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Crucial Processes' Interaction During the Renewal of Articular Cartilage: the Mathematical Modeling

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Abstract. The article is an attempt to explain the renewal of the articular cartilage in normalcy and osteoarthritis development by principles of mathematical modeling. Such models help to develop advanced methods of prevention, detection and treatment of osteoarthritis including molecular biotechnologies based on tissue engineering conception. We used histological images to perform structural analysis to discover the signs of active system and its states. Received data are useful to develop research protocols in cartilage tissue engineering.

Keywords: articular cartilage; osteoarthritis; cartilage tissue engineering; mathematical modeling.

Introduction

Osteoarthritis is a wide-spread degenerative disease of joints associated with a large social and economic burden. Its incidence rates are greater than 100/100,000 person-years (rates from Fallon Community Health Plan in Massachusetts (USA), Dutch Institute for Public Health (RIVM)) [1]. Ten percent of people who are older than 55 suffer knee osteoarthritis, and 2,5% become disabled. So, the restoration of damaged and lost tissues of the articular cartilage is one of the most important problems of modern regenerative medicine [2, 3].

Biological and social reasons of this are well observed [4, 5]. There are initially low cartilage regenerative capability, increasing age and quality of life, as consequence, quick ageing of population with active lifestyle, “traumatic” plague in connection with technology expansion to all areas of professional activity, and extremism challenges.

Classical approaches assume defects substituting by auto- or homological material or stimulating own regenerative ability of cartilage. Such approaches have a number of irremovable limitations and do not guarantee full cartilage restoration for a long time. The satisfactory solution seems to be within the physical-chemical biology and current molecular tissue engineering technologies [6, 7, 8]. Such approach combines advanced bio-compatible chondroinductive materials with controlling mechanisms of all processes needed to remodel articular cartilage, similar to native one. Tissue engineering together with regenerative medicine, based on stem cells usage, is interdisciplinary scientific area has being developed for a bit more than 25 years. It is based on engineering principles and techniques and uses the latest achievements of material science, chemistry, biology and bioinformatics for biological substitutes, which restore, maintain and improve injured tissue functions. These engineering constructions should be biomimetic, have proper physical-chemical properties and, ideally, should be replaced by organism’s self-tissue within some reasonable period [3, 5]. The development and implementation of such materials requires from a researcher to understand interaction of key processes, underlying articular cartilage matrix remodeling.

There are different approaches to simulate articular cartilage as a biological system. Some researchers handle it as molecular-biological system. Article [9] introduced mathematical model of interactions in system “chondrocytes – (pro-/anti-) inflammatory cytokines”, which had properly described cellular response to some signal molecules. Another common approach was applied in the paper [10]. It introduced 3D diffusion model of locomotion elements destruction and, in particular, articular cartilage, which is described as a mechanical system.

This articles implements active systems theory approach in cartilage tissue remodeling. During the quantitative morphological analysis, we try to discover meaningful measures, which qualify active system states.

Modeling the articular cartilage

We start from point of view about articular cartilage to be an active system, which response to controlling actions might be determined adequately. Therefore, such system modeling problem reduces to finding dependencies that reflect principles of its functioning. However, taking into account the current level of our visions of cartilage renewal, such problem becomes unsolvable because of great variety of evolution factors which affect this organ. For example, more than one hundred genes are directly involved in osteoarthritis development. That is why articular cartilage could not be handled as a black box – the number of probable system state indicators is vast, and there is no way to get values of most of them non-invasively. Thus, we introduce articular cartilage as an active system (fig. 1).

