

Influence of autoclave sterilization on the surface parameters and mechanical properties of six orthodontic wires

C. Pernier*, B. Grosogeat*, L. Ponsonnet**, G. Benay* and M. Lissac*

*LEIBO, UFR d'Odontologie, Université Claude Bernard Lyon I, France and **CEGELY, CNRS-UMR 5005, Ecole Centrale de Lyon, France

SUMMARY Orthodontic wires are frequently packaged in individual sealed bags in order to avoid cross-contamination. The instructions on the wrapper generally advise autoclave sterilization of the package and its contents if additional protection is desired. However, sterilization can modify the surface parameters and the mechanical properties of many types of material.

The aim of this research was to determine the influence of one of the most widely used sterilization processes, autoclaving (18 minutes at 134°C, as recommended by the French Ministry of Health), on the surface parameters and mechanical properties of six wires currently used in orthodontics (one stainless steel alloy: Tru-Chrome® RMO; two nickel–titanium shape memory alloys: Neo Sentalloy® and Neo Sentalloy with Ionguard® GAC; and three titanium–molybdenum alloys: TMA® and Low Friction TMA® Ormco and Resolve® GAC).

The alloys were analysed on receipt and after sterilization, using surface structure observation techniques, including optical, scanning electron and atomic force microscopy and profilometry. The mechanical properties were assessed by three-point bending tests.

The results showed that autoclave sterilization had no adverse effects on the surface parameters or on the selected mechanical properties. This supports the possibility for practitioners to systematically sterilize wires before placing them in the oral environment.

Introduction

Orthodontic wires are frequently packaged in individual sealed bags in order to avoid cross-contamination. The instructions on the wrapper generally advise autoclave sterilization of the bag and the wire if additional protection is required. It is therefore important to know if this sterilization technique can be carried out with all types of orthodontic wire, whatever the alloy, and to see whether this process has any adverse effect on the surface structure and mechanical properties.

Since the late 1980s, studies have been undertaken to investigate the possibility of changes in orthodontic wires resulting from sterilization. The studies have mainly concerned the mechanical properties, assessed by bending and tensile tests. The results obtained have been contradictory, some concluding that sterilization results in the alteration of the mechanical properties, while others have reported no differences (Buckthal *et al.*, 1986; Buckthal and Kusy, 1988; Mayhew and Kusy, 1988; Kapila *et al.*, 1991, 1992; Smith *et al.*, 1992; Staggers and Margeson, 1993; Crotty *et al.*, 1996).

Surprisingly, surface topography, which is of importance for corrosion behaviour, some mechanical properties, and the performance of sliding mechanics, has not been extensively studied. Some authors have investigated titanium and nickel–titanium disks, but not orthodontic archwires. Their studies have shown that sterilization procedures alter the surface topography, resulting in modifications of surface roughness, discolouration,

contamination, and decreased levels of cell attachment and spreading (Keller *et al.*, 1990; Stanford *et al.*, 1994; Vezeau *et al.*, 1996; Thierry *et al.*, 2000).

The aim of this investigation was, therefore, to re-assess the consequences of the sterilization of orthodontic wires, as there have been considerable changes in the disinfection and sterilization protocols and the wires used (chemical composition, thermomechanical production processes, surface treatment), notably titanium alloys (shape memory wires, ion implantation, etc.).

Materials and method

The alloys

Six different wires currently used in orthodontics were investigated: one stainless steel alloy with a cross-section of 0.019 × 0.025 inches: Tru-Chrome® (Rocky Mountain Orthodontics, Denver, Colorado, USA); two nickel–titanium shape memory alloys with a cross-section of 0.018 × 0.025 inches: Neo Sentalloy® and Neo Sentalloy with Ionguard® (GAC International, Inc., Central Islip, New York, USA); three titanium–molybdenum alloys with a cross-section of 0.019 × 0.025 inches: TMA® and Low Friction TMA® (Ormco Corp., Glendora, California, USA) and Resolve® (GAC).

The terms 'Ionguard' and 'Low Friction' refer to the same ion implantation process, which hardens the alloy surface and thus reduces the friction coefficient of the wires.

All wires were autoclaved for 18 minutes at 134°C and examined before and after sterilization using different surface analysis techniques [optical, scanning electron (SEM), atomic force microscopy (AFM) and profilometry] and three-point bending tests.

In order to assess the bacteriological condition of the orthodontic wires as received from the suppliers, three different microbiological cultures were undertaken.

The first included 12 wires (three TMA®, three Low Friction TMA®, three Resolve® and three Neo Sentalloy®), cultured in Schaedler's broth (Biomérieux SA, Marcy L'Etoile, France), in an oven for 72 hours at 37°C (Jouan SA, St Herblain, France), in a CO₂-enriched atmosphere (Genbox CO₂, Biomérieux). The second concerned six wires (three Neo Sentalloy with Ionguard® and three Tru-Chrome®), cultured in aerobic conditions, for 72 hours at 37°C, in Schaedler's broth, and the third 18 wires (three TMA®, three Low Friction TMA®, three Resolve®, three Neo Sentalloy®, three Neo Sentalloy with Ionguard® and three Tru-Chrome®), cultured in aerobic conditions, for 5 days at 37°C, on Schaedler's sheep blood agar (Biomérieux).

