EXPERIMENTAL MEASUREMENTS OF FRICTION FORCES ON THE TISSUE IN PRABABILISTIC VIEW

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Abstract

This paper is devoted to stochastic determination of values of friction forces generated during hydro-dynamical flow of viscid-elastic nutrition liquid in thin boundary layer present on surface of human joint cartilage sample during its growing in a bio-reactor.

Taking into account the need for tissue cultivation, this paper presents the detail results of measurements of friction forces occurring on the cartilage sample during the tissue cultivation. Results of irregularities of human joint cooperating surfaces are applied to the friction simulation during the cartilage cultivation in bioreactor. This paper has been prepared based on the objective of European Project MTKD-CT-2004-517226 to represent the methodology and goal of the idea described in and make possible wider discussion on this subject for further developments during the realization. Stages of joint cartilage development and growth in bio-reactor of random character, analysis of joint cartilage surface nano- roughness, the used cartilage of thigh bone head of human hip joint, distributions of probability density function of the random changes in height of nutrition liquid boundary layer on surface of the tissue growing in bio-reactor, control of friction forces and geometrical parameters of natural cartilage surface are presented in the paper.

Keywords: *Friction forces, bioreactor, micro and nano scale*

1. Introductory remarks

In the growing element of joint cartilage, randomly varying heights of surface roughness are taken into account. Before commencing the growing process of cartilage elements in bio-reactor, the roughness heights of healthy and ill (used) surfaces of the cartilages were measured by means of laser and mechanical gauges. Random changes of the height and geometrical form of cartilage surface roughness induce changes in superficial layer height (from 1 μm to 2μm) of growing tissue as well as changes in boundary liquid layer (from 0.01 μm to 1μm) adhering to the cartilage cell surface. Also, forms of pores present in the growing tissue superficial layer, change randomly, that has an influence on changes of amount of nutrition liquid leaking to the most active -in the course of growing- superficial layer of tissue surface. The cross section of the cartilage cell has value from 90 μm^2 to 400μm^2.

Changes in heights of liquid boundary layer are generated as a result of the frequencies and amplitudes of non-stationary motions of bio-reactor casing, deliberately so selected as to ensure an optimum growing process. However, the changes of liquid boundary layer heights can be generated also in a random way as a result of randomly varying roughness of co-operating joint surfaces, which not necessarily must be favourable to the growing process. Hence a problem arises

of control and optimum selection of random changes of liquid boundary layer heights in the course of tissue growing.

As a result of the sustained non-stationary effects, within a very short time period having the order of microseconds, crucial changes in heights of the liquid boundary layer adhering to tissue surface appear. Such changes – in opinion of bio-tribologists -significantly influence permanent or temporary growth changes in the cartilage growing in bio-reactor. Values of friction forces due to changes of liquid boundary layer heights, which appear within time interval of several micro-seconds, often decide on a character of tissue growth. Knowledge on the changes as well as parameters associated with tissue growing process in random conditions in bio-reactor, as well as knowledge on successive phases of tissue growth is of decisive importance for correctness of information dealing with reproduction of cell matrix and next with proper use of the information to tissue transplant processes. As the so decisive period within which the above mentioned processes of changes in tissue growth character occur, lasts only for several or a few dozen micro-seconds, hence their measurement is very expensive, difficult and even hardly possible. For this reason the analytical – numerical determination of randomly varying values of velocity of nutrition liquid flow in thin boundary layer adhering to growing tissue, and next the determination of values of friction forces with accounted for random effects occurring within several or a few dozen micro-seconds, is – in opinion of this author - very purposeful as being associated with taking into account the measured joint cartilage roughness.

The problem of growing the human joint cartilage in bio-reactor with accounting for random conditions has not been so far considered at all in such literature sources as [1], [2], [3], [4], [5], [6], [7], [8].

