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Application of the thin electrolyte layer technique to corrosion testing of dental materials.

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APPLICATION OF THE THIN ELECTROLYTE LAYER TECHNIQUE TO CORROSION TESTING OF DENTAL MATERIALS

by

MARTIN LEDVINA

A DISSERTATION

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Proper simulation of the oral environment for the corrosion testing of dental materials is crucial for determining corrosion rates and mechanisms. In this study, the thin electrolyte layer technique (TET) was characterized and employed to investigate the importance of the chemical composition of the testing environment on the outcome of electrochemical tests. The thickness of the electrolyte layer in TET is only 0.5 mm and contains only 20 µl of electrolyte. This arrangement simulates the physical characteristics of the oral environment and facilitates testing in human saliva. Oxygen availability for reduction on the sample surface was determined, using cathodic polarization of Pt in borate buffer, to be lower in TET than in traditional (bulk electrolyte) techniques. Appreciable differences were found during polarization experiments on 316 L SS in saline and artificial saliva. Oxygen content was found to play a significant role in the corrosivity of various species contained in artificial saliva. Potentiodynamic polarization employing human saliva in TET on 316L SS proved to be very different from tests performed in artificial saliva. This was believed to be due to the presence of organic species, specifically proteins, contained in human saliva. This was further confirmed by cyclic polarization and corrosion current measurements of four commercial nickel-chromium (NiCr) alloys with varying amounts of Be. For this phase of the experiment, artificial saliva (AS), AS with 1% albumin, AS with 1% of mucin, and

parotid human saliva were employed as electrolytes. The results obtained in the various electrolytes depended on the composition, microstructure, stability of passive film, and presence of casting porosity of the alloys tested. Proteins had insignificant effect on alloys with highly stable passive films, whereas corrosion rates increased substantially in those alloys with compromised passive film formation. Proteins, especially mucin, lowered the activity of pores and seemed to produce an inhibitive action against localized corrosion. The same trends were observed in human saliva. To clarify the mechanisms of protein-surface interaction, electrochemical impedance spectroscopy (EIS) was employed with the same alloy-electrolyte combinations. Based on the results, it was hypothesized that proteins are adsorbed to the anodic areas where pits may be forming or casting porosity exists. The electrostatic interaction and affinity of proteins for metallic ions plays a significant role. The absorbed macromolecules physically block transport of reactants to and from the interface and slow down the corrosion reaction appreciably. Overall, this investigation contributed to the further understanding of the electrochemistry of the oral environment, particularly the contribution of proteinaceous species.

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INTRODUCTION

The success of a material to be used in the human body depends on many factors. When a new material for such an application is being developed, one of the major issues is the reaction of the human organism to this element. Biocompatibility studies of materials involves testing of toxicity, *in vitro* and *in vivo* release of potentially harmful species, reaction of different types of cells on the presence of such species, and an understanding of how the physical and chemical surface character may influence the reaction of the living system upon initial of insertion of the material into the body.

One of the most common ways to test metallic biomaterials for release of ionic species is electrochemical analysis. Ideally, the studies should be carried out in the same environment in which the material is projected to function. However, since most of the time this approach is problematic, researchers use simulated *in vitro* conditions for testing. In such cases, proper modeling of the *in vivo* state becomes an important issue. In the field of dental materials, simulation of the oral environment presents a challenge not only because of complex salivary chemistry but also because of the physical character of the saliva layer.

Development of new materials with the aim to improve their function and ease of manufacturing is a continuous effort. Sometimes improvement of one property compromises performance of material in another aspect. This situation applies to nickel-chromium dental casting alloys where beryllium has been added to improve casting properties but has compromised the environmental stability of these materials.

In this study, modeling of the oral environment will be evaluated and a novel technique for electrochemical testing of dental materials will be introduced and characterized. Furthermore, this technique will be used to study the influence salivary species have on the mechanism of corrosion of nickel-chromium alloys.

Simulation of the Oral Environment

When evaluating the biocompatibility of a material, one of the most important factors is correctly estimating the release of potentially harmful species to the human body. In addition, by understanding the mechanism of such release, the behavior of the material can be modified by changing production methods. Ideally, all biocompatibility related tests should be carried out in the same environment as the intended application, *in vivo*. However, such testing is usually complicated, and studies are, therefore, for the vast majority, performed under simulated, *in vitro* conditions. Because the results of biocompatibility studies strongly depend on the testing environment, it becomes important to choose one that simulates the *in vivo* conditions as closely as possible. The oral environment is very complex. Saliva contains a variety of inorganic and organic compounds[1]. The composition is also individual from person to person and is influenced by diet, time of day, physical condition of the individual and many other factors[1, 2].

Traditionally, corrosion tests of dental materials have been performed in electrochemical cells that use relatively large volumes of electrolyte. Due to the difficulties associated with problematic collection of saliva and its instability, synthetic solutions have been developed[3]. Substantial effort has been devoted to development of such electrolytes to simulate the complex electrochemistry of human saliva as closely as possible[3-7]. In spite of the relative complexity of these solutions, the few studies that compared results obtained in

both artificial and natural electrolytes continue to show significant differences in corrosion behavior of some dental materials[8-10]. These differences could be attributed to the organic species missing in artificial saliva formulations[9].

The oral cavity environment also possesses specific physical characteristics that have not been addressed before. Saliva is present on the tooth surface as a film approximately 0.1 mm thick[11]. In steady state conditions, salivary flow has been reported to be approximately 0.5 ml/min. This corresponds to a linear velocity of 0.8 to 8 mm/min[12, 13]. The movement of the interface layer is further restrained by adsorption of salivary proteins onto the surface. This implies the presence of a relatively still, thin film of saliva on the tooth superstructure.

Thin films of electrolyte are known to exhibit an appreciably different electrochemical behavior compared to bulk electrolytes. It was discovered that the corrosion rate and corrosion potential of iron and steel change appreciably when decreasing the thickness of the electrolyte layer on the specimen from 1 to 0 mm[14]. Furthermore, it has been reported that the cathodic polarization curves on a platinum electrode and AC impedance data of iron varied significantly with differing thicknesses of the electrolyte from 10 to 1000 μm[15]. Mass transport due to convection seems to be difficult in a thin electrolyte layer, and, subsequently, the amount of oxygen available on the material's surface under a thin film is expected to be different than in a bulk electrolyte. It was previously shown that the concentration of oxygen does significantly affect the corrosion behavior of surfaces of dental materials[16].

Considering the above, it can be speculated that not only the chemistry of the environment but also the thickness of the electrolyte layer are important factors in the oral environment simulation for *in vitro* testing of dental materials. Therefore, it would be

beneficial to employ a technique requiring a lower volume of electrolyte, enabling the use of human saliva and artificial solutions with protein content as a thin layer of electrolyte.

A suitable technique was developed at IBM's T.J. Watson Research Center. The thin electrolyte layer technique (TET) has been used, with an advantage, for electrochemical measurements in electrolytes of low conductivity[17, 18]. The TET employs a unique design of sample-electrolyte arrangement[17-20]. Even though the technique has been used in several corrosion studies, detailed comparison of TET to bulk electrolyte techniques has not been performed.

Effects of Salivary Proteins

Use of human saliva in corrosion testing of dental materials and investigating the effects of organic constituents on saliva electrochemistry has been limited due to the large variety of substances present and the lack of a suitable low volume technique. As a result, the number of studies in this area has been small. Polarization behavior of several dental amalgams has been examined in human saliva and Ringer's solution[10]. Differences between the results obtained in the respective electrolytes were discovered to depend on the type of amalgam. The addition of albumin and mucin to Ringer's solution appeared to cause similar results to human saliva[21]. It was suspected that proteins do not take part in the electrochemical reactions. Rather, by their adsorption to the surface the kinetics of the reactions is slowed down. In this study, various possibilities for the adsorption mechanisms were discussed but no specific conclusion was made. The role of the individual salivary constituents on its electrochemical behavior was reviewed and the knowledge of protein influences was concluded to be far from complete[9].

More data, however, are available on corrosion effects of serum proteins on orthopedic biomaterials. It was found that serum albumin does not affect passivation of nickel[22]. However, the presence of serum proteins was reported to influence the primary passivation mechanism of 316L stainless steel[23]. The effect of serum proteins on corrosion rates of various pure metals was also investigated[24]. It was reported that the corrosion rate of aluminum and titanium exhibited no significant change due to the presence of proteins in the electrolyte. The rate of chromium and nickel release increased, while that of molybdenum substantially decreased when compared to an environment without proteins. Another investigation indicated that the corrosion products of 316L SS produced by saline with serum additions were organometallic in character[25]. Furthermore, the proteinaceous species were shown to have different binding strengths to chromium and nickel[26].

Previously, it was hypothesized that adsorption of proteins onto a metallic surface is a significant factor in the electrochemistry of protein-surface interaction. However, the importance of the different mechanisms was not addressed[21]. Protein adsorption and binding strength was demonstrated to depend on the metal in question[27]. Recently, the adsorption of salivary proteins was investigated in connection with the formation of dental plaque on implant surfaces. Both hydrophobic and hydrophilic mechanisms of binding were reported to be significant on model surfaces of silicon[28]. Albumin was found to be the most prevalent protein adsorbed onto titanium powder[29].

Another important mechanism of protein binding was shown to be based on electrostatic interaction. Salivary mucins are known to contain side-chains often ending with sialic acid residue[2]. The carboxyl group of sialic acid is fully ionized at physiological pH values and confers a strong negative charge on the macromolecule. Therefore, electrostatic

attraction involving these species and positively charged surface areas can be possible. This was reported for hydroxylapatite[30, 31]. Furthermore, the adsorption of salivary mucin was found dependent on the pH of the environment[32]. However, such mechanisms have not been discussed for metallic surfaces.

Some dental materials, like nickel-chromium casting alloys, possess heterogenous microstructures[33] and, therefore, can give rise to a nonuniform surface charge distribution when exposed to a conductive solution. It is conceivable that such a situation could cause preferential adsorption of proteinaceous species. This would in effect influence the electrochemical behavior of such surfaces.

Nickel-Chromium Dental Casting Alloys

Nickel-chromium (NiCr) casting alloys have been developed as a substitute for precious alloys and used in crown and bridge restorative dentistry[34]. Because of the less noble nature of these alloys and, therefore, possible reactivity concerns in nickel-sensitive patients[35], their corrosion properties have been studied extensively by a variety of methods. For example, in one study, 12 commercial NiCr alloys were compared based on anodic and cathodic polarization in an artificial saliva solution[36]. The potentiostatic deaeration method that enables the measurement of small corrosion currents generated by a NiCr alloy was applied[37]. Later, this experimental technique was extended to a variety of NiCr alloys[38].

Beryllium was introduced to the NiCr alloy systems in an effort to improve its casting properties[39]. Beryllium additions resulted in the formation of a NiBe eutectic phase embedded in the NiCr matrix[33, 40, 41]. This phase, however, was found to detrimentally influence the corrosion resistance of the alloy beyond minimum additions of Be[40]. When

examining the surface properties, it was shown that the eutectic surface film contained lower oxygen and chromium concentrations and was therefore concluded to be less protective[33]. The influence of Be on the corrosion resistance of Ni-based experimental alloys with 25% Cr, 10% Mo, and Be content ranging from 0 to 2.1% was studied by potentiodynamic polarization and impedance spectroscopy in artificial saliva at 25°C[40]. Microstructural characterization revealed increasing amounts of the NiBe eutectic with increasing Be content. It was reported that alloys with more than 0.6% Be were highly susceptible to localized corrosion. All of the reviewed studies were carried out in artificial saliva solutions.

Based on the available information, it could be hypothesized that the electrochemical activity of NiCr alloy surfaces would be significantly altered by the addition of albumin and mucin to artificial saliva. Furthermore, it can expected that these effects will be influenced by the microstructure of these systems.

Specific Hypotheses

Proper simulation of the oral environment could be a function of both the chemistry and physical conditions. Therefore, it is of interest to apply and characterize an electrochemical technique that would enable the use of human saliva and proteinaceous solutions in small volumes, present as a thin film on the sample surface. This approach would make the investigation of protein effects on the corrosion behavior of dental materials more accessible. Also, using electrochemical techniques, it would be possible to gain insight into the mechanisms involved in the corrosion processes influenced by protein-surface interactions. Nickel-chromium alloys, due to the variability of microstructure and therefore

surface properties within the same class of dental materials, are suitable and realistic models to be used in this investigation.

Based on the literature, the following hypotheses for this research were postulated.

1. Electrochemistry of a dental material surface will be significantly different under a thin film of electrolyte, as opposed to bulk solutions. This will be, for the most part, due to a different amount of oxygen available on the sample surface in both techniques.

2. The results obtained in human saliva will be different from those obtained in artificial solutions. The differences will be attributed to the organic, mainly proteinaceous, content of saliva. Furthermore, the variances will most likely be different from alloy to alloy.

3. Different protein species will affect the surface electrochemistry differently.

4. The magnitude of protein activity will depend not only on the surface chemistry but also on the microstructure and, in effect, on the surface charge distribution.

Experimental Approach

The TET represents a method which will alleviate most problems formerly associated with the use of saliva in corrosion experiments. Only 20 µl of testing solution is required in TET testing. Previous investigators encountered discontinuous current transients in electrochemical testing in saliva, probably due to the blocking of ionic flow through a salt bridge by proteinous sediment[10]. The TET does not use a salt bridge and the electrode is 0.5 mm from the sample surface. This arrangement prevents the problems faced previously.

The first goal of this study was to characterize the TET technique. It has been noted that reduction of oxygen is the rate determining step in the corrosion reaction of dental materials[16]. Therefore, in order to determine the relative oxygen concentration on the sample

surface, cathodic polarization of a platinum electrode in borate buffer (pH 6.8) under both TET and bulk electrolyte conditions (BET) was performed. Open circuit potential (OCP), polarization resistance and potentiodynamic polarization data of 316L stainless steel (SS) obtained in 0.9% saline of differing oxygen content, using both BET and TET, were utilized to further characterize the TET system. Even though 316L SS is not a frequently used dental material anymore, it has been characterized in many cases involving serum proteins[24,25]. Additionally, its relatively homogenous microstructure and passive layer makes 316L SS a good reference material with an expected high reproducibility of results. The TET experiments were run in an enclosed chamber, enabling the maintenance of proper atmosphere, temperature and humidity during testing.

Secondarily, artificial saliva (AS) was employed with both BET and TET, providing information on the corrosivity of the individual species present in AS. The AS employed was reported as having a similar content of inorganic species as human saliva. Its behavior (buffering capacity, pH) was found to be close to that of saliva[3]. In this second segment, human saliva (HS) was employed as an electrolyte with TET. Comparison of the results with those obtained in AS provided information on the effect of organic species contained in HS. In these experiments, the sample was 316L SS. Testing was carried out at 25°C.

The third phase of this investigation employed four commercial nickel-chromium (NiCr) dental casting alloys (Neptune, Rexalloy, Regalloy T, and Vera Bond). The surfaces of these alloys have been characterized previously using cyclic polarization and Auger spectroscopy[33]. Samples for this investigation were cast in a commercial lab according to the manufacturer's specifications and exhibited differing amounts of casting porosity. Four different electrolytes were used: AS, AS with 1% of albumin, AS with 1% of mucin, and HS.

Cyclic polarization profiles were recorded for each alloy-electrolyte combination under the TET conditions. This data together with corrosion current values provided information for evaluating the individual protein influences, as well as for comparing human and artificial saliva electrolytes.

In the final component of this study, electrochemical impedance spectroscopy (EIS) was carried out in order to support the hypotheses raised by the previous research. Scans were performed at a slight overpotential (vs. OCP) for Regalloy and VeraBond and in the middle of the passive region in Neptune and Rexalloy. The polarization resistance values were determined from the Nyquist plots and the trends compared to the corrosion data obtained by the direct current (DC) techniques. Furthermore, the capacitance of the double layer data together with the phase shift plots provided insight into the mechanism of the protein-surface interaction. Both DC and EIS testing in this phase of the study were performed at 37°C.

THE THIN ELECTROLYTE LAYER APPROACH TO CORROSION TESTING OF DENTAL MATERIALS - CHARACTERIZATION OF THE TECHNIQUE

by

M. LEDVINA AND E.D. RIGNEY

Biomaterials in press

ABSTRACT

An innovative technique for corrosion testing of metallic dental materials is introduced. The thin electrolyte layer technique (TET) simulates the physical characteristics of the oral environment by employing a still, thin layer of an electrolyte, in contrast to bulk electrolyte techniques (BET) which utilize relatively large quantities of fluid. Limiting current density tests on a platinum electrode revealed a lower surface oxygen content for TET. Borate buffer (pH 6.8) was employed as an electrolyte. The effect of lower oxygen content in TET on passivation and polarization characteristics of 316L stainless steel (SS) in 0.9% saline was investigated. The results revealed differences in the polarization resistance and open circuit potential development with time, as well as in anodic and cathodic polarization behavior. Lower O_2 concentration in TET was attributed to different electrolyte convection characteristics under both testing conditions. Additionally, use of the TET resulted in better data reproducibility. Overall, this investigation led to a deeper understanding of the electrochemical processes inherent in thin electrolytes such as those found in the oral environment.

INTRODUCTION

Electrochemical *in vitro* investigation has been a useful tool to assess corrosion properties of metallic dental materials and to compare their stability in the oral environment. As these materials are used in the human body, correct estimation of *in vivo* ionic release rates is important. Consequently, proper modeling of the oral environment in *in vitro* testing is required to obtain results similar to the clinical situation. A close approximation of the *in vivo* conditions is also beneficial when the mechanism of environmental degradation is of

interest. The rationale for this study was based on analysis of the oral environment and comparison of it to the standard testing procedure.

Both the chemical and physical aspects of the oral environment are complex. Saliva is composed of many inorganic and organic compounds[1]. In addition, its composition is unique to each individual and is influenced by a number of variables, including time of day, diet, and physical condition of the individual[1]. However, using natural saliva as the electrolyte for testing has several limitations. Saliva is unstable and difficult to collect in large quantities. To alleviate some of the problems, several synthetic solutions have been developed and used for corrosion testing[2]. In spite of the development of synthetic electrolytes of complex compositions, studies continue to show differences in corrosion behavior between samples tested in natural and artificial saliva solutions[3-5]. A significant need for expanded research in this area has been expressed[4].

From the physical point of view, saliva is present on the tooth surface as a film approximately 0.1 mm thick[6]. In steady state conditions, salivary flow has been reported to be approximateiy 0.5 mL/min. This corresponds to a linear velocity of 0.8 to 8 mm/min[7,8]. The movement of the interface layer is further restrained by adsorption of salivary proteins onto the surface. This implies the presence of a relatively still, thin film of saliva on the tooth superstructure.

Previous studies concentrated on the simulation of the oral environment through changes in artificial saliva composition[2]. To the best of our knowledge, researchers have not investigated the effects of the physical character of the salivary film on the electrochemistry of the oral cavity. The basic physical character of the oral environment was neglected as each case employed relatively large volumes of an electrolyte in electrochemical tests[9]. Thin films

of electrolyte are known to exhibit an appreciably different electrochemical behavior as compared with bulk electrolytes. It was discovered that the corrosion rate and corrosion potential of iron and steel changed when decreasing the thickness of the electrolyte layer on the specimen from 1 to 0 mm[10]. Subsequently, using standard bulk electrolyte techniques may provide inaccurate information in determining the corrosion behavior of metallic dental materials. It would, therefore, be of interest to use a technique in which the electrolyte is present on the sample surface as a thin layer. Determining if and how those conditions affect the electrochemistry of the corrosion processes would also be beneficial.

A suitable technique was developed at IBM's T.J. Watson Research Center. The thin electrolyte layer technique (TET) has been used, with an advantage, for electrochemical measurements in electrolytes of low conductivity[11,12]. The TET employs a different design of sample-electrolyte arrangement compared with the standard (bulk electrolyte) techniques (BET). Even though the technique has been used in several corrosion studies[11-14], detailed comparison of TET and BET has not been performed.

The aim of the first part of this study was to apply the TET to corrosion testing of dental materials and address possible differences in electrochemical processes between TET and BET. Assuming that the TET testing conditions better simulate the oral environment, results of this study shall lead to deeper understanding of the *in vivo* processes. Oxygen is the major reducing species in the electrochemical degradation of dental materials, and its concentration plays a significant role[15]. It was hypothesized that the difference in the physical character of the sample-electrolyte arrangement between the TET and BET could cause a difference in the oxygen supplied to the metal surface. To address the hypothesis, cathodic polarization of a platinum electrode in borate buffer (pH 6.8) was performed. To test the

importance of these differences in practical application, the electrochemical behavior of 316L SS was investigated in electrolytes with different oxygen concentrations.

This goal was approached by examining the open circuit potential and polarization resistance development with time and the cathodic and anodic polarization behaviors of 316L SS. Stainless steel was selected for ease of data interpretation. The simple and homogeneously passivating microstructure of 316L SS eliminates the complexity of corrosion reactions usually associated with multiphase materials such as amalgams or NiCr dental casting alloys. Additionally, the surface behavior of 316L SS in biological environments has been investigated and described in several previous studies[16-18].

MATERIALS AD METHODS

Thin Electrolyte Technique (TET)

The TET setup (*Figure 1*) consists of a sample covered by a PVC masking tape (3M, USA) that exposes a working area of 0.32 cm^2 [11]. Platinum mesh serves as a counter electrode and the reference electrode is the Hg/Hg_2SO_4 electrode. The system is set up in the following manner. A sample is placed on a laboratory jack, and the working area is covered with a circular piece of filter paper the same size as the working area. Platinum mesh is placed over the covered area. The sample is raised toward the center of an opening of a clamped fitting that seals the working area from the area of the specimen covered by the tape. A second piece of filter paper is dropped into the fitting. Twenty microliters of an electrolyte (corresponding to an electrolyte thickness of 0.5 mm) is then injected using a micropipet. The reference electrode is inserted into the fitting, and the system is connected to a potentiostat just before the start of an experiment.

Platinum Polarization

Cathodic polarization of a bright platinum electrode was performed to determine the relative oxygen concentration in TET. Immediately before each experiment, the electrode was polished using 1 μm alumina, cleaned in an ultrasonic bath, washed with methanol, and dried. The BET experiments were performed using borate buffer with three different oxygen concentrations: oxygenated, continuously aerated before and during the experiment (aerated), and aerated just before the test with air (saturated) at 37°C. The buffer was purged with the respective gas for 40 min before the start of an experiment and during the test, except for the saturated condition. Only air saturated electrolyte was employed with TET. Exact oxygen concentrations in the electrolytes were not measured, because that was not necessary for relative measurement using the limiting current densities.

Experiments Using 316L SS

For the experiments on 316L SS, cylindrical samples (diameter 15.5 mm, thickness 3mm) were used. The samples were polished through a series of 120, 240, 360, and 600 grit SiC paper and repolished on 600 grit SiC paper before each test. After grinding, the samples were rinsed in distilled water and methanol, dried in a dry air stream, and immediately tested.

The samples were subjected to a series of consecutive electrochemical tests at 25°C designed to follow the passivation process and provide information on the cathodic and anodic polarization behaviors in electrolytes of different oxygen concentrations. The experimental sequence used was executed in the following order: (1) open circuit potential (OCP) vs. time for a duration of 1 min, (2) polarization resistance, (3) OCP vs. time for 5 min, (4) polarization resistance, (5) OCP vs. time for 5 min, (7) polarization resistance, (8)

OCP vs. time for 1 min, and (9) potentiodynamic polarization (-250 to 500 mV vs. OCP at 1 mV/s). Polarization resistance was performed in the range ±20 mV vs. OCP at 1 mV/s. This approach was similar to that used in previous studies[10,11]. All BET experiments used 1 L of electrolyte and a salt bridge. The reference electrode used in both TET and BET was the standard Hg/Hg_2SO_4 electrode.

To keep the number of the redox reactions in the corroding system to a minimum, 0.9% saline was used as an electrolyte in this study. The BET experiments were performed using saline with four different oxygen concentrations: deaerated, oxygenated, continuously aerated, and saturated with air. Preparation of these solutions was the same as described above with the borate buffer. Again, only the nonagitated solution was used in TET (from here on referred to as the TET experiment). Since the cathodic polarization of Pt used the same treatments of the electrolyte, the relative differences in oxygen concentration were assumed to be the same. All testing was performed on a PARC model 273 potentiostat using model 352 Softcorr II corrosion analysis software.

The values of polarization resistance were determined from the linear portions of the polarization resistance curves. The OCP data reported in *Table 1* represent the ending potentials from each OCP vs. time test. Each experiment was repeated three times and the standard deviation was computed. All potential data are expressed in millivolts vs. saturated calomel electrode (SCE).

RESULTS

Determination of Oxygen Concentration

The cathodic polarization profiles using the platinum electrode in borate buffer of different oxygen concentrations exhibit different oxygen limiting current density values (OLCD). The values were taken at -300 mV vs. SCE (*Figure 2*). In the oxygenated solution, the OLCD value was found to be 7.9×10^{-4} A/cm^2. In the aerated solution, it was 1.25×10^{-4} A/cm^2, and in the saturated solution, it was 4.26×10^{-5} A/cm^2. The OLCD value in the TET experiment was 2.64×10^{-5} A/cm^2.

Equilibration of 316L SS

Table 1 contains OCP and polarization resistance (R_p) data collected, with standard deviations. The 316L SS sample reached its lowest and highest OCP values in the electrolytes containing the lowest and highest oxygen concentration, respectively. OCP values for TET, continuously aerated, and saturated with air solutions, are very similar and do not statistically differ from each other. The R_p values for the experiments using continuously aerated, saturated with air, and oxygenated solutions were also not statistically different. As can be seen in *Table 1*, the reproducibility, inversely related to the statistical deviation, of the OCP and R_p data is much better using TET than BET techniques. The development of OCP with time in the first minute of the experiment is shown in *Figure 3*. The change of potential under the TET condition has a negative slope as opposed to all BET conditions which approach a positive one.

Table 1 Equilibration kinetics--open circuit potential and polarization resistance

	Electrolyte									
	Deareated		Saturated with air		Aerated		Oxygenated		TET	
	Average	St. Dev.†	Average	St. Dev.†	Average	St. Dev.†	Average	St. Dev.†	Average	St. Dev.†
E1*	-441.3	23.6	-240.0	11.4	-244.0	7.1	-212.0	7.9	-236.3	20.3
E2	-506.7	41.0	-199.7	12.3	-200.7	5.7	-177.0	7.1	-201.0	2.9
E3	-512.7	46.6	-182.0	11.4	-183.0	5.4	-162.7	7.3	-185.7	0.5
RP1#	34.8	3.0	37.6	2.8	35.2	2.8	58.0	0.7	30.0	4.5
RP2	34.1	16.0	65.5	4.0	61.8	2.2	57.9	4.4	54.8	1.8
RP3	55.7	11.4	86.7	7.4	84.7	2.3	81.2	6.5	70.8	0.9

* E1, E2 and E3--open circuit potential (OCP) value 60, 380, and 700 s respectively, from the beginning of the experiment; [mV vs. SCE]; † standard deviation.

#RP1, RP2 and RP3--polarization resistance value 60, 380 and 700 s respectively, from the beginning of the experiment; [kΩ/cm^2].

Potentiodynamic Polarization of 316L SS

Examples of potentiodynamic curves are given in *Figure 4.* Cathodic kinetics in oxygen containing environments is mixed with both oxygen and hydrogen ion reduction taking place. Cathodic polarization curves exhibit OLCDs, increasing directly with the oxygen content of the electrolyte from approximately 6.3 x 10^{-6} to 1.6 x 10^{-4} A/cm^2. The TET experiment produced the lowest value of O_2 diffusion current density. The OLCD values were taken at -500 mV vs. SCE. In the anodic part of the polarization curves all BET experiments, regardless of oxygen content, exhibited breakdown potentials (E_b) of approximately 100 mV vs. SCE. The samples tested using TET consistently produced breakdown potentials of 340 mV vs. SCE.