In the first series of cultures, only one wire (Low Friction TMA®) induced growth of bacterial colonies. Microscopically, this contamination was due to Gram-positive, catalase-positive cocci. In the second series, two wires induced growth of germ colonies: one Neo Sentalloy with Ionguard® (contamination with Gram-positive, catalase-positive cocci) and one Tru-Chrome® (contamination with Gram-positive, catalase-positive, sporulated bacilli). In the last series, only one wire (Neo Sentalloy®) induced proliferation of bacterial colonies. The contamination was due to Gram-positive, sporulated bacilli.

The bacterial experiments thus showed that the orthodontic wires were not sterile, as 12 per cent of the wires developed germ colonies when they were cultured in their as-received condition. This contamination was low, but if total protection is desired, sterilization via an autoclave, as recommended by the manufacturers, should be carried out.

The sterilization technique

Autoclave sterilization was chosen because it is a technique frequently used in orthodontic practice and is recommended by the manufacturers.

Since 2001, the French Ministry of Health has recommended a treatment at 134°C for at least 18 minutes when autoclave sterilization is required (recommendation no. DGS/5C/DHOS/E2/2001/138 of the French Ministry of Health, 14 March 2001).

The autoclave used was an MS 61 NE/AUT (Colussi Srl, Pordenone, Italy). In order to comply with current norms, the wires had previously been placed in 5 per cent Micro 10+® solution (detergent, bactericide,

fungicide, virucide solution; Unident SA, Geneva, Switzerland), in an ultrasonic bath (Bandelin-Sonorex super RK 255 H; Bandelin Electronic, Berlin, Germany) for 10 minutes, followed by rinsing in distilled water, drying on absorbent paper, and placement in a sealed bag.

Assessment of the wires

Surface parameters.

Optical microscopy.

A Vanox optical microscope (Olympus Optical Co. Ltd., Tokyo, Japan), equipped with an Olympus digital camera system, was used. For each alloy, 10 wires were observed before and after sterilization at four different enlargement ratios: x5, x10, x20 and x40.

SEM.

The same observations were then made on the same number of wires with a SEM (Philips, Eindhoven, The Netherlands), used at 30 kV acceleration tension. The enlargements selected were x50, x100, x200, x500 and x1000.

Profilometry.

This device mechanically scans the surface of a sample at a constant speed. The tip, which is vertically mobile, remains in contact with the surface to establish the profile. The irregular vertical movements of the tip are plotted against the constant horizontal movement to establish the profile of the surface explored.

The profiles were established with a Perthen C 5 D Perthometer (Mahr-Perthen, Göttingen, Germany), a compact electronic device used to determine different criteria of roughness and to provide the profiles of milled parts. The palper used was a RFHTB-50 (Mahr-Perthen) with a 5 µm radius tip and a cone angle of 90 degrees.

The perthometer bases its profiles on the Deutsches Institut für Normung (German Institute for Standardization) roughness norm 'R' and determines 'Ra', which is the arithmetic mean roughness, expressed in microns. It is the arithmetic mean of the departures of the roughness profile from the mean line:

$$Ra = (1/lm) \int_0^{lm} |y| dx$$

where *lm* is the length of the palp.

A series of five measurements (*lm* = 4.8 mm) on four different wires per alloy was carried out before autoclave sterilization. Four other wires from the same sample were also profiled after sterilization. It was not possible to use the same wires before and after sterilization, as profilometry is considered an invasive technique which can alter the surface studied.

The results obtained were analysed using Student's *t*-test.

AFM.

AFM provides topographical three-dimensional (3D) images in real space with high resolution (to the atom) by recording the vertical movements of a microtip mounted on a cantilever scanning the surface of a sample. AFM is based on an optical approach that consists of reflecting a laser beam by means of a mirror on to the cantilever and then to a segmented four photodiode detector which generates an 'error' signal from which the topographic image is produced.

For this study, an AutoProbe CP (Park Scientific Inc., Sunnyvale, California, USA) with 0.6 μm cantilevers was used (X - Y resolution at the atomic level, $Z < 0.5\text{\AA}$). It was equipped with PSI ProScan version 1.5 software (Park Scientific) for capturing and processing images, thus allowing 3D reconstructions and measurements of the surface parameters calculated on the scanned surface to be obtained.

This research focused on one of these parameters: the arithmetic average roughness R_{ave} (equivalent to Ra obtained in profilometry) where

$$R_{\text{ave}} = \sum_{n=1}^N |z_n - \bar{z}| / N$$

where \bar{z} is the average value of z

This value was obtained on $100 \times 100 \mu\text{m}$ surfaces. For each alloy, a series of 18 measurements per wire was undertaken on three different wires before sterilization and on three other wires from the same batch after sterilization. The results obtained were analysed with Student's *t*-test.

In contrast to profilometry, AFM is a non-invasive technique, scanning a surface (not a line) with a higher resolution.