Fig. 1 shows the process of clustering the single cells which is the first stage of the tissue engineering process. Next, the three-dimensional growth of the clustered cells is observed. Then the grown cells form the cell matrix. The so far equal single cells start to differ to each other [11]. Between the vasculature cells the friction forces appear in nano-scale. The forces greatly affect further growth of the cells. The above presented stages develop in random [11].

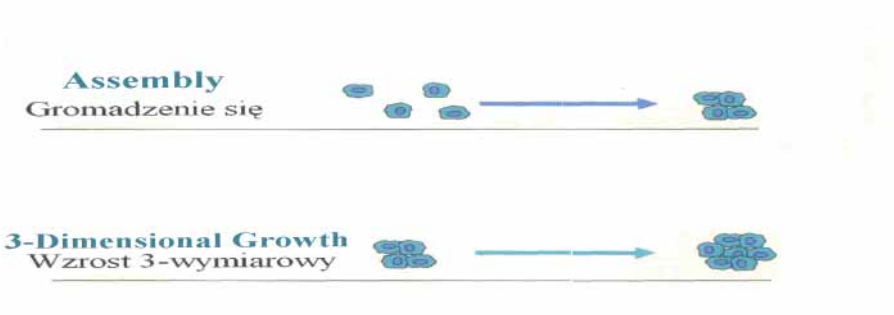


Fig. 1. Stages of joint cartilage development and growth in bio-reactor of random character

2. Height of liquid boundary layer

The dimensionless height of liquid boundary layer ε_{T1} depends on two geometrical variables x, z on the layer surface, as well as on the time t, and is composed of two parts described by the following formula [10], [13]:

$$\varepsilon_{T1}=\varepsilon_{T1s}(x_1,z_1,t_1)+\delta_1(x_1,z_1,\xi), \tag{1}$$

where : ε_{T1s} stands for the dimensionless total height of liquid thin layer constrained by hypothetically smooth surfaces. This part of liquid boundary layer height contains dimensionless corrections of the height, caused by hyper-elastic deformations of the superficial layer of growing

cartilage or tissue. The symbol δ_1 stands for the dimensionless random part of changes of the liquid boundary layer height, due to vibrations, external non-stationary excitations of bio-reactor casing, as well as roughness asperities of joint cartilage surface, measured from a conventional mean level. The symbol ξ describes a random variable characterizing the roughness spread over the tissue area.

The mathematical expectation operator can be defined as follows [12], [13]:

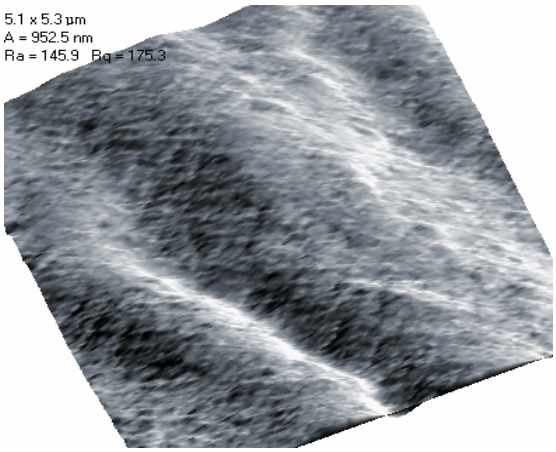
$$E(*) = \int_{-\infty}^{+\infty} (*) \times f_k(\delta_1) d\delta_1, \tag{2}$$

where : f_k – dimensionless probability density function.

