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Research Article

Impact of Autoclave Sterilization on Microstructure of Two Niti Endodontic Rotary File Systems: A Differential Scanning Calorimetric Analysis.

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ABSTRACT

Introduction: Autoclaving is the most common and most efficient sterilization method used to sterilize endodontic files. But the effect of heating and cooling cycle's occurring during sterilization on the physical and mechanical properties of niti files has not been clearly stated. Nitinol alloys exhibit two unique features that are relevant to endodontics: shape memory (SM) and super elasticity (SE). Both these features are as a result of reversible transformation of the austenite (parent phase) to the marten site (daughter phase). This phase transformation is thermo elastic. It can be induced by cooling or application of stress. The reverse transformation takes place, when the metal is heated or stress is released. Phase transformations are associated with significant changes in mechanical properties of the alloy. Thus the structure of NiTi is important to the clinical performance. Some studies have been done in the past which report about changes in properties of the NiTi instruments after heat sterilization.

Aims and objectives: The aim and objectives of this study are to analyze the microstructure and phase changes of Protaper and Race NiTi instruments at 0 and 5 cycles of moist heat sterilization by using differential scanning calorimetry.

Materials and Methods: A total of 20 NiTi rotary instruments belonging two different brands were selected for this study. The 20 instruments were divided into 2 groups. Group I contained 10 Protaper F_2 25 mm, tip size 25 NiTi rotary files [Dentsply – Tulsa dental]. Group II contained 10 Race 6 % 25 mm, tip size 25 NiTi rotary files [FKG Dentaire].Of the 10 samples from each group, 5 samples were kept for observation in as received condition (subgroup a) and 5 samples were submitted for 5 cycles of sterilization in autoclave (subgroup b). The instruments were arranged in an endobox and submitted for sterilization. The sterilization cycles were conducted in an autoclave [Uniqueclave C-79, Confident dental equipments, India] at 121^0 C (250 ^{0}F) under 15psi for 20minutes. Samples were allowed to cool to room temperature for at least 30 minutes between cycles.10 instruments of each brand were examined - five samples in the as received condition [subgroup a] and the other five after 5 cycles of sterilization [subgroup b]. The measurements were conducted with a DSC 2910 device (TA instruments, New Castle, Delaware (USA)) over a temperature range from -100 to 150^0C. A liquid nitrogen cooling accessory was used to achieve subambient temperatures. During the measurements, the DSC cell was purged with dry nitrogen. Temperature calibration of the DSC apparatus was done with n-pentane, deionized water and indium. The linear heating or cooling rate was 10^0C per min. For each analysis, the specimen was first cooled from room temperature to-100^0C, then heated to 150^0C to obtain the heating DSC curve and subsequently cooled from 150^0C back to -100^0C. To check the reproducibility of the measurements, the same heating– cooling cycle was repeated a further two times. The sample specimens were sectioned with a water-cooled, slow-speed diamond saw to minimize mechanical stresses that might change the proportions of the austenitic and martensitic NiTi

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phases from those in the 'as-received' instruments. Each sample specimen consisted of three segments of the same file, each approximately 4–5 mm in length. The specimens were placed in an aluminium crucible and an empty aluminium crucible was used as the inert control specimen. The weight of the samples was approximately 10 mg.

Temperature at which the phase changes occur and the enthalpy changes for the transformations were obtained from the DSC thermographs. Means between the groups were compared with the paired t-test and values within the groups were compared with student t test for independent samples.

Results: The present study shows that the phase transformation within NiTi alloy of the instruments is reversible. The reversibility is not affected after repeated heating and cooling in the same instrument, after 5 sterilization cycles. From the temperature values, significant differences are revealed between files of two brands, except for Af (austenite finish) temperature. Between new and sterilized files of the same brand, all values are significant except for Af value of Race instruments. The enthalpy values of heating and cooling curves of both brands, does not show any significant difference. The enthalpy changes in the heating and cooling cycles of both brands of new and sterilized instruments range from 2.37 j/g to 3.941 j/g. These calculated values lie within the range of 1.7 to 19.2 j/g, which has been reported for nickel - titanium orthodontic wires.

