Surface Micromorphology and Crystalline Structures of Hard Dental Tissue Treated by Atmospheric Pressure Plasma Jet

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Abstract

In this paper, the results of helium plasma jet treatment of hard tissues of natural human teeth including enamel, dentin, and cementum are presented. Changes in surface morphology and structural properties have been investigated using scanning electron microscopy (SEM) in connection with multiscale image analysis, X-ray diffraction (XRD) with the aid of the Rietveld method and Williamson-Hall plots, energy dispersive X-ray (EDX) and Raman spectra analysis. Despite initial differences in scaling behaviors of the surfaces of freshly extracted tissues, spatial characteristics of their plasma-treated counterparts appeared similar taking into account the fractal measures. On the other hand, despite improvement in crystalline structure of enamel due to the plasma treatments, the structure of cementum has been found intact.

Keywords: Hard dental tissue, Plasma jet treatment, Fractal analysis, Micromorphology, Crystalline Structures.
1. Introduction

Multicomponent structure of natural teeth makes them one of the most intriguing substances in the human body containing hard tissues (enamel, dentin and cementum) joined together with soft ones (dental pulp). Enamel includes hexagonal hydroxyapatite crystals [1, 2] while dentin, under the enamel layer, has tubular microstructure [3] and cementum exhibits organic and inorganic components as proteins and apatite which cause lower hardness comparing to dentin [4]. Far from the disadvantages of traditional methods of data analysis, if a structure-property-performance triangle is considered in dental science for various materials, successful clinical applications are obtained. For example, Sanches et al. studied the effect creating micro-porosities on the tooth surface before dental restoration with adhesive composites [5] but standard statistics were not enough for discovering geometric irregularities. On the other hand, Marshall et al. [6] and Poggio et al. [7] focused on the demineralization and remineralization processes and surface roughness of enamel and dentin, respectively.

Plasma is produced by excitation of neutral gas particles and forms a source of heavy ionized atoms [8, 9]. Depending on the process of plasma production, it can be formed in two ways: thermal plasma and non-thermal plasma (e.g. cold atmospheric plasma – CAP). In the first type, heavy particles and electrons are in thermodynamic equilibrium, while in the latter one electrons are hotter than the remaining heavy particles [10]. Several different sources of energy including heat, electricity and light can be used to produce plasma. There are several methods for CAP generation such as Plasma Needle, Atmospheric Pressure Plasma Jet (APPJ), and Dielectric Barrier Discharge (DBD). Nitrogen, argon, oxygen, hydrogen and their mixture with various proportions are mainly applied for the source gas. Among many applications, modification of surface properties of materials, such as chemical corrosion resistance and hardness [11] along with their application in dentistry including sterilization of equipment and increasing the tooth porosity [12-15] are some potential applications of CAP due to its ability in penetrating thin hollows [16] along with harmful microorganisms destruction [17].

As an initial application of plasma in dentistry, it was applied for producing dental instruments or being used in their disinfection process since Stoffels et al. introduced possible therapeutic in dentistry [18]. Nowadays, among several uses of plasma jet in dentistry treatment of devices and surfaces is very important. Another significant application of direct plasma is therapeutic purpose in or on human body [19]. Over the past decade, intensive researches have been carried out to study the properties of molar teeth and their modifications [20-24]. In the present work, the effect of plasma jet treatment with He source on morphology and structural characteristics of dental tissues is investigated through energy dispersive X-ray (EDX), X-ray diffraction (XRD), scanning electron microscopy (SEM), and Raman spectra analysis.