DISCUSSION

Determination of Oxygen Concentration

Figure 2 shows a 6.3-fold difference in limiting current densities between oxygenated and aerated solutions. This value roughly corresponds to the difference in oxygen content between air and pure oxygen. Consequently, the OLCD approach was considered a satisfactory method for estimating the oxygen concentration ratio between the TET and BET conditions. Using this technique, TET exhibits 1.7 times lower oxygen concentration than a saturated BET condition. The relative difference between TET and aerated conditions is 5.5 times less oxygen available for reduction in TET. These data contradict a previously held belief that TET would allow higher oxygen content on the sample surface compared with BET[12]. The differences are likely to exist because of more powerful convection in the bulk

electrolyte cell arrangement, taking into account the distinctive geometries of the TET and BET setups.

The platinum electrode exhibited substantially lower OCP under TET conditions (254 mV vs. SCE) as compared with all bulk electrolyte situations (saturated 348 mV, aerated 335 mV, and oxygenated 340 mV vs. SCE). One argument for such behavior could be alkalization of the electrolyte during the experiment. This pH change was estimated by the following method. The redox currents for the Pt/borate buffer system were measured using polarization resistance values determined after 20, 40, and 60 min of equilibration. The average current I flowing through the cell was 0.5 μA. Calculation of the buffering capacity of borate buffer at pH = 6.8 using a buffer modeling software (SEQS, CET Research Group) yielded a value of $\beta = 0.0134$. Subsequently, in 20 μL of electrolyte, 2.68×10^{-4} mol of OH^- would cause a pH change of 1 unit. Considering that the prevailing cathodic reaction has the form

$$O_2 + 2H_2O + 4e^- = 4OH^-,$$

the molar quantity of OH^- (n) produced in 1 h of equilibration (t = 3600 s) can be calculated using the Faraday law

$$n = \frac{It}{F} = \frac{0.134 \times 10^{-6} \times 3600}{96500} = 5 \times 10^{-9}\ mol.$$

Assuming linear behavior of pH vs. $[OH^-]$ in the vicinity of pH = 6.8, the pH change after 1 h of equilibration is negligible; $\Delta pH = 1.87 \times 10^{-5}$.

The low OCP value in TET may be explained using simple principles of electrochemistry. *Figure 5* shows a schematic model of anodic and cathodic reactions in the

Pt/buffer system in the form of a current vs. potential graph. Oxygen reduction reaction produces different values of diffusion limited current densities, as determined experimentally. The anodic and cathodic currents must be equal at OCP. Subsequently, the differences in the oxygen polarization profiles give rise to a nonlinear distribution of OCP values with respect to oxygen concentration in the solution.

Equilibration of 316L SS

The final OCP values of the 316L SS sample (*Table 1*) exhibited a different trend than those recorded on platinum. Only the deareated condition produced statistically different OCP values from all other situations, TET included. This would not indicate a different oxygen content in TET. However, the Rp values in TET and BET were statistically different.

Realizing the different polarization character of reactions occurring on Pt and 316L SS surfaces, this discrepancy can be explained in the following manner (*Figure 6*). It is recognized that in approximately neutral solutions, reduction of O_2 is the controlling cathodic reaction, and reduction of H^+ ions plays only a minor role [19]. Also, the cathodic curve is a synergistic combination of all reduction reactions which can have different thermodynamic (equilibrium potential) and kinetic (exchange current density, polarization characteristics) parameters. According to the mixed potential theory, even a small concentration of O_2 will result in partial concentration control of the cathodic reaction, and the OCP will be positioned closer to $E(O_2/OH^-) = 0.613$ mV vs. SCE. However, if the concentration of O_2 is low enough, as in a deareated electrolyte, depolarization caused by the H^+ reduction takes over. The activation control of the overall cathodic reaction becomes more pronounced, and

the mixed cathodic potential is shifted abruptly to more negative values, close to the equilibrium potential for H^+/H_2 and/or H_2O/OH^- reactions (-676 mV vs. SCE).

Overall corrosion current also depends on anodic properties. These include, for passivating materials, the oxide film quality and thickness. Subsequently, corrosion currents will be higher in environments with lower concentrations of O_2 because of the formation of a passive film with less protective properties. This effect was not seen in Pt because it does not develop a passive layer on the metal (*Figures 5* and *6*). The difference in oxygen concentration influence on OCP values between 316L SS and platinum exists also due to substantially lower equilibrium potentials for anodic processes on 316L SS. These potentials will polarize the oxygen reduction reaction closer to a region of limiting current behavior. On the other hand, the electrochemical processes on platinum take place at more positive potentials where the currents associated with oxygen reduction are lower.

Therefore, it can be hypothesized that O_2 concentration influences the electrochemical reactions on passivating surfaces in two ways. First, varying O_2 contents change the mixed potential of the cathodic reactions. This change consequently accounts for the abrupt shift of OCP when decreasing the O_2 concentration in the electrolyte. Secondly, the available oxygen supply affects the quality of the passive film which controls the Rp value in more gradual fashion.

The shape of the OCP vs. time curve from the first minute of the experimental sequence can also provide information on the surface state change and, indirectly, on the relative amount of O_2 available for oxide formation. It can be seen (*Figure 3*) that the OCP of the sample under TET conditions decreases with time. The OCP of samples under BET in both air and O_2 become more positive with time. In the electrochemical system under

consideration, the positive slope of the equilibration curve is associated with the process of passivation, whereas a negative slope is not. Overall, the data collected in this part of the study serve as evidence that the oxygen concentration difference between TET and BET has a practical impact on the results of electrochemical tests.

Potentiodynamic Polarization of 316L SS

As expected, the cathodic portions of the curves generated during potentiodynamic polarization (***Figure 4***) reflect the concentration of oxygen at the specimen surface. Comparing the shapes of the cathodic curves obtained from both oxygen containing and deareated solutions shows that hydrogen ion reduction and oxygen reduction contribute to the overall reduction reaction. The oxygen diffusion current density (ODCD) taken at -500 mV vs. SCE was lowest in TET (6.3×10^{-6} A/cm^2), followed by saturated (4×10^{-5} A/cm^2), aerated (7×10^{-5} A/cm^2), and oxygenated (1.6×10^{-4} A/cm^2) conditions. The proportions among these values qualitatively agree with those found in the platinum/borate buffer system.

The polarization curve obtained using TET exhibits substantially higher breakdown potentials compared with BET. Because the values of BET breakdown potentials are consistent regardless of oxygen content, low O_2 concentration in TET is probably not a cause of this behavior. The BET experiments used Teflon washers with knife-edged seals to define the working area. It was suspected that a gradual increase of current beyond the BET breakdown potentials is associated with crevice corrosion development along the seal. The BET specimens showed more pronounced and frequent signs of crevice attack, as was determined by optical microscopy evaluation of the specimens after each test. On the other hand, the severity of crevice attack in TET samples was negligible. Therefore, it is

conceivable that the masking tape used in TET offers much better protection against the development of crevice corrosion.

The noise detected in the anodic part of the curves may be caused by breakdown and repassivation effects. The anodic currents in all BET experiments increase with decreasing oxygen concentration. This is not surprising considering the positive influence of rising oxygen content on the protectiveness of the passive film. The reproducibility of the curves is best using the TET setup.

The results of the potentiodynamic polarization curves reinforced the conclusions drawn from the previous parts of this study. Using the TET decreases the availability of oxygen on the sample surface. This affects the electrochemical processes that are possible in the system. The higher oxygen concentration in BET is probably caused by convection-induced fluid movement. This movement narrows the oxygen diffusion profile and makes transport of oxygen from the bulk of the solution toward the specimen surface easier. The linearity of the polarization curves close to the OCP is in agreement with basic kinetic laws of electrochemistry. These results confirm findings of previous research and justify TET as a valid electrochemical tool[11,12].

CONCLUSIONS

The present investigation revealed new information on the electrochemistry of corrosion processes under the conditions of a thin electrolyte. The differences between TET and BET were determined and analyzed. It was found that the lower oxygen concentration in TET, caused by different convection characteristics of the TET and BET systems, is the major cause of those differences. It is believed that the results of this study contribute to the

further understanding of corrosion processes in the oral environment, as the electrolyte layer in TET is much closer to that found in *in vivo* salivary films than would be employed by traditional BETs. Other advantages of the TET include excellent reproducibility of results, reduced susceptibility to crevice corrosion, simplicity of use, and substantially smaller volumes of electrolyte needed for testing. The TET appears to be another step toward obtaining a better model of the oral environment for electrochemical testing of dental materials. The TET can also be used to address the influence of proteins and biofilms on the electrochemical behavior of metallic surfaces and can be used in other situations where application of BET is impractical.

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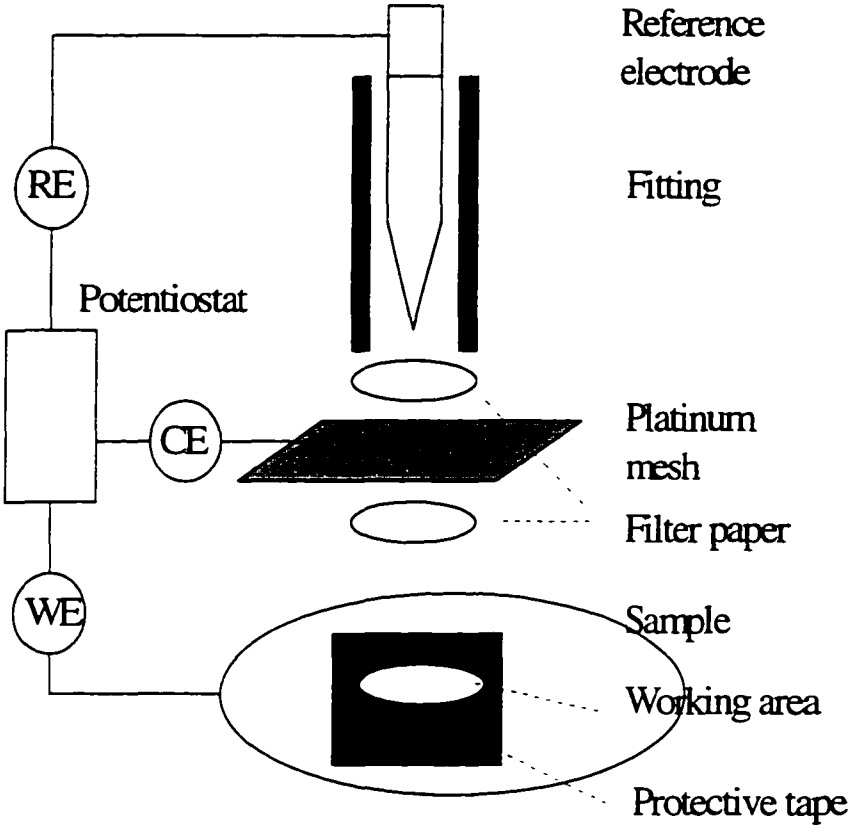

Figure 1 Thin electrolyte technique (TET) setup. Schematic drawing. (From Ref. 11, with permission.)

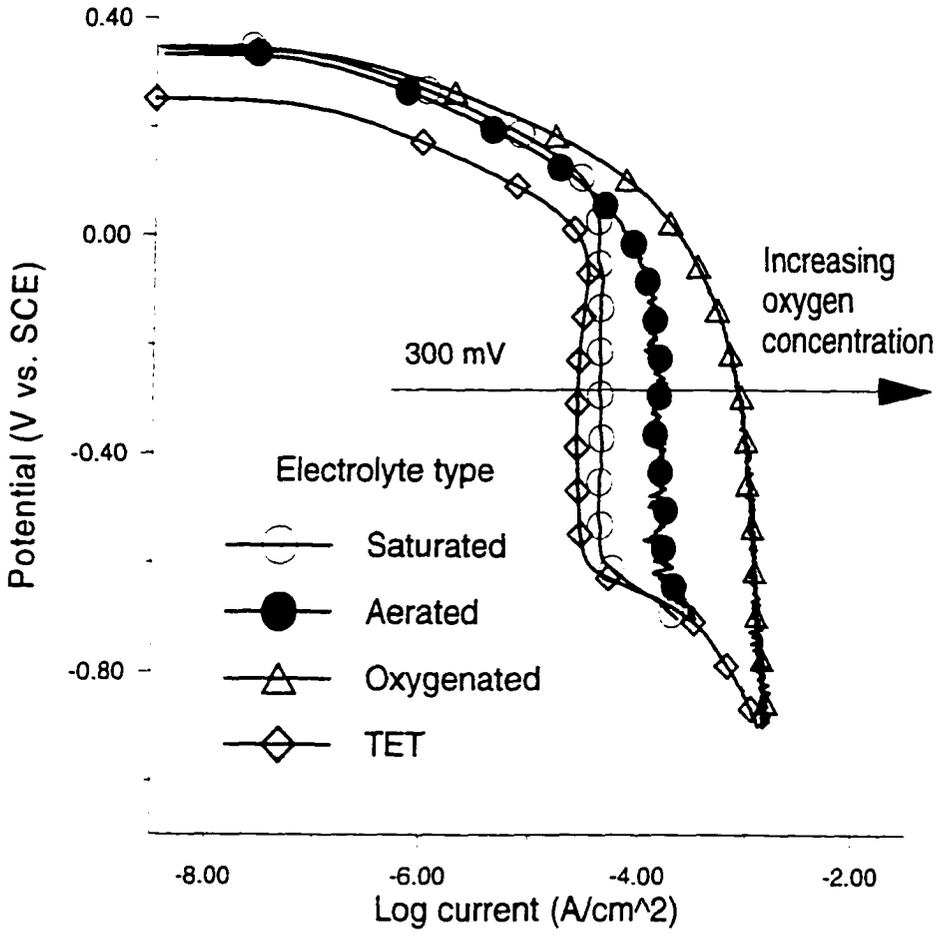

Figure 2 Cathodic polarization of platinum electrode in borate buffer (pH = 6.8). Values of oxygen limiting current density (OLCD) were taken at -300 mV vs. SCE. Using BET, the oxygenated, aerated, and saturated with air solutions yielded OLCD values of 7.9 x 10^{-4}, 1.25 x 10^{-4}, 4.26 x 10^{-5} A/cm^2, respectively. The OLCD in the TET experiment was 2.64 x 10^{-5} A/cm^2.

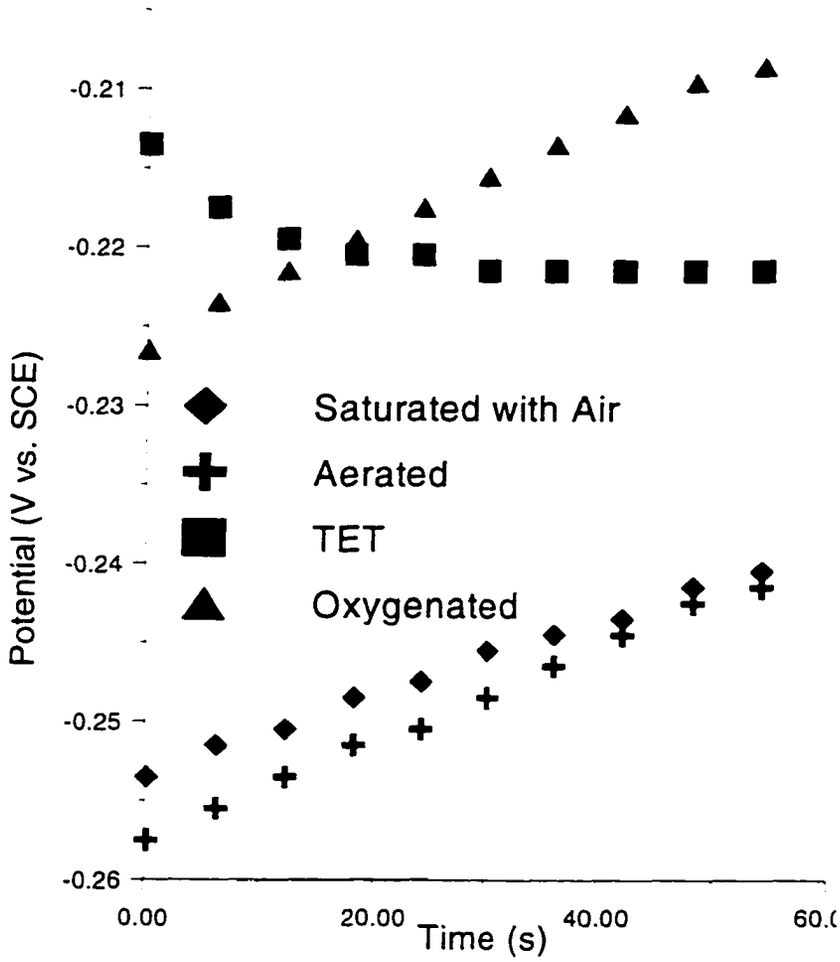

Figure 3 Development of the open circuit potential (OCP) of 316L SS in 0.9% saline in the first minute of the experimental sequence. Only the TET conditions gave rise to negative slope of the potential equilibration curve, suggesting that 316L SS did not undergo further passivation in TET. This tendency was not observed in all the BET situations.

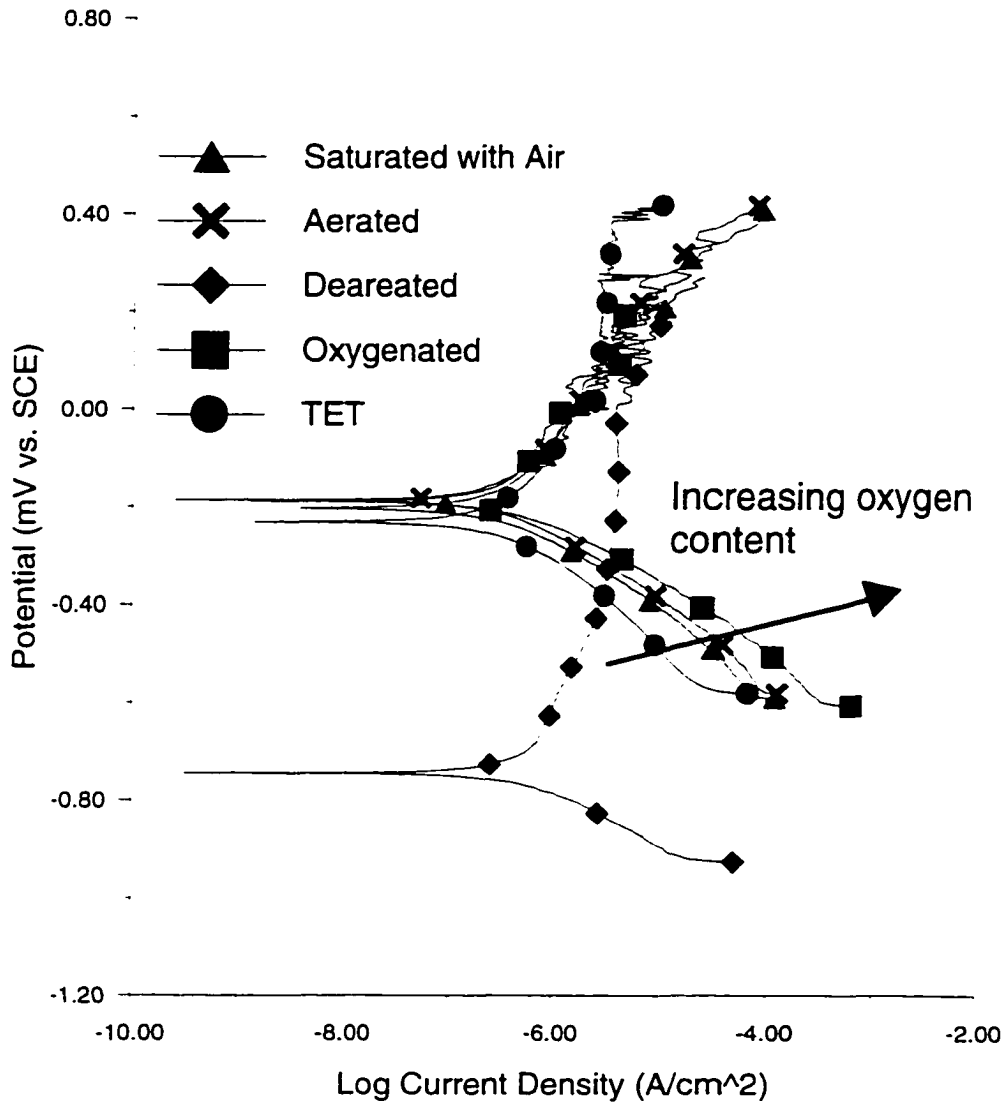

Figure 4 Potentiodynamic polarization of 316L SS in 0.9% saline. The sample experiences lowest oxygen limiting current density in TET.

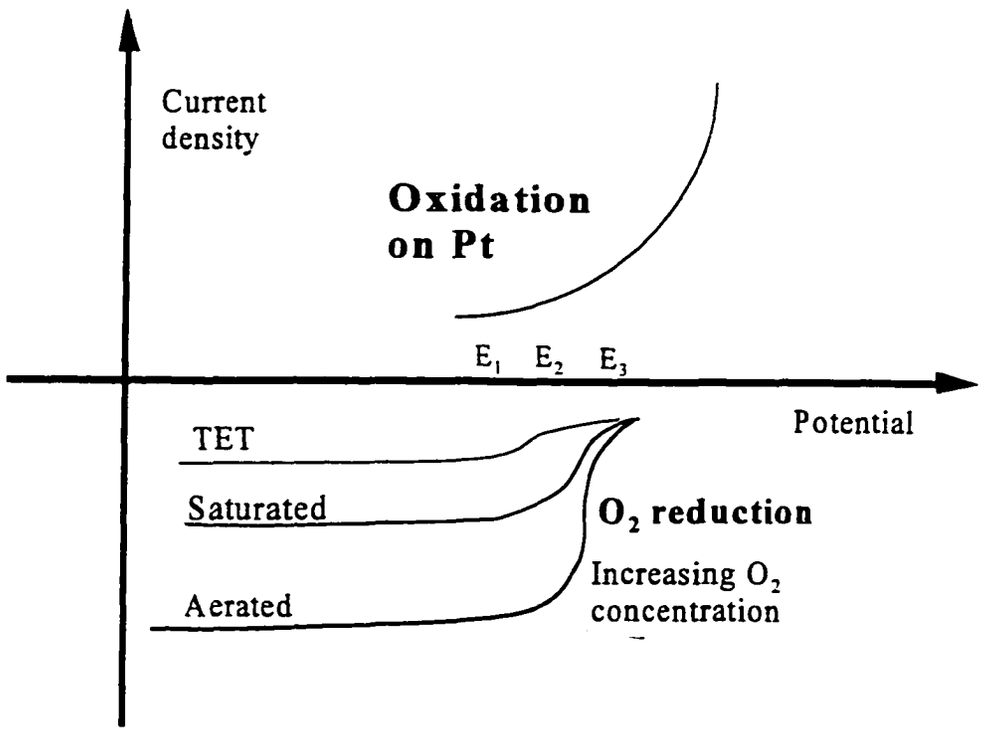

Figure 5 Influence of oxygen concentration on open circuit potential (OCP) value in the system Pt/borate buffer (pH = 6.8). Mixed OCP potentials E_1, E_2, and E_3 relate to TET, saturated, and aerated solutions respectively. Schematic drawing.

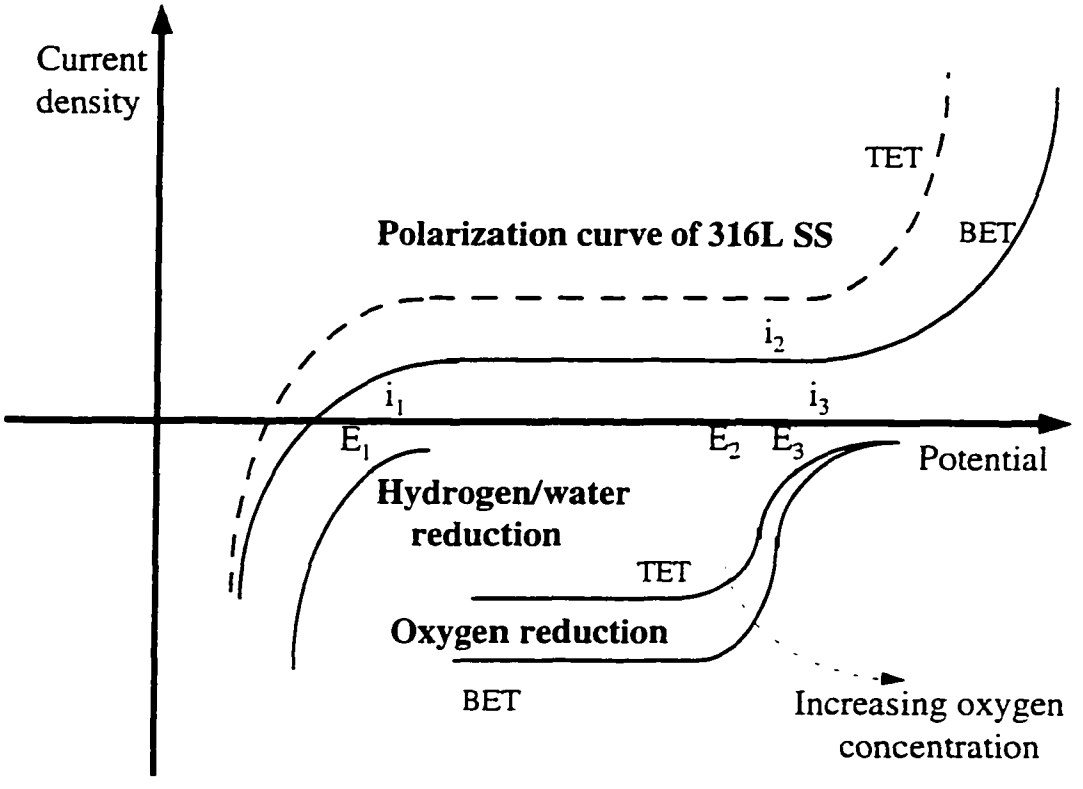

Figure 6 Influence of oxygen concentration on anodic and cathodic processes on 316L SS in 0.9% saline. Mixed potentials E_1, E_2, E_3, and corrosion currents i_1, i_2, and i_3 are associated with deareated, saturated with air, and oxygenated electrolyte, respectively. Schematic drawing.

THE THIN ELECTROLYTE LAYER APPROACH TO CORROSION TESTING OF DENTAL MATERIALS - IMPLEMENTATION OF HUMAN SALIVA

by

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ABSTRACT

The goal of this study was to implement human saliva as a testing electrolyte in corrosion investigation of dental materials and to compare this natural electrolyte to artificial saliva solution. The thin electrolyte layer technique (TET) is a suitable technique for this purpose due to low volume requirements and the geometry of the cell. 316L stainless steel (SS) samples were tested in artificial saliva under both TET and bulk electrolyte technique (BET) conditions to determine the effect of different oxygen concentrations on the electrochemistry of the system. Substantial differences between TET and BET were analyzed based on the individual role of species present in the system. Phosphates and lactic acid were hypothesized to increase corrosivity due to their chemical activity and adsorption to the surface. Tests performed in human saliva using TET showed significant differences in open circuit potential and in the polarization characteristics of the sample, as compared to the artificial saliva experiments. Known complexing properties of some saliva proteins to chromium and the slowing of oxygen transport toward the surface due to adsorbed proteins were assumed to be reasons for lower values of open circuit potential in the natural electrolyte. During potentiodynamic polarization, substantially higher breakdown potentials as well as the presence of current transients were noticed in saliva. A theory for localized corrosion inhibition in this system was proposed. Glycoproteins contained in saliva, carrying a strong negative charge, get absorbed inside forming pits, thus blocking the transport of chloride anions to the pit. The presence of proteins hinders the normal autocatalytic process of pitting corrosion.

