THE EFFECT OF 5% SODIUM HYPOCHLORITE, 17% ETHYLENE DIAMINE TETRA ACETIC ACID AND TRIPHALA ON TWO DIFFERENT ROTARY NICKEL-TITANIUM INSTRUMENTS: AN ATOMIC FORCE MICROSCOPY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY ANALYSIS

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CERTIFICATE

This is to certify that this dissertation entitled "**The effect of 5% Sodium hypochlorite, 17% Ethylenediaminetetraacetic acid and Triphala on two different rotary Nickel-Titanium instruments : an Atomic Force Microscopy and Energy Dispersive X-Ray Spectroscopy analysis**" is a genuine work done by **DR. PRAMOD S PRASAD** under my guidance during his postgraduate study period between 2010 - 2013.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY in CONSERVATIVE DENTISTRY AND ENDODONTICS – BRANCH IV.** It has not been submitted partially or fully for the award of any other degree or diploma.

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LIST OF ABBREVATIONS USED

AFM	**Atomic Force Microscope**
CCD	**Charged Couple Device**
EDTA	**Ethylene diamine tetraacetic acid**
EDS	**Energy Dispersive X-Ray Spectroscopy**
GTP	**Green Tea Polyphenols**
LCF	**Low-Cycle Fatigue**
MTAD	**Mixture of Tetracycline, Acid and Detergent**
Ni-Ti	**Nickel-Titanium**
NaOCl	**Sodium hypochlorite**
SEM	**Scanning Electron Microscope**
SMA	**Shape Memory Alloys**
SPSS	**Statistical Package for Social Sciences**
Ra	**Roughness average**
Rq	**Root Mean Square (RMS) deviation of roughness profile**
Rc	**Mean height of the roughness profile elements**

CONTENTS

Introduction

The advent of nickel-titanium (NiTi) alloys in the field of dentistry has revolutionized the speciality in many ways. Nickel-Titanium alloy was developed in the early 1960's by W. F. Buehler, a metallurgist at the Naval Ordnance Laboratory in Silver Springs, Maryland, USA[1]. Considering its property of superior elasticity and resistance to tortional fracture, nickel-titanium endodontic rotary files were introduced to the field of endodontics[2]. Nitinol has a greater strength and low modulus of elasticity when compared with stainless steel[3,2]. This helps Ni-Ti instruments to negotiate and prepare curved root canals without early permanent deformation unlike stainless steel files.

The Ni-Ti alloys used for manufacturing the endodontic files contain approximately 56% (wt) nickel and 44% (wt) titanium[4]. Though NiTi instruments have many advantages, instrument separation is not an uncommon occurrence in clinical use[5,6]. Fracture of NiTi instruments used in rotary motion occurs in two different ways: fracture because of torsion and fracture because of flexural fatigue[7,8]. Torsional fracture occurs when a part of the instrument is locked in the canal while the shank still continues to rotate, ie; the torque exerted by the handpiece exceeds the elastic limit of the metal[9]. The generation of tension / compression cycles at the point of maximum flexure when the instrument freely rotates in a curvature leads to flexural fatigue fracture[10].

Surface alterations of NiTi instruments can occur even if they are in short-term contact with endodontic irrigants[11]. Sodium hypochlorite is the most widely used endodontic irrigant whose antimicrobial effectiveness is on account of its high pH. The hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) produced by the ionization of NaOCl leads to aminoacid degradation and the hydrolysis of organic tissues. The antibacterial and tissue dissolution action of sodium hypochlorite increases with an increase in concentration and proportionally there is also an increase in its toxicity[12].

Nygaard and Ostby introduced EDTA to the field of endodonticcs in the year 1957. They recommended the use of a 15% EDTA solution having a pH of 7.3[13]. EDTA has been the most commonly used chelating solution which reacts with calcium ions in dentin and forms a soluble chelate[14]. The sequential use of sodium hypochlorite and EDTA has been the most recommended to remove endodontic smear layer[15].

The constant increase in antibiotic resistant strains and side effects caused by the use of synthetic drugs has prompted researchers to look for more biocompatible and efficient herbal alternatives. Triphala is an Indian ayurvedic herbal formulation consisting of the dried and powdered fruits of three medicinal plants Terminalia bellerica, Terminalia chebula, and Emblica officinalis[16].

Different methods are being employed to evaluate the surface characteristics of NiTi rotary instruments. Scanning Electron Microscopy has been the most common method used for surface analysis of NiTi rotary endodontic instruments[5,17,18]. Recently, Atomic Force Microscopy (AFM) as a technique is gaining in popularity as a valuable research tool for the evaluation of the surface topography of various biomaterials[19]. Many of the recent research to evaluate the surface topography of endodontic instruments has been done with the help of AFM[11,20-23]. Unlike the scanning electron microscope which provides a two-dimensional projection or a two-dimensional image of a sample, the AFM provides a three-dimensional surface profile. Samples viewed by AFM do not require any special treatment protocols (such as metal/carbon coatings) that would irreversibly change or damage the sample. It is also seen that the AFM can provide a higher resolution than SEM.

The Atomic Force Microscope works on the principle of the scanning tunneling microscope developed by Gerd Binnig and Heinrich Rohrer in early 1980s at IBM Research, Zurich for which they were awarded the Nobel Prize for Physics in the year 1986. The first Atomic Force Microscope was invented by Quate and Gerber in 1986. Energy-Dispersive X-ray Spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on the investigation of an interaction of some source of X-ray excitation and a sample. Each element has a unique atomic structure allowing unique set of peaks on its X-ray spectrum. Many studies have been conducted to analyze the surface of rotary NiTi endodontic files using EDS[17,24].

This in-vitro study evaluated the surface characteristics of two different rotary NiTi files on treatment with three different irrigants using Atomic Force Microscope and Energy-Dispersive X-Ray Spectroscopy.

Aim of the study

The aim of the present study is to evaluate the effect of 5% Sodium hypochlorite (NaOCl), 17% Ethylenediaminetetraacetic acid (EDTA) and Triphala as irrigating solutions on the nano-structural surface of Protaper and iRaCe rotary NiTi endodontic files using Atomic Force Microscope (AFM) and Energy-Dispersive X-ray Spectroscopy (EDS).

Review of Literature

Sen BH et al.[15] reviewed the role and significance of Ethylene Diamine Tetraacetic Acid in the management of smear layer. During instrumentation of endodontic therapy, a layer of material composed of dentin, remnants of pulp tissue and odontoblastic processes, and sometimes bacteria isalways formed on the canal walls. It has been shown that this layer is not a complete barrier to bacteria and it delays but does not abolish the action of endodontic disinfectants. If smear layer is to be removed, EDTA and NaOCl solutions have been shown to be effective, among various irrigating solutions and techniques, including ultrasonics that have been tested. The role of calcium ions on dentin and the mechanism of chelation is described depicting the significance of EDTA as an important irrigant in the cleaning and shaping procedures. The authors stress on the need for further studies to establish the clinical importance of the absence or presence of smear layer.

Heling I et al.[34] investigated sodium hypochlorite (with and without EDTA), chlorhexidine and hydrogen peroxide in varying concentrations when used in sequence or in combination as endodontic irrigants. Sterile saline served as control. Six standardized bovine incisor root specimens, which had been infected with enterococcus faecalis were exposed to each solution. Dentin powder samples were incubated and the quantity of bacteria present was assessed using spectrophotometry. All irrigant regimens were more effective than saline in killing bacteria. The result showed that chlorhexidine and NaOCl were similarly effective. At specific concentrations a synergestic effect was noted when mixtures of chlorhexidine and hydrogen peroxide were tested.

Siedlecki CA, Marchant RE[19] in their research summarized that atomic force microscopy (AFM) has provided mechanistic insights into the molecular level interactions that occur at the biomaterial interface. Several unique operational modes have been developed which utilize intermittent contact with the sample and decrease applied shear forces. These dynamic modes also can be used to study the role of different structural components on biomaterial micromechanical properties. Force detection techniques allow molecular level studies of force mapping for determining structure/function relationships. Advancements in tip manufacturing, image processing techniques, the use of model surfaces and labeling all have contributed to the advancement of the AFM as a state-of-the-art research instrument. In this report, they examined the applicability of the AFM to the study of biomaterials and cell/molecular interactions.

Sattapan B et al.[7] analyzed the type and frequency of defects in nickel-titanium rotary endodontic files after routine clinical use over a period of six months. Almost 50% of the files showed some visible defect; 21% were fractured and 28% showed other defects without fracture. Fractured files could be divided into two groups according to two groups according to the characteristics of the defects observed. Torsional fracture occurred in 55.7% of all fracture files, where as flexural fracture occurred in 44.3%. The results indicated that torsional failure, which may be caused by using too much apical force during instrumentation, occurred more frequently than flexural fatigue.

Thompson SA.[4] reviewed the history, metallurgy, structure, phase transformations, uses of nickel-titanium alloys and described briefly on the manufacturing of Nitinol alloy and fabrication of root canal instruments. The nickel-titanium alloy has been used in the manufacture of endodontic instruments in recent years because these have greater strength and a lower modulus of elasticity compared with stainless steel alloys. The super-elastic behavior of Nitinol wires means that on unloading they return to their original shape following deformation. These properties are of interest in endodontology as they allow construction of root canal instruments that utilize these favourable characteristics to provide an advantage when preparing curved canals. The instrument design has to be ground into the Nitinol blanks. Further difficulties during production include elimination of surface irregularities (milling marks) and metal flash (roll-over) on the cutting edges that may compromise the cutting ability of the instruments and potentially cause problems with corrosion. In this review he gives an insight into the importance and advantages of nickel-titanium rotary endodontic files over stainless steel files.

Bayranoglu G et al.[48] determined the effects of the oral environment's pH on the corrosion of dental metals and alloys that have different compositions, using electrochemical methods. The corrosion rates and the cathodic Tafel slopes were obtained from the current potential curves. The effect of pH on the corrosion of dental metals and alloys was dependent on their composition. Dissolution of the ions occurred in all of the tested pH states. The dissolution was moderately low for samples containing titanium because its surface was covered with a protective layer, whereas the dissolution was maximum for the samples containing tin and copper. Addition of cobalt and molybdenum to the alloys improved their corrosion resistance, these cobalt and molybdenum alloys were not affected by changes in the pH. Dissolution of the precious metal alloys increased as the percentage of noble metals increased.

Martins RC et al.[17] analyzed the surface irregularities on ProFile nickel-titanium rotary instruments before use, after sterilization by two different methods and after instrumentation of molar root canals. The analysis were carried out by scanning electron microscopy(SEM) and X-ray energy dispersive spectroscopy (EDS). The results showed the deposition of more dentin materials on the surface of instrumented files. This study indicates the need to re-evaluate the process employed for machining the nickel-titanium instruments and to review the cleaning methods currently in use.

Grawehr M et al.[35] evaluated the interactions of ethylenediamine tetraacetic acid with sodium hypochlorite. Amounts of available chlorine were determined in the EDTA/NaOCl solutions with an iodine/thiosulphate titration method. Calcium chelation capacity was titrated with a pure calcium solution using a murexide indicator. The study concluded that EDTA retained its calcium-complexing ability when mixed with NaOCl, but EDTA caused NaOCl to lose its tissue dissolving capacity and virtually no free chlorine was detected in the combinations. Clinically, this suggests that EDTA and NaOCl should be used separately and as an alternating regimen, copious amounts of NaOCl should be administered to wash out remnants of the EDTA.

O'Hoy PYZ et al.[28] evaluated the effect of repeated cleaning procedures on fracture properties and corrosion of nickel-titanium (NiTi) files. New NiTi instruments were subjected to 2, 5 and 10 cleaning cycles with the use of either diluted bleach or Milton's solution as disinfectant. Files were then tested for torsional failure and and flexural fatigue, and observed for evidence of corrosion using scanning electron microscope. The study concluded that files can be cleaned up to ten times without affecting fracture susceptibility or corrosion, but should not be immersed in NaOCl overnight. Milton's solution is much more corrosive than bleach with the same NaOCl concentration.

Alapati SB et al.[18] used Scanning Electron Microscope to compare the appearances of the tip sections of ProFile 0.04 taper and Lightspeed 25-mm long, ISO size 25 nickel-titanium rotary instruments. These files were used to prepare mesial canals of extracted mandibular molars. For the used ProFile instruments, there was some flattening of the characteristic material rollover and minor apparent wear at the edges of the flutes, but there was little change in the tip regions of the used Lightspeed instruments. Deposits on the surface of the instruments were attributed to the manufacturing process and the in vitro preparation of root canals in the extracted teeth. The simulated canal use did not cause substantial changes in the regions of these two brands of rotary instruments that are involved in the clinical preparation of root canals.

Martin B et al.[9] evaluated the effect of rotational speed and the angle and radius of curvature of root canals on the fracture of two types of nickel-titanium rotary instruments namely K3 and ProTaper. A total of 240 extracted human maxillary and mandibular molars were divided into two groups according to the angle of the canal curvature. Instrumentation was done at three different rotational speeds and more fracture was noted on greater curvature roots and with high r.p.m. Each instrument in the subgroup was used a maximum of 20 times and at one rotational speed only. The study concluded that instrument fracture was associated with rotational speed and the angle of curvature of the canal but with no significant differences between the files or radii of canals.

Darabara M et al.[26] evaluated the pitting and crevice corrosion characteristics of stainless steel and NiTi endodontic files in R-EDTA and NaOCl irrigating solutions. The cutting flutes of 12 files of each material were embedded in epoxy resin, polished, exposed to the irrigating solutions and used as an electrode. Results implied that pitting or crevice corrosion are not likely to occur for all the materials examined in both irrigating solutions. In NaOCl all materials showed significantly higher corrosion potential as well as lower corrosion current density compared with R-EDTA reagent. The study concluded that none of the tested materials is susceptible to pitting or crevice corrosion in R-EDTA and NaOCl solutions .

Michael A Baumann[49] reviewed the manufacturing, corrosion and sterilization of nickel-titanium alloys. The lattice organization can be altered either by temperature or stress. Although temperature changes are used during the manufacturing process, root canal treatment causes stress to NiTi files and a stress induced martensitic transformation phase. The environment of the mouth causes corrosion of NiTi alloys. Corrosion pits in products rich in titanium were also described. More studies on endodontic instruments indicate that there are changes but that they are not seen as clinically relevant. Dry heat and steam autoclave decreased the flexibility of stainless steel and NiTi files. With sodium hypochlorite, there is a hint of pitting corrosion after sterilization and exposure to 5% sodium hypochlorite. In this review the author discussed the chronology of nickel-titanium use in endodontics, the international standards organization recommendations, the features of nickel-titanium files and the new approaches and challenges in the manufacturing and design of nickel-titanium rotary files.

Peters OA[10] reviewed the factors that influence shaping outcomes with nickel-titanium rotary endodontic files, such as preoperative root-canal anatomy and instrument tip design, operator experience, rotational speed, and specific instrument sequence. Implications of various working length definitions and desired apical widths are correlated with clinical results. Despite the existence of one ever-present risk factor, dental anatomy shaping outcomes with nickel-titanium rotary instruments are mostly predictable. Nickel-titanium rotary instruments require a preclinical training period to minimize separation risks and should be used to case related working lengths and apical widths. This article enumerates the different fracture modes of the nickel-titanium instruments and the influence of core dimensions on fracture resistance of files.

Caroline RA Valois et al.[20] evaluated the topography of conventional stainless-steel files and both hand and rotary nickel-titanium files by using AFM. Stainless steel K-files (Dentsply and Moyco), NiTi hand files (Nitiflex and Greater Taper), and NiTi rotary files (Greater Taper and Quantec) were analysed and Root Mean Square (RMS) parameters for contact mode imaging microscopy variations were measured. All instruments showed topographic irregularities distributed on surface. The hand NiTi Greater Taper, rotary NiTi Greater Taper, and rotary NiTi Quantec showed greater values of vertical amplitude topography compared to K-Densply and NiTiflex files. The study also concluded that the

AFM proved to be a valuable research tool in the investigation of endodontic files topography.

Ullmann CJ et al.[8] evaluated the static fracture loads of ProTaper nickel-titanium instruments that had been subjected to various degrees of cyclic fatigue. Torque and angle of failure of new instruments and instruments that had been stressed to 30, 60 or 90% of their cyclic fatigue rotations in a simulated canal were tested. Rotations were continuously measured with a memocouple and the fracture patterns were analyzed with scanning electron micrographs. With unused ProTaper instruments, resistance to cyclic fatigue decreased with an increase in diameter. Torque at failure showed a strong linear relationship to instrument diameter while the angle at failure was weakly related to the diameter. The study found that the resistance to cyclic fatigue decreased with increase in diameter and recommended that the larger instruments that have been subjected to some cyclic fatigue should be used with great care or discarded.

Alapati SB[5] examined numerous discarded ProFile GT, ProFile, and ProTaper nickel-titanium rotary instruments obtained from two graduate endodontic clinics using Scanning Electron Microscope. These instruments had an unknown history of clinical use and had fractured or experienced considerable permanent torsional deformation without complete separation. The failure process generally exhibited substantial ductile character, evidenced by a dimpled rupture fracture surface. Crack propagation at grain boundries and cleavage surfaces indicative of transgranular fracture were observed for some specimens. The instruments were classified as with no evidence of visible deformation, evident permanent deformation and fractured. In this study they suggested the different methods for improving the fracture resistance of rotary endodontic files.

Alexandrou G et al.[47] evaluated the effect of repeated dry heat sterilization on surface characteristics and microstructure of Mani nickel titanium rotary instruments. Thirty-three new Mani NRT instruments, size 30, taper 0.04 and 25 mm in length were examined. Twenty-seven instruments were divided into three groups for surface characterization by scanning electron microscopy (SEM). The study concluded that machining defects and structural imperfections of new Mani instruments are indicative of the difficulty in manufacturing nickel–titanium endodontic instruments and suggested that Mani instruments are capable of superelastic behavior under clinical conditions.

