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ToF-SIMS images and spectra of biomimetic calcium silicate-based cements after storage in solutions simulating the effects of human biological fluids

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ABSTRACT

ToF-SIMS images were obtained from a section of a tooth, obturated by means of a new calcium-silicate based cement (wTCF) after storage for 1 month in a saline solutions (DPBS), in order to simulate the body fluid effects on the obturation. Afterwards, ToF-SIMS spectra were obtained from model samples, prepared by using the same cement paste, after storage for 1 month and 8 months in two different saline solutions (DPBS and HBSS). ToF-SIMS spectra were also obtained from fluorine-free cement (wTC) samples after storage in HBSS for 1 month and 8 months and used for comparison. It was found that the composition of both the saline solution and the cement influenced the composition of the surface of disks and that longer is the storage greater are the differences. Segregation phenomena occur both on the cement obturation of the tooth and on the surface of the disks prepared by using the same cement. Indirect evidences of formation of new crystalline phases are supplied.

1. Introduction

The dental pulp communicates with the periodontal tissues through the apical foramen and via lateral canals. Exposure of the dental pulp or the periradicular tissues to microorganism results in the development of pulpal and periradicular pathosis. For this reason the sealing of the communication canals with the oral cavity is of fundamental importance. A number of materials have been used to fill the root apex during surgical apical root removal, including amalgam, zinc-oxide-eugenol and glass ionomer cements, epoxy resins and calcium-silicate Portland-based cements. Many of them showed important clinical limitations that reduced the success rate [1,2] and none of them has fulfilled all the criteria for an ideal root filling [2]. Material for root-end filling procedures should be easy to use, stable, biologically inert or bioactive. Several studies proposed the use of hydraulic cement to seal the apical root end thanks to their ability to set in humid and wet conditions. Later, preliminary *in vivo* and clinical studies suggest that calcium-silicate cements may present better results than the other materials [3–5]. At present, new calcium-silicate materials are under development for clinical use as root-end filling materials and for other endodontic clinical application [6–9].

Calcium-silicate cements are hydraulic materials able to set in the presence of moisture such as blood and fluid contamination [10–14]. Calcium-silicate cements, such as MTAs (mineral trioxide aggregate) and white Portland, showed adequate or excellent root marginal adaptation and sealing ability [14].

Preliminary investigations suggested that calcium-silicate materials may induce the formation of a superficial biocoating, mainly composed by apatite, when immersed in solutions, such as the Dulbecco's Phosphate Buffered Solution (DPBS), that simulate the effects of the body fluids [14].

The samples used in this paper are part of a wider project concerning the formulation of material that can be used in different biological surroundings.

Due to thermodynamic reasons, most of the composite materials may suffer from diffusion and/or segregation phenomena that give rise to a surface chemical composition sometimes remarkably different with respect to the bulk of the material. Since the reactions with the surrounding tissues start at the contact surfaces, it is of great importance to obtain detailed chemical information on the surfaces and interfaces involved in the interaction with biological tissues. Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is an analytical technique very suitable for this purpose [15]. It indeed gives the molecular information typical of mass spectrometric techniques from the uppermost layer of a material. In addition, due to its high spatial resolution, the ToF-SIMS allows us to obtain detailed information on the distribution of a given element/molecule on the surface and/or within the bulk of the material, in other words to obtain a chemical map. The information gathered by this technique will allow us to gain a better knowledge of the chemical interactions at the interfaces between

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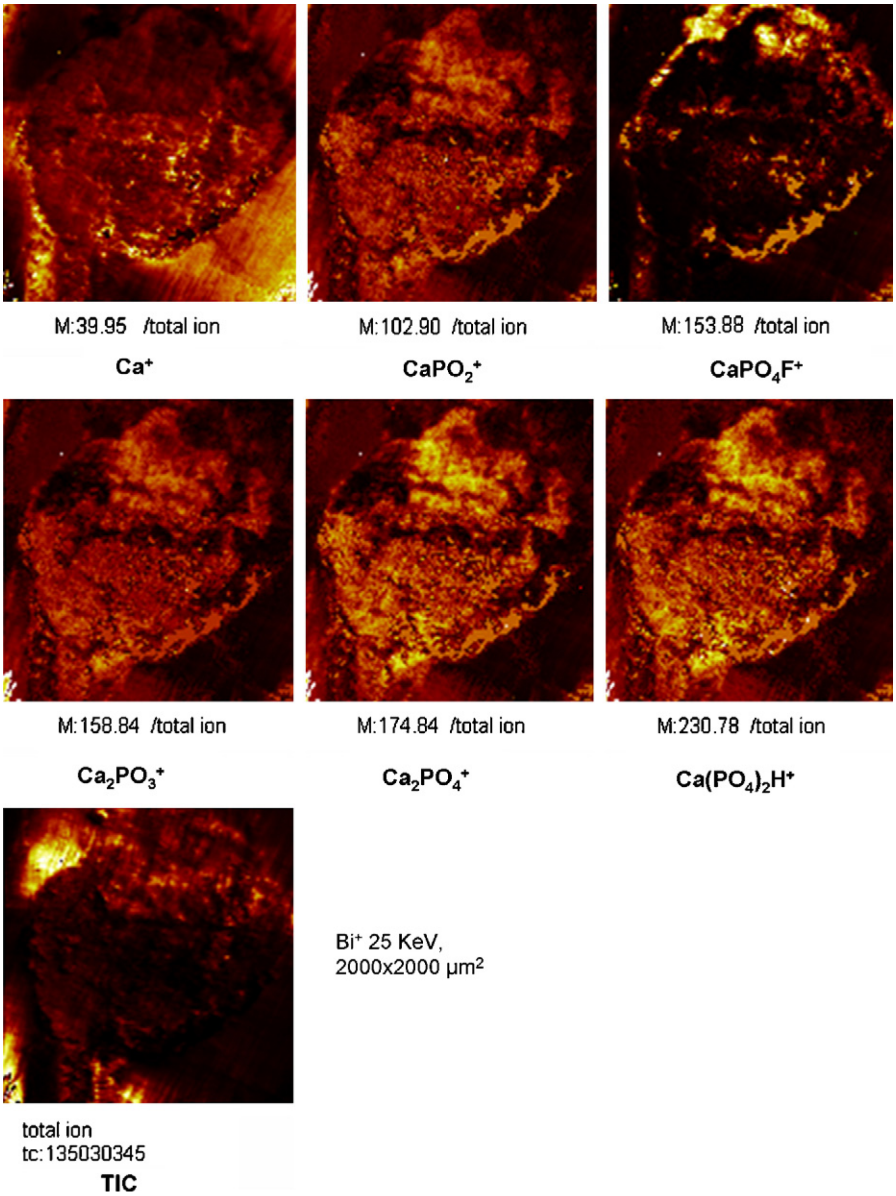

Fig. 1. ToF-SIMS positive ion maps of Ca-containing fragments obtained from the portion of the tooth obturated with the wTCF cement paste after immersion in the DPBS buffer solution for 1 month. All the images are normalised to the total ion current map (TIC).

cements and tissues. ToF-SIMS has been already used for the characterisation of hydroxyapatites [16] or to study the interaction of metallic prostheses with biological tissues [17,18].

In the present paper we report the results of some preliminary studies concerning the possibility to follow by means of ToF-SIMS technique the chemical modification that occurs both in model and in real samples prepared by using new cement pastes.

2. Experimental

2.1. Preparation of section of apical filled roots of the tooth

Briefly, upper incisors were selected from a pool of extracted human permanent teeth extracted for surgical reasons and stored in distilled water for no more than 1 month at 4 °C. Roots were optically inspected for crack formation or resorption areas. The coronal portion of each tooth was removed to reduce the dimension of the samples. Apical portion (approximately 3 mm) was then removed with a diamond bur working under water at 20,000 rpm. A small root-apical cavity (3 mm diameter and depth) was then prepared according with conventional surgical procedures using a small diamond bur and gently washed with water and EDTA for 20 s. One operator was responsible for the entire procedure.

After the cavity preparation had finished, the wTCF experimental sealer was prepared as previously described and immediately placed in the root-apical cavity to complete the filling procedure. A sustained push, lasting for several seconds, was carried out with a stainless plugger. Each sample was exposed to Rx analysis to check the placement of material to the working length, to verify the thickness of the apical filling, and to detect the presence of voids inside the filling material. The filled root was washed with distilled water for 5 s, and then lightly dried using two paper points for several seconds. The filled root was then placed in the DPBS solutions and stored at 37 °C for 1 month.