Conclusion: From the study it was concluded that, phase transformation within the NiTi matrix of both Protaper and Race NiTi instruments is reversible and the reversibility is not affected by repeated heating and cooling cycles. Both Protaper and Race NiTi instruments are completely austenite in the oral environment and hence are capable of super elastic behavior during clinical use.

Keywords: (endodontics, endodontic instruments, niti files, niti rotary, protaper, autoclave sterilization, differential scanning calorimetry, microstructure)

Introduction

Autoclaving is the most common and most efficient sterilization method used to sterilize niti endodontic files. But the effect of heating and cooling cycles occuring during sterilization on the physical and mechanical properties of niti files has not been clearly stated.

Nitinol alloys used for manufacturing endodontic instruments contains approximately 55% nickel and 45% titanium. These alloys exhibit two unique features that are relevant to endodontics: shape memory (SM) and superelasticity (SE). ***Shape memory effect*** - the metal deformed in an apparently permanent way, recovers its original shape when heated. ***Superelasticity*** -the metal deformed in an apparently permanent way, recovers after the removal of stress. Both these features are as a result of reversible transformation of the austenite (parent phase) to the martensite (daughter phase).

This phase transformation is thermoelastic,. It can be induced by cooling or application of stress. The reverse transformation takes place, when the metal is heated or stress is released.

In the case of temperature induced martensite transformation which results when the alloy is cooled through a critical transformation temperature range (TTR), the stable, cubic lattice crystal structure (austenite) changes to a monoclinic structure (martensite). This phenomenon starts at temperature Ms (martensite start) and ends at temperature Mf (martensite finish). It can be reversed by heating the alloy above the TTR, in the reverse transformation temperature range or RTTR, giving rise to the shape memory effect. Sometimes, the direct transformation from austenitic to martensitic NiTi includes an intermediate structure, called R-phase, which is a hexagonal lattice.

In the case of stress induced martensitic transformation, austenite is transformed to martensite as a result of the application of stress and reverts back to austenite when unloaded which gives superelasticity. This property of superelasticity is more relevant in endodontics, since stress occurs when the instrument is introduced inside the root canal.

Phase transformations are associated with significant changes in mechanical properties of the alloy. Thus the structure of NiTi is important to the clinical performance. Some studies have been done in the past which report about changes in properties of the NiTi instruments after heat sterilization.The purpose of this study is to assess the effect of moist heat sterilization on microstructure of two different brands of NiTi rotary instrument systems using differential scanning calorimetry (DSC).

AIMS AND OBJECTIVES

The aim and objectives of this study are to analyze the microstructure and phase changes of Protaper and Race NiTi instruments at 0 and 5 cycles of moist heat sterilization by using differential scanning calorimetry.

MATERIALS AND METHODS

A total of 20 NiTi rotary instruments belonging two different brands were selected for this study. The 20 instruments were divided into 2 groups. Group I (n=10) Protaper F_2 25 mm, tip size 25 NiTi rotary files [Dentsply – Tulsa dental]. Group II (n=10) - Race 6 % 25 mm, tip size 25 NiTi rotary files [FKG Dentaire]

Of the 10 samples from each group, 5 samples were kept for observation in as received condition (subgroup a) and 5 samples were submitted for 5 cycles of sterilization in autoclave (subgroup b). The instruments were arranged in an endobox and submitted for sterilization. The sterilization cycles were conducted in an autoclave [Uniqueclave C-79, Confident dental equipments, India] at 121^{0} C (250 ^{0}F) under 15psi for 20minutes. Samples were allowed to cool to room temperature for at least 30 minutes between cycles.

