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ORIGINAL ARTICLE

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

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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.

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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, Lai et al[2] first discovered that *Streptococcus sanguis* (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. sanguis* and *Actinomyces viscosus*, *Actinomyces naeslundii*, *Bacterionema matruchotii*, and *Fusobacterium nucleatum*.[3–5]

In 1983, Lancy et al[5] 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 prosthesis. 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 allows 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.