Electrochemical behaviour of microbes on orthodontic wires

S. Maruthamuthu*, A. Rajasekar, S. Sathiyanarayanan, N. Muthukumar and N. Palaniswamy

Corrosion Science and Engineering Division, Central Electrochemical Research Institute, Karaikudi 630 006, India

The aim of the present study is to investigate the electrochemical behaviour of orthodontic wires in artificial saliva with and without bacteria. Four factors were considered to characterize the bacteria present in the saliva, viz. urban and rural groups of vegetarians and non-vegetarians. The results showed that among the heterotrophic bacterial isolates, Gram-positive species dominated over the Gram-negative species in the ratio of 4 : 1. The samples also confirmed the presence of sulphate reducing bacteria. Moreover, chemolithotrophs like manganese and iron oxidizing bacteria and acid producing bacteria were isolated and identified in the saliva. The bacterial strains were isolated and identified based on their morphological, physiological and biochemical characterstics. *Nesseria sicca*, *Eikenella corrodens*, *Pseudomonas* sp., *Escherichia coli*, *Wolinella* sp., *Campylobacter* sp., *Kurthia* sp., *Lactobacillus acidophilus*, *Rothia dentocariosa*, *Streptococcus salivarius*, *Arachnia propionica*, *Stomatococcus mucilogenosus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus roseus*, *Corynebacterium maturochotii*, *Brocothrix* sp., *Listeria* sp., *Staphylococcus* sp., *Gallionella* sp., *Legionella* sp., and *Thiobacillus* sp. were noticed in the saliva. Polarization and impedance studies were carried out for orthodontic wires, investigating the electrochemical behaviour of stainless steel in the presence of microbes. On the basis of electrochemical investigations, it was concluded that NiTi-0.016 and SS-26 gauge were better candidate material for dental applications.

Keywords: Bacteria, corrosion, electrochemical behaviour, orthodontic wires, saliva.

MOUTH is the portal entry of the human body. Saliva has several viruses, bacteria, yeast and fungi and their products, such as organic acids and enzymes, epithelial cells, food debris and components from gingival crevicular fluid[1]. Moreover, saliva is a hypotonic solution[2] containing bioactonate, chloride, potassium, sodium, nitrogenous compounds and proteins. The pH of saliva varies from 5.2 to 7.8. Many Gram-negative and Gram-positive bacterial species form a major part of the dental plaque around the teeth and also colonize the mucosal surfaces. These bacterial dental plaque are responsible for initiating two of the most common human afflictions: caries and periodontal disease. Generally, orthodontic wires are used to correct irregularities in the arrangements of teeth (Figure 1). Stainless alloys have been used as orthodontic wires with a wide range of applications in both the fixed and removable appliances[3]. Corrosion on orthodontic materials may be caused by an electrolyte such as saliva. Factors such as temperature, quantity and quality of saliva, plaque, pH, protein, physical and chemical properties of food and liquids and oral health conditions may influence corrosion[4]. Matasa[5] explained the corrosion of orthodontic appliances which may be uniform, localized or pitting, crevice and intergranular. During the past few years, there has been a broadening of interest in the use of implantable materials, viz. metals, ceramics and polymers, and devices in reconstructive oral surgery[6,7]. Hence, the orthodontic wires were evaluated by employing chemical and mechanical factors by various investigators[8–15].

In India, food habitats are entirely different between the rural and urban population. Though some studies[12–15] have been carried out on electrochemical behaviour of orthodontic wires in artificial saliva by the developed countries, there is no literature available on electrochemical behaviour of orthodontic wires in the presence of microbes. The present study aims to investigate the electrochemical behaviour of orthodontic wires in the presence of dental microbes using a mixed culture of rural and urban people from Tamil Nadu, India.

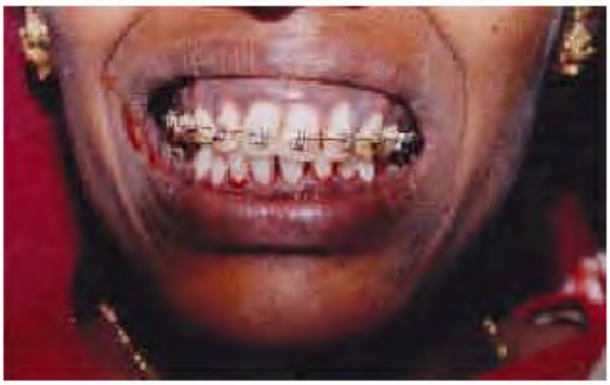

Figure 1. Appliances of orthodontic wires for a rural woman.

*For correspondence. (e-mail: biocorr@cecri.res.in)