2. Experimental Details

2.1. Preparation of dental samples

Ten natural teeth were selected, extracted and stored in saline and then transferred to the laboratory for cleaning by standard dentifrice and tooth brush. Afterward, they placed in ultrasonic bath containing acetone and alcohol in order to remove impurities and finally and dried in air. To investigate the effect of plasma treatment on the structure and morphology of teeth, helium plasma jet was applied. Samples were treated with a single-electrode multiple-harmonic AC driven APPJ
composed of a copper wire electrode with a diameter of 0.1 mm placed inside of a 75 mm long and 0.9 mm inner diameter borosilicate glass capillary tube [24]. The end of the electrode wire was 0.5 mm away from the end of the capillary tube. The tube was mounted on a PTFE (Polytetrafluoroethylene) housing. Electrode was connected to a high voltage power supply (3.5 kV, 25 kHz). The working gas (Helium) was leaked through the glass tube at a flow rate of 2 slm. Helium purity was 99.999% and its flow was controlled using Alicat MC-5SLPM-D mass flow controller. The distance between the sample surface and the electrode tip was kept at 1 cm and all hard tissue of the teeth i.e enamel, dentin and cementum were treated by plasma. Due to similar results, the results of a typical molar tooth of a 35 man which were irradiated by He plasma for 8 min were chosen. The schematic of sample treatment with He plasma jet is presented in Figure 1. EDX was applied for elemental analyzing, (KYKY- EM3200) and SEM for investigation of morphology. Moreover, Raman spectra analysis were used to provide information about the structural quality and stresses and variations of crystallinity while XRD (STOE-XRD) was used to determine the crystal structure of the specimen by using a diffractometer with CuKα radiation (λ=0.15406 nm) in the range of 20 from 10 to 70 degree.

![Figure 1. The schematic of sample treatment with He plasma jet](image)

2.2. Methods of structural characterization

Multiscale spatial characteristics of surface morphology of the samples were obtained from SEM images making use of the allometric scaling dependence of the height data on the wavelength. SEM images with gray-scale pixels were processed using numerical routine that begins with calculation of the autocorrelation function according to the formula [25]:

$$R_{mm} = \frac{1}{(N-m)(N-n)} \sum_{p=1}^{N-m} \sum_{q=1}^{N-n} z_{(p+m)(q+n)} \cdot z_{pq}$$  \hspace{1cm} (1)

where (mn) are discrete coordinates of the shifted duplicate with respect to the source image. The ratio of the extreme decay lengths $\tau$ is taken as a measure of the surface anisotropy ratio $S_{tr}$ [26]:

...
\[ S_n = \frac{\tau_{a1}}{\tau_{a2}} \]  

where: \( a1 \) and \( a2 \) – are the directions of the fastest and the slowest autocorrelation decay, respectively.

Afterwards, autocorrelation function is converted into the structure function according to the formula:

\[ S_{mn} = 2S_q^2 (1 - R_{mn}) \]  

where \( S_q \) is the surface roughness (RMS). Mean profile of the structure function averaged around origin is known to follow specific scaling behavior:

\[ S(\tau) = K\tau^{2(2-D)} \]  

where, \( D \) – is the fractal dimension, and \( K \) – pseudo-topothesy. In general, \( D \) and \( K \) define the ways, how the relative and absolute amplitudes of surface height variations depend upon the changes in the wavelengths, respectively. On the other hand, the corner frequencies \( \tau_c \) are thresholds, at which the profile plots change the slope.

Physical properties of the materials under investigation are also studied making an insight into the arrangement of the crystal lattice. To this end, X-ray diffraction techniques are invaluable. However, materials with apparent mosaicity produce spectra with overlapping peaks, hence the proper crystal structure is hardly determined using Bragg’s law solely. In order to reconstruct accurate crystal lattice and determine the relative contributions of various structural components, the Rietveld method was used. In addition, the relative deformations of crystal lattices are determined using the Williamson-Hall plots. Based on the basic Scherrer equation, Williamson and Hall proposed the formula for the line broadening including effects of finite crystallite size (mosaic domains) \(<D>\) and uniform microstrains \( \varepsilon \) [27]:

\[ \beta_{hkl} \cdot \cos(\theta) = \frac{k\lambda}{\langle D \rangle} + 4\varepsilon \sin(\theta) \]  

Using the least-squares method straight line can be found, the slope and intercept of which correspond to the strain level and particle size, respectively. Finally, to estimate the degree of crystallinity, Crystalline Quality Factor (QF) is used as a normalized ratio of a difference between total and baseline integral spectra:

\[ Q_F = \frac{A-B}{A} \cdot (0.812)^{-1} \]  

where: \( A \) – is the total area below the XRD curve, \( B \) – is the area under the baseline, and 0.812 is the normalization factor established for the reference (perfect HAP structure).