3. Optimization of standard deviation of the height of nutrition liquid boundary layer depending on kind of joint cartilage surface

Description of real changes in the height of liquid boundary layer depends on surface changes taking place in growing joint cartilage or tissue. Random changes in cartilage surface are described by probability density functions determined on the basis of comparisons between results of the author's experiments and other experimental results achieved by S. Chihzik [11], see Figs : 2a, 2b, 3a, 3b, and 3c.

a)



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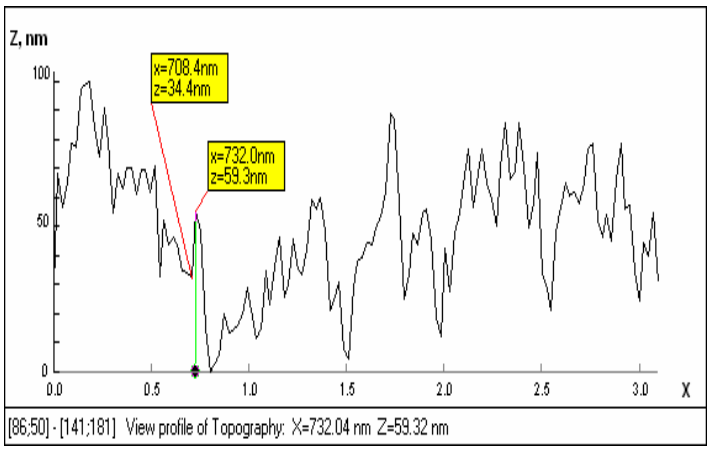
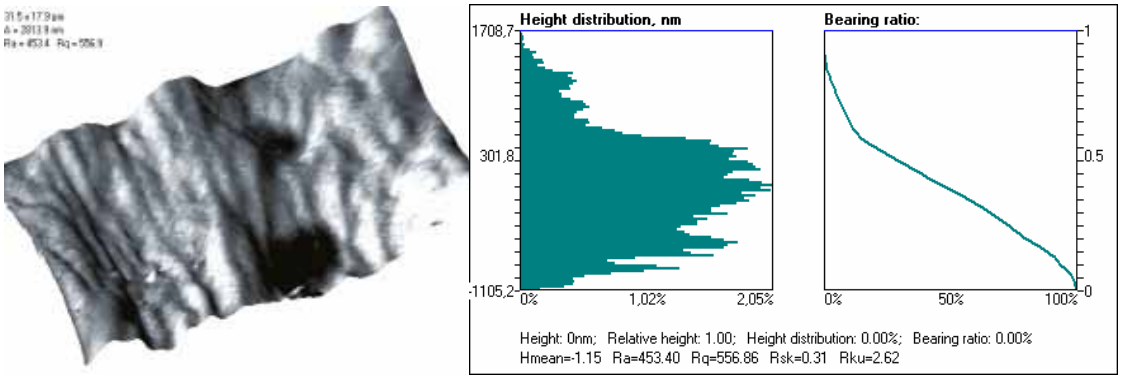
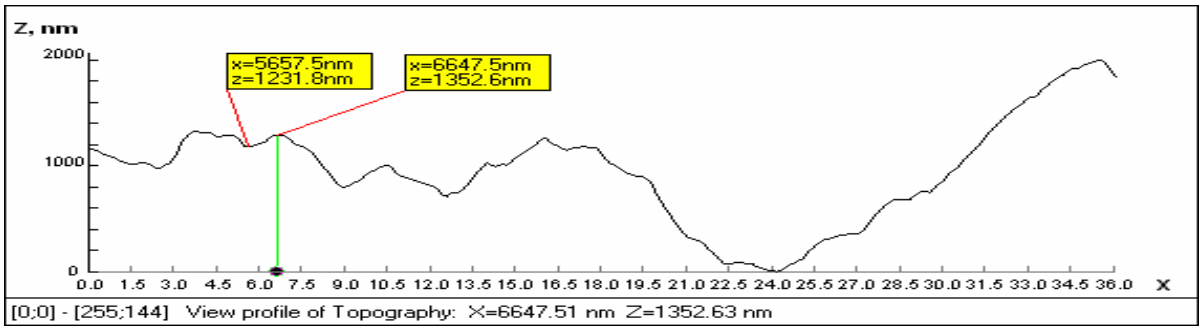


Fig. 2. Analysis of joint cartilage surface nano- roughness by using a microscope of atomic forces: a) topography ;b) cross-section profile of the surface



a) b)



c)

Fig. 3. Analysis of joint cartilage surface nano- roughness by using a microscope of atomic forces: a) topography; b) result of statistical analysis ; c) cross-section profile of the surface [7]

Nano-scale measurements of value of changes in surface roughness of joint cartilage sample, made with the use of a scanning microscope, are presented in Fig. 2a and 2b. where the topographic diagram is shown of the roughness height of about 1000 nm on the sample of ~5 x 5 μm area. No distinct anisotropic structure of the surface is observed, whereas the size of the network structure of cells amounts to 100 - 200 nm, and the diameter of fibres reaches 20 nm.

