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The Effect of Some Fluids on Surface Oxidation and Amount of Released Iron of Stainless Steel Endodontic Files

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Summary: Endodontic files come in contact with blood, infected pulp tissue, and irrigating solutions during root canal therapy. Some instruments such as stereomicroscopy and scanning electron microscopy are used to observe corrosion of endodontic files which are complicated and dependent on preparation methods. Having knowledge of the corrosion and ion release of endodontic files can help in drawing firm deductions as to which files would perform better in the clinical scenario. Therefore, we have used energy dispersive X-ray analysis and an atomic absorption spectrophotometer to track oxygen on the surface and iron in the exposed media to observe the oxidative rate of the media. In this study, corrosion by blood was higher than other biological fluids, but less than with sodium hypochlorite (NaOCl). Observations of energy dispersive X-ray analysis and atomic absorption spectrophotometer results demonstrated that after exposure the amount of oxygen on the surface and surrounding areas increased. Therefore, the files should be rinsed as soon as possible during and after use to hinder the oxidation rate, but blood may produce a different behavior and it might be considered as a decreased risk of broken stainless steel files remaining in the root canal after treatment. SCANNING 34: 309–315, 2012. © 2012 Wiley Periodicals, Inc.

Key words: endodontic file, corrosion, blood, sodium hypochlorite, PBS, deionized water, atomic absorption spectrophotometer

Introduction

Endodontic files come in contact with blood, infected pulp tissue, and irrigating solutions during the process of chemomechanical preparation of the root canal system (Haapasalo *et al*., 2005; Saghiri *et al*., 2009). Presoaking endodontic instruments in disinfectant solutions is a recommended and accepted method for removal of organic debris, prior to sterilization (Haapasalo *et al*., 2005). In addition, broken files may remain in root canals for years and release corrosion products that may lead to unwanted inflammatory reactions (Viana *et al*., 2006). The corrosion behavior of endodontic files has been studied extensively (Viana *et al*., 2006; Madarati *et al*., 2008), and it is known that 5.25% sodium hypochlorite, which is the most commonly used irrigant and instrument disinfectant, causes corrosion of endodontic instruments (Sirtes *et al*., 2005). However, no study has determined the corrosion potential of blood. This is clinically relevant considering the environment in which root canal files are used. It is possible that corrosion of instruments may be initiated by blood, prior to being worsened by sodium hypochlorite (NaOCl). Atomic absorption spectrophotometry provides accurate

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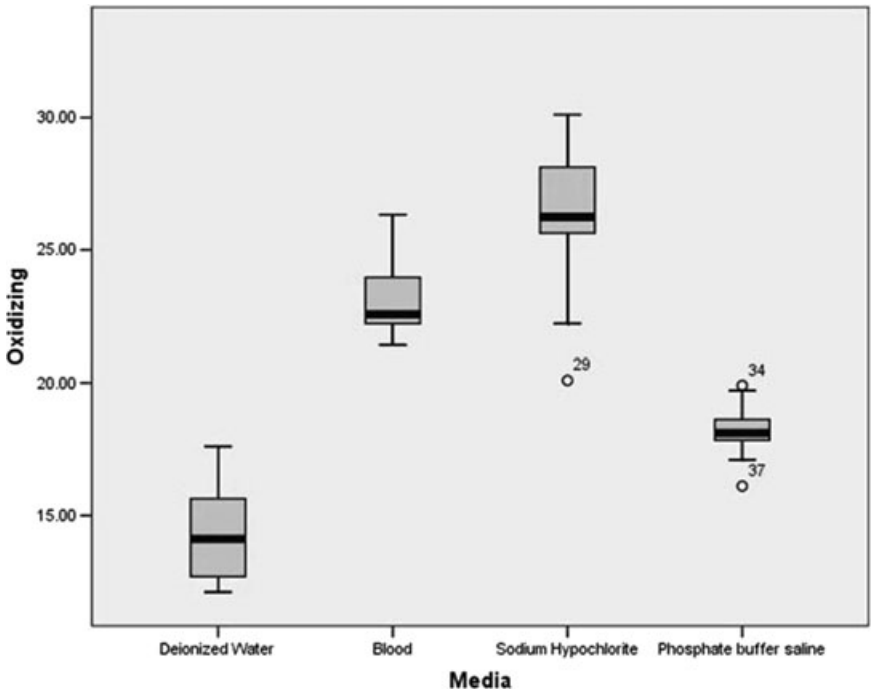