Materials and methods

Bacterial enumeration and identification

Samples were collected from four different persons considering two factors, either rural or urban and vegetarian or non-vegetarian, of age group between 15 and 30 years. Samples were collected from the respective persons early morning (6 a.m.) using 5 ml sterile mineral water and a sterile brush in a sterile screw-capped tubes. Uniform timing was maintained for brushing and nature of brushing was also kept uniform. The samples were then stored in a refrigerator at 4°C until further use. One ml of each saliva sample was transferred to 99 ml of sterile distilled water. The samples were serially diluted up to 10^{-10} dilution, using 9 ml sterile distilled water blanks and the samples were plated by pour-plate technique. The nutrient agar (Hi-media) medium was used to enumerate heterotrophic bacteria. Manganese agar and iron agar (Hi-media) were used for iron and manganese depositing/oxidizing bacteria. Thiobacillus agar (Hi-media) was used to isolate the acid-producing bacteria (AP). The heterotrophic bacterial plates were incubated for 24–48 h at 37°C and the plates for chemolithotrophic and AP bacteria were incubated for 48–72 h at 37°C. Duplicate plates were also maintained and counts were made after the incubation. Plates containing 30 to 300 bacterial colonies were selected and total bacterial counts were made. Bacterial populations were expressed as colony forming unit (CFU) per ml of the sample used. API broth (Hi-media) was prepared for cultivation of sulphate reducing bacteria (SRB). Ten ml of the broth was suspended in screw-capped tubes and sterilized by autoclaving at 121°C for 15 min. Three tubes were taken and 1, 0.1 and 0.01 ml of the sample was added to each tube. About 2 to 3 ml of sterile paraffin oil was added to all these tubes to create anaerobic condition. All these tubes were incubated for 28 days at 37°C. After incubation, development of black colouration was noticed, which confirms the growth of SRB due to sulphate reduction.

Morphologically dissimilar colonies were selected randomly from the nutrient agar plates. The well-isolated colonies were purified using appropriate medium employing streaking methods. The pure cultures were maintained in specific slants at 4°C to keep the microbial strain viable. A loopfull of bacterial culture was inoculated for characterization of bacterial strains into sterile nutrient broth and incubated overnight. The fresh overnight broth culture was subjected to the following microscopic, physiological and biochemical tests for their differentiation, characterization and identification. The isolated bacterial cultures were identified up to genus level by their morphological and biochemical characterization, viz. Gram staining, motility, indole, methyl red, Voges-Proskauer test, citrate test, H_2S test, carbohydrate fermentation test, catalase test, oxidase test, starch, gelatin, lipid hydrolysis, etc. according to the key described in Bergey's Manual of Determinative Bacteriology[16–18].

Electrochemical studies

The materials NiTi-0.016, stainless steel (SS) round wire – 0.016 and SS-26 gauge were used for electrochemical evaluation. The appropriate composition of wires could not be got from the manufacturers. The wires were mounted with araldite in the plastic straws, leaving only small fixed area (Table 1) for exposure to the medium; electrical contact was taken from the other end.

The open circuit potential was monitored for a period of 5 h in the presence/absence of bacteria with respect to saturated calomel electrode (SCE) as the reference electrode, using a digital multimeter of high impedance. The specimen were immersed for 12 h at 37°C in sterile as well as mixed bacteria-inoculated artificial saliva. Polarization was done using the above wires. Conventional three-electrode cell assembly was used for polarization measurements. Prosthetic wire specimens as the working electrode, a large area platinum foil as the counter electrode and SCE as the reference electrode respectively, were used for polarization study. Polarization measurements were carried out using a computer-controlled potentiostat (Model PGP 201). Steady-state polarization was carried out from open circuit potential to –1000 (cathodic) and +1000 mV (anodic) at the scan rate of 1 mV/s. I_{corr} values were obtained from the plot of E vs log i. Impedance studies were carried out using computer-controlled EG & G electrochemical impedance analyser (Model M6310) with software M 398. After attainment of a steady state potential, an AC signal of 10 mV amplitude was applied and impedance values were measured for frequencies ranging from 0.01 to 100 kHz. The values of R_{ct} were obtained from the Bode plots.

Results and discussion

Lin *et al.*[19] studied the effect of fluoride concentration on the corrosion behaviour of NiTi orthodontic wires and concluded that passive range and breakdown potential decreased on increasing the sodium fluoride (NaF) concentration, while passive current density increased on increasing NaF concentration. The effect of niobium content on corrosion behaviour of spot-welded dental materials

Table 1. Surface area of orthodontic wires used for electrochemical studies

Wire	Area (cm^2)
NiTi-0.016	1.257×10^{-3}
Round wire SS-0.016	1.422×10^{-3}
SS-26 gauge	1.591×10^{-3}

was studied by Lee *et al.*[20]. It was concluded that performance of dental bracket materials could be improved by addition of niobium. Release of metal ions from the galvanically coupled orthodontic wires has been studied by Ijima *et al.*[21], who suggested that coupling of different alloys in the oral environment accelerates the amount of released metal ions from the alloy. Thompson *et al.*[22] studied the corrosion behaviour of 2205 duplex stainless steel though electrochemical and immersion tests in 37°C with 0.9% sodium chloride solution. They found that SS-2205 had a longer passivation range than SS-316L. The corrosion rate of SS-2205 was 0.416 mpy, whereas for SS-316L it was 0.64 mpy. The effect of temperature changes and frictional forces on phase transformation in orthodontic wires was studied by Lee *et al.*[20] and Park *et al.*[23]. Kao and Huang[24] studied the corrosion behaviour of orthodontic metal brackets at various pH. A comparative evaluation of growth of microorganisms on the surface of various orthodontic wires was made by Uppendar Kumar *et al.*[25]. They suggested that α-hemolytic streptococci was the principle causative organism in decalcification. Two types of microorganisms – SRB (*Bacteriodes corrodens*) and AP (*Streptococcus mutans*) have been discussed with corrosion of dental alloys. However, no study has been carried out on the electrochemical behaviour of microbes and corrosion behaviour of orthodontic wires. In the present study, manganese and iron oxidizers are reported in saliva and electrochemical behaviour of mixed cultures isolated from rural and urban areas has been investigated.

