Interaction of slow highly charged ions with hard dental tissue: studies of fluoride uptake and remineralization efficacy

A Daskalova$^{1,4}$, G Kasperski$^2$, P Rousseau$^3$, A Domaracka$^3$ and A Lawicki$^3$

$^1$Acad. E. Djakov Institute of Electronics, Bulgarian Academy of Sciences, 72 Tsarigradsko Chaussee, 1784 Sofia, Bulgaria
$^2$Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk Wrzeszcz, Poland
$^3$CEA-CIMAP, GANIL, BP 5133, F-14070 Caen Cedex 5, France

E-mail: a_daskalova@code.bg

Abstract. TOF-SIMS mass spectroscopy data are presented on ion irradiation of hard dental tissue using a beam of $^{129}$Xe$^{20+}$ (15 kV) ions delivered in the ARIBE facility by an ECR source. The investigation was focused on the mass distribution of the fragment ions. A comparison is made between the mass spectra from hard dental tissue treated by olaflur-(C$_{27}$H$_{60}$F$_2$N$_2$O$_3$) and untreated hard dental tissue obtained under irradiation by low-energy highly-charged ions (HCIs). We found significant differences between the mass spectra of enamel after introducing amine fluoride (olaflur) and the mass spectra of pure untreated enamel. Further, we separated out the effects caused by radiation induced in the tooth enamel from those induced in dentin, which has not been performed before. In order to conduct a further detailed analysis, it is necessary to extend the research scope to include the influence of fluorine compounds on enamel and dentin.

1. Introduction
Collision induced fragmentation using highly charged ions from an ECR source has been demonstrated to be a powerful method providing information complementary to that of other experimental methods. The specificity of experiments with an ECR source is related to the large choice of the type and charge of the ion, the easy scan and the high selectivity of the kinetic energy [1]. Slow highly-charged ions, while approaching a surface, can capture electrons and (in addition to projectile kinetic energy deposition by elastic and inelastic collisions) a strong localized electronic excitation is induced close to the surface. Various studies have shown that due to their low kinetic energy, slow HCIs do not penetrate deeper layers, thus opening new possibilities for surface analysis. Secondary ion mass spectrometry (SIMS) has been proved to be a powerful tool for microanalytical characterization of biomineralized tissues. The technique permits quantitative analysis of more than 40 elements in tooth tissues [2]. In some earlier studies, secondary ion mass spectrometry was used to determine the distribution of Na, K, Sr, F and Cl in the buccal enamel of human deciduous incisors [3]. The work of Lundgren et al. [4] allowed the estimation of the incorporation of some critical elements into the enamel in correlation with the differentiation stages of the ameloblasts throughout the whole tooth.

$^4$ To whom any correspondence should be addressed.
The elemental composition pattern of dental enamel was examined and a picture was presented describing a limited region. Later on Jaelevik et al. [5] studied the hypomineralization in children’s first molars with respect to the chemical composition of the disturbed enamel. The concentration gradients for F, Cl, Na, Mg, K and Sr in hypomineralized enamel were examined by means of secondary ion mass spectrometry and were compared to normal enamel. Gotliv and colleagues have utilized the technique of time-of-flight secondary ion mass spectrometry (TOF-SIMS) to map the distribution of positive and negative inorganic ions, as well as the organic components in the fully mineralized intact peritubular dentin (PTD) structure in bovine tooth cross-sections [6].

The present study aims at obtaining data on the fundamental interaction processes of multiply charged ions with a hard biological tissue (teeth). Slow multiply charged ions can be a powerful tool for biological damage studies due to the localized energy deposition.

We have previously acquired good experimental results on ultra-short laser ablation of hard tissue (bone and tooth). These preliminary studies have provided initial information on how to alter the chemical composition of molecules of biological origin [7-9]. However, for a full understanding of the processes of the molecular fragmentation pathways, it is especially important to study the interaction process by slow multiply charged ions. Most approaches to studying the ion-induced damage to biomolecules involve mass-spectrometric techniques. In this study, a comparison is performed between the mass spectra from treated with olafur and from untreated tooth samples obtained under irradiation with low-energy highly-charged ions (HCIs). Previous experiments have shown the possibility of avoiding the effect of charging the insulator samples by wrapping them with a metallic wire, as this is an important problem in studying insulator targets. Moreover, the HCI-induced mass spectrum will be a reference of the blank sample before fluorine covering.