Three-point bending tests. The mechanical properties of the wires before and after sterilization were tested on a three-point bending bench with a Deltech LC 102 TC captor (Deltech, Corbas, France), with a measurement scope (EM) of $\pm 2 \text{ daN}$ and a sensibility of $\pm 2623 \text{ mV/V}$ for EM.

This system, linked to a graph plotter, produces a load/displacement curve enabling the elastic modulus for the different wires before and after sterilization to be established, using the formula:

$$E = P/f \times L^3/4bh^3 = \text{slope} \times L^3/4bh^3$$

where P = load, L = distance between the two plots, b = wide side of wire, h = narrow side of wire, f = displacement.

The displacements studied were 3 mm, at a speed of 0.3 mm/mn. This study was carried out on three wires of each type before sterilization, and on the same number after sterilization.

Results

Surface parameters

Optical and SEM. The images obtained by optical and SEM could be compared with the 3D reconstructions obtained by AFM (Figures 1 and 2). The results showed the great variability in the surface parameters of orthodontic wires, resulting from the different production processes involved. Tru-Chrome[®] appeared to have the most heterogeneous surface parameters, alternating between smooth stretches and rough surface, with hollows and random striation. Neo Sentalloy[®] alloys had the roughest surface and even more so when they had been submitted to ion implantation treatment. The TMA[®] wires, with or without surface treatment, were clearly striated, which may be due to the wire drawing process. Finally, the Resolve[®] wire had the most homogenous surface structure parameters.

Optical and SEM did not reveal any variation in the surface parameters after sterilization for any of the wires studied.

Profilometry. The profilometry results are shown in Figure 3.

Before sterilization, Tru-Chrome[®] was the smoothest wire, closely followed by Resolve[®], then by the two TMA[®] alloys, and finally by the two Neo Sentalloy[®] wires. In all cases, the wires that had undergone ion implantation were found to be rougher than those without surface treatment. Finally, for the Tru-Chrome[®] and Neo Sentalloy Ionguard[®] wires, the measurements obtained covered a wide range of roughness, consistent with the heterogeneous surface structure parameters observed by microscopy.

Concerning the possible change in surface structure parameters after sterilization, the profilometry study did not identify statistically significant roughness differences ($P > 0.05$) before or after autoclave sterilization.

AFM. AFM measurements established a classification of the wires by their average roughness (Ra) very similar to that obtained by profilometry. The only notable exception was for Neo Sentalloy[®] without ion implantation, which appeared much lower and was comparable with the Ra of Resolve[®] and TMA[®] (Figure 4).

Student's *t*-test demonstrated a statistically significant difference in roughness ($P < 0.05$) before and after sterilization for TMA[®] and Neo Sentalloy wires with Ionguard[®].

