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IN SILICO ANALYSIS OF AN ARTICULAR CARTILAGE REGENERATIVE REHABILITATION UNDER CONDITIONS OF MESENCHYMAL STEM CELLS IMPLANTATION AND THEIR MECHANICAL STIMULATION

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Abstract. *One of the most important tasks of modern medicine is the development of effective technologies for the treatment of joint diseases caused by damage to the articular cartilage. The results of experimental studies and a number of successful clinical practices indicate that its solution can be found within the framework of a new medical direction - regenerative rehabilitation, which synergistically combines the methods of regenerative and rehabilitation medicine. In particular, regenerative rehabilitation of articular cartilage defects involves the use of cellular technologies, the effectiveness of which is enhanced by mechanical stimulation of chondrogenic cells, which accelerates their proliferation, differentiation, and formation of an extracellular matrix. The simulation results indicate that its outcome depends not only on a set of parameters determined by the state of the tissue in the defect aria, but also on their combination.*

One of the main goals of this work is to find the best combination of parameter values that are practically achievable in the process of articular cartilage regenerative rehabilitation using cellular technologies and mechanical stimulation of cells. Its solution is based on the study of a regenerative tissue rehabilitation mathematical model, the state parameters set of which is determined by the Sobol-Statnikov method, based on a systematic study of the parameter space uniformly distributed in a multidimensional cube. The practical significance of the results of the work lies in the fact that they can be used to evaluate the effectiveness of mechanical stimulation various methods of articular cartilage defects in the process of regenerative rehabilitation.

Key words: *Articular cartilage, Osteoarthritis, Stem cell implantation, Mechanical cell stimulation, Regenerative rehabilitation, Mathematical model*

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1. INTRODUCTION

It is known that the current mechanical and biophysical properties of the cellular tissue microenvironment depend on the mechanical impact experienced by cells in the past and largely determine their future fate [1]. In particular, mechanical impacts on mesenchymal stem cells (MSC), which have a high regenerative potential, affect their ability to synthesize elements of the extracellular matrix (ECM), including fibronectin, laminin, collagen and proteoglycans, and form the microenvironment necessary to increase their own viability, proliferation and differentiation [2,3].

Taking into consideration these facts, a hypothesis was formulated according to which, by artificially creating a certain cellular microenvironment, it is possible to control the life cycle of MSCs implanted in the area of articular cartilage defect, stimulate their proliferation and differentiation in the desired direction in order to ensure the regeneration of damaged tissue. This hypothesis is confirmed by the results of a number of studies *in silico* [4–7], *in vitro* [8–10], and *in vivo* [11–13]. There is an opinion that the regenerative rehabilitation methods of cartilage tissue defects, which have been intensively developed in recent years, have great potential for its successful practical implementation [14]. However, these methods still need to be improved. One of the main issues to be resolved on the way to their widespread introduction into medical practice is how exactly it is necessary to influence MSC implanted in the area of articular cartilage defect in order to achieve the best effect of its restoration. This is an extremely complex task, the solution of which depends on many factors, the influence of which on the achievement of the final results is ambiguous and is characterized by a high degree of uncertainty.

