Comparison of the adhesion of *Streptococcus sanguinis* to commonly used dental alloys stratified by gold content

Hung Te Hung a, Dong Qing Ye a*, Chern Hsiung Lai b**

a Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Mei-San Road, Hefe 230032, Anhui, China
b Department of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, San-Ming District, Kaohsiung, Taiwan

Received 8 April 2016; Final revision received 25 July 2016
Available online 9 November 2016

**KEYWORDS**
adhesion; dental prosthetic alloys; gold content; *Streptococcus sanguinis*; surface roughness

**Abstract**  
*Background/purpose:* *Streptococcus sanguinis* is an early colonizer of biofilm and plays a key role in the process of adhesion to prosthetic surfaces by facilitating the adhesion of later colonizers. The main aim of this study was to determine if *S. sanguinis* is affected by the gold concentration dental prosthetic alloys.

**Materials and methods:** Five commonly used alloys with varying degrees of gold concentration were selected for this study. We evaluated the ability of *S. sanguinis* ATCC strain 10556 to adhere to each of these alloys by counting the number of cells that adhered to each of the tested alloys. Each alloy was also assessed for cell adherence using scanning electron microscopy. One-way analysis of variance and Student–Newman–Keuls comparison test were used for statistical analysis based on cell counts from each well for the test and control groups.

**Results:** The highest concentration of bacterial cells adhered best to pure gold alloy (458 ± 8) followed by 88.4% gold Je alloy (382.33 ± 2), 56% gold Wi alloy (269 ± 4), 2% gold Es alloy (212.33 ± 2), and nongold Re alloy (183 ± 3). Based on the cell counts and scanning electron microscopy observations, there was a clear correlation between gold concentration and *S. sanguinis* adherence.

**Conclusion:** The findings of this study suggest that alloys with a lower gold concentration may result in lower bacterial colonization rates and may reduce the risk of invasive infections. When choosing an alloy, low gold concentrations may be a better clinical choice.

Copyright © 2016, Association for Dental Sciences of the Republic of China. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* Corresponding author. Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Mei-San Road, Hefe 230032, Anhui, China.
** Corresponding author. Department of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, San-Ming District, Kaohsiung, Taiwan

E-mail addresses: baohe2359334@yahoo.com.tw (H.T. Hung), ydq@ahmu.edu.cn (D.Q. Ye), chern@kmu.edu.tw (C.H. Lai).
Introduction

Dental plaque is a form of biofilm that contains a number of different species of disease-causing bacteria. They are the main source of the toxins that cause a variety of dental diseases such as dental caries, gingivitis, periodontitis, root canal infections, and peri-implantitis. Biofilm forms on the hard and soft structures in the oral cavity, and the biofilm formation can be classified into the following four phases: (1) adherence to the surfaces of structures, (2) the building up of attachments, (3) absorption, and (4) accumulation.1

Among oral species, Streptococcus sanguinis plays a pioneering role as well as an assisting role in biofilm formation. In 1973, Lacey et al2 first discovered that Streptococcus sanguinis (former species name for S. sanguinis) strain M5 from dental plaque carried hair-like filamentous processes extending up to 55 nm from the surface of the cell wall.

Subsequently, a number of investigations have been published on coaggregation between S. sanguinis and Actinomyces viscosus, Actinomyces naeslundii, Bacterionema matruchotii, and Fusobacterium nucleatum.3–5

In 1983, Lancy et al6 demonstrated that S. sanguinis can combine with B. matruchotii and F. nucleatum to form corncob structures and attach itself to the surfaces of teeth and teeth roots resulting in oral diseases. After S. sanguinis attaches itself to a tooth or the fillings of a tooth, other types of bacteria can then subsequently attach themselves onto S. sanguinis. For example, Streptococcus mutans and Porphyromonas gingivalis, among others, can attach onto S. sanguinis, resulting in tooth decay or periodontal disease.6,7 The attachment of oral bacteria to fillings and teeth surfaces has been shown to be a significant cause of dental diseases.7