The differential scanning calorimetry (DSC) testing was performed at the Central leather research institute, Guindy, Chennai. 10 instruments of each brand were examined - five samples in the as received condition [subgroup a] and the other five after 5 cycles of sterilization [subgroup b].

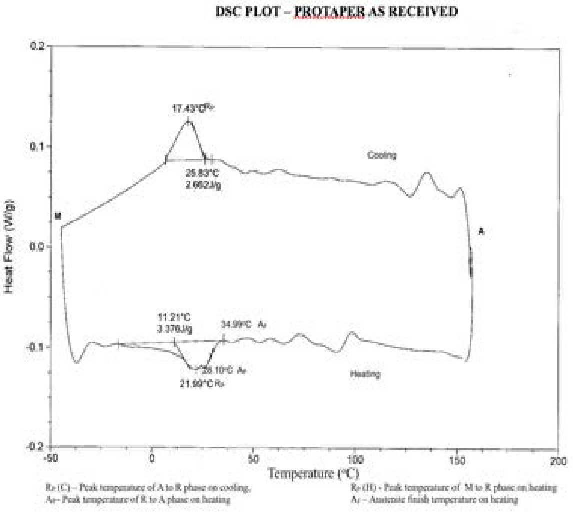
DSC accurately measures the difference in thermal power supplied to a sample specimen and an inert control specimen heated at the same rate. Structural transformations within the matrix of NiTi alloys are revealed as endothermic peaks on the heating DSC curves and as exothermic peaks on the cooling DSC curves. The measurements were conducted with a DSC 2910 device (TA instruments, New Castle, Delaware (USA)) over a temperature range from -100 to 150^{0}C. A liquid nitrogen cooling accessory was used to achieve subambient temperatures. During the measurements, the DSC cell was purged with dry nitrogen. Temperature calibration of the DSC apparatus was done with n-pentane, deionized water and indium. The linear heating or cooling rate was 10^{0}C per min. For each analysis, the specimen was first cooled from room temperature to-100^{0}C, then heated to 150^{0}C to obtain the heating DSC curve and subsequently cooled from 150^{0}C back to -100^{0}C. To check the reproducibility of the measurements, the same heating– cooling cycle was repeated a further two times. The sample specimens were sectioned with a watercooled, slow-speed diamond saw to minimize mechanical stresses that might change the proportions of the austenitic and martensitic NiTi phases from those in the ‘as-received’ instruments. Each sample specimen consisted of three segments of the same file, each approximately 4–5 mm in length. The specimens were placed in an aluminium crucible and an empty aluminium crucible was used as the inert control specimen. The weight of the samples was approximately 10 mg.

Temperature at which the phase changes occur and the enthalpy changes for the transformations were obtained from the DSC thermographs. Means between the groups were compared with the paired t-test and values with in the groups were compared with student t test for independent samples.

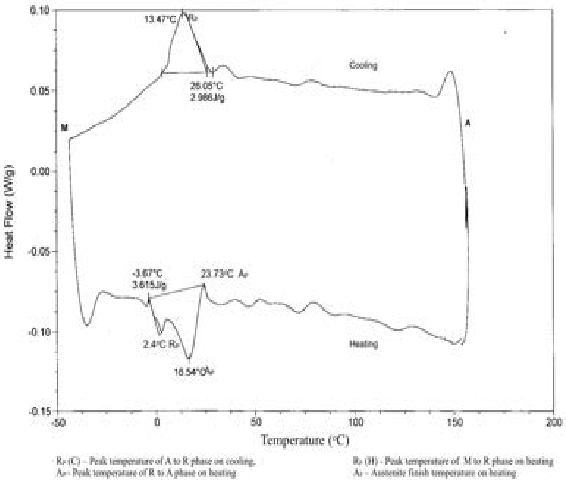
RESULTS

The DSC thermographs for Protaper and Race niti files in the as received condition and after