INTRODUCTION

In the previous part of this study, the authors introduced the thin electrolyte layer technique (TET) to the field of corrosion testing of metallic dental materials[1]. The TET uses a 0.5 mm thick electrolyte layer and thus approximates the physical aspects of the oral environment and improves its simulation compared with the standard bulk electrolyte technique (BET). Using the limiting current density method, the concentration of oxygen on the sample surface was found to be approximately half that in BET. Additionally, the authors characterized variations in the electrochemical behavior of 316L stainless steel (SS) under TET and BET conditions. The variations were attributed to a lower oxygen content under TET situations caused by thermal convection. Apart from improved oral environment simulation, testing in TET offers other advantages, for example, excellent reproducibility and increased resistance to crevice corrosion during anodic polarization.

To simplify data interpretation, 0.9% saline solution was used as an electrolyte in the previous study[1]. However, in addition to chlorides, human saliva contains a number of active corrosion species, both inorganic (chlorides, phosphates, carbonates) and organic (proteins, lactates, urea, enzymes, and other types)[2]. Chlorides can cause a passive film breakdown[3]. Phosphates are known to have inhibitive action and form phosphate films on steel surfaces[4]. Carbon dioxide and carbonates generally increase corrosive attack[5]. Investigating the effects of organic constituents on saliva electrochemistry has been limited due to the large variety of substances present and the lack of a suitable low volume technique. As a result, the number of studies in this area has been insignificant. The role of the individual constituents of saliva on its electrochemical behavior was reviewed, and the knowledge of protein effects was concluded to be far from complete[6].

More data, however, are available on corrosion effects of serum proteins. It was found that serum albumin does not affect passivation of nickel[7]. In another study, the presence of serum proteins was noticed to influence the primary passivation mechanism of 316L SS[8]. The effect of serum proteins on corrosion rates of various metals was also investigated[9]. The corrosion rate of aluminum and titanium exhibited no significant change due to the presence of proteins in the electrolyte. The rate of chromium and nickel release increased, while that of molybdenum substantially decreased when compared to an environment without proteins. Another investigation indicated that the corrosion products of 316L SS produced by saline with serum additions were organometallic in character[10].

In order to closely simulate the chemistry of the oral environment and subsequently address the role of the organic constituents, testing should be ideally carried out in human saliva. However, human saliva is unstable and difficult to collect in large quantities. These limitations have precluded routine use of saliva in dental materials testing. Previous studies concentrated on the simulation of the oral environment through development of various artificial saliva solutions and the search for a composition which would simulate the electrochemical properties of human saliva[11-14]. How closely these synthetic environments simulate *in vivo* conditions has been one of the major unresolved questions in corrosion research in biological environments[6]. An extensive comparison of natural and synthetic saliva has not been performed. It was pointed out that synthetic electrolytes do not accurately represent the complex electrochemistry of saliva[6,15]. This was attributed to the absence of organic, mainly proteinaceous, substances[16]. Therefore, the objective of this study was to obtain data, which would improve the understanding of the differences in electrochemical

behavior between natural and artificial saliva and possibly help to clarify the effects of organic species.

The TET can alleviate most problems formerly associated with the use of saliva in corrosion experiments. Only 20 µl of testing solution is required in TET testing. Therefore, the TET offers a means for implementing human saliva as an electrolyte. Previous investigators encountered discontinuous current transients in electrochemical testing in saliva, probably due to blocking of ionic flow through a salt bridge by proteinous sediment[16]. The TET does not use a salt bridge, and the electrode is 0.5 mm from the sample surface. This arrangement prevents the problems faced by other investigators.

A review of the literature clearly reveals that the effect of serum proteins on corrosion kinetics is individual to the material in question. A similar dependency on chemistry and state of surface can be expected for saliva proteins; however, the compositional differences of saliva and serum may influence the quantity and perhaps even quality of the effects. The material characterized in this study is 316L SS. Although, at present, this material is not often used as a dental material, it has been extensively studied in both industrial and biological environments and, therefore, represents a wealth of comparative literature. Another advantage of using 316L SS is relative surface homogeneity.

TET provides a higher degree of oral environment simulation. Its use is justified by differences in the TET and BET test results produced by lower oxygen availability on the sample surface in TET. A comparison of TET and BET techniques using saline yielded very different electrochemical behavior in 316L SS[1]. Most corrosion testing of dental materials is performed using artificial saliva solutions. It is known that oxygen concentration significantly influences the activity of various species contained in the artificial saliva[4,17].

Therefore, it would be of interest to investigate how the lower oxygen content in TET influences the results of corrosion tests. Consequently, experiments involving artificial saliva in this study were carried out in both TET and BET.

MATERIALS AND METHODS

Sample Preparation

The methodology used in this study is similar to that applied in the previous research by these authors[1]. Cylindrical samples of 316L SS were polished through a series of 120, 240, 360, and 600 grit SiC paper and repolished before each subsequent test on 600 grit SiC paper. After grinding, the samples were rinsed in distilled water and methanol, dried in a dry air stream, and immediately tested.

Electrochemical Testing

Both the TET and BET experiments involved the same sequence of electrochemical tests. The tests were performed in the following order and with the following parameters: (1) open circuit potential (OCP) vs. time (1 min), (2) polarization resistance, (3) OCP vs. time (5 min), (4) polarization resistance, (5) OCP vs. time (5 min), (6) polarization resistance, (7) OCP vs. time (1 min), and (8) potentiodynamic polarization (-250 to 500 mV vs. OCP, rate of polarization 1 mV/s). Linear polarization was performed in the range ±20 mV vs. OCP at rate 1 mV/s. The first part of the experimental sequence was designed to provide information about equilibration electrochemistry and passive film formation. The second part addressed cathodic and anodic polarization behavior.

Electrolytes

Three different types of electrolytes were used to address the effect of the electrolyte composition: saline (SA), artificial saliva (AS), and human saliva (HS). Saline was selected as a simple control electrolyte; the results have been discussed previously[1]. The selected artificial saliva solution did not contain any proteinaceous constituents, although, among all synthetic electrolytes used, it has been shown to be most similar to human saliva in its electrochemical behavior[18]. Human saliva as an electrolyte contains many organic constituents as well as inorganic compounds. The compositions and other characteristics of the electrolytes are shown in *Table 1*. The two synthetic electrolytes were employed in both TET and BET tests; human saliva was used in TET tests only.

Table 1 Composition and pH of electrolytes

Electrolyte	pH	Composition [g/liter of solution]	Pretreatment
Saline (SA)	6.3	9 NaCl	40 min air
Artificial saliva (AS)	6.8	1.5 KCl, 1.5 $NaHCO_3$, 0.5 KSCN, 0.5 NaH_2PO_4, 0.9 lactic acid	40 min air+10%CO_2
Human saliva (HS)	7.1	chlorides, bicarbonates, phosphates, proteins, glucose, amylase, lactate, urea, uric acid*	none

*Chemical analysis of human saliva was not part of this study, the qualitative composition was taken from Lentner [2].

Forty minutes prior to the start of an experiment, saline was aerated and artificial saliva was purged with a mixture of air with 10 vol% of CO_2. Human saliva was collected

at least 2 h after meal intake from one subject but no longer than 5 min before start of an experiment to increase reproducibility of the electrolyte composition. The BET testing was performed in a conventional 1 l glass cell (EG&G Princeton Applied Research, Princeton, NJ, USA) with a salt bridge. Prior to the gas treatment, the cell was placed inside a water bath (Fisher Scientific, Pittsburgh, PA, USA) on a rack above a 7 cm water level. The system was enclosed by a plexiglas cover and equilibrated at 25°C. Right before insertion of the specimen, the cover was removed and, immediately after connecting the electrical leads to the electrodes, the whole system was again enclosed. The system was not airtight, since a small opening in the cover allowed for pressure equilibration. For saline experiments, the chamber was supplied with humidified air at a rate of 1 l/min for the duration of the experiment. For AS and HS experiments, the atmosphere inside of the system was treated to maintain the initial pH value and buffering capacity of the electrolytes. Initially, right after enclosing the system, 4 l of humidified CO_2 (temperature 25°C) was introduced to the chamber in the first minute to produce 10 vol% CO_2 in the chamber. For the rest of the experiment, a mixture of 10 vol % CO_2 and air was fed to the chamber at a rate of 1 l/min. The gasses flowing to the chamber were humidified by passing through water in a series of wash bottles equipped with glass frits. The temperature inside of the chamber as well as the temperature of the incoming gasses was held by at 25°C. After each run involving HS, the Hg/Hg_2SO_4 reference electrode tip was cleaned with RENOVO X cleaning solution (Radiometer Analytical, Denmark), following the manufacturers instructions.

All testing was performed using a PARC model 273 potentiostat and model 352 Softcorr II corrosion analysis software (EG&G Princeton Applied Research, Princeton, NJ, USA). The measured parameters describing the electrochemical properties of the electrolytes

include OCP, development of OCP values with time (ΔOCP), polarization resistance (R_p), shape of the polarization curves, and breakdown potential value. The ending potentials from each OCP vs. time tests are reported as OCP data. The values of polarization resistance were determined from the linear portions of the polarization resistance tests. Each experiment was repeated three times, the means for each parameter and the standard deviation were computed. All potential data are expressed in mV vs. saturated calomel electrode (SCE).

RESULTS AND DISCUSSION

The OCP, ΔOCP, and R_p data including standard deviations are shown in *Table 2*. It can be seen that both the OCP and R_p values move in a negative direction with increasing complexity of the electrolyte. The discussion of the results is presented according to the type of electrolyte tested. The artificial saliva results are compared to saline and presented by test format. For human saliva, the outcome of the tests is discussed with respect to artificial saliva and saline results in TET alone.

Saline (SA)

The differences between the BET and TET results were presented and discussed previously[1]. The OCP values in BET (-182 mV) and TET (-186 mV) were not found to be statistically different. The polarization resistance values in TET were lower (70.8 Ω/cm^2) than those recorded from BET experiments (86.7 Ω/cm^2). The dissimilarities were attributed to oxygen content in TET.

Table 2 Open circuit potential (OCP) and polarization resistance (Rp) values of 316L SS in saline, artificial saliva, and human saliva

	Saline				Artificial Saliva				Human Saliva	
	BET		TET		BET		TET		TET	
	Average	St. Dev.	Average	St. Dev.	Average	St. Dev.	Average	St. Dev.	Average	St. Dev.
OCP1*	-240.0	11.4	-236.3	20.3	-258.7	6.9	-252.3	4.8	-278.7	12.0
OCP2	-199.7	12.3	-201.0	2.9	-230.3	6.6	-247.3	2.9	-276.3	8.2
OCP3	-182.0	11.4	-185.7	0.5	-216.3	5.0	-244.7	3.4	-273.0	7.9
ΔOCP			50.0				8.0		6.0	
Rp†	54.6	2.1	30.0	4.5	16.2	2.4	44.2	1.5	23.6	0.2
Rp2	65.5	4.0	54.8	1.8	31.5	0.2	47.1	2.6	44.3	1.9
Rp3	86.7	7.4	70.8	0.9	44.3	1.0	60.9	1.6	57.5	1.3

*OCP1, OCP2, and OCP3--Open circuit potential value 60, 380, and 700 s respectively, from the beginning of the experiment; ΔOCP--difference between initial and last OCP measurement.

†Rp1, Rp2 and Rp3-- Polarization resistance value 60, 380 and 700 s respectively, from the beginning of the experiment.

Artificial Saliva (AS)

Comparing the AS parameters with those obtained in SA (*Table 2*), it can be seen that in AS samples exhibited a more negative final OCP and lower R_p in both TET and BET. To explain this phenomena, the role of the electrolyte species in both anodic and cathodic processes must be discussed. Artificial saliva contains 0.5g/l of NaH_2PO_4. Phosphates act as inhibitors of corrosion when applied to some alloys, due to the formation of a phosphate layer on the metal surface. Significant protection properties of NaH_2PO_4 in concentration of 0.1 wt.% have been reported for carbon steel[19]. Evidence exists to indicate a strong affinity of the phosphate ion for iron. In the area of dental materials, a beneficial effect by phosphates on the corrosion reduction of dental amalgam has been reported[13]. The effect of electrochemically active species is specific to the material surface. In stainless steel, it is conceivable, that iron phosphate formation competes with chromium oxide development and alters the quality of the protective film in a negative manner. Thus, the uniform attack would be increased compared with an environment that does not contain phosphates. The experimental results support this hypothesis.

Lactic acid may influence the electrochemistry of the system by actively taking part in the charge transfer reactions and by altering the double layer structure due to surface adsorption. It has been reported that lactic acid forms thermodynamically stable compounds with iron[20]. Therefore, it is conceivable that the formation of iron lactate would partially contribute to the lowering of the R_p value in AS. The kinetics of equilibration in the AS system was significantly faster than that containing saline, judging from smaller ΔOCP values (*Figure 1, Table 2*). Probably, this phenomena is caused by a quickly established stable double layer at the electrode-electrolyte interface due to adsorption of molecules of

lactic acid. Potentials of maximum adsorption for iron and nickel are -0.56 and -0.46 V vs. SCE, respectively[21]. It was shown that, in many electrode-electrolyte systems, the magnitude of adsorption does not change significantly in the interval from ±0.2 to ±0.5 V around the potential of maximum adsorption. This depends on the concentration of organic species in the electrolyte. Therefore, it is reasonable to assume that, at the OCP measured in this system (-244 mV in TET), the magnitude of adsorption is still significant. The adsorbed layer may effectively slow down oxygen diffusion to the surface. This would result in a slower passive film formation and eventually lower the OCP value, as noticed experimentally (*Table 2*). In addition, the R_p value could decrease as a direct result. This too was confirmed experimentally.

The breakdown potential value in SA is substantially lower than in AS (*Figure 2*). This is not surprising because artificial saliva contains only 1.5g/l of NaCl, compared with 9g/l in the saline solution (*Table 1*). However, this trend did not hold true for uniform corrosion where significantly lower R_p values were observed in artificial saliva (*Table 2*).

The difference in OCP values in TET and BET was found to be 28.4 mV in AS compared to an insignificant variation in saline (*Table 2*). In order to explain the phenomenon, one needs to consider the effects of reaction species on the potentials of the cathodic and anodic reactions. In neutral oxygen containing electrolytes, the main cathodic reaction causes deacidification of the environment and has the following form:

$$O_2 + 2H_2O + 4e^- = 4OH^-. \qquad (1)$$

One possible cause of the potential shift during the experiment can be a change in the pH (ΔpH) of the electrolyte as can be seen from the Nernst equation for reaction (1), assuming a partial pressure of oxygen $p(O_2) = 0.9$ atm:

$$e = e^{o} + \frac{0.059}{4}\log(p_{O_2})^2 - 0.059pH = e^{o} - 0.01 - 0.059pH. \qquad (2)$$

The pH change can be considered negligible in BET because of the large volume of electrolyte. In TET, however, the pH change of the electrolyte could be substantial in the low electrolyte volume. The change can be estimated based on a knowledge of the buffering capacity of the electrolyte and the measured current flowing through the cell. The buffering capacity of the AS electrolyte was determined from the slope of the neutralization curve (*Figure 3*) to be approximately 0.053 at the pH of testing. In order to quantify the determining change in the electrolyte chemistry occurring during the experiment, the same approach for ΔpH presented in the previous study was used[1]. This yielded a value for ΔpH = 0.042 for 1 h of sample equilibration. Using this estimate, the theoretical change of the equilibrium cathodic reaction potential is only 2 mV. Therefore, some other mechanism must be responsible for the observed shift in OCP of approximately 30 mV. The kinetics of oxygen transport are 1.7 times slower in TET due to larger diffusion layer thickness[1]. Therefore, the quality of the passive film must be affected negatively, causing the equilibrium potential for the anodic reaction to be more negative. This in turn causes the mixed potential in TET to be more negative than in BET. A graphical representation of this is shown in *Figure 4*.

Polarization resistance values measured for both experimental setups in artificial saliva exhibited an opposite trend from that found in 0.9% saline (*Table 2*). This can be attributed to the action of phosphate and lactic acid which are not present in simple saline. The protectiveness of the oxide formed in AS is less effective than in SA. The corrosion mechanism is apparently altered and oxygen concentration in the electrolyte plays the opposite role in AS as compared to SA. DC corrosion measurements alone, however, are insufficient to determine the exact nature of this effect.

Human Saliva (HS)

The OCP and R_p values obtained in saliva are even more negative than the ones measured in artificial saliva, although the magnitude of the differences is not comparable to that observed between artificial saliva and saline (*Table 2*). Human and artificial saliva are very similar in composition with regard to inorganic species[12]. However, human saliva contains more and larger varieties of organic compounds. Therefore, it can be assumed that the shift in the measured parameters is caused by the presence of the organic species. The following discussion attempts to deconvolute the various effects of the organic species that are suspected to be active in the corrosion process.

Human saliva has been used as an electrolyte in only a very limited number of published studies. Finkelstein and Greener examined the polarization behavior of several dental amalgams in human saliva and Ringer's solution[15]. They reported that the differences found in OCP and overall polarization behavior of the amalgams in the two electrolytes were strongly dependent on the type of amalgam. In two cases out of three, the OCP was more negative in HS than in Ringer's solution. Another publication suggested that the OCP value

of amalgams decreases with the addition of salivary proteins to Ringer's solution[16]. In the current study, similar trends in the OCP values were observed. The OCP of 316L SS was more negative in HS than in AS, although the difference of 28 mV was smaller than that reported between Ringer's solution and human saliva on amalgam by Finkelstein and Greener. The reason for this difference can be attributed to the different composition of the synthetic electrolyte used in this study and/or due to the difference in surface chemistry between amalgam and 316L SS.

In a subsequent search for an electrolyte which would be more representative of saliva, Finkelstein and Greener investigated the role of mucin and albumin in saline on the polarization of dental amalgam[16]. The effect of these proteins on the corrosion of dental amalgam was summarized as inhibiting the corrosion process. One mechanism proposed by the authors suggests that the organic constituents absorb onto the surface, slowing down both the cathodic and anodic reactions. However, in the present study, the kinetics of corrosion in saliva was found to be faster than in the artificial electrolyte (*Table 2*). A different mechanism of protein action on the 316L SS surface must be taking place. Woodman *et al.* reported that chromium and nickel release rates from 316L SS were approximately ten-fold higher in serum than in saline[10]. It was proved that both metals are bound to complexes by a protein class which includes albumin, a protein found both in serum and in saliva. The fact that the magnitude of the corrosion rate increase discovered here is much lower in saliva than that reported in serum is not surprising since the content of active proteins in saliva is substantially lower than that of serum.

A similar mechanism of adsorption, discussed previously for artificial saliva, may be responsible for the negative shift of the OCP value in human saliva. Adsorption of proteins

and other organic species contained in HS could decrease the rate of oxygen transport to the metal surface and therefore slow down the passivation process. This eventually causes the material to come to a lower OCP. To what extent each of these proposed mechanisms dominate cannot be determined from the collected data. However, comparing the results in AS and HS with the electrochemical properties of the electrolytes, it can be proposed that both mechanisms play an important role.

The potentiodynamic polarization curve obtained in HS exhibits several distinctively different features compared to the curve obtained in AS (*Figure 2*). A definite anodic peak was observed in AS at 100 mV ($4.5x10^{-3}$ A/cm^2). This was not the case in saliva. However, the origin of this maximum could not be determined. Additionally, the passive current density is slightly lower in HS. The major difference among the three curves is the breakdown potential (E_b) value. It was found to be lowest in SA (310 mV), followed by AS (490 mV), and HS (710 mV). The difference between SA and AS can be attributed to different chloride ion concentrations (9g/l in SA and 1.5g/l in AS). The reported Cl^- concentration in HS is between 17 and 23 mmol[2]. The concentration in artificial saliva was 20 mM. Therefore, another mechanism must be considered to explain the high E_b value in HS. It can be noticed that the HS curve contains current transients in the range of potentials close to the breakdown potential in AS (*Figure 2*). Suter and Bohni recorded current micro-transients while investigating the passivity of stainless steel using a microelectrochemical method[22]. The transients were associated with local breakdowns and repassivations of the passive film. However, the transients observed in this study were several orders of magnitude higher than those observed in the cited study.

Considering the above, the following hypothesis could explain the high E_b values and the mechanism of transient formation in HS. Glycoproteins have been reported as a major proteinaceous constituent of saliva[2,23]. The side chains of these proteins often end with a sialic acid residue (*Figure 5*). The carboxyl group of sialic acid is fully ionized at physiological pH values and confers a strong negative charge on the macromolecule. In pitting attack, it is well documented that the local dissolution which occurs within the pit produces an excess of positive charge in the pit area, resulting in the migration of negatively charged species to the pit to maintain electroneutrality. It is conceivable that not only Cl^- ions but also the molecules of glycoproteins are attracted to the inside of the pit, effectively blocking the continued transport of Cl^- ions. Furthermore, it is accepted theory that a growing pit has to be countered with sufficient area for the cathodic reaction to consume electrons released by metal dissolution[24]. It can be expected that adsorption of organic species in HS is quantitatively greater than in AS. The proteins adsorbed onto the pit may hinder access to oxygen by the cathodic areas surrounding the place of attack. This may be an additional reason why the growth of the pit stops and the surface of the pit repassivates, thus giving rise to the current transient and eventually to higher breakdown potential. The current transient behavior was not reported in Finkelstein and Greener's study of dental amalgam in human saliva[15]. This can be explained by the difference in the surface nature and chemistry of dental amalgam compared to stainless steel.

CONCLUSIONS

This study had a two-fold purpose. The first goal was to employ human saliva as an electrolyte and to clarify the effect of species contained in both artificial and human saliva

on the electrochemistry of the electrolyte. The second goal was to justify use of the TET by showing the existence of significant differences in the electrochemical properties of artificial saliva in TET as compared to BET.

The discussion of results offered an explanation of influences of individual species contained in human and artificial salivas on the electrochemical behavior of 316L SS. Phosphates and lactic acid were believed to have negative influence on the corrosion resistance of this passivating, iron-based alloy.

Human saliva was successfully used as an electrolyte and showed notable differences in the test results as compared to the artificial solution, especially in the breakdown potential value. It was concluded that proteins contained in saliva are capable of forming complex compounds with the metallic ions, although the effect of individual species could not be quantified based on the results of this study. Furthermore, negatively charged proteins (glycoproteins) were hypothesized to be active in decreasing the susceptibility of 316L SS to localized corrosion. It is conceivable that selective adsorption of the protein molecules to the inside and/or immediate vicinity of forming pits is a reason for such behavior.

The authors are aware that the composition of human saliva is highly individual and changes even during the day. By collection of saliva from one subject at a particular time of the day with respect to meal intake, the variations in saliva compositions were limited, illustrated here by the small deviations obtained. A chemical analysis of the saliva was not performed, and it was assumed that the composition fell within margins widely reported for healthy individuals. The purpose of this study was to show qualitative differences between the electrochemistry of human and artificial saliva, but not to address possible concentration

effects of individual species. However, it should be stated that TET, because of the low electrolyte volume required to perform a test, is a suitable technique for such a determination.

It should be noted also that each class of dental materials possesses different surface and electrochemical properties and therefore are affected differently by the same species. However, the similarity of 316L SS surface electrochemistry with some materials currently in use (nickel-chromium casting alloys) provides incentive to study these materials in the future. Further work will address the influence of individual species on saliva corrosivity in a quantitative manner and provide information on the proposed mechanism of pit termination due to protein adsorption.

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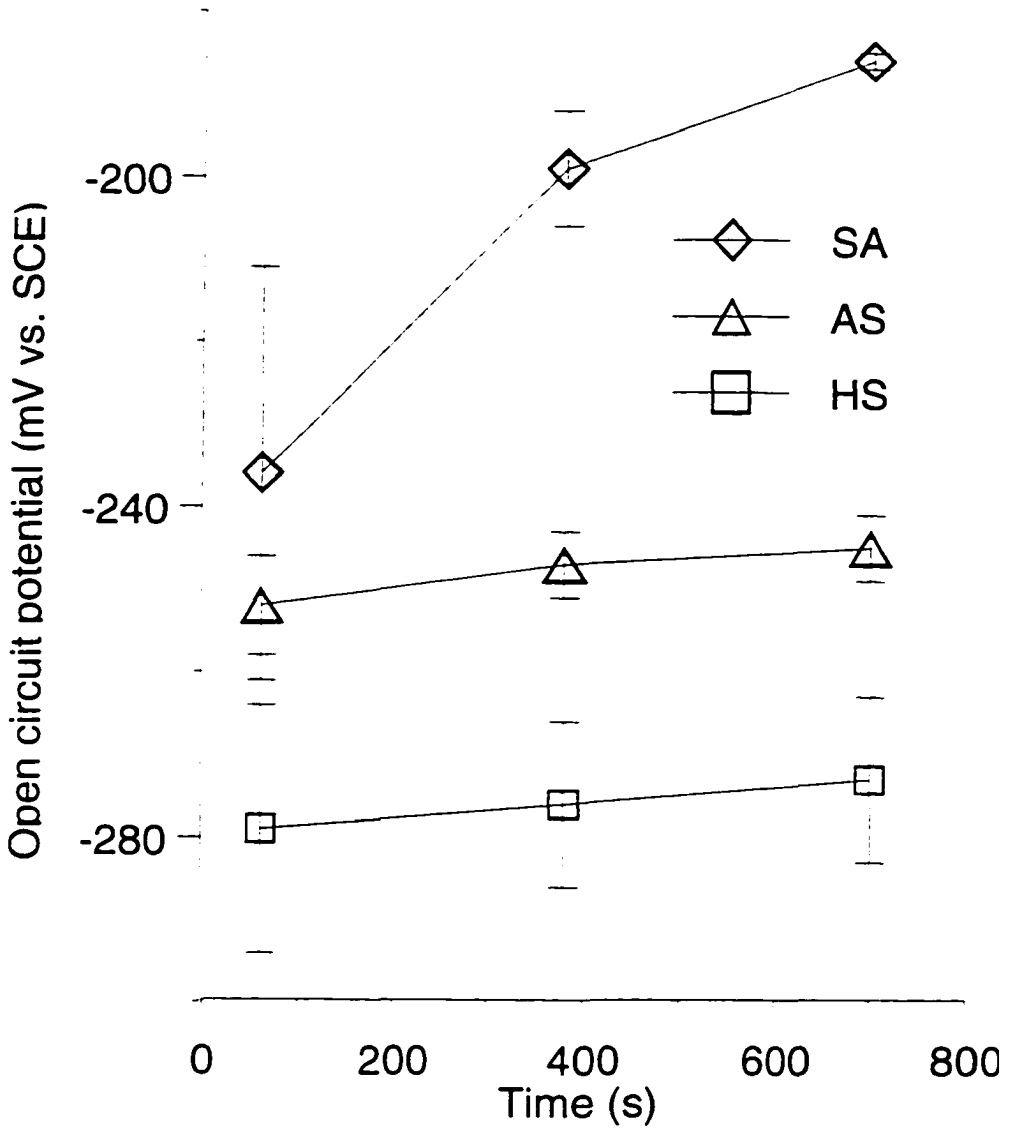

Figure 1 Development of the open circuit potential during the experimental sequence. SA (saline), AS (artificial saliva), HS (human saliva). The OCP change decreases with increasing complexity of the electrolyte from SA to AS to HS.