The average infection window of S. sanguinis in humans starts in infancy at approximately 9 months. The aggregation of S. sanguinis is closely related to dental growth. After a new tooth has erupted into the oral cavity, the level of S. sanguinis detected in the saliva is significantly higher.8 Approximately 11.4% of the Streptococcus species detected in newborns are S. sanguinis.9

The oral cavity seems to be the main habitat of S. sanguinis. It is the most obvious type of bacteria found in dental plaques and lives most suitably on the flat surfaces of teeth. S. sanguinis can also be isolated feces and can cause up to 31.9% of cases of endocarditis.10

In a clinical setting, dentists also find oral biofilms adherent to dental metal prostheses. We endeavored to assess if the concentration of gold in the dental alloys used in metal dental prosthesis was associated with levels of S. sanguinis adhesion.

Materials and methods

To determine the adherence capability of oral bacterial, the most commonly used precious and nonprecious dental alloys were selected for this study. They included Remanium CS (Re; Dentaurum GmbH & Co., Ispringen, Germany), Esteticor Biennor (Es; CM Dental Cendres & Metaux SA, Biel-Bienne, Switzerland), Williams W (Wi; Ivoclar Vivadent Inc., Amherst, NY, USA.), Jelenko Diamond (Je; Jelenko Dental Alloys, San Diego, CA, USA.), and pure gold (Gd; 9999 gold bar; King Fook Holdings, Hong Kong, China). The precious alloys included Gd with 99.99% of gold, Je with 88.4% of gold, and Wi with 54% of gold. Two nonprecious metals were included in this study: Es with 2% of gold only and Re with no gold content. All of the other metal elements are listed in Table 1.

S. sanguinis ATCC strain 10556 was used for this study to evaluate the adhesion capability to the above precious and nonprecious dental alloys.

S. sanguinis was grown on Brucella Blood Agar plates supplemented with 5% of sheep blood cells in the MACS-MG-500 anaerobic workstation in an atmosphere that contained 85% of nitrogen, 10% of hydrogen, and 5% of carbon dioxide at the temperature of 35°C for 24 hours.11 Standard bacterial suspensions containing 1 × 10^6 cfu/mL in Brain Heart Infusion broth (Difco, Becton, Dickinson and Co., Hunt Valley, MD, USA) were added to each of the 12 wells of tissue culture plates. Each well was placed on one of the alloy disk as described above. Three disks of each precious and nonprecious alloy were used as the test groups. Three wells containing equal concentrations of S. sanguinis cells without alloy disk served as a positive control for microbial cell counts. Each 12-well tissue culture plate was incubated under the same conditions as the blood agar plates for the same 24-hour period.

The number of S. sanguinis cells adhering to each metal plate was determined by the total bacterial cell numbers in the positive control well minus the number of cells in the broth of each tested well with precious or nonprecious alloy disks. After cell counts were determined, the plates remaining in the wells were then processed by three rinses with phosphate-buffered saline (pH 7.2) solution, followed by two fixations with 2% glutaraldehyde fixative solution and five dehydration procedures for scanning electron microscopy (SEM) examinations.

Statistical analysis

A statistical software program (SPSS for Window, version 10.01; SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

One-way analysis of variance (ANOVA) and Student–Newman–Keuls comparison test were used for statistical analysis based on the cell counting from triplicated testing and control groups.

Results

The indirectly measured average counts and standard deviations of the number of S. sanguinis cells that adhered to the alloy in three wells for each of the alloys in order of increasing gold content are shown in Figure 1. There is a clear correlation between gold content and S. sanguinis adherence. One-way ANOVA statistical analysis and comparisons test among five homogeneous subsets of dental alloys was performed using Student–Newman–Keuls (SNK-Q) comparison test (P < 0.005) among the five groups of tested dental alloys demonstrated statistical significance.
Adhesion of *S. sanguinis* to common dental alloys

Table 1  Lists of ingredients and % of metal elements in tested dental alloys.