The tooth enamel is a hard substance covering the crown of the tooth (figure 1); it consists of 98 % of non-organic compounds, mainly hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\), magnesium, carbonate, sodium, calcium and fluorine. The remaining 2 % are organic compounds and water.

Fluorine, which is introduced from an outside source, combines with the enamel thus increasing the enamel’s resistance to acids; fluorine also facilitates the enamel’s remineralization and accelerates the growth of new, greater hydroxyapatite crystals. However, too high an amount of fluorine may lead to osteoporosis. Hydroxyapatite is mainly used as a biomaterial for covering orthopedic or dental prostheses and producing bones substitutes. Up to the present, studies concerning the enamel have been focused on the influence of fluorine compounds (e.g. MgF_2, CaF_2 and ions Mg^{2+}, Sr^{2+}, Ba^{2+}) on changes in its physico-chemical properties [10]. However, research is lacking on the influence of the interaction between carbonate–fluorine compounds (with relatively low dissociation energy) and the hydroxyapatite contained in the enamel.

In view of the above, we studied the sputtering process of hard biological tissue under various ion beam parameters (velocity and charge state, i.e. potential energy) as the results could form the basis of optimizing the desorption process. The sputtered species were characterized using time-of-flight SIMS, yielding information on the physical processes involved during exposure to ionizing radiation.

![Figure 1](image-url)
The interaction of keV ions with biomolecules or clusters can depend on a variety of properties of both the projectile and the target.

The results of the measurements performed may contribute to the better understanding of enamel remineralization mechanisms and to determining the mechanisms of increasing the immunity acquired (to caries decay) by the fluorization process (enamel assimilation of fluorine); interest in such studies have been declared by many pharmaceutical companies. The results may also be beneficial to optimizing the processes of producing new composite mixtures used in hydroxyapatite-containing implants. Further, it is our believe that this study’s results will contribute to a better recognition of the origin of osteoporosis, thus leading to its prevention.

2. Materials and methods

Freshly extracted human third molars with clinically sound enamel were provided by dentists and were stored in an olaflur solution. From each tooth, three longitudinal enamel or dentine samples were prepared by using a water-cooled diamond saw. The separation planes of the halves were ground with a successively decreasing grain size. This procedure ensured optimal surface flatness. Then they were brushed with non-fluoridated pumice powder and rinsed with distilled water. Surfaces without any visible defects were only selected for the treatment procedure. The fluoridation process was performed by treating the samples for three days with an amine fluoride solution – olaflur (C_{27}H_{60}F_{2}N_{2}O_{3}) for three minutes. Subsequently, the samples were rinsed in distilled water for 30 s, air dried at room temperature and stored in closed plastic bags.

The principle of the experimental procedure is given in figure 2. The experiments were performed in an ultra-high vacuum chamber at a base pressure of 1.3x10^{-6} Pa. The samples were irradiated using an electron gun.

The biological sample under investigation was bombarded by ions and the interaction products were analyzed by the time-of-flight method. The ions generated in the collision region were extracted through an electric field lens system into a Wiley-McLaren time-of-flight (TOF) spectrometer and detected by a microchannel plate (MCP) detector. We used an electron cyclotron resonance ion source to generate ions with charge states q ranging from 1 to 30 at kinetic energies from 1 keV to 15 keV. A crucial feature of the experiment was cooling the UHV chamber by liquid nitrogen. The base pressure during experiments was thus kept around 1.3x10^{-6} Pa and the contributions of the residual gas to the experimental data were negligible. The charging effect being the main issue when insulator targets are studied, we avoided it by wrapping the insulator samples with a metallic wire.