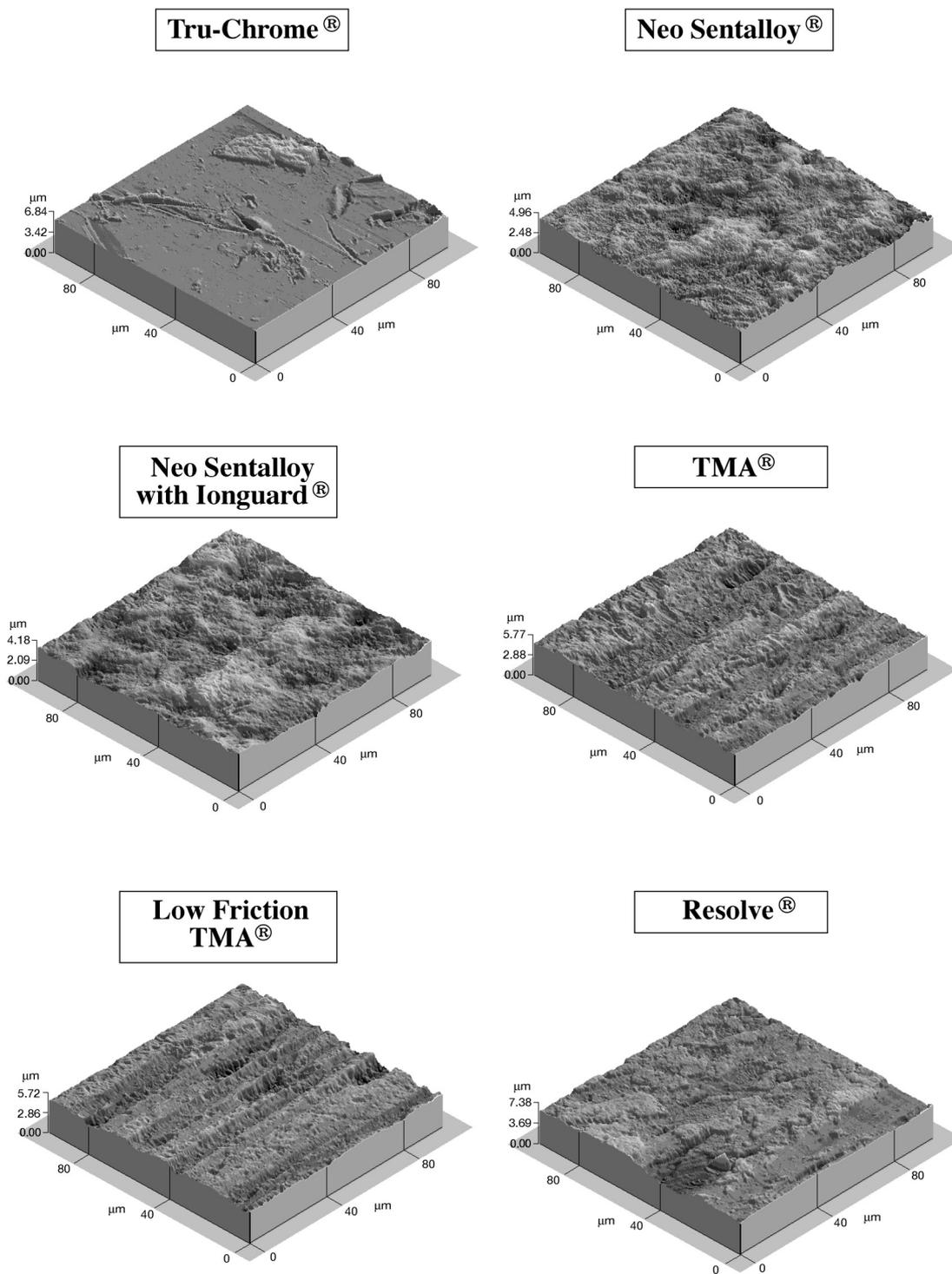


Figure 1 Three-dimensional reconstructions obtained by atomic force microscopy, before sterilization.

The 3D reconstructions obtained by AFM (Figures 1 and 2) confirmed the optical and SEM observations concerning the considerable variability in the surface structure parameters of the wires. Tru-Chrome® was again characterized by alternating areas of smoothness and areas with clearly defective surface structure

parameters, often in the form of hollows. The Neo Sentalloy® wires had a very irregular surface with humps and hollows, especially visible on the surface of the wires having undergone ion implantation. TMA® alloys, with or without surface treatment, were deeply striated, probably because of the wire drawing