| Alloys | Au | Pt | In | Zn | Pd | Mn | Ag | Pb | Sn | Ga | Cu | Ru | Ni | Cr | Mo | Si |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Re     | 0  |    |    |    |    |    |    |    |    |    |    |    | 61 | 26 | 11 | 1.5|
| Es     | 2  | 1  |    |    |    |    |    |    | 77.8| 8  | 11 | 0.2|    |    |    |    |
| Wi     | 54 | 1.5| 26.4| 15.5|    |    |    |    | 2.5 |    |    |    |    |    |    |<1.0 |
| Je     | 88.4| 9.5| 0.3 | 0.6 | 1.0|    |    |    |    |    |    |    |    |    |    |    |
| Gd     | 99.99|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Discussion

The actual environment in the oral cavity is complicated, containing more than 700 types of bacteria. Many factors have been reported to affect bacterial adherence including surface roughness, surface characters of the materials, the hydrophobicity of the surface, surface free energy, salivary pellicle, albumin level, oral environment, and metal toxicity.

In the oral cavity, many bacteria can only survive by adhering to hard surfaces or soft tissues. Early colonizers...
(such as S. sanguinis) play a key role in the process of adhesion because they attach directly to the surface and facilitate the adhesion of later colonizers.2

S. sanguinis can adhere to metal in two main ways: specifically or nonspecifically. Specific adhesion means that S. sanguinis can adhere to specific types of adhesion-receiving metals. When S. sanguinis adheres to metal nonspecifically, it usually depends on static or gravity to maintain adhesion. Morris and McBride28 discovered that, for S. sanguinis, two types of adhesion-receiving metals exist in hydroxyapatite after the latter is covered with saliva, and that there are sialic acid lectins on S. sanguinis that link to hydroxyapatite.29 Mergenhagen et al30 discovered that the Actinomyces lectins of S. sanguinis consist of many repeats of hex saccharides, which contain Gal NAc endings. Stinson and Jinks31 proposed that S. sanguinis is specifically linked to saliva. Gong and Herzberg32 asserted that S. sanguinis is apparently 150 kDa and consists of two domains that can adhere to metals. They also claimed that high levels of secretory IgA and a-amylase can be found in S. sanguinis, which is in high concentrations in saliva.33 The adhesion ability of different strains of S. sanguinis varies: those cells that are surrounded by fiber with hair-like structures can adhere relatively better than those with clusters of fiber hair.34

Based on the results of this study, there is a clear association between higher gold content in commonly used dental alloys and the ability of S. sanguinis to adhere to metal alloy surfaces (Tables 1 and 2; Figure 1). Pure gold disks (Gd group) had the highest number of S. sanguinis cells adhering to them. The number of S. sanguinis cells adhering to the other four groups of alloys showed a decreasing tendency as the percentage of gold content decreased—that is, the precious pure gold Gd metal disks accounted for the highest number of S. sanguinis cells (458 ± 8.50) followed by 88.4% gold Je precious alloy (382.33 ± 2.52), 54% gold Wi precious alloy (269 ± 4.00), 2% gold Es nonprecious alloy (212.33 ± 2.52), and no gold Re nonprecious alloy (183 ± 3). On SEM, the surface of pure gold Gd and 88.4% gold Je precious alloy appeared to be quite rough with scratches and uneven surfaces (Figures 5 and 6) when compared with nonprecious Es or Re alloys (Figures 2 and 3). It is also important to note that on SEM, the rougher the surface and the more scratched grooves and pitting were present, the more S. sanguinis cells adhered to the surface (Figure 5).

Also, it is known that metal alloys with higher gold contents are increasingly soft. In the dental laboratory, all dental prosthesis, including crowns, bridges, inlays, and onlays, are polished as smooth as possible prior to being delivered to dental clinics for final restoration in the oral cavities of patients. The appearances of scratches and uneven surfaces may result from polishing processes in the dental laboratory. The harder dental prosthetic materials may have a smoother surface and hence may reduce bacteria adhesion and colonization activities as shown in the results of present study.