3. Results and discussion

Amine fluoride is a surface-active fluoride beneficial to dental plaque control and contained in a number of complex formulations of oral hygiene products. Amine fluorides such as olaflur and deactaflur are used in tooth pastes. Fluoride ions can combine rapidly with calcium in dental enamel to form calcium fluoride [11, 12]. The uptake of fluoride into the almost insoluble crystal structure of the tooth is a very slow process, but it binds the ions very tightly. The enamel is, therefore, also called the “stable” fluoride reservoir. The surfactant characteristics of amine fluorides promote also a homogeneous distribution of the calcium fluoride globules. Thus, Nelson et al. [13] suggested that the particle size of CaF_2 crystallites may be an important factor in determining the effectiveness of a topical fluoride agent. Therefore, it could be assumed that the larger globules observed after treatment...
with low fluoride concentrations will dissolve more slowly and serve as a reservoir for more extended periods of time contributing to a caries preventive effect. This process is called dental remineralization with fluorides.

The choice of elements for analysis in this study was based on the assumption that they might have a role during the remineralization of the hard dental tissues [14]. The secondary ion mass spectrometric analyses only showed differences in the elemental composition between normal and oflur treated enamel for Ca, C, F, but no notable differences in the elemental composition for Mg, Na.

The SIMS mass spectrum of a tooth dentin sample (figure 3) exhibits dominating peaks of Na⁺ and Ca⁺ ion at m/z = 23, 40. The peaks at m/z = 52, 56 do not originate from the biological sample, but rather from Cr and Fe, these compounds being present in the metallic wires used to prevent charging of the organic sample.

| Mass number | Ion species               |
|-------------|---------------------------|
| 12          | C                         |
| 16          | O                         |
| 19          | F                         |
| 23          | Na                        |
| 24          | Mg                        |
| 31          | P                         |
| 39          | K                         |
| 40          | Ca                        |
| 56, 57      | CaO, CaOH                 |
| 59          | CaF, CaO, CaOH            |
| 60          | CO₃                        |

Figure 4 shows a typical mass spectrum obtained with ¹²⁹Xe²⁰⁺ projectiles colliding with tooth enamel (table1).

The principal inorganic constituent of enamel is hydroxyapatite Ca₁₀(PO₄)₆(OH)₂, which tends to incorporate a number of trace elements. Some of the elements present in the mass spectrum here might reflect events that occur during hard tissue formation where huge a number of ions are transported through the odontoblast or ameloblast layers. One possible role for some of the different inorganic elements, other than Ca⁺, might be as co-transport ions in the plasma-membrane transporters of the mineralizing cells. Sodium, for example, is transported in parallel to phosphorus in many cell types. Other elements are probably exogenous in origin and accumulate principally by ionic exchange at the tooth surface.

Differences between normal and treated enamel are expressed in the elemental composition and in the Ca⁺, F⁺, CO₃⁺, CaOH⁺ peaks ratios. In treated enamel there was a considerable increase in fluorine content (figure 5a). In the present study, the higher concentration of fluorine in the treated enamel
areas might point to the possibility that deeper penetration of exogenous elements from the surface had been facilitated by the porosity of the enamel.

The content of C$^+$ increased in irradiated dentin in contrast to enamel (figure 5b). Carbon is a component of olaflur - (C$_{27}$H$_{60}$F$_2$N$_2$O$_3$). A possible explanation could be that these ions are incorporated into the dentin as a consequence from the treatment with olaflur.

![TOF-SIMS mass spectrum of tooth enamel + olaflur](image)

![TOF-SIMS mass spectrum of tooth dentin + olaflur](image)

Figure 5. a) TOF-SIMS mass spectrum of tooth enamel + olaflur; b) TOF-SIMS mass spectrum of tooth dentin + olaflur.