The mean statistical height of the roughness amounts to 22.7 nm. In the cross-section shown in Fig. 2b the characteristic vertical and lateral asperities of the roughness, reaching the dimensions of 20 – 30 nm, can be observed. The values correspond to the dimensions of collagen fibres.

In Fig. 3 are shown results of the measurements of swine knee joint cartilage, made with the use of the scanning microscope (ACM). It was NT-206 measuring instrument made by Micro-test-machine Co., Belarus. The topographic diagram of 32x18 μm surface element, shown in Fig. 3a, reveals a surface anisotropy in sliding motion direction. Fig. 3b shows that the statistical analysis of the roughness height gives its mean standard deviation amounting to 0.45 nm. The level of the roughness height deviations is presented in Fig. 3c. The greatest deviations reach about 0.3 - 0.6 μm, whereas their lateral dimensions - 5 - 7 μm. The roughness peaks and hollows reach about 60 - 100 nm, and their lateral dimensions are of 0.5 - 1 μm.

In Fig. 4 is presented the geometrical structure of the sample of used joint cartilage of thigh bone head. The measurements were performed by means of a laser gauge. It can be observed that the unevenness height exceeds 100 micrometers i.e. over a hundred times greater than that of joint cartilage surface sample shown in Fig. 3.

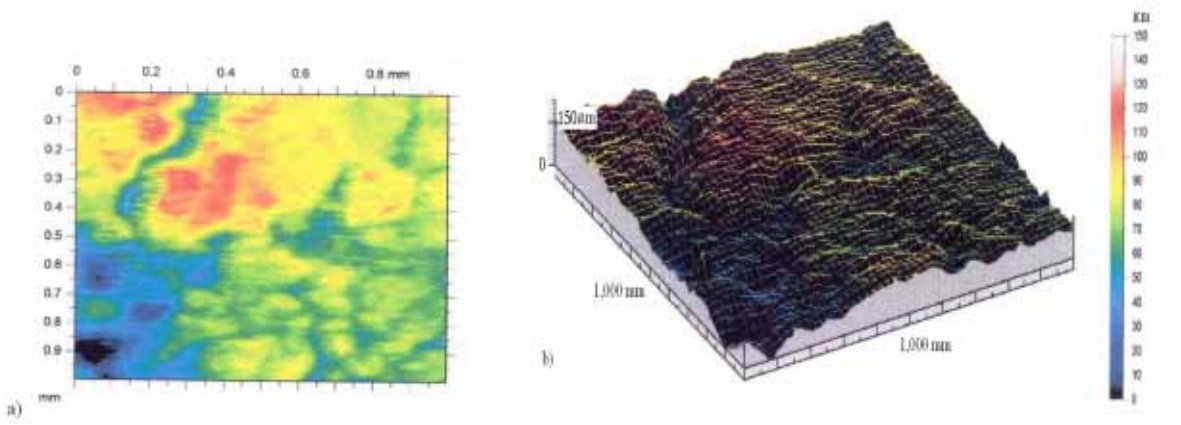


Fig. 4. The used cartilage of thigh bone head of human hip joint

The above described geometrical structures of joint cartilage samples grown in bio-reactors to a large extent form the height of the nutrition liquid boundary layer which adheres to those rough surfaces.