### 3. Results and discussion

Elemental content of enamel and cementum including Oxygen (O), Phosphorus (P), Calcium (Ca), Carbon (C), Chlorine (Cl) and Carbon (C) [20] are analyzed through EDX spectra and the results are summerized in Table 1 which confirms the higher mineralization of enamel with more content of mineral elements. The C contents of enamel changes from enamel with ~4% carbon to
cementum with ~50% carbon [21]. Ca/P ratio in Table 1 is an important criteria of appetite crystals which is 1.7-1.8 for both enamel and cementum while other researcher report valus near 2. This value results in non-stoichiometric hydroxyapatite (HAP) crystals in the tissues, either Ca-abundant or P-deficient.

Table 1. Relative elemental mass compositions of the enamel and cementum tissues.

<table>
<thead>
<tr>
<th>Element</th>
<th>Enamel</th>
<th>Cementum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>3.96</td>
<td>50.06</td>
</tr>
<tr>
<td>Oxygen</td>
<td>42.63</td>
<td>41.55</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>16.08</td>
<td>3.24</td>
</tr>
<tr>
<td>Calcium</td>
<td>37.33</td>
<td>5.15</td>
</tr>
</tbody>
</table>
Figure 2. SEM images of surfaces of A) cementum (reference), B) enamel (reference), C) plasma-treated cementum, and D) plasma-treated enamel. The images are 30 µm in side length.

Figure 2A and B represent SEM images of surfaces of freshly extracted dental tissues, namely cementum and enamel, respectively, while Figure 2C and D show SEM images of the same tissues after treatment with the plasma. Figure 2A reveals non-homogenously calcified structure of untreated cementum that appears wavy with large number of shapeless lumps on it with the diameter in the range from several up to tens of micrometers. Unfortunately, no alignment can be seen in Figure 2A specific of fibrous microstructure of mineralized ends of extrinsic and intrinsic fibers. Fig. 2B presents the surface of untreated enamel, which is found apparently smoother than
that of cementum. After the plasma treatment, surface height variations in cementum largely decreases (Figure 2C), although deep cracks appear probably due to dehydration of the material. In contrast, any visible changes to the overall surface morphology of plasma-treated enamel can be hardly spotted in Figure 2D, although tiny lumps around 1 micrometer in size appear sharper and hence highlighted on the micrograph in Figure 2D compared to Figure 2B.