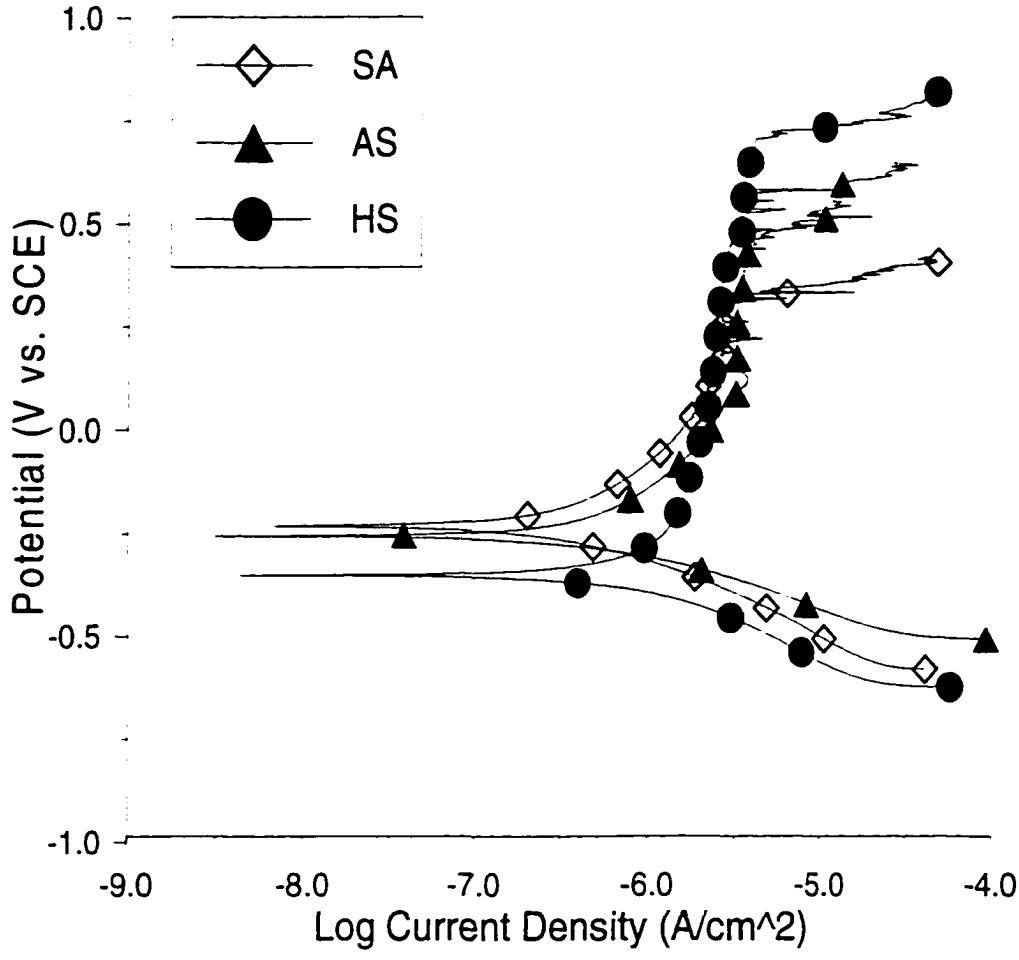

Figure 2 Potentiodynamic polarization of 316L SS in saline (SA), artificial saliva (AS), and human saliva (HS). The breakdown potential was lowest in SA (310 mV), followed by AS (490 mV) and HS (710 mV).

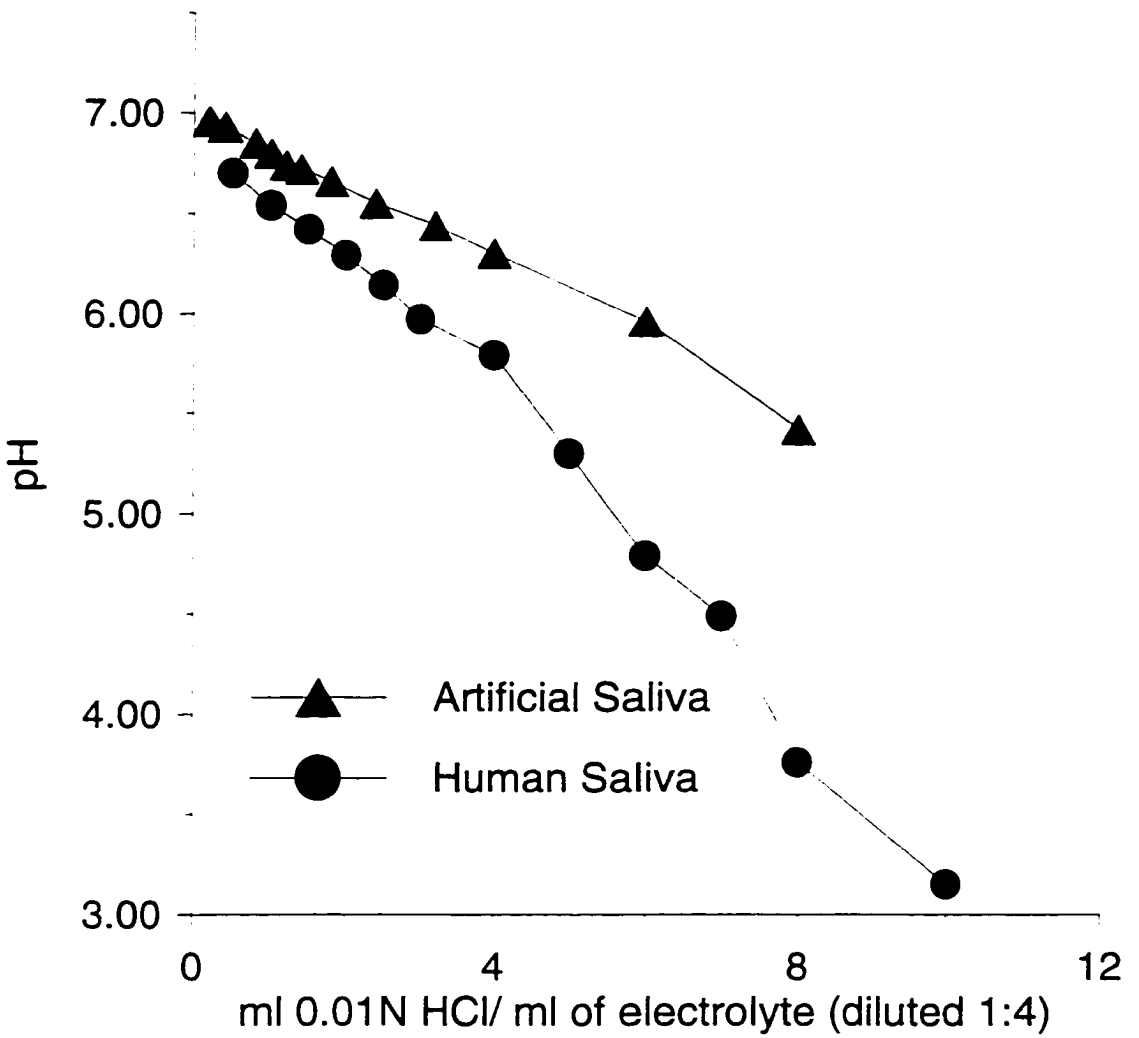

Figure 3 Neutralization curves of artificial and human saliva. The buffering capacity of the electrolytes was determined from the slope of the the curves.

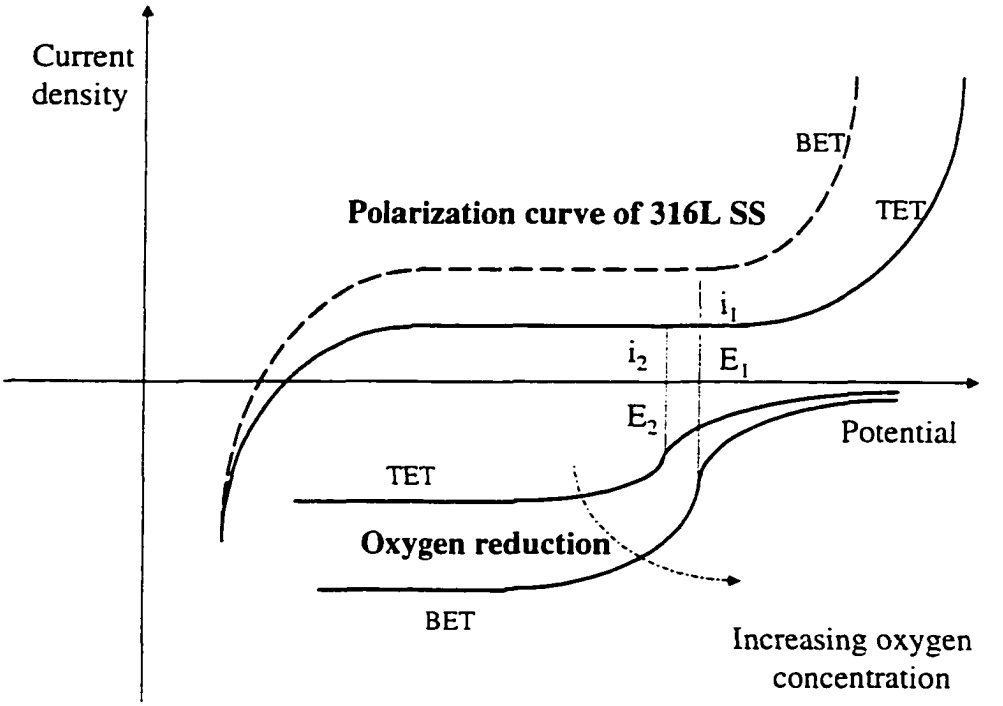

Figure 4 Influence of oxygen concentration on anodic and cathodic processes on 316L SS in artificial saliva. Mixed potentials E_1 and E_2 and corrosion currents i_1 and i_2 are associated with TET and BET experiments respectively. Amount of oxygen in TET is lower than in BET. Passive currents increase with increasing oxygen concentration (BET). That forces the anodic current to rise (i_1).

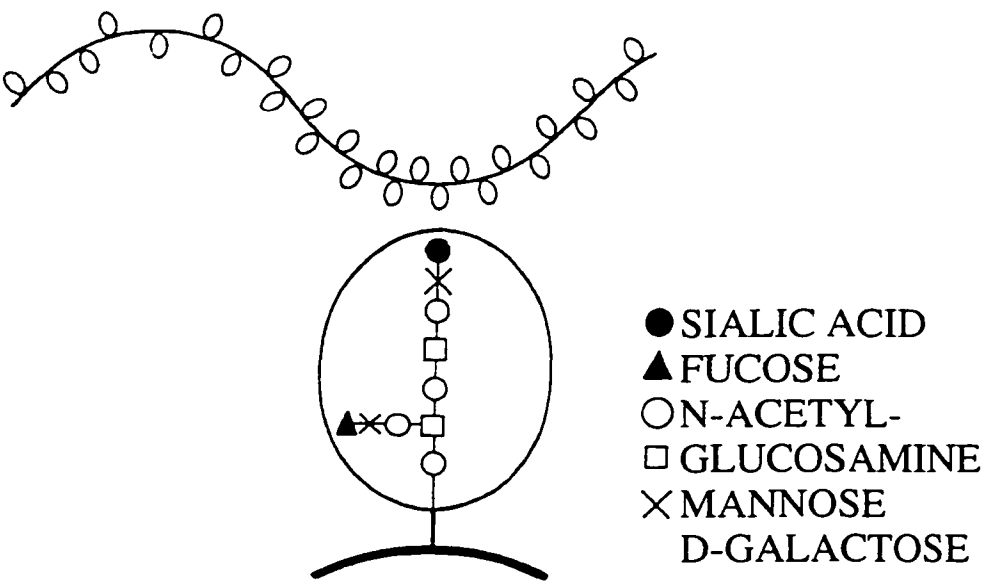

Figure 5 Schematic drawing of a typical glycoprotein molecule. The side chains often end with sialic acid that confers a strong negative charge on the end of the sidechain and the molecule itself.

EFFECT OF SALIVARY PROTEINS ON THE ELECTROCHEMICAL BEHAVIOR OF NICR DENTAL CASTING ALLOYS

by

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ABSTRACT

The goal of this study was to investigate the effect of proteins contained in saliva on the electrochemical behavior of dental casting alloys. Tests were carried out in artificial saliva (AS), artificial saliva with 1% albumin (AL), artificial saliva with 1% mucin (MU), and human saliva. Open circuit potential (OCP), corrosion current (i_{corr}) and cyclic polarization data of four commercial nickel-chromium alloys (Neptune, Rexalloy, Regalloy T, and Vera Bond) were collected using the thin electrolyte layer technique (TET). Significant differences in behavior of the alloy-electrolyte combinations were reported. Corrosion current was lowered by the addition of AL and MU and by the use of HS as compared to AS with no protein additions in alloys with typical casting porosity. The corrosion resistance of alloys with high quality passive films was not influenced significantly by the presence of proteins in the electrolyte. Alloys with lesser tendency to passivation (Vera Bond) showed inferior behavior in AL, MU, and HS. Polarization behavior was influenced only in alloys with heterogenous surface charge distribution (Regalloy T and Vera Bond). The results were supportive of the hypothesis of localized adsorption of proteins based on electrostatical attraction. Additionally, it was found that the corrosion behavior of HS is far more complex than that of simple electrolytes with protein additions.

INTRODUCTION

Proper modeling of the *in vivo* environment plays an important role in corrosion testing of materials that are designed for use in the human body and where correct approximation of ion release and mechanisms of deterioration are of high interest. In the area of dental biomaterials, substantial effort has been devoted to the simulation of oral

environment. Many questions still remain unanswered[1,2]. In previous research it was shown that not only simulation of the chemistry of the oral environment but also thickness of the electrolyte layer can have a significant effect on corrosion test results[1]. Therefore the authors introduced and characterized a novel technique for electrochemical investigation of dental alloy systems[1]. The thin electrolyte layer technique (TET) allows experiments to be performed under a 0.5 mm layer of electrolyte. Only 20 µl of liquid is required for testing. The need for such a low volume of solution makes this technique highly suitable for examining the effects of rare salivary components, as it facilitates the use of human saliva in experimentation.

A variety of chemical solutions have been developed by investigators wishing to accurately simulate the oral environment. Many of these efforts have been reviewed in earlier work[3]. Considering the complexity of human saliva, artificial solutions are quite a simplification of reality. Most of them have no protein content. Furthermore, information on how well these artificial electrolytes simulate the complex electrochemistry of human saliva has been scarce. A previous study on mercury amalgams presented several hypotheses on how albumin and mucin, proteins contained in saliva, could affect behavior of dental amalgams[4]. However, no in-depth explanation of the observed effects was offered. Our previous research showed that significant differences exist in electrochemical behavior of 316L SS when tested in artificial and human saliva under a thin film of electrolyte[3]. It was hypothesized that proteins may influence the aggressiveness of electrolytes in terms of the susceptibility of materials to localized forms of corrosion. A theory involving selective, charge-induced adsorption of protein molecules into the forming pits hindering the autocatalytic mechanism of pitting was proposed.

From the literature available on corrosion of orthopaedic materials, it is obvious that the effects of proteins on corrosion are dependent on the material in question[5, 6]. It can be expected that the same would apply to dental materials, especially if relatively active ones such as NiCr dental casting alloys are considered.

Nickel-chromium casting alloys were developed as a substitute for precious alloys and used in crown and bridge restorative dentistry[7]. Because of the less noble nature of these alloys and possible toxicity concerns for Ni-sensitive patients[8], the corrosion properties have been studied extensively by a variety of methods. For example, in one study, 12 commercial NiCr alloys were compared based on anodic and cathodic polarization in an artificial saliva solution[9]. The potentiostatic deaeration method that enables the measurement of small corrosion currents such as those generated by a NiCr alloy was applied recently[10]. Later, this experimental technique was extended to a wide variety of NiCr alloys[11].

Beryllium was introduced to the NiCr alloy systems in an effort to improve its casting properties[12]. Beryllium additions resulted in the formation of a NiBe eutectic phase embedded in the NiCr matrix[13-15]. This phase was found to have detrimental influence on the corrosion resistance of the alloy beyond certain concentrations of Be[13]. When examining the surface properties, it was shown that the eutectic surface film contained lower oxygen and chromium concentrations and was therefore determined to be less protective[14]. The influence of Be on the corrosion resistance of Ni-based experimental alloys with 25% Cr, 10% Mo, and Be content ranging from 0 to 2.1% was studied by potentiodynamic polarization and impedance spectroscopy in artificial saliva at 25°C[13]. Microstructural characterization revealed increasing amounts of NiBe eutectic with rising Be content. It was noticed that

alloys with more than 0.6% Be were highly susceptible to localized corrosion. All of the reviewed studies were carried out in artificial saliva solutions.

Based on available information, it was hypothesized that the electrochemical activity of the NiCr alloy surfaces would be significantly affected by the addition of albumin and mucin to artificial saliva. Furthermore, it was expected that these effects would be influenced by the surface microstructure of these systems. Therefore in this study, four commercial NiCr alloys (two with and two without Be content) were examined using the TET technique in artificial saliva with and without albumin and mucin. The open circuit potential (OCP), corrosion current, and cyclic polarization data were compared to those obtained in human saliva.

MATERIALS AND METHODS

Samples

Four commercial NiCr alloys were used: Regalloy T (RG), Neptune (NP), Rexalloy (RX), and Vera Bond (VB). The composition of the alloys as reported by the respective manufacturers is summarized in *Table 1*. Samples were cast into cylinders (diameter 14 mm, height 5 mm) at a commercial dental lab according to manufacturer's directions. The samples were first polished down to 0.5 μm alumina, etched with a mixture of 10 mL HF and 100 mL HNO_3 and characterized by light microscopy. For electrochemical investigation, the castings were polished down to 600 grit SiC, washed with distilled water, ultrasonically cleaned in methanol and dried in a stream of air prior to testing.

Electrolytes

All four alloys were tested in four different electrolytes: (1) artificial saliva - AS (1.5g KCl, 1.5g $NaHCO_3$, 0.5g KSCN, 0.5g NaH_2PO_4, and 0.9g lactic acid per liter of solution), (2) AS with 1g/l bovine serum albumin (AL), (3) AS with 1g/l bovine submaxillary mucin (MU), (4) stimulated parotid saliva. All synthetic electrolytes were frozen in small vials (5 ml of solution) and stored in a freezer immediately after preparation. Stimulated human parotid saliva was collected from healthy donors using Lashley cups[16], as previously described[17]. After collection, saliva was distributed to vials in the same volume as the synthetic solutions. All electrolytes were allowed to defrost at room temperature about 10 min before the start of each experiment. Twenty µl of electrolyte was injected into the cell using an Eppendorf pipette.

Table 1 Composition of NiCr alloys used (in weight %)

Alloy	Ni	Cr	Mo	Fe	Al	Be	Other	Manufacturer
Neptune (NP)	63	21	8.4	1.7	0.2	-	4Nb	Jeneric/Pentron, USA
Rexalloy (RX)	67	13	6.8	5.2	-	-	7Ga	Jeneric/Pentron, USA
Regalloy T (RG)	71	16	4.5	0.1	3.3	0.6	4Mn	Dentsply, USA
Vera Bond (VB)	77	12	4.8	0.1	2.8	1.7	-	Aalba-Dent, USA

Techniques

All experiments were performed using the TET. Its rationale for use as well as description of the setup has been described previously[1,3]. The TET cell uses only 20 µl of electrolyte in 0.5 mm thick layer. Tests were performed at 37°C in a controlled atmosphere

of humidified air with 10 vol% CO_2 to maintain the pH and buffering capacity of the electrolytes.

Each test consisted of recording the OCP for 40 min followed by cyclic polarization (from -250 mV vs. OCP to threshold current of 0.003 A/cm^2 for RG and VB, and 0.03 A/cm^2 for NP and RX). OCP, corrosion current (i_{corr}), breakdown potentials (E_b), and the shapes of polarization curves were the observed parameters. After each run, the Hg/Hg_2SO_4 reference electrode tip was cleaned with RENOVO X cleaning solution (Radiometer Analytical, Dennmark).

All testing was performed using a PARC model 273 potentiostat and model 352 Softcorr II corrosion analysis software (EG&G Princeton Applied Research, Princeton, NJ, USA). Each experiment was repeated three times, and the means for each parameter and the standard deviation were computed. All potential data are expressed in millivolts vs. saturated calomel electrode (SCE).

RESULTS AND DISCUSSION

Characterization of Microstructure

After etching, the samples were examined using light microscopy. Observed microstructures of NP, RX, RG, and VB are presented in *Figure 1*. The surfaces revealed differing amounts of second phase and casting porosity present. The appearance of the surfaces is in good agreement with those published previously[14]. These authors examined the microstructures using Auger electron microscopy including depth profiling and surface mapping. Intermetalic compounds were found to be precipitated in NP and RX, whereas NiBe eutectic imbedded in α-NiCrMo matrix dominated the microsturctures of RX and VB.

These findings were confirmed by X-ray diffraction in another study[18]. The amount of the NiBe eutectic increases rapidly with rising Be concentration. Casting porosity was found in Rexalloy and Regalloy T microstructures as a consequence of sample manufacturing *(Figure 1)*.

For the purposes of this study, the alloy surfaces can be divided into three groups according to the their apparent chemical and physical homogeneity:

1. High homogeneity: Surface of Neptune contained intermetallic compounds but no casting porosity was apparent. The second phase particles were relatively small. It was shown previously that the passive film covering the surface is coherent[14].

2. Medium homogeneity: Both surfaces of Rexalloy and Regalloy T exhibited significant amounts of casting porosity and second phases.

3. Low homogeneity: Surface of Vera Bond was covered by the least coherent passive film layer[14].

Open Circuit Potential

NP and VB exhibited a significantly more positive OCP in AS with respective additions of AL and MU than in the other two electrolytes (*Table 2*). This is in agreement with results of a previous study on mercury amalgams where similar differences were not noted between the OCP of dental amalgam in Ringer's solution with and without the addition of albumin and mucin[4]. However, the trend was reversed for Rexalloy and Regalloy T, alloys with higher levels of porosity, although the magnitude was mostly within the margins of standard deviation. The effect on OCP does not seem to be the result of composition,

specifically the Be content. Rather, there seems to be a strong correlation between the presence of porosity with the interaction of proteins on the surfaces.

Table 2 Open circuit potential in mV vs. SCE experienced by NiCr alloys in examined electrolytes

	Neptune (NP)	Rexalloy (RX)	RegalloyT (RG)	VeraBond (VB)
Artificial Saliva	-253 ± 24	-268 ± 31	-322 ± 27	-289 ± 15
AS + 1%	-43 ± 20	-202 ± 22	-409 ± 18	-192 ± 8
AS + 1% Mucin	-60 ± 16	-189 ± 12	-461 ± 29	-215 ± 12
Human Saliva	-239 ± 11	-181 ± 21	-395 ± 24	-262 ± 6

In the porous alloys, it can be assumed that the part of the corrosion current generated during the degradation at OCP is due to the activity of pores. If selective adsorption of proteins to the areas of pores is taking place as described previously[3], this decreases the current generated by the surface and in effect decreases corrosion potential. However, additional processes are probably involved in the human saliva system since the OCP of Neptune and Rexalloy was not significantly different for AS and HS even though HS contains proteinaceous substances.

Corrosion Currents

The corrosion currents (i_{corr}) at OCP, as determined from the Tafel portions of the polarization curves are presented in *Figures 2, 3, 4,* and *5* for NP, RX, RG, and VB.

The addition of AL and MU proteins were shown to lower the corrosion rate on surfaces of medium homogeneity containing casting porosity (RX and RG). Results obtained in human saliva followed the same trend (*Figures 3* and *4*). This corresponds with the OCP trend for these alloy systems (*Table 2*). RX and RG experienced significantly higher corrosion currents than NP. That can be expected considering the higher Cr and Mo content in NP compared with RX and RG (*Table 1*).

Contrary to expectations, the magnitude of corrosion currents generated by RX and RG were higher than those measured in VB. This can easily be attributed to the porosity present in RX and RG as a result of the standard casting procedure. It is also likely that part of the corrosion current is generated by the activity of pores and/or localized second phases. Subsequently, the decrease of corrosion currents upon addition of proteins can be explained by selective adsorption of proteins into the areas of porosity[3].

Surfaces with extremely high homogenity (NP), where discrete anodic and cathodic sites are not present and uniform corrosion occurs by diffusion of ions through the passive film, exhibited no major changes when proteins were added. However, HS was found to increase the i_{corr} value substantially (*Figure 2*). Therefore, it is likely that human saliva contains other, probably organic, species that are active in the corrosion process when the surface of the material is covered with a homogenous passive layer.

In VB, the least microstructurally homogenous alloy system, albumin and mucin additions had the opposite effect. The additon of the proteins as well as testing in HS caused the corrosion current to rise substantially. It was shown previously that in VB the protective oxide film is poor or not present on the NiBe phase[14]. This finding was further verified by the measurement of Ni release from an alloy containing comparable amounts of the NiBe

phase found in VB using atomic adsorption spectroscopy[13]. Yang and Black reported recently on the high affinity of proteins to Ni and easy formation of its organometallic compounds[19]. Based on this knowledge, it can be hypothesized that the kinetics of the corrosion process on VB in protein containing environments is accelerated by removal of Ni atoms facilitated by the affinity of proteins to Ni ions.

Cyclic Polarization

Alloys without Be (Neptune and Rexalloy). During cyclic polarization, both Neptune and Rexalloy exhibited a wide passive region in all electrolytes *(Figures 6* and *7*). The passive current varied insignificantly between the four electrolytes, within the margin of an error. Even the breakdown potentials were largely independent of the electrolyte used. This observation is in agreement with previous research examining the influence of mucin and albumin on the polarization behavior of mercury amalgams[4], although some differences were observed between the reported behavior in Ringer's solution and human saliva. This difference could be due to organic substances[20]. Overall, the selection of electrolyte did not have a large influence on the polarization behavior of NP and RX, the more corrosion resistant alloys out of the four examined here.

It was reported in a previous paper that changes in polarization behavior on 316L SS in human saliva were observed in the potential range of 500 to 750 mV vs. SCE[3]. For NP and RX systems, the breakdown potential was around 1000 mV vs. SCE. At such high potentials the situation is complicated by the oxidation of Cr^{3+} to Cr^{6+}. Therefore, it is conceivable that the variances in the polarization behavior caused by the presence of proteins around the breakdown potential for 316L SS would be diminished in the NP and RX alloys.

Alloys with Be (Regalloy T and Vera Bond). Alloys containing beryllium exhibited significantly different polarization behavior as compared to alloys without Be. The anodic polarization profiles did not exhibit passive regions, but rather a steady increase in current with increasing potential was observed (*Figures 8* and *9*). Additionally, the presence of proteins and use of human saliva caused significant differences among the polarization profiles of these alloy systems.

The four electrolytes used here produced many variations in the shapes of polarization curves of RG. In AS, after a very short passive region, the system gave rise to a broad anodic peak at approximately 100 mV vs. SCE (0.03 A/cm^2).This peak was much less pronounced in HS and AS with AL ($8x10^{-5}$ A/cm^2) and was completely missing in AS with added MU. In order to explain these differences, one has to consider possible protein-surface interactions that can lead to the observed phenomena. Since no major differences in the cyclic polarization results were observed in NP and RX, it is first important to point out the microstructural differences between the alloys with and without Be. Even though second phases are present in both NP and RX (*Figure 1*), the surfaces possess relative homogeneity in terms of chemical composition and relatively even passive film (protective oxide) thickness[14]. This implies an even distribution of surface charge during general corrosion. On the other hand, the microstructure of RX (*Figure 1*) shows the presence of a NiBe eutectic phase embedded into the NiCr matrix. It was shown previously that the quality of a passive film present on these phases is inferior to that covering the matrix phase[14]. Such compositional variances will give rise to uneven surface charge distribution with anodic and cathodic areas clearly defined.