In olaflur treated enamel and dentin, the general tendency was a slight reduction in calcium content in relation to untreated enamel. However, despite the considerable variation, the Ca ratio was rather constant throughout. The presence of magnesium is closely related to calcium. It is also possible that some magnesium ions had been excluded during crystallite formation and had finally become located on crystallite surfaces, in hydration layers or as parts of a separate phase. There might also be a correlation between magnesium and the enamel protein content. However, there is a need for further studies of normal and treated enamel and dentin and for further evaluation of HCI SIMS method in hard tissue research.

4. Conclusions
We conclude that the treated enamel analyzed here by secondary ion mass spectrometry has a higher amount of fluorine. The concentration of Ca level stays proportional in both treated enamel and dentin. We demonstrated the sensitivity of the HCI SIMS method and its capability in examining hard dental tissue. We report obtaining secondary ion mass spectra of untreated (pure) enamel, pure enamel with olaflur and pure dentin with olaflur under the impact of Xe$^{20+}$ ions. Conducting further detailed analysis would necessitate extending the research scope to include the influence of fluorine compounds on enamel and dentin (i.e. amine fluoride - fluorinol and pure fluoride protector).

Acknowledgements
This experiment was performed at the distributed LEIF-Infrastructure at ARIBE, GANIL, France, supported by Transnational Access, under a grant by the European Project P09073A.

References
[1] Briand J-P, Giardino G, Borsoni G, Le Roux V, Béchu N, Dreuil S, Tüske O and Machicoane 2000 The interaction of slow highly charged ions on surfaces Rev. Sci. Instrum. 71 627
[2] Lodding A, Fischer P, Odelius H, Norén J, Sennerby L, Johansson C, Chabala J and Levi-Setti R 1990 Secondary ion mass spectrometry in the study of biomineralizations and biomaterials Analytica Chimica Acta 241 314
[3] Norén J, Lodding A, Odelius H and Linde A 1983 Secondary ion mass spectrometry of human deciduous enamel distribution of Na, K, Mg, Sr, F and Cl Caries Res. 17 502

[4] Lundgren T, Persson L G, Engström E, Chabala J, Levi-Setti R and Norén J 1998 A secondary ion mass spectroscopic study of the elemental composition pattern in rat incisor dental enamel during different stages of ameloblast differentiation Archives of Oral Biology 43 841–8

[5] Jälevik B, Odelius H, Dietz W and Noren J 2001 Secondary ion mass spectrometry and X-ray microanalysis of hypomineralized enamel in human permanent first molars Archives of Oral Biology 46 247

[6] Gotliv B and Veis A 2008 The composition of bovine peritubular dentin: matching TOF-SIMS, scanning electron microscopy and biochemical component distributions Cells Tissues Organs 189 19

[7] Daskalova A and Husinsky W 2004 Ultra-short laser ablation of biological tissue Proc. SPIE 5830 473-8

[8] Daskalova A and Husinsky W 2006 Detection of biological molecules from hard biological tissue using ion-beam-induced desorption and femtosecond laser ablation Plasma Process. Polym. 3 257-61

[9] Daskalova A and Husinsky W 2007 Ionization of biomolecules in high-intensity laser fields Proc. SPIE 6604 66042G1-5

[10] Weir M D, Chow L C and Xu H K 2012 Remineralization of demineralized enamel via calcium phosphate nanocomposite J. Dental Res. 10 979-84

[11] Holler B E, Friedl K H, Jung Hiller K A and Schmalz G 2002 Fluoride uptake and distribution in enamel and dentin after application of different fluoride solutions Clin. Oral Investig. 6 144

[12] Schemehorn B R, Wood G D, McHale W and Winston A E 2011 Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources J. Clin. Dent. 22 51-4

[13] Nelson D, Jongebloed W and Arends J 1983 Morphology of enamel surfaces treated with topical fluoride agents: SEM considerations J. Dent. Res. 62 1201-24

[14] Roveri N, Battistella E, Bianchi C, Foltran I, Foresti E, Iafisco M, Lelli M, Naldoni A, Palazzo B and Rimondini L 2009 Surface enamel remineralization: biomimetic apatite nanocrystals and fluoride ions different effects J. Nanomater. http://dx.doi.org/10.1155/2009/746383