Measurements of surface layer of the articular cartilage using microscopic techniques

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Abstract. The articular cartilage is the structure that directly cooperates tribologically in biobearing. It belongs to the connective tissues and in the joints it assumes two basic forms: hyaline cartilage that builds joint surfaces and fibrocartilage which may create joint surfaces. From this fibrocartilage are built semilunar cartilage and joint disc are built as well. The research of articular cartilage have been done in macro, micro and nano scale. In all these measurement areas characteristic features occur which can identify biobearing tribology.

The aim of the research was the identification of surface layer of articular cartilage by means of scanning electron microscopy (SEM) and atom force microscopy (AFM) and the analysis of topography of these layers. The material used in the research of surface layer was the animal articular cartilage: hyaline cartilage and fibrous (articular meniscus) coming from normal knee joint.

1. Introduction
The atomic force microscopy (AFM) is a technique bringing three-dimensional images of sample surface topography with the resolution similar to that obtained in the electron microscopy (SEM). The advantage of using AFM as compared with SEM is that the technique is able to study samples structures in different conditions like in ambient air (room temperature and atmospheric pressure) or a fluid environments (for example physiological conditions). The latter one is particularly important for biological samples since it enables the imaging of samples in their natural or close to natural conditions. In addition, the sample preparation for AFM measurements does not need to cover them with thin layer of gold or carbon as it takes place in SEM. Therefore, taking into account the AFM advantages, it was decided to use of AFM to imagine the surface of articular cartilage, both hyaline and fibrous (articular meniscus) coming from normal knee joint.

The principle of AFM operation is following: an AFM tip, with the end composed of few atoms, is placed at the end of thin, elastic cantilever and then, it is moved in a close proximity to the surface. Forces that act between the AFM tip and surface causes a deflection of the cantilever bringing directly the information about the magnitude and type of the interaction forces and indirectly about the surface topography.

The force $F$ acting between the atoms of the AFM tip and surface deflects the cantilever according to Hooke’s law: $F = k \cdot x$, where $x$ is the cantilever deflection and $k$ is the cantilever spring constant.

The deflection of the cantilever can be precisely measured by means of the optical system composed of a laser and photodiode (fig.1). The laser beam is focused at the end of the cantilever i.e. above the tip and the photodiode register the location of the reflected laser beam. The cantilever deflection can be measured precisely with the measurement error lower than the size of single atoms. The lower detection limit is defined by the smallest possible deflection to be detected (practically, of the order of tenth of nanometers). Such small cantilever deflections cause a displacement of the reflected laser beam enabling the measurement of a force with accuracy down to tens of piconewtons.
The investigated sample is placed on the AFM moving system composed of piezoelectric stepping motor and scanner which work on the basis of inverse piezoelectric phenomenon. The system allows both easy and convenient sample manipulation, particularly important during sample exchange, and fine approaching and scanning without crushing the tip on the sample surface.

![Figure 1](image1.png)

Figure 1. a) Schematic view of the atomic force microscope equipped with the “liquid-cell” setup. b) The photo of the home-built AFM working at the Institute of Nuclear Physics in Cracow. The locations of sample, laser and photodetector corresponds to the scheme in part a).

The atomic force microscopy used for these studies was a home-built device working in contact mode at the Institute of Nuclear Physics in Cracow (fig. 1b).

2. Research of surface topography

The cartilage samples were prepared directly before AFM measurements (fig. 2). First, they were taken after opening of the joint capsule cutting method from joint surface and articular meniscus and immediately placed in physiological buffer (0.9% NaCl). Afterwards, samples were placed in the “liquid cell” setup which was fulfilled with the same buffer and measured.

![Figure 2](image2.png)

Figure 2. The cartilage samples were prepared directly before AFM measurements

The images of the surface topography of the hyaline and fibrous cartilage in physiological salt solution were obtained by means of AFM. The scan size was 100 x 100 μm, 50 x 50 μm and 10 x 10 μm. Figure 3 presents the exemplary images of the surface topography and its 3D-representation for three chosen samples of fibrous cartilage of the knee joint.
The measurements, performed using AFM, showed that the surface of the knee joint is covered by filamentous structures. In order to quantify them, the sections along and crosswise were determined. Figure 4 presents the way of sectioning through single filament and the definitions of filament height, \( h \), and width, \( d \) taken at the half of the height.

The average value of the width was of 40.5 ± 8.1 \( \mu \text{m} \) whereas the mean height was of 30.1 ± 7.2 \( \mu \text{m} \) (in both cases \( N = 30 \)). The determined errors are standard deviations. Their relative large values (correspondingly, 20% and 24 %) suggested the certain degree of diversity of filaments.
Figure 5. SEM images of cartilage surfaces from head of animal femoral bone in different magnifications.