Enumeration

Heterotrophic aerobic bacterial population

The counts of aerobic bacteria were very high in urban non-vegetarian and vegetarian samples, it was in the range between 2.7×10^{13} and 2.1×10^{13} CFU/ml. The aerobic bacterial population in the case of rural non-vegetarian and vegetarian samples was 4×10^{11} and 3.2×10^{10} CFU/ml respectively (Table 2).

Iron oxidizing bacteria

The count was very high in rural non-vegetarian and vegetarian samples, it was 5.2×10^{7} and 6.0×10^{7} CFU/ml respectively, compared to the urban non-vegetarian and vegetarian samples was 3.1×10^{7} and 3.2×10^{6} CFU/ml respectively (Table 2).

Manganese oxidizing bacteria

The counts showed no significant difference between the samples (Table 2). The counts in urban non-vegetarian and vegetarian, and rural non-vegetarian and vegetarian were 3.7×10^{4}, 2.1×10^{4}, 4.8×10^{4} and 1.5×10^{4} CFU/ml respectively.

Acid producing bacteria

The counts were 3.8×10^{3} CFU/ml in urban non-vegetarian sample, 1.2×10^{3} CFU/ml in urban vegetarian sample, 5.9×10^{3} CFU/ml in rural non-vegetarian sample and 4.1×10^{2} CFU/ml in rural vegetarian sample (Table 2).

Enumeration of SRB

SRB counts were 1.2×10^{3}, 1.0×10^{3}, 1.4×10^{3} and 1.1×10^{3} cells/ml in urban non-vegetarian, urban vegetarian, rural non-vegetarian and rural vegetarian samples respectively (Table 2).

Identification of bacterial isolates

Tables 3 and 4 give the results of the bacterial strains isolated and identified from the rural and urban, vegetarian and non-vegetarian samples. Based on their morphological, physiological and biochemical characteristics, 27 strains were identified. *Neisseria sicca*, *Eikenella corrodones*, *Pseudomonas* sp., *Escherichia coli*, *Wolinella* sp., and *Campylobacter* sp., were Gram-negative bacteria. Gram-positive bacteria identified were *Kurthia*, *Lactobacillus acidophilus*, *Rothia dentocariosa*, *Streptococcus salivarius*, *Archnia propionica*, *Stomatoccus mucilogenosus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus roseus*, *Corynebacterium maturochotii*, *Brocothrix*, *Listeria* and *Staphylococcus* sp. Among the heterotrophic bacterial isolates, Gram-positive bacteria were found to be more dominant than Gram-negative bacteria, in the ratio 4 : 1. It can be assumed that the presence of lysozyme enzyme in the saliva has antibiotic characteristics, which may be the reason for the lower number of negative strains. Manganese, iron oxidizing bacteria and AP identified were *Gallionella* sp., *Legionella* sp., *Bacillus* sp., *Pseudomonas* sp. and *Thiobacillus* sp. (Table 5). Gram-negative bacteria dominated over Gram-positive bacteria in the ratio 6 : 1. This distribution of bacterial genera depends upon the salivary flow rate, pH of the saliva, temperature, organic matter, oral hygienicity, plaque, etc.[26].

Electrochemical behaviour of orthodontic wires

Potential measurement

NiTi-0.016: Figure 2 shows the potential vs time behaviour of steel wire immersed in artificial saliva over a period of 5 h. It can be seen that in the case of NiTi-0.016 wire immersed in sterile saliva system, the potential is initially in the order of –200 mV vs SCE and the potential value

Table 2. Distribution of bacterial population in saliva samples

Sample	Total bacterial population (CFU/ml)				
	HB	MOB	IOB	AP	SRB
Urban non-vegetarian	2.7×10^{13}	3.7×10^{4}	3.1×10^{7}	3.8×10^{3}	1.2×10^{3}
Urban vegetarian	2.1×10^{13}	2.1×10^{4}	3.2×10^{6}	1.2×10^{3}	1.0×10^{3}
Rural non-vegetarian	4.0×10^{11}	4.8×10^{4}	5.2×10^{7}	5.9×10^{3}	1.4×10^{3}
Rural vegetarian	3.2×10^{10}	1.5×10^{4}	6.0×10^{7}	4.1×10^{2}	1.1×10^{3}

HB, Heterotropic bacteria; MOB, Manganese oxidizing bacteria; IOB, Iron oxidizing bacteria; AP, Acid producing bacteria; SRB, Sulphate reducing bacteria.

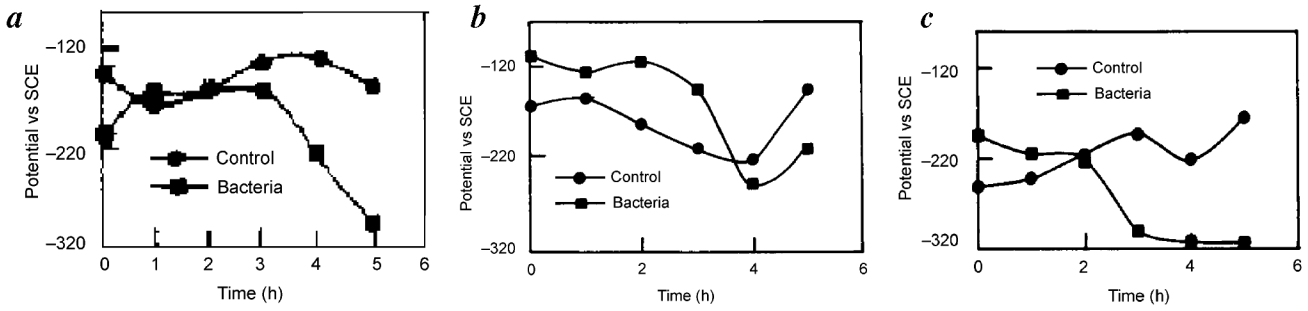

Figure 2. Potential vs time for (*a*) NiTi-0.016; (*b*) round wire SS-0.016 and (*c*) SS-26 gauge.