Fig 1. Box plot depicts of oxidizing rate in each group stored in deionizer water, blood, sodium hypochlorite, and phosphate buffer saline.

quantitative analyses for metals in water, sediments, soils, or rocks (samples are analyzed in solution form, so solid samples must be leached or dissolved prior to analysis). Metals include Fe, Cu, Al, Pb, Ca, Zn, Cd, and many more. This instrument can easily detect concentration range in low mg/L (ppm) range (Gatehouse and Willis, '61). In clinical application, when files break and remain in the canal, they may release metal ions to the surroundings, which has been found to cause toxic effects on the tissues (Madarati and Watts, 2008).

The aim of this study was to evaluate the surface topography, oxidation rate (OR), and to measure the amount of iron ion as a product of corrosion of stainless steel endodontic files in blood, deionized water (DW), 5.25% NaOCl, and phosphate buffer saline (PBS) by scanning electron microscopy (SEM) equipped with energy dispersive X-ray spectroscopy (EDX) and an atomic absorption spectrophotometer. The null hypothesis was that there was no difference in the oxidation rate and release of iron ion as a product of corrosion of stainless steel files in the four fluids tested.

Materials and Methods

V-range files Hedstrom files (n = 40) (V-Range, Dentsply, EU) were divided into four groups (n = 10) and immersed in one of the following solutions (5 mL), in 10 mL glass vials, for 30 min. Group 1: Deionized water (DW); Group 2: blood; Group 3: 5.25% NaOCl; Group 4: PBS.

Human blood was collected under Research Ethics Committee of Kamal Asgar Research Center approval (protocol no. KARC/20C2010-41-0) by a vein puncture needle 25 × 7 (Vacutainer) in a 5-mL tube with the 5% percentage by weight anticoagulant EDTA.

Scanning Electron Microscopy

After 30 min of immersion, all specimens were immersed in a super-sonic water bath (Supersonic Single Tank Cleanin`g Machine YTC 300, Banciao, Taiwan) for 5 min to remove remnants (contamination) on the surface of the file for each group before electron microscope analysis. The specimens were air dried for 24 h in a desiccator. SEM (TESCAN model VEGA II LSH, coupled with EDX [X-ray detector from Brüker]) and a custom made jig/slab was used for imaging and determining the composition at the same position. All analyses were carried out at 15 kV. The oxygen percentage on the surface was assessed with EDX in 2 mm length of each file. Average percentage of oxygen was captured for each file as shown in Figure 4. Subsequently, one file from each group was randomly selected and surface topography was analyzed using SEM at $\times 30g$ magnification. For high magnification, one sample from each group was randomly chosen and gold sputtered; for the blood