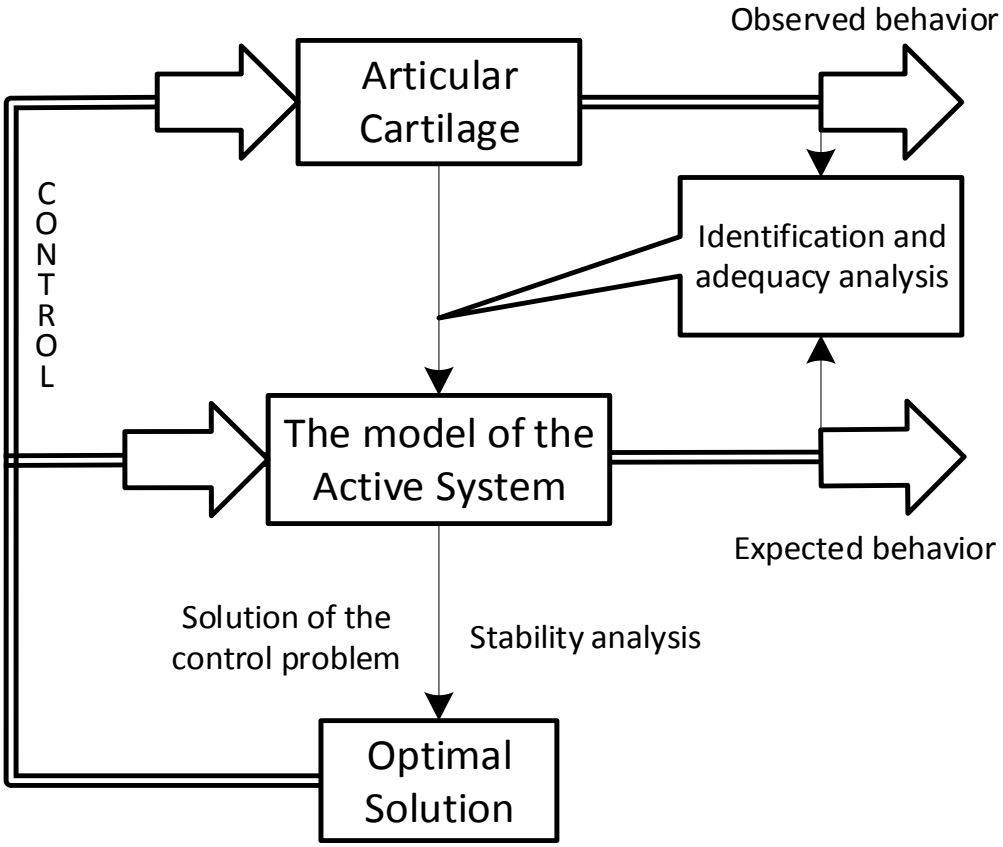

Fig. 1. Steps to the articular cartilage model

Living cells reactions on controlling actions are well studied already. It is difficult to determine or predict the intensity of such reactions, but proposed approach does not require accurate data from this point of view.

In the next section, we consider articular cartilage and its endogenous processes to determine active system components, structure and feasible set of states. We also construct a number of measures, which could be useful for understanding the work of the system.

Articular cartilage endogenesis

Articular cartilage is an amazingly complex of biomaterials. It has high rigidity, compression, strength, stability and amortization indices. Extracellular matrix of cartilage contains about 60% of collagen and 25% of proteoglycans. Another 15% is a widest range of matrix proteins. Collagen net provides cartilage structure sustainability [11].

Chondrocytes maintain physiological regulation and biological consistency of cartilage, although they are widely spaced. Articular cartilage suffers wide range of loads, including shifting, squashing and stretching, because of its location on the surface of the joint. These loads are distributed across all cartilage matrix and are absorbed by its biomolecules. At nanoscale level mechanical loads scatters and then transmits to chondrocytes, which in their turn translate these signals into biochemical signaling molecules [4, 12]. These molecules then start anabolic and catabolic processes. Therefore, chondrocytes are the most suitable candidates to be principals of an active system.

Although osteoarthritis is a chronic inflammatory disease, involving homotypic structural alterations, such alterations are caused by specific decay-accelerating factors. Chondrocytes have independent abilities to initiate and carry on response to cartilage tissue injury. All osteoarthritis course instantiated by two consequent stages: when chondrocyte is trying to restore injured cartilage tissue (1), and when extracellular matrix is obliterated by enzymes produced by

chondrocytes (2). During second stage the matrix synthesis inhibits and, in consequence, articular cartilage erosion occurs.

All these considerations allow us to construct a set of the feasible system states $A = \{a_h, a_r, a_d\}$, where a_h is stationary state (non-injured cartilage), a_r is cartilage remodeling state (synthesis processes are prevalent), a_d is tissue degradation state.