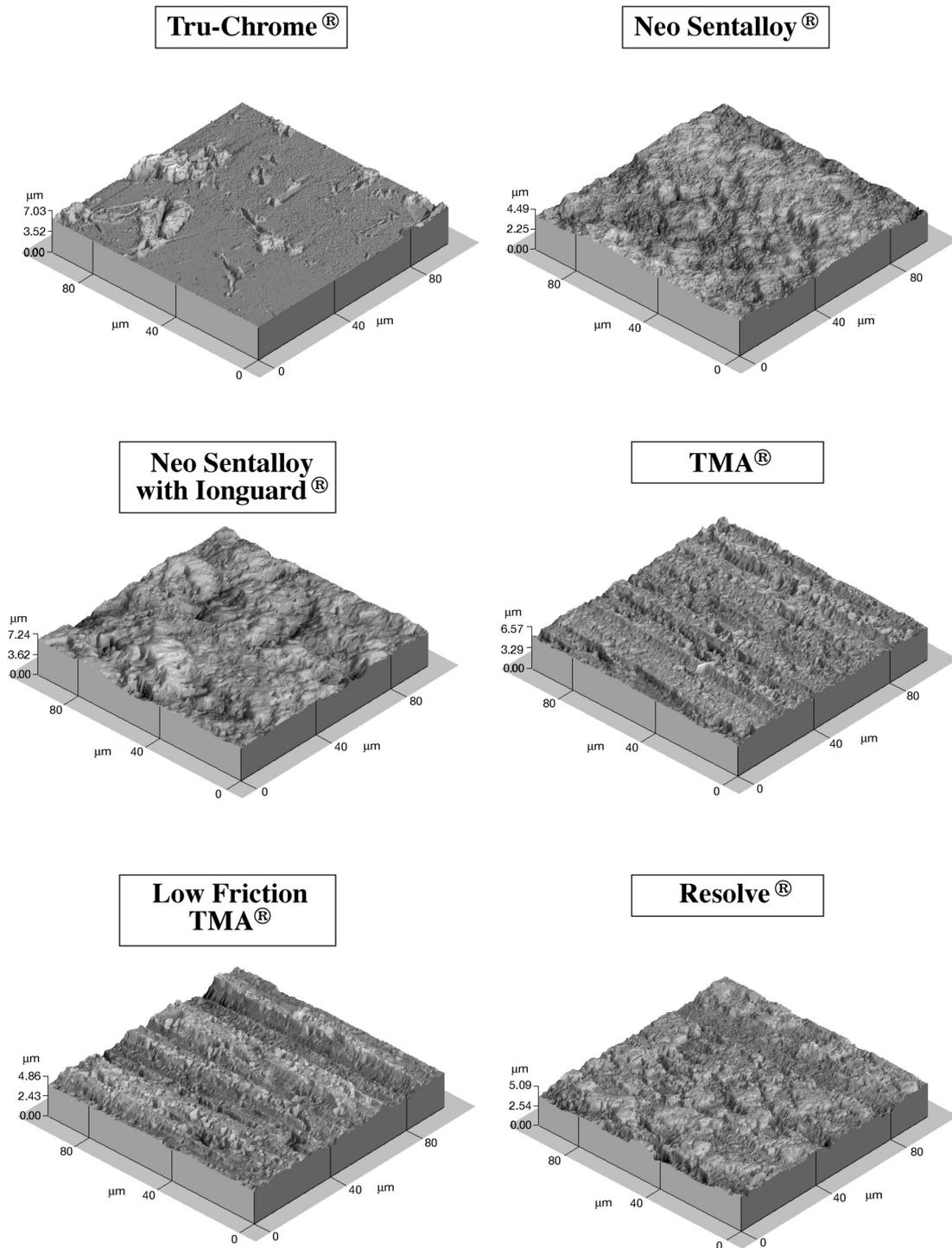


Figure 2 Three-dimensional reconstructions obtained by atomic force microscopy, after sterilization.

process used. Observation of the 3D reconstructions did not, however, provide as clear a picture of the difference between the two TMA alloys as that provided by roughness measurements (roughness of TMA Low Friction® greater than that of TMA®). Finally, Resolve® can be seen to be characterized by surface parameters situated between those of the

Neo Sentalloy® and TMA® wires, with the smoothness impaired by striations, and by humps and hollows, but less so than the features observed in the Neo Sentalloy® and TMA® wires.

For all the wires studied, the 3D reconstructions did not show a clear difference in surface structure parameters after autoclave sterilization, even for TMA®

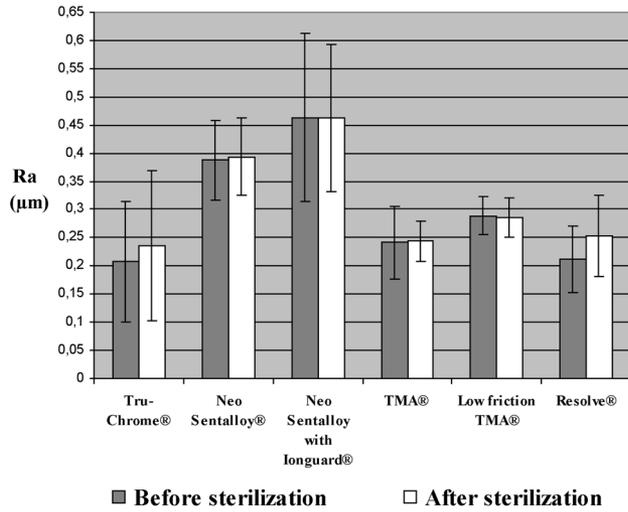


Figure 3 Average roughness R_a (μm), obtained by profilometry, before and after sterilization.

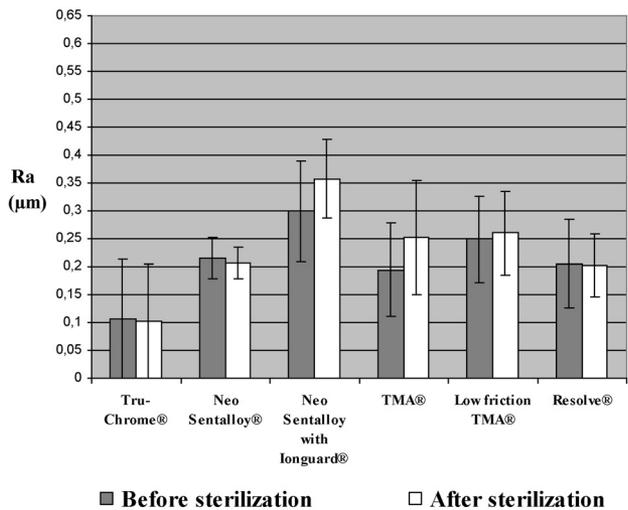


Figure 4 Average roughness R_a (μm), obtained by atomic force microscopy, before and after sterilization.

and Neo Sentalloy with Ionguard®, for which roughness measurements had shown a difference in smoothness.

Three-point bending tests

The three-point bending tests enabled the determination of the elastic modulus for each of the alloys tested (Figure 5), based on the load/displacement curves (Figure 6). For the stainless steel wire (Tru-Chrome®) and the titanium–molybdenum alloys (TMA®, Low Friction TMA® and Resolve®), the measurements were made under loading conditions, whereas for the nickel–titanium alloys (Neo Sentalloy® and Neo Sentalloy with Ionguard®), the measurements were carried out under

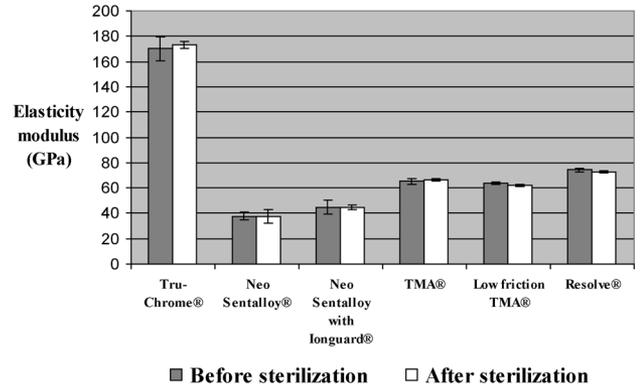


Figure 5 Average elastic modulus (GPa), before and after sterilization.

both loading and unloading, as their characteristics are different during these two stages. It should also be noted that for these alloys the calculation of the elastic modulus was made from the graph representing the elastic deformation of the austenite. The results correspond to the elastic modulus in the austenite state.