Proteins generally contain polar groups on their side-chains, making them likely to take part in charge-induced adsorption phenomena. Of the two proteins used as an additive to AS, mucin molecules exhibit excess negative charges[21]. The proteinaceous components may adsorb onto the anode surface and form a diffusion barrier preventing migration of corrosion products from the site, thus slowing down the kinetics of the reaction. It is also possible that proteins adsorb onto the cathodic areas, thereby preventing the cathodic reaction (reduction of oxygen) from occurring. Which one of these effects plays the major role cannot be easily determined, but it is conceivable that the charge of the organic molecule is active in local adsorption phenomena, judging from the differences observed in the polarization curves in AL and MU for RG.

The polarization profiles of VB did not exhibit statistically significant variations for AS, AL, and MU electrolytes. On the other hand, VB demonstrated significantly higher E_b in HS than in the other three solutions. Furthermore, the HS curve also showed more pronounced passivity-breakdown behavior while the current in the other electrolytes was steadily increasing with potential. These results are different from those obtained on RG. The surface of VB is more active than that of RG[14], with much larger surface areas occupied by the NiBe etutectic. Additionally, VB contains less Cr than RG (*Table 1*). Under such conditions, the "protective" effect of proteins is diminished. It is possible that the corrosion phenomena in no longer localized but uniform where both anodic and cathodic reactions are taking place in large areas.

Previously it was hypothesized that negatively charged proteins will get adsorbed inside of the pits based on electrostatic forces and thus block the transport of chloride ions into the pit[3]. Such a mechanism would hinder the kinetics of the autocatalytic process of

localized corrosion. Based on the results of this study, this mechanism is valid only for certain types of surfaces and depends on an overpotential. Beyond the breakdown potential the protein-surface interaction that influences the electrochemical behavior diminishes as is apparent from the similar shape of the repassivation curves independent of the electrolyte used. This would suggest that, once above the breakdown potential, the protein-surface interaction is disturbed. Results from this study suggest that this is one of the probable mechanisms. However, the effect of proteins is probably more complex and dependent on a number of variables.

CONCLUSIONS

Differences between the electrochemical behavior of NP, RX, RG, and VB dental alloys using human and artificial saliva with and without mucin and albumin additions exist and seem to be dependent on the alloy microstructure, composition, presence of porosity, and overpotential. The influence of proteins is apparent not only at higher overpotentials but also at OCP for alloys exhibiting porosity and therefore high corrosion currents. The mechanism of protein-surface interaction is not straightforward and should be investigated further.

Although the addition of proteins to inorganic artificial electrolytes achieves higher simulation of the electrochemistry of human saliva, substantial differences were still noticed in this study. Human saliva is a complex system and contains other species that can be active in the degradation process in a variety of ways. Using the TET technique provides an economical means of investigating the influence of these substances.

The differences among the results obtained in the four electrolytes were not uniform from alloy to alloy. Therefore, the use of human saliva as an electrolyte for corrosion studies

of dental alloys should be considered, especially when the mechanism of degradation is of interest. Future studies should address the importance of pH and compositional differences of saliva collected from different individuals on the corrosion test results.

Porosity seems to play an important role in the electrochemical behavior of NiCr casting alloys, especially in the natural electrolyte. The presence of pores seems to diminish corrosion resistance at OCP. Based on this information it would seem that restorative material processing should be optimized to avoid porosity in NiCr alloys.

ACKNOWLEDGMENTS

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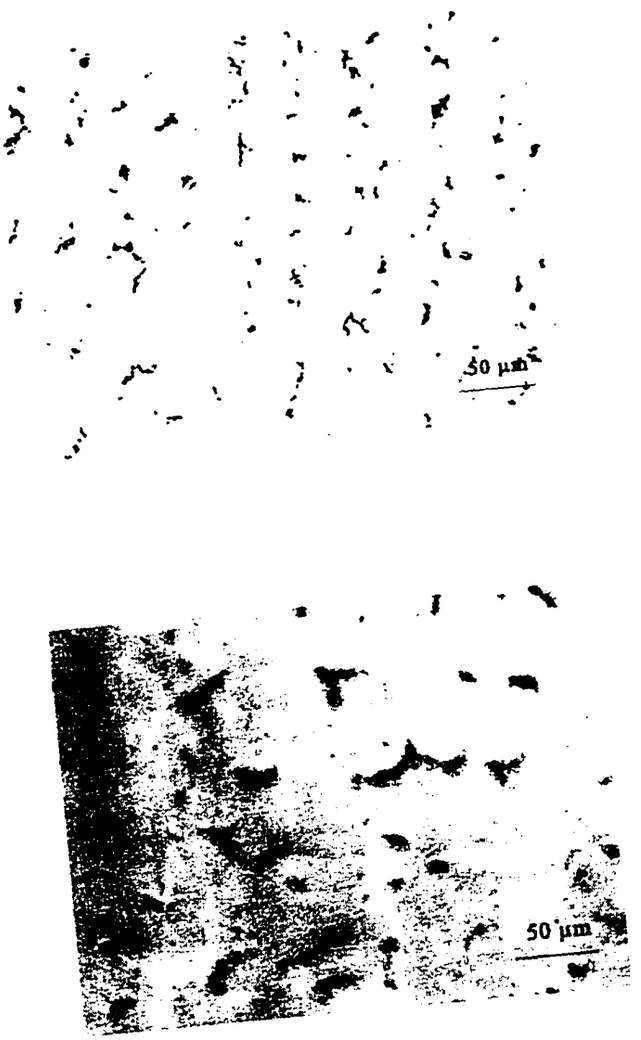

Figure 1 Microstructures of Neptune (above) and Rexalloy (below). Magnification 200X.

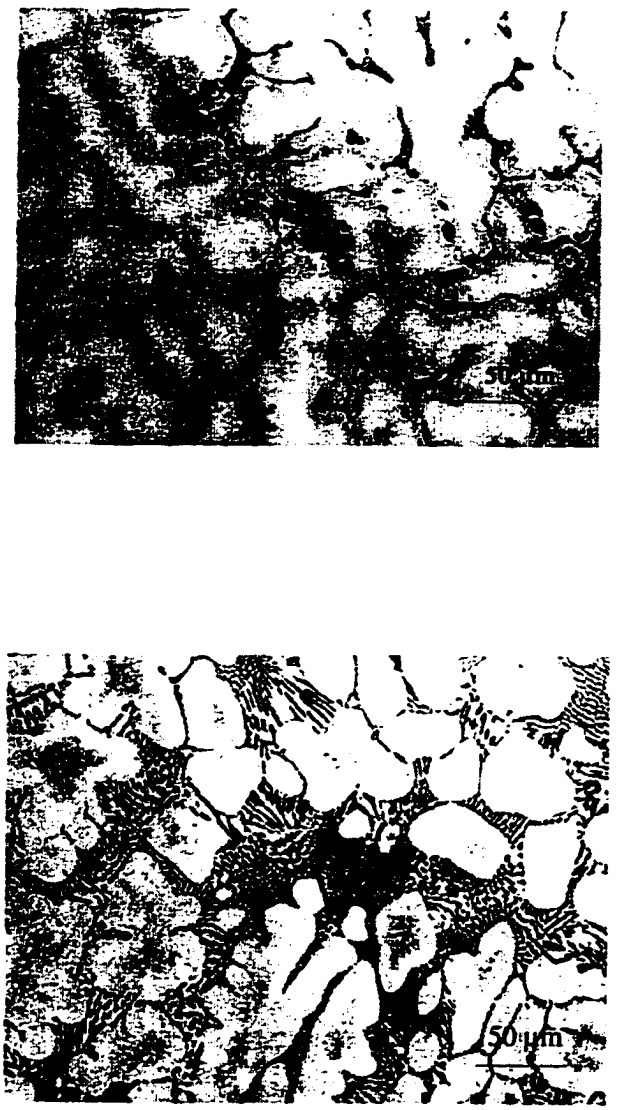

Figure 2 Microstructures of Regalloy T (above) and Vera Bond (below). Magnification 200X.

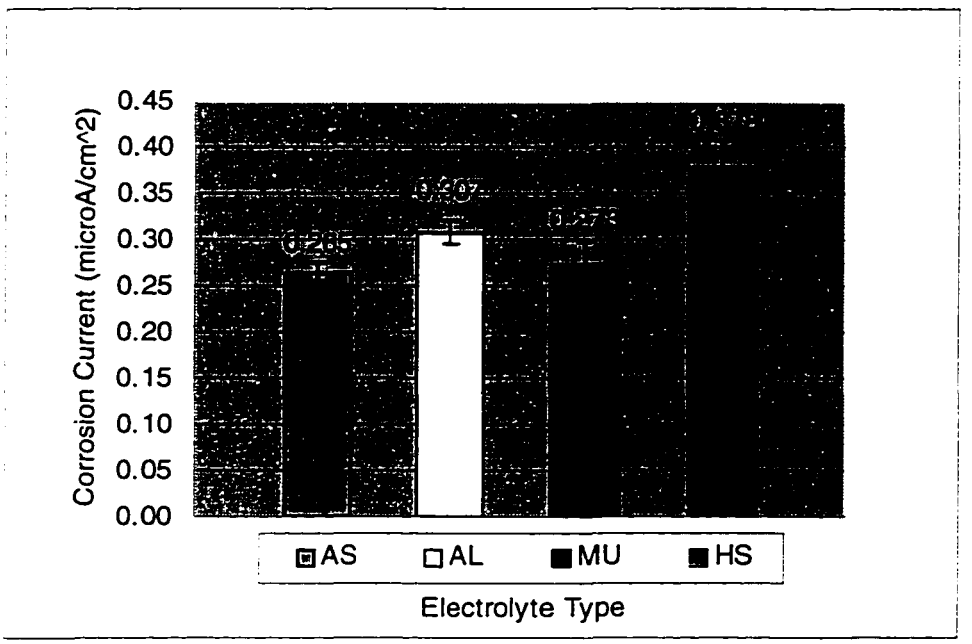

Figure 3 Corrosion currents of Neptune in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

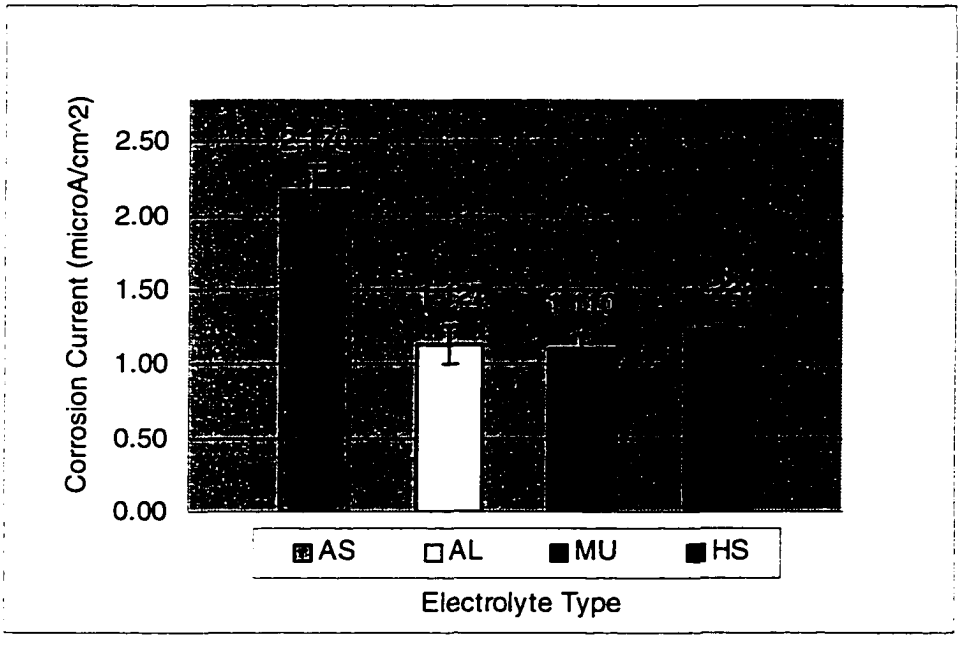

Figure 4 Corrosion currents of Rexalloy in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

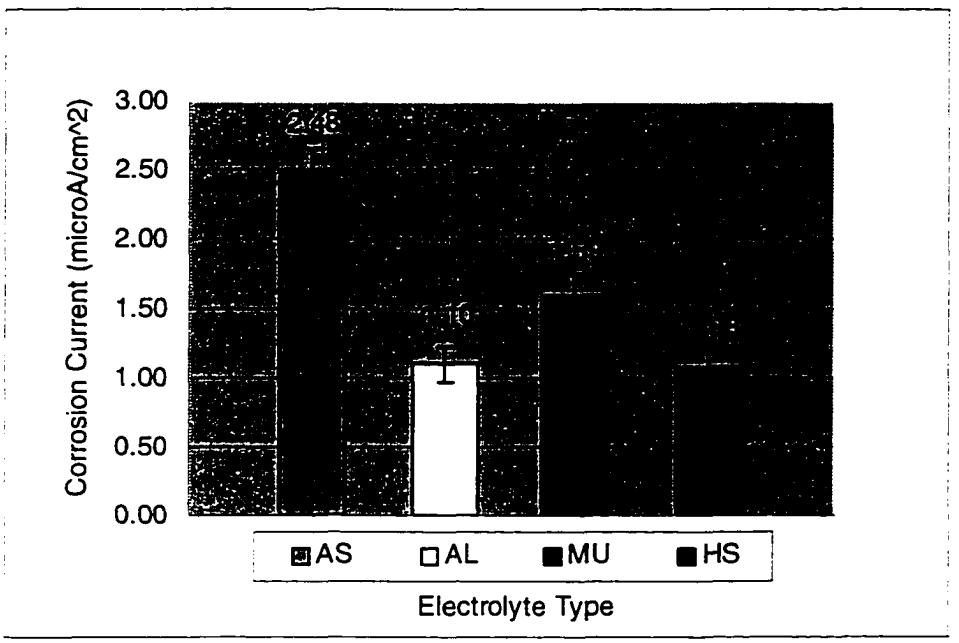

Figure 5 Corrosion currents of Regalloy T in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

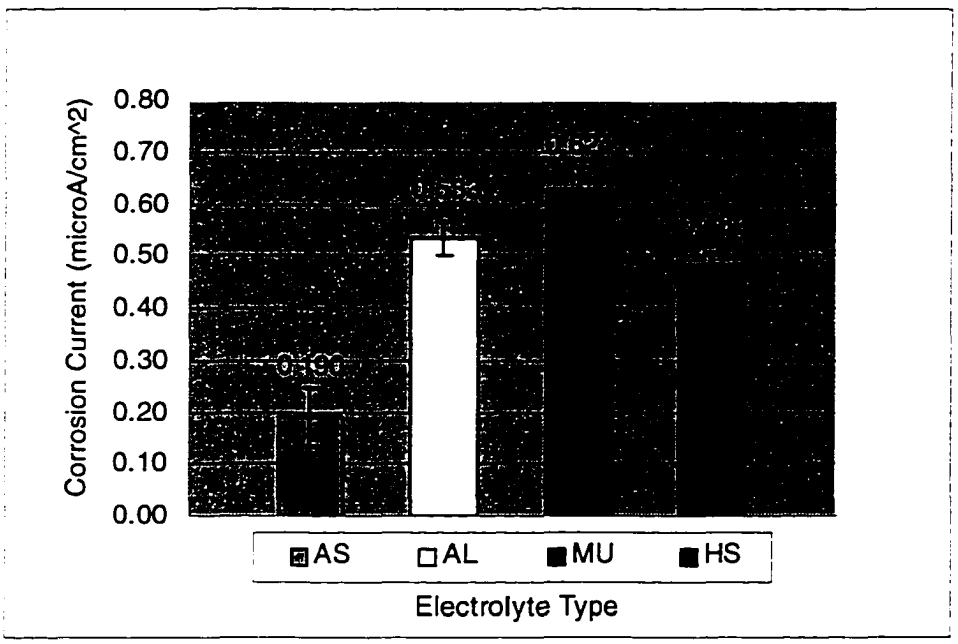

Figure 6 Corrosion currents of Vera Bond in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

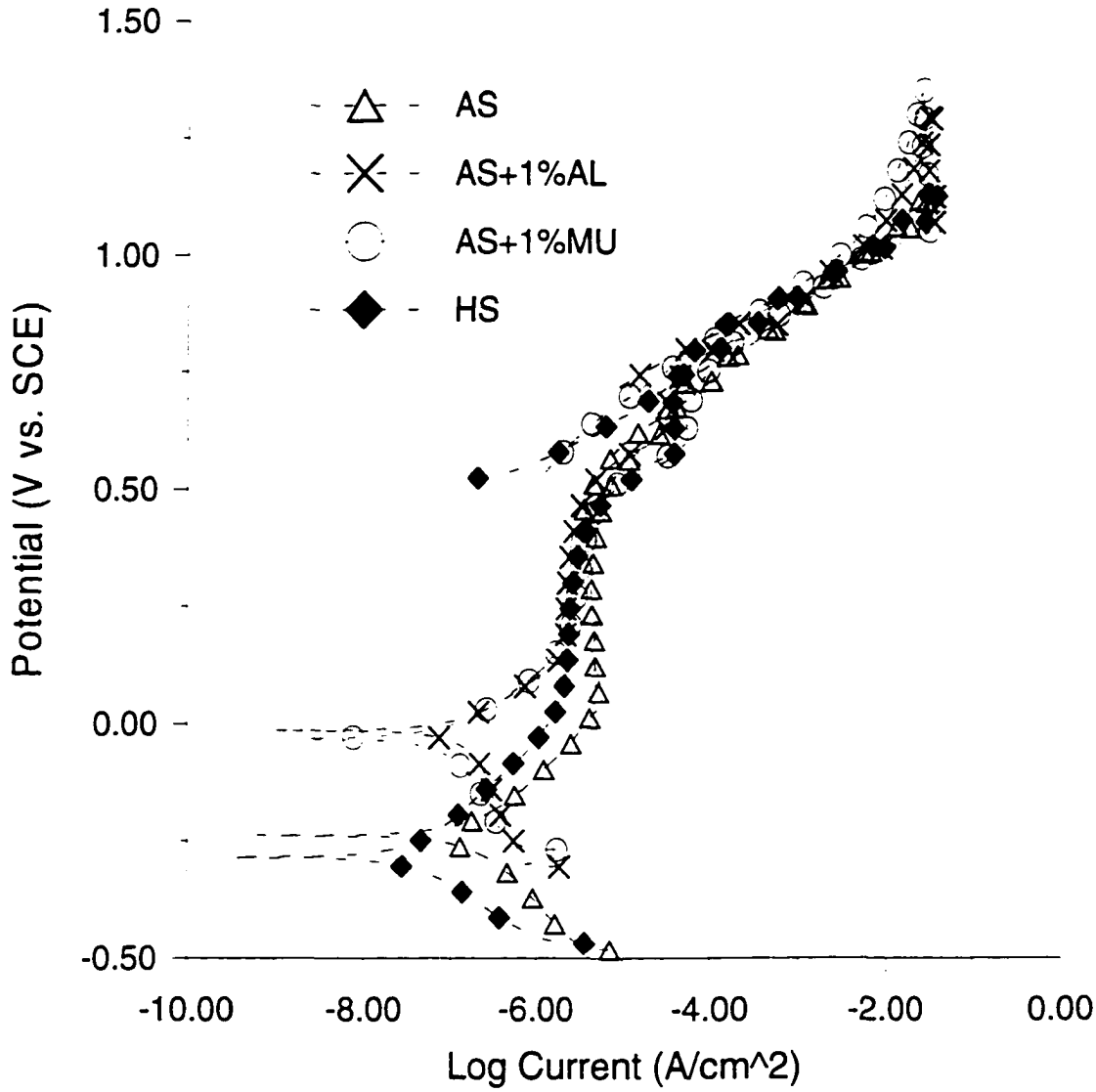

Figure 7 Cyclic polarization of Neptune in artificial saliva (AS), artificial saliva with 1% albumin (AL), artificial saliva with 1% mucin (MU), and human saliva (HS).

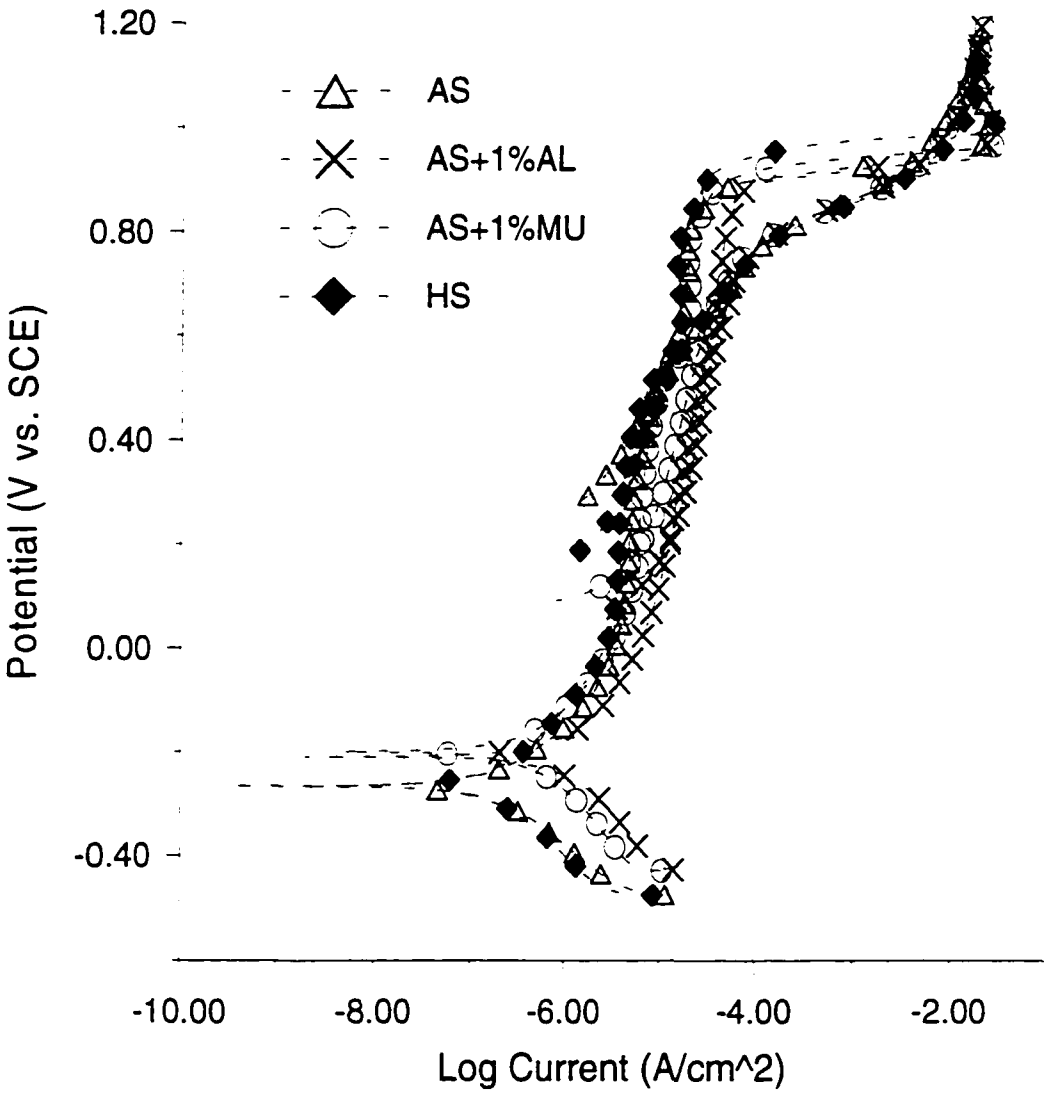

Figure 8 Cyclic polarization of Rexalloy in artificial saliva (AS), artificial saliva with 1% albumin (AL), artificial saliva with 1% mucin (MU), and human saliva (HS).

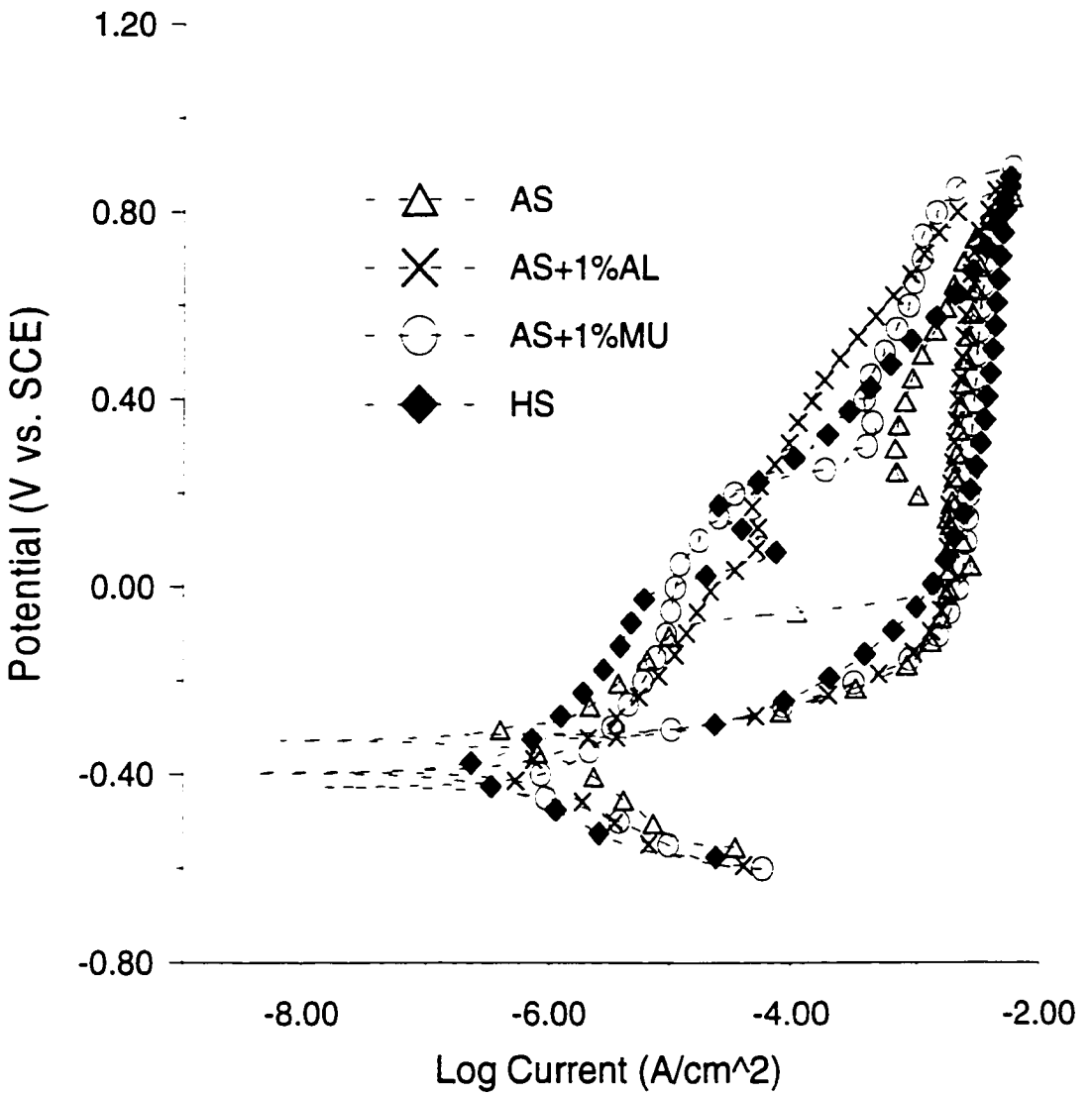

Figure 9 Cyclic polarization of Regalloy T in artificial saliva (AS), artificial saliva with 1% albumin (AL), artificial saliva with 1% mucin (MU), and human saliva (HS).