The correct description of random changes in height of the boundary layer depends on an appropriate choice of probability density function. As an assessment criterion the standard deviation has been chosen. The probability density functions concern cartilage surface - and simultaneously –the changes in nutrition liquid boundary layer height resulting from vibrations and surface roughness.

It is assumed that the dimensionless probability density distribution of random changes in joint gap height takes the form of the sequence terms presented in papers [13].

- If unsteady forced vibrations of bio-reactor as well as unsteady effects of suction of nutrition liquid to pores of tissue superficial layer, and also its unsteady growth generate random changes in the height of nutrition liquid boundary layer then the range of every probability density function describing the gap height changes has different limits, as shown in Fig. 5a.

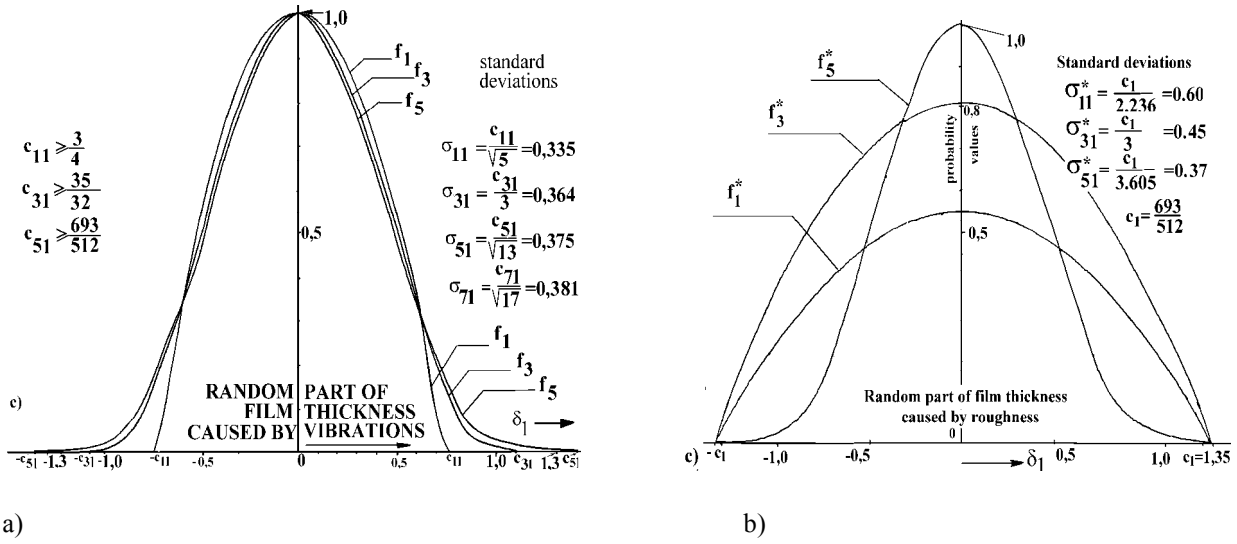


Fig. 5. Distributions of probability density function of the random changes in height of nutrition liquid boundary layer on surface of the tissue growing in bio-reactor, forced by: a) vibrations and unsteady loading on the presented surface of growing cartilage, b) protrusions of the presented rough cartilage surface

Each probability density function reaches the value of 1 in one of the points of its domain, that was discussed in detail in papers [13]. Hence the following probability density function of the smallest standard deviation, is obtained [13]:

$$f_1(\delta_1) \equiv \begin{cases} \left[1 - \left(\frac{4\delta_1}{3}\right)^2\right] & dla \quad |\delta_1| \le +3/4 \\ 0 & dla \quad |\delta_1| > 3/4 \end{cases} \qquad \sigma_{11} = \frac{3/4}{\sqrt{5}} = 0,336 \ . \tag{3}$$

• If random changes in height of nutrition liquid boundary layer are generated by unevenness of joint cartilage rough surface then the range of every probability density function describing the gap height changes has the same limits, as shown in Fig. 5b.