The results obtained enabled the classification of the different wires, the most rigid being Tru-Chrome®, with an elastic modulus of around 170 GPa, followed by Resolve® (~73 GPa), TMA® (~65 GPa), Low Friction TMA® (~63 GPa), Neo Sentalloy with Ionguard® (~45 GPa), and Neo Sentalloy® (~38 GPa).

The three-point bending tests did not reveal any variation in the load/displacement curves after sterilization for any of the wires studied.

Discussion

Surface parameters

The means used to study the surface parameters of the wires all contribute to show that there are considerable differences between the wires. The surface topography is a result of the choice of the alloy, the complex manufacturing processes, and the surface treatment.

The results allowed the classification of the wires according to their average roughness, from the smoothest to the roughest, as follows: Tru-Chrome® (stainless steel), Resolve® (titanium–molybdenum), TMA® (titanium–molybdenum), Low friction TMA® (titanium–molybdenum + ion implantation), Neo Sentalloy® (nickel–titanium), Neo Sentalloy with Ionguard® (nickel–titanium + ion implantation).

This classification is close to that of Prosocki *et al.* (1991) and Bourauel *et al.* (1998), who found that stainless steel wires had the smoothest surfaces, that the TMA® alloy was fairly rough, and that the nickel–titanium alloys had the most widely dispersed results. The more unexpected finding concerns Neo Sentalloy®.