2.2. Cements preparation

The experimental calcium-silicate cements were identified as wTC and wTCF. The powder constituted [7,8] a white Portland cement (CEM I, Aalborg, Denmark) subjected to thermal

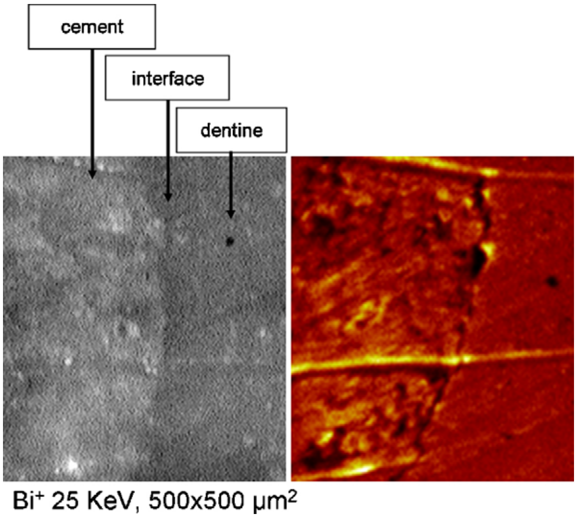

Fig. 2. Optical (left side) and TIC (right side) images obtained of a portion of the tooth after a cleaning by rastering a mild Ar^+ ion beam (1 keV, 14 nA, 180 s).

and mechanical treatments, and added with calcium chloride, anhydrite ($CaSO_4$), montmorillonite [$(Na,Ca)_{0.33}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot n(H_2O)$]. Small quantities (1%, w/w) of sodium fluoride were added in the composition of wTCF. Both cements were mixed with DPBS (liquid/powder ratio of 0.3) and layered on a plastic cover-slip of 13 mm diameter (Thermanox Plastic, Nalge Nunc International, NY, USA) to obtain standard disks. The exposed surface area of the disks was $1.9 \pm 0.1\ cm^2$ and their thickness approximately 0.9 mm. Mechanical vibrations were used to obtain an almost macroscopically flat and regular surface. Immediately after their preparation all disk samples were placed in 5.2 mL soaking solutions (DPBS and HBSS) at 37 °C for 1 month and 8 months and then analyzed. The Dulbecco's modified Phosphate Buffered Solution and the Hank's Balanced Salt Solution (HBSS) were supplied by Lonza Walkersville Inc. (USA). The concentrations of the main components, calculated on the basis of the supplied compositions, are: DPBS (K^+ 4.18 mM, Na^+ 152.9 mM, Cl^- 139.5 mM, $H_2PO_4^-$ 1.5 mM, HPO_4^{2-} 8.06 mM); HBBS (Ca^{2+} 1.27 mM, K^+ 5.82 mM, Na^+ 142.1 mM, Mg^{2+} 0.81 mM, SO_4^{2-} 0.81 mM; Cl^- 145.1 mM, $H_2PO_4^-$ 0.44 mM, HPO_4^{2-} 0.34 mM, HCO_3^- 4.17 mM, glucose 1 g/L).

2.3. ToF-SIMS analysis

The ToF-SIMS ion images and spectra were obtained by means of a ToF-SIMS IV instrument (ION-TOF, Muenster, Germany) equipped with Bi^+ LMIG and Ar^+ gas primary ions sources. In particular, the ion maps were recorded by using a 25 keV Bi^+ ion source, in bunched mode with a measured primary current of 0.7 pA; the sizes of the analyzed area are indicated in each figure. The Ar^+ beam was used for a mild (1 keV, 14 nA, 180 s) sputtering of the tooth surface. The spectra were obtained by using a 25 keV Bi^+ ion primary beam rastered over an area of 100 × 100 square micrometers, always maintaining the static conditions (ion dose $<1 \times 10^{12}\ ions/cm^2$). During the acquisition of both ToF images and spectra, in order to reduce the effect of charging-up caused by the impinging of the primary ions on highly insulated materials, a flooding of the surfaces was achieved by means of a low energy electrons flux. The vacuum in the analysis chamber was maintained in the range of 10^{-8} torr. The surface of the tooth section was gently scraped by means of a razor blade before the analysis. Before ToF-SIMS analysis, the cement disks, drawn out from the buffer solutions, were dipped in milliQ water and afterwards dried under a gentle nitrogen flux.

2.4. XRD analysis

The XRD analysis were performed on the cement disks by means of Rigaku Ultima IV Type III In-plane diffractometer, by using the following conditions: X-ray Copper Kα, 40 kV, 40 mA; Scanning Mode 2Theta/Theta; Start Angle 20°, Stop Angle 120°, Step width 0.02°, Scan Speed 0.300 °/min.

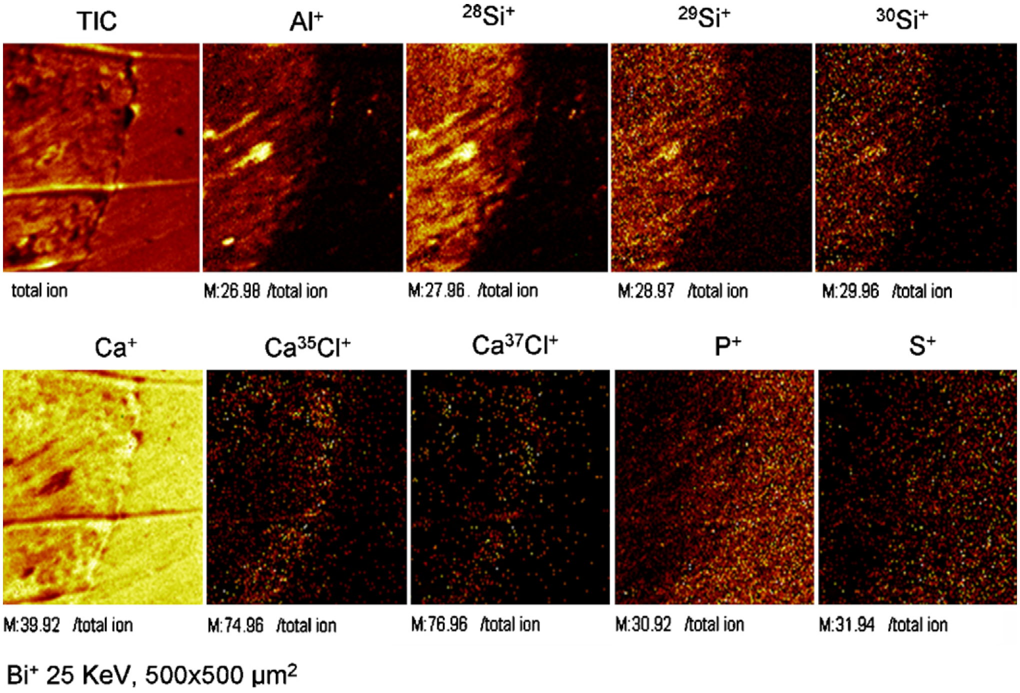

Fig. 3. ToF-SIMS positive ion maps of selected fragments obtained from the portion of the tooth shown in Fig. 2. The ion images are normalised to the total ion current maps (TIC) that is reported for comparison.

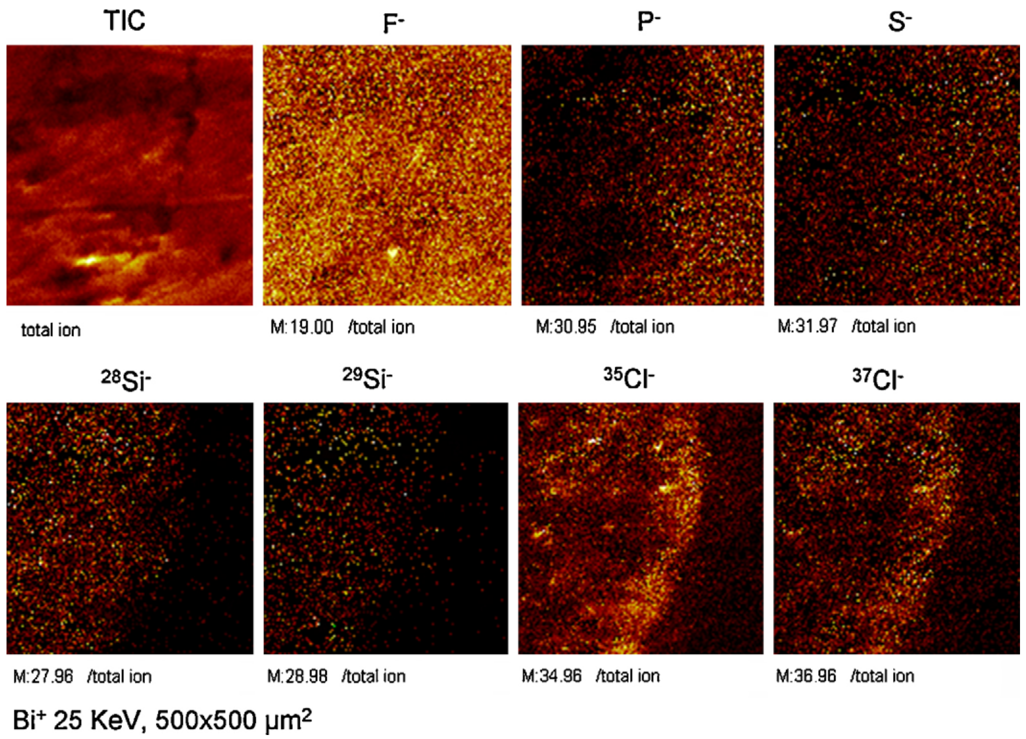

Fig. 4. ToF-SIMS negative ion maps of selected fragments obtained from the portion of the tooth shown in Fig. 2. The ion images are normalised to the total ion current maps (TIC) that is reported for comparison.