The dimensionless form of probability density function as well as its standard deviation can be presented as follows [13]:

$$f_5^*(\delta_1) \equiv \begin{cases} \frac{693}{512\,c_1}\left(1 - \frac{\delta_1^2}{c_1^2}\right)^5 & \text{dla} \ -c_1 \le \delta_1 \le +c_1 \\ 0 & \text{dla} \quad |\delta_1| > c_1 \end{cases} \qquad \sigma_{51}^* = \frac{c_1}{\sqrt{13}} = 0,375397 \ . \tag{4}$$

4. Comparison of the obtained results with experimental data

There are two reasons which determine validity of geometrical measurements and mechanical features of the cartilages growing in bio-reactors.

The first of them concerns the necessity to achieve preliminary data for the mathematical model describing the tissue growth process in bio-reactor. The next reason justifying the undertaken research is to obtain complex criteria for bio-reactor operation and control of joint cartilage growing process carried out in it. The most important characteristics resulting from the measurements are values of joint cartilage roughness given in micro- and nano-scale as well as mechanical features of the tissue, especially such as Young modulus of elasticity, nutrition liquid dynamic viscosity, friction coefficients in the tissue superficial layer and liquid boundary layer of a few dozen nanometers in height. The use of AFM microscope of atomic forces for complex technical investigations to a large extent has facilitated carrying-out the measurements of tissue surface features and control of its growing process in bio-reactor.

During conventional nano-scale measurements of surface topography, the AFM atomic microscope makes it possible to simultaneously map local lateral forces and oscillatory phases of translations which may be interpreted as a representation of local friction forces and local adhesive forces as well as values of elasticity modules [1],[9]. The spectroscopic forces determined by means of the AFM provide information on local values of elasticity modules as well as those of dynamic viscosity of the liquid contained in the thin layer on tissue surface [5].The complex data derived by applying the AFM microscope may be used for imaging the investigated surfaces [12] that simultaneously makes it possible to visualize and qualitatively characterize geometrical and local physical - chemical diversities of joint surface superficial layer. In Fig. 6 the schematic pictures of structure of swine joint cartilage surface of 12 x 12 μm area are presented on the basis of the topography and image of lateral forces derived from the scanning performed by means of the AFM microscope [11].