Extracellular matrix regeneration rate is strict – it is delicate balance between synthesis and destruction. Osteoarthritis means broken balance with catabolic processes prevalence. In normal and pathologic conditions cartilage matrix homeostasis depends on autocrine and paracrine control mechanisms. These mechanisms regulate anabolic and catabolic ways of control of chondrocytes quantity and extracellular matrix volume.

Chondrocytes produce structural molecules of articular cartilage, such as collagen and proteoglycans, which help to form cartilage tissue. Simultaneously cells produce different metalloproteinases, which regulate the composition of cartilage tissue. On molecular scale such regulation is based on growth factors such a transforming growth factor β (TGF-β), insulin-like growth factor 1 (IGF-1) and bone morphogenetic proteins 2 and 7 (BMP-2, BMP-7). These factors stimulate chondrocytes to produce structural macromolecules (anabolic pathways). At the same time cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) stimulate chondrocytes to secret proteinases, which cause extracellular matrix degradation (catabolic pathways) [13, 14].

The entire process can be divided into two stages. At the first stage trigger impact on the articular cartilage activates T-lymphocytes and synovial macrophages. At the second stage with the help of chondrocyte receptors IL1R1 they activate the transcription factors NF-kB of the corresponding genes with IL-1β [15].

On molecular genetic level, this process is the result of certain genes' expression and suppression. Such genes belong to a limited set and are responsible for cell cycle, metabolism and intercellular communications in cartilage tissue. Therefore, the set of feasible messages U contains some kind of elements $u_1^+, u_2^+, u_3^+, \ldots, u_1^-, u_2^-, u_3^-, \ldots$, where $u_1^+, u_2^+, u_3^+, \ldots$ stimulate chondrocyte to secrete growth factors (TGF-β, IGF-1, OP-1, physical loads [7]), and $u_1^-, u_2^-, u_3^-, \ldots$ cause matrix degradation (chondrocytes are affected by cytokines IL-1, IL-6, TNF-α, etc.).

The next step to representing the articular cartilage as an active system is to construct the functional $\Phi(\eta, y)$, where $\eta \in U, y \in A$. This leads to necessity to find a way to estimate control efficiency. The ideal physiological indicator is mechanoreceptors signals, which is transmitted into the brain. If such signals are different from the expected ones, an organism suffers pain, which is clinical sign of osteoarthritis. Unfortunately, such indicators are impossible to be used because of the current diagnostic techniques. Therefore, we need indicators that are more suitable.

Search for informative indicators

Currently, expert assessments of histological material and MRI examinations are the 'gold standard' of articular cartilage diagnostics. This approach does not presuppose any kind of numeric values. Therefore, we propose the following approach.

The current state of the specific cartilages is judged by different parameters. Tissue density arrangement is very promising numeric indicator. We have determined the value of density in different zones of cartilage. Specific agent toluidine blue has been used for staining the articular cartilage samples of 5-7 μm depth. It easily revealed extracellular matrix proportionally the concentration of glycosaminoglycans [16]. In such case digital image pixels' brightness inversely relates to extracellular matrix density in corresponding area. Articular cartilage tissue has natural inhomogeneity, which can cause unexpected measurement fluctuations. To compensate this we measured average brightness in rectangular areas of histologic image with equivalent size 50 x 80 μm.

Fig. 2 illustrates one-dimensional distribution of extracellular matrix density $D(\tau)$ in samples of healthy (continuous line) and injured (dashed line) cartilages. The axis of abscissas contains values of the parameter $\tau \in [0,1]$, which indicates current point relative position between bone marrow ($\tau = 0$) and synovial fluid. The ordinate axis contains extracellular matrix density values.

