Application of argon plasma sheet in the etching process of calcium carbonate crystals for AFM tests

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This paper presents utilization of argon plasma for gradual etching of calcium carbonate crystals. The plasma treatment has been chosen as it appears to be the technique that enables removal of following material layers, thus, the access to the inside of crystals is possible. Examples of investigations of the morphology and mechanical properties of surfaces of calcium carbonate crystals are presented. The impact of plasma treatment has been verified in terms of roughness and volume changes investigated using atomic force microscopy technique in a multi-step experiment. Therefore, we were able to observe the crystal degradation process, revealing the spatial inhomogeneity of the calcium carbonate crystals resulting from their core-shell structure.

Keywords: atomic force microscopy; calcium carbonate crystals; microwave plasma; plasma sheet

1. Introduction

Biominerals such as bones, teeth, shells and otoliths are composites of calcium carbonate or calcium phosphate and organic matrix. They differ significantly from their inorganic counterparts. Organic matrix, which represents from 0.02 % to 20 % of dry weight, determines the size, shape, polymorph, hierarchical structure and mechanical properties of the composite [1–3]. It is still not fully understood how living organisms incorporate the biological material into crystals to design structures of desired properties.

Otolith matrix macromolecule – 64 (OMM-64) was found in the inner ear of rainbow trout [4]. It is involved in mineralization of otolith, which is a calcium carbonate crystal responsible for gravity sensing. This protein influences the shape and polymorph of the crystal but its effect on the internal structure and mechanical properties is still not recognized [5]. In this study, a recombinant protein was used to produce calcium carbonate crystals (CaCO₃) in vitro.

The effect of matrix proteins on crystal formation, structure and properties is extensively investigated to provide more insight into biomineralization process and its pathology [6–8]. Moreover, it gives guidelines on how to mimic a nature and design artificial materials of specific properties [9]. Especially promising seem to be the techniques that allow us to look deep inside the crystals. A good candidate for that appears to be a plasma sheet [10, 11].

Investigations of the influence of plasma treatment on calcium carbonate have been performed using scanning calorimetry, tensile tests and dynamic mechanical analysis [12]. The analysis of variations of decomposition and attrition rates of calcite with plasma operating conditions were also investigated [13]. Recently, the surface treatment using discharge plasma for commercially available CaCO₃ nanoparticles was used for investigation...
of filler particle activation, and appearance of agglomerates was observed using scanning electron microscopy [14].

Atomic force microscopy (AFM) has been used with success in investigations of the influence of a particular medium on specific materials in order to obtain information about structure, resistance or homogeneity [15, 16]. AFM technique is particularly useful when samples are of micrometer size, and/or the changes that need to be recorded are in nanometer range, which excludes the use of macroscopic methods [17].

In this work, we proposed an investigation of morphology and surface mechanical properties of CaCO$_3$ crystals treated by plasma sheet using AFM. The impact of the plasma treatment was verified in terms of roughness changes while the aim of this process was exposing deeper layers of the crystals. Investigated CaCO$_3$ crystals exposed to plasma were gradually etched, allowing to carry out measurements of morphological and mechanical properties of their inner areas. This opened a possibility of investigation of their entire structure and understanding the properties which determine their functioning in the natural environment. According to our knowledge, such an approach has not been reported so far.

2. Materials and methods

2.1. In vitro calcium carbonate mineralization

In vitro calcium carbonate mineralization was carried out using modified diffusion method [4], used previously for precipitation of crystals in the presence of Stm and Stm-like proteins [7, 8]. This method is based on decomposition of ammonium hydrocarbonate and slow CO$_2$ diffusion into calcium dichloride solution [18]. Calcium carbonate precipitation in the presence and absence of recombinant otolith matrix macromolecule – 64 (rOMM-64) was carried out for 24 h in a closed desiccator. 200 µl of 10 mM CaCl$_2$ was placed in 96-well plates above 2 mg of solid (NH$_4$)$_2$HCO$_3$ covered with Parafilm with 5 holes. A circular cover glass of a 5 mm diameter was placed on the bottom of each well. Calcium dichloride solution was supplemented with a final concentration of 0 µg/ml and 60 µg/ml rOMM-64, respectively. After 48 h incubation at room temperature, the crystallization solution was removed, crystals were washed twice with 200 µL distilled water and air-dried. Fig. 1 presents optical microscope images of the calcium carbonate crystals.