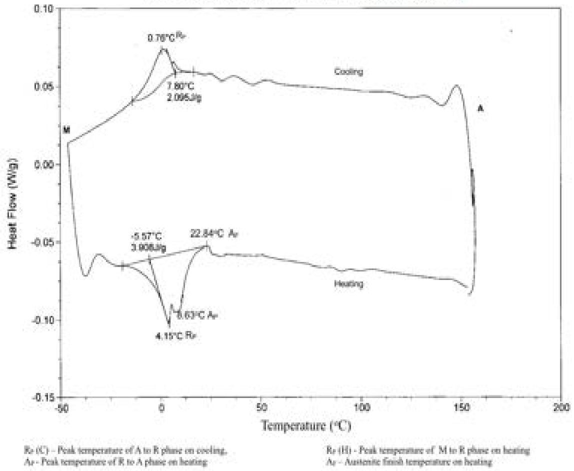
5 cycles of sterilization are given in figure 1.



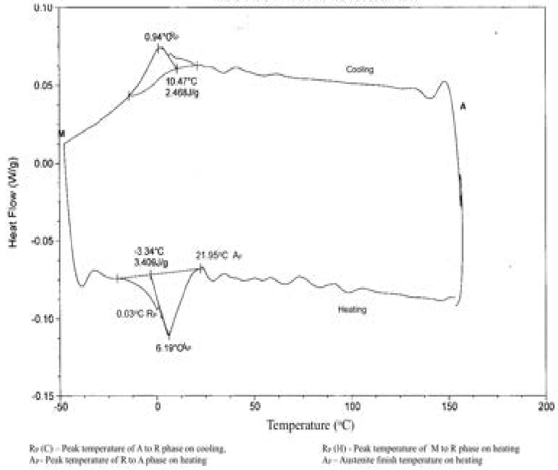
DSC PLOT : PROTAPER – 5 CYCLES OF STERILIZATION



DSC PLOT : RACE – 5 CYCLES OF STERILIZATION



DSC PLOT – RACE AS RECEIVED



The values obtained from DSC thermograms for the Protaper and Race samples at 0 and 5 cycles of sterilization are given in table I and II respectively.

TABLE 1:

Group I Samples	R_P Heating ^{O}C		A_P Heating ^{O}C		A_F Heating ^{O}C		R_P Cooling ^{O}C		ΔH Heating J/G		ΔH Cooling J/G	
	0	5	0	5	0	5	0	5	0	5	0	5
1	21.99	2.4	26.1	16.54	34.99	23.73	17.43	13.47	3.376	3.615	2.662	2.98
2	20.43	2.32	25.87	16.79	33.63	22.69	16.23	13.45	3.075	3.351	2.193	2.489
3	19.88	1.92	24.73	16.09	32.59	21.77	15.72	12.97	3.206	3.519	2.357	2.678
4	20.75	2.76	25.62	17.13	33.49	23.24	16.71	13.83	3.159	3.437	2.219	2.532
5	25.58	3.21	26.79	17.19	35.28	24.54	18.42	14.92	3.321	3.597	2.436	2.753
Mean	**21.13**	**2.52**	**25.82**	**16.75**	**33.99**	**23.19**	**16.90**	**13.73**	**3.227**	**3.504**	**2.373**	**2.686**

TABLE 2:

Group II Samples	R_P Heating ^{O}C		A_P Heating ^{O}C		A_F Heating ^{O}C		R_P Cooling ^{O}C		ΔH Heating J/G		ΔH Cooling J/G	
	0	**5**	**0**	**5**	**0**	**5**	**0**	**5**	**0**	**5**	**0**	**5**
1	0.03	4.15	6.19	8.63	21.95	22.84	0.94	0.76	3.409	3.908	2.468	2.095
2	0.14	4.36	6.59	8.79	22.35	23.04	1.07	0.97	3.476	3.892	2.453	2.597
3	1.24	4.87	7.25	9.27	23.08	29.32	2.54	1.98	3.516	3.973	2.519	2.653
4	1.43	5.14	7.76	9.39	23.57	24.73	2.87	2.09	3.573	4.017	2.57	2.793
5	0.73	4.59	7.08	9.09	22.79	23.12	1.59	1.25	3.541	3.917	2.497	2.603
Mean	**0.71**	**4.62**	**6.97**	**9.03**	**22.74**	**23.61**	**1.80**	**1.31**	**3.503**	**3.941**	**2.501**	**2.548**