3. Results and discussions

3.1. ToF-SIMS imaging

Fig. 1 shows the ion images of Ca-containing fragments obtained from the portion of the tooth obturated with the WTCF paste after immersion in the Dulbecco's Phosphate Buffered Saline (DPBS) solution for 1 month. All the reported ion images are normalised pixel by pixel to the total ion current, in order to reduce the topographical effects. The calcium ion image is reported for comparison. All the images show a central portion, whose shape is almost circular, that represent the obturated part of the tooth. In particular, the calcium ion image shows a nearly closed black ring, representing the lowest ion emission (low Ca content), in a pretty homogeneous and more intense picture. It seems complementary to this image the one obtained from 154 Da nominal mass, attributed to $CaPO_4F^+$ and/or $CaHPO_4(H_2O)^+$ fragments. This images indeed shows a yellowish-sand coloured ring immersed in black neighbourhood. This distribution indicates the formation of this specie just at the border of the tooth obturation, at the interface between the cement paste and the tooth's dentine. The remaining ion images reported in Fig. 1 refer to some calcium phosphate fragments that are distributed mainly on the cement paste (obturation) zone. This indicates a chemical modification that the cement paste undergoes after immersion in the DPBS solution, leading to the formation of phosphate-containing products.

In order to reduce matrix effects and to check the composition of the underlying layers, the tooth surface was *cleaned* by rastering a mild (1 keV, 14 nA, 180 s) Ar^+ ion beam over a portion of the above image. Fig. 2 shows the optical (left side) and the total ion (right side) of this part. The arrows in the optical image label the different zones. Figs. 3 and 4 show the ion distribution of selected positive and negative ions respectively, obtained from the area reported in Fig. 2. As above, the selected ions' spatial distributions are normalised to the total ion, whose image is reported for comparison. The positive ToF-SIMS images show a quite uniform distribution of the calcium ion, while aluminium and silicon are clearly distributed in the cement paste zone. This is not surprising because the latter are components of the cement paste, while the former is present both in the cement paste and in the dentine. The phosphorus and sulphur ions are distributed mainly in the dentine zone, even if the former shows a not negligible intensity also at the interface region. In particular, this region shows the presence of chlorine-containing Ca species that are preferentially distributed along this interface, indicating a segregation of the $CaCl_2$ contained in the cement, even if one cannot exclude the formation of further $CaCl_2$ during the storage due to the relative high content of chlorine ions in the DPBS solution. Similar comments can be derived for the negative ToF-SIMS images, that show an almost uniform distribution of fluorine ions, while the ion maps of the remaining masses reflect those observed for the positive ions.

Summarizing:

- the surface of the obturation before the mild etching shows several Ca-containing phosphorous fragments that suggest a chemical interaction between the calcium of the cement paste and the phosphate group present in DPBS solution;
- one of these fragments, attributed to $CaPO_4F^+$ and/or to $CaHPO_4(H_2O)^+$, shows a distribution around the obturation at the border of dentine zone;
- after mild ion etching, as expected, the intensity of the polyatomic fragments is strongly reduced so that mostly monoatomic ions maps have been revealed; nevertheless they show the presence of three zones related to cement paste, to dentine and to the interface between these two respectively, confirming the interaction between cement paste, DBBS solution and dentine. In particular the $CaCl^+$ (in positive mode) and Cl^- (in negative mode) seem to be distributed just along this interface suggesting a preferential migration and/or a selective formation of calcium chloride-containing species.

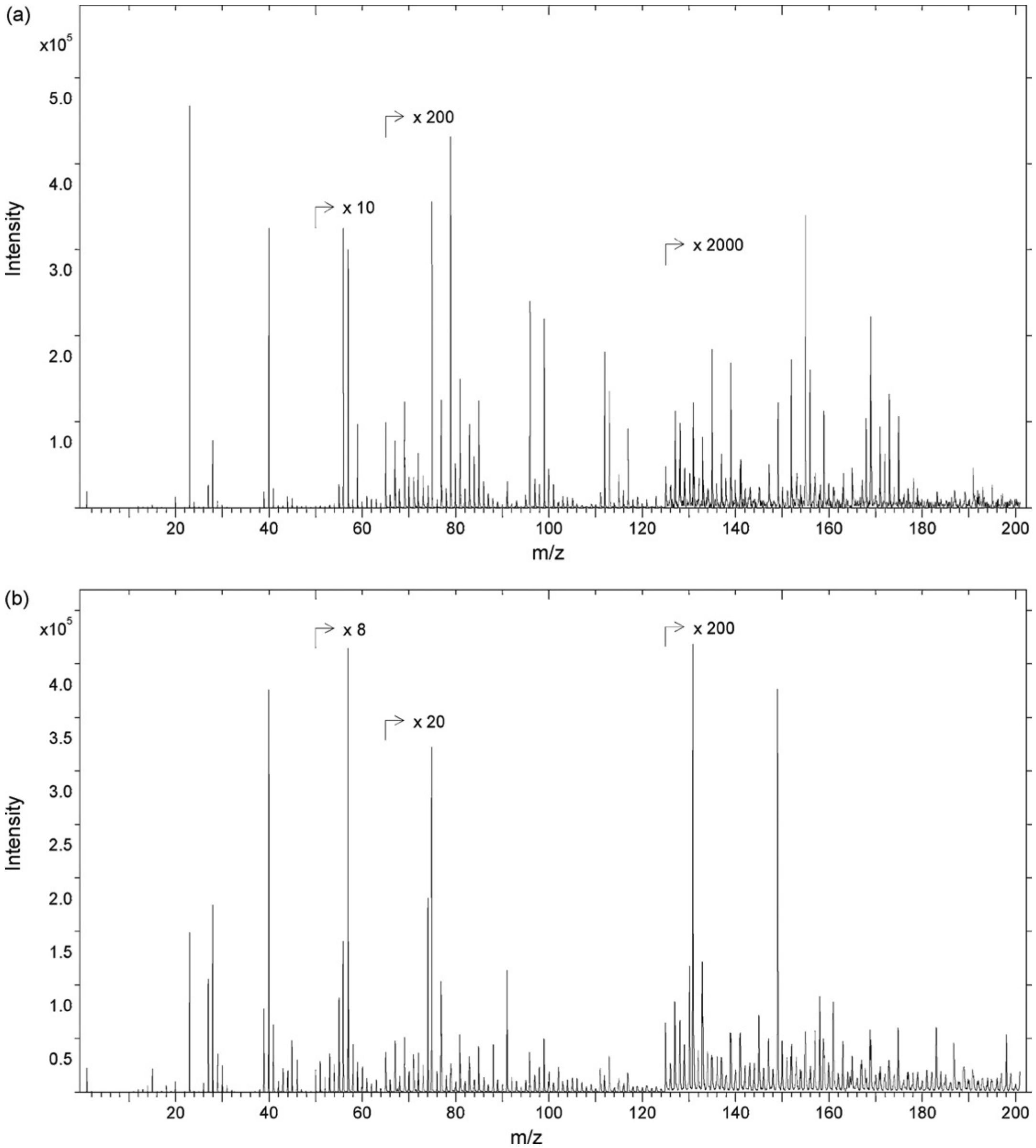

Fig. 5. ToF-SIMS positive spectra of wTCF cement paste after immersion in DPBS buffer solution for different times: (a) 1 month; (b) 8 months.

3.2. ToF-SIMS spectra

The difficulty to obtain intense ion map from inherently nonuniform and highly insulated surface pulled us to study model systems based on disks made by the cement paste before used for the obturation of the tooth. Afterwards, these disks were soaked in two different buffer solutions in order to simulate the effects of the interaction between the cement paste and human biological fluids. The times of the immersion were 1 month and 8 months.