Berutti E et al.[50] evaluated the influence of immersion in sodium hypochlorite on resistance to cyclic fracture and corrosion of ProTaper NiTi rotary instruments. In this study, a total of 120 new ProTaper NiTi rotary files were randomized and assigned into different groups. In control group, the shaft was excluded and in other two groups, the files were immersed completely and excluding the shaft for 5 min. All instruments were then tested for cyclic fatigue, recording the time in seconds to fracture.

Micromorphological and microchemical analysis were also completed by means of a field emission scanning electron microscopy (SEM). The study concluded that if rotary NiTi instruments operate immersed in a NaOCl solution contained in the pulp chambers of the teeth restored with metals or alloys having different electrochemical nobility values, galvanic corrosion may occur.

Kuber Sood et al.[24] assessed the effect of repeated cleaning and sterilization procedures on 3 brands of Nickel-Titanium endodontic file using Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS). A total of 105 new NiTi endodontic files from three different manufacturers namely Protaper, RaCe and ENDOWAVE were subjected to 2, 5 and 10 cycles of cleaning and sterilization procedure. Files were pre soaked in 2.5% NaOCl for 15 min and 1 hour followed by sterilization process using steam autoclave at 121°C at 15 psi pressure for 15 minutes. Then all these files were observed for evidence of corrosion such as pitting or deposition of corrosion products using SEM. EDS was conducted to analyze the chemicalelements in corroded and non corroded areas of the file. All the files showed mild to severe corrosion with 1 hour immersion in NaOCl. Protaper showed maximum corrosion followed by RaCe and least corrosion in ENDOWAVE rotary files. The study concluded that this can be due to the various surface treatment included during the manufacturing process of these files.

Troian CH et al.[6] evaluated the deformation and fracture of nickel-titanium RaCe and K3 size 25, 0.04 taper instruments using Scanning Electron Microscope. Canal preparations were done in simulated root canals with each set of instruments and scanning electron microscopic evaluation was done before and after the preparations. Three observers scored images of the instruments after each use for distortion of the spirals (no distortion, distortion of one spiral or distortion of more than one spiral), wear (no wear, small, moderate or severe wear) and fracture (yes or no). A significant difference was found between RaCe and K3 in terms of deformation and fracture of size 25, 0.04 taper instruments; K3 instruments had more favorable results.

Novoa XR et al.[39] evaluated the corrosion resistance of nickel-titanium endodontic rotary instruments immersed in 5.25% sodium hypochlorite solution. The corrosion performance of proTaper NiTi instruments was evaluated using commercial 5.25% sodium hypochlorite solution and the same solution partially neutralized. Electrochemical measurements were carried out using a potentiostat equipped with a five channel zero resistance ammeter for galvanic current measurements. The instruments were sectioned into three parts (cutting part, noncutting part and the shank) and degreased with acetone and rinsing with demineralized water prior to being immersed in sodium hypochlorite solution for testing. Nine instruments were employed to check the reproducibility of the electrochemical measurements. The study concluded that the corrosion resistance of NiTi alloy was enhanced by lowering the pH of sodium hypochlorite solution to 10.1, which allows the system to reach the stability domain of the passivating species TiO_2 and NiO_2.

Cheung GSP et al.[45] compared the low-cycle fatigue (LCF) behavior of electropolished and nonelectropolished nickel-titanium (NiTi) instruments of the same design in hypochlorite. Forty-five electropolished and 62 nonelectropolished NiTi engine files were subjected to rotational bending at various curvatures in 1.2% hypochlorite solution. Number of revolutions to failure, crack-initiation sites, extent of slow crack extension into the fracture cross-section, and surface strain amplitude were noted. A linear relationship was found between LCF life and surface strain amplitude for both groups with no discernable difference between the two. No nonelectropolished instrument showed more than one crack origin, significantly fewer than for the nonelectropolished instruments. The square root of crack extension and strain amplitude were inversely related. The study concluded that, although surface smoothness is enhanced by electropolishing, this did not protect the instrument from LCF failure.

Anderson ME et al.[44] investigated the effect of electropolishing on cyclic flexural fatigue and torsional strength of rotary nickel-titanium endodonticinstruments. Electropolished and nonelectropolished ISO size 30(0.04 taper) EndoWave (J Morita Corporation, Osaka, Japan), ProFile (Dentsply Maillefer, Ballaigues,Switzerland), and RaCe (FKG, La-Chaux De Fonds, Switzerland) instruments from the same manufacturing batches were investigated. The number of rotations to fracture and torque at fracture were determined and compared among the instruments tested. The study concluded that electropolishing may have beneficial effects in prolonging the fatigue life of rotary NiTi endodontic instruments. The benefits of electropolishing are likely to be caused by a reduction in surface irregularities that serve as points for stress concentration and crack initiation.

Ove A Peters et al.[42] investigated the effect of immersion in sodium hypochlorite on torque and fatigue resistance of two nickel-titanium files. ProFile and RaCe files were immersed in 5.25% NaOCl for one and two hours at two different temperatures. Torque and angle at failure were measured. The resistance to cyclic fatigue was determined by counting rotations to breakage. Resistance to cyclic fatigue decreased significantly for ProFile and RaCe instruments after immersion in sodium hypochlorite. The study concluded that NiTi rotary files have reduced resistance to cyclic fatigue after contact with heated sodium hypochlorite and may then be considered single-use instruments.

Ugur Inan et al.[21] evaluated and compared the topography of new and used ProTaper and rotary nickel-titanium (NiTi) instruments by using atomic force microscope. Four new and four used S1, S2, F1, F2 instruments were used. New and used instruments were analyzed on eleven points along a three millimeter section at the tip of the instrument. Quantitative measurements according to the topographic deviations (root mean square) were recorded. Mean root mean square values for used ProTaper instruments were higher than the new ones, and the difference between them was statistically significant. The study concluded that used ProTaper instruments demonstrated more surface deformation and wear.

Bui TB at al.[46] investigated the effect of electropolishing ProFile nickel titanium rotary instruments on torque resistance, fatigue resistance, and cutting efficiency. Cyclical fatigue was determined by counting rotations until breakage with an applied 30°, 45°, and 60° curve with a 5-mm radius. Torque and angle at failure were measured by rotating clamped files at 2 rpm until breakage. Cutting efficiency was determined by measuring the velocity of file advancement into plastic blocks with 100-g constant force for 5 seconds. The study concluded that electropolishing significantly reduced resistance to cyclic fatigue but did not affect torsional resistance. However, electropolishing reduced the angle at failure and amount of unwinding. Electropolishing did not significantly affect the cutting efficiency of ProFile instruments.

Antonoi Bonaccorso et al.[43] evaluated the pitting corrosion resistance of nickel-titanium rotary instruments with different surface treatments in 17% ethylenediaminetetraacetic acid and sodium chloride solutions. Electropolished RaCe, non-electropolished RaCe and physical vapor deposition –coated Alpha files were used. Electrochemical measurements were carried out using a potentiostat for galvanic current measurements. On the basis of electrochemical test, no localized corrosion problems are to be expected in EDTA. In NaCl, pitting potential occurred at higher values for the electropolished and PVD instruments, indicating an increased corrosion resistance. The study concluded that there appears to be a risk of corrosion for NiTi instruments without surface treatments in contact with sodium chloride. NiTi files with PVD and electropolishing surface treatments showed an increase corrosion resistance.

Antonio Bonaccorso et al.[32] chemically analyzed the rotary nickel-titanium instruments with and without electropolishing after cleaning procedures with sodium hypochlorite. Electropolished and non-electropolished RaCe files were used for this study and the surface analysis before and after cleaning in NaOCl was done using Energy dispersive x-ray analysis, Auger electron spectroscopy and Scanning Electron Microscopy. The nonelectropolished files showed marked presence of sodium chloride deposits in the machining marks and microcracks. As regards the chemical nature of the surface, the electropolished files had an oxide increase compared with the low oxide concentration (mainly TiO_2) before cleaning. The nonelectropolished files already possessed higher oxides concentration (TiO_2 and NiO) before NaOCl cleaning. Sodium hypochlorite treatment affects the chemical composition of the surface and in particular for nonelectropolished instruments, of the bulk exposed through machining marks and fabrication microcracks.

Fabiola Ormiga GB et al.[41] analyzed the influence of electrochemical polishing on flexural fatigue and torsional properties of K3 nickel-titanium endodontic rotary instruments. New files and polished files were tested for flexural fatigue and for resistance to fracture by twisting. The result showed no significant statistical difference existed between these groups. Scanning electron microscopy analysis showed no significant differences caused by electrochemical polishing between these groups. The conclusion from the results presented in this article is that electrochemical polishing does not increase the mechanical resistance of NiTi K3 endodontic files. This electrochemical treatment affects neither resistance to flexural fatigue nor torsional resistance of these files. According to SEM analysis, the surface of K3 file is not affected by the electrochemical polishing.

Topuz O et al.[23] investigated the effect of sodium hypochlorite on the surface characteristics of RaCe rotary nickel-titanium instruments using Atomic Force Microscopy. Four new RaCe instruments were used in this study. One 30.06 and one 30.02 instrument were immersed in sodium hypochlorite solution for 5 min. The instruments were analyzed on eleven points along a three millimeter section. Surface topography of the instruments were evaluated using the AFM. Root Mean Square values were used to compare the topographic variations. Statistically significant differences were found between immersed and new files. The study concluded that NaOCl causes deterioration on the surface of RaCe instruments and should be used with care during clinical use because of the risk of unexpected failure.

Caroline RA Valois et al.[22] evaluated the surface of rotary NiTi files after multiple autoclave cycles. They used ProFile and Greater Taper rotary NiTi files were attached to a glass base. AFM evaluation was done after 1, 5, and 10 autoclave cycles. The analysis was performed on fifteen different points. AFM was selected for this study because it is a sensitive and reliable technique that offers a suitable means for acquisition of qualitative and quantitative data concerning surface topography of rotary NiTi files. The same files before autoclave were taken as control. The arithmetic mean roughness, maximum height and root mean square parameters were recorded. All parameters were higher for both Greater Taper and ProFile after 10 cycles. Their results indicated that the multiple autoclave cycles increase the depth of surface irregularities located on rotary NITi files.

Zahed Mohammad[12] reviewed the different aspects of sodium hypochlorite use in endodontics. The elimination of pulpal debris which act as sources of infection may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of root canal between treatment sessions. In order to reduce or eliminate bacteria and pulpal tissue remnants, various irrigation solutions have been suggested to be used during treatment. Sodium hypochlorite is the most common irrigating solution used during root canal therapy. In this article the author describes the history mechanism of action, the antibacterial, antifungal, buffering actions of sodium hypochlorite. Tissue dissolving property and its effect on dentine and on endodontic instruments are also reviewed on this article.

Prabhakar J et al.[31] evaluated the antimicrobial efficacy of Triphala, green tea polyphenols, MTAD and 5% sodium hypochlorite against E.faecalis biofilm formed on tooth substrate. Extracted human teeth were biomechanically prepared, vertically sectioned, placed in the tissue culture wells exposing the root canal surface to enterococcus faecalis to form a biofilm. Qualitative assay with three week biofilm showed complete inhibition of bacterial growth with triphala, MTAD and sodium hypochlorite except GTP and saline, which showed presence of bacterial growth. Qualitative assay with six week biofilm showed growth when treated with triphala, GTP and MTAD where as sodium hypochlorite has shown complete inhibition. The study concluded that 5% NaOCl had the maximum antibacterial activity against enterococcus faecalis biofilm formed on tooth substrate. Triphala, green tea polyphenols and MTAD showed statistically significant antibacterial activity. The use of herbal alternative as a root canal irrigant might prove to be advantageous considering the several undesirable characteristics of sodium hypochlorite.

Ametrano G, D'Anto et al.[11] evaluated the effects of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) on the surface characteristics of ProTaper rotary nickel-titanium instruments using Atomic Force Microscopy. Twenty protaper instruments were divided into five groups: no immersion, immersion in 5.25% NaOCl for 5 or 10 min and immersion in 17% EDTA for 5 or 10 min. The topographic irregularities were evaluated at the nanometric scale and found that the RMS and Ra values of instruments treated with NaOCl and EDTA solutions were statistically higher than that of the new ones. They also concluded that AFM is a suitable method for quantifying and evaluating the surface of endodontic instruments and the effects of irrigates.

Madhu Pujar et al.[51] evaluated the antibacterial efficacy of Triphala, Green tea polyphenols and sodium hypochlorite against E.faecalis biofilm formed on tooth substrate. Human extracted tooth were biomechanically prepared, vertically sectioned and placed in wells containing enterococcus faecalis to form a biofilm. After two weeks all groups were treated for ten minutes with test solutions and were analyzed quantitativelyThe colony forming units were counted and the results showed NaOCl with maximum antibacterial activity. Triphala and GTP have shown significantly better antibacterial activity. They concluded that herbal alternatives can be used as root canal irrigants, considering the undesirable effects of sodium hypochlorite.

Gutmann JL [25] reviewed the perspectives on the uniqueness of the nickel-titanium alloys, the properties of nitinol in clinical applications. The expanded use of nickel-titanium rotary instruments in root canal procedures has led to the development of a wide variety of shapes, designs and applications. Root canal anatomy has not changed, however, and the same challenges exist in both initial treatment and the revision of unacceptable treatment. These challenges include application with high levels of achievement and low to no levels of adverse effects, such as instrument fracture, root canal ledging. The author emphasized on the different surface treatments like implantation and electropolishing of NiTi instruments. Effect of twisting and machining of the NiTi instrument, the heat treatments done during the manufacturing process were also discussed in this review.

Materials and Methods

Files and irrigants used

A total of eight new nickel-titanium rotary endodontic files, four files each of ProTaper – S2 (DENTSPLY, Switzerland) (Fig.1a) and iRaCe – R3 (FKG DENTAIRE, Switzerland) (Fig.1b) were used for this study. The irrigants used were 5% NaOCl (Azure Research Lab Pvt. Ltd, India) (Fig2a), 17% EDTA (Prime Dental Products Pvt. Ltd, India) (Fig.2b) and Triphala (IMPCOPS, Chennai, India) (Fig.2c).

Methodology

The cutting flutes of the three files each from ProTaper and iRaCe were immersed seperately in three beakers containing the three different irrigating solutions for 5 minutes. These immersed instruments were then let to air dry at room temperature. One file each from the ProTaper group and the iRaCe group were evaluated without immersion in the irrigants and were kept as control.

The files were then attached to the metal holder on the sample stage of AFM using adhesive tape (Fig.5a, 5b). Nine areas of the surface were analyzed on a 3-mm section taken at the middle portion of the files. The AFM images of the tested samples were then recorded using the non-contact mode of the AFM under ambient conditions.

A total of nine perfect squares of 2x2, 5x5 and 10x10 micrometer were examined on every sample which were then unitized to 1x1 micrometers for statistical analysis. The Roughness average (Ra), Root Mean Square (RMS) and the Mean Height of Roughness Profile Elements (Rc) of the scanned profiles were then recorded (Table.1). These parameters depict the vertical topography of the instrument surface and an increase in these values reflects the alterations caused by the used irrigants.

Atomic Force Microscope

Atomic Force Microscope (5500-Agilent Technologies, USA) (Fig.3a, 3b) was used for evaluating the surface topography of the study samples. The Atomic Force Microscope (AFM) consists of a cantilever tip which probes the specimen surface. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to a deflection of the flexible cantilever in the z-direction. A photodiode detects the deflection of the cantilever through a laser beam focused on and reflected from the rear of the cantilever (Fig.4). A computer processes the electrical differential signal of the photodiode which is obtained from each point on the instrument surface and generates a feedback signal for the piezoscanner in order to maintain a constant force on the tip. The processed data of the samples are then recorded in digital form as sets of x, y and z values. These help to evaluate the vertical amplitude of a surface topography. The three dimensional topographic image is then captured by the CCD camera and these images were then processed using the Picoimage software.

Energy Dispersive X-Ray Diffraction Spectroscopy

After the AFM analysis, the same samples were then analyzed with the Energy Dispersive X-Ray Diffraction Spectroscopy (EDS) for chemical characterization on the instrument surface (Fig.6a, 6b). The files to be analyzed are mounted on to the EDS machine (Fig.6c). The EDS works when the incident beam falling on the sample excites an electron in an inner shell, ejecting it from the shell. An electron from an outer, higher-energy shell then fills the hole, and the difference in energy between the higher-energy shell and the lower energy shell will be released in the form of an X-ray. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. As the energy of the X-rays are characteristic of the difference in energy between the two shells, and of the atomic structure of the element from which they are emitted, this allows the elemental composition of the specimen to be measured in weight percentage.

The data obtained for statistical analysis are the Arithmetic Mean Deviation of roughness profile (Ra), Root Mean Square *(RMS)* Deviation of roughness profile (Rq) and Mean Height of roughness profile elements (Rc).

Data was then analyzed using the computer software, Statistical Package for Social Sciences (SPSS) version 10. Data derived is expressed in the mean and standard deviation. Student's t test was used to compare the mean values between the ProTaper and the iRaCe groups. Analysis of variance (One Way ANOVA) was performed as parametric test to compare different treatments. Duncan's Multiple Range Test was also performed along with ANOVA as post-hoc test to elucidate multiple comparisons between treatments. For all statistical evaluations, a two-tailed probability of value < 0.05 was considered as significant.

Results

The three-dimensional AFM images of the surfaces of all the Protaper and iRaCe instruments including new and those immersed in the three different irrigants showed topographic irregularities at the nanometric scale. The control group images of Protaper files significantly differed in surface topography when compared to iRaCe control sample files (Figs. 7, 11). Ra, RMS and Rc values of new Protaper files were significantly lower compared to new iRaCe files (Table-4, Chart III) ($P < 0.05$). Except for the EDTA immersed, the three other samples of Protaper files (new, NaOCl and Triphala immersed files) showed no significant difference (Table-2, Chart I).

The Ra and RMS values of new and triphala immersed iRaCe files did not differ significantly, but had a very highly significant difference with NaOCl and EDTA immersed iRaCe files ($P < 0.001$) (Table-3, Chart II). All treated Protaper and iRaCe instruments image revealed an increase in roughness compared to their respective controls. Though both the Protaper and iRaCe shows significant surface roughness properties, iRaCe group showed higher values of surface roughness (Ra, RMS and Rc) than the Protaper group (Table 1). Among the immersed files, the maximum Ra value was observed for NaOCl immersed iRaCe files and minimum Ra values were observed for triphala immersed Protaper files. The increase in Ra, RMS and Rc values were manufacturer and irrigating solution dependent.