R_P (C) – Peak temperature of A to R phase on cooling,
R_P (H) - Peak temperature of M to R phase on heating
A_P - Peak temperature of R to A phase on heating
A_F – Austenite finish temperature on heating
ΔH(C) – Total enthalpy change during cooling
ΔH (H) – Total enthalpy change during heating

STATISTICAL ANALYSIS

TABLE 3: Values for DSC Analysis (Statistical analysis was done to compare the values within the group using student t test for independent samples.)

Group I	0 cycles		5 cycles		p value
	Mean	**SD**	**Mean**	**SD**	
R_P Heating	21.73	2.29	2.52	0.49	< 0.001**
A_P Heating	25.82	0.75	16.75	0.45	< 0.001**
A_F Heating	34.00	1.12	23.19	1.05	< 0.001**
R_P Cooling	16.90	1.06	13.73	0.73	< 0.001**
ΔH Heating	3.23	0.12	3.50	0.11	< 0.001**
ΔH Cooling	2.37	0.19	2.69	0.20	< 0.001**

Group II	**0 cycles**		**5 cycles**		**p value**
	Mean	**SD**	**Mean**	**SD**	
R_P Heating	0.71	0.63	4.62	0.39	< 0.001**
A_P Heating	6.97	0.61	9.03	0.32	< 0.001**
A_F Heating	22.75	0.63	24.61	2.74	0.164^{NS}
R_P Cooling	1.80	0.87	1.41	0.60	0.035*
ΔH Heating	3.50	0.06	3.94	0.05	< 0.001**
ΔH Cooling	2.50	0.05	2.55	0.27	0.684^{NS}

**	-	denotes significance at 1% level
NS	-	Non significance

TABLE 4: Values for DSC Analysis (Statistical analysis was done to compare the values between the groups using paired t test.)

No. of cycles of Sterilization	**Traits**	**Group I**		**Group II**		**p value**
		Mean	**SD**	**Mean**	**SD**	
0 cycles	R_P Heating	21.73	2.29	0.71	0.63	< 0.001**
	A_P Heating	25.82	0.75	6.97	0.61	< 0.001**
	A_F Heating	34.00	1.12	22.75	0.63	< 0.001**
	R_P Cooling	16.90	1.06	1.80	0.87	< 0.001**
	ΔH Heating	3.23	0.12	3.50	0.06	0.002**
	ΔH Cooling	2.37	0.19	2.50	0.05	0.181^{NS}
5 cycles	R_P Heating	2.52	0.49	4.62	0.39	< 0.001**
	A_P Heating	16.75	0.45	9.03	0.32	< 0.001**
	A_F Heating	23.19	1.05	24.61	2.74	0.312^{NS}
	R_P Cooling	13.73	0.73	1.41	0.60	< 0.001**
	ΔH Heating	3.50	0.11	3.94	0.05	< 0.001**
	ΔH Cooling	2.69	0.20	2.55	0.27	0.376^{NS}

**	-	denotes significance at 1% level
NS	-	Non significance

DISCUSSION

Research about the metallurgic properties of Niti files and analysis of the external stresses placed on the files will enable the dentists to improve the quality of root canal treatment.

The ADA council on dental therapeutics, Council on dental practice (1988, 1996) [1] has recommended that heat sterilization be used for all instruments that can withstand repeated exposure to required sterilization temperature.

In 1988, Walia et al recommended nitinol for the manufacturing of endodontic instruments, since the files made from nitinol were three times more flexible and resistant to fracture than the conventional stainless steel files [2].