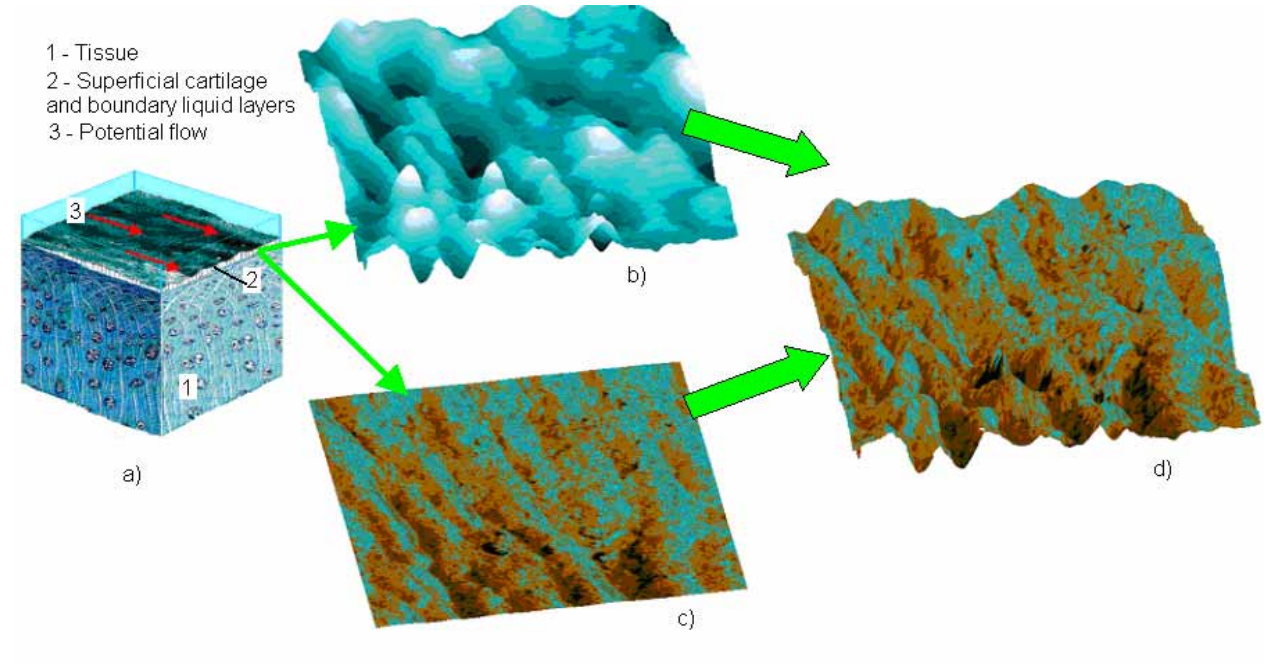


Fig. 6. Control of friction forces and geometrical parameters of natural cartilage surface, carried out by means of the AFM microscope of atomic forces; a) scheme of location of the AFM, b) topography and outward appearance of swine knee joint cartilage (the maximum roughness of 870 nm in height), c) image of tangential forces over the same area, d) combined topography and image of tangential force

This fact shows the diversity of distributions of friction forces over the investigated surfaces in micro- and nano-scale.

The prospect for investigations with the use of the AFM microscope is deemed in determining the tissue surface friction forces that makes it possible - from a general point of view - to ensure a greater stability of measurement results. After some modification of the AFM scanning microscope units such measurements could be performed directly in a bio-reactor. The proposed technique will make it possible to characterize more sensitively friction features in the conditions very close to those usually met in natural joints [4].

One of the important advantages of the application of AFM microscope in the area of investigations with the use bio-reactors is the possibility of carrying out measurements directly in liquid, namely that which surrounds the cells.

5. Conclusions and comparisons

In this chapter the new model for determining the friction forces which act on human joint cartilage surface in the course of its growing in bio-reactor, was presented. In the experimental investigations, were obtained the joint cartilage surface images taken by means of the AFM microscope in order to elaborate a computer model of contact zone between tissue outer layer and liquid boundary layer in micro- and nano-scale.

The transplantation which consists in removal of ill bone tissue or cartilage from human joints can be successfully performed many times in contrast to the transplantation of artificial joint or acetabulum. The replacement of human artificial joint is possible to be done at most three times during human lifetime. Therefore the growing of natural human tissue in bio-reactor is very important for contemporary research. During the growing process in bio-reactor the growing tissue is poured down with a biological liquid. Optimum growth of the tissue depends on an appropriate value of the friction forces resulting from the volumetric rate of poured- down liquid. During growing process each tissue requires friction forces appropriate for it. Therefore values of the friction forces should be controlled during tissue growing. The electronic instruments used for measuring the friction forces are very expensive. Moreover, taking the measurements in the course

of tissue growing does not influence favourably the tissue growing process in bio-reactor. Hence there is a greatly justified need to use the non-invasive numerical simulation of flow parameters including the friction forces which occur during the tissue raising accompanied by its growing in bio-reactor.

For the assumed values of:

- the nutrition liquid density $\rho=O(1000 kg/m^3)$,
- the boundary layer height ε from 0.01μm to 0.1μm,
- the tissue outer layer height ε_o from 1μm to 2μm,
- nutrition liquid viscosity $\eta=0(10^{-3} Pas)$,
- the mean value of tissue growing time t_o=1000s,
- the length L=O(10μm) and breadth D=O(10μm) of cells and poured-down tissue about 200μm long,
- the coefficient of nutrition liquid penetration into tissue outer layer, $c_k=O(10^{-12}m^2)\approx 1(\mu m)^2$,
- the Galileo number Ga=0(0.01),
- the Strouhal number $Str=0(10^{-8})$,
- the volumetric flow rate of the nutrition liquid delivered to bio-reactor $Q_o=0.10 mm^3/s$,

we obtain the resulting data as follows:

- the horizontal components of nutrition liquid velocity vector : $v_x=0(50\mu m/s)$, $v_z=0(50\mu m/s)$,
- the characteristic value of stresses in tissue outer layer : from 0.1Pa to 1.0 Pa,
- the values of the horizontal components of friction forces, F_x, F_z : from 10μN to 60μN,
- the characteristic value of tissue growing rate :from 0.010μm/s to 1μm/s.