The EDS analysis has shown that the surface of new ProTaper and iRaCe files had almost similar amount of nickel, titanium and oxygen (Figs.15,19). A small amount of tantalum was also present on the surface of new iRaCe file (fig. 19). The NaOCl and EDTA immersed group of files have shown a decrease in weight percentage of nickel and titanium on the alloy surface (Figs. 16,17,20,21). The presence of sodium and chlorine was observed on the surface of NaOCl immersed files.

Table 1. Ra, Rq and Rc values (nm) at 2x2, 5x5 and 10x10 (µm areas)

	Ra (nm)			Rq (nm)			Rc (nm)		
	2 x2 µm	5x5 µm	10x10 µm	2 x2 µm	5x5 µm	10x10 µm	2x2 µm	5x5 µm	10x10 µm
Protaper (New)	0.95 1.04 1.13	1.58 1.78 1.98	2.54 2.26 1.98	1.17 1.25 1.33	1.96 2.07 2.18	2.70 2.83 2.96	2.76 3.00 3.24	6.64 6.32 6.oo	7.00 7.08 7.16
Protaper(NaOCl)	0.410 0.594 0.778	2.30 2.64 2.98	5.10 5.26 5.32	0.69 0.75 0.81	3.06 3.17 3.28	5.18 6.88 8.58	0.740 0.941 1.142	7.50 7.61 7.72	13.5 14.2 14.9
Protaper(EDTA)	1.50 1.62 1.74	9.50 10.4 11.3	17.7 16.6 15.5	2.00 2.01 2.02	10.5 12.0 13.5	21.7 20.9 20.1	3.05 2.82 2.59	22.9 20.8 18.7	65.1 59.6 54.1
Protaper (Triphala)	0.98 0.88 0.78	1.93 2.67 3.41	3.01 2.78 2.55	0.91 1.08 1.25	2.94 3.15 3.36	3.81 3.45 3.09	1.59 2.83 4.07	11.1 11.3 11.5	7.51 9.38 11.25
i-Race(New)	1.09 2.16 3.23	9.16 6.78 4.40	11.15 8.18 5.21	2.04 2.80 3.56	7.53 8.10 8.67	9.50 10.4 11.3	8.39 7.19 5.99	24.9 19.9 14.9	21.79 20.90 20.01
i-Race(NaOCl)	7.71 7.15 6.59	17.7 22.1 26.5	30.8 27.5 24.2	10.43 9.47 8.51	30.6 29.2 27.8	33.9 36.5 39.1	21.36 18.36 19.96	59.50 58.20 56.90	90.2 87.8 85.4
i-Race(EDTA)	6.18 5.57 4.96	19.0 18.1 17.2	24.7 23.3 21.9	8.43 7.74 7.05	22.9 22.5 22.1	35.0 33.3 31.6	9.08 8.29 7.50	55.4 57.2 59.0	79.9 78.4 76.9
i-Race (Triphala)	0.87 1.23 1.59	7.51 7.06 6.61	12.01 9.67 7.33	1.62 1.51 1.40	10.06 8.59 7.12	12.01 12.30 12.59	5.75 3.95 2.15	27.9 35.2 42.5	31.9 38.5 45.1

Ra ; *Arithametic Mean Deviation of roughness profile*
Rq ; *Root Mean Square(RMS) Deviation of roughness profile*
Rc ; *Mean Height of the roughness profile elements*

Table 2. Analysis of variance comparing different treatment in ProTaper

Parameter	Treatment	Mean	+ SD	F value	P value
RA	New	0.37^{a}	0.15	7.745	< 0.01
	NaOCl	0.45^{a}	0.13		
	EDTA	1.52^{b}	0.65		
	Triphala	0.42^{a}	0.13		
RMS	New	0.44^{a}	0.17	8.644	< 0.01
	NaOCl	0.57^{a}	0.16		
	EDTA	1.83^{b}	0.73		
	Triphala	0.51^{a}	0.14		
RC	New	1.16^{a}	0.41	3.851	< 0.05
	NaOCl	1.14^{a}	0.58		
	EDTA	3.84^{b}	2.29		
	Triphala	1.54^{ab}	0.67		

a, b – Means with same superscript in each parameter do not differ each other (Duncan's Multiple Range Test)

Chart I

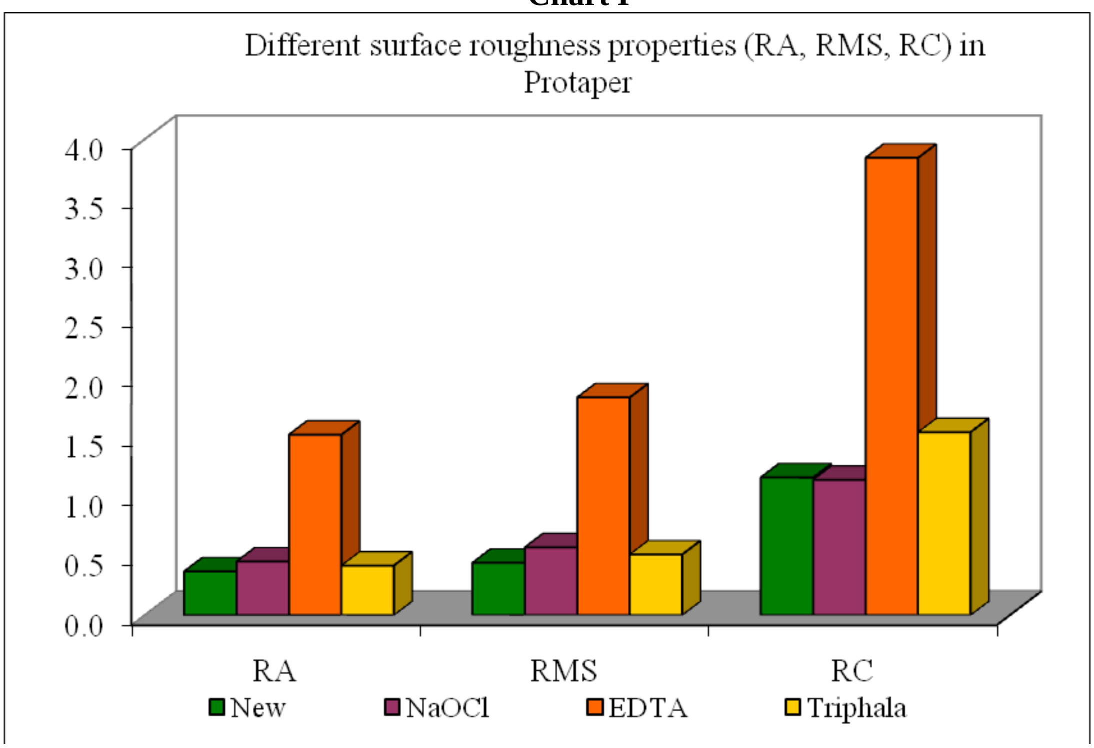


Table 3. Analysis of variance comparing different treatment in iRaCe

Parameter	Treatment	Mean	± SD	F value	P value
RA	New	1.08^{a}	0.27	15.006	< 0.001
	NaOCl	3.58^{c}	0.84		
	EDTA	2.91^{b}	0.65		
	Triphala	1.00^{a}	0.40		
RMS	New	1.35^{a}	0.29	20.487	< 0.001
	NaOCl	4.74^{b}	1.09		
	EDTA	3.90^{b}	0.58		
	Triphala	1.23^{a}	0.48		
RC	New	3.22^{a}	1.00	4.932	< 0.05
	NaOCl	10.21^{b}	2.02		
	EDTA	7.81^{ab}	3.65		
	Triphala	4.29^{a}	2.56		

a, b, c – Means with same superscript in each parameter do not differ each other (Duncan's Multiple Range Test)

Chart II

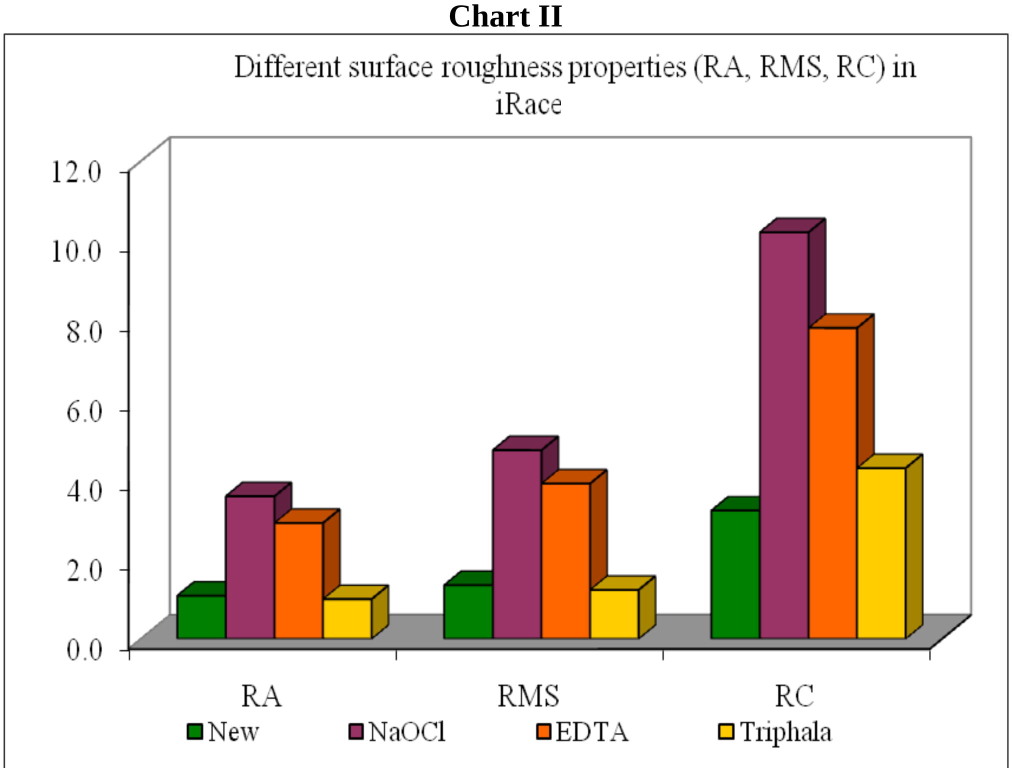


Table 4. Comparison of different surface roughness properties between new ProTaper and iRaCe

Parameter	Treatment	Mean	± SD	t value	P value
RA	Protaper	0.37	0.15	- 4.051	< 0.05
	i Race	1.08	0.27		
RMS	Protaper	0.44	0.17	- 4.651	< 0.05
	i Race	1.35	0.29		
RC	Protaper	1.16	0.41	- 3.316	< 0.05
	i Race	3.22	1.00		

Chart III

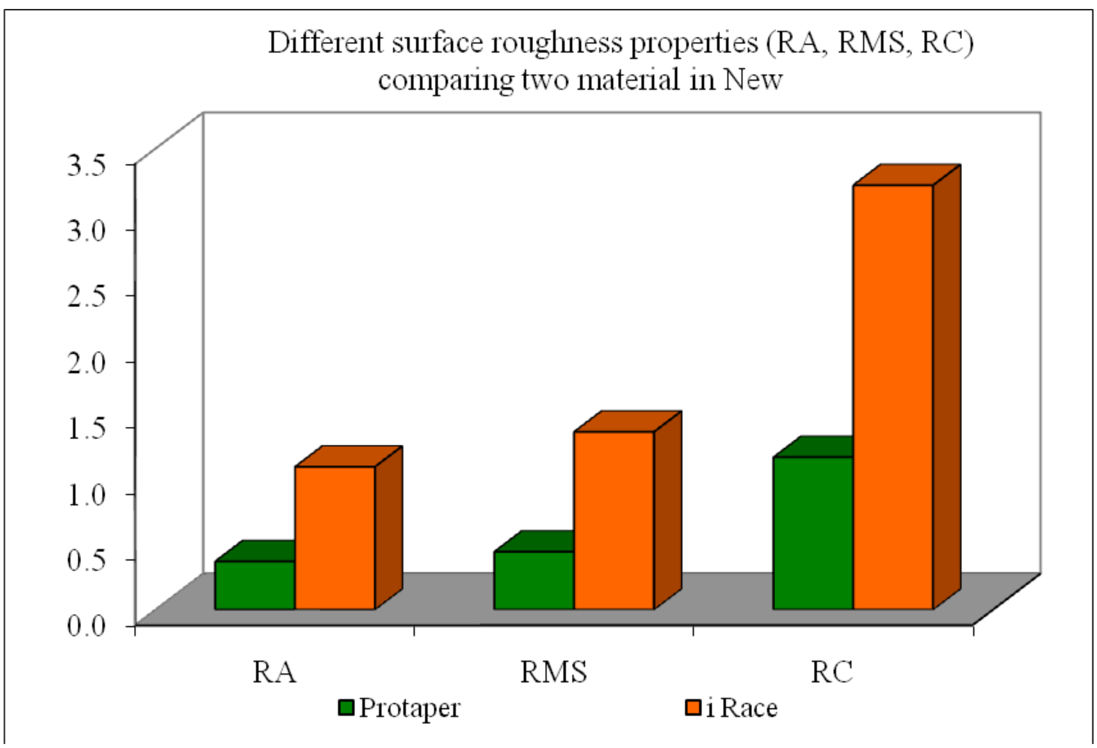


Table 5. Comparison of different surface roughness properties between ProTaper and iRaCe in NaOCl

Parameter	Treatment	Mean	± SD	t value	P value
RA	Protaper	0.45	0.13	- 6.415	< 0.01
	i Race	3.58	0.84		
RMS	Protaper	0.57	0.17	- 6.531	< 0.01
	i Race	4.74	1.09		
RC	Protaper	1.14	0.58	- 7.888	< 0.01
	i Race	10.21	2.02		

Chart IV

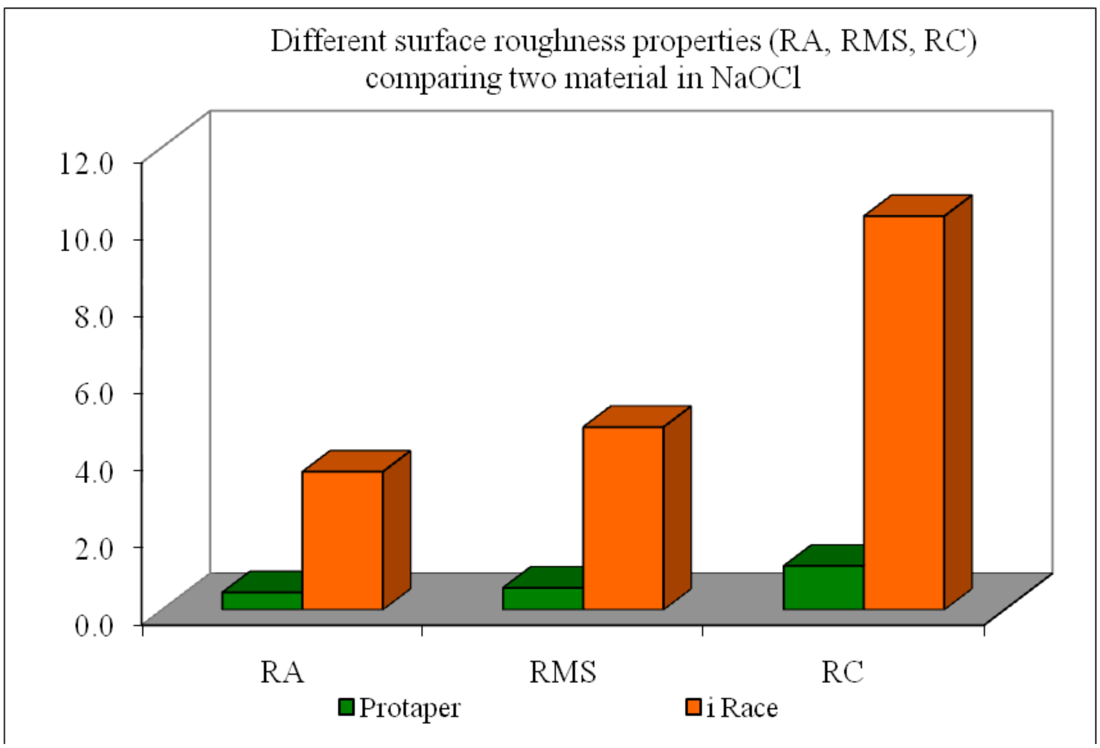


Table 6. Comparison of different surface roughness properties between ProTaper and iRaCe in EDTA

Parameter	Treatment	Mean	± SD	t value	P value
RA	Protaper	1.52	0.65	- 2.626	< 0.05
	i Race	2.91	0.65		
RMS	Protaper	1.83	0.73	- 3.821	< 0.05
	i Race	3.90	0.59		
RC	Protaper	3.84	2.29	- 2.592	< 0.05
	i Race	7.81	3.65		