Table 3. Bacteria identified from urban non-vegetarian/vegetarian samples

Urban non-vegetarian	Urban vegetarian
Rothia dentocariosa (only species)	*Rothia dentocariosa* (only species)
Pseudomonas sp.	*Pseudomonas* sp.
Escherichia	*Escherichia*
Streptococcus	*Streptococcus*
Lactobacillus	*Lactobacillus*

Table 4. Bacteria identified from rural non-vegetarian/vegetarian samples

Rural non-vegetarian	Rural vegetarian
Bacillus sp.	*Kurthia* sp.
Eikenella corrodens (only species)	*Lactobacillus* sp.
Micrococcus sp.	*Rothia dentocariosa* (only species)
Corynebacterium sp.	*Neisseria* sp.
Bacillus sp.	*Streptococci* sp.
	Arachnia propionica (only species)
	Stomatococcus muciloinosus (only species)
	Staphylococcus sp.

gradually shifts to the positive side to about –120 mV with time. In the presence of mixed culture in saliva, the initial potential is –140 mV and the potential value rapidly continues to move to the negative direction throughout the study period. At the end of fifth hour, the potential is more negative by 160 mV, i.e. –300 mV vs SCE.

Round wire SS-0.016: In the case of round wire SS-0.016 (Figure 2 *b*) in the presence of microbes, the initial potential is –116 mV vs SCE. It gradually shifts to the negative side to about –220 mV vs SCE with time. In the absence of microbes, round wire SS-0.016 shows an initial value of –180 mV vs SCE and it slowly goes to the negative side up to the fourth hour, and at the fifth hour it goes to the positive side at the value of –140 mV.

SS-26 gauge: Figure 2 *c* shows the potential time behaviour of SS-26 gauge in presence and absence of microbes in artificial saliva. In the presence of microbes in artificial saliva the potential is –200 mV; it gradually goes to the negative side to about –310 mV. In the absence of microbes, the initial potential is about –250 mV. It gradually goes to the positive side and at the end of fifth hour, the potential is –180 mV.

Polarization study

NiTi-0.016: Figure 3 *a* and *b* shows the anodic and cathodic polarization curve for NiTi-0.016 immersed in artificial saliva with and without microbes. It can be seen that the anodic polarization curve shows higher breakdown potential of about 880 mV in the presence of microbes, whereas the control curve shows lesser breakdown potential of about 280 mV. This indicates that microbes improve the passivity of NiTi-0.016. Besides, it can also be observed that i_p is also lesser in the presence of microbes. The corrosion current obtained from these anodic polarization

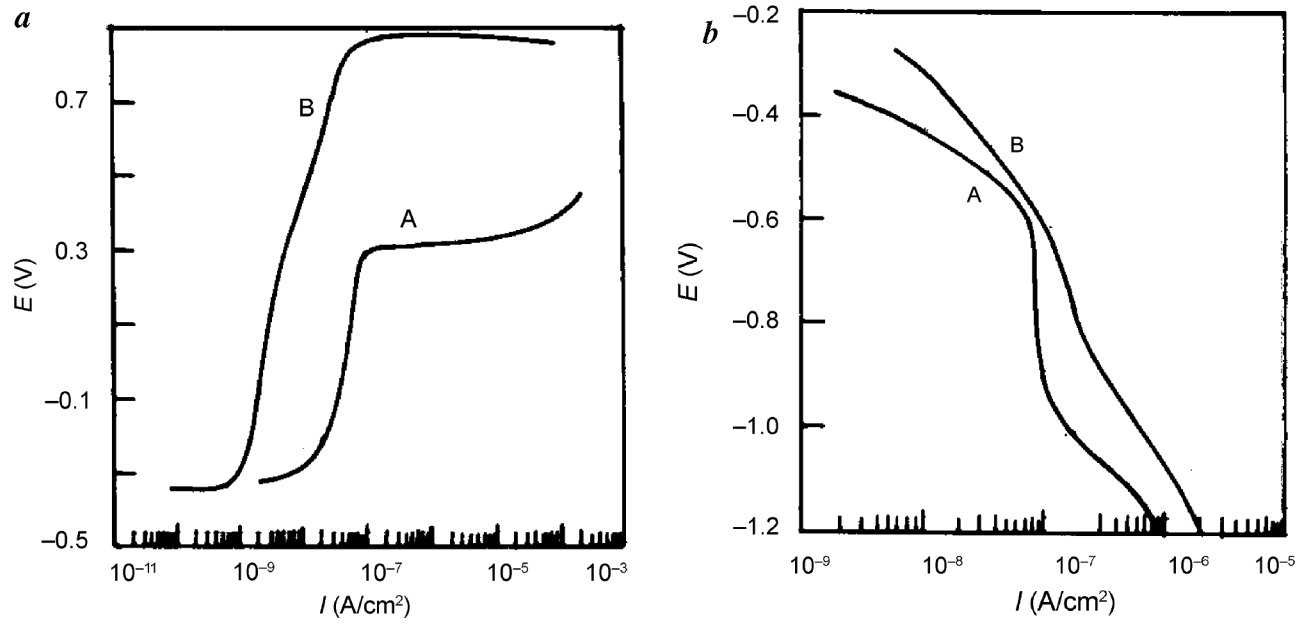

Figure 3. Anodic (*a*) and cathodic (*b*) polarization curves for NiTi-0.016 in presence and absence of microbes in artificial saliva. A, Control; B, Bacteria inoculated.