We used a wTCF cement paste and DPBS and HBSS buffer solutions (see also the experimental part). In addition, samples prepared by using a wTC cement paste and soaked in HBSS buffer solution were used for comparison. All of this gives rise to six different samples from which ToF-SIMS mass spectra were obtained both in positive and in negative mode.

All the spectra show peaks up to maximum 300 Da, in spite of the use of Bi^+ ions as primary projectiles in order to increase the ion yield [19]. The limitation of the mass range is mainly due, in our opinion, to the high electrical insulation of the cement disks caused by their composition and thickness in spite of the electron flooding of the surfaces.

Figs. 5 and 6, report the positive mass spectra up to about 200 Da of the wTCF samples after immersion in DPBS and in buffer solutions respectively. Fig. 7 shows the positive mass spectrum of wTC sample after immersion in HBSS solution. The part (a) of all these figures refers to mass spectra obtained after an immersion time of 1 month, while the part (b) refers to mass spectra recorded after an immersion time of 8 months. The spectra are dominated at low *m*/*z* ratio region mainly by $^{23}Na^+$, $^{39}K^+$and $^{40}Ca^+$ ions peaks. Other less abundant peaks, attributed to $^{24}Mg^+$, $^{27}Al^+$, $^{28}Si^+$ and to CaO^+ (56 Da) and $CaOH^+$ (57 Da) are also present. All the above peaks are mainly due to components of the cement pastes used. Starting at 70 Da a decay of the spectrum intensity is observed; this decay becomes very strong beyond 120 Da. In this region the main peaks are attributed to phosphate-containing peaks and most of them contain calcium as counterion. Since the mass spectrum contains a lot of chemical information, sometimes not easily understandable, statistical multivariate techniques are widely used [20–22] when

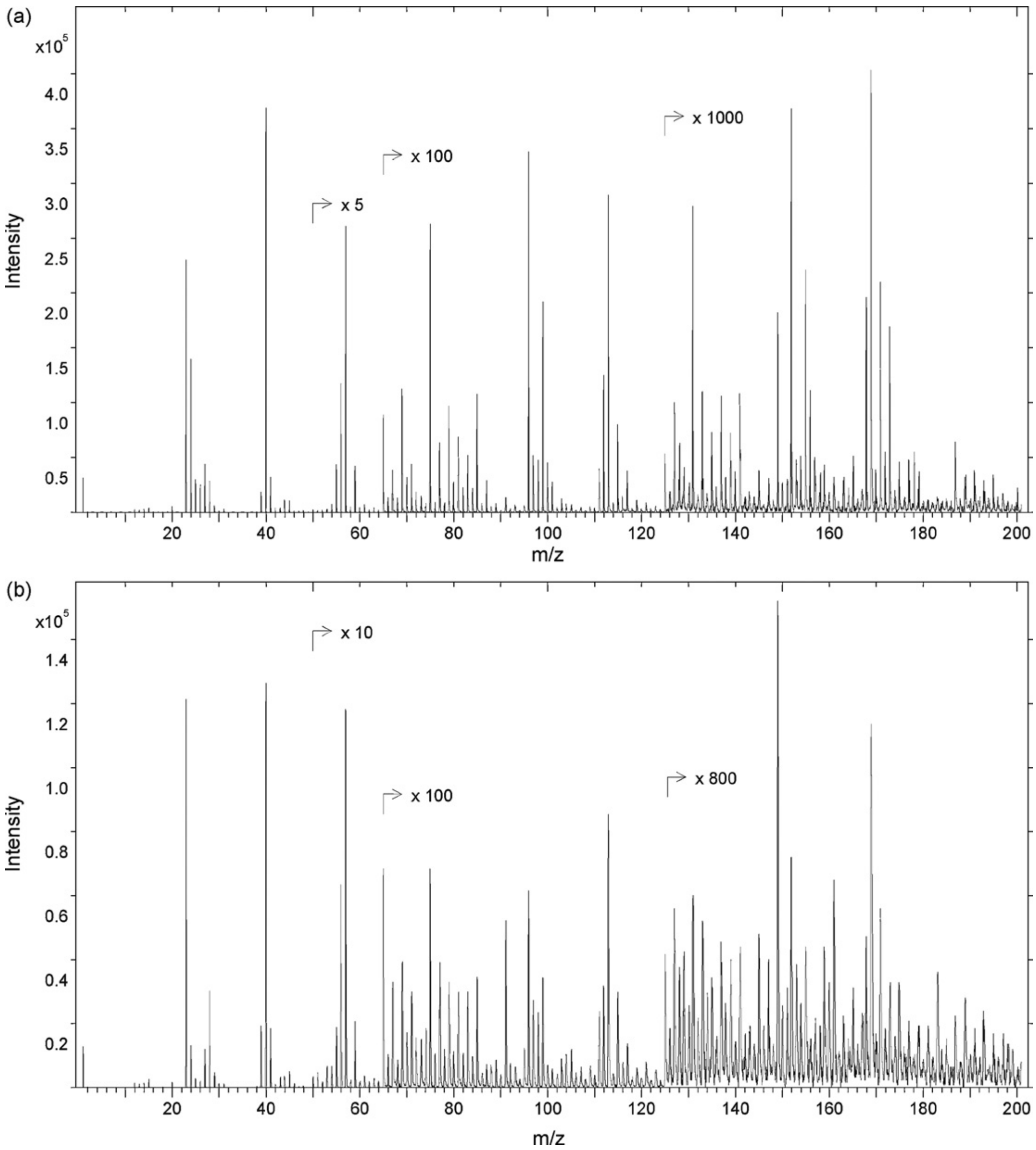

Fig. 6. ToF-SIMS positive spectra of wTCF cement paste after immersion in HBSS buffer solution for different times: (a) 1 month; (b) 8 months.

the discrimination of the most significant data, among complex and numerous patterns, as those represented by our mass spectra, is requested. The principal components analysis (PCA) is probably the simplest and most widely used multivariate technique. To obtain a PCA graph we build matrices from ToF-SIMS positive spectra by using the data obtained from four mass spectra for each sample. In order to avoid loss of significance due to neglect the less intense peaks, for each mass spectrum, the area of each peak was normalised to the total ion current. Next, these normalised data were auto-scaled (i.e., mean centred and scaled to their variance) by means of a routine of the program used for multivariate analysis (SIMCA-P+ from Umetrics AB, Sweden).

Fig. 8 reports the PCA scores plot of the first two principal components. Each ellipse groups together spectra pertaining to the same sample. The radii of the ellipses are a measure of the compactness of the different groups. It can be easily seen that, while the wTFC sample immersed for 1 month in HBSS gives rise to an extraordinarily compact group, where often all the points overlap each other, the same sample, after 8 months of immersion, produces a quite large distribution. More in general, the samples after 8 months of immersion give rise to a larger distribution, with respect to the same samples after 1 month of treatment. We can observe that the lower part of the PCA 1–2 scores plain contains the samples after 1 months of treatment, while the upper part the ones after 8 months of immersion. Among the samples treated for 1 month, the ones that do not contain fluorine in the cement paste are well separated from the ones that have fluorine; in addition, the latter give rise to two compact groups (one for each buffer solution) very close each to other. This suggests that, at this stage of the treatment, the two different buffer solutions used did not give rise to substantially different products or, even better, that 1 month of treatment do not produce very strong modification in the cement paste. This interpretation is supported by the fact that the samples after 8 months of immersion, in spite of a larger distribution with respect to those that undergo 1 month of treatment, give rise to groups far away from each other. This indicates, in our opinion, that the longer the treatment the greater becomes the difference among the different samples. In order to acquire more information on the chemical composition and or on the modification that occurs in the samples at different treatment times, we produce the plot of the loadings,