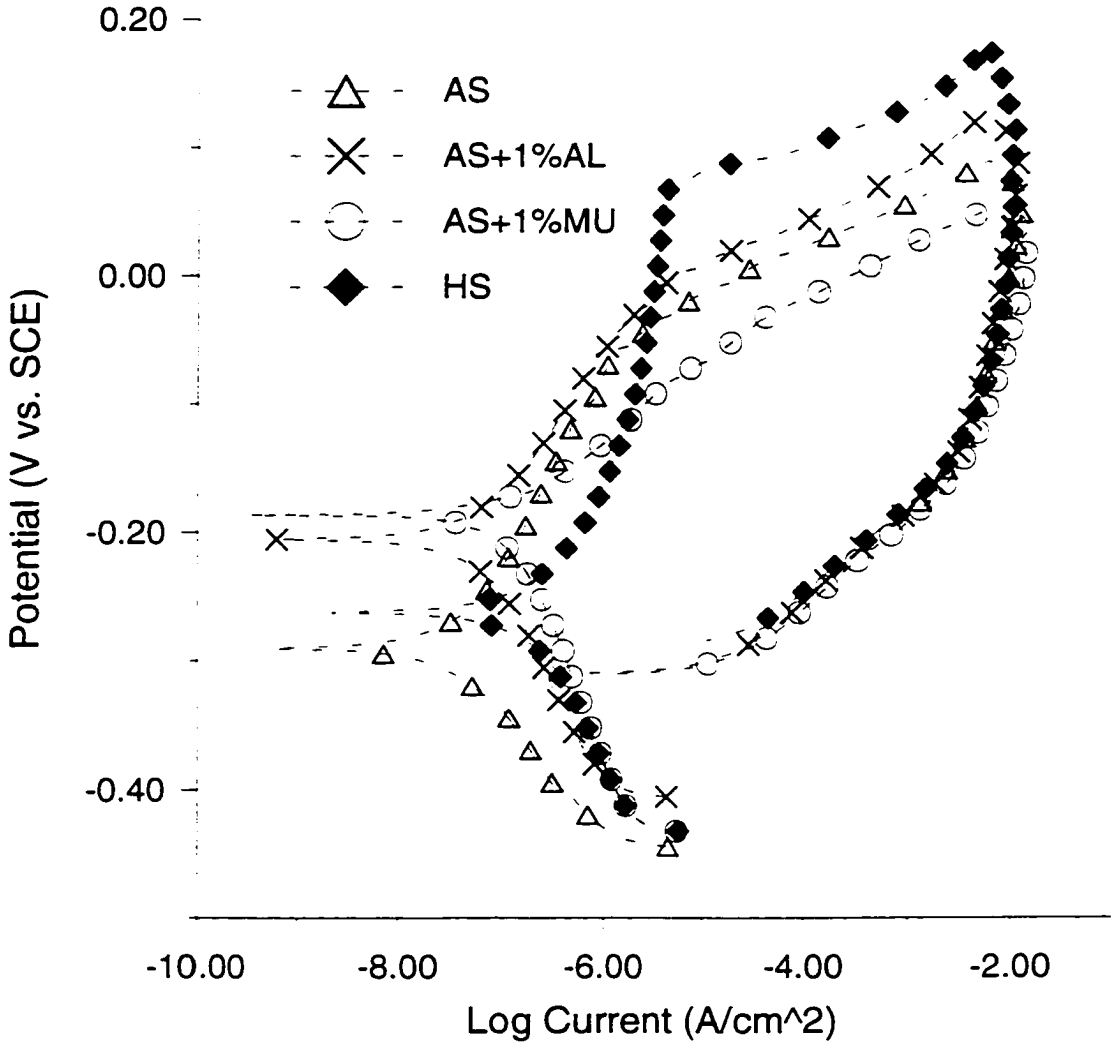

Figure 10 Cyclic polarization of Vera Bond in artificial saliva (AS), artificial saliva with 1% albumin (AL), artificial saliva with 1% mucin (MU), and human saliva (HS).

IMPEDANCE STUDY OF PROTEIN INFLUENCE ON CORROSION OF NICKEL-CHROMIUM DENTAL CASTING ALLOYS

by

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ABSTRACT

The goal of this study was to investigate the effects of proteins on the electrochemistry of dental materials. Four commercial NiCr dental casting alloys (Neptune, Rexalloy, Regalloy T, and Vera Bond) were investigated using the electrochemical impedance spectroscopy (EIS) under a thin layer of electrolyte. Artificial saliva (AS), AS with 1% of albumin (AL), AS with 1% of mucin (MU), and human saliva were the electrolytes. Polarization resistance and double layer capacitance values determined from the Nyquist plots were found to be strongly dependent on the composition and microstructure of the alloys. The addition of proteins to AS was shown to have the biggest influence on the results of alloys containing porosity. Furthermore, the results of this study reinforced the theory of preferential protein adsorption due to electrostatic interaction presented in previous work based on direct current data. Results presented in Nyquist plots indicate that corrosion was either diffusion or activation controlled depending on the alloy electrolyte combination. Equivalent circuit models were developed to describe the observed EIS behavior. This investigation provided further insight into the understanding of the corrosion mechanisms in environments containing proteins.

INTRODUCTION

In a previous study, the authors examined the effects of salivary proteins on the electrochemical behavior of four commercial NiCr dental casting alloys (Neptune, Rexalloy, Regalloy T, and Vera Bond) using direct current (DC) techniques[1]. The different alloy compositions (especially in terms of Be content) gave rise to very different microstructures. The samples also contained varying amounts of casting porosity. Artificial saliva (AS),

artificial saliva with 1 % of albumin (AL), artificial saliva with 1 % mucin (MU), and human saliva were employed as electrolytes. Significant differences were noted in corrosion current values and cyclic polarization characteristics between the four electrolytes. Experimental results were strongly dependent on the alloy composition and microstructure.

Earlier, it was shown that proteins play a significant role in electrolyte-sample corrosion interactions[1]. The presence or absence of porosity played a significant role in this relationship. It was hypothesized that proteins are attracted to the pores blocking the transport of species involved in the corrosion reaction. However, more mechanistic information is required to reinforce this theory.

Adsorption of salivary proteins was shown to influence the formation of acquired pellicle and dental plaque on enamel surfaces[2]. Recently, with the widespread use of oral implants, studies have focused on adsoption of salivary proteins to metallic surfaces. It was shown that the adsorption of salivary proteins is a very rapid process on both hydrophilic and hydrophobic surfaces of treated silicon[3]. However, the proteinaceous materials were reported to be adsorbed with varying binding strengths. Poorly coherent information exists on the relationship between the material composition and the resultant protein binding power. A study performed on dental alloys showed that protein adsorption was independent of the material used[4]. However, other research showed that protein adsorption and desorption varies for different metals[5]. Albumin was found to be the most prevalent protein adsorbed onto titanium powder[6]. In some specific cases, protein adsorption may be based on electrostatic interaction of proteins with the surface[7,8]. It follows logically that proteins should have an effect on the electrochemistry of the metallic surfaces, especially when there is variable surface charge distribution in the electrolyte.

Electrochemical impedance spectroscopy (EIS) is a suitable tool for determining both the corrosion rates[9] and the corrosion mechanisms for many systems[10]. The primary advantage of EIS over DC techniques is that by using EIS it is possible to characterize a system without disturbing the sample-electrolyte equilibrium. The technique has been used extensively in the field of biomaterials[11, 12]. In the area of dental materials, fitting the EIS data to a model has brought insight into the corrosion mechanisms of dental amalgam[13]. EIS data of an experimental NiCr alloy with differing amounts of Be illustrated the effects of localized corrosion of these materials in artificial saliva[14].

The DC data collected earlier suggested that the influence of proteins is dependent not only on composition but also on porosity[1]. In order to gain better insight into the mechanisms of the protein-surface interaction and its infuence on the electrochemical behavior of surfaces, EIS experiments were performed using the same alloy-electrolyte combinations as previously described under a thin layer of electrolyte[1]. This research investigates the EIS behavior of the electrode/electrolyte system with a focus on hypothesizing about the behavior of the double layer capacitance, the electrode polarization resistance and subsequently any adsorption/desorption mechanisms. Equivalent circuit models that schematically represent this behavior are presented.

MATERIALS AND METHODS

The NiCr alloy samples of Neptune (NP), Rexalloy (RX), Regalloy T (RG) and Vera Bond (VB) and their preparation and composition have been discussed previously[1]. Artificial saliva (AS), AS with 1% of bovine submaxillary mucin (MU), AS with 1% of serum albumin (AL), and human saliva (HS) were used as the electrolytes[1]. The thin electrolyte technique

(TET) was employed with the same setup as that used previously[15, 16]. The temperature and atmosphere of the experiments were the same as in the preceding investigation[1]. EIS experiments were performed using a model 6310 Impedance Analyzer (EG&G Princeton, Princeton, NJ, USA).

After placement into the TET cell, the samples were equilibrated at open circuit potential (OCP) for 20 min before running the EIS experiments. Scans were initiated at 100 kHz and terminated at 0.1 Hz, and the wave amplitude was $\pm$ 5 mV. Since a variety of conditions can exist in the oral environment, causing variations in the *in vivo* potential, both the protein influence at the OCP and under elevated potentials were of interest; therefore, the alloys were tested at two different overpotentials. RG (high porosity) and VB (low porosity) have been noted to lack a distinct passive behavior[1] and were therefore tested at + 20mV vs. OCP. The slight overpotential was imposed to increase the stability of the system. NP (low porosity) and RX (high porosity) were tested at 350 mV vs. saturated calomel electrode (SCE), roughly in the middle of the passive region for these alloys[1].

Triplicate experiments for each alloy-electrolyte combination were carried out. Polarization resistance (R_p) and double layer capacitance (C_{dl}) values were determined by circle fit to Nyquist plots using the M398 and Equivckt™ software. Averages with standard deviations were computed. The trends in the polarization resistance (R_p) data were compared to values of corrosion current (i_{corr}) obtained previously from DC testing[1]. Bode and Nyquist plots obtained for all the alloy-electrolyte configurations served as a base for discussion.

RESULTS AND DISCUSSION

Polarization Resistance and Double Layer Capacitance

Vera Bond (VB) exhibited the lowest R_p value at OCP in the MU electrolyte (*Figure 1*). This corresponded to the largest i_{corr} observed for this alloy in MU during the DC testing (*Table 1*). Experimentation in all protein-based electrolytes (AL, MU and HS) resulted in significantly lower R_p values than in the nonprotein-based AS electrolytes. Even though the average $1/C_{dl}$ value was the largest in the MU electrolyte, the spread of data present in the HS electrolyte precludes any estimation of statistically significant differences (*Figure 1*). The microstructure of VB contained the largest amount of second phases out of the four alloys tested[1, 17]. Furthermore, it was shown that the protectivity of the passive film covering the NiBe eutectic phase is inferior[17]. High binding ability of proteins to metallic ions, especially to those of Ni, has been reported by others[18]. Considering the high activity of the surface and therefore low energy necessary to remove Ni and Be from the eutectic surface of VB, it is conceivable that the metastable equilibrium gained in AS (and accompanied by high R_p values) is disturbed by the affinity of proteins to Ni. The result is higher i_{corr}. Higher values of $1/C_{dl}$ in MU compared to AL could be caused by massive adsorption of mucin based on charge attraction between negatively charged protein molecules and the anodic sites on the surface.

Another alloy tested near its OCP was RG. It exhibited a significantly decreased R_p value when tested in the AS electrolyte (*Figure 2*). The highest R_p values were exhibited in the MU electrolyte, and there was no significant difference between the values exhibited in the AL and HS electrolytes. This rank order again agrees with the one observed in the i_{corr} measurements made during DC testing (*Table 1*). The alloy also exhibited a significant deviation in phase angle measured at low frequencies (*Figure 3*) indicating a profound

susceptibility to localized corrosion[14, 19]. The calculated value of $1/C_{dl}$ (thickness of double layer) was also the lowest when tested in the AS electrolyte (*Figure 6*).

Table 1 Corrosion current of Neptune, Rexalloy, Regalloy T, and Vera Bond alloys exhibited in artificial saliva (AS), AS with 1% albumin, AS with 1% mucin, and human saliva (HS)[1]

Alloy	Neptune	Rexalloy	Regalloy T	Vera Bond
Electrolyte	Corrosion Current at OCP (μA/cm^2)			
AS	0.26 ± 0.01	2.48 ± 0.17	2.17 ± 0.16	0.25 ± 0.06
AS + 1% albumin	0.31 ± 0.01	1.10 ± 0.13	1.12 ± 0.13	0.53 ± 0.03
AS + 1% mucin	0.27 ± 0.03	1.59 ± 0.15	1.11 ± 0.1	0.62 ± 0.05
HS	0.38 ± 0.03	1.08 ± 0.11	1.22 ± 0.04	0.48 ± 0.02

The surface of Regalloy T has been characterized previously. Its microstructure revealed a significant presence of a second phase and casting porosity. Also previously, it was hypothesized that the mucin moleclules exhibit an active interaction with positively charged areas (anodes) on the alloy surface based on electrostatic forces[1]. This theory is reinforced by significantly larger $1/C_{dl}$ (proportional to double layer thickness) values in MU compared to AL that is not known to possess a strong charge (*Figure 2*). The $1/C_{dl}$ value in HS was found to be between those for MU and AL but not significantly different from each other. There is probably no heavy accumulation of protein of the surface when only AL is present judging from the minor differences in $1/C_{dl}$ and R_p values between the AS and AL electrolytes.

The data for Neptune (NP) were collected at 350 mV vs. SCE, approximately in the middle of the passive range[1]. No notable difference was found among the R_p values obtained

in AS, AL and MU (*Figure 4*). This corresponds very well with the trend in i_{corr} at OCP obtained previously using DC techniques (*Table 1*). The inverse capacitance ($1/C_{dl}$) values for NP are also significantly lower in HS (*Figure 4*). Graphically, it is obvious from the Bode phase angle plot for NP (*Figure 5*). The HS curve presents a very strong deviation from the capacitive behavior at lower frequencies. The decreased R_p value connected with the low frequency phase behavior implies that this alloy is more susceptible to degradation in HS. There is a question if this would imply higher susceptiblity to localized corrosion since no differences were observed in the cyclic polarization curve for both cases[1]. The alloy probably experiences higher passive current density due to character of the HS electrolyte.

Comparing the i_{corr} values at OCP with the R_p data (*Figure 4* and *Table 1*) at 350 mV vs. SCE, it could be that the increased potential does not have an influence on the relative magnitude of the protein effects on surfaces with a homogenous passive layer. However, the low R_p values in HS signal that increased potential has an effect on the aggressiveness of species other than AL and MU also present in HS.

Rexalloy, tested at 350 mV vs. SCE and containing casting porosity[1] manifested lower R_p values in AS then in the other electrolytes, where there was no significant difference among the values (*Figure 6*). This trend once more corresponds to the icorr values found in DC tests (*Table 1*). The capacitance values (*Figure 6*) had a substantial spread, probably due to the nonuniformity of the porosity distribution. This occurred even though care was taken to place the cell at the exact location on the sample surface. The phase shift plot for RX (*Figure 7*) showed a slightly larger decrease in phase angle in the capacitive region (low frequency end), indicating the presence of a localized form of corrosion[14, 19]. Considering the porosity in RX, this tendency can be translated into an elevated activity of the pores in AS.

This trend, however, is very slight and was not observed on the Bode impedance plot (*Figure 7*). It can be hypothesized that, at higher overpotentials, the effects of proteins on the surface with porosity diminish and are probably overcome by increased driving force for localized corrosion even in the passive state.

Probable Mechanisms/Models

The Nyquist plots for Regalloy T and Vera Bond (*Figure 8*) show a deviation from ideal charge transfer control behavior. Both alloys exhibit a large amount of the eutectic NiBe phase in their microstructure. Regalloy T also has minimal porosity in the second phase. Considering the microstructure of these alloys as well as the results obtained, it could be appropriate to describe the EIS behavior of these systems based on a the presence of strongly adsorbed intermediate species. A model for this is shown in *Figure 9* and was proposed by a number of authors[20-23]. It has been hypothesized that highly charged proteins may adhere to specific anodic sites on the microstructure during corrosion testing[1, 16]. The larger $1/C_{dl}$ values observed in the MU and HS electrolytes for both these alloys indicate a protein layer may be adsorbed onto the samples. The adsorbed layer might contribute to the deviation from the ideal charge transfer behavior projected by the Randles equivalent circuit model.

Nyquist plots for Neptune in AS, AL and MU exhibit a very significant diffusion control behavior in the low frequency segment (*Figure 10*). The Nyquist plot for the alloy in HS exhibits a very reduced R_p and no diffusion control. The Nyquist plots for Rexalloy exhibit what appears to be a transmission boundary diffusion control behavior at the low frequency segment (*Figure 11*). The behavior of both these alloys (except for Neptune in HS)

can be summarized to be a single step charge transfer reaction in the presence of diffusion. The model shown in (*Figure 9*) might be very representative of the behavior exhibited by the systems[24].

CONCLUSIONS

The influence of proteins depends not only on the composition and microstructure of NiCr alloys but also on porosity. The presence of proteins on porous surfaces leads to higher impedance at low frequencies, indicating lower susceptibility of the alloy to localized corrosion. Electrostatic interaction probably plays an important role in the protein-induced inhibitive mechanism judging from the pronounced effects in the MU electrolyte.

The presence of proteins in the electrolyte does not have a significant effect on alloys with relatively homogenous microstructures and stable passive films. However, for alloys with a second phase that leads to relatively low passive layer stability, the corrosion resistance drastically decreases with the addition of proteins. It is highly probable that, under such circumstances, the affinity of proteins to certain ions adds a link to the transfer of corrosion products away from the surface.

In some cases, the behavior of HS cannot be fully explained from results obtained in AS with protein additions. Therefore, other species that have the ability to influence the aggressiveness of the electrolyte must be present in saliva. Subsequently, using artificial electrolytes can lead to errors when trying to determine the exact ion release rates or study the mechanism of corrosion reactions on dental materials.

Equivalent circuit models were found in the literature that are appropriate for modeling the behavior of the sample-electrolyte interface of NiCr alloys. Subsequent

investigation will attempt to fit the data into the model and further confirm the conclusions of this study. Future efforts in this area will also involve direct observation of protein adsorption using the atomic force microscopy.

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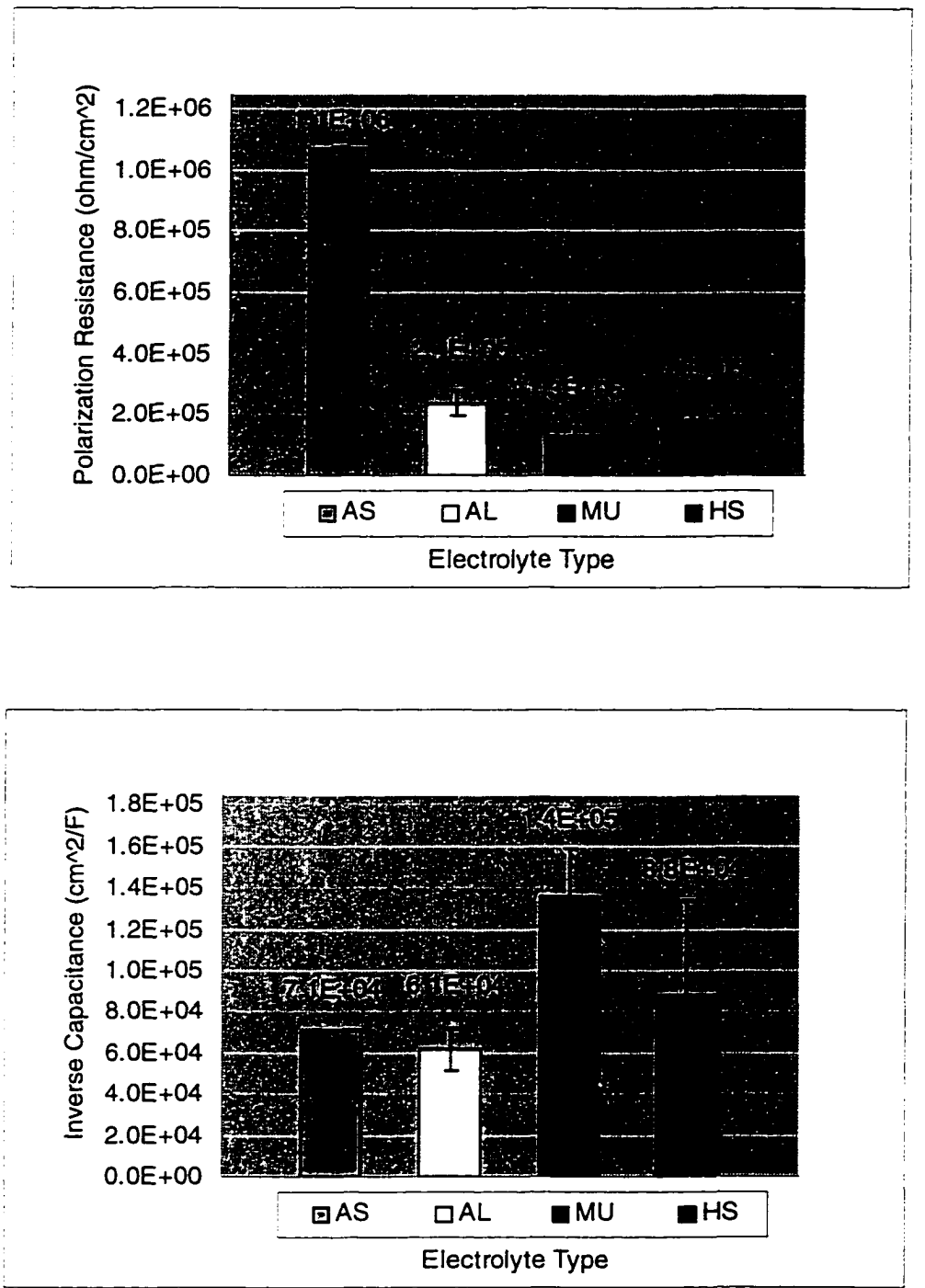


Figure 1 Polarization resistance (above) and inverse capacitance (below) values of Vera Bond in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

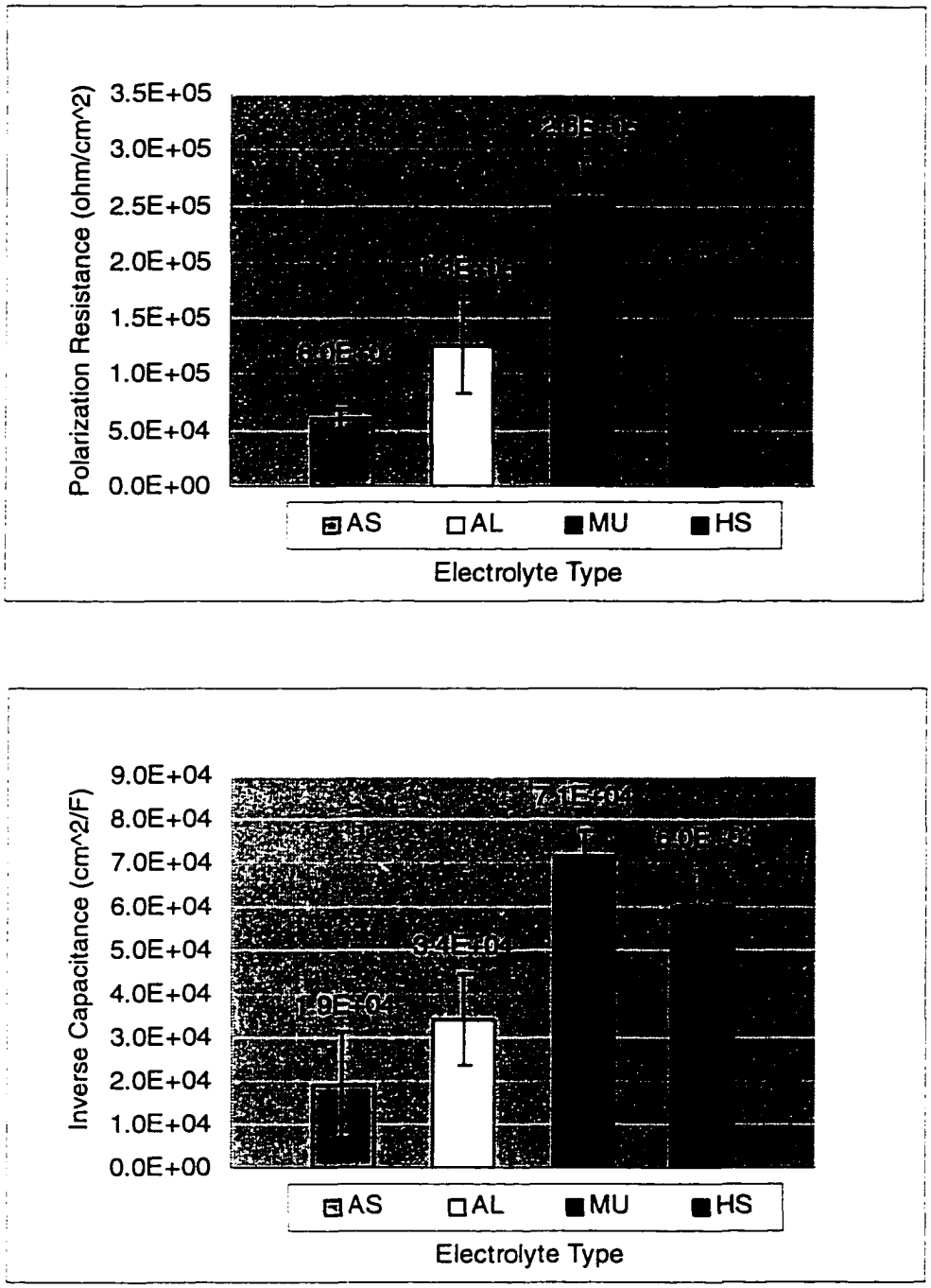


Figure 2 Polarization resistance (above) and inverse capacitance (below) values of Regalloy T in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

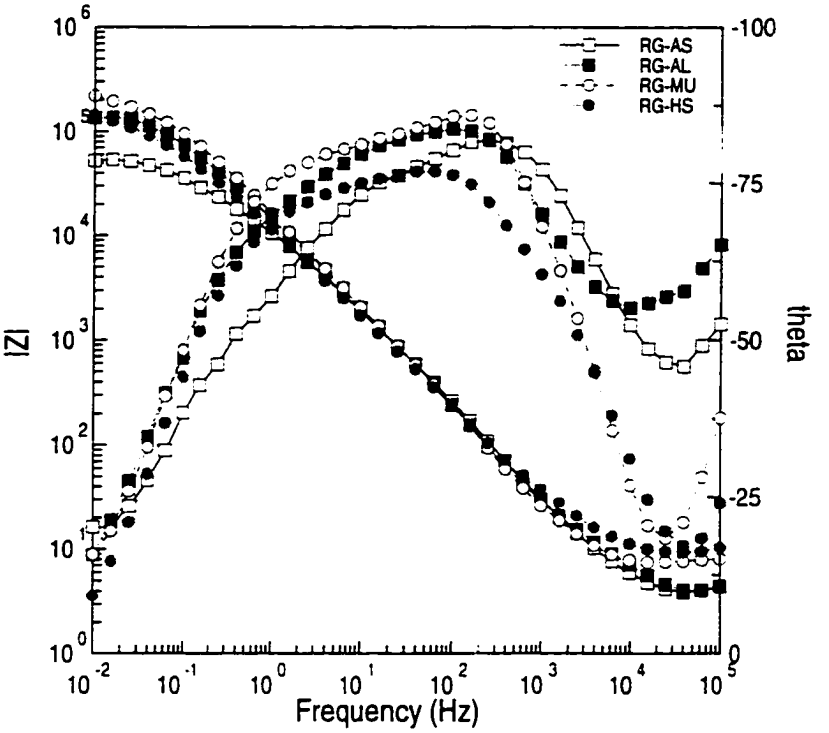
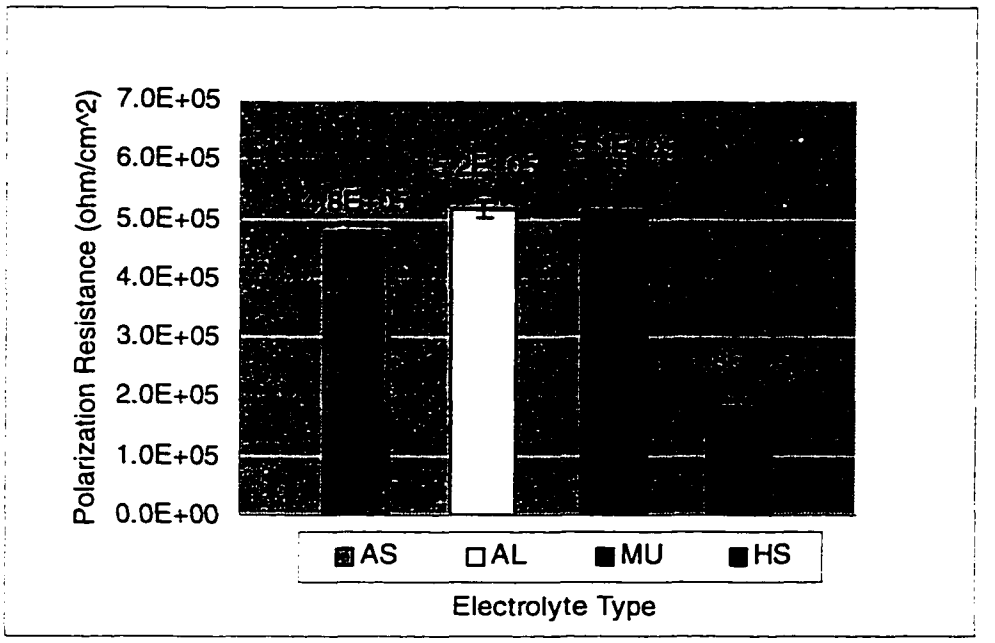


Figure 3 Bode impedance and phase shift plots of Regalloy T in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).