Table 2. Statistical and fractal characteristics of surface topography of the samples under investigation: Str – anisotropy ratio, D – fractal dimension, \( \tau_c \) – corner frequency.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Str</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>( \tau_{c1} ) [( \mu m )]</th>
<th>( \tau_{c2} ) [( \mu m )]</th>
<th>( \tau_{c3} ) [( \mu m )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cementum</td>
<td>0.63</td>
<td>-</td>
<td>2.71</td>
<td>2.86</td>
<td>-</td>
<td>1.09</td>
<td>3.73</td>
</tr>
<tr>
<td>Plasma-cementum</td>
<td>0.75</td>
<td>2.66</td>
<td>2.78</td>
<td>2.88</td>
<td>0.257</td>
<td>1.23</td>
<td>3.24</td>
</tr>
<tr>
<td>Enamel</td>
<td>0.73</td>
<td>2.67</td>
<td>-</td>
<td>2.88</td>
<td>0.182</td>
<td>-</td>
<td>3.24</td>
</tr>
<tr>
<td>Plasma-enamel</td>
<td>0.83</td>
<td>2.80</td>
<td>-</td>
<td>2.88</td>
<td>0.220</td>
<td>-</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Table 2 summarizes spatial characteristics of surface morphology of cementum and enamel samples using statistical parameters brought by correlation analysis (anisotropy ratio \( S_{tr} \)) and scale-invariant parameters specific of fractal analysis (fractal dimension \( D \) and corner frequency \( \tau_c \)). In general, the surfaces under investigation become more isotropic upon plasma treatment, as the anisotropy ratio increases from 14 to 20 per cent depending on the sample. On the other hand, fractal analysis reveals multiscale spatial structure of the samples associated with the presence of geometrical objects of various sizes. More specifically, cluster structure of cementum is found composed of small units 1 \( \mu m \) in size (primary structure) that aggregate to form much larger blocks almost 4 \( \mu m \) in their diameter (secondary alignment). Obtained fractal dimensions clearly indicate that changes in relative amplitudes of surface height variations upon scaling are smaller within sub-units compared to the entire clusters. Interestingly, plasma uncovers additional sub-primary structure of the surface geometry of cementum associated with the presence of tiny lumps of material few hundreds nanometers in size that are less sensitive to changes in local surface roughness upon scaling relative to larger clusters and super-clusters. On the other hand, spatial characteristics of aforementioned higher-order structures of cementum remain almost unchanged after plasma treatment. Similar conclusion can be drawn upon comparison of fractal parameters of both samples of enamel. Note, however, that primary structure of that material is related to much smaller elements around 200 nm in size, that agglomerate to form 3 \( \mu m \) clusters. The scaling behavior of both component structures significantly differs in untreated enamel due to large gap in fractal dimensions, whereas in plasma-treated sample both fractal dimensions are found similar.
Figure 3. XRD spectra taken from surfaces of cementum and enamel before and after the plasma treatment, normalized with respect to the highest peak in the range. The upper spectrum of perfect HAP structure is shown for reference to identify most prominent peaks with respective Miller indices.

Figure 3 shows diffraction spectra of the samples under scrutiny normalized to the highest peak in the 2theta range. As in previous studies, XRD spectra of both cementum samples are found quite poor with only a few noisy and diffuse lines specific of amorphous structure of the material or a very small volume of coherently-diffracting domains. Note that plasma treatment makes pronounced (211) peak to appear in the spectrum of cementum at the expense of very broad (213) line. Unlike cementum, XRD spectra of enamel are of much better quality being comprised of sharp, well-distinguished lines, which resemble those in a perfect HAP structure. Interestingly, enamel undergoes structural change due to plasma treatment similar to cementum. More specifically, broad and probably overlapped (211) peak in untreated enamel splits into a series of well-separated peaks in plasma-treated samples indirectly proving that some sort of ordering of the crystalline structure has occurred.

Table 3. Crystal properties derived from XRD spectra using Rietveld and Williamson-Hall methods: $a_0$, $c_0$ – lattice constants, $\gamma_0$ – lattice angle, $<D>$ – mean size of coherently-diffracting domains (CDD), $\varepsilon$ – relative lattice distortions, $Q_F$ – crystalline quality factor.

<table>
<thead>
<tr>
<th></th>
<th>$a_0$ [Å]</th>
<th>$c_0$ [Å]</th>
<th>$\gamma_0$ [°]</th>
<th>$&lt;D&gt;$ [Å]</th>
<th>$\varepsilon$ [%]</th>
<th>$Q_F$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cementum</td>
<td>9.41</td>
<td>6.90</td>
<td>120</td>
<td>110</td>
<td>0.127</td>
<td>62</td>
</tr>
<tr>
<td>Plasma-cementum</td>
<td>9.39</td>
<td>6.85</td>
<td>120</td>
<td>110</td>
<td>0.125</td>
<td>30</td>
</tr>
<tr>
<td>Enamel</td>
<td>9.48</td>
<td>6.87</td>
<td>120</td>
<td>212</td>
<td>0.003</td>
<td>70</td>
</tr>
<tr>
<td>Plasma-enamel</td>
<td>9.48</td>
<td>6.88</td>
<td>120</td>
<td>375</td>
<td>0.110</td>
<td>56</td>
</tr>
</tbody>
</table>
Table 3 presents various parameters characterizing crystallinity of the samples, including: lattice constants, size of diffracting domains, the level of lattice distortions and quality factor, derived from their XRD spectra using a number of numerical methods (the Rietveld refinement algorithm, Williamson-Hall method). In general, obtained results support previous conclusions drawn from cursory observation of characteristic features in XRD spectra that cementum is of poorer crystallinity than enamel considering much smaller sizes of CDD domains and higher levels of relative lattice strains. Moreover, plasma treatment hardly affects the structure of cementum, but substantially improves that of enamel. Surprisingly, however, the quality factor $Q_F$ exhibits the opposite trend, that is deterioration in the entire crystalline structure of the materials. Possible reason of observed discrepancy might be associated with the way the quality factor is calculated. Very broad, but weak lines in noisy spectra of cementum contribute to significantly large integral spectra leading to non-zero $Q_F$ values.

