, National University of Singapore Faculty of Dentistry, Singapore*