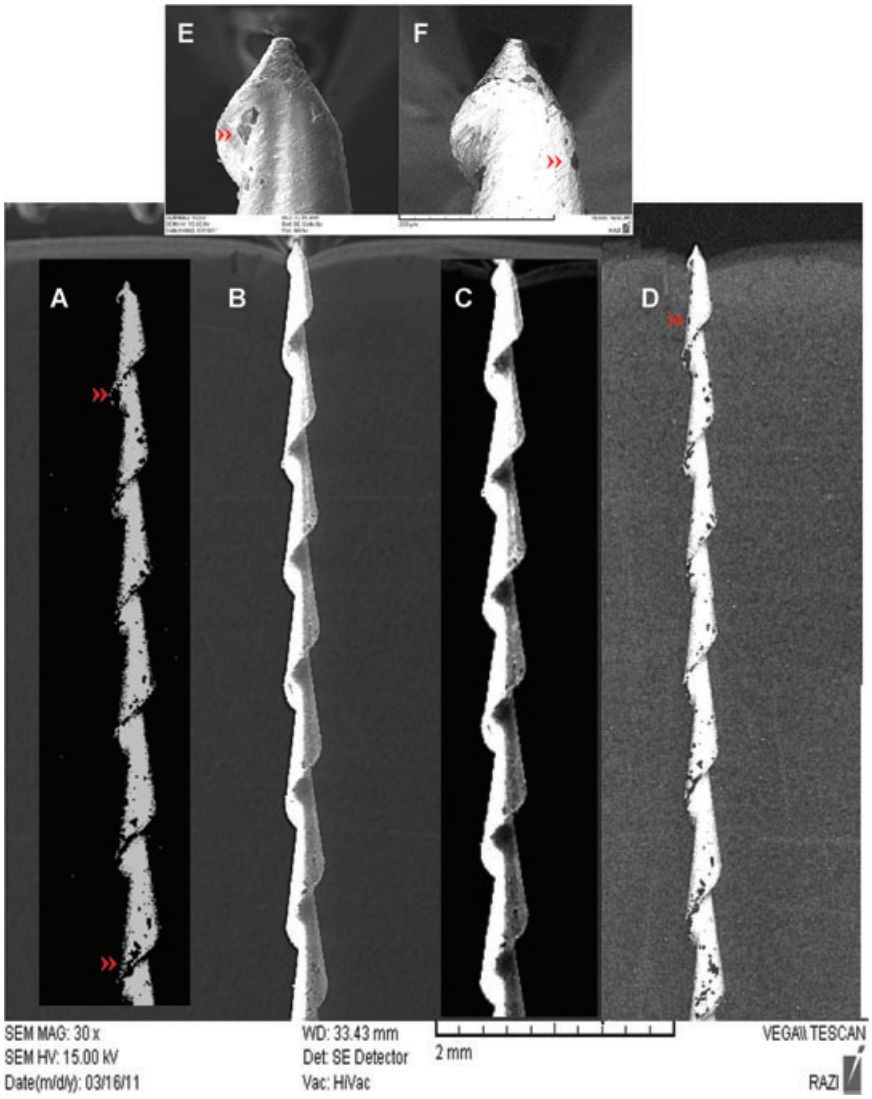

Fig 2. SEM micrographs of specimens stored in blood (**A, E**), deionized water (**B**), phosphate buffer saline (**C**), and sodium hypochlorite (**D, F**). More spots (≫) can be seen on the surface of the file stored in sodium hypochlorite and blood. Original magnification ×30 and ×2,500*g*.

group before the file was placed in 2% glutaraldehyde for 24 h and then rinsed three times with a sodium cacodylate buffered solution (0.1M, pH 7.2). After incubation in osmium tetroxide for 1 h, the files were then dehydrated with ascending concentrations of ethyl alcohol (30–100%), placed in a desiccator for 24 h, and afterwards the file was sputter coated with gold.

Statistical analysis of the record of oxygen was performed by one-way ANOVA followed by Tukey's HSD test, with the significant level set at $p < 0.05$.

Atomic Absorption Spectrophotometry

This study was similar to those carried out previously (Lugowski *et al.*, '87; Correia *et al.*, 2002). In brief, an atomic absorption spectrophotometer (Shimadzu 6300) was used in the present study. Before immersing the files into the solutions, the amount of iron ion was measured using an atomic absorption spectrophotometer in order to see if there was already any amount of iron ion component in these solutions. In addition, after immersion of the files in the selected environment, the solutions were gathered and analyzed separately. All samples were numerically coded to make the study blind.