Table 5. Bacterial species identified from saliva samples

Gram-positive species	Gram-negative species	Manganese oxidizing bacteria/acid producing bacteria
Kurthia	*Nesseria sicca*	*Gallionella* sp.
Lactobacillus acidophilus	*E. corrodens*	*Legionella* sp.
R. dentocariosa	*Pseudomonas*	*Bacillus* sp.
Streptococcus salivarius	*Escherichia coli*	*Pseudomonas* sp.
A. propionica	*Wolinella*	*Thiobacillus* sp.
S. mucilogenosus	*Campylobacter*	
Staphylococcus epidermidis		
Bacillus suffins		
Bacillus cereus		
Micrococcus roseus		
Corynebacterium metaurahotti		
Brocothrix		
Listeria		
Staphylococcus		

Table 6. Anodic polarization characteristics of orthodontic wires in artificial saliva

	Control			Test		
Orthodontic wire	E_{corr} (mV)	b_a (mV/decade)	I_{corr} (A/cm^2)	E_{corr} (mV)	b_a (mV/decade)	I_{corr} (A/cm^2)
NiTi-0.016	−323	33	0.80×10^{-5}	−339	55	7.72×10^{-7}
Round wire SS-0.016	−240	720	6.29×10^{-6}	−430	339	1.93×10^{-5}
SS-26 gauge	−334	High	6.27×10^{-6}	45	358	1.04×10^{-6}

curves is 7.72×10^{-7} and 0.80×10^{-5} A/cm^2 respectively, in the presence and absence of microbes (Table 6). Corrosion current from the cathodic polarization curve for NiTi-0.016 immersed in mixed culture containing artificial saliva is shown in Figure 3 *b*. It can be seen that the polarization behaviour changes drastically in the presence of microbes. The reduction current at various potentials is higher in the presence of microbes. In the control system the limiting current can be clearly seen in the range between 625 and 825 mV, whereas in the presence of

Table 7. Cathodic polarization characteristics of orthodontic wires in artificial saliva

Orthodontic wire	Control			Test		
	E_{corr} (mV)	b_a (mV/decade)	I_{corr} (A/cm^2)	E_{corr} (mV)	b_a (mV/decade)	I_{corr} (A/cm^2)
NiTi-0.016	–357	126	2.05×10^{-6}	–248	271	6.48×10^{-6}
Round wire SS-0.016	–94	286	1.46×10^{-6}	–142	360	1.41×10^{-5}
SS-26 gauge	–203	133	2.29×10^{-7}	36	363	0.93×10^{-6}

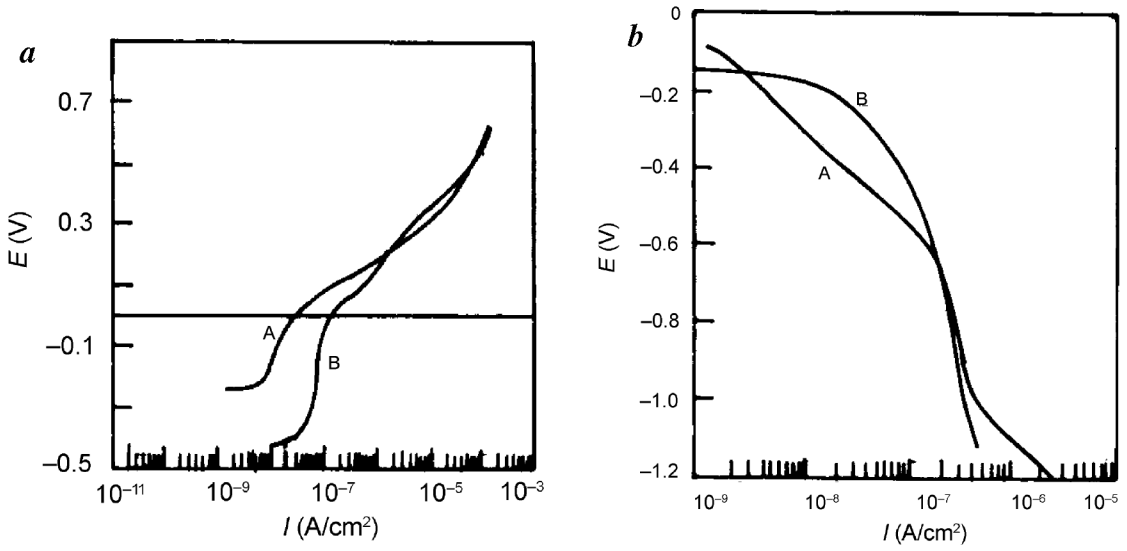

Figure 4. Anodic (*a*) and cathodic (*b*) polarization curves for round wire SS-0.016 in presence and absence of microbes in artificial saliva. A, Control; B, Bacteria inoculated.