In summary, the results of this study show that gold concentration is strongly associated with increasing S. sanguinis adhesion to dental prosthetic metal alloys, which

| Groups   | N  | $\chi \pm s$ | $F$  | $P$  |
|----------|----|--------------|------|------|
| Blank control | 3  | 585 ± 8$a$  | 2361.0 | <0.0001 |
| Re       | 3  | 183 ± 3$a$   |      |      |
| Es       | 3  | 212 ± 3$a$   |      |      |
| Wi       | 3  | 269 ± 4$a$   |      |      |
| Je       | 3  | 458 ± 9$a$   |      |      |
| Gd       | 3  | 585 ± 8$a$   |      |      |

*a Statistically significant differences: post hoc test with Student–Newman–Keuls comparison test ($P < 0.005$) among five groups of tested dental alloys.

S.s. = Streptococcus sanguinis.

*a Analysis of variance: statistically significant differences ($F = 2361.0$, $P < 0.0001$) among five groups of tested dental alloys and blank control.
may serve as a future springboard for adhesion of other pathogenic anaerobic and aerobic bacteria. This may be attributable to the increasing softness of the alloy that gold confers, which can increase rough surfaces with pits and fissures, a beneficial factor for bacterial adhesion. These findings contradict prior impressions that precious alloys are a better choice than nonprecious alloy. The authors suggest that nonprecious dental restorative alloys are a better choice than precious alloys, particularly with the content of gold. Reducing precious alloy use will decrease the cost of manufacturing dental prosthetics with consequent economic advantages, an increasingly important issue given the increasing concerns over the rising cost of healthcare.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

The authors thank the team members in the laboratory of Professor Chern H. Lai (director of the Research & Diagnostic Laboratory for Anaerobic and Oral Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan) for providing excellent technical support and valuable contributions: Cheng Pan Kuo and Wen-Teng Wu. The authors also thank Professor Fa Ming Pan (Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Anhui, China) for assistance with the statistical analysis.