Results

The mean ± SD values for the different groups are shown in Figure 1. The highest oxidation rate was found in the 5.25% NaOCl group and the least in the DW group. The mean ± standard deviation of

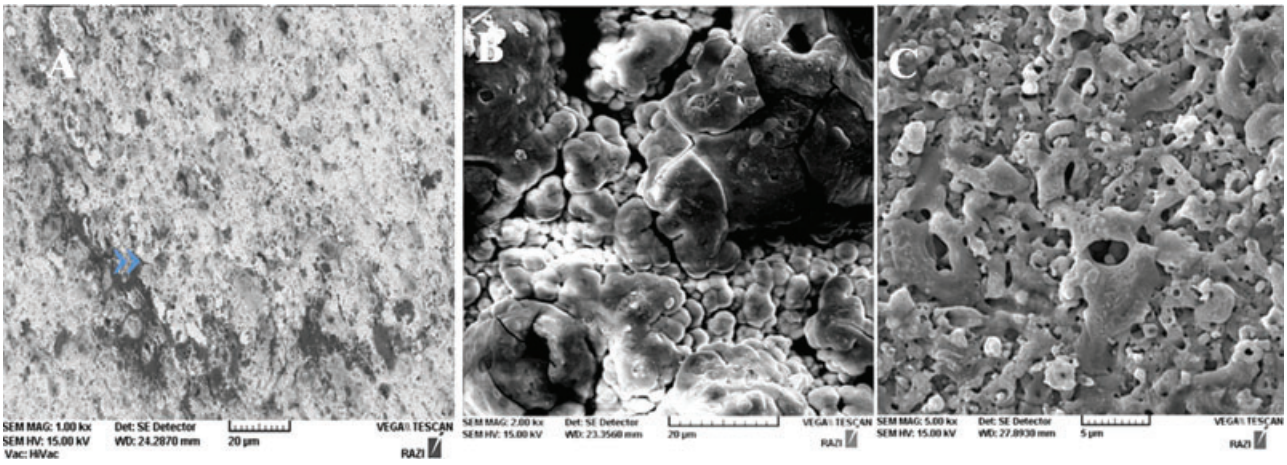

Fig 3. SEM micrographs of files stored in deionized water (**A**) and in blood (**B, C**) after gold coating. Deposition of some biological material over the surface is evident. The intact file also does not have a smooth surface (**A**) and some roughness can be easily detected. Original magnification ×1,000; ×2,000; and ×5,000*g*.

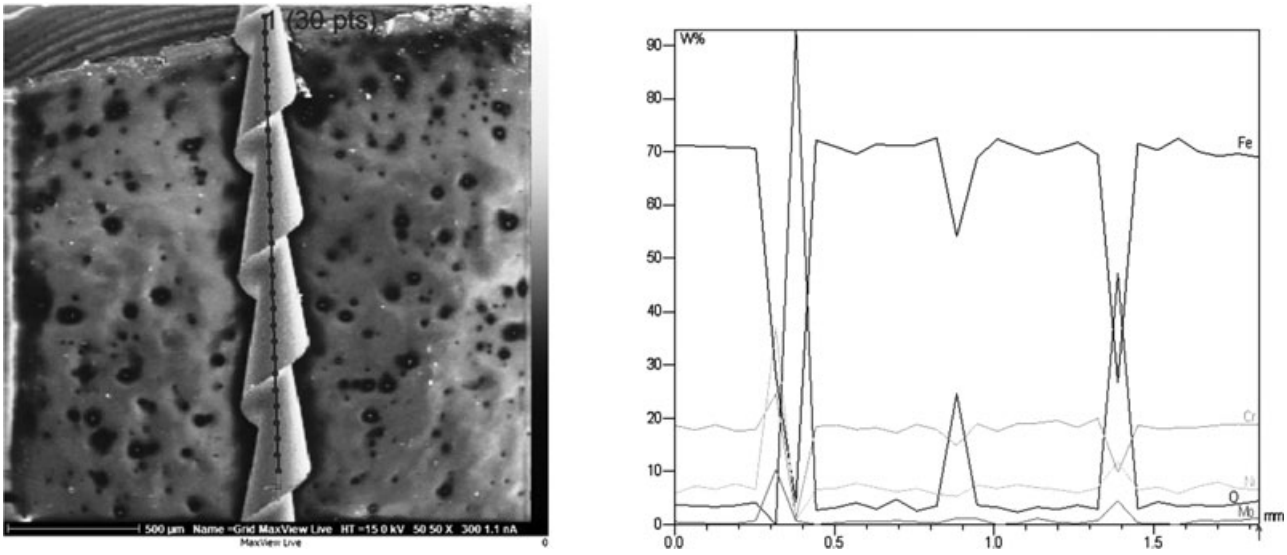

Fig 4. Micrograph and line EDX of file stored in deionized water. This group has a minimal percentage of oxygen over the surface (Original magnification ×30*g*).