Chart V

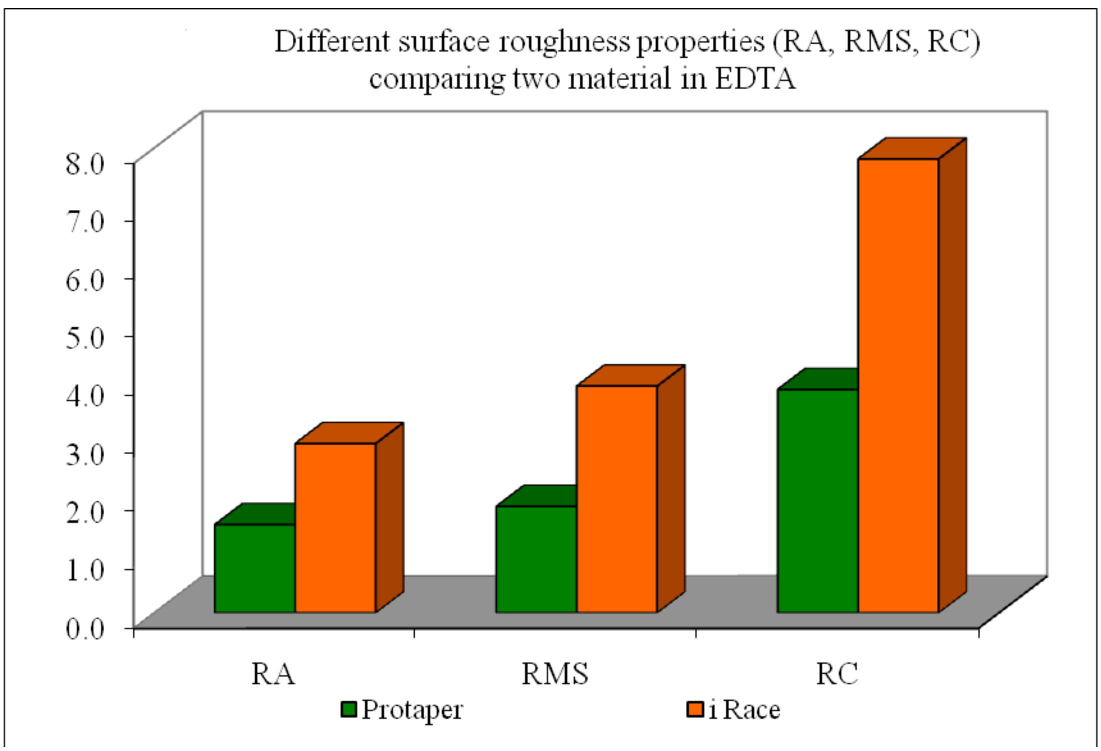
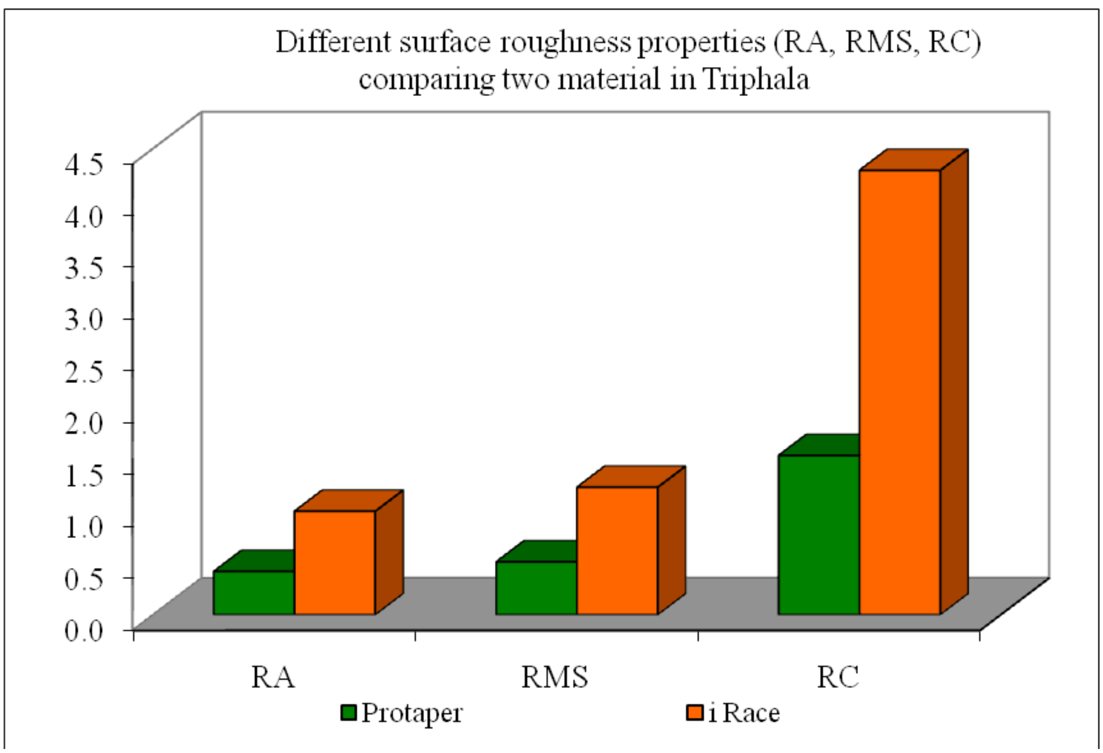


Table 7. Comparison of different surface roughness properties between ProTaper and iRaCe in Triphala

Parameter	Treatment	Mean	± SD	t value	P value
RA	Protaper	0.42	0.13	- 2.543	< 0.05
	i Race	1.00	0.40		
RMS	Protaper	0.51	0.15	- 2.511	< 0.05
	i Race	1.23	0.48		
RC	Protaper	1.54	0.67	-2.801	< 0.05
	i Race	4.29	1.56		

Chart VI



Discussion

The mechanical instrumentation of root canals alone cannot sufficiently disinfect the canal space regardless of whether stainless steel or NiTi instruments are used. The ideal irrigant or combination of irrigants should destroy the bacteria, dissolve the necrotic tissue, lubricate the canal, remove the smear layer and not irritate the healthy tissues[33]. Sodium hypochlorite, as an irrigant encompasses many desirable properties required of a ideal root canal irrigant. Therefore, it has been advocated as the most ideal of the available irrigating solutions[12].

Sodium hypochlorite is used in varying concentrations from 0.5% to 6%. It is used as an unbuffered solution having a pH of 11 and a buffered solution (bicarbonate) having a pH of 9. The high pH of sodium hypochlorite interferes with the cytoplasmic membrane integrity causing an irreversible enzymatic inhibition, biosynthetic alterations in the cellular metabolism and also causing phospholipid degradation. In higher concentrations, sodium hypochlorite results in the dissolution of both necrotic and vital tissues.

Ethylenediamine Tetra – Acetic Acid (EDTA) creates a stable calcium complex with the dentin mud, smear layer or the calcific deposits present along the canal walls. It softens the dentin, particularly peritubular dentin and especially in the coronal and middle aspects of canals[34]. EDTA also helps to prevent apical blockage and aids in the disinfection process by improving the penetration of the irrigating solution mainly by the removal of the smear layer. EDTA exerts its strongest effect when used synergistically with NaOCl. It has been shown that EDTA retained its ability to chelate calcium in the presence of NaOCl, but the tissue dissolving ability of NaOCl was reduced. So it was concluded that both the solutions should be used separately[35].

The constant increase in antibiotic resistant microbial strains and the side effects caused by the use of synthetic drugs and also the hazards associated with irrigants such as NaOCl has prompted researchers to look for safer, more biocompatible and patient friendly herbal alternatives. Among the herbal alternatives, triphala has been the most promising[51]. Triphala has shown the most significant antibacterial efficacy against the enterococcus faecalis biofilm which is present on the tooth surface. Triphala has been proven to be safe, containing active constituents that have beneficial physiologic effect and also having curative properties such as being antioxidant, anti-inflammatory and radical scavenging[36].

The manufacture of NiTi endodontic instruments is a far more complex procedure compared to that of stainless steel instruments, as the NiTi files have to be machined rather than twisted. The Nitinol used in the manufacture of endodontic instruments contain approximately 56% (wt) nickel and 44% (wt) titanium. In some NiTi alloys, a small percentage (< 2% wt) of nickel can also be substituted by cobalt. The resultant combination is a one – to – one atomic ratio (equiatomic) of the major components and as with the other metallic systems, the alloy can exist in various crystallographic forms[4].

The most striking feature of NiTi alloys is their property of shape memory and superelasticity. Shape-memory alloys (SMA) have a unique capability to recover to their original shape after undergoing large deformation through heating. Besides Shape-memory effects, NiTi alloys also exhibit superelastic (SE) behaviour that allows them to return to their original shape upon unloading. The manufacturers of nickel-titanium (NiTi) root canal instruments have come up with different methods which focus on altering the surface of the alloy as well as altering the alloy microstructure in order to enhance the efficacy and the longevity of the NiTi instruments[25]. NiTi as an alloy, has a high level of biocompatibility along with it possessing excellent corrosion resistance property[26].

The protaper NiTi rotary endodontic files are available in different series labeled as S1, Sx, S2, F1, F2 and F3. The protaper S2 series file used in our study has a tip diameter which corresponds to ISO size 015 along with having a progressive taper. The iRaCe rotary files are available as R1, R1a, R1b, R2 and R3. The iRaCe R3 file used in our study has a tip diameter which corresponds to ISO size 030 having a taper of .04. The middle region of the cutting flutes was selected for evaluation based on the ease of specimen mounting and also for focusing on the atomic force microscope. Since the aim of our study was to evaluate the effect of irrigants on the instrument (ProTaper and iRaCe) surface, the region to be evaluated on the cutting flutes was assumed to be of less significance.

One important surface characteristic, as claimed by the manufacturers of iRaCe, is that of electropolishing. Electropolishing, is a method commonly employed for the surface finishing of most metallic medical equipments. In this process, the instruments which are connected to an anode are immersed together with another electrode in a temperature-controlled bath of electrolytes followed by the passing of direct electric current into the solution. The metal at the anode is dissolved into the solution, whereas a reduction reaction will occur at the cathode. This process alters the surface composition and texture of the instrument thereby rendering it a more homogeneous surface oxide layer that acts as a protective film, having less defects and residual surface stress. By this process of electropolishing, the corrosion resistance of the metal is enhanced along with corresponding improvement in the surface characteristics[25].

The EDS analysis of our study specimens revealed the presence of tantalum (Ta) on the surface of new iRaCe files which was not present on the surface of new ProTaper files. The presence of this noble metal on the surface of iRaCe files may be attributed to the electropolishing procedure as claimed by the manufactures.

Nickel-Titanium instruments come into contact with sodium hypochlorite when the irrigant is present in the pulp chamber and also during root canal instrumentation. Sodium hypochlorite is known to be corrosive to metals causing, selective removal of nickel from the NiTi instrument surface leading to micro pitting. It is supposed that these microstructural defects can lead to areas of stress concentration, crack formation and finally leads to the weakening of the instrument surface[27,50]. There was a significant corrosive phenomenon observed in NiTi instruments exposed to 1% NaOCl for up to ten cleaning cycles[28]. Corrosive patterns were found when light speed NiTi rotary instruments were used along with 5% NaOCl for a time period of thirty or sixty minutes[29].

EDTA has been the most commonly used chelating agent which reacts with the calcium ions present in dentin leading to the formation of a soluble chelate[14]. The cutting efficiency of stainless steel files is significantly reduced by EDTA as a result of slight corrosion[30]. Triphala has shown statistically significant antibacterial activity against a 6-week enterococcus feacalis biofilm advocating its use as an effective irrigant[31].

In the present study, AFM was used for evaluation of surface topography of ProTaper and iRaCe files. Scanning electron microscopy has been the most widely used method to image microscopic features of sample surfaces. However, AFM provides a number of advantages over the conventional microscopy techniques. Electron microscopic methods often require sophisticated sample preparation methods, such as etching techniques or metal evaporation into the samples, which can lead to artifacts and loss or change of detectable information of the sample structure and properties. Atomic force microscopes which can be used in either an ambient or a liquid environment require neither a vacuum environment nor any special sample preparation, thus leaving the material in its native state[23].

AFM was selected for the present study because it is a sensitive and reliable technique that offers a suitable means for the acquisition of qualitative and quantitative data concerning the surface topography of rotary NiTi files. SEM and AFM are both able to resolve structure down to the nanometric scale, but the image formation mechanisms are quite different. SEM uses an electron beam operated under vacuum to produce a two-dimensional 'photographic' image of the samples but cannot provide any quantitative data. In contrast, AFM reconstructs a three-dimensional image of the surface topography in real time. These data sets can be analyzed with dedicated digital software to provide all the data pertaining to the examined surface in a quantitative form. Moreover, AFM provides appropriate topographic contrast with greater detail, direct measurements in all three dimensions[11].With these advantages, AFM has proved to be a valuable research tool in the field of endodontics and has been used in research studies[20-22].

In the present study, both the untreated ProTaper (Ra-0.37, RMS-0.44,Rc-1.16nm) and iRaCe files (Ra-1.08, RMS-1.35, Rc-3.22nm) showed surface deterioration at the nanometric scale. It is assumed that the presence of surface irregularities on NiTi files make them more prone to corrosion and fracture. Manufacturing defects present in NiTi files can also play a role in the process of corrosion. In our study ProTaper files have demonstrated considerable amount of instrument surface deterioration which is attributed to the milling marks present on the file surface which act as crevices and thereby initiate corrosion[40].

During the grinding of the cutting edges, small scratches and grooves are created on the surface of the instrument, this might limit their fatigue life and their corrosion resistance to sodium hypochlorite[38]. A SEM investigation done by Fabiola et al on electropolished and non-electropolished files suggest that electropolishing has no influence on the fracture resistance of K3 rotary NiTi instruments[41]. The electropolished RaCe instruments had a subjectively smoother surface compared to the nonelectropolished ProFile files and also there was no difference in the corrosion patterns between RaCe and ProFile files[42].

In our study, iRaCe has shown higher surface roughness values compared to the ProTaper files despite the fact that IRaCe files were electropolished. The process of electropolishing may have a beneficial effect in prolonging the fatigue life of rotary NiTi endodontic instruments. An earlier study done by Cheung et al to compare the low-cycle fatigue (LCF) behavior of electropolished and nonelectropolished nickel-titanium files has shown that the surface smoothness was enhanced by electropolishing but the process did not protect the instrument from LCF failure[45]. The benefit of electropolishing results in the reduction of the surface irregularities that serve as points for stress concentration and crack initiation[44].

The present study showed higher surface roughness values for new iRaCe (Ra-1.08nm) files when compared with the new ProTaper files (Ra-0.37nm). The fact that both files were from different manufacturers could also have been a reason for such minute disparity in the surface roughness values between the files. An investigation done on the effect of electropolishing Profile nickel-titanium rotary instruments has shown that it did not significantly affect the cutting efficiency[46]. Electropolishing only affects the surface of NiTi instruments but the unaltered core will still contain inclusion particles such as titanium carbonitride and nickel titanium oxide from the manufacturing process of NiTi alloy[47]. A significant increase in surface roughness has been shown for NaOCl (Ra-3.58nm) and EDTA (Ra-2.91nm) immersed iRaCe when compared to the NaOCl (Ra-0.45nm) and EDTA (Ra-1.52nm) immersed ProTaper sample files. The initial roughness profile of the study NiTi files has proportionally changed in intensity due to the immersion in the different irrigants.

The irrigant immersed ProTaper and iRaCe instrument images revealed an increase in the roughness compared to their respective new files. This shows that all the three study irrigants can cause alterations to the surface of ProTaper and iRaCe endodontic files. The present study data reveals that EDTA (Ra-1.52nm) immersed ProTaper files have shown significant differences in the surface topography when compared with the untreated (Ra-0.37nm) ProTaper study samples. Both the EDTA and NaOCl immersed iRaCe files have shown very highly significant difference when compared with untreated iRaCe files ($P < 0.001$). The values obtained in our study are in contradiction to most of the other studies which state that sodium hypochlorite is more corrosive than EDTA[26,32,42]. Darabara et al used the cyclic potentiodynamic polarization method to evaluate the pitting and crevice corrosion characteristics of NiTi instruments exposed to 17% EDTA and 5.25% sodium hypochlorite solutions, the study demonstrated high corrosion resistance of the instruments to both irrigating substances. However the data from the above mentioned studies were based on different contact times between files and irrigants and on SEM analysis instead of AFM which might explain the difference between the results.

A previous study done by Ametrano et al used AFM to evaluate EDTA and sodium hypochlorite immersed ProTaper files have shown significant surface alterations on the surface of EDTA immersed files when compared to NaOCl immersed study files[11]. This result is in accordance with our study signifying high surface deterioration potential of EDTA when compared to NaOCl which may be attributed to the lower pH of EDTA. Corrosion of endodontic instruments can occur during chemomechanical preparation, chemical disinfection or sterilization as a result of the chemical substances used[37]. It has been reported that topographic irregularities may have a considerable impact on the resistance to fracture by endodontic instruments[5,17].

As of date, there are no published data regarding the use of AFM to evaluate the effect of any herbal irrigant on rotary endodontic files. In our study, sodium hypochlorite (Ra-3.58nm) and EDTA (Ra-2.91nm) immersed iRaCe files have shown a significantly higher surface roughness values when compared to the Triphala immersed iRaCe files (Ra-1.00nm). The fact that triphala is a less reactive hydrocarbon compound when compared to other study irrigants could be a reason for this difference. In a study done by Topuz et al the immersion of RaCe NiTi files in 5.25% NaOCl for 5 minutes caused localized surface pitting and cracks which lead to the modification in the integrity and resistance to fracture of NiTi instruments[23]. Long term immersion of NiTi files in irrigants leads to an increased level of surface deterioration.

In the present study, only the cutting flutes were immersed in the irrigants so as to avoid any bias that may occur due to the interaction of different metals which are present in the shank of the instrument. The energy dispersive x-ray microanalysis (EDX) has shown that the cutting and noncutting sections of ProTaper files was made of NiTi alloy, while the shank was made of gold-plated brass[39]. The corrosion resistance of ProTaper NiTi instruments to NaOCl was evaluated at two different pH levels and it was found that the corrosion resistance of NiTi alloy was enhanced by lowering the pH[39,48].

Energy Dispersive X-Ray Diffraction Spectroscopy (EDS) was used in the present study for the chemical characterization on the instrument surface. The incident beam falling on the sample will excite an electron in an inner shell, ejecting it from the shell. An electron from an outer, higher-energy shell then fills the hole, and the difference in energy between the higher-energy shell and the lower energy shell will be released in the form of an X-ray. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. As the energy of the X-rays are characteristic of the difference in energy between the two shells, and of the atomic structure of the element from which they are emitted, this allows the elemental composition of the specimen to be measured in weight percentage. Many studies have been conducted to analyze the surface of rotary NiTi endodontic files using EDS[17,24].