References

1. Watnick P, Kolter R. Minireview: biofilm, city of microbes. J Bacteriol 2000;182:2675–9.
2. Lai C, Listgarten M, Rosan B. Serology of Streptococcus sanguis: localization of antigens with unlabeled antisera. Infect Immun 1973;8:475–81.
3. Cisar JO, Kolenbrander PE, McIntire FC. Specificity of coaggregation reactions between human oral streptococci and strains of Actinomyces viscosus and Actinomyces naeslundii. Infect Immun 1979;24:742–52.
4. Mouton C, Reynolds HS, Gasiecki EA, et al. In vitro adhesion of tufted oral streptococci to Bacterionema matruchotii. Curr Microbiol 1979;3:181–6.
5. Lancy P, Dirienzo JM, Apelbaum B, et al. Corn-cob formation between Fusobacterium nucleatum and Streptococcus sanguis. Infect Immun 1983;40:303–9.
6. Falke WA, Burger BW. Microbial surface interactions: reduction of the hemagglutination activity of the oral bacterium Fusobacterium nucleatum by adsorption with Streptococcus and Bacteroides. Arch Oral Biol 1981;26:1015–25.
7. Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–44.
8. Caufield PW, Dasanayake AP, Li Y, et al. Natural history of Streptococcus sanguinis in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun 2000;68:4018–23.
9. Pearce C, Bowden GH, Evans M, et al. Identification of pioneer viridans streptococci in the oral cavity of human neonates. J Med Microbiol 1995;42:67–72.
10. Douglas CW, Heath J, Hampton KK, et al. Identity of viridans streptococci isolated from cases of infective endocarditis. J Med Microbiol 1993;39:179–82.
11. Lai CH, Listgarten MA, Rosan B. Immuno-electron microscopic identification of Streptococcus sanguis with peroxidase labeled antibody: localization of Streptococcus sanguis in intact dental plaque. Infect Immun 1975;11:200–10.
12. Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005;43:5721–32.
13. Rodriguez-Hernandez AG, Juarez A, Engel E, et al. Streptococcus sanguinis adhesion on titanium rough surface: effect of shot-blasting particles. J Mater Sci Mater Med 2011;22:1913–22.
14. Burgers R, Gerlach T, Hahnel S, et al. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res 2010;21:156–64.
15. Siegrist BE, Brexv MC, Gusberti FA, et al. In vivo human dental plaque formation on different supporting substances: a scanning electron microscopic and bacteriological study. Clin Oral Implants Res 1991;2:38–46.
16. Capopresco S, Cerroni L, Frangini S, et al. Bacterial adhesion to dental alloys. The role of the surface and composition. Minerva Stomatol 1999;48:509–23.
17. Frojd V, Chavez de Paz L, Andersson M, et al. In situ analysis of multispecies biofilm formation on customized titanium surfaces. Mol Oral Microbiol 2011;26:241–52.
18. Satou J, Fukunaga A, Satou N, et al. Streptococcal adherence on various restorative materials. J Dent Res 1998;67:588–91.
19. Satou J, Fukunaga A, Morikawa A, et al. Streptococcal adherence to uncoated and saliva-coated restoratives. J Oral Rehabil 1991;18:421–9.
20. Steinberg D, Sela MN, Klinger A, et al. Adhesion of periodontal bacteria to titanium and titanium alloy powders. Clin Oral Implants Res 1998;9:67–72.
21. Hannig M. Transmission electron microscopy of early plaque formation on dental materials in vivo. Eur J Oral Sci 1999;107:55–64.
22. Kato J. Influence of surface condition upon cytotoxicity of copper–gold alloy. Shika Rikogaku Zasshi 1976;17:63–78.
23. Wataha JC, Hanks CT. Biological effects of palladium and risk of using palladium in dental casting alloys. J Oral Rehabil 1996;23:309–20.
24. Kaga M, Seale NS, Hanawa T, et al. Cytotoxicity of amalgam alloys and their elements and phases. Dent Mater 1991;7:68–72.
25. Gebel T, Lantsch H, Plessow K, et al. Genotoxicity of platinum and palladium compounds in human and bacterial cells. Mutat Res 1997;389:183–90.
26. Buenger J, Staldberg SJ. Cyto- and genotoxic effects of coordination complexes of platinum palladium and rhodium in vitro. Int Arch Occup Environ Health 1996;69:33–8.
27. Wierda D, Pazdernik TL. Toxicity of platinum complexes on hemopoietic precursor cells. J Pharmacol Exp Ther 1979;211:531–8.
28. Morris EJ, McBride BC. Adherence of Streptococcus sanguis to saliva-coated hydroxyapatite: evidence for two binding sites. Infect Immun 1984;43:656–63.
29. Högst AH, Dankert J, Feijen J. Adhesion of Staphylococcus epidermidis and Staphylococcus saprophyticus to hydrophobic biomaterials. J Gen Microbiol 1985;131:2485–91.
30. Mergenhagen SE, Sandberg AL, Chassy BM, et al. Molecular basis of bacterial adhesion in the oral cavity. Rev Infect Dis 1987;9:467–74.
31. Stinson MW, Jinks DC. Adherence of *Streptococcus mutans* and *Streptococcus sanguis* to salivary components bound to glass. *Infect Immun* 1981;32:583–91.

32. Gong K, Herzberg MC. *Streptococcus sanguis* expresses a 150-kilodalton two-domain adhesion: characterization of several independent adhesion epitopes. *Infect Immun* 1997;65:3815–21.

33. Gong K, Mailloux L, Herzberg MC. Salivary film expresses a complex, macromolecular binding site for *Streptococcus sanguis*. *J Biol Chem* 2000;275:8970–4.

34. Wyatt JE, Hesketh LM, Handley PS. Lack of correlation between fibrils, hydrophobicity and adhesion for strains of *Streptococcus sanguis* biotypes I and II. *Microbios* 1987;50:7–15.