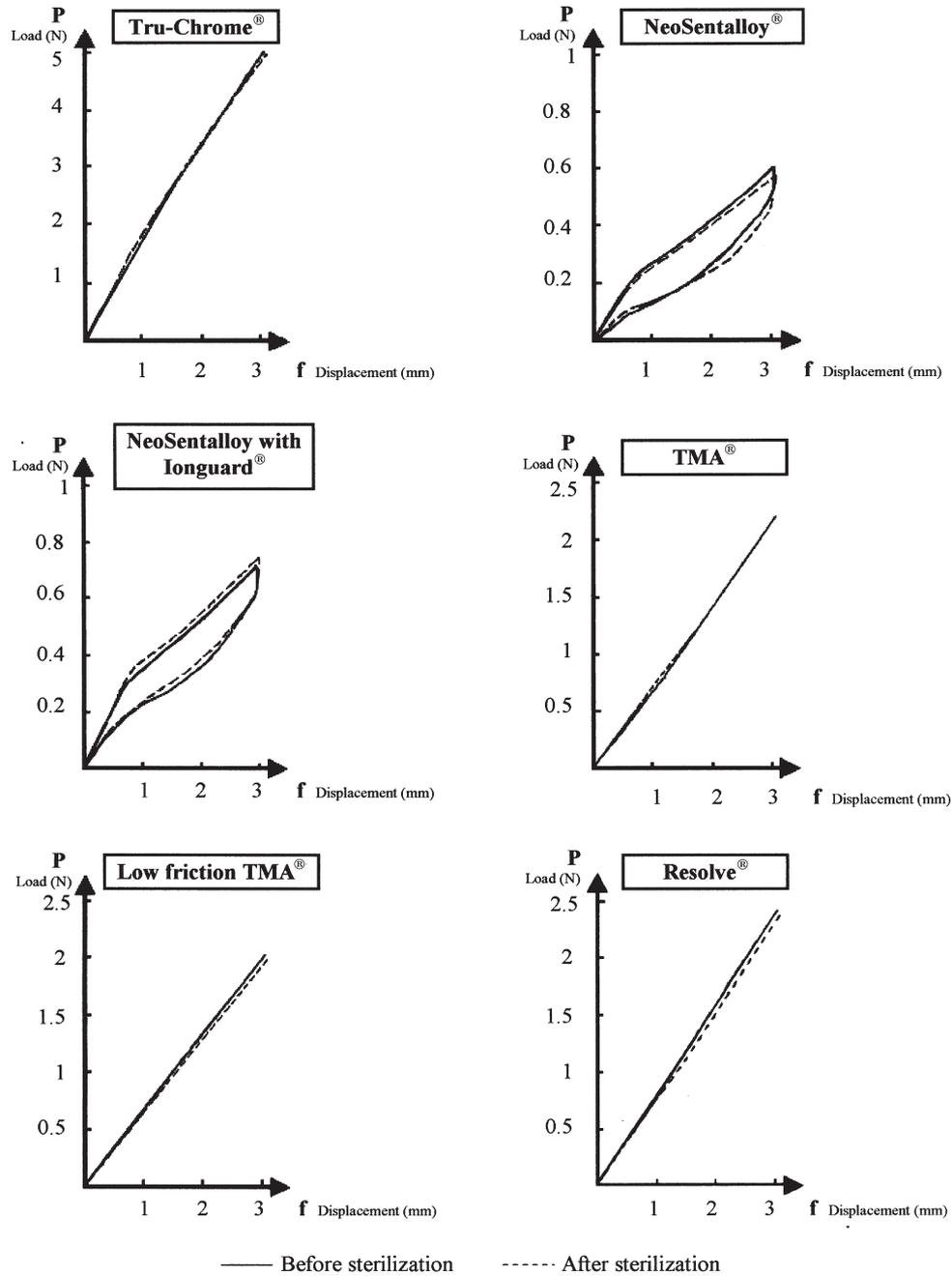


Figure 6 Load displacement curves, before and after sterilization.

Bourauel *et al.* (1998) calculated an average roughness of 0.65 μm by profilometry (arithmetic roughness mean) and of 0.62 μm by AFM (geometric roughness mean), whereas the results of this study were 0.39 and 0.21 μm , respectively (arithmetic roughness means). It should, however, be borne in mind that although in the 1998 study Neo Sentalloy® was by far the roughest of the orthodontic wires examined, this may have encouraged the manufacturers to improve the surface features of this product.

The surface structure of orthodontic wires is an essential feature, known to influence the aesthetic result, the resistance to corrosion, and the biocompatibility of the device. However, the relationship comprising roughness and the frictional forces existing between the wire and brackets is more complex and not yet totally clear.

In orthodontics, the teeth are moved along a wire, and this creates frictional forces between the wire and the bracket adhering to the tooth. If the frictional forces

are large, the speed at which the tooth can be moved diminishes and the movement becomes unpredictable. It is therefore a balance to reduce these forces which depend on a number of factors linked to the wires (composition, surface features, geometry, etc.), to the brackets (composition, design, dimension, etc.), to the ligatures, and to saliva.

With such a complex phenomenon, it is not surprising that there should be so many conflicting views in the literature. It is, however, currently assumed that the frictional forces are not as great as when stainless steel wires are used, and that they increase with cobalt–chrome alloys, a little more with nickel–titanium alloys, and even more so with titanium–molybdenum alloys (Bourauel *et al.*, 1998).

It has not been possible to correlate this classification with the average roughness of these wires. It is important to realize that a very smooth surface can generate friction just as much as a rough surface, as the contact areas increase with the smoothness. It would appear that the roughness of orthodontic wires is at a level where it is a parameter that has little influence on the frictional forces produced. This view of roughness and friction is confirmed by the results obtained by Kusy and Whitley (1988), who showed that a smooth surface is not a sufficient condition to generate low friction coefficients (Prosocki *et al.*, 1991).

The observations in this study concerning Low Friction TMA[®] and Neo Sentalloy with Ionguard[®] would also appear to corroborate this point of view. These wires, widely considered to generate less friction than their non-implanted counterparts, were shown to have a higher average roughness than their non-implanted counterparts (+0.05 μm on average, Figures 3 and 4). This increased roughness could be linked to the ion implantation or to the pre-implantation preparation of these wires. Ion implantation is a surface treatment in which different elements (in this case nitrogen and oxygen) are ionized and then accelerated towards a target, where they impact the surface and form a hybrid layer enriched with TiN and TiO. This outer layer is hard and generates large compressive forces inside the material at the atomic level. This improves the material's ductability and resistance to fatigue, and reduces the friction coefficient of these wires (Burstone and Farzin-Nia, 1995).

For all the wires tested, the average roughness values were lower when measured with AFM than by profilometry. This difference was small for TMA[®], Low Friction TMA[®], and Resolve[®], but was found to be very large for Tru-Chrome[®], Neo Sentalloy[®] and Neo Sentalloy with Ionguard[®] (Figures 3 and 4). These differences between profilometry and AFM can be attributed to the different sensibilities of the two measurement devices (10^{-2} μm for the profilometer and Å for AFM) and the scales used, the former scanning

approximately a 5 mm line, whereas the latter scans a 0.1×0.1 mm area.

These differences are all the more noticeable when the surfaces studied are heterogeneous with alternating surfaces of different roughness (Tru-Chrome[®], Figures 1 and 2) or with large surface defects (Neo Sentalloy[®] and Neo Sentalloy with Ionguard[®], Figures 1 and 2). On the contrary, the differences are smaller when the roughness of the surfaces is due to regular, shallow striation and pits (TMA[®], Low Friction TMA[®], and Resolve[®], Figures 1 and 2).