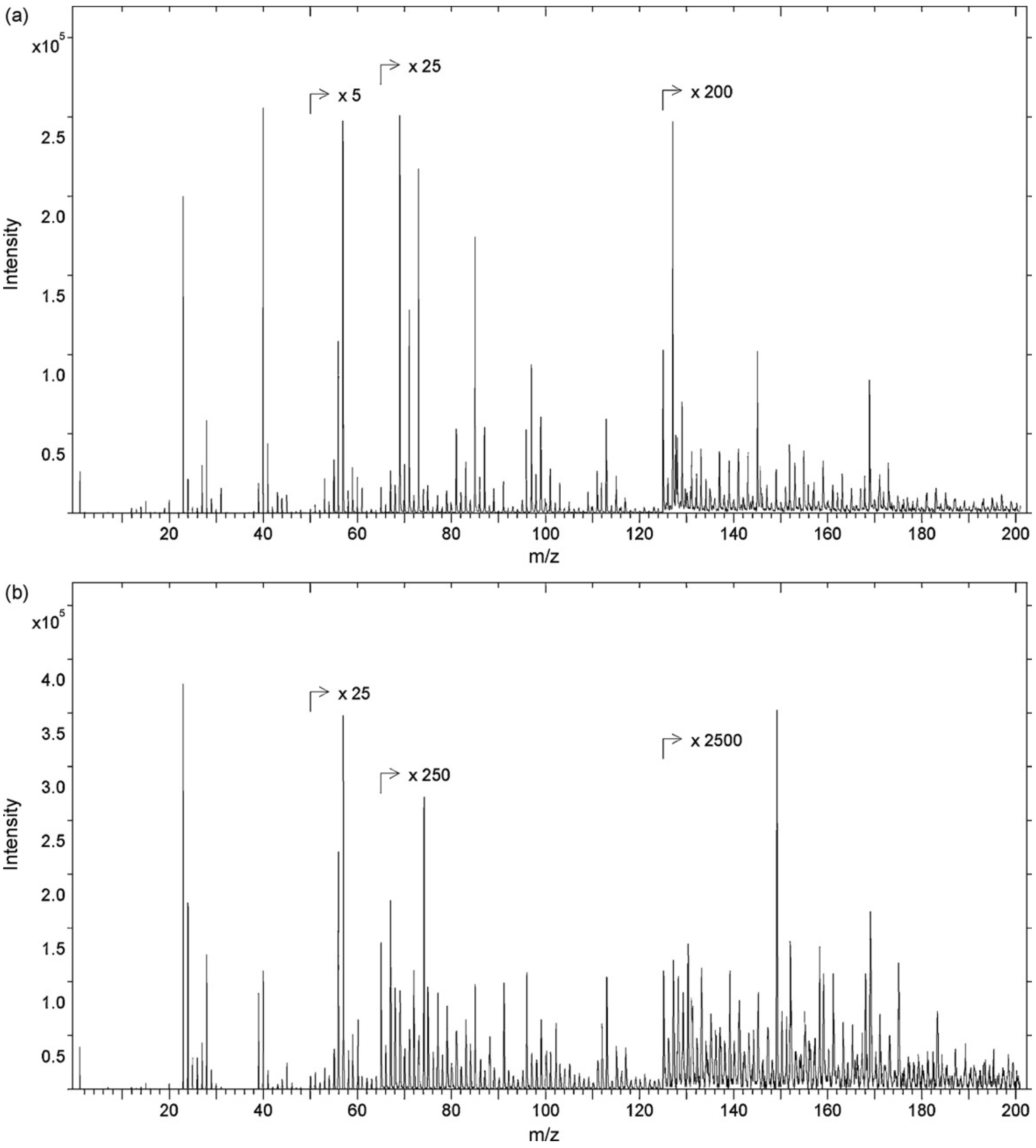

Fig. 7. ToF-SIMS positive spectra of wTC cement paste after immersion in HBSS buffer solution for different times: (a) 1 month; (b) 8 months.

namely the plot in the PCA plane of the peaks of the mass spectra that are characteristic of each group in the loading plot. Unfortunately, taking into account the richness of the mass spectra and the fact that we build the matrices with auto-scaled data, we were unable to recognise indubitably the most significant peaks. On the other hand, the building of the matrices by using non auto-scaled data does not produce a PCA plot with a separation as good as that obtained with the auto-scaled ones. In addition, the most abundant peaks, i.e., ^{23}Na, ^{24}Mg, ^{27}Al, ^{28}Si, ^{39}K, ^{40}Ca, and so on, obviously are the most significant in the loadings plot.

For this reason, we decided to examine the peaks present in the mass spectra one by one, in order to find, in any, the most apparent differences among the various samples. The comparison of the peak intensities has been made by normalizing each peak to the total ion current in order to take into account the different yield of each spectrum. This allows a mild qualitative comparison between the same fragments along the various spectra. However, we have firmly in mind that these comparison might fail when applied to different peaks, because the ion yields difference that occurs between the various species. For this purpose, in Fig. 9 we report the bar plot of the ratio between the intensity values recorded at 8 months to the ones recorded at 1 month of the most intense peaks of positive ToF-SIMS spectra. The intensity value for each peak is obtained averaging those obtained from four spectra, taken on different areas of the surface sample, and normalised each one to the related total ion current.

The positive spectra of all 8-month samples show an increase of the ^{28}Si intensity with respect to the 1-month-treated samples ranging about between 1.6 and 3.2 times. The Ca, and CaOH ratios show values close to the unit in both the wTCF samples, while the wTC sample shows values about 5 times lower with respect to the 1-month sample. This suggests for the 8-month WTC sample a strong depletion at the surface of the Ca species. This depletion is counterbalanced by the strong increase of Mg that reaches in the 8-month sample a value about 6 times higher than that found in the 1-month-treated sample. The magnesium is a constituent both

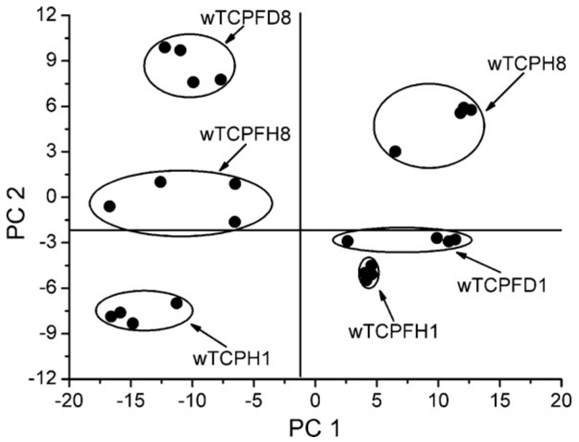

Fig. 8. PCA scores plot obtained from ToF-SIMS positive spectra of wTC and wTCF samples after immersion in HBSS and DPBS solution for 1 and 8 months. The final letters H and D indicate the HBBS and the DPBS buffer respectively; while the numbers 1 and 8 indicate the soaking time (months).

of the cement paste used as well as of the HBSS buffer solution, so we cannot in principle discriminate between these two Mg sources. However, on making a comparison between the wTC paste with the wTCF one, both treated in HBSS, in order to decide whether the Mg increase was due to the HBSS solution or to a diffusion of Mg present in the pristine cement, we found that in the 8-month wTCF sample the relative amount of Mg decrease was about 5 times with respect to the 1-month one. This suggests that the different behaviour of the two HBSS treated samples is mainly due to a different surface reorganisation of the cement itself, giving rise, perhaps, to the formation of new crystalline phases magnesium-rich. It is interesting that the behaviour of the Al^+ ratio remains close to the unit in the HBSS treated samples, while it increases about 4 times in the DPBS treated sample for 8 months with respect to the 1 month. This fact, together with the increase in the Si^+ ratio suggests the formation of a new phase, rich of aluminium and silicon, in the DPBS treated sample. As to the ratio of Na^+ and K^+, they are components of both buffer solutions then, in our opinion are not particularly diagnostic, taking into account also their very high ionisation yield.

The wTCF samples immersed for 8 months in DPBS solution show a $CaCl^+$ ion (75 and 77 Da) content about 15 times higher than the 1-month-treated samples, while this increase did not occur in samples treated with the HBSS solution, and in particular in the wTC sample a decrease of about 40% is observed, in agreement with the lowering of Ca-containing species above reported. This is in spite of the fact that both the buffer solutions have a nominal content of chlorine ion very close each to other. We cannot discriminate whether the increase of $CaCl^+$ ions should be due to segregation of the $CaCl_2$ contained in the cement paste or to formation of new $CaCl_2$ considering the relative high amount of chlorine in the buffer solutions. However, considering the different behaviour of the two buffer solutions, we are inclined to attribute this fact mainly to segregation phenomena. In this respect, it should be remembered that, in the real tooth the $CaCl^+$ ion was mainly distributed at the interface region, as discussed above. Moreover, we note an increase in the $CaOCaCl^+$ peak (*m*/*z* 131–133) in the spectrum obtained from the wTCF sample immersed 8 months in the DPBS solution, with respect to the 1-month-treated sample (compare the upper spectrum of Fig. 5 with the bottom one). This might suggest the formation of species grown on the bulk of the cement as the treatment time increases.