Energy dispersive X-ray spectroscopy revealed that 5.25% NaOCl affected the chemical composition of the NiTi file surface after 1-4 hours and also the exposed bulk of the instruments through the machining and fabrication microcracks[26]. EDS was used in our study to determine the surface composition of the corroded areas seen on these study files. It is important to study the correlation between peak amplitudes on EDS and the concentration of chemical elements in the analyzed area.

The EDS analysis showed that the surface of new ProTaper and iRaCe files had almost the similar amount of nickel, titanium and oxygen components. A small weight percentage of tantalum was also present on the surface of new iRaCe files. The presence of this noble metal may be attributed to the electropolishing of iRaCe files as claimed by the manufacturers. To enhance the performance, durability and safety of root canal instruments, alteration in the inherent metallic properties like heat treatment can be considered in preference to electropolishing[49].

The qualitative EDS spot analysis of NaOCl immersed iRaCe files showed surface irregularities which contained principally sodium and chlorine. NaOCl immersed ProTaper file have also shown trace amounts of both sodium and chlorine. The sodium and chlorine elements present on the surface of these NiTi files are due to the immersion in sodium hypochlorite. The EDS result also showed a corresponding decrease in the weight percentage of nickel and titanium where as there was an increase in the presence of sodium and chlorine elements on the instrument surface.

The Atomic force microscopy is a nondestructive, practical method for evaluating endodontic file surface in a quantitative way. The analysis on the nanoscale supplied evidences of the necessity of manufacturers to adjust processing parameters to minimize and prevent the irregularities on the surface of these instruments. This quality control measurement can certainly improve the efficacy of the NiTi endodontic file’s clinical performance. NaOCl and EDTA are considered as the most promising irrigants with the property of tissue dissolution and chelation respectively. Their use in endodontics cannot be compromised for the sake of the minimal surface deterioration it may cause on the rotary NiTi files. Measures should be taken at the manufacturing stage itself to improve the physical properties of the NiTi files. Apart from electropolishing, other microstructural heat treatment procedures, ion implantation and plasma immersion techniques should also be studied and considered for increasing the clinical efficacy of NiTi rotary endodontic files.

Conclusion

Within the limitations of this study it can be concluded that:

1. Short-term contact with 17% EDTA and 5% NaOCl can cause significant surface deterioration on the surface of ProTaper and iRaCe rotary NiTi endodontic files.
2. Triphala showed no significant surface deterioration on iRaCe and ProTaper rotary NiTi endodontic files.
3. Electropolished iRaCe files did not show any resistance to surface deterioration when compared to nonelectropolished ProTaper files on immersion in irrigants.
4. Atomic Force Microscope can be considered as a suitable method for quantifying and evaluating the surface characteristics of endodontic instruments.

Summary

The aim of this in-vitro study was to evaluate the effect of sodium hypochlorite (NaOCl), ethylenediamine tetraacetic acid (EDTA) and triphala on surface characteristics of ProTaper and iRaCe rotary nickel-titanium instruments using Atomic Force Microscope (AFM) and Energy Dispersive X-Ray Diffraction Spectroscopy (EDS).

A total of eight nickel-titanium rotary endodontic files, four files each of ProTaper – S2 (Dentsply, Switzerland) and iRaCe – R3 (FKG dentaire, Switzerland) were used. Three files each from ProTaper and iRaCe were immersed in 5.25% sodium hypochlorite, 17% ethylene diamine tetraacetic acid and triphala separately for five minutes. One file each from ProTaper and iRaCe were evaluated without immersion in irrigants. Nine perfect squares of 2x2, 5x5 and 10x10 micrometer were examined on a 3mm section taken from the middle portion of the files using Atomic Force Microscope (AFM). The Roughness average (Ra), Root Mean Square (RMS) and Mean Height of Roughness Profile Elements (Rc) of the scanned profiles were then recorded. The same samples were then evaluated using Energy Dispersive X-Ray Spectroscopy (EDS) to get the elemental characterization of the instrument surface. Data were analyzed by means of Student's 't' test, One Way ANOVA and Duncan's Multiple Range Test.

The three-dimensional AFM images of the surfaces of all the Protaper and iRaCe instruments including the new and those immersed in the three different irrigants showed topographic irregularities at the nanometric scale. Ra, RMS and Rc values of new Protaper files were significantly lower compared to iRaCe files ($P < 0.05$). Except for the

EDTA immersed, the other three samples of Protaper files (new, sodium hypochlorite and triphala immersed files) showed no significant difference in their surface roughness. The NaOCl and EDTA immersed iRaCe files have shown a very highly significant increase in surface topography when compared to the new and triphala immersed iRaCe files ($P < 0.001$). The treated Protaper and iRaCe instruments image revealed an increase in the surface roughness compared to their respective controls. Among the immersed files, the maximum Ra value was observed for the NaOCl immersed iRaCe files and the minimum Ra value was observed for the triphala immersed Protaper files.

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MATERIALS USED

Fig. 1a. ProTaper NiTi rotary file *(DENTSPLY, SWITZERLAND)***)**

Fig. 1b. iRaCe NiTi rotary file *(FKG DENTAIRE, SWITZERLAND)*

MATERIALS USED

Fig. 2a. 5% NaOCl *(AZURE RESEARCH LAB, Pvt. Ltd)*

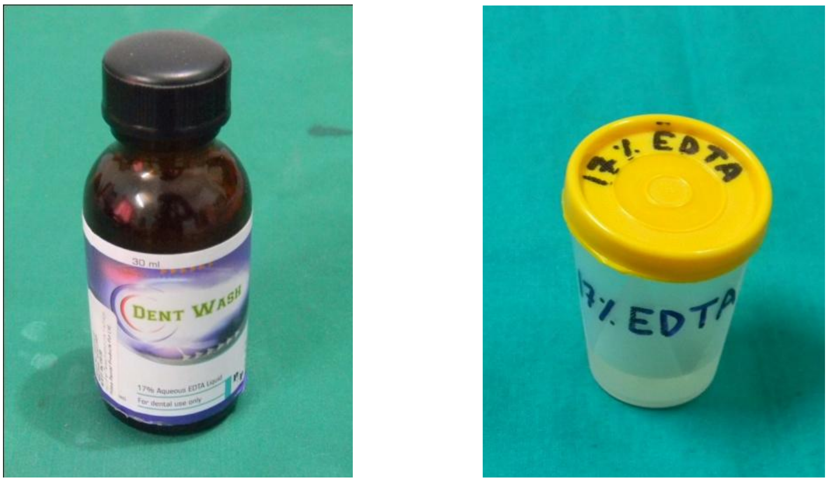


Fig. 2b. 17% EDTA (*PRIME DENTAL PRODUCTS, INDIA)*

Fig. 2c. Triphala *(IMPCOPS, CHENNAI, INDIA)*

EQUIPMENTS USED

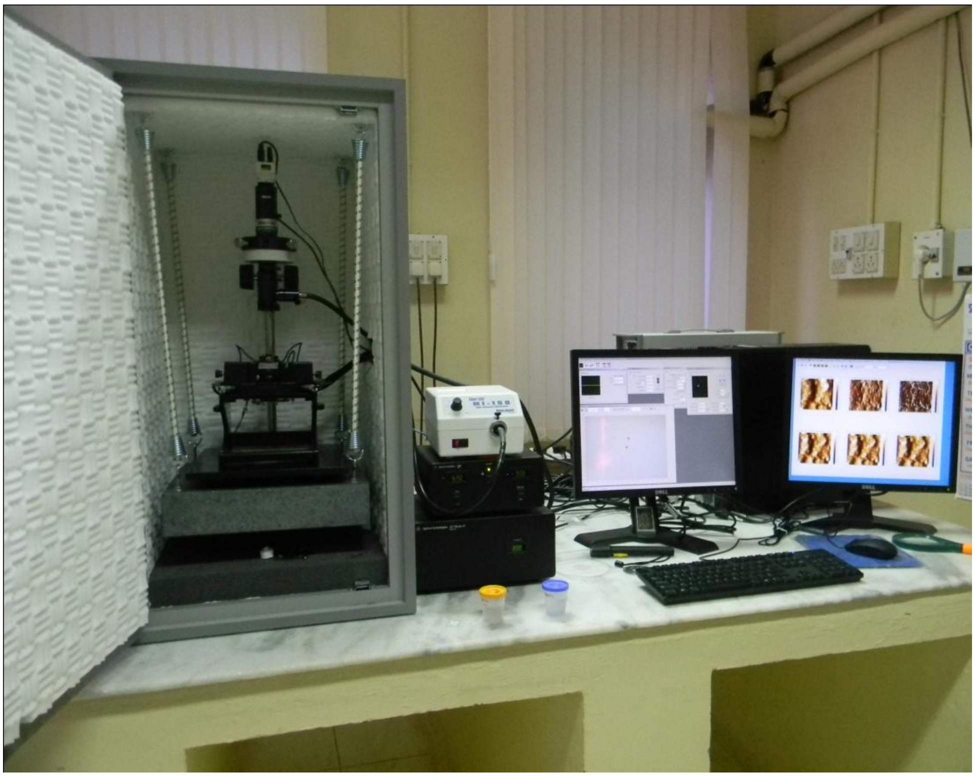


Fig. 3a. AFM unit with Picoimage software

Fig. 3b. AFM - 5500 *(AGILENT TECHNOLOGIES,USA)*

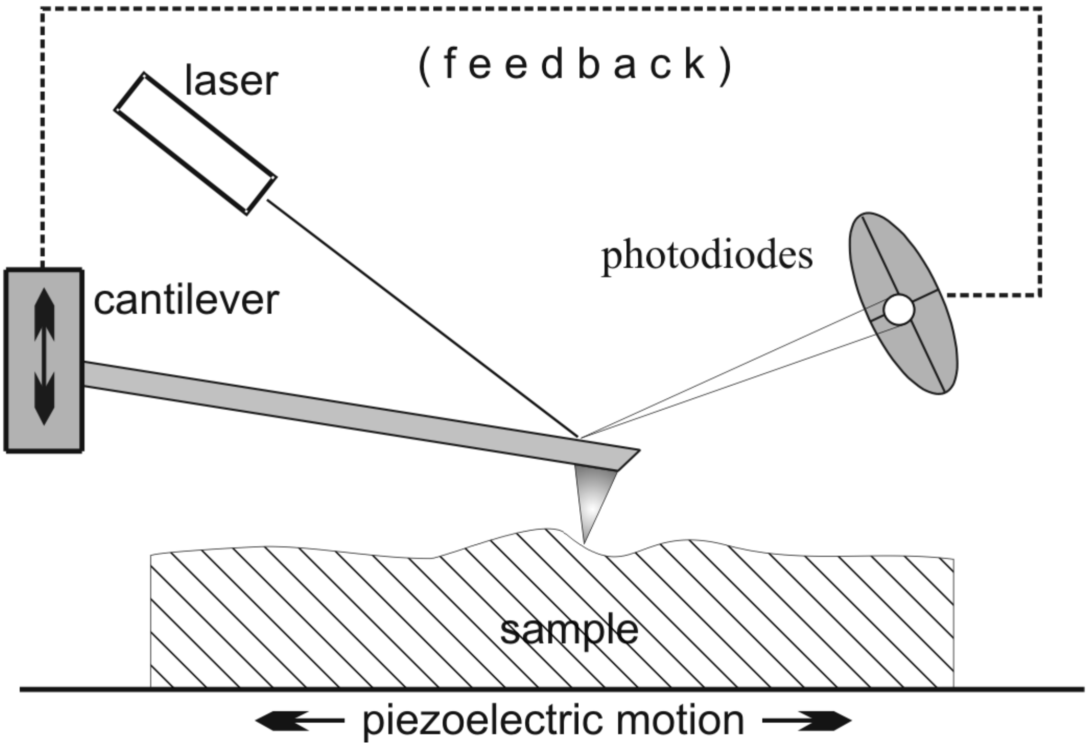


Fig.4. Working principle of AFM

EQUIPMENTS USED

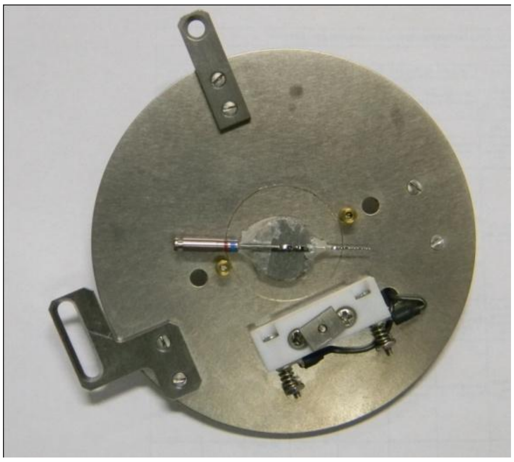


Fig. 5a. iRaCe file on sample stage

Fig. 5b. ProTaper file on sample stage

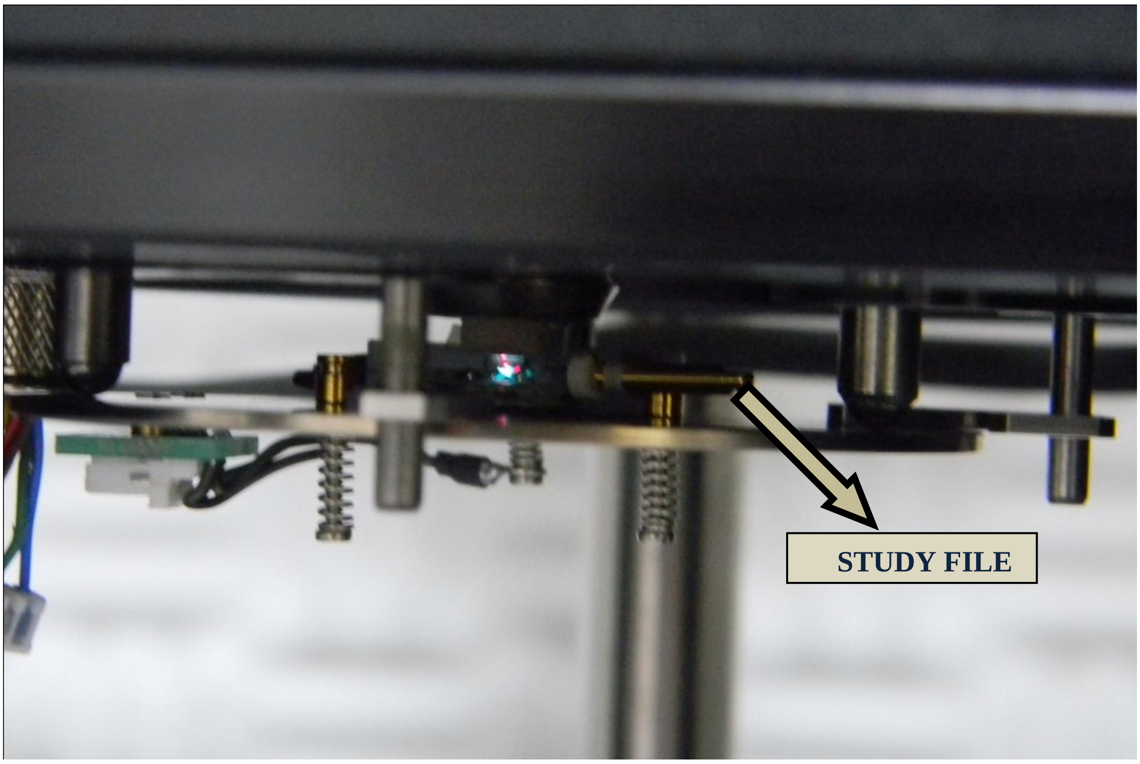


Fig. 5c. Sample stage with file relative to scanner of AFM

EQUIPMENTS USED

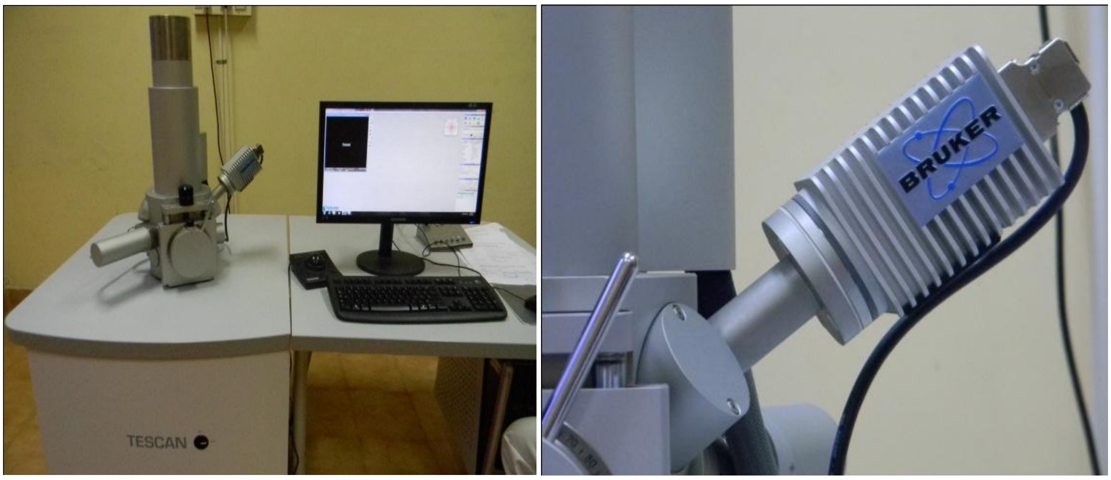


Fig.6a. Energy Dispersive X-Ray Spectroscopy (EDS) *(BRUKER, USA)*

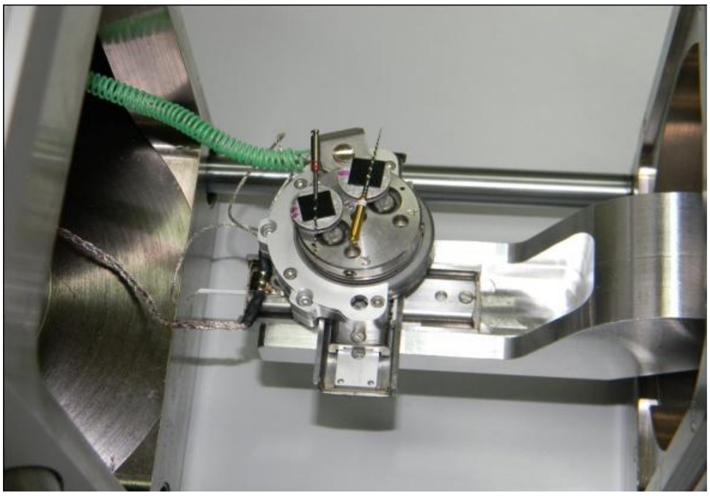
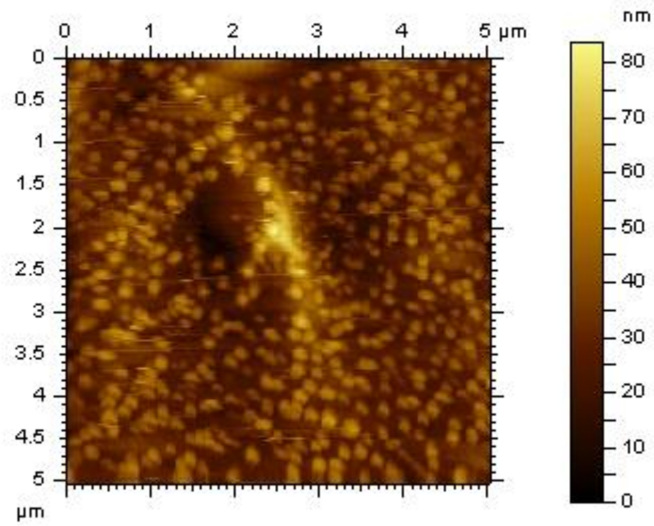