microbes it cannot be noticed. The corrosion current from the cathodic polarization curve in the presence and absence of microbes is 2.05×10^{-6} and 6.48×10^{-6} A/cm^2 respectively (Table 7). The reduction of i_p and higher value of breakdown potential indicates the increased passivity of NiTi alloy in the presence of microbes. It is well known that the presence of microbes in the oral cavity leads to fermentation and subsequent accumulation of trace metals from the food. The fermented products may improve the passivity of NiTi alloy, whereas the accumulated cations increase the corrosion current during cathodic reduction. The microbes present in biofilm consortia also produce peroxide and it also determines the cathodic reduction current. From cathodic polarization experiments, potential between –620 and –700 mV indicates increasing current in the presence of microbes. It can be assumed that Cr^{++} reduction may be possible at this potential range and oxygen reduction or biogenic hydrogen peroxide reduction may accelerate the cathodic current.

Round wire SS-0.016: Figure 4 *a* and *b* shows the anodic and cathodic polarization of round wire SS-0.016 in the presence and absence of microbes. The i_p of the material in the presence of microbes is higher than that of the control (see B, Figure 4 *a*). Cathodic polarization shows higher current at –300 mV (see B, Figure 4 *b*), whereas there is no significant difference in limiting current. These curves (anodic and cathodic polarization) reveal that microbes enhance corrosion. The anodic current is 1.93×10^{-5} A/cm^2 in the presence of microbes, whereas in the absence of microbes, the current is 6.29×10^{-6} A/cm^2 (Table 6). The cathodic current is 1.46×10^{-6} and 1.41×10^{-5} A/cm^2 in control and test system respectively (Table 7).

SS-26 gauge: Figure 5 *a* shows that SS-26 gauge has higher breakdown potential in the presence of microbes. In control system, i.e. in the absence of microbes, the breakdown potential is lesser; at the same time the passivation potential range is higher when compared to the experimental system. It is also interesting that limiting current in the control system is higher when compared to the bacterial system at –600 mV (Figure 5 *b*). This reveals that bacteria consume oxygen in the electrolyte, which is reflected in the cathodic polarization curve. The corrosion current from the anodic and cathodic polarization curves is 1.04×10^{-6} and 0.93×10^{-6} A/cm^2 respectively, in the presence of microbes. The current obtained from anodic and cathodic curves is 6.27×10^{-6} and 2.29×10^{-7} A/cm^2

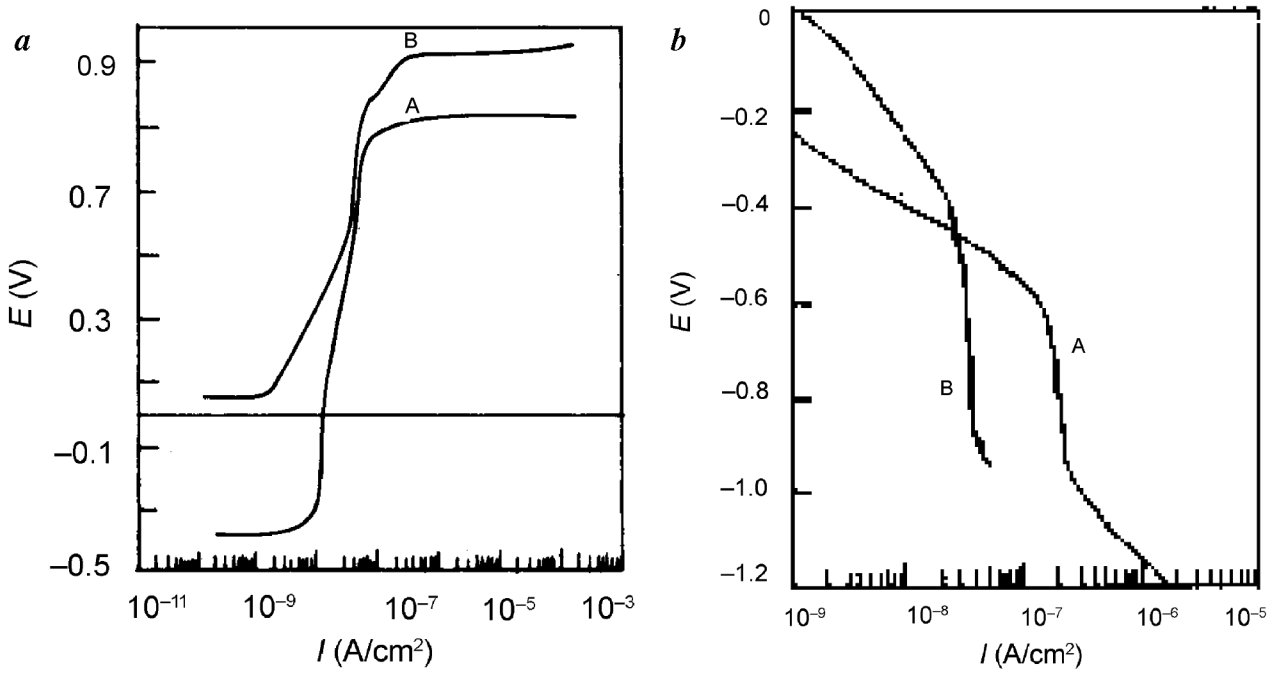

Figure 5. Anodic (*a*) and cathodic (*b*) polarization curves for round wire SS-26 gauge in presence and absence of microbes in artificial saliva. A, Control; B, Bacteria inoculated.