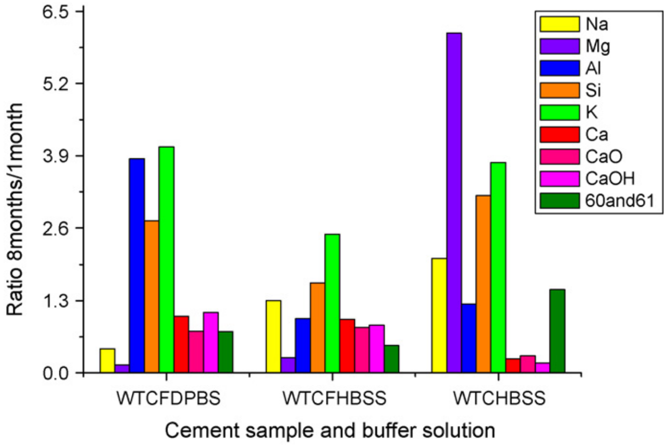

Fig. 9. Bar plot obtained from ToF-SIMS positive spectra reporting the ratio between the normalised intensities of selected ions recorded on the reported samples after 8 months and 1 month of immersion in the indicated buffer solutions. All the reported ions are positively charged except the ones at *m*/*z* 60 and 61 that are negative.

The mass spectrum of wTCF after 8 months of immersion DPBS sample shows an increase in the 149 Da ion peak with respect to 1-month sample of about 24 times. In the wTCF sample treated with the HBSS the increase is about 5 times, while the wTC sample, treated in the same buffer solution, shows a decrease of about half time. We do not have at the moment a unique attribution of this peak. This because the spectra obtained in the samples immersed for 8 months show a worsening of the resolution that does not allow us to decide between quasi-isobaric fragments. However, among the hypothesised structures mostly contain organic-based compounds.

In this respect it should be noted that the wTCF sample immersed 8 months in the DPBS solution shows a noticeable content of organic fragments in the range 15–150 Da. Most of these peaks contain nitrogen and should be related to the growth of bacteria. In a lesser extent a similar behaviour is also found for the other two 8 months treated samples. The presence of organic fragments is also supported by the negative spectra, in particular by the fragment at *m*/*z* 26 that will be discussed below. Since most of the bacteria grow in the presence of phosphate, the amount of bacteria on the surface of the cement paste disks might constitute an indirect marker of the amount of phosphate-containing species built on the surface. It should be taken into account that the surfaces of the cement disks were washed with Millipore® quality water before the analysis, so that most of the adventitious contamination, as well as the inorganic species, precipitated on the surface, and not the ones grown on it, were removed.

Additional information can be obtained from the comparison of the CaOH/CaO intensity ratio taken from the mass spectra of the various compounds at 1 month and at 8 months. The CaOH peak has been used as marker of hydroxyapatite [23] while CaO peak might be used as marker of the cement itself. In our case the CaOH peak might be also due to $Ca(OH)_2$ (portlandite), hydration product of CaO [24]. However, making a comparison between the cement samples at 1 month with the ones at 8 months, the CaOH/CaO ratio decreases about 60% in wTC in HBSS samples, shows a slight increase (20%) in the wTCF in HBSS treated samples and a more relevant one (40%) in the wTCF in DPBS treated ones. Namely, it seems that fluorine plays a more important role in the formation of CaOH than the composition of the buffer solution. Perhaps, the observed CaOH peak might be related also to fluoroapatite formation.

This interpretation is supported by the similar trend of the OH^-/O^- ratio, as obtained from the negative spectra of the studied samples reported in Figs. 10–12, with respect to the $CaOH^+/CaO^+$ one as obtained from the positive of Figs. 5–7. In particular, Figs. 10 and 11, report the negative mass spectra up to about 200 Da of the wTCF samples after immersion in DPBS and in HBSS solutions

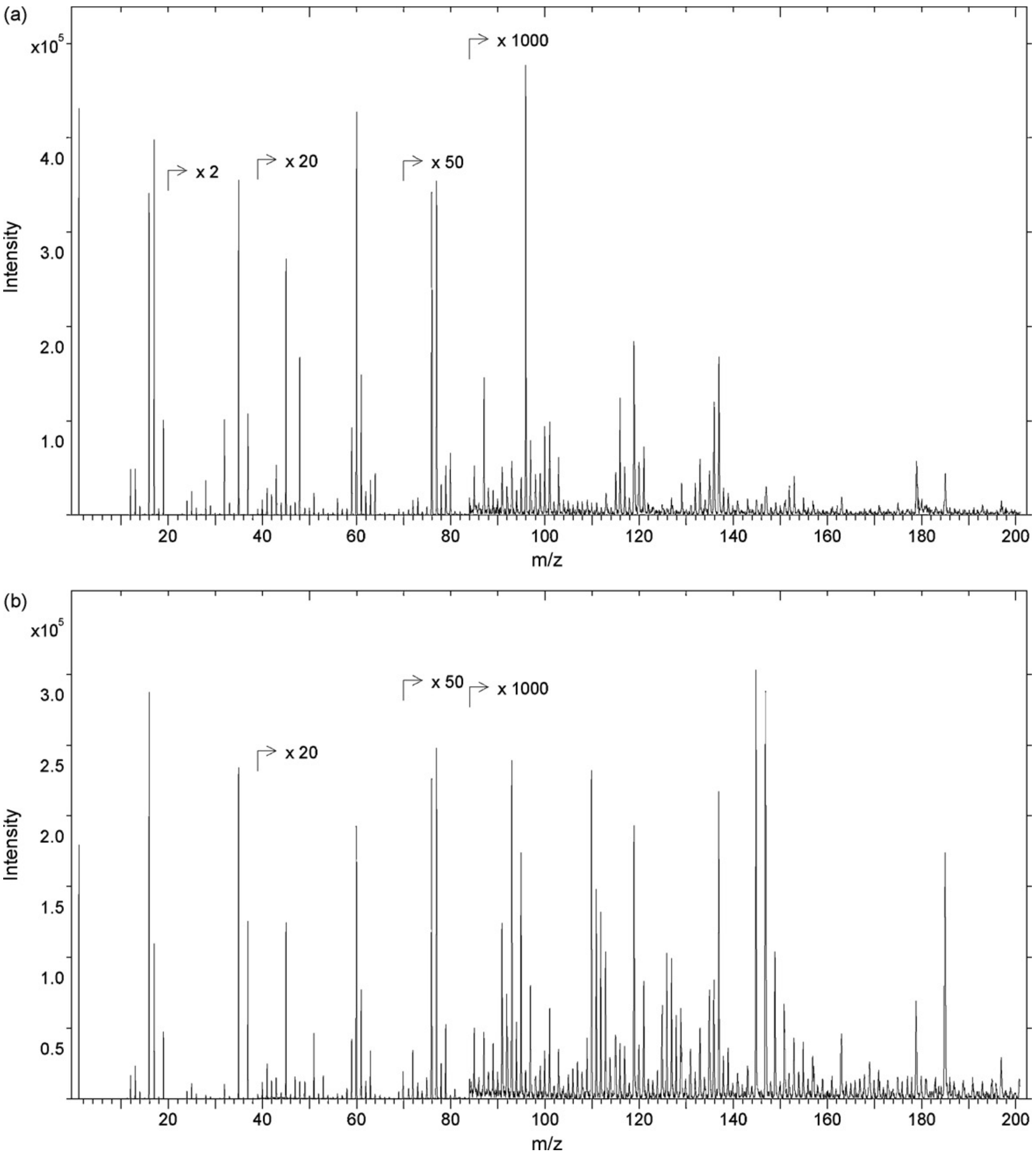

Fig. 10. ToF-SIMS negative spectra of wTCF cement paste after immersion in DPBS buffer solution for different times: (a) 1 month; (b) 8 months.

respectively, while Fig. 12 shows the negative mass spectra of wTC sample after immersion in HBSS solution.

The OH^-/O^- ratio, indeed, decreases with the treatment time of about 50% (wTC in HBSS), is nearly constant (wTCF in HBSS), while increases of about 30% (wTCF in DPBS). This hypothesis would find a further support in the graph of Fig. 13, where the differences of the OH^-/O^- ratios found between the 8 months treated samples and the 1 month ones are plotted versus the $CaOH^+/CaO^+$ ratios found between the 8 months treated samples and the 1 months ones. This graph shows a nearly linear trend, confirming the strict relationship existing among these data.

Moreover, the negative spectra confirmed what we observed in the positive ones. In particular, the bottom part of Fig. 10 reveals the relative higher content of $CaCl_2^-$ and $CaCl_3^-$ calcium-chlorinated ions in the wTCF samples after 8 months of immersion in DPBS (as confirmed by their isotopic distribution at 110, 112, 114 Da and 145, 147, 149, 151 Da respectively) with respect to the other samples. In particular, the relative increase of these species is about 40 times with respect to the sample treated for 1 month in the wTCF-DPBS samples and should be compared with the figures of about 8 times found in the wTCF and wTC sample immersed in HBSS. This in spite of the fact that the relative abundance of the ^{35}Cl and ^{37}Cl peaks is comparable in all the samples.

