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Estimation of Cartilage Mechanical Properties by Microindentation

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Introduction

Articular cartilage represents a composite heterogeneous material whose function is maintenance of stable movement with low friction under various loading conditions. Behavior of the cartilage under load is defined by its structure. There are distinguished four zones in articular cartilage that are different in matrix morphology and biochemistry – superficial, transitional, middle and calcified [1–4].

Each zone has its specific function but all they have to resist to compression when the joint is under load. Structural features of each zone define their contribution to the cartilage deformation and recovery. To trace distribution of mechanical characteristics, e.g. elastic modulus, across the cartilage depth, measurements should be done for as thin cartilage slices as possible. For example, the superficial zone important for the compressive strength of the matrix has thickness 0.2–0.6 mm in human cartilages [1–5]. It is the thinnest cartilage zone and therefore it would be reasonable to test 0.2 mm thick cartilage slices. However, the thinner indented sample, the more influence of the substrate on the measurement results [3, 5].

Obviously, smaller indentation depth can reduce influence of rigid substrate. In this case, reasonable correlation between the sample thickness and indentation depth can help to obtain reliable data. These conditions may be provided at microindentation of thin cartilage slices.

Instrument and technique

To realize such scheme, a specialized instrument was developed (Fig. 1).

The instrument used stainless steel ball indentor of diameter 4 mm pressed to the examined sample in static or dynamic mode under computer control. A measurement circuit estimated indentation depth with nanometer accuracy that allowed applying minimum loads to the sample. Small loads and indentation depth enabled examination of thin layers under pressure below the yield stress.

Nanometer accuracy of the indentation depth measurement was reached with the help of atomic force microscope probe and corresponding system of the displacement detection. Calibration of the indentation depth measurement circuit was fulfilled according the routine accepted for surface probe microscopes.

Loading system used an electromagnet controlled by host computer via specialized electronic unit (Fig. 1). The instrument measurement and feedback circuits allowed indentation either under constant load or for the preset depth. The loading system was calibrated under the scheme of dead weight compensation.

For static loading, the sample was subjected to single indentation cycle: load was applied gradually so that indentation rate was about 100 micron/min, then it was kept at maximum level for 60 s and after that the load was gradually decreased. In the case of dynamic loading, cycles of the load increase and successive sharp drop were repeated necessary number of times. Frequency of a loading/unloading cycle was about 0.1 Hz. Maximum indentation depth under both operation modes did not exceed 200 microns. Force applied by indentor to the sample reached 16 N at static loading and 3.5 N under dynamic mode.

The instrument design provided also for the sample placing in liquid medium that enabled investigations of influence of different media on the sample deformational characteristics.

Host computer registered indentation depth and load and saved the data in a file on hard disk. The results were processed and analyzed with help of spreadsheet software.

Using the instrument, cartilage samples of pork knee joint were tested for compression and recovery in different media. The cartilage samples were taken not later than 12 hr *post mortem* and tested without storing. The samples were prepared as follows. Cylinder of diameter 6 mm was cut off

medial menisci in the thickest area (about 3 mm). Then each cylinder was sliced parallel to articular surface for samples with thickness 0.5 mm starting from the surface. So, cartilage slices from the depth 0, 0.5, 1.0, 1.5 and 2.0 mm under the articular surface were tested.