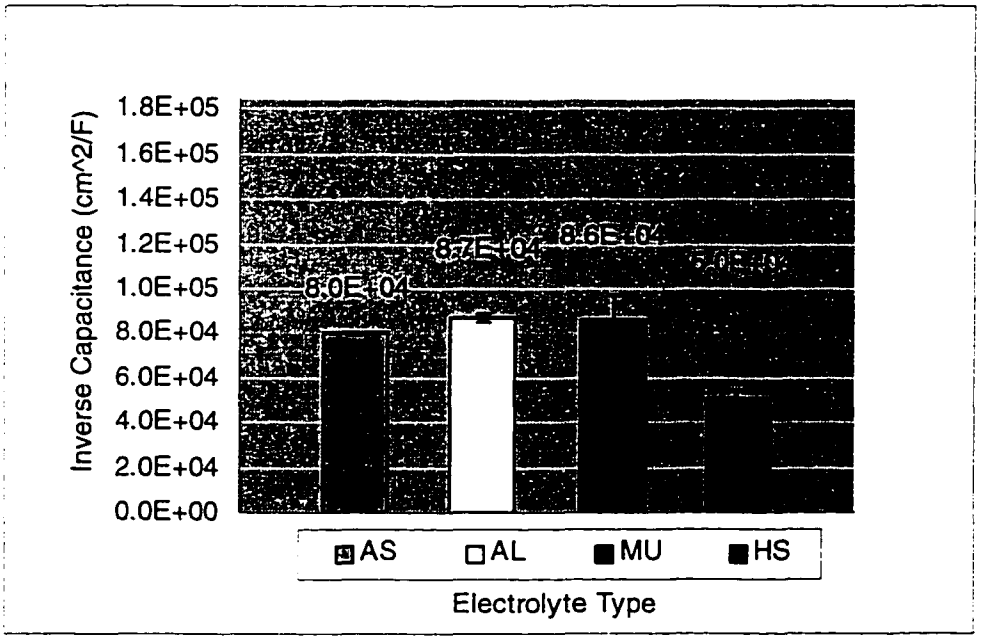


Figure 4 Polarization resistance (above) and inverse capacitance (below) values of Neptune in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

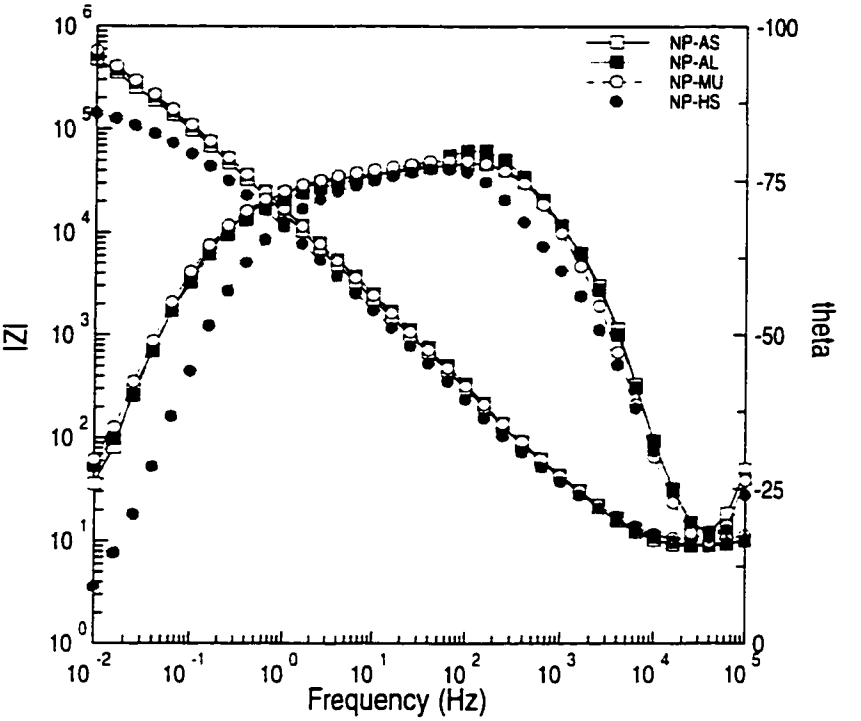
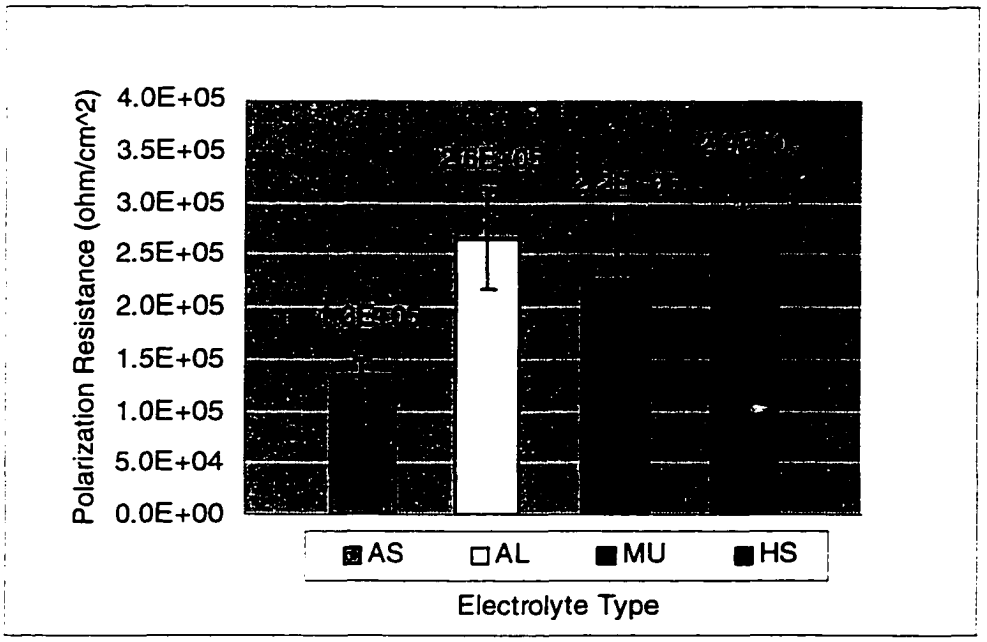


Figure 5 Bode impedance and phase shift plots of Neptune in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).



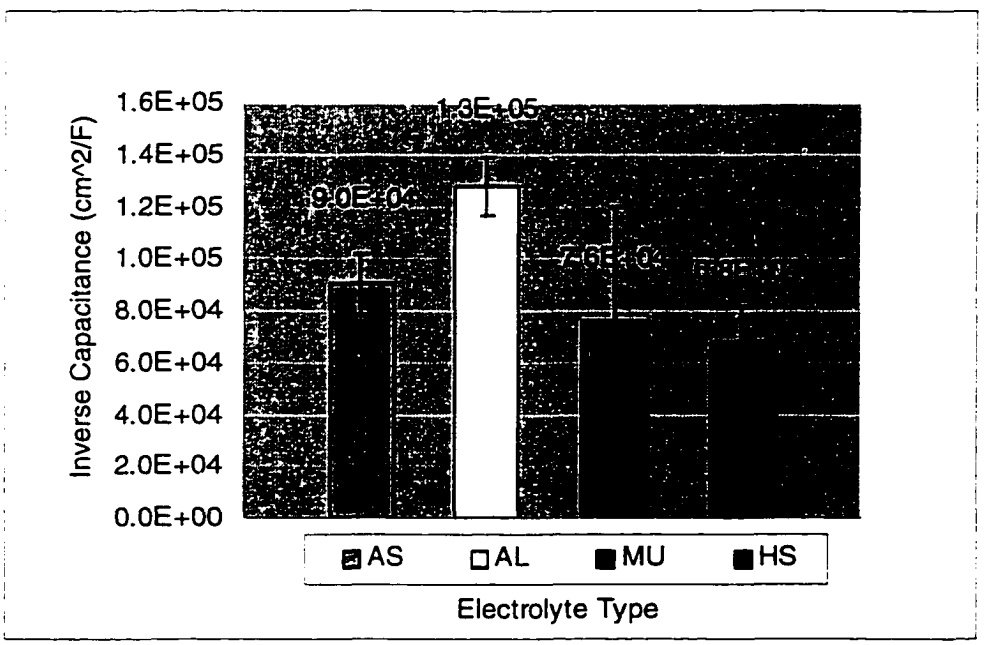


Figure 6 Polarization resistance (above) and inverse capacitance (below) values of Rexalloy in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

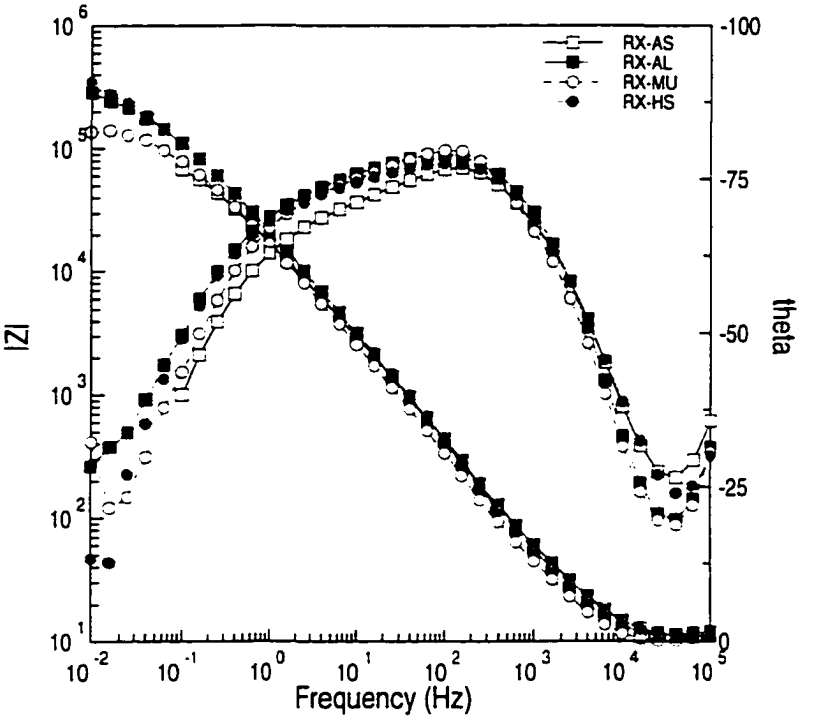


Figure 7 Bode impedance and phase shift plots of Rexalloy in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

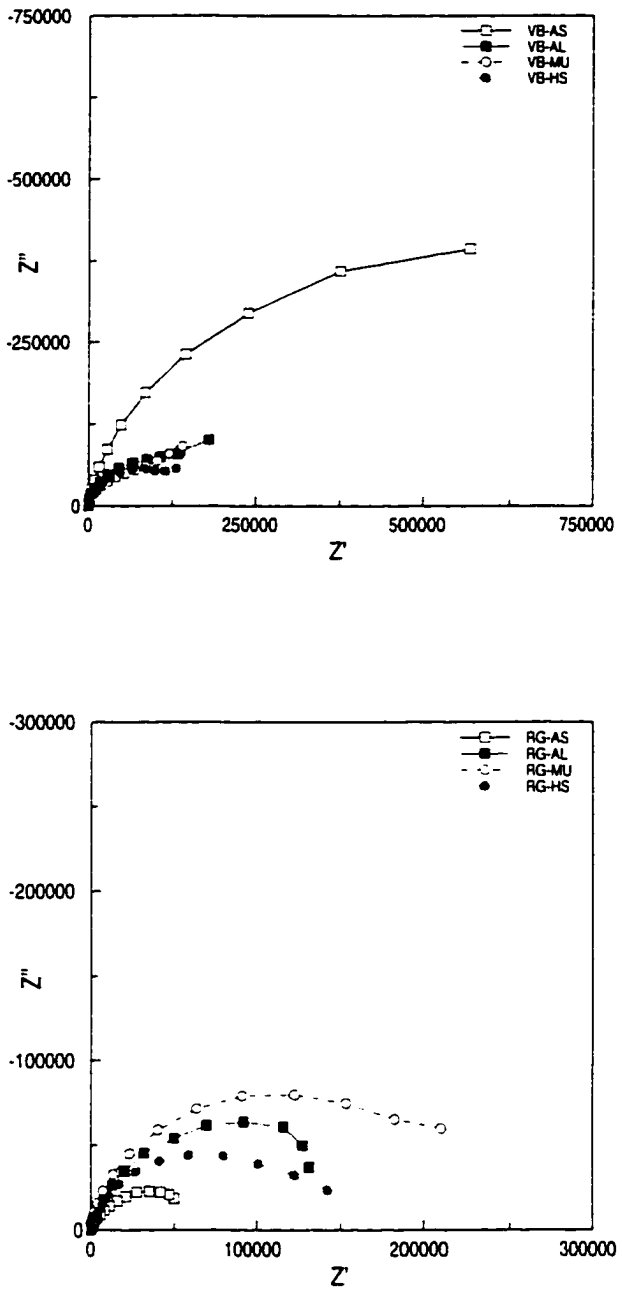


Figure 8 Nyquist plots of Vera Bond (above) and Regalloy T (below) in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

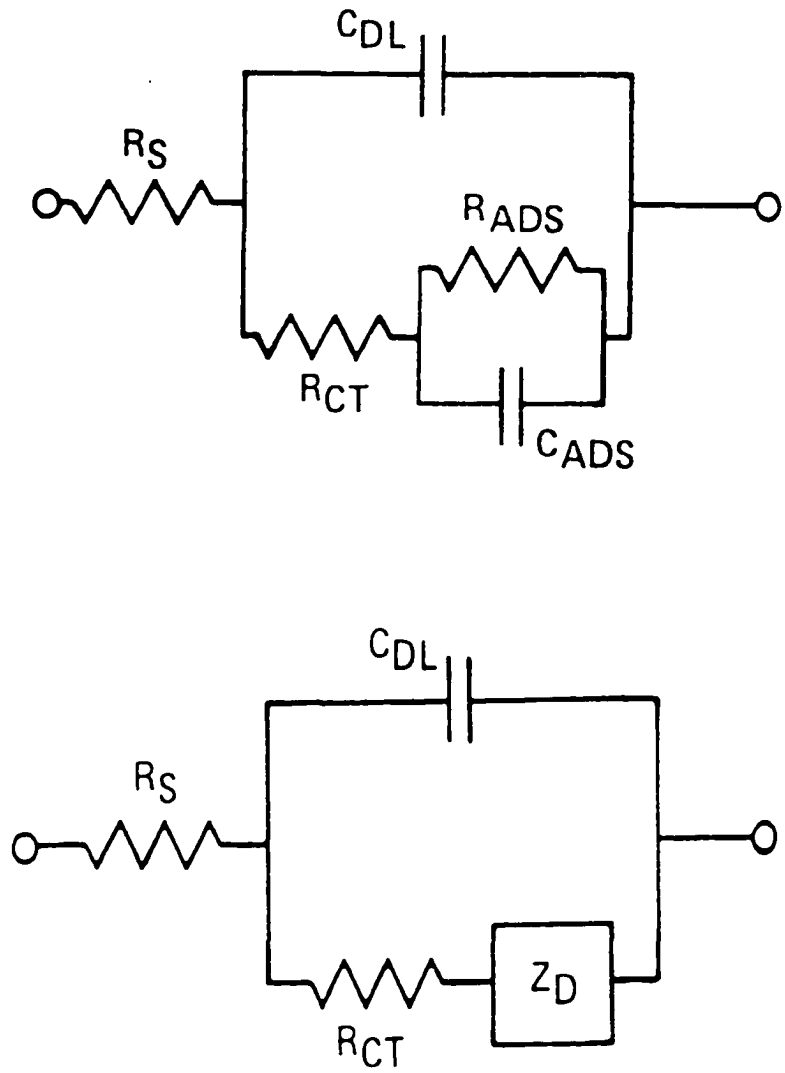


Figure 9 An equivalent circuit describing reaction involving a strongly adsorbed intermediate (above) and Randles equivalent circuit involving a single-step charge-transfer process with diffusion of reactants and/or products to the interface. R_S - solution resistance, C_{DL} - double layer capacitance, R_{CT} - charge transfer resistance, R_{ADS} and C_{ADS} - resistance and capacitance due to adsorbed species respectively, and Z_D - diffusion impedance.

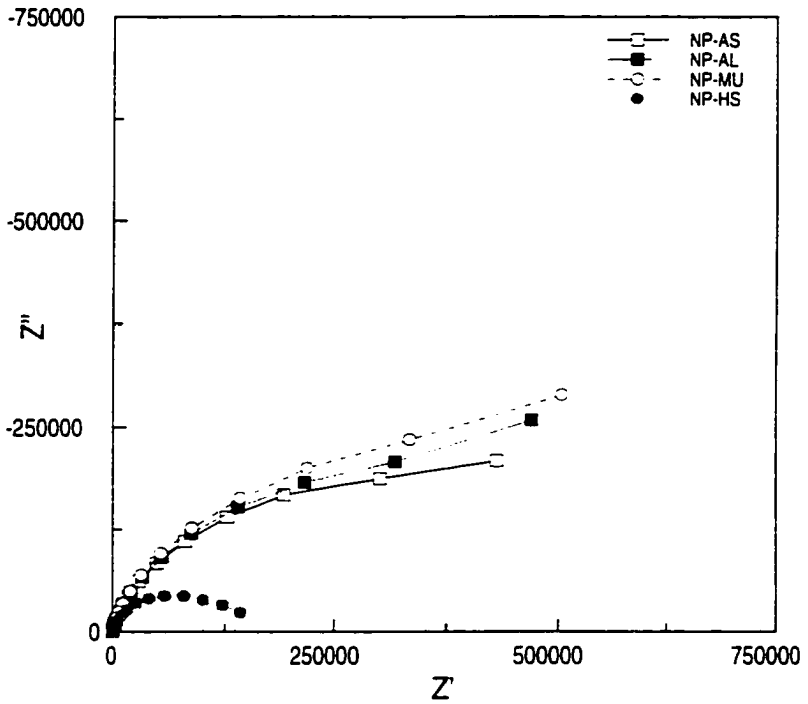


Figure 10 Nyquist plot of Neptune in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

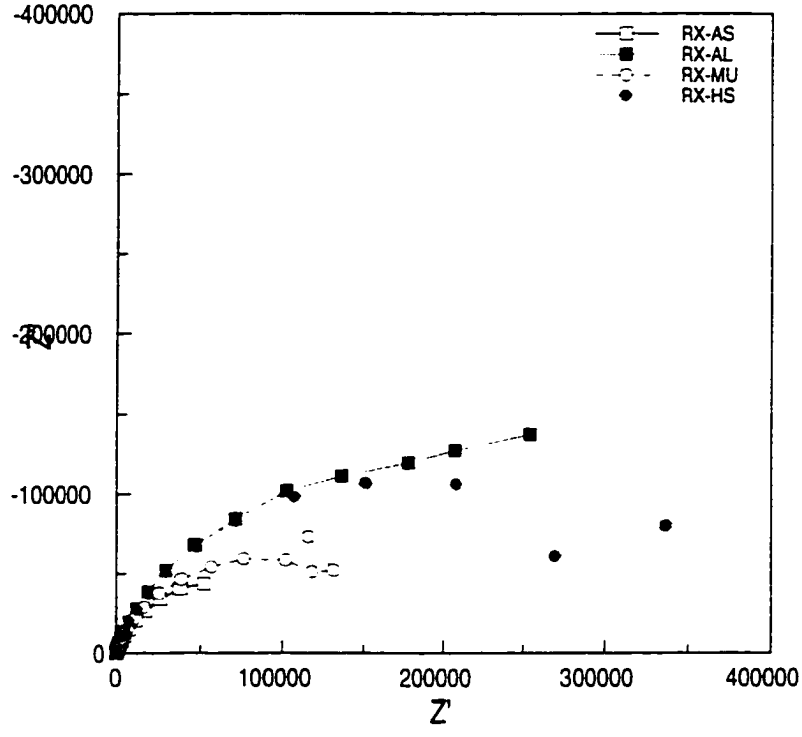


Figure 11 Nyquist plot of Rexalloy in artificial saliva (AS), AS with 1% albumin (AL), AS with 1% mucin (MU), and human saliva (HS).

DISCUSSION

The overall goal of this study was to better simulate the oral environment in electrochemical testing of dental materials. A number of previous studies concerning the electrochemistry of metals under atmospheric conditions indicated that the corrosion behavior of ferrous materials was very different under a thin electrolyte layer as opposed to complete immersion in a bulk electrolyte[14,15].

Similarly, dental casting alloys could be expected to exhibit different behavior in the oral cavity than those measured in the typical testing environment of 1 l of electrolyte. Additionally, a low volume technique would eliminate problems associated with the use of human saliva in a classical testing setup[10].

Thin Electrolyte Layer Technique--Characterization

The technique described in the Introduction and Paper I of this study--the TET--has been determined as suitable for the purpose of this study. Even though TET has been used before in the area of atmospheric corrosion research [17-20], electrochemical characterization of the TET cell has not been performed. Because oxygen is the major reducing species in physiological environment corrosion[16], the first objective of the study was to determine the oxygen concentration on the sample surface under the TET conditions. Cathodic polarization of a bright Pt electrode was performed in both TET and BET situations in a borate buffer solution (pH = 6.8). In the BET experiments, buffer of different oxygen concentrations was

used. This technique was chosen because it had been proven in the literature[15] and did not require direct measurement of oxygen concentration.

The OLCD values for each electrolyte-technique combination were determined from cathodic polarization curves (Paper I, *Figure 2*). The ratio of OLCD values under TET and BET (no aeration during the experiment) was found to be 1.7. That means that there is roughly half the oxygen available for reduction under the TET conditions, using the same electrolyte. This result has a practical importance. The two-fold difference in oxygen availability can result in significantly different corrosion values. This was clearly indicated in the data obtained from electrochemical experiments on 316 L SS surfaces. The OCP development with time, OCP values, and potentiodynamic profiles (Paper I, *Figures 3* and *4*) of 316L SS differed significantly when using TET and BET in saline of the same initial oxygen concentration.

One of the obvious concerns when using small electrolyte volumes for electrochemical testing is the possible pH change experienced by the electrolyte during the experiment due to electrochemical reactions. Therefore, experiments should be ideally carried out in buffered solutions. In order to estimate the pH change during an experiment, the molar quantity of OH^- ions formed during the experiment was calculated from Faraday's law. The calculation was based on an average current flowing through the cell and the length of the experiment. The pH change was estimated to be 1.87×10^{-5} for borate buffer and 0.042 for artificial saliva. Even though these changes are negligible, attention should be paid to electrolyte selection and knowledge of its buffering capacity.

Cathodic polarization as a tool for determining oxygen concentration is quite precise and has been used by others[15]. Supplemental data, possibly having higher quantitative

meaning, could be obtained by using an oxygen sensitive microelectrode. This technique, however, was found to be cost prohibitive and therefore was not used in this study.

Implementation of Human Saliva

Once TET was characterized, the next logical step in the oral simulation improvement was to implement human saliva as an electrolyte and compare the results to those obtained in artificial saliva solution. Because plentiful research data have been collected for 316 L SS in biological environments containing serum proteins[22-26], this material was used for this part of the study. Artificial saliva used throughout this study was reported to exhibit very close behavior to human saliva[3]. As for the human saliva, the exact composition was not determined, but it was assumed that its content was within the limits reported elsewhere[1,2]. In order to increase the buffering capacity of both artificial and human saliva and to mimic conditions inside an oral cavity, the TET setup was enclosed inside a chamber with a regulated atmosphere of humidified air containing 10 vol% CO_2 at 37°C.

The experimental data showed more OCPs and lower polarization resistance values (R_p) in human saliva than in artificial saliva (Paper II, *Table 1*). The effect on the OCP value was similar to that observed previously when mercury amalgams were tested in bulk human saliva[10].

Finkelstein and Greener listed a series of possible reasons for the observed differences between results obtained in Ringer's solution and Ringer's solution containing proteins[42]. One possible mechanism suggests that organic molecules (proteins) adsorb onto the surface, slowing down both the anodic and cathodic reactions. No further details of this mechanism were described. However, in this study, the corrosion rate of 316L SS was found

to be faster in artificial than human saliva, suggesting that protein action on an alloy surface is very complex. This suggestion was also proposed by Marek based on a wide literature review[9]. Potentiodynamic profiles obtained in artificial and human saliva were found to be significantly different in this study, especially in breakdown potential values. This finding is in contradiction with the results of Finkelstein and Greener who did not report any notable differences in polarization behavior of mercury amalgams in solutions with and without protein additions[42]. These results indicate that, in alloy systems, proteins are active not only at OCP but also at elevated potentials. Because potentials of dental restorations can assume different values depending on the environment and possibility of galvanic contact in the oral cavity, this finding is of a practical importance.