The comparison between the wTCF samples immersed in HBSS and DBBS solution for 1 month and for 8 months shows a decrease in the relative content of SiO_x^- species. On the contrary, the wTC sample shows an increase of about twice of the SiO_x^- species. This finding seems to be in contrast to the trend of ^{28}Si peak found in the positive spectra, where we observed an increase in the silicon intensity after 8 months of treatment in comparison with the 1-month-treated samples, irrespective of the compositions both of the sample and of the buffer solution. In order to clarify these anomalous findings, we plotted the intensity of the sum of the peaks at m/z 76 and 77, obtained from negative ToF-SIMS spectra and attributed respectively to SiO_3^-, SiO_3H^-, versus the $^{28}Si^+$ peak intensities obtained from the positive ToF-SIMS spectra of

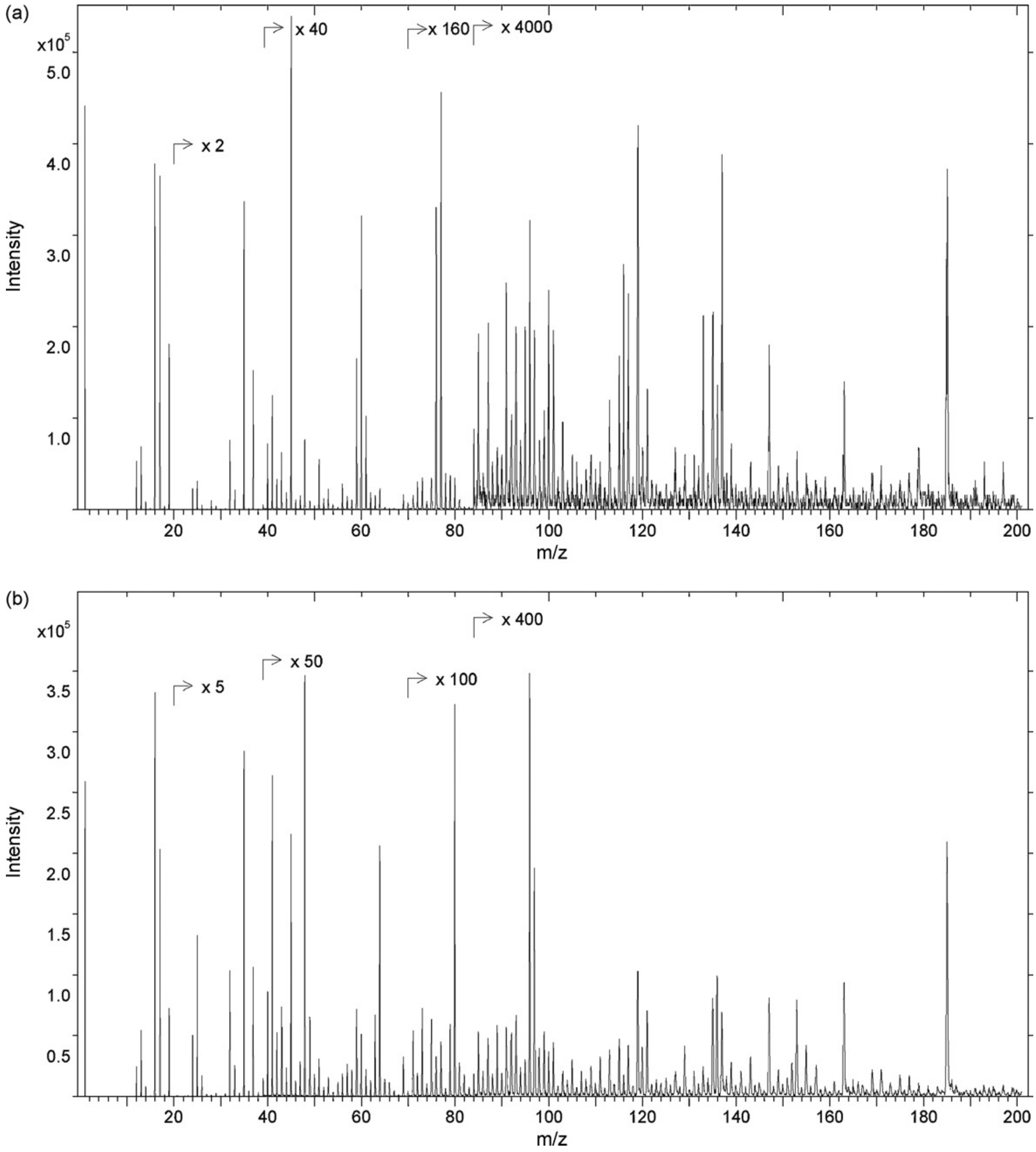

Fig. 11. ToF-SIMS negative spectra of wTCF cement paste after immersion in HBSS buffer solution for different times: (a) 1 month; (b) 8 months.

the different samples. We obtained a very good linear correlation between the data obtained from the samples treated for 8 months as reported in Fig. 14, while the data obtained from the samples treated for 1 month show a worsening of the correlation even if within the dispersion of data. What is of particular interest, in our opinion, is the different slope of the two linear fits: about 2 times higher in the 1 month samples with respect to the 8 months treated samples. This difference should be related to changes in the composition in the 8 months treated samples with respect to the 1 month ones that does affect in a different way the ion yield of the above species. This interpretation supports the above hypothesis of different crystalline phases appearing at the highest treatment time. Moreover, the 8 months HBSS treated samples show a strong (more than 9–14 times) relative enrichment of $MeH_xP_yO_z$ based species (peaks at 119, 135, 136, 137, 185 Da mainly, were Me = Na and/or Ca) with respect to the 1-month samples, while in the DPBS sample this increase is as high as 3 times only. In principle, this fact is surprising since the content of phosphate species, that might lead to hydroxyapatite formation, is 14 times higher in DPBS solution with respect to the HBSS one. However, it should be taken into account that we are considering ratios and not absolute values so what we report are relative variations. This means that the content of phosphate-containing species increased quite rapidly [25,26] on the surface of the samples treated with the DPBS solution, while the ones treated in HBSS required longer treatment time. The more intense peaks 80, 81, 96 and 97 Da, observed in the negative spectra, were not included in the above calculation, since the scarce resolution, due both to the roughness of the surface of the samples and to the high insulation of the cement disks, does not allow us to discriminate among quasi-isobaric fragments. In particular, we are unable to distinguish among phosphorus-containing species, namely HPO_2^-, $H_2PO_2^-$, HPO_3^-, $H_2PO_4^-$ and sulphur-containing fragments, namely SO_3^-, HSO_3^-, SO_4^-, HSO_4^- having the same nominal mass, i.e., 80, 81, 96, 97 respectively. How-

