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Original Article

Electrochemistry of calcium precipitating bacteria in orthodontic wire

P. Dhandapani [a], M. MuraliKannan [b], B. Anandkumar [c], S. Maruthamuthu [a,*], S.P. Manoharan [a]

[a] Corrrosion Division, CSIR – Central Electrochemical Research Institute, Karaikudi 630 006, India
[b] School of Chemical Engineering & Bioengineering, University of Ulsan, 680 749, South Korea
[c] Department of Biochemistry and Biotechnology, Sourashtra College, Madurai 625 004, India

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ABSTRACT

Background: Calculus composed of inorganic and organic components with bacteria formed on teeth gets deposited on orthodontic wires. The reason for calculus formation and impact of calcium precipitating bacteria (CPB) on orthodontic wire were studied. A pilot study on electrochemical characterization of CPB on orthodontic wires was done.
Methods: CPB were isolated from orthodontic patients and identified by molecular techniques. The electrochemical behavior of two isolates (CPB-1 and CPB-3) on orthodontic wires was studied by employing polarization and impedance techniques. The CPB morphology by scanning electron microscopy and chemical characterization of CPB and tooth pulp stone were studied by Fourier transform infrared (FTIR) and X-ray diffraction (XRD).
Results: The two isolates *Bacillus megaterium* (CPB-1) and *Paenibacillus* sp. (CPB-3) identified with 16S rRNA sequencing method increased pH of B4 medium from 5.32 to 8.3. The carboxylic acid and phosphate groups identified in FTIR analysis acted as nucleation sites for calcium deposition. The biogenic crystal phases identified in teeth pulp stone by XRD were similar to bacterial isolates cultured in the laboratory. The electrochemical studies with two CPB species revealed that biogenic calcium phosphate species act as cathodic inhibitors on orthodontic wire.
Conclusion: The present study concluded that teeth pulp stone formation is due to CPB and high pH determines the mineralization process. Diffusion process and dispersive capacitive behavior indicate that the chloride ions may penetrate through calcium deposits and initiate pitting corrosion on orthodontic wire which may enhance the leaching of toxic elements in saliva.
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1. Introduction

Dental plaque is a complex biofilm consisting of 500 or more microbial species [1,2]. Its mineralization with calcium and inorganic phosphate [P_i] derived from saliva and crevicular fluid results in supragingival and subgingival calculus, respectively [3]. Oral fluids are the major source of nutrients for dental plaque [4]. Understanding of calculus formation and its effects is particularly important for communication with endemic, generalized, periodontal disease associated with early calculus deposition [5]. Calculus is composed of minerals as well as inorganic and organic components along with bacteria on teeth [6]. The calculus formation can be noticed on orthodontic wire and the influence of calculus on orthodontic wire is unknown.

Stainless steel alloys have been used as orthodontic wire with a wide range of applications in both fixed and removable appliances [7]. Orthodontic stainless-steel appliances are considered to be corrosion resistant, but localized corrosion has been observed in the oral cavity [8]. In the oral cavity, factors such as temperature, quantity and quality of saliva, presence of chloride, pH, protein, and physical and chemical properties of food can potentiate conditions favorable to corrosion. The corrosion of dental alloy may be due to oxidation, dissolution, or electrochemical reaction created by oral environmental biofilms [9].

In vitro and *in vivo* experiments have been carried out to monitor the release of metal ions from orthodontic appliances made of stainless steel which can be a source of risk to chromium and nickel leaching [10]. Sfondrini et al. [11] monitored the release of nickel ions from new stainless steel brackets, recycled stainless steel brackets, and nickel-free orthodontic brackets immersed in artificial saliva at different pH values including 4.2, 6.5, and 7.6. Higher chromium ion release from brackets was observed at acidic pH, when compared to neutral conditions. Fixed orthodontic appliance therapy for an average period of 16 months can lead to increased levels of nickel and chromium ion release into the saliva of patients as noted by Amini et al. [12]. Ortiz et al. [13] reported from the toxicity assessment of metal ions released from stainless steel orthodontic brackets that they caused severe damage to

* Corresponding author. Tel.: +91 4565 227770; fax: +91 4565 227779.
E-mail address: biocorrcecri@gmail.com (S. Maruthamuthu).

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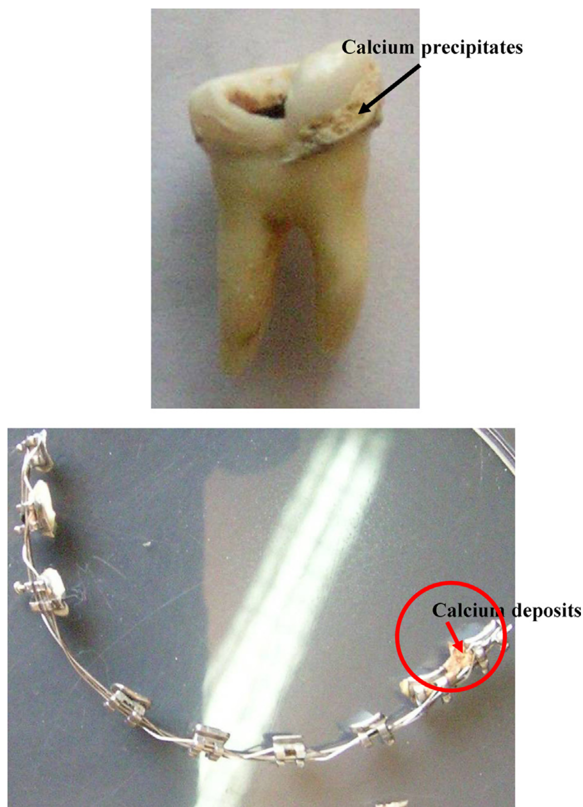