Table 8. Impedance parameter for orthodontic wires in artificial saliva

	R_{ct} (kΩ cm^2)		C_{dl} (Faraday)	
Orthodontic wire	Control	Test	Control	Test
NiTi-0.016	1.63	7.04	5.17×10^{-7}	3.14×10^{-7}
Round wire SS-0.016	0.74	0.43	3.17×10^{-6}	4.43×10^{-6}
SS-26 gauge	0.65	6.59	2.92×10^{-6}	2.79×10^{-7}

respectively, in the absence of microbes (Tables 6 and 7). The nature of the curves suggests that bacteria do not affect the material significantly where it improves the passivity of SS-26 gauge.

Impedance spectroscopy

The bode plots for control and experimental systems for NiTi-0.016 are given in Figure 6 *a* and the impedance data are summarized in Table 8. It can be seen that R_{ct} value for NiTi-0.016 is lower (1.63 kΩ cm^2) in control system compared to experimental system (7.04 kΩ cm^2), i.e. in the presence of microbes (Figure 6 *a*). There is no significant variation in resistant values of round wire SS-0.016 (control: 0.74 kΩ cm^2; test: 0.43 kΩ cm^2; Figure 6 *b*). R_{ct} value for SS-26 gauge is 0.65 kΩ cm^2 for control, while it is 6.59 kΩ cm^2 for the experimental system (Figure 6 *c*). Impedance data support the results observed from the polarization data; so it can be assumed that metabolic activity of microbes may influence the resistance of the oxide film[27]. Table 8 shows the interfacial double layer capacitance C_{dl} values for various materials in the presence and absence of microbes. NiTi-0.016 and SS-26 gauge have capacitance of 3.14×10^{-7} and 2.79×10^{-7} respectively, in the presence of microbes, which is lesser than that of the control. Besides, C_{dl} value is slightly higher in the experimental system than control in round wire SS-0.016. It can be assumed that negatively charged microbes may get adsorbed on the passive film of stainless alloys and reduce the capacitance of the oxide film. Adsorption may enhance the passivity of the stainless alloys, which supports the resistance of the materials observed in the impedance technique. Maruthamuthu *et al.*[27] also noticed lower capacitance values in the biofilmed samples compared to the control. This result may be explained in terms of the production of anions by bacterial metabolism, which could reduce the donor concentration of the oxide film by neutralization with excess cations present in the oxide film of stainless alloys. Christopher *et al.*[26] also made an electrochemical impedance study and noticed passivation in the presence and absence of dissolved oxygen with lactic acid. They suggested that chlorides are the most aggressive ions when compared to other constituents of the solution in the corrosion behaviour.

Microbial corrosion in orthodontic wires

The large surface area provided by the tooth surface along with the orthodontic wire and accumulation of food debris provide favourable condition for growth of biofilm

on the surface[28]. A complex mechanism (Figure 7) of interaction occurs between the chemoorganotrophs and chemolithotrophs existing in the various zones, i.e. aerobic, facultative and anaerobic, favouring the corrosion process. Aerobic bacteria utilize the simple sugars, enter into glycolysis and TCA cycle releasing carbon dioxide[29]. The facultative chemoorganotrophs enter into the fermentative pathway utilizing the simple sugars to produce organic acids, alcohols and CO_2. The formation of organic acids reduces pH, thus favouring corrosion. Chemorganotrophs in the anaerobic zone, viz. SRB, utilize the lactate as carbon source and reduce sulphate to sulphide. Sulphide combines with iron to form ferrous sulphide as the corrosion product. The sulphide produced by SRB enters into the interface of the anaerobic and facultative zones, where it gets oxidized by sulphate oxidizing bacteria to sulphate. Sulphuric acid is also formed which reduces the pH, and favours the decalcification of teeth and corrosion of metallic implants, because of its corrosive nature. The low pH creates favourable environment for aerobic chemolithotrophs like manganese and iron oxidizing bacteria[30]. These microbes oxidize manganese and iron and the reaction products, viz. MnO_2, FeO, Fe_2O_3, $MnCl_2$, $FeCl_3$ favour further corrosion of orthodontic wires. Due to deposition of the biofilm, the metal surface beneath the biofilm and the other areas are exposed to different amounts of oxygen, which leads to differential aeration. The less-aerated zone acts as an anode, which undergoes corrosion, releasing metal ions into the saliva. These metal ions combine with the end-products of the bacteria, along with the chloride ion in the electrolyte (saliva) to form more corrosive products like ferric chloride ($FeCl_3$), manganese chloride ($MnCl_2$), etc. favouring further corrosion (Figure 7). This leads to metal leaching, release of nickel and chromium into the body. Moreover, the corrosive products also cause decalcification of teeth[28,31], dental caries and soft-tissue damage, which leads to secondary infection. Nickel and chromium induce type-IV hypersensitivity reaction in the body, and act as hapten, carcinogen and mutagen. Man-