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References

[1] Ahn, H.-S., Chizhik, S.A., Dubravin, A.,M., Kazachenko, V.P., Popov, V. V., *Application of phase contrast imaging atomic force microscopy to tribofilms on DLC coatings*, Wear 2001.

[2] Burnham, N. and Colton, R. J., *Measuring the nanomechanical properties and surface forces of materials using an atomic force microscope*, J. Vac. Sci. Technol. A7 2906-13, 1989.

[3] Chizhik, S. A., Huang, Z., Gorbunov, V. V., Myshkin, N. K. and Tsukruk, V. V., *Micromechanical properties of elastic polymeric materials as probed by scanning force microscopy,* Langmuir 14, pp. 2606-09, 1998.

[4] Chizhik, S. A., Ahn, H.-S., Chikunov, V.V., Suslov, A. A., T*uning fork energy dissipation nanotribometry as option of AFM,* Scanning Probe Microscopy. pp. 119 - 121, 2004.

[5] Chizhik, S.A., Gorbunov, V.V., Myshkin, N.,K., *The analysis of molecular scale roughness effect on contact of solids based on computer modelling*, Precision Engineering, 17 pp. 186-191, 1995.

[6] Chizhik, S. A., Gaiduk, N. B., Myshkin, N. K., *Computer simulation of precision contact with account of microgeometrical and mechanical heterogeneity of surfaces, Metrology & properties of engineering surfaces*, in Proceedings of the 8th International conference. UK, pp. 65 - 69 April 2000.

[7] Chizhik, S. A., Gorbunov, V. V., Myshkin, N. K., *The analysis of molecular scale roughness effect on contact of solids based on computer modelling,* Precision Engineering. V. 17, 3, pp. 186-191. 1995.

[8] Chun-Yuh, Huang, Soltz, M., A., Kopacz, M., Mow Van, C., Ateshian, Gerard A., E*xperimental Verification of the Roles of Intrinsic Matrix Viscoelasticity and Tension-Compression Nonlinearity in the Biphasic Response of Cartilage*, Trans.of ASME Vol.125, p. 84 February 2003.

[9] Giselbrecht, S., Giezelt, T., Gottwald, E., Guber A E., Trautmann, C., Truckenmüller, R., Weibezahn K. F., *Microthermoforming as a novel technique for manufacturing scaffolds in tissue engineering Cell Chipds*, IEE Proc. Nanobiotechnology, Vol.151, No.4, August 2004.

[10] Lin J.R. (2001): *The Effects of Couple Stresses un the Squeeze Film Behaviour Between Isotropic Rough Rectangular Plates*, J. of Applied Mechanical Engineering, pp.1007-1024 Vol. 6, No.4.

[11] Shasholko D. I., Suslov A. A., Chizhik S. A., *Joint analysis of topography and contrast SPM images with the software package*, Surface View // Scanning Probe Microscopy. pp. 252–254, 2002.

[12] Wierzcholski, K., *Asperities and deformations of hyper elastic joint cartilages in cultivation aspects* (in English), Zeszyty Naukowe Katedry Mechaniki Stosowanej Technical University of Gliwice pp.469-475, 2005.

[13] Wierzcholski, K., *Hip joint lubrication after injury for stochastic description with optimum standard deviation,* Acta of Bioengineering and Biomechanics, Vol 7,No.2, pp.13-40, 2005.