oxidation rate was 14.2 ± 1.8, 18.2 ± 1.0, 23.1 ± 1.5, and 26.2 ± 3.0 for DW, PBS, blood, and NaOCl, respectively. ANOVA demonstrated significant differences among groups ($p < 0.001$). Tukey's test also revealed significant differences between blood and DW or PBS groups ($p < 0.05$).

SEM micrographs of each group are shown in Figure 2, revealing that blood and NaOCl produced black spots on the surface and may have penetrated into the surface reacting with the alloy.

The mean ± SD of iron released from the files were −2.08 ± 0.66, 0.007 ± 0.00, 0.002 ± 0.00, and 0.080 ± 0.03 in blood, DW, NaOCl, and PBS, respectively. The negative sign in the blood group means that the file adsorbed iron after immersion in blood. ANOVA test revealed significant differences in the amount of iron present in the solution after immersion in different solutions ($p < 0.001$). Homogeneity of variance was not shown by the Levene test. Therefore, Dunnett C test was used to evaluate any significant differences between groups. This test revealed that there were significant differences between all solutions in the amount of iron present in the solution.

Discussion

Corrosion adversely affects metallic surfaces by causing pitting and porosity, and decreases the cutting efficiency of endodontic files (Neal *et al.*, '83). Fracture caused by fatigue failure mechanism occurs due to crack initiation at the cutting surfaces and propagation toward the axial center of the file (Stokes *et al.*, '99). It is well known that environmental conditions

can modify both the crack initiation and propagation processes. The term "corrosion fatigue" has been used to describe the phenomenon of cracking in materials under the combined actions of cyclic loading and a corrosive environment (gaseous or aqueous). Irrigating solutions appear to have a major impact on endodontic files, as far as this phenomenon is concerned (Sprowls, '87; Stokes *et al*., '99).

The present study evaluated the amount of oxygen as a product of corrosion by EDX. This method is accurate and provides quantitative analyses in a nondestructive manner (Fischmeister, '88; Angelidis and Sklavounos, '95).

The initial stages of oxidation (including oxygen adsorption and incorporation, oxide islands nucleation, and growth into a continuous film) have been actively studied and are now understood relatively well. Since EDX can detect oxygen, it is easy to determine whether or not iron oxide has formed (Saghiri *et al*., 2012). Despite other destructive methods such as inductive coupled plasma (ICP) or X-ray diffraction (XRD), EDX analysis only detects oxygen as components of the deposit and is not able to determine the crystallographic structure as a nondestructive method. The solutions used in this study represent the simplest biologically relevant media with respect to endodontic files (Saghiri *et al*., 2009); also, previous studies (Cheung *et al*., 2007; Peters *et al*., 2007) illustrated that dynamic exposure employing mechanical force may accelerate the crack and surface of exposure of endodontic files. So, in the current study, an immersion period of 30 min was followed to simulate the clinical scenario.

NaOCl, a chlorine-containing solution that is routinely used for root canal irrigation in endodontic treatment as well as for disinfection of root canal files, is a highly aggressive agent to NiTi materials (Lasley *et al*., 2004). From the results of our study, the same appears to be true for stainless steel files. Immersion of endodontic files in NaOCl for up to 1 h leads to superficial corrosion (Busslinger *et al*., '98), although it appears to have little effect on the overall mechanical properties of the instrument (Haïkel *et al*., '98; Zhang *et al*., 2010). An important finding of this study, which has important clinical implications, was the corrosion of instruments following contact with blood. The contact of endodontic files with blood is inevitable. Blood caused more corrosion than DW and PBS. It might be attributed to the different kinds of enzymes in blood and oxidative agents released from leucocytes, which may result in a powerful oxidative effect of blood on the surface of stainless steel files (Zhang *et al*., 2010).