Fig.6b. Sample files mounted on the EDS machine

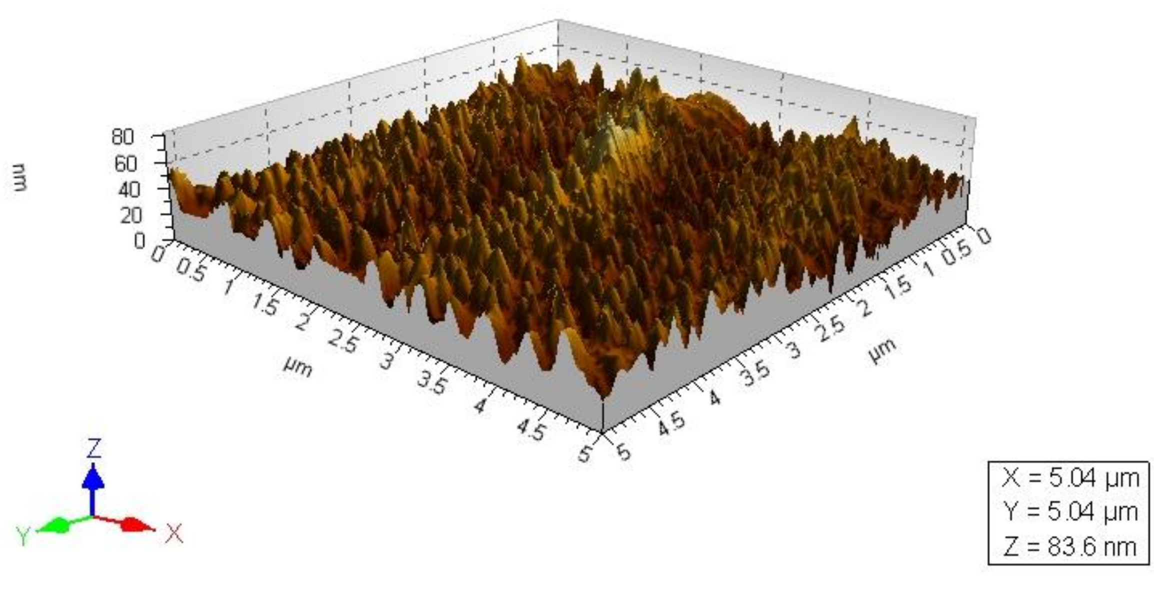
AFM IMAGES

iRaCe – New (Fig.11)

2D TOPOGRAPHY



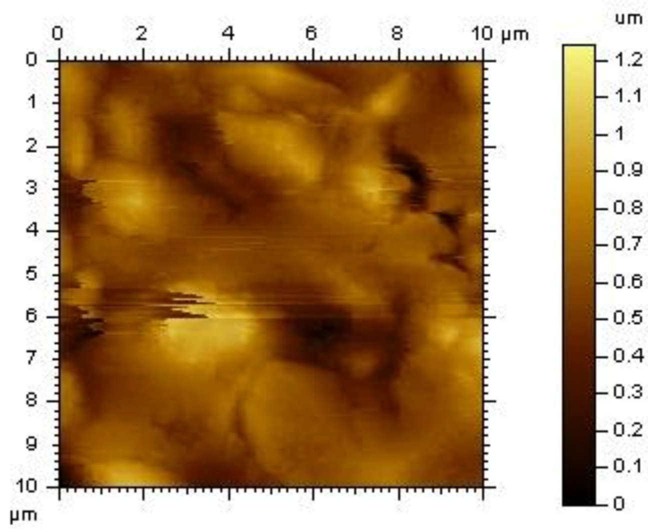
3D TOPOGRAPHY



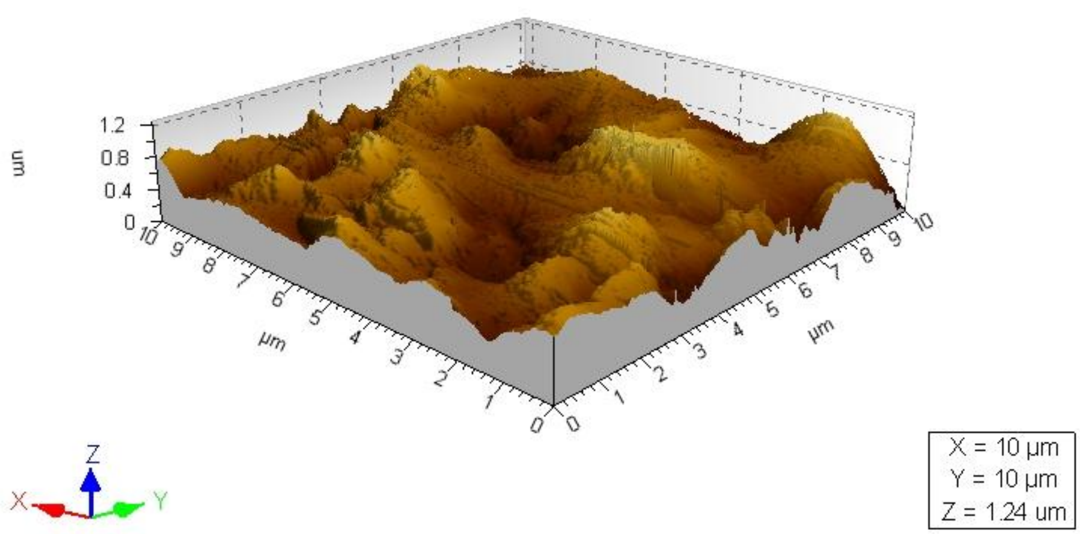
AFM IMAGES

iRaCe – 5% NaOCl (Fig.12)

2D TOPOGRAPHY



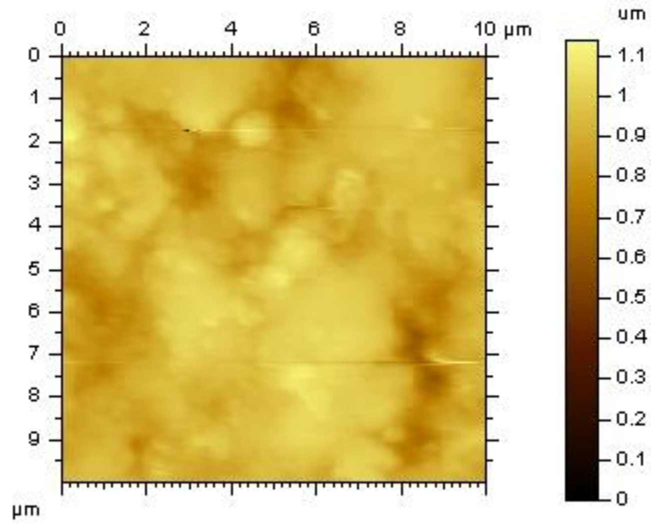
3D TOPOGRAPHY



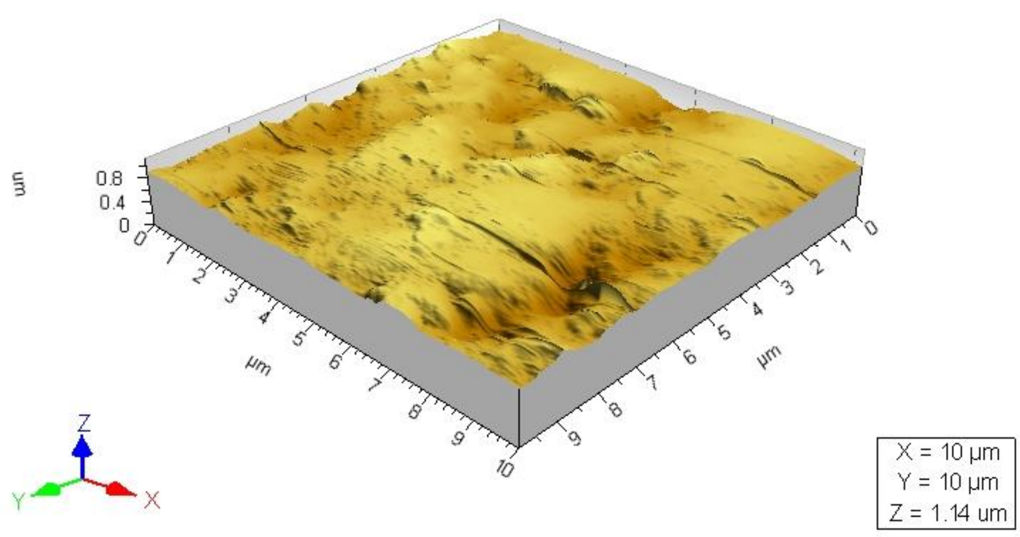
AFM IMAGES

iRaCe – 17% EDTA (Fig.13)

2D TOPOGRAPHY



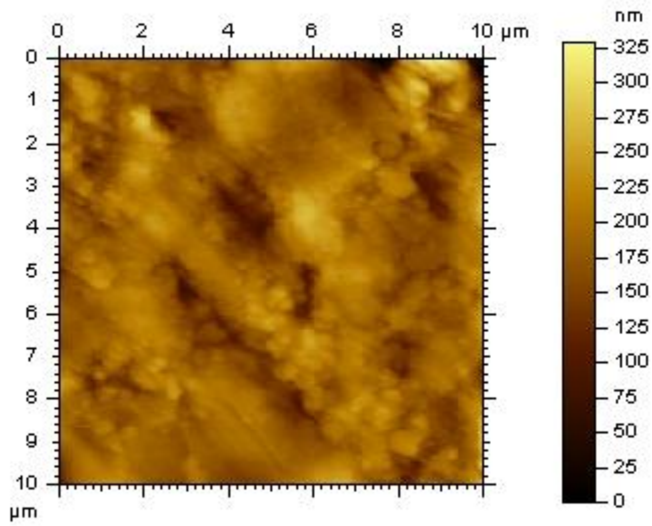
3D TOPOGRAPHY



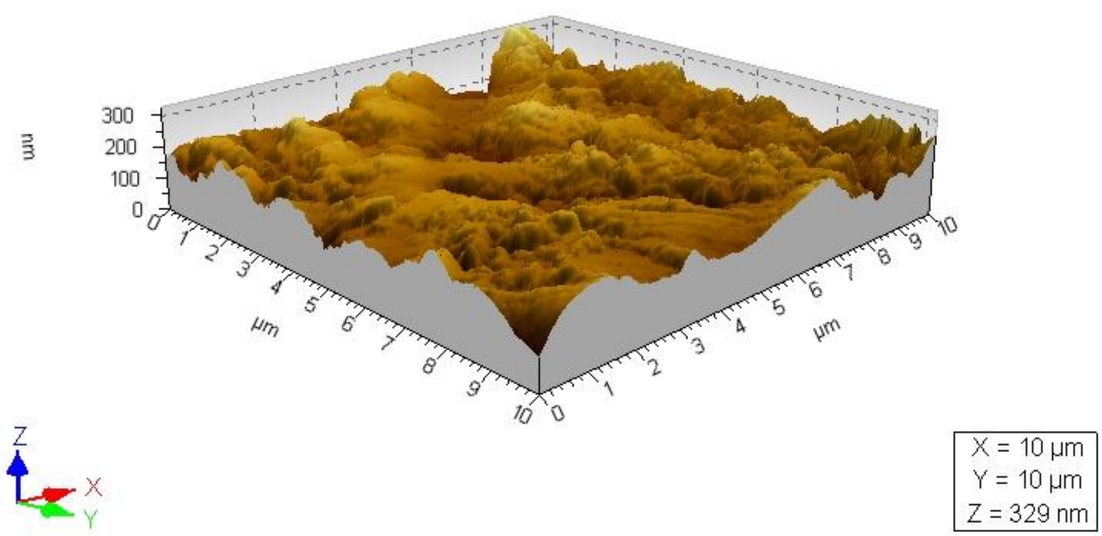
AFM IMAGES

iRaCe – TRIPHALA (Fig.14)

2D TOPOGRAPHY



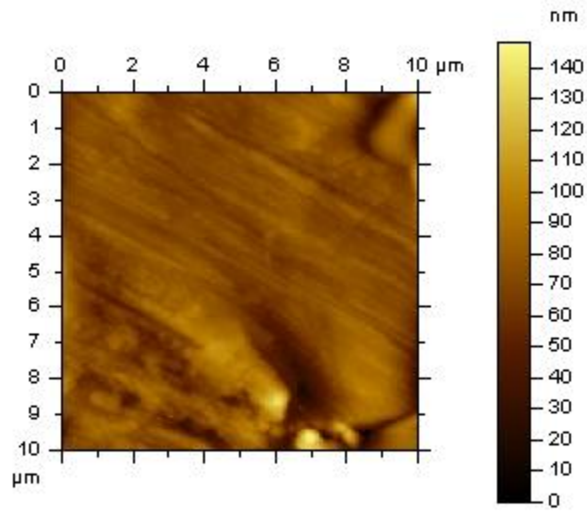
3D TOPOGRAPHY



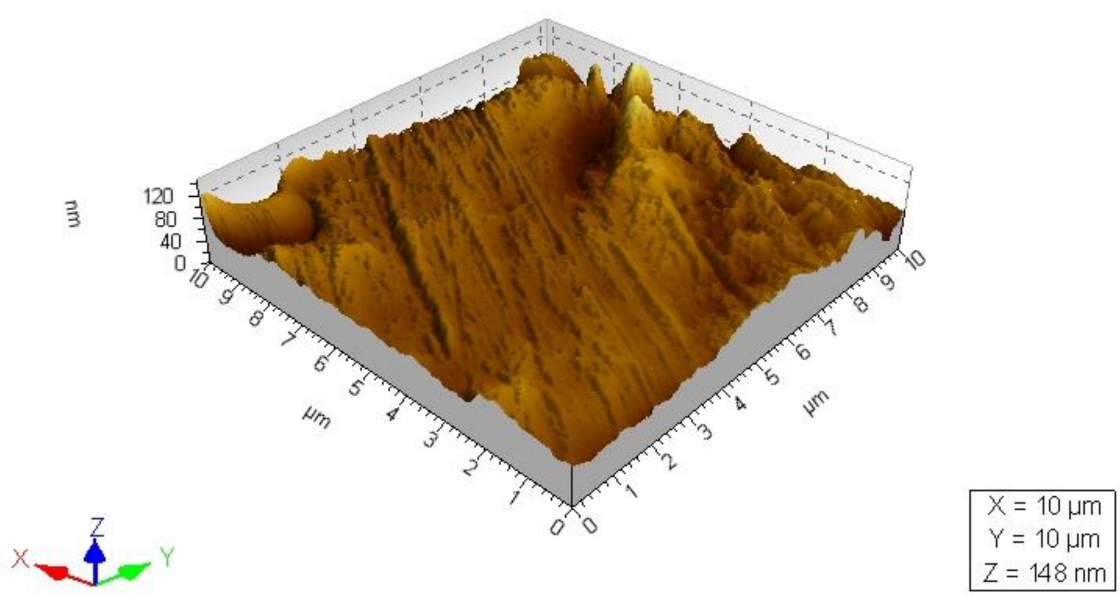
AFM IMAGES

ProTaper – NEW (Fig.7)

2D TOPOGRAPHY



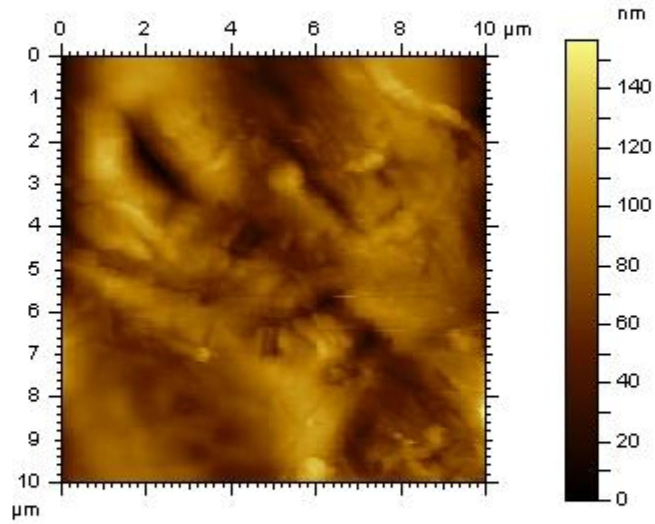
3D TOPOGRAPHY



AFM IMAGES

ProTaper – 5% NaOCl (Fig.8)