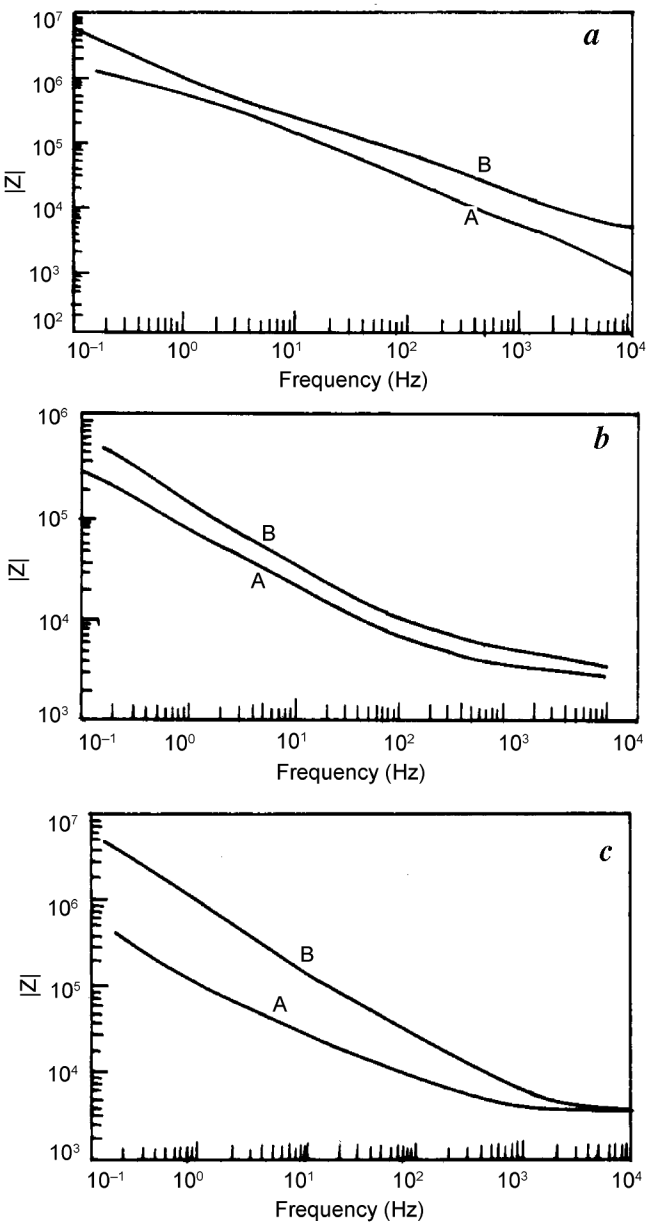

Figure 6. Impedance spectroscopy for NiTi-0.016 (*a*), round wires SS-0.016 (*b*) and SS-26 gauge (*c*).

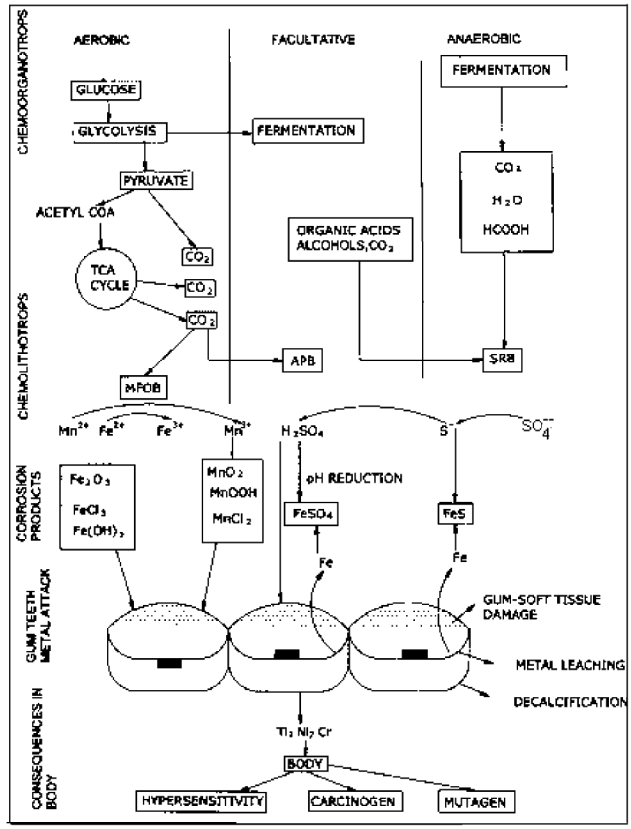

Figure 7. Mechanism of biocorrosion on orthodontic wires in oral cavity.

ganese from the alloy is also consumed with the saliva, which produces toxicity leading to nervous, skeletal disorders, etc. Hence, awareness about corrosion of orthodontic wires in the oral environment is needed, where the microbes have a significant role in the corrosion process. On the basis of polarization and impedance studies, the following conclusions can be made.

(1) Bacteria improve the passivity of some stainless alloys, viz. NiTi-0.016 and SS-26 gauge. Brushing and attachment of microbes on wire may disturb the passivity of passive metals.
(2) Bacteria slightly reduce the resistance and increase the corrosion current for round wire SS-0.016.
(3) The present results indicate that NiTi-0.016 and SS-26 gauge are better for dental applications. Since material compositions are not presented here, an intensive study is needed to correlate the metal composition with bacterial activity.
(4) Leaching of manganese, chromium, nickel and iron from the wires may be due to the availability of manganese oxidizers, iron oxidizers and heterotrophic bacteria in the saliva. Further explorations are needed on the relationship between orthodontic wires and distribution and activity of manganese and iron oxidizers.

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