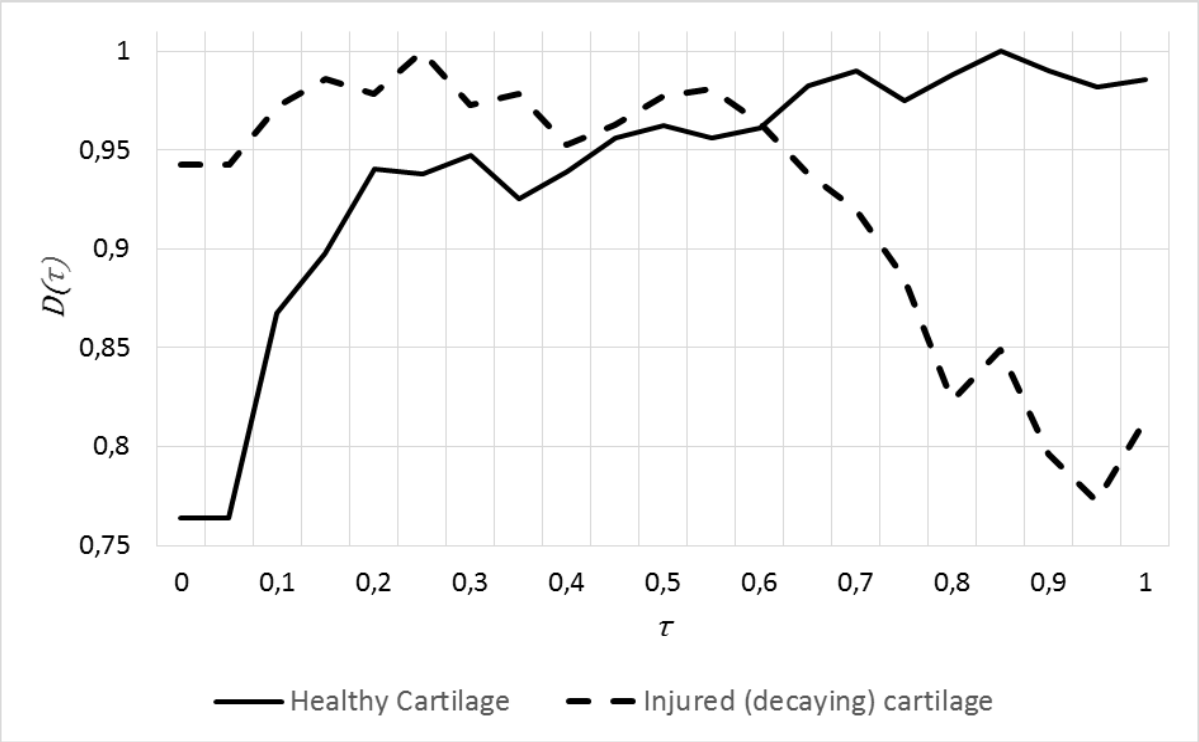

Fig. 2. The distribution of representative extracellular matrix density

These graphs show that slight variety and high value of extracellular matrix density are the good characteristics of healthy cartilage. Accordantly, the reduced density with its additional decreasing at articular surface is typical for injured cartilage. Density values at left part of the graph are lower because of plenty of chondrocytes in cartilage area adjacent to subcartilaginous structures. In consequence of reduced chondrocytes life cycle and physical stress, injured cartilage becomes thin, extracellular matrix density goes down and erosion occurs.

Fig. 2 also demonstrates density distribution of completely healthy cartilage and cartilage with an explicit pathology. In fact, the problem of cartilages differentiation on the basis of certain indicators is extremely complicated. We processed digital photographs of microscopic slides derived from cartilages of six certain dogs. The total quantity of samples was 30. Expert conclusion about status of cartilage and the measurements of tissue density in different sample areas using specialized software were conducted in parallel independent regimen. Finally, we got 74 different density distribution data sets.

We calculated values of eight different indicators for every data set (Table 1).

Table 1: Indicators used to designate articular cartilage processes

Indicator	Evaluation formula	Process or phenomenon reflected by indicator
Cartilage matrix density mean value	$\overline{D} = \sum_{1}^{20} D\left(\frac{i\tau}{20}\right) \Big/ 20$	Current balance of cartilage matrix synthesis and decay
Cartilage matrix volume	$\int_{0}^{1} D(\tau) d\tau$	Prevail cartilage loads volumes
Measured density values variance	$\frac{1}{20}\sum_{1}^{20}\left(D(\tau) - \overline{D}\right)^2$	Differences of rates of cartilage matrix synthesis and decay
Maximum deviation from 1	$\max_{\tau}(1 - D(\tau))$	Maximum matrix decay intensity