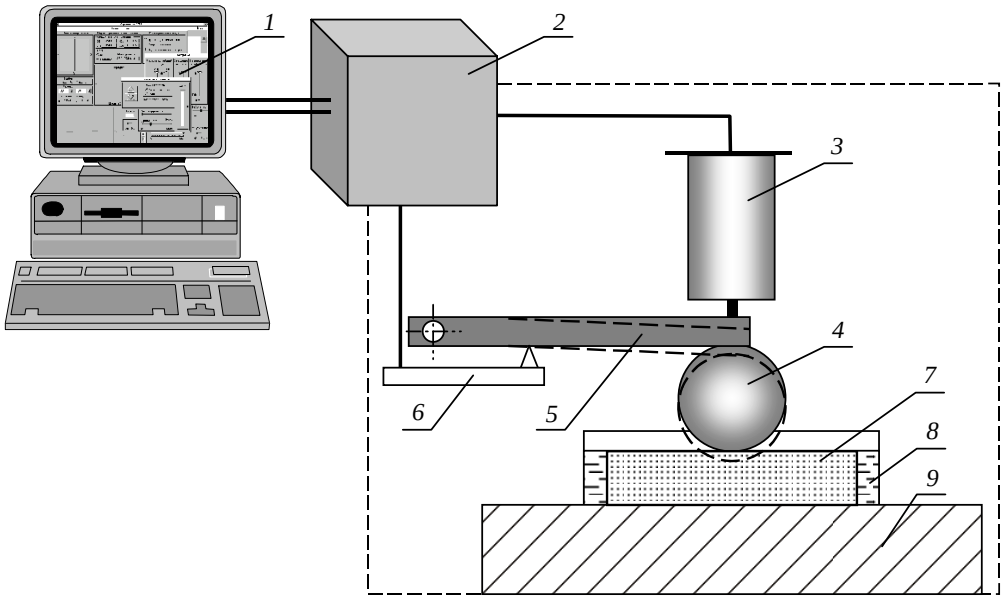

Fig. 1. Schematic diagram of the instrument for cartilage microindentation: *1* – a host computer; *2* – control electronic unit; *3* – an electromagnet for loading the indentor; *4* – spherical indentor; *5* – an arm for the indentor supporting; *6* – a piezoresistive AFM probe; *7* – sample; *8* – liquid medium; *9* – lifting platform

In the experiments, the following fluids were used as liquid media: (1) natural synovia taken not later than 12 hr *post mortem* and tested without storing; (2) pseudosynovia prepared according [6] and representing 2 wt.% water solution of carboxymethyl cellulose sodium salt with addition of inorganic salts; (3) pseudosynovia as in medium 2 with addition of 2 wt.% liquid-crystalline cholesterol esters [3, 7]; (4) physiological solution. Tested samples were immersed in medium so that the fluid did not cover cartilage slice. Experiments were conducted at ambient temperature of 22–24 °C.

Static loading

Indentation of the cartilage samples under static mode was fulfilled for the preset depth. The applied load and indentation depth were registered and then were to calculate elastic modulus. Elastic modulus for the case of spherical indentor of radius r was defined according to Hertzian equation:

$$E = \frac{3}{4}\frac{P}{\sqrt{r\delta^3}}$$

where P is applied load; δ is indentation depth.

Analysis of the experimental results (Fig. 2) shows that rheological characteristics of the surrounding medium effect the cartilage matrix reaction on the applied load. Among the tested fluids, natural synovia imparted the highest resistance to the samples. Pseudosynovia doped with liquid crystals showed the closest to synovia properties. That may be a confirmation for the theory proposed in [3] stating that liquid-crystalline compounds of natural synovia are responsible for the cartilage matrix deformational and tribological properties.

Highest elastic modulus was observed for the cartilage surface layers. Deeper in the cartilage, stiffness of the matrix was lower than in surface layers. This result agrees well with known data on the elastic properties of cartilage zones [3, 5]. Collagen fibres and flattened chondrocytes forming the

superficial zone are oriented in the cartilage along the articular surface. Such structure imparts high tensile and compression resistance to this area of the matrix. In contrast to superficial layers, collagen fibres and other matrix components in inner cartilage zones are either randomly arranged or oriented perpendicular to the articular surface [2, 4]. Therefore transitional and middle (radial) zones are softer and exert lower resistance to the compression in comparison with matrix of the superficial zone.

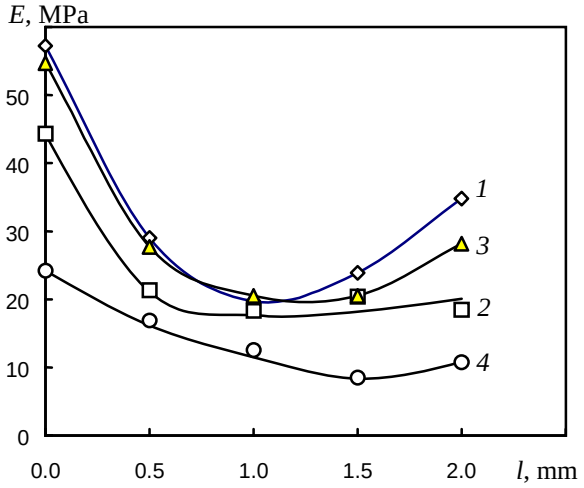

Fig. 2. Distribution of elastic modulus values across the cartilage depth at static loading for different media: *1* – natural synovia; *2* – pseudosynovia; *3* – pseudosynovia doped with liquid crystals; *4* – physiological solution