![Fig. 1. Example images of (a) pure calcium carbonate crystals and (b) calcium carbonate crystals doped with rOMM-64.](image)

2.2. Plasma sheet

For the study, we have used a waveguide-based microwave source for generation of argon plasma sheet at atmospheric pressure [10, 11]. This novel microwave source operated in argon under atmospheric pressure and delivered plasma in the form of a plasma sheet. In this source, the microwave...
power was supplied to the discharge region via
a rectangular waveguide. A flat quartz box was
inserted into the waveguide through the rectangu-
lar slits which were cut in the wider waveguide
walls, perpendicularly to the narrow walls. The mi-
crowave discharge was generated inside the flat
quartz box and extended beyond the box. Addition-
ally, a movable table for specimens allowed for tun-
ing the time of plasma treatment procedure. Fig. 2
presents the waveguide-based microwave source
for the generation of Ar plasma sheet. The CaCO_3
crystals were treated with plasma microwave power
of 1300 W at Ar flow rate of 20 L/min, for 1 s
in 20 courses.

Fig. 2. The waveguide-based microwave source for the
generation of Ar plasma sheet (a) the schematic
of the device (1 – base, 2 – housing, 3 – mag-
netron, 4 – waveguide, 5 – plasma guide, 6 –
argon inlets, 7 – sample holder) and (b) the pho-
thograph of plasma sheet.

The influence of plasma on CaCO_3 crystals
topography was investigated using a commercial
Innova AFM system from Bruker Company. The
measurements were done at ambient conditions
using a force modulation and tapping modes.

The whole procedure with plasma treatment and
AFM investigation was repeated 3 times. For each
step of the experiment, AFM images were recorded
for two specific areas on two different crystals. The
data were processed using SPIP software from Im-
age Metrology Company and the roughness pa-
rameters, including S_q (root mean square) and S_a
(average roughness) were calculated [19]. By ob-
serving the impact of the process on specific ar-
eas, using precise sample positioning ability in
AFM, we could obtain the results with high degree
of confidence [20].

3. Results and discussion

The CaCO_3 crystals are supposed to have a
core-shell structure and this may be the reason of
inhomogeneous morphology and mechanical prop-
erties. Fig. 3 presents the mechanical differences
between the steps on the surface recorded us-
ing the FMM (force modulation microscopy) tech-
nique [21]. One can see the subtle differences in
mechanical properties at various steps. This inho-
mogeneity of mechanical properties may affect the
different reaction to the plasma treatment.

The examples of topography measurements are
shown in Fig. 4. Images for a sample before and
after three individual steps of plasma treatment are
shown sequentially. It is observed, that the removal
process of the top layer of CaCO_3 crystal pro-
ceded in inhomogeneous fashion.

Table 1 and Table 2 show the values of the
roughness parameters S_a and S_q obtained for two
specific areas on two different crystals before and
after three procedures of plasma treatment. Based
on the results of roughness measurements given in
the tables, one can observe the changes after each
exposure to the plasma. The process is not only
connected with removal of material but also with
migration of material on the surface. One should
remember that plasma also generates high tem-
perature which additionally complicates the anal-
ysis of the phenomena occurring on the CaCO_3
crystal surface.
Based on the obtained AFM images, the cross-section analysis was performed. The locations of cross-sections are indicated on the AFM images in Fig. 4 with the dashed line. The difference between the cross-section area before and after III plasma treatments are shown in Fig. 5. The material loss in this particular cross-section was calculated as 0.14 µm$^3$.

4. Conclusions

In this paper, we have presented the preliminary results of utilization of argon plasma in etching of CaCO$_3$ crystals as a method of outer layer removal, in order to access the inner areas and to investigate their morphological and mechanical properties. Promising outcome of the experiments is encouraging to perform further works, as the complexity and small size of the investigated objects strongly limits the range of available investigation methods.
Table 1. The roughness parameters for crystal 1.

<table>
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<tr>
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<th>Before</th>
<th>I plasma</th>
<th>II plasma</th>
<th>III plasma</th>
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<tbody>
<tr>
<td></td>
<td>$S_a$</td>
<td>$S_q$</td>
<td>$S_a$</td>
<td>$S_q$</td>
</tr>
<tr>
<td>area 1</td>
<td>204.76</td>
<td>209.17</td>
<td>242.69</td>
<td>249.94</td>
</tr>
<tr>
<td>area 2</td>
<td>160.73</td>
<td>173.06</td>
<td>176.63</td>
<td>190.36</td>
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</table>

Table 2. The roughness parameters for crystal 2.

<table>
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<th>III plasma</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$S_a$</td>
<td>$S_q$</td>
<td>$S_a$</td>
</tr>
<tr>
<td>area 1</td>
<td>186.72</td>
<td>187.18</td>
<td>167.88</td>
</tr>
<tr>
<td>area 2</td>
<td>62.39</td>
<td>62.58</td>
<td>26.27</td>
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and gaps on the CaCO$_3$ crystals surfaces may be noticed. The accumulation of material on the surface is also visible. This is related to the process of removing the top layer of CaCO$_3$ crystal which is inhomogeneous.

Further optimization of the etching process parameters is planned as a certain outcome quality is expected in terms of intended experiments. By enabling this processing method one should obtain the access to quasi tomography approach. In addition, the application of this approach will be tested for other materials and structures, such nanocomposites or tissue.

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