2D TOPOGRAPHY



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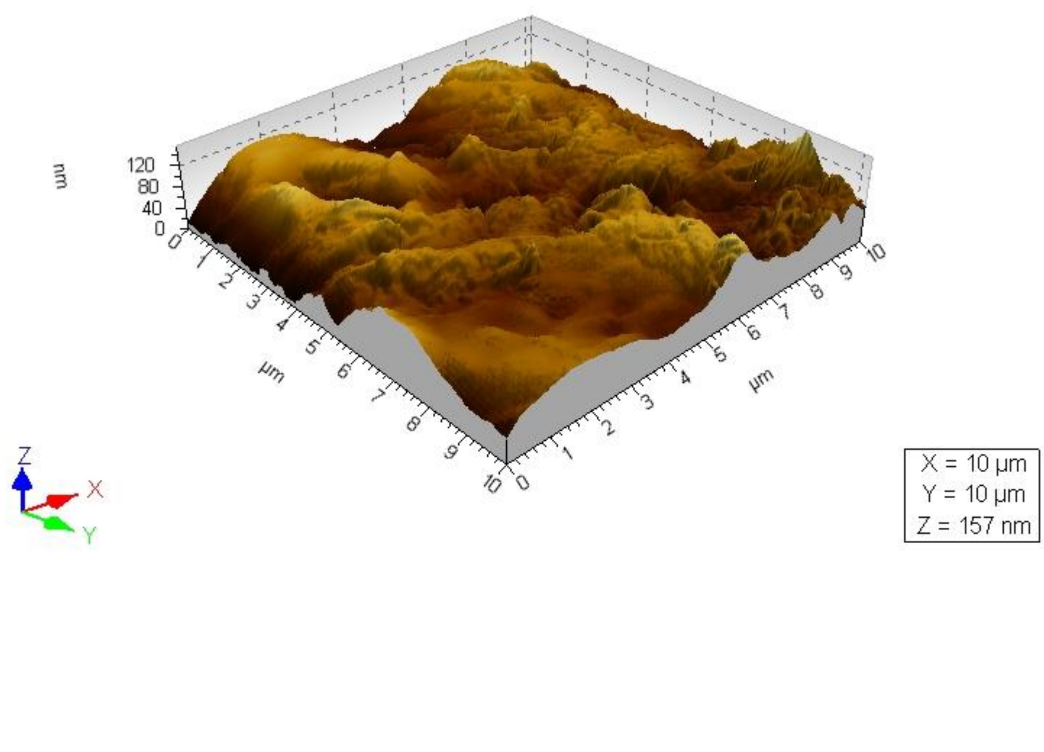
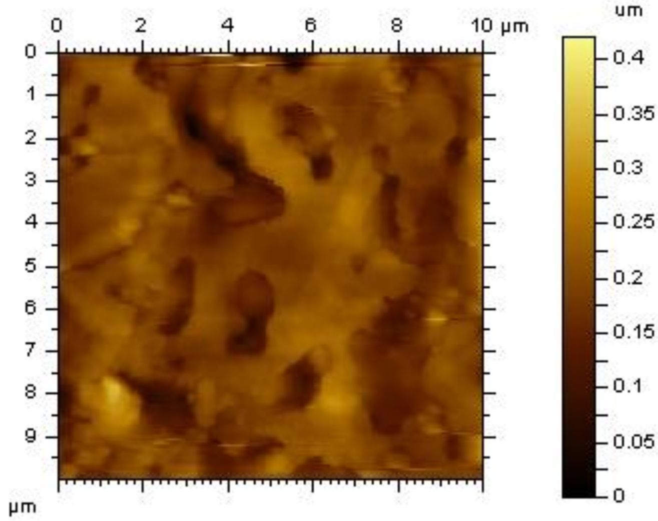


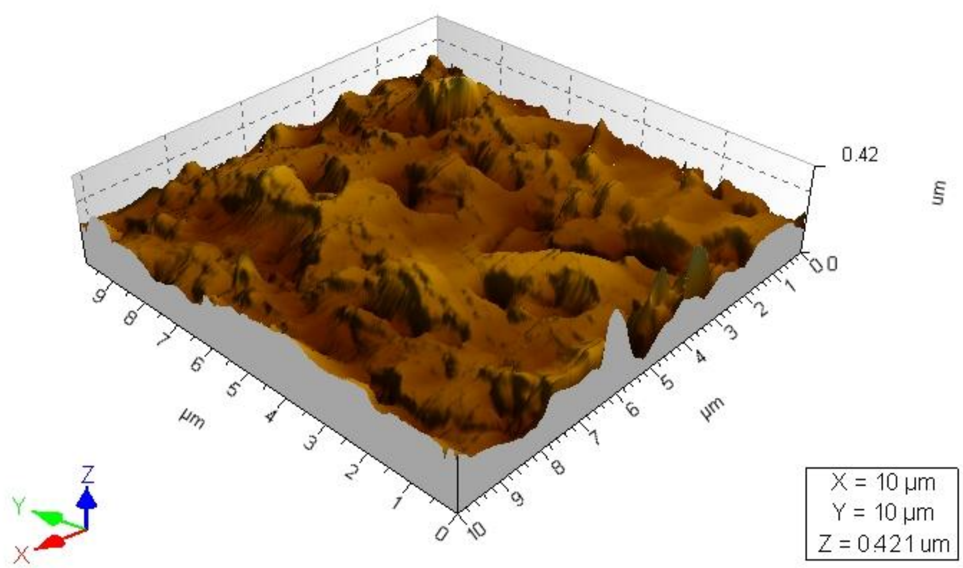
AFM IMAGES

ProTaper – 17% EDTA (Fig.9)

2D TOPOGRAPHY



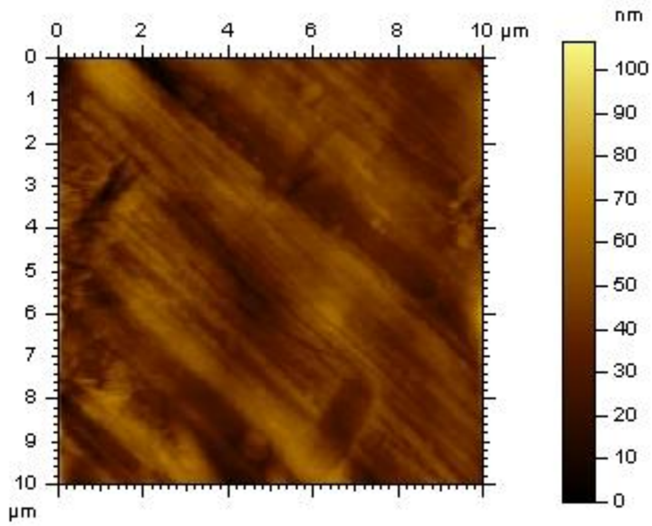
3D TOPOGRAPHY



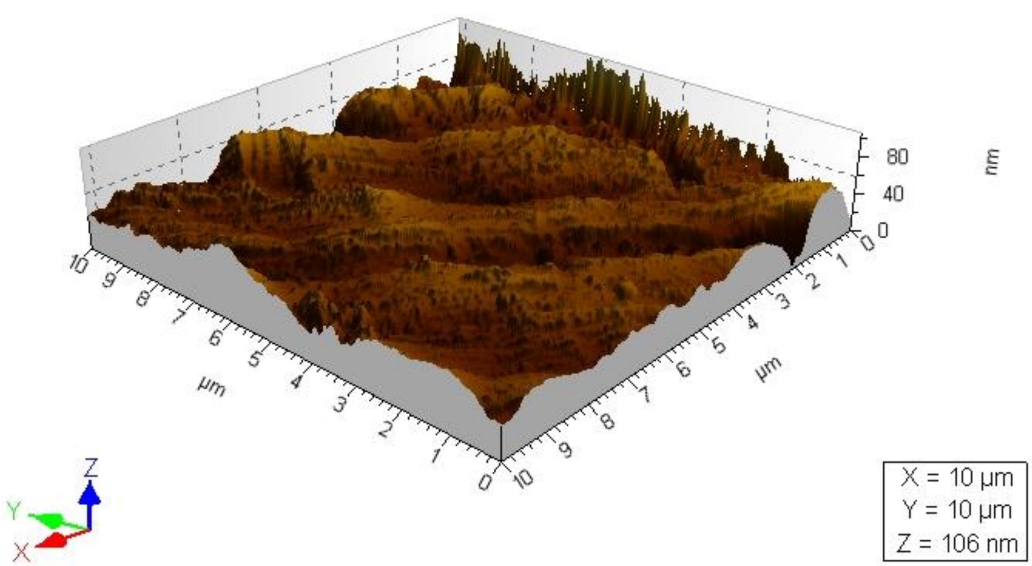
AFM IMAGES

ProTaper – TRIPHALA (Fig.10)

2D TOPOGRAPHY

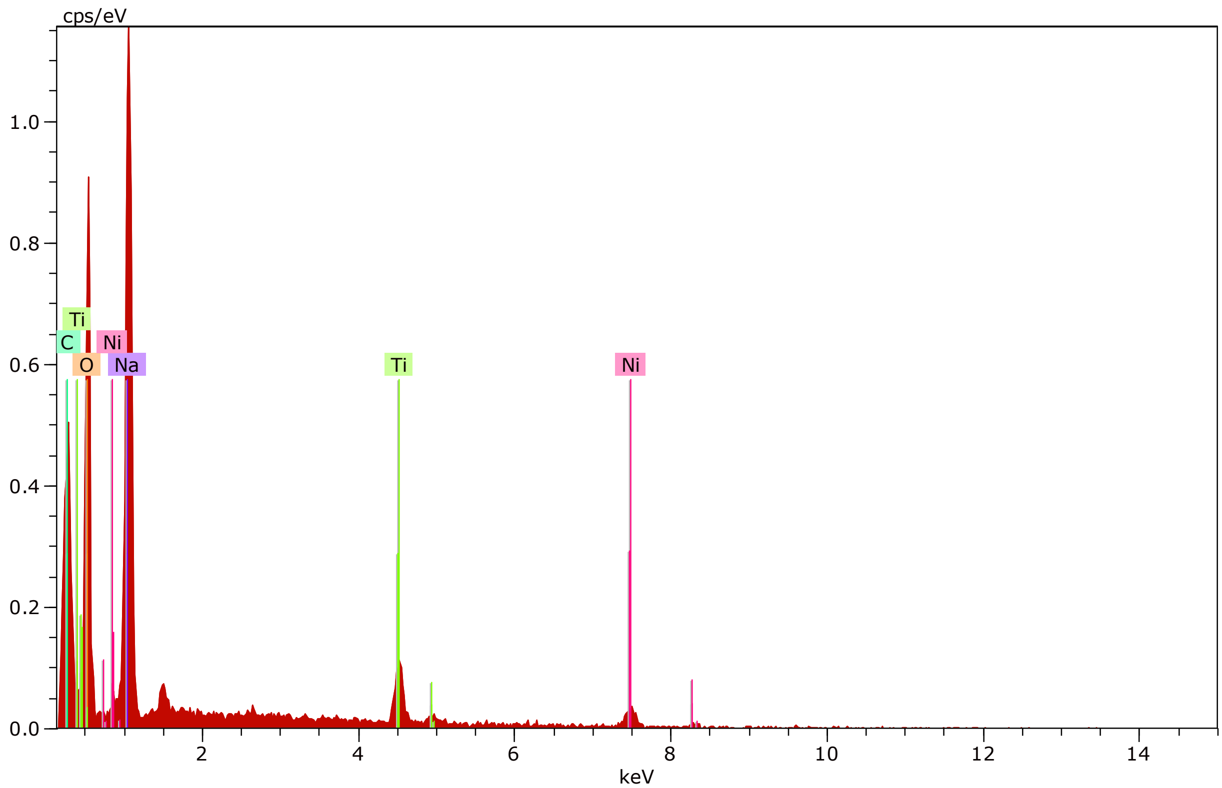


3D TOPOGRAPHY



EDS ANALYSIS

iRaCe – EDTA (Fig.21)

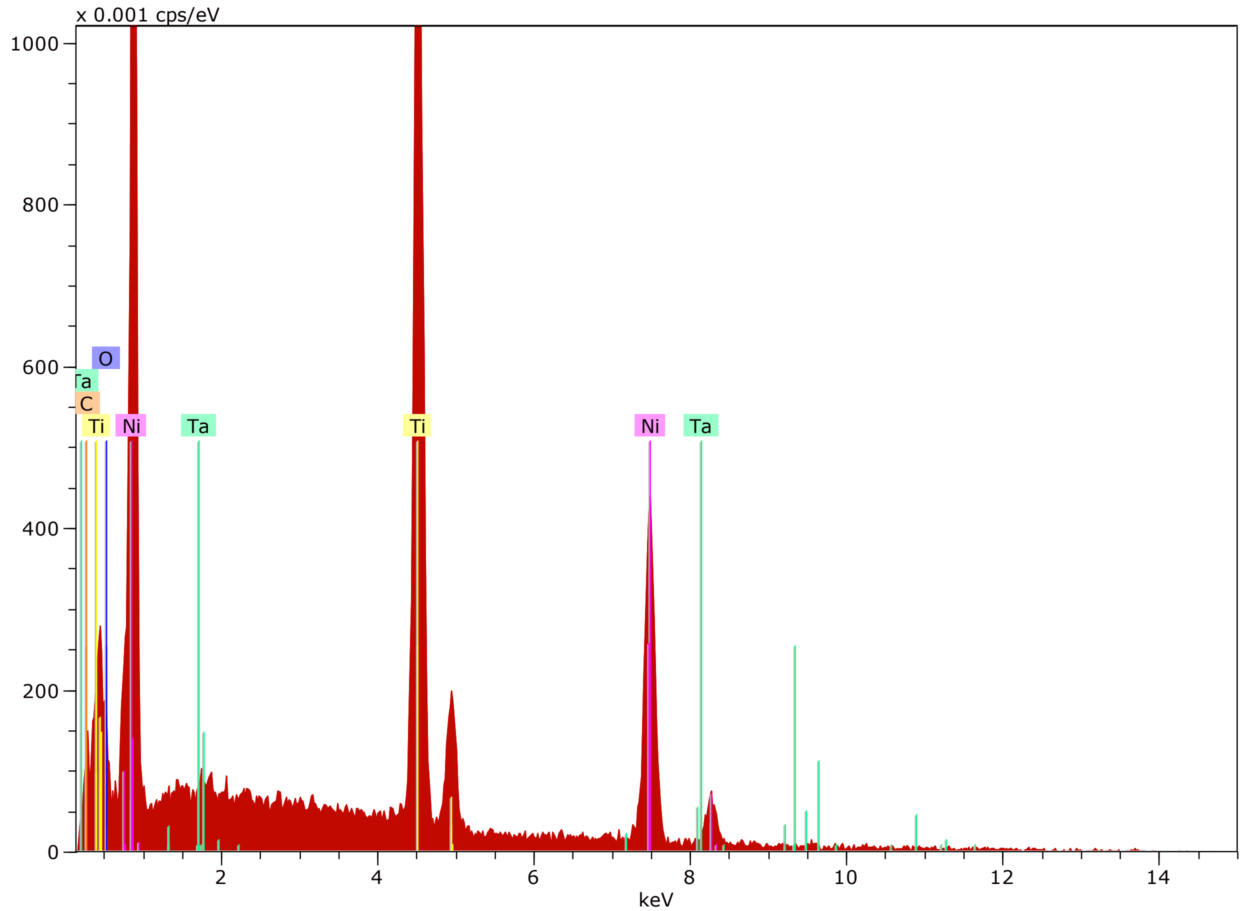


Spectrum: Acquisition

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Na	11	K-series	26.89	48.09	48.89	Na2O	64.83	36.24	1.79	0.425	1.131	1.000	1.001
O	8	K-series	15.49	27.72	40.48		0.00	0.00	2.57	0.189	1.469	1.000	1.000
Ni	28	K-series	7.33	13.10	5.22	NiO	16.68	9.32	0.54	0.087	1.413	1.000	1.064
Ti	22	K-series	6.20	11.09	5.41	TiO2	18.50	10.34	0.32	0.062	1.711	1.000	1.038
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	55.91	100.00	100.00								

EDS ANALYSIS

iRaCe – New (Fig.19)