Optical and SEM showed no significant differences after sterilization. This is contrary to the findings of Keller *et al.* (1990), Vezeau *et al.* (1996), and Thierry *et al.* (2000), who found contamination (hydrocarbon, Cl, Fe, Na, Ca, etc.) and discolouration on the surface of titanium or nickel–titanium disks after autoclave sterilization. These differences were, however, due to residual matter remaining on the surface after condensation of the water used in the sterilization process, and this reinforces the necessity of using absolutely pure water to produce the steam. Moreover, those investigations were carried out on highly polished disks with a roughness of 3–5 nm, which is far less than the roughness values of the orthodontic wires used in this study. The surface structure defects of the wires may have enabled possible contamination and discolouration to go undetected, or the absence may be due to the use of perfectly pure water for sterilization in the present research.

Similarly, profilometry values revealed no difference in the average roughness of the wires before or after sterilization (Figure 3). On the contrary, for some wires, the more precise AFM measurements showed a statistically different average roughness before and after autoclaving (Figure 4). The wires in question were TMA[®] and Neo Sentalloy with Ionguard[®] with, in both cases, an average roughness increase of approximately 0.05 μm after sterilization. This is in agreement with the results of Thierry *et al.* (2000), showing that the surface structure of nickel–titanium disks was rougher after dry heat or autoclave sterilization (Ra : 5 to 25 nm).

It is, however, necessary to stress that the increase in surface roughness was extremely small (+0.05 μm) and it is most unlikely that it would have any clinical effect. The differences are only perceptible when an extremely precise measurement tool is used, and are only found on the surface of certain wires (TMA[®] and Neo Sentalloy with Ionguard[®]); other wires of similar composition not being affected.

This increase in roughness can perhaps be explained by the fact that sterilization removes debris or traces of grease which might have remained on the wires after their production, which would have made the real surface roughness of the wires before sterilization more difficult to measure. It is to be remembered that the first stage of

the sterilization protocol imposed cleaning by detergent (micro[®] 10+) in an ultrasonic bath. This stage is indispensable, as it clears any organic or lipid traces which could remain on the wires and harbour micro-organisms.

The first part of the research has thus shown that autoclave sterilization by the latest protocols does not affect, or affects insignificantly, the surface features of the six alloys tested.

Elastic modulus

The three-point bending tests carried out on the six alloys before sterilization enabled the classification of the alloys from the most rigid to the most supple as follows: Tru-Chrome[®] (stainless steel) $E \approx 170$ GPa; Resolve[®] (titanium–molybdenum) $E \approx 73$ GPa; TMA[®] (titanium–molybdenum) $E \approx 65$ GPa; Low Friction TMA[®] (titanium–molybdenum + ion implantation) $E \approx 63$ GPa; Neo Sentalloy with Ionguard[®] (titanium–molybdenum + ion implantation) $E_a \approx 45$ GPa; Neo Sentalloy[®] (nickel–titanium) $E_a \approx 38$ GPa.

This classification and the values of the elastic modulus are in accordance with those obtained for the same or similar wires (Kusy and Greenberg, 1982; Drake *et al.*, 1982; Asgharnia and Brantley, 1986; Muraviev *et al.*, 2001). Similarly, the load displacement curves are a true reproduction of the characteristic properties of the different alloys, and notably of the nickel–titanium alloys which show different behaviour under increasing and decreasing loading. The results obtained after sterilization did not modify the above classification and the elastic modulus values were similar for sterilized and unsterilized wires from the same batch.

Taking into account the small number of wires tested mechanically (six wires of each alloy from the same batch, before and after sterilization), no satisfactory statistical analysis of the results could be carried out, and the findings should be interpreted with caution. Nevertheless, it appears that autoclave sterilization at 134°C for 18 minutes does not modify either the load displacement curve or the elastic modulus for the six alloys tested.

While these results were to be expected for the stainless steel and titanium–molybdenum alloys, it was much less the case for the nickel–titanium alloys. The conclusions of the study are in accordance with the findings of Buckthal and Kusy (1988), Mayhew and Kusy (1988), Smith *et al.* (1992), and Crotty *et al.* (1996), but in contrast with the results obtained by Kapila *et al.* (1991, 1992), and Staggers and Margeson (1993). Even more clearly, the results of the study confirm what the inventor of these so-called ‘Japanese NiTi alloys’ claimed as early as 1986, i.e. that the effect of temperature on these alloys is negligible up to 400°C, above which the super-elasticity decreases and practically disappears

at 600°C, even with very short exposure times (Miura *et al.*, 1986).

For this reason, and in spite of the small number of samples, it can reasonably be concluded that the tendency, under three-point bending, is a valid indication of reality, and that autoclave sterilization following the latest protocols does not alter the mechanical properties of the alloys tested.

Conclusion

1. Profilometry, optical, SEM, and AFM did not provide clear evidence of any significant change in the surface features of the alloys tested. The few changes observed after sterilization were minimal (+0.05 µm), all showing a slight increase in roughness which would not have any effect in everyday clinical use of the wires.
2. The three-point bending tests also showed no change in behaviour after sterilization for movements up to 3 mm.

For dental practitioners who want to guarantee maximum safety for their patients, sterilization of orthodontic wires before placement does not alter the properties of the alloys.

Address for correspondence

Claire Pernier
17 rue de la Charité
69002 Lyon
France
Email: claire.pernier@mageos.com

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