The position of the point of maximum	$\tau_{\max} = \max \tau : D(\tau) = 1$	Cartilage matrix synthesis and decay balance
The position of beginning point of falling density	$\tau_f : \forall \tau > \tau_f \ D(\tau) < D(\tau_f)$	Balance point of diffusion flows of subcartilaginous structures and synovial fluid.
The position of point of cartilage matrix volumes equality	$\tau_0 : \int_0^{\tau_0} D(\tau)d\tau = \int_{\tau_0}^{1} D(\tau)d\tau$	Dynamic balance of matrix synthesis and decay
Coordinates difference of beginning points of falling density and volumes equality	$\tau_f - \tau_0$	Matrix remodeling process acceleration or deceleration

Hereafter we plan to use these indicators for searching of informative conditions enabling us to differentiate and classify samples. Every examined cartilage is in one of feasible active system states, which belong to set *A*. We believe that informative conditions can be found using statistically different indicators for elements of *A*. Therefore, we calculate these indicators values for samples belonging to each of three separated groups. Obtained series were tested with Wilcoxon-Mann-Whitney criteria. Results are shown in Table 2.

Obviously, the series differentiate slightly by most of indicators. However, the point of maximum density position is different for healthy and regenerating cartilage (p = 0,1311). Moreover, the position of the point of cartilage matrix volumes equality for certain cartilages difference is statistically confident.

Table 2: Series concurrence probability in groups of cartilages is in certain remodeling states for distinct indicators

Indicator	**p-value of Wilcoxon test for series corresponding to cartilages in the state of...**		
	regeneration and homeostasis	**homeostasis and degradation**	**regeneration and degradation**
Cartilage matrix density mean value	0,6265	0,3778	0,6265
Cartilage matrix volume	0,7843	0,5430	0,8314
Measured density values variance	0,7843	0,5034	0,2478
Maximum deviation from 1	0,8552	0,6926	0,5430
The position of the point of maximum	0,1311	0,2736	0,5090
The position of beginning point of falling density	0,7578	0,8484	0,4042
The position of point of cartilage matrix volumes equality	0,0194	0,2871	0,4202
Coordinates difference of beginning points of falling density and volumes equality	0,9394	0,5430	0,6926

The point of maximum position (p = 0,2736) along with the point of cartilage matrix volumes equality (p = 0,2871) allow to differ degrading and healthy cartilage. The variance values of measured density (p = 0,24777) are significantly different for regenerating and decayed cartilage.

All these results show the possibility to discover certain cartilage regeneration states (stationary matrix regeneration, synthesis prevalence and matrix growth, decay prevalence and matrix loss) using spatial distribution of some quantitative morphologic indicators in cartilage tissue.

Conclusion

This paper describes the first step to developing the model of articular cartilage. The main obstacle which does not allow to finish it is the unsolved problem of cartilage samples differentiation using conditions based on quantitative morphometric indicators. Nevertheless, we expect to solve this problem after revealing more informative indicators of cartilage tissue remodeling. By now, we examined only some small part of them.

Certainly, such indicators will be found in the design of molecular, molecular-genetic research and metabolomics. Such markers will lead to developing research protocols which describe not only the current cartilage state, but also the prognosis of articular cartilage remodeling due to application of modern tissue engineering constructions.

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Взаимодействие ключевых процессов при обновлении матрикса суставного хряща: математическая модель

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Аннотация. Авторы предприняли попытку объяснить закономерности регенерации и обновления суставного хряща с позиций теории активных систем. Такое понимание, и основанное на нем математическое моделирование процессов, происходящих в суставном хряще, является важнейшим шагом на пути к формированию новых методов профилактики, диагностики и лечения заболеваний суставов, прежде всего – остеоартроза. На основе структурного анализа гистологических изображений выявлены признаки здорового и дегенерирующего хряща, в результате чего получены индикаторы, которые необходимо достигать при использовании тканеинженерных конструкций.

Ключевые слова: суставной хрящ; тканевая инженерия; остеоартроз; математическое моделирование.