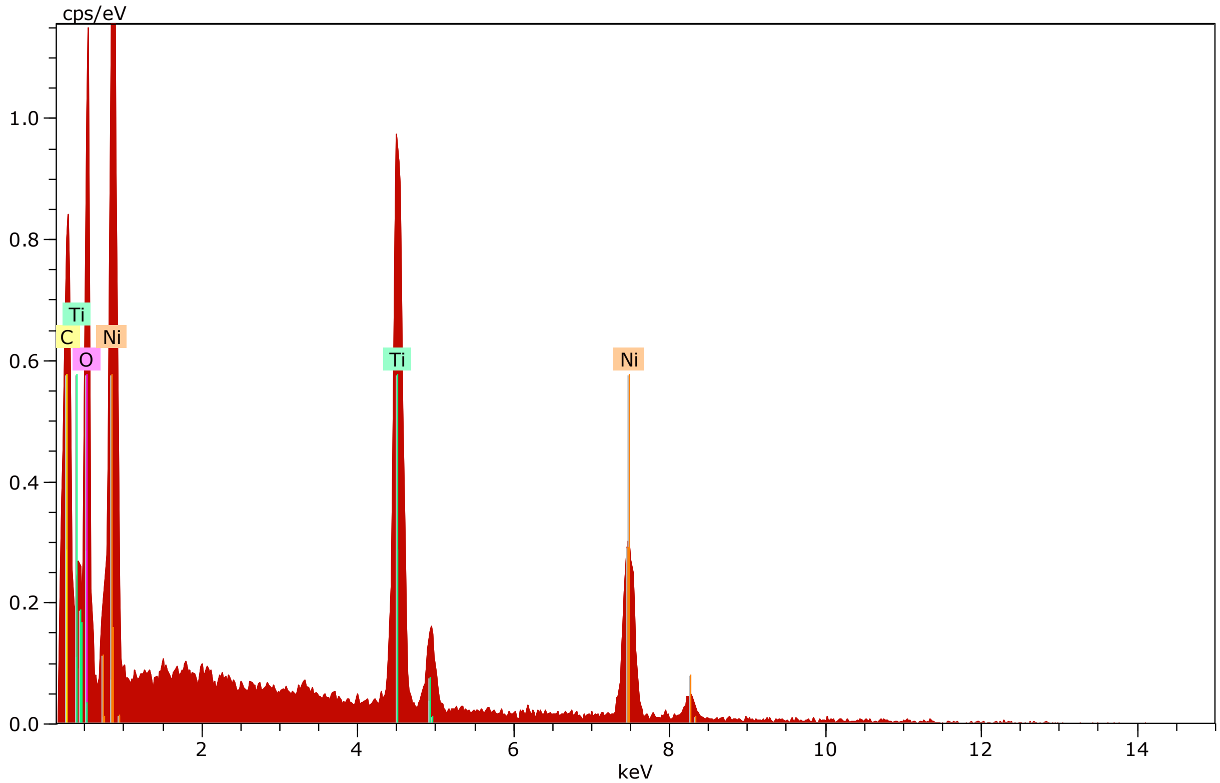


Acquisition Date:5/14/2012 4:16:35 PM HV:15.0kV Puls th.:0.95kcps

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Ni	28	K-series	48.17	38.07	20.47	NiO	48.45	61.30	1.73	0.377	0.968	1.000	1.042
O	8	K-series	38.23	30.22	59.61		0.00	0.00	11.22	7.674	0.039	1.000	1.000
Ti	22	K-series	37.56	29.69	19.57	TiO2	49.53	62.66	1.18	0.238	1.197	1.000	1.043
Ta	73	L-series	2.56	2.02	0.35		2.02	2.56	0.32	0.039	0.492	1.000	1.043
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	126.52	100.00	100.00								

EDS ANALYSIS

iRaCe –Triphala (Fig.22)

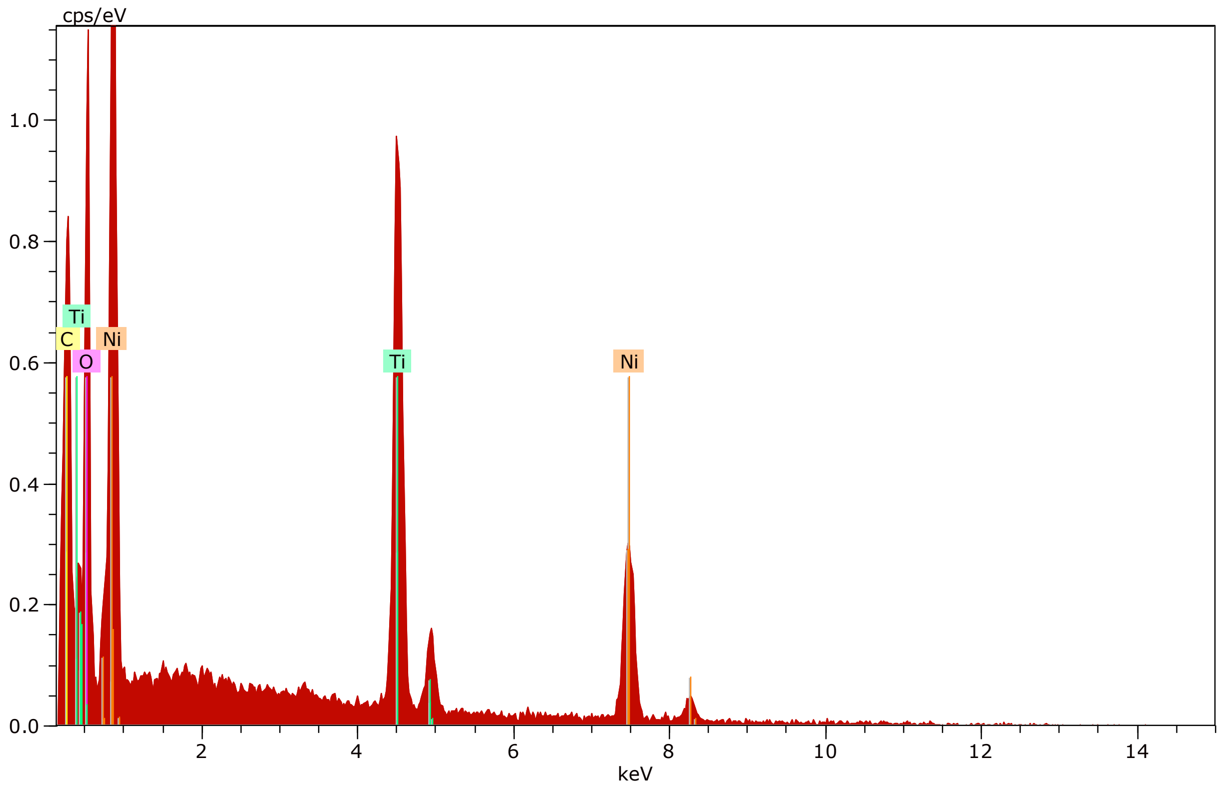


Spectrum: Acquisition

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Ni	28	K-series	37.86	38.11	20.08	NiO	48.50	48.17	1.43	0.409	0.896	1.000	1.040
O	8	K-series	30.82	31.02	59.97		0.00	0.00	4.90	0.582	0.533	1.000	1.000
Ti	22	K-series	30.67	30.87	19.94	TiO2	51.50	51.16	0.99	0.269	1.101	1.000	1.042
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	99.34	100.00	100.00								

EDS ANALYSIS

iRaCe –Triphala (Fig.22)

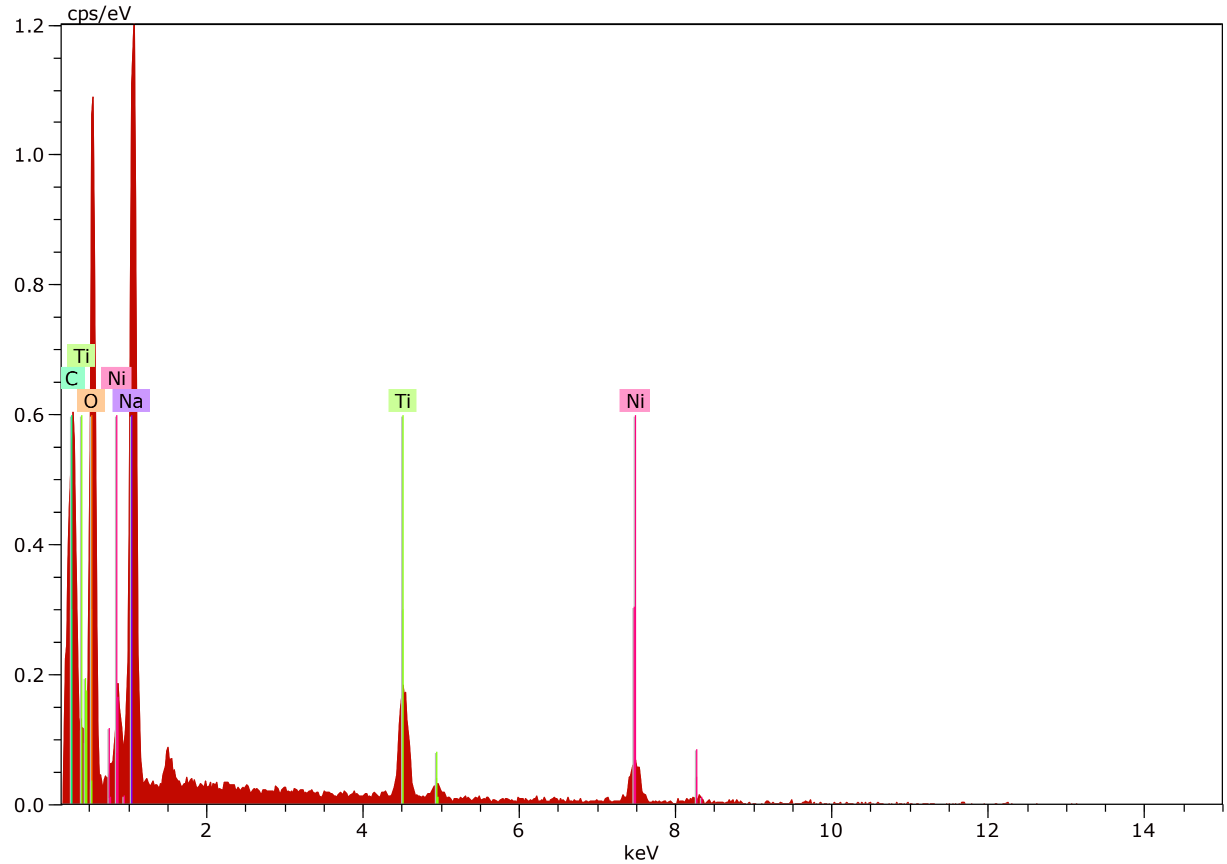


Spectrum: Acquisition

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Ni	28	K-series	37.86	38.11	20.08	NiO	48.50	48.17	1.43	0.409	0.896	1.000	1.040
O	8	K-series	30.82	31.02	59.97		0.00	0.00	4.90	0.582	0.533	1.000	1.000
Ti	22	K-series	30.67	30.87	19.94	TiO2	51.50	51.16	0.99	0.269	1.101	1.000	1.042
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	99.34	100.00	100.00								

EDS ANALYSIS

ProTaper –EDTA (Fig.17)

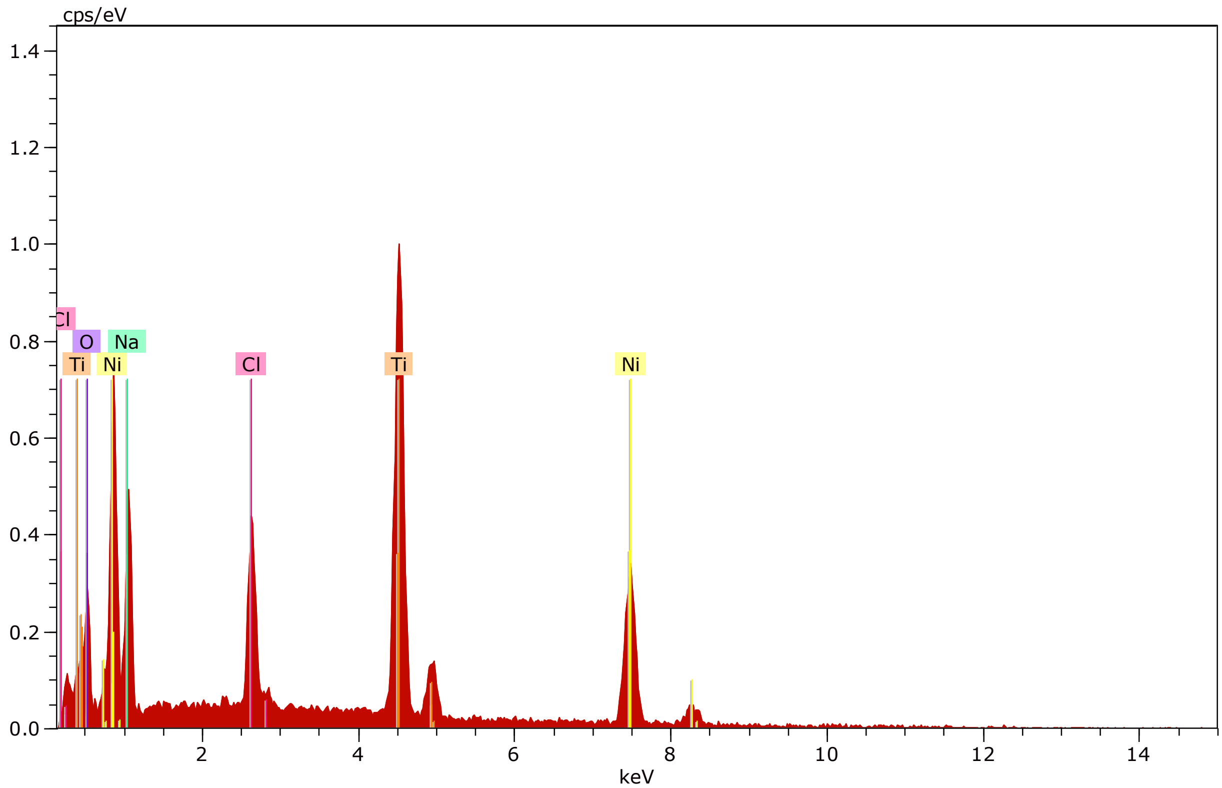


Acquisition Date:5/14/2012 5:02:14 PM HV:15.0kV Puls th.:0.53kcps

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Na	11	K-series	32.46	39.77	42.41	Na2O	53.62	43.76	2.15	0.506	0.785	1.000	1.001
O	8	K-series	22.83	27.97	42.85		0.00	0.00	3.58	0.281	0.997	1.000	1.000
Ni	28	K-series	15.33	18.78	7.84	NiO	23.90	19.51	0.85	0.184	0.970	1.000	1.055
Ti	22	K-series	11.00	13.48	6.90	TiO2	22.48	18.35	0.48	0.110	1.178	1.000	1.040
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	81.62	100.00	100.00								

EDS ANALYSIS

ProTaper –NaOCl (Fig.16)

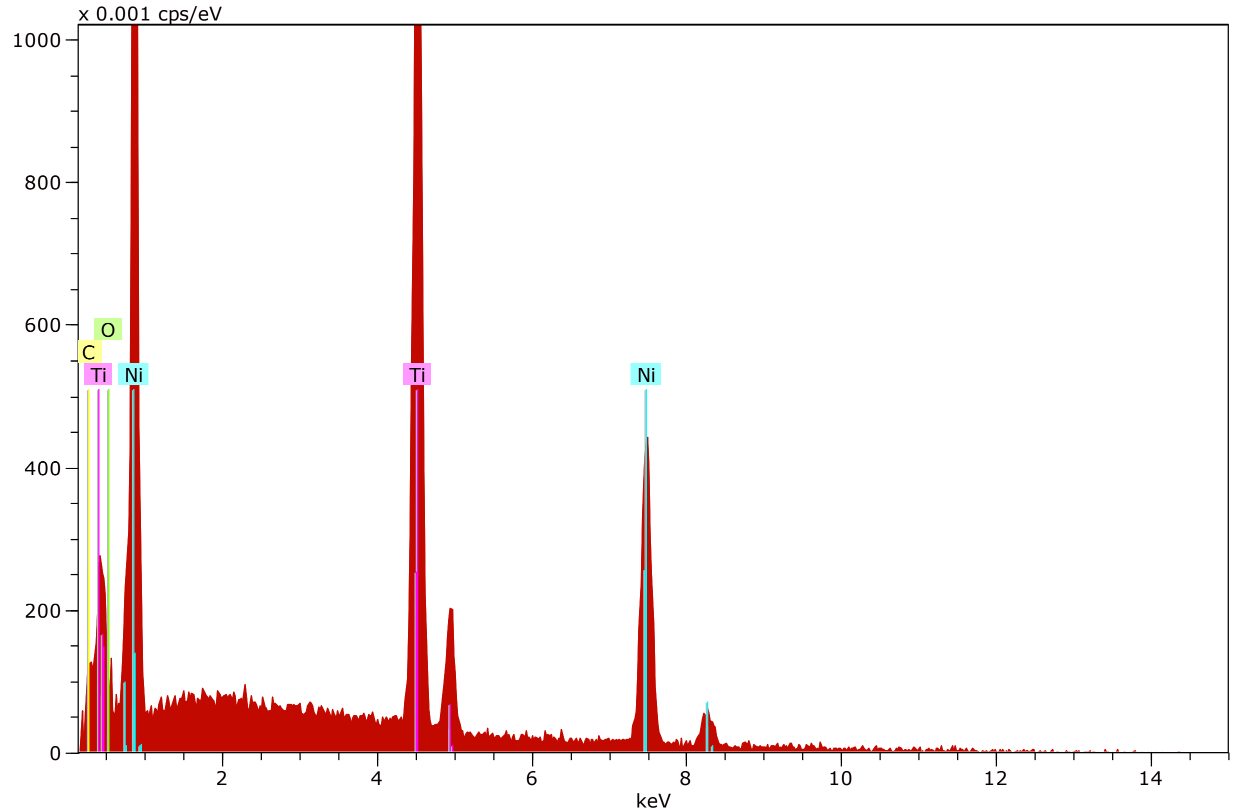


Spectrum: Acquisition

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Ni	28	K-series	42.04	33.73	17.07	NiO	42.93	53.50	1.57	0.335	0.967	1.000	1.042
O	8	K-series	36.29	29.12	54.07		0.00	0.00	7.99	0.000	0.000	1.000	0.000
Ti	22	K-series	32.33	25.94	16.09	TiO2	43.28	53.93	1.04	0.210	1.185	1.000	1.040
Na	11	K-series	9.28	7.44	9.62	Na2O	10.03	12.50	0.68	0.092	0.804	1.000	1.001
Cl	17	K-series	4.69	3.76	3.15		3.76	4.69	0.22	0.028	1.312	1.000	1.021
		Total:	124.62	100.00	100.00								

EDS ANALYSIS

Protaper – New (Fig.15)



Acquisition Date:5/14/2012 4:04:34 PM HV:15.0kV Puls th.:0.99kcps

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Compound	Comp. C [wt.%]	norm. Comp. C [wt.%]	Error (1 Sigma) [wt.%]	K fact.	Z corr.	A corr.	F corr.
Ni	28	K-series	46.16	38.62	20.40	NiO	49.15	58.75	1.65	0.361	1.029	1.000	1.040
O	8	K-series	36.93	30.90	59.87		0.00	0.00	12.67	12.228	0.025	1.000	1.000
Ti	22	K-series	36.43	30.48	19.73	TiO2	50.85	60.78	1.15	0.231	1.264	1.000	1.042
C	6	K-series	0.00	0.00	0.00		0.00	0.00	0.00	1.000	1.000	1.000	0.000
		Total:	119.52	100.00	100.00								