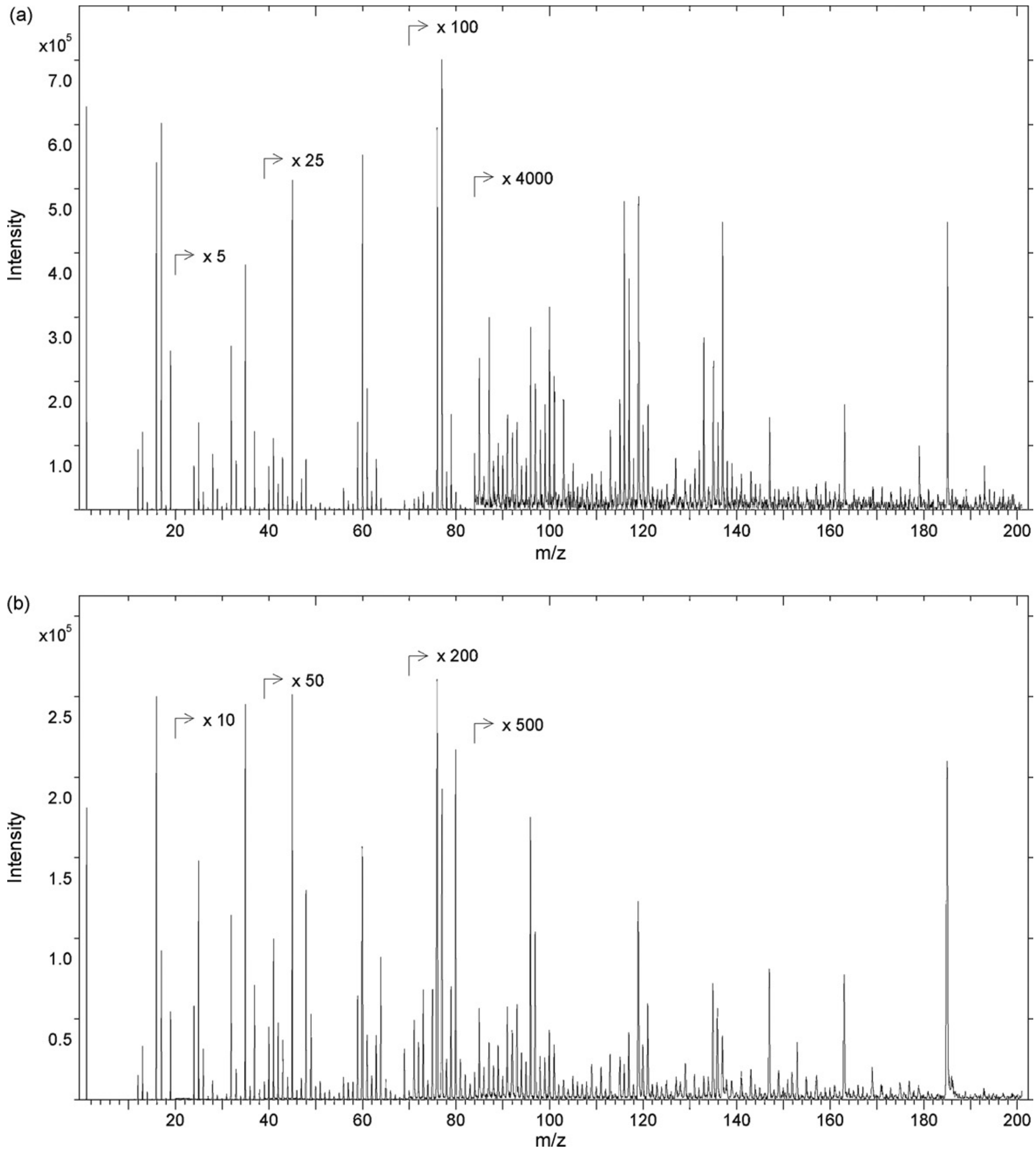

Fig. 12. ToF-SIMS negative spectra of wTC cement paste after immersion in HBSS buffer solution for different times: (a) 1 month; (b) 8 months.

ever, in the case of wTC samples treated in HBSS for 8 months, where a great increase of $^{24}Mg^+$ ions was observed in the positive spectra, the above fragments may be attributed mainly to sulphur-containing species, taking into account that the HBSS solution contains about 8.1×10^{-4} mol/L of $MgSO_4 \cdot 7H_2O$. Similarly, the peaks at 60 and 61 Da were not included, because we cannot make an undisputable choice among SiO_2^-/SiO_2H^- and CO_3^-/HCO_3^-.

All of this suggests different pictures for the surface of the samples immersed in HBSS and the ones treated with DPBS solutions. In particular, the samples immersed in HBSS undergo an enrichment of phosphorus-containing species at the expense of CaO ones, while the cement disks treated with DPBS show a rapid enrichment of phosphate species followed by segregation and/or formation of chlorine-containing species at the expense of CaO ones. We reasonably suppose that the CaO group originally present in the cement is partially hydrated and then transformed in phosphate-containing species and/or chlorine-containing species with a lower ion yield with respect to Ca and CaO species.

The presence at the surface of the 8 months treated samples of different chemical species with respect to the 1 month ones, could give rise to new mineralogical phases detectable, for instance, by means of X-ray diffraction measurements. Actually, preliminary XRD results show strong differences between the samples due both to cement composition and to the treatment time. Work is in progress and the results will be reported and discussed in a forthcoming paper.

Finally, we would like to discuss the behaviour of the peak at mass 26 Da, attributed to CN^- ion on the basis of its exact mass; this peak shows a relative increase of 2.5 (wTCF + DPBS), 3 (wTCF + HBSS) and 4 (wTC + HBSS) times in the samples after 8 months of treatment with respect to the ones after 1 month. In our opinion the CN^- specie might be due bacteria growth

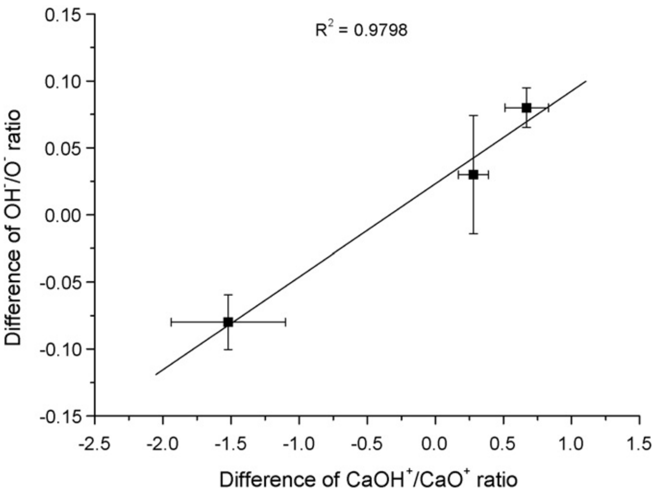

Fig. 13. Plot of differences between 8 months and 1 month samples of the OH^-/O^- ratios versus the differences between 8 months and 1 month samples of the $CaOH^+/CaO^+$ ratios.

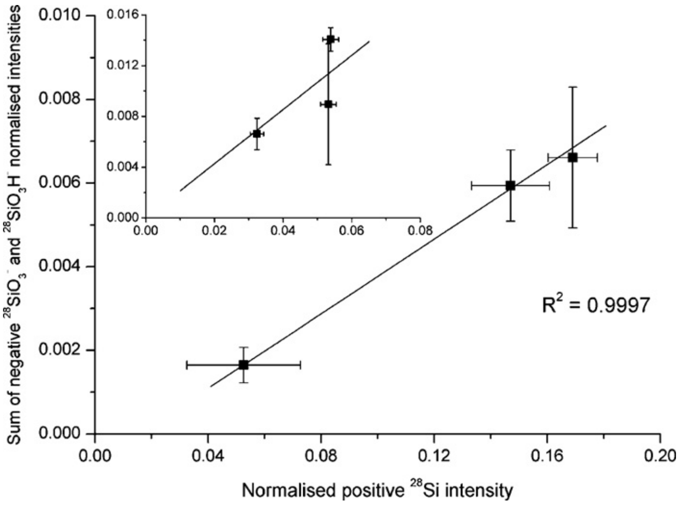

Fig. 14. Plot of the sum of 76 ($^{28}SiO_3{}^-$) and 77 ($^{28}SiO_3H^-$) normalised intensity versus $^{28}Si^+$ normalised intensity obtained from the negative and the positive ToF-SIMS spectra respectively, of the cement samples after 8 months of immersion in the buffer solutions. The similar plot, obtained from the cements sample after 1 month of immersion in the buffer solutions, is reported in the inset at the top-left of the figure.

and could be related to the formation of phosphate-containing species.

Summarizing:

- After 1 month treatment the compositional differences (presence of fluorine) seem more important than the kind of buffer solution used.
- After 8 months the differences among the three groups of samples become larger and also the dispersion within the same group appears to be larger.
- The samples after 8 months of treatment show a complex modification of their composition with respect to the 1-month-treated samples. The enrichment of phosphate-containing components, for instance, seems one of the most important phenomena observed together with the segregation of $CaCl_2$ in the wTCF samples stored in DPBS solution. All the data support the hypothesis of structural changes with formation of new crystallographic phases.
- The increase of the N-containing peaks seems to be related with bacteria growth and supports the observed increase of phosphate fragments content.

4. Conclusions

The ToF-SIMS study of a actual tooth sample has clearly shown the existence of an interface, between the dentine (tooth) and the obturation (cement) whose composition is different with respect to that of both the last two. The composition of the interface as well as the one of obturation indicates that a process of biomineralization occurs and that this process is not very fast but proceeds with time.

The ToF-SIMS study of the cement disks, used as model systems, reveals a very complex phenomenology. The differences of the composition (even if slight) of both the cement pastes and of the two buffer solutions here used, seem to influence the evolution of the systems under study, leading, after 8 months of treatment, to quite differentiate surfaces, as revealed by PCA of positive ToF-SIMS mass spectra.

The information gathered from the study of the mass spectra of each sample, both in positive and negative mode, suggests the formation of new crystalline phases with time, whose composition cannot be obtained from ToF-SIMS data only. In this respect, preliminary data obtained by means of XRD analysis, confirms this hypothesis.

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