PBS is a standard buffer salt solution with only inorganic species that mimics the ion strength of human blood (Alvarez *et al*., 2008). We believe that this exposure may bring about morphological changes in the stainless steel files because of acceleration of the oxidation process and the deposit of some oxidation layer on the surface of endodontic files.

Instrument fractures during root canal treatment can complicate the clinician's success. This affects the long-term prognosis of root canal treatment negatively (Madarati and Watts, 2008). The exposed metal surface undergoes an electrochemical dissolution of material at a finite rate, due to interactions with the surrounding environment. Upon exposure, the initiation of corrosion can be the result of interaction of ingredients of blood such as water, complex organic compounds, dissolved oxygen, sodium, chloride, bicarbonate, potassium, calcium, magnesium, phosphate, amino acids, proteins, plasma and lymph with the file surface, whether it is the formation of localized electrochemical cells resulting in pitting attack, or crevice corrosion that may ultimately result in a cascade of events leading to increased concentrations of local and systemic trace metal (Suter *et al*., 2005; Zhang *et al*., 2010).

The biocompatibility of metallic materials is closely related to their corrosion behavior. Large amounts of metal ions released from the broken endodontic file are generally harmful to the apical zone. The release of ions may cause unwanted reactions and corresponds with its corrosion rate. Corrosion resistance of endodontic files and its alloys relies on the presence of a passive film on the surface. In NiTi base files, this thermodynamically stable oxide film prevents the matrix from corroding and provides good corrosion resistance and excellent biocompatibility in physiological environments, but in stainless steel files this layer is not stable and may wash out easily from the surface (Trepanier *et al*., 1995). In addition, the release of metal ions also takes place accompanied by the growth of the oxide film over the surface (Wataha and Lockwood, 1998).

In spite of the oxidation rate, blood impeded release of iron ion into the media. The most abundant cells in vertebrate blood are red blood cells (Vaupel *et al*., 2005). These cells contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen. In our experiment, the amount of iron release in blood media decreased significantly after immersion despite other groups and oxidation rate results. Upon contact with the blood, platelets become activated, induce fibrin to crosslink, and form a platelet-fibrin rich layer over the texture of stainless steel files during immersion into the blood (Figure 3); this film may jeopardize release of iron ion form the surface (Werner *et al*., 2007).

Although blood groups illustrate the highest rate of oxygen over the surface, the authors believe that immediately after exposure some blood cells containing iron are adsorbed on the surface that may be extracted from the media, so this surface may be difficult to penetrate by oxygen and may corrode the surface

of endodontic files. The major factor that governs the corrosion process of metallic biomaterials is the kinetic barriers that prevent corrosion not by energetic mechanisms, but by the physical limitation of the rate at which oxidation or reduction processes can take place.

Many studies reported that an unusual foreign-body reaction to particulate products of corrosion and wear was present in the tissue around broken files. Some of these reactions may be originated from corrosion products coming from files that had contact with blood (Gettlemann *et al*., '91; Saunders *et al*., 2004).

Conclusions

Within the limitations of the present study, the following could be concluded. Corrosion of stainless steel files by blood was higher than with DW and PBS but less than with NaOCl. Therefore, the files should be discarded or rinsed as soon as possible during and after use, to prevent fracture due to corrosion. In addition, despite the lack of significant differences in oxidation rate of blood and NaOCl, the amount of iron release of both groups revealed significant differences. These findings are probably due to the influence of protein ingredients of blood and may create a biological film over the surface of files during exposure to the media, which may affect the release of iron to some extent. Collectively, observations in initial oxidation rate and iron release of blood group suggest that it might be considered as a decreased risk of tissue reaction to stainless steel files remaining in the canal. Because there is no published report on these issues, further investigation is recommended for thrombogenicity and hemocompatibility of surface broken stainless steel.

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Conflict of interest

We affirm that we have no financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript and deny any conflicts of interest related to this study.

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