Measurement of the samples recovery immediately after unloading showed that the less hysteresis was obtained when synovial fluid washed the cartilage slices (5–10%). In medium of pseudosynovia doped with liquid crystals, the closest result was reached (10–15% hysteresis). Hysteresis of cartilage slices in pseudosynovial fluid made 20–30%. The highest values of the parameter were obtained with physiological solution: some samples showed more than 50% hysteresis.

So, the best compression characteristics for the cartilage slices were obtained with natural synovial fluid as medium. Natural components of cartilage synovial medium work together and provide the best performances to the entire joint.

Dynamic loading

Indentation of the cartilage slices under dynamic mode was done at constant load. Indentation depth increased with each loading/unloading cycle as cartilage matrix recovery lagged and electronics traced these changes to maintain constant load. Experiments were stooped when indentation depth reached 200 microns.

Results of the cartilage slice compression under dynamic mode were practically the same as at static loading (Fig. 3). However, calculated values of elastic modulus were lower, especially for the surface layers. That may be caused by slow recovery of the cartilage matrix after the unloading resulting in deeper and deeper indentation with each cycle. So, total hysteresis accumulated during all previous cycles contributed to the final depth used then for calculations of elastic modulus values.

It should be also mentioned that distribution of the elastic modulus values across inner layers of cartilage differs from that observed at static loading: the deepest zones showed slower recovery of the matrix in this area.

For the surface layers, a ratio between maximum indentation depth δ_{max} and the pit depth after the sample unloading δ_{rec} is plotted in Fig. 4.

Natural synovia provided faster and more full recovery of the surface cartilage layers under cyclic loading and unloading than other media. Pseudosynovia doped with liquid crystals gave the

closest results again. It should be mentioned that $\delta_{max}/\delta_{rec}$ ratio increased with time that means that resistance of the cartilage rose with compression. However, this regularity was not kept when physiological solution served as a medium. Probably, high viscosity and non-Newtonian properties of first three fluids contributed significantly to the observed behavior of the cartilage samples.

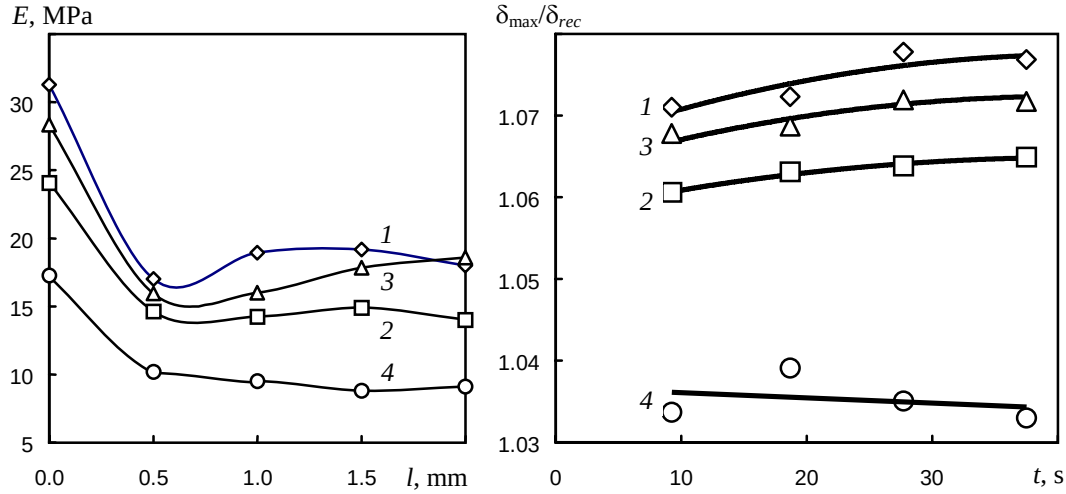

Fig. 3. Distribution of elastic modulus values across the cartilage depth at dynamic loading in: *1* – natural synovia; *2* – pseudosynovia; *3* – pseudosynovia doped with liquid crystals; *4* – physiological solution

Fig. 4. Kinetic of changes of ratio between maximum indentation depth and the pit depth after the unloading at microindentation under dynamic loading in: *1* – natural synovia; *2* – pseudosynovia; *3* – pseudosynovia doped with liquid crystals; *4* – physiological solution

So, analysis of cartilage behavior under loading/unloading cycles allows checking the artificial lubricants adequacy to natural synovia. Such analysis may be done from the data obtained at microindentation of the cartilage samples in tested medium. From all the viewpoints, synovia remains ideal fluid for the joint and should be used as a standard for model media.