The differences in filaments heights of the fibrous cartilage determined by SEM (fig. 5) and AFM (fig. 3,4) can result from the protocol of the sample preparation, especially for SEM measurements. The AFM measurements were carried out for the surface immersed in physiological saline and therefore the filament height delivered form such conditions can be attributed to the true height of filaments in native conditions.

The examples of cross sections along the filaments are shown in fig. 6. The profiles were separated for better visualization.

The analysis of heights of the surface structures taken along the filaments (fig 6) suggested that their surfaces are relatively flat. The height of the surface structures varied a little in the range of 0–25 nm, what may indicate certain extend of differentiation between filaments.

The measurements and analysis of the surface of knee joint articular meniscus was performed analogously. The obtained images were significantly different – there were no filamentous structures visible. Figures 10 – 18 present a) the surface topography of fibrous cartilage – articular meniscus from animal knee joint measured in the physiological saline, b) 3D surface representation and c) the surface after applications of the edge filter, obtained for different scan sizes (100, 50, 10 μm) corresponding to three different probing resolution: 398, 200 i 40 nm, respectively.

The surface of fibrous cartilage (articular meniscus) was quantitavely described with the use of surface parameters such as peak-to-peak, average surface roughness and surface root-mean-square values (for calculations 10 AFM images were taken, all having the same scan size of 100 x 100 μm):

1. Peak-to-peak value S_y:

   \[ S_y = z_{max} - z_{min} \]

2. Average surface roughness: S_a:

   \[ S_a = \frac{1}{N^2} \sum_{i=1}^{N} \sum_{j=1}^{N} |z(i, j)| \]

\[ 4 \]
3. Surface root–mean–square (RMS): $S_q$:

$$S_q = \sqrt{\frac{1}{N^2} \sum_{i=1}^{N} \sum_{j=1}^{N} z^2(i, j)}$$

assuming that AFM delivers a set of $N \times N$ topographic data $z(i, j)$ where $i$ and $j$ are plane coordinates. The calculated values are presented in Table 1.

Table 1.

| Probe no: | $S_a$ [nm] | $S_s$ [nm] | $S_q$ [nm] |
|-----------|------------|------------|------------|
| 1         | 575.2      | 41.0       | 59.7       |
| 2         | 579.1      | 63.5       | 81.7       |
| 3         | 502.2      | 54.1       | 68.7       |
| 4         | 724.3      | 83.6       | 103.4      |
| 5         | 572.4      | 80.0       | 99.1       |
| 6         | 435.6      | 48.8       | 62.9       |
| 7         | 800.3      | 79.7       | 98.6       |
| 8         | 678.1      | 82.6       | 102.0      |
| 9         | 564.3      | 40.2       | 58.2       |
| 10        | 483.0      | 38.3       | 53.4       |

For all studied 10 images (i.e. different surface locations) of the articular meniscus, the surface parameters, $S_a$, $S_s$, and $S_q$ (RMS), fluctuated. The peak-to-peak values were in the range of 435 to 800 nm, the average surface roughness varied from 38 to 83 nm and the surface root–mean–square (RMS) values – 53 to 103 nm. The surface parameters describe the diversity of structures present on the surface of the hyaline cartilage.
3. Summary
Hyaline and fibrous cartilage joint are used in processes of joint lubricating, loads carriage in static and dynamic conditions and in movement shock-absorption. It is important to recognize the organizational structure of this cartilage in order to identify tribological processes, which take place during cooperation of joint surfaces.

Maximum absolute values of roughness in both kinds of cartilage surfaces are small (800 to 900 nm) and similar. It can be seen that this surfaces are very smooth with rounded peaks and valleys. Analysis of morphological structure of fibrous cartilage shows, that it is a tissue characterized by more concentrated construction than hyaline tissue.

Fibrous cartilage in knee joints are exposed to huge reduced strains. Articular meniscus and articular disc – are exposed to stresses, that are generated by joint surfaces, that are getting closer to each other (additionally separated from each other by synovial fluid) when starting the tribological contact.

Hyaline cartilage has a structure that is more resistant to stresses because they are intercepted by cartilaginous and osseous layers.

Characteristic shape of outer layers of hyaline and fibrous cartilage, in aspect of lubricating mechanism can constitute geometrical conditions of synovial fluid adsorption in working surfaces of joint. Examination with use of AFM technique has a significant meaning in determining of spatial compatibility of synovial fluid structure with cartilage and articular meniscus structure, and during creation of nano lubrication wedges, that are able to generate normal strains in conditions of tribological extortions.

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The research has been supported by State Committee for Scientific Research (KBN) within the framework of grant No.4083/B/T02/2008/34