Figure 4 indicates Raman spectra of enamel exposed to mechanical wear and cementum hidden in the gums both belong to the applied molar tooth and are similar in their large variety of lines. The widths of these lines which extend up to hundreds of reciprocal centimeters confirm a solid with a complex and highly disordered structure. From EDX, the value of Ca/P for enamel and cementum obtain as 2.2 and 2.1, respectively which confirm that enamel is the most mineralized tissue with hydroxyapatite content. Moreover, although both tissues have similar types of contents, the percentage of these elements is fewer in cementum. These results are compatible with the results of Raman spectra bands so that hydroxyapatite lattice look similar with more intensity in enamel. Comparing to previous works (e.g. Kirchner., 1997), the peak at 960 cm$^{-1}$ is related to the vibrations of the PO$_4$ functional group with weaker peaks around from 900 cm$^{-1}$ to 1200 cm$^{-1}$ which are hardly observed in Figure 4. As can be seen, the spectrum of cementum in this range is wider. The similarities of the shape and widths of phosphate bands in enamel and cementum are against their crystalline structure in XRD and can be justified by aging-induced demineralization or the anisotropy of the light scattering during measurements so that the peaks may depend on the sample orientation versus incident light. Another group of peaks at 1245 and 1450 cm$^{-1}$ belong to organic materials. The vibrations of a double C-O bonds in 1600-1700 cm$^{-1}$ interval and N-H groups centered at 1240 cm$^{-1}$ are also observable. The wide double band in the range from 1200 to 1500 cm$^{-1}$ assigned to distorted amide bonds within NH and CH$_2$ groups.
4. Conclusion

In this paper, hard tissues of natural human teeth including enamel, dentin, and cementum have been treated with helium plasma jet to study changes in their morphology and structural characteristics. SEM images revealed non-homogenously calcified structure of untreated cementum with large number of shapeless lumps that turned upon plasma treatment into a surface with deep cracks, although otherwise smooth. Moreover, the surface of untreated enamel, smoother than that of cementum, did not show visible changes upon plasma treatment except the size of tiny lumps which appear sharper. In general, the surfaces became more isotropic in terms of statistical measures of height variability. Multiscale spatial structure was deduced from fractal analysis. Small units cluster structure of cementum aggregated into larger blocks. However, because of the presence of tiny lumps that are less sensitive to changes in local surface roughness, plasma jet was found to reveal sub-primary structure of cementum leaving higher-order structures almost unchanged due to plasma treatment. Despite several differences in the scaling behaviors of untreated enamel and cementum, their plasma-treated counterparts were found similar in this aspect. Very weak XRD spectra of cementum can be concluded with poor crystallinity or even amorphous structure of this tissue, while sharp and well-distinguished patterns observed for enamel can be related too much larger CDD domains. Also, highly disordered and complex structure of dental tissues can be deduced from Raman spectra. Thanks to EDX, the Ca/P ratio in enamel and cementum are found at 2.2 and 2.1, respectively. Together with Raman spectra this means the presence of functional groups specific of hydroxyapatite with the larger content in enamel.
Conflict of interest
Neither author has a financial or proprietary interest in any material or method mentioned. All authors read and approved the final manuscript. The authors have no conflicts of interest to disclose.

References