Other applications

The obtained results indicate an ability of the proposed microindentation technique to characterize compression behavior of heterogeneous objects on the example of articular cartilage and also to estimate its mechanical properties. However, it is possible to use the developed technique for other purpose. We tested soft polyurethane and rubber to study their viscoelastic behavior.

The samples of 2 mm thickness were subjected to microindentation under static mode. For loading, a hardened steel spherical indentor with diameter 14.3 mm was employed. It was pressed into the samples by dead weight of 0.75 g that made 7.35 mN. Time dependences of the indentation depth obtained at the tests were used to analyze viscoelastic behavior of the samples.

The analysis of curves (Fig. 5, a) showed that the behavior of the polymers could be described by Voigt viscoelastic element as

$$E = E_0 - \mu E_0(1 - e^{-t/t_0}),$$

where E_0 is a momentary elastic modulus; μ is scale parameter; t_0 is characteristic time of relaxation.

Comparison of the experimental results for polyurethane samples with data obtained at nanoindentation according technique from [8] (Fig. 5, b) indicated that they agree well being of one order of magnitude. Besides, extrapolation of the nanoindentation data to loads of about 10 mN gives the result for elastic modulus achieved by microindentation technique (about 40 MPa).

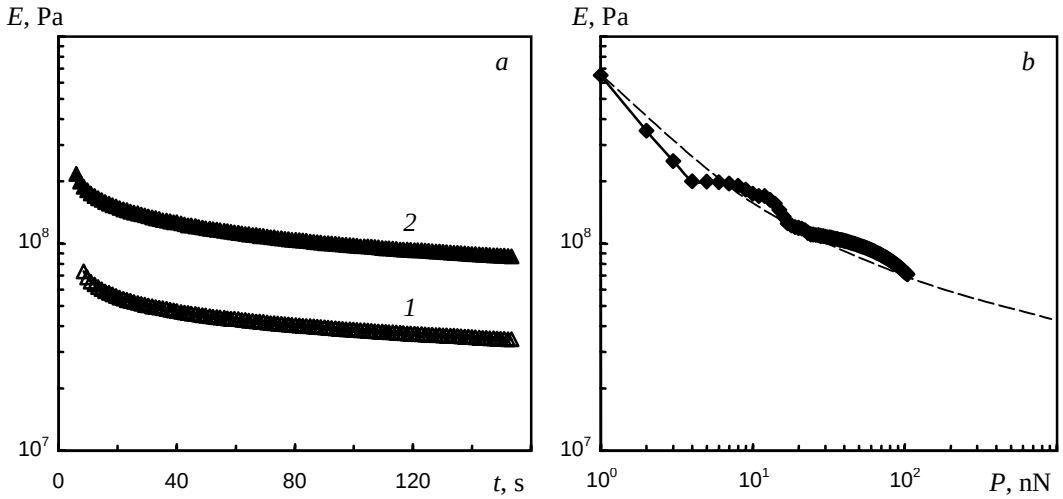

Fig. 5. Curves of viscoelastic behavior of polyurethane (*1*) and rubber (*2*) samples calculated from microindentation data (*a*) and elastic modulus of polyurethane obtained at nanoindentation (*b*)

Conclusion

A possibility of cartilage mechanical characteristics measurement using microindentation technique has been shown. For that purpose, an instrument was designed that features nanometer accuracy at the indentation depth measurement. With help of the instrument, distribution of elastic modulus values across the cartilage depth has been obtained. Analysis of cartilage compression and recovery in different media allows comparison of artificial lubricants with natural synovial fluid. The developed technique can be successfully applied for testing prosthetic materials as well.

Using the instrument, mechanical characteristics of homogenous polymeric materials have been measured. Obtained data agree with the results of the materials nanoindentation.

Acknowledgement

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