Fig. 1. Teeth pulp stone and orthodontic wire collected from dental hospital.

cellular DNA and concluded that chromium and nickel ions are also genotoxic and cytotoxic, and may also be a cause for the generation of allergic reactions. Moreover, microbial corrosion on orthodontic wire by iron oxidizing bacteria, manganese oxidizing bacteria, acid producing bacteria, and sulphate reducing bacteria were also noticed [14,15], but there is no report available on the influence of calcium precipitating bacteria (CPB) on orthodontic wire.

In the present study, CPB were isolated from orthodontic appliances and identified by 16S RNA gene sequencing. The electrochemical behavior of CPB on stainless steel wire was studied by polarization and impedance techniques.

2. Materials and methods

2.1. Sample collection

Scales on orthodontic wire were collected from Karaikudi Dental Hospital, Karaikudi, India (Fig. 1). The scales on six samples were scraped into sterile containers and used for the microbial characterization. The collected samples were stored in an ice box and transported to Biocorrosion laboratory, CSIR-CECRI, Karaikudi, India for microbiological and electrochemical studies.

2.2. Isolation of bacteria

Scale samples of 0.1 g were removed aseptically and transferred to 9 ml of sterilized 1% peptone water and stirred for 1 h at 37 °C. After 1 h of stirring, the samples were subjected to serial dilution and plated by using B4 medium (Himedia Laboratories, Mumbai, India) consisting of the following ingredients (g/l): calcium acetate 2.5; yeast extract 4.0; glucose 10.0 as described previously [16,17]. The plates were incubated at 37 °C for 2–3 weeks. The total viable bacterial counts were enumerated and the bacterial population was expressed as colony forming units per gram (CFU/g).

2.3. Partial biochemical characterization of the isolates

Morphologically dissimilar dominating isolated colonies were selected randomly, further streaked on B4 medium agar plates, and purified. The pure cultures were maintained in B4 medium agar slants at 4 °C to keep the microbial strain viable. The morphological and biochemical characterization were done to identify the bacterial isolates up to genus level according to *Bergey's Manual of Determinative Bacteriology* [18]. Biochemical characterization of the isolates was carried out by employing Himedia biochemical test kit.

2.4. Molecular identification of bacteria

Genomic DNA of the bacterial isolates was extracted according to Ausubel et al. [19] and amplification of genes encoding small subunit ribosomal RNA was carried out [20]. The amplified product was purified using GFX^{TM} PCR DNA and Gel Band Purification kit (Amersham Biosciences, Chalfont St. Giles, UK) and cloned in pTZ57R/T vector according to the manufacturer's instruction (InsT/Aclone^{TM} PCR Product Cloning Kit, MBI Fermentas, Vilnius, Lithuania), and transformants were selected on LB medium containing ampicillin (100 μg/ml) and X-gal (80 μg/ml). DNA sequencing was carried out using ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). For sequencing reaction, Big Dye Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Waltham, MA, USA) was used.

2.5. Phylogenetic analysis of the isolates

The sequences obtained were analyzed with BLAST search version 2.2.20 [21] and tools of Ribosomal Database Project II Release 10 (http://rdp.cme.msu.edu) for taxonomic hierarchy of the sequences. Multiple sequence alignments were performed using CLUSTAL X2 [22] with a collection of taxonomically-related sequences obtained from the National Center for Biotechnology Information Taxonomy Homepage (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/) and Ribosomal Database Project-II Release 10 [http://rdp.cme.msu.edu]. Phylogenetic and similitude analyses were done with the common 16S rRNA gene regions, and all alignment gaps were treated as missing data. The paired similitude and pairwise distance calculations using the transversion/transition weighting ($R = s/v$) and the Kimura two-parameter model [23] were performed with the MEGA version 4.1 program [24]. The phylogenetic trees were constructed (neighbor-joining method), and 1000 bootstrap replications were carried out to validate internal branches [25]. MatGAT v2.01 software [26] was used to calculate the similitude percentages among sequences.

2.6. Growth rate and pH measurements

The continuous cultures of CPB in B4 medium were used for the growth study. Sterile B4 medium was added once in 3 days to maintain bacterial culture viable. The bacterial growth was evaluated for 20 days by following changes in the optical density (OD) at a wavelength of 620 nm by UV-Visible spectra meter (Model: specord 50 ANALYTIK JENA, Jena, Germany). The pH was also measured with time using a digital pH meter: Model-Eutech instruments pH 510 (pH/mV/°C meter) (Thermo Scientific, Waltham, MA, USA).

2.7. Characterization of biofilm and orthodontic wire surface

2.7.1. Scanning electron microscope analysis

The small stainless steel coupons of size 1 cm × 1 cm were mechanically polished to mirror finish and then subjected to immersion in nitric acid for 5 min at 60 °C in a water bath followed by gentle cleaning with trichloroethylene. These coupons

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