The most effective methods for sterilizing endodontic instruments are the steam autoclave, dry heat oven and ethylene oxide [3]. In the present study, sterilization in steam autoclave was chosen because it is one of the most accessible methods. Dry heat oven is sometimes preferred, but dry heat is not as penetrating as steam autoclave sterilization and also requires higher temperature and longer times [4, 5]. Only proper steam autoclaving can reliably produce completely sterile instruments [6].

Any sterilization procedure should not significantly alter the physical properties of endodontic instruments [7]. The choice of maximum five sterilization cycles in this study was based on the literature reports, which state that Ni-Ti rotary instruments can be utilized safely to shape 10 curved root canals [8, 9]. Thus, five cycles of sterilization correspond to clinical use during the average useful life of the instrument.

Differential scanning calorimetry is the technique that is used to study the microstructure of NiTi alloys. It is a method that allows the identifications of NiTi phase at a given temperature and it also provides temperature ranges and enthalpy changes for the phase transformations. This analysis was previously commonly used for analysis of microstructure of superelastic Niti wires used in orthodontics [10-12] and has also been used successfully to investigate phase transformation within NiTi matrix of endodontic instruments.

DSC study by Brantley et al in 2002 is the first in endodontic literature for using DSC to investigate the structure of NiTi rotary instruments [13]. They state that DSC is a very powerful tool for material characterization of NiTi rotary instruments. DSC is able to provide direct information about the NiTi phases which are responsible for the clinical behavior of the NiTi files, which is not readily available from other analytical techniques.

Recently DSC analysis has been used to investigate phase relationships within NiTi matrices of endodontic instruments - Profile, Lightspeed, Quantec and Hero in the as-received condition and after clinical or simulated clinical use [13-15]. Alexandrou et al have used DSC to investigate Mani NRT, Profile and Flexmaster instruments after sterilization [16,17].

No information exists in the literature for DSC analysis of Protaper or Race instruments. Interpretations of the DSC plots in this study were based on previous studies of NiTi alloys in orthodontics [10] and previous studies of endodontic instruments [13,16,17] .

The present study shows that the phase transformation within NiTi alloy of the instruments is reversible. The reversibility is not affected after repeated heating and cooling in the same instrument, after 5 sterilization cycles.

From the temperature values, significant differences are revealed between files of two brands, except for Af (austenite finish) temperature. Between new and sterilized files of the same brand, all values are significant except for Af value of Race instruments. The enthalpy values of heating and cooling curves of both brands, does not show any significant difference.From the temperature values of all specimens, it is evident that the transformation from martensitic to austenic NiTi is completed below the oral environment temperature (~37^0C). Since all the specimens are completely austenite in the oral environment, these differences are not expected to have clinical impact. According to Duering and Pelton, superelasticity occurs when austenite is present, between As temperature (Austenite start) and Md (defined as the temperature above which stress-induced martensite can no longer be formed, about 25 to 50^0C higher than Af), suggesting that all examined specimens of both brands are considered capable of superelastic behavior [18]. This means that these instruments could undergo superelastic behaviour during use in the root canal, where the imposition of stress upto 8% strain causes transformation to martensitic phase [19].When the instrument is unloaded; the alloy reverts back to the original austenitic phase.

The enthalpy changes in the heating and cooling cycles of both brands of new and sterilized instruments range from 2.37 j/g to 3.941 j/g. These calculated values lie within the range of 1.7 to 19.2 j/g, which has been reported for nickel - titanium orthodontic wires [10,11].

CONCLUSION

From the study it was concluded that, phase transformation within the NiTi matrix of both Protaper and Race NiTi instruments is reversible. The reversibility is not affected by repeated heating and cooling cycles. Both Protaper and Race NiTi instruments are completely austenite in the oral environment and hence are capable of super elastic behavior during clinical use.

CONFLICT OF INTEREST NOTIFICATION

I, Dr.B.Hemasathya hereby certify that I have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

I declare that the above information is true and correct.

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