The results can be explained based on the ability of proteins to form complexes with metallic elements frequently contained in biomedical alloys. This suggestion is founded on information in the literature regarding the electrochemical behavior of orthopaedic alloys in serum containing environments and in similar compositions of serum and saliva[22-26]. Comparing the results of this part of the study with available references, it was assumed that the action of proteins most likely depends on factors such as chemical composition, presence of second phases in the microstructure, electrochemical differences between the phases and subsequent surface charge distribution. Authors were aware that the chemical complexity of human saliva prohibits deconvolution of the effects of any individual organic species contained in saliva and therefore places limitations on data analysis. Therefore, the next part of this investigation involved testing in well characterized proteinaceous solutions as well as in human saliva.

Electrochemical Behavior of NiCr Dental Alloys in Human Saliva and Proteinaceous Artificial Saliva

Samples of NiCr alloys (Neptune, Rexalloy, Regalloy T, and Vera Bond) were cast according to the manufacturer's recommendation at a commercial dental lab. Samples were found to contain varying amounts of porosity as a result of the casting procedure and subsequent processing. This resulted in another variable, in addition to compositional and microstructrual differences among the alloys. It is apparent that casting porosity is a frequent occurrence in these dental casting systems, and its influence has not been fully addressed. In the data analysis, the porosity was treated as a microstructrual feature giving rise to inhomogeneous charge distribution on the surface during corrosion.

From the analysis of the results, it appeared that proteins have a positive influence on the corrosion rate in alloys that exhibited porosity. The corrosion rate of Neptune, an alloy with homogenous passive film[33], was not affected by the addition of proteins (Paper III, *Figure 3*), and exposure of Vera Bond that was shown to be covered with an unstable passive film[33] in artificial saliva containing proteins resulted in an increased corrosion rate (Paper III, *Figure 6*). The potentiodynamic polarization tests showed inconsistencies among the behavior of the four alloys (Paper III, *Figures 7-10*). Alloys without Be did not seem to be influenced by the addition of proteins, whereas proteins had a positive effect on the corrosion properties of alloys containing Be.

The samples used in this study were of slightly different bulk composition. Surface porosity was not characterized, nor the surface charge measured. Therefore, quantitative results could not be obtained. However, the strength of qualitative conclusions of protein-surface interactions made at this point can be based on the assumptions listed below.

First, kinetics of electrochemical reactions in protein containing environments partially depends on adsorption of the proteins to the surface in question. However, adsorption of albumin (thought to be an important factor in the corrosion process) was not shown to be significantly different on hydrophobic and hydrophilic surfaces[43]. Additionally, the quantity of protein adsorption was not very different when studied on various dental materials[44].

Secondly, the surface energy on which the protein adsorption depends would not change significantly from one examined NiCr alloy to another, considering their similar composition and surface finish. Nevertheless, it remains questionable whether the negative trends in protein containing solutions found in the case of Vera Bond are due to differences in surface tension or due to the fact that surface atoms are exposed to protein molecules and therefore readily form complexes. Future investigations can include clarification of the relationship between the quality of passive film, surface energy, and the protein effects on the electrochemistry of the system.

The amount of porosity on the sample surface was uneven and not quantified. Therefore, no direct quantification of the way proteins interact with the sample surface was possible. Future studies should be ideally carried under more well-characterized conditions using an alloy of one composition with differing amounts of porosity, quantified, for example, by means of quantitative microscopy. The results in terms of corrosion current or breakdown potential could then be directly compared with the quantity and size of the porosity present on the surface.

The mechanism of protein-surface interaction proposed in Paper II and Paper III of this study needed to be reinforced by means of a suitable technique. Electrochemical

impedance spectroscopy (EIS) has been shown to provide information on the mechanism of corrosion processes in biological environments[45-47]. EIS was used in the final part of this research with the goal to confirm the direct current (DC) measurements and to gain an insight into the proposed mechanisms.

Impedance Studies of Protein Influences on Corrosion of NiCr Alloys

Electrochemical impedance spectroscopy was selected to confirm the mechanism of protein-surface interaction proposed in previous parts of this study. The samples as well as the electrolytes used were the same as in the previous part of this investigation.

The inverse value of double layer capacitance ($1/C_{dl}$) is proportional to the thickness of the double layer and protein containing electrolyte's thickness of adsorbed protein layer. The higher the value of $1/C_{dl}$, the bigger the amount of adsorbed protein on the surface. It was also expected that, if the theory of protein-pore interaction presented in Paper II and III is correct, the buffering action of proteins against localized corrosion will be highest in mucin-containing environments due to the strong negative charge that these protein molecules carry.

Regalloy T (Be, porosity) as expected revealed the biggest differences shown by the AC data. Polarization resistance measured in artificial saliva containing mucin was highest, corresponding to highest value of inverse capacitance (Paper IV, *Figure 2*). Albumin containing solutions did not show that significant effect and values for HS were between those for AL and MU. Both R_p and $1/C_{dl}$ values were lowest in AS. Phase angle plot of Regalloy T in the four tested electrolytes showed significant deviation in phase angle measured at lower frequencies in AS (Paper IV, *Figure 3*). According to Pan *et al.* this

signifies a much higher degree of localized corrosion susceptibility of this alloy in AS than in the protein containing electrolytes. The lowest susceptibility was found in MU[40]. In Rexalloy (porosity, no Be) this trend was not as well defined as in Regalloy T (Paper IV, *Figure 6*). This could be explained by the absence of Be in Rexalloy, leading to lower activity of the pores and subsequently to less defined charge distribution on the Rexalloy surface.

In Vera Bond (Be, no porosity) even though the inverse capacitance value was highest in MU, showing high protein accumulation, the polarization resistance value was the lowest. That contrasted with a several-fold difference in R_p value in AS compared to MU (Paper IV, *Figure 1*). This observation can be explained by considering the weak performance of the passive film on Vera Bond as reported earlier[33]. Such behavior serves as evidence that the activity of proteins depends intensely on the state of the surface. Neptune showed insignificant differences in R_p and $1/C_{dl}$ values in AS, AL, and MU, whereas these values were lower in HS, suggesting that other corrosion active species, apart from AL and MU, must be present in saliva, significantly influencing the results.

Mechanism of Protein-Surface Interaction in NiCr Alloys

As determined from this investigation, the protein-surface interaction in general depends on the bulk composition, state of surface (passive film properties), existence of porosity, and surface charge distribution. For NiCr casting alloy systems studied, the following mechanism of protein-surface interactions can be proposed. It should be noted that the boundaries between the alloy groups' properties as described below are not sharp and that

the different mechanisms described below will become dominant, depending on the prevailing state of the alloy surface.

Alloys with stable passive film and no porosity. The surface of such alloys is electrochemically homogenous and protected by a coherent passive film layer with high protective properties. Additions of albumin and mucin to the electrolyte have neither negative nor positive effects on the metal dissolution rate. EIS data collected in electrolytes with and without proteins are essentially the same, suggesting a similar mechanism of dissolution whether the proteins are present or not. Results obtained in human saliva were very different from the results obtained in the other three electrolytes. This suggests that, apart from albumin and mucin, there are other species in human saliva involved in the corrosion reactions, influencing the kinetics and mechanism of the controlling reaction. Since it is unknown which substances may be responsible, future research should concentrate on decoding the effects of individual species contained in human saliva. However, since the composition of human saliva is very complex, this can be a very tedious task. Therefore, it is suggested that researchers routinely use human saliva, characterized by its pH and buffering capacity, in conjunction with the thin electrolyte layer technique.

Alloys with unstable passive film and no porosity. The corrosion mechanisms of these alloys is controlled by the thermodynamic ability of the atoms to be transferred into an ionic stage and limited, in most cases, by diffusion of species to and from the surface. This group of NiCr alloys reflected most their stable behavior in solutions that did not contain any proteins. It is conceivable, considering the metal-binding power of the tested proteins, that

the presence of proteins in the electrolyte decreases the overall thermodynamic barrier for transfer of atoms from the surface to solution. This effectively enables the corrosion reaction to proceed at a much faster rate, as seen from the data. The effect is even enhanced when negatively charged protein molecules (mucin) are present in the electrolyte. That is logical considering that more protein molecules get attracted to the positive anodic areas and leave them once their charge is neutralized with an available metal ion. This would also suggest higher accumulation of such protein on the surface as shown by higher inverse capacitance values. From the results of this study it is not clear which ions are attracted to albumin and mucin molecules, however, it is known from previous research that Ni possesses such properties. In future investigation, atomic absorption spectroscopy studies could provide a quantitative answer to this issue. However, the experiments would have to be carried out in bulk solution.

Alloys containing casting porosity with or without beryllium addition. Surface porosity acts as places where localized corrosion starts to occur immediately after the sample is immersed in an electrolyte containing chlorides. Chloride ions migrate inside the pores where Ni ions are available (*Figure 1*). A hydrolysis reaction involving Ni^{2+} and Cl^- ions and water molecules produces excess H^+, resulting in localized acidification of the electrolyte inside the pores. The change of pH causes the aggressiveness of the electrolyte to rise, leading to further dissolution of the metal. Electrons produced in this reaction are transferred to the surface and consumed in oxygen reduction. The surface adjacent to the pores is therefore charged negatively, whereas the inside of the pore, where Ni^{2+} abounds, becomes charged positively. These reactions continue with speed dependent on the system conditions

and sooner or later result in the material failure. In an electrochemical cell, the kinetics of the reactions depends on overpotential, as well. This is a well known self-propagating mechanism for localized corrosion, applied to the NiCr system.

When proteins carrying a negative charge are present in the electrolyte (*Figure 2*), the protein molecules start to see the positive charges of the pores. This leads to accumulation of protein around the pore opening. Such activity can have several effects, resulting in a lower dissolution rate inside the pores. Protein accumulation can hinder the mass transport in and out of the pores, including Cl^- and Ni^{2+} migration (*Figure 3*). That tends to restore the thermodynamic equilibrium inside the pores, lower the kinetics of the electrochemical reactions and possibly even enhance the probability of the pore repassivation.

This mechanism seems to be active to a certain degree even at elevated potentials as illustrated in the polarization curves for Regalloy T (Paper III, *Figure 9*). Nevertheless, from the results of this study it is evident that beyond certain potentials, corresponding to relatively high driving forces for localized corrosion to propagate, this mechanism loses its effect. Such potentials, however, are not usually experienced by materials in the oral cavity.

Suggestions for Future Research

This investigation produced evidence that electrolyte thickness plays an important role in electrochemical testing of dental materials and ultimately affects the results of the tests, significantly. Additionally, this study brought an insight to the influences of albumin and mucin on corrosion behavior of NiCr dental casting alloys and determined that proteinaceous artificial saliva solutions are not always a suitable replacement for human saliva in corrosion testing. Porosity was shown to have a profound effect on the

electrochemical behavior of alloys and on the protein-surface interaction. Even though the results of this study brought a significant amount of new knowledge, not all questions were answered and multiple possibilities for future research were opened.

Future research may include further investigation of the role salivary species play in the corrosion processes. This may include the deconvolution of corrosion effects of individual salivary species and understanding the importance of differences in saliva compositions among individuals in terms of corrosion test results. The future efforts could ideally lead to establishing the use of TET and saliva as a standard system for corrosion testing of dental and possibly even orthopaedic materials.

This investigation opened further possibilities to explore the effect of proteins on other classes of metallic materials used in dentistry and orthopaedics. The TET could also be used with advantage for testing in situations involving thin biofilms grown on metallic surfaces. Additional research can be devoted to examination of the casting porosity effects in dental materials, including quantification of the porosity and confirmation of dissolution rates by means of atomic adsorption spectroscopy. An implicit proof of some theories outlined in this investigation can be achieved by direct observation of selective protein adsorption by *in situ* atomic force microscopy and correlation of the electrochemical test results with surface tension measurements.

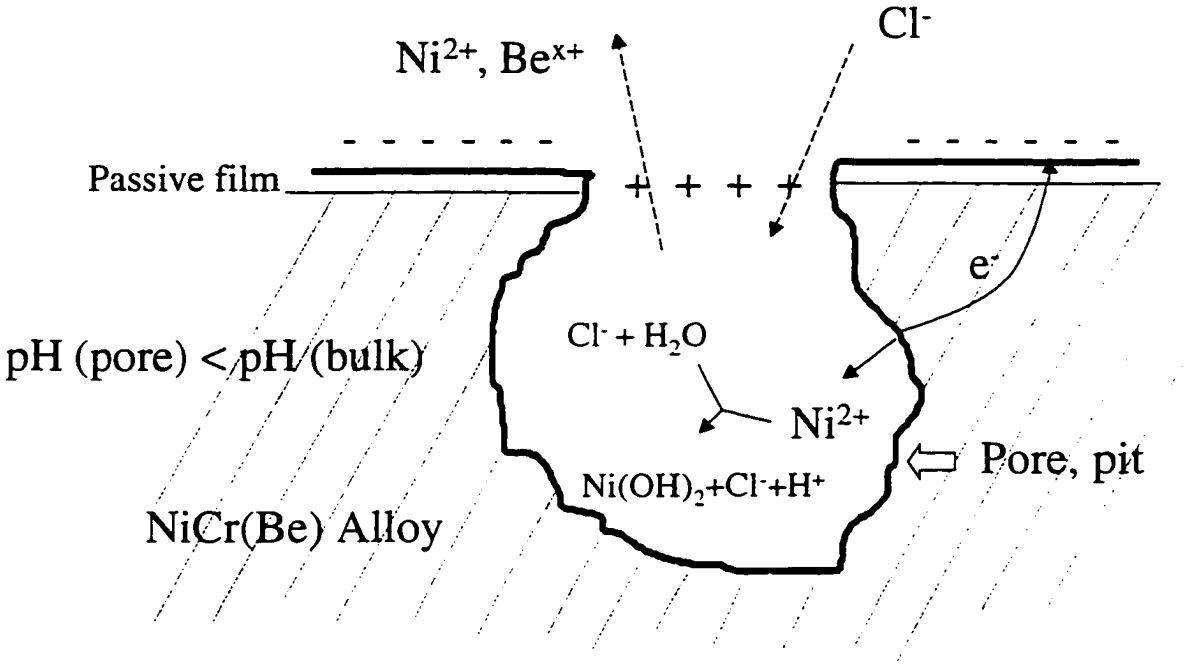


Figure 1 Mechanism of localized corrosion of a NiCr dental alloy exhibiting casting porosity. Electrolyte contains Cl^- ions.

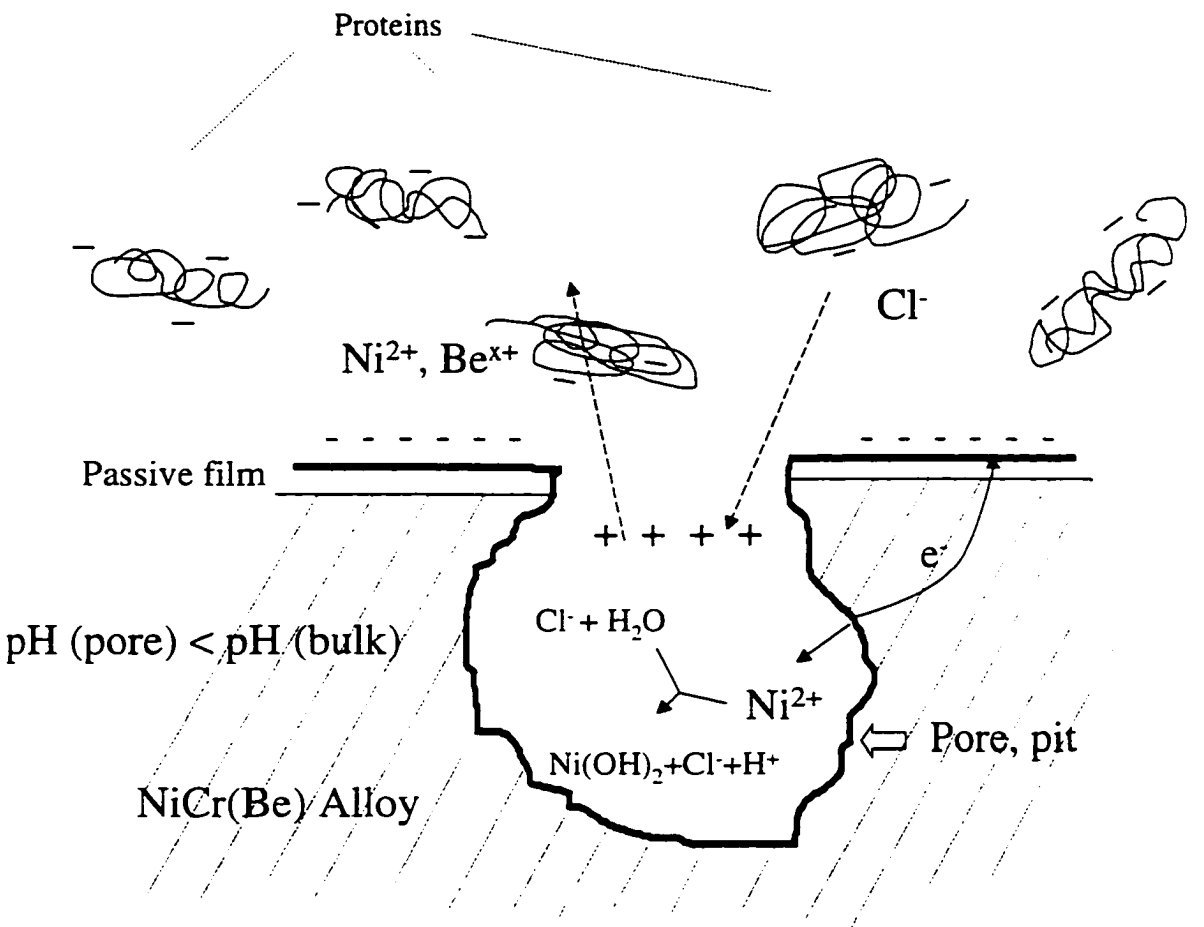


Figure 2 Mechanism of localized corrosion of a NiCr dental alloy exhibiting casting porosity in a solution containing negatively charged protein molecules. Electrolyte contains Cl^- ions.

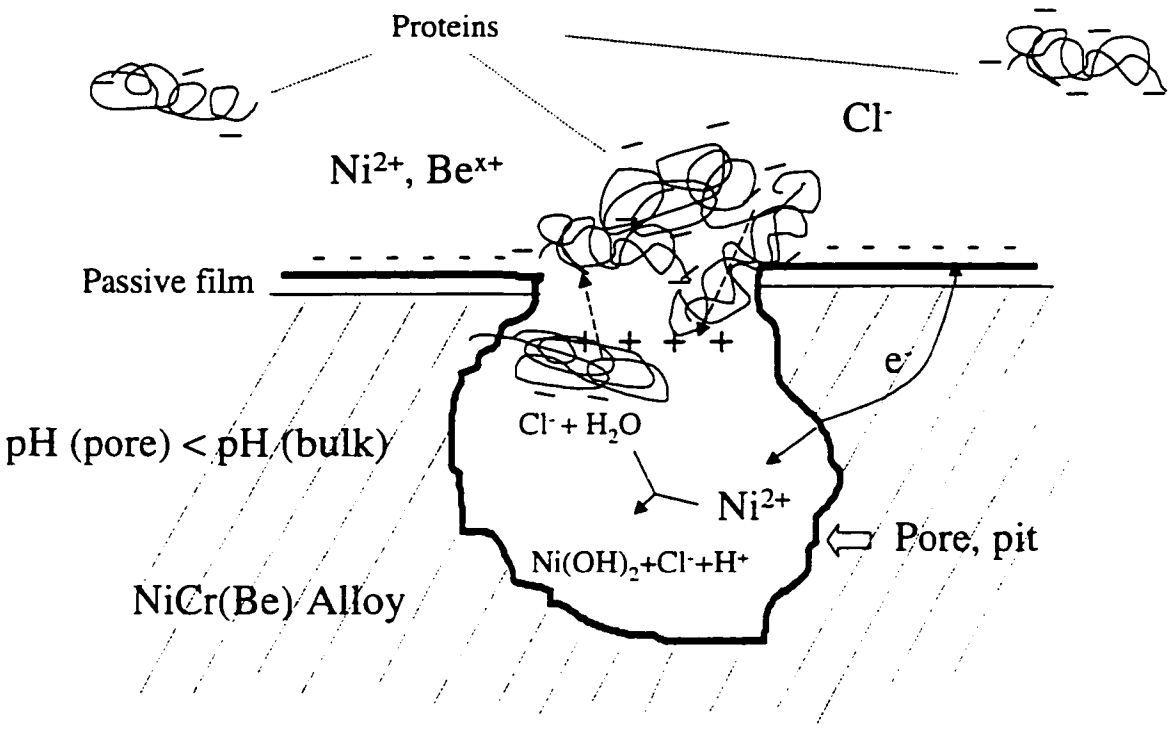


Figure 3 Inhibiting effect of negatively charged proteins on localized corrosion propagation in a NiCr alloy system exhibiting casting porosity. Electrolyte contains negatively charged protein molecules and Cl^- ions.

CONCLUSIONS

The physical character of the testing setup in terms of the electrolyte thickness has a major influence on the electrochemistry of the system and therefore on the outcome of the tests. The amount of oxygen available to the surface is one of the major reasons for such differences. Thus, the TET presents a better means of modeling the oral environment than the traditional BET.

The aggressiveness of corrosion important species contained in artificial saliva electrolytes depends on oxygen concentration and, therefore, is different under TET compared with BET conditions. TET can be easily used to evaluate the influence of these species on the corrosion resistance of dental materials.

Human saliva can be easily employed in electrochemical studies of dental materials using the TET technique. Electrochemical behavior of human saliva is quite different from that of artificial solutions on 316L SS. The organic salivary components are responsible, their influence being most significant at breakdown potential. The difference between human and artificial saliva behavior is caused mainly by the presence of proteins in human saliva, although it is not the only factor.

The addition of albumin and mucin to artificial saliva produces changes in the electrochemical behavior of NiCr dental casting alloys. These changes depend on the composition, microstructure, and porosity of the castings. In alloys with highly stable passive layers the effect of protein addition is negligible; whereas, in alloys with low quality passive films, proteins increase corrosion rates significantly. Alloys containing casting porosity,

however, exhibit substantially improved resistance to localized corrosion with proteins present in the electrolyte.

Solutions containing albumin did not exhibit exactly the same behavior as solutions with mucin addition. Therefore, adsorption based on electrostatic interaction between protein molecules and the surface is an important factor in the inhibitive effect of proteins on microstructurally inhomogeneous and porous surfaces.

The results of NiCr alloy testing in human saliva exhibit trends similar to those obtained in artificial saliva containing proteins, with some exceptions existing. This implies that the addition of proteins to artificial saliva does not guarantee complete simulation of the oral environment for *in vitro* testing.

Based on the results, we recommended using human saliva in combination with TET in electrochemical experiments addressing dental materials, especially if the corrosion mechanism of the materials in the oral environment is of interest. However, a study examining how the differences in diet, age, sex, and other factors influence the aggressiveness of human saliva would be beneficial. Further studies should concentrate additional research on the mechanism of charge-induced protein adsorption on surfaces using direct observation techniques like atomic force microscopy.

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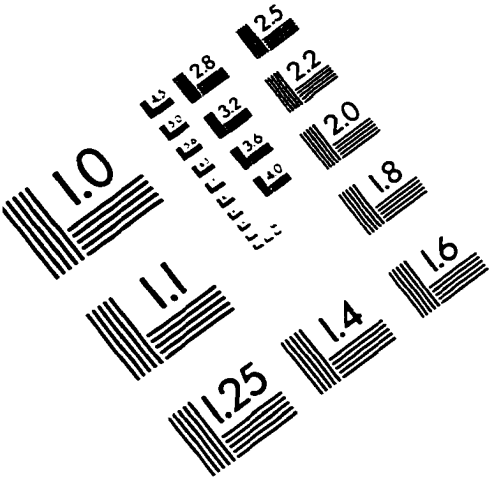

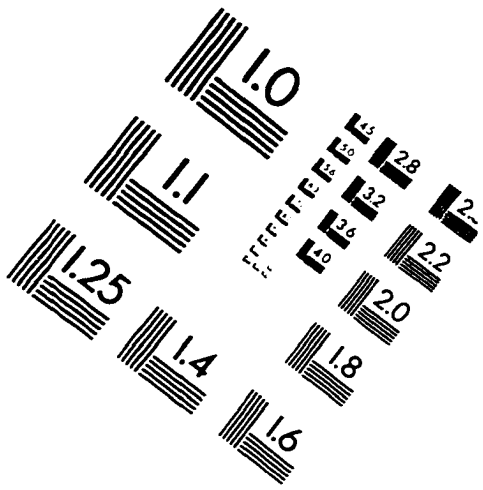

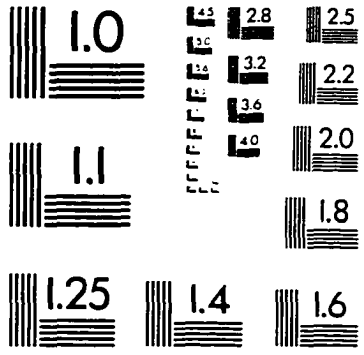

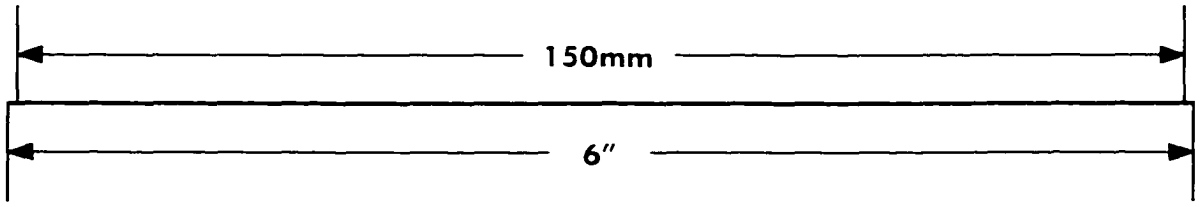

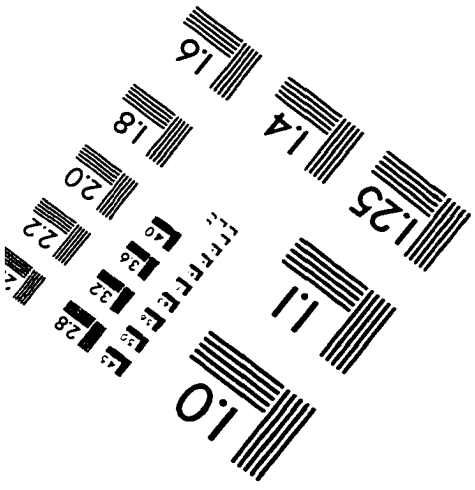

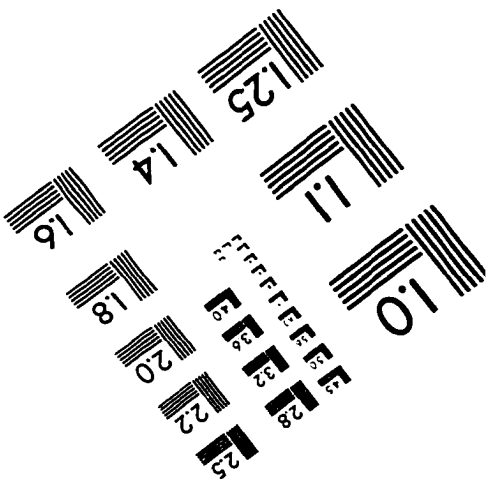

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