At the same time, it is known that the mechanical and biophysical state of articular cartilage is characterized by many measurable parameters that change as a result of mechanical stimulation of the tissue. These include: the rates of diffusion, proliferation, differentiation and death of cells, the effect of growth factors and nutrients on the deposition and degradation of ECM, the degree of reproduction and degradation of growth factors, the rate of consumption of nutrients by cells, etc. The question arises: what are the optimal values of the above, as well as other parameters that ensure a stable, irreversible and final process of tissue regeneration? In addition, no less important (and perhaps even more important) is the question of how to ensure the creation of a cellular microenvironment corresponding to optimal parameters. In other words: how to carry out mechanical stimulation of cells, providing the best conditions for the regeneration of damaged tissue? The answer to this question, if it exists, can be obtained by comparing the results of in vivo experiments with the results of in silico studies performed on the basis of adequate mathematical models for tissue regenerative rehabilitation. It is clear that this is an extensive way, but, unfortunately, currently there are no other ways apparently to find the optimal solution to the multiparametric problem of repairing articular cartilage defects. Known methods of its treatment cannot be considered not only optimal, but also sufficiently reliable, because in most cases they do not lead to positive results. This is largely due to the peculiarities of the cartilage tissue, which is practically devoid of a vascular network, as a result of which the nutrition of its cells is carried out mainly due to the diffusion of nutrients. Therefore, unlike, for example, bone tissue, hyaline articular cartilage after destruction in the vast majority of cases is not able to fully recover. After reparative regeneration, at best, it is transformed into hyaline-like cartilage [15]. At the same time, it should be noted that cases of complete restoration of local articular cartilage defects as a result of MSC cell therapy followed by physiological procedures are known. It can be assumed that such outcomes are possible as a result of achieving an optimal cellular microenvironment in the tissue defect area as a result of

its optimal mechanical stimulation. With this in mind, the most important task, the solution of which is necessary for organizing effective processes of articular cartilage defects regenerative rehabilitation, is to determine the optimal parameters of the cellular microenvironment, which must be achieved by mechanical stimulation of cells.

Articular cartilage has a very complex structure, represented by solid and liquid phases, and its rigidity is determined to the greatest extent by the fibrous structure of the extracellular matrix and the incompressibility of the interstitial fluid [16]. When loaded, the stiffness of cartilage changes along with a change in the ratio of solid and liquid phases. At the same time, at the initial stage of loading, the degree of hydration of the matrix does not change, since osmotic pressure prevents the release of interstitial fluid. Its extrusion from the matrix begins only when the load acting on the joint will lead to an intra-articular pressure greater than the osmotic pressure. When the load is removed, the reverse process occurs - the absorption of interstitial fluid by cartilage. The viscous component of the synovial fluid, consisting of molecules larger than the pores of the matrix, always remains in the joint space and the low-viscosity component moves from the joint space to the cartilage and back during loading/unloading of the joint. This promotes the transfer of cells and nutrients into the cartilage and the removal of metabolic products from the cartilage into the joint cavity.

The state of articular cartilage under conditions of mechanical loading (stimulation) is characterized by a number of parameters, estimates of which can be obtained as a result of in vivo experiments, thanks to modern methods and analysis tools. At the same time, the measured values of the key parameters α_i, which have the greatest impact on the course of cellular processes, are within some segments $\alpha_i^{\min} \le \alpha_i \le \alpha_i^{\max}$. When modeling the processes for regenerative rehabilitation of articular cartilage, taking into account the personalized characteristics of the tissue, the boundary values of the parameters $\alpha_i^{*} = \alpha_i^{\min M}$ and $\alpha_i^{**} = \alpha_i^{\max M}$ may be deliberately chosen to be larger than $\alpha_i^{\min}$ and $\alpha_i^{\max}$, respectively, to be able to evaluate as many methods of mechanical stimulation of cells as possible.

Obviously, the simulation results will depend on which point $A_k=(\alpha_{k1}, \alpha_{k2}, \ldots, \alpha_{kn})$ in the parameter space $A=(\alpha_1, \alpha_2, \ldots, \alpha_n)$ will be investigated. Theoretically, it is possible to simulate an infinite number of variants for regenerative rehabilitation processes, if we assume that the parameters α_i are continuously distributed on the segments $[\alpha_i^{*}, \alpha_i^{**}]$. If the parameters α_i are represented as discrete sets, then a finite number of models can be investigated. But in any case, in order to obtain the most complete information about the processes of regenerative rehabilitation, the parameter space A should be systematically investigated.

One of the main objectives of this work is to search for the values of the cellular microenvironment key parameters, which are practically achievable using cellular technologies and mechanical stimulation of cells, under which the best conditions for regenerative rehabilitation of articular cartilage defects are achieved. To solve it, a mathematical model of regenerative tissue rehabilitation is used, the combination for state parameters of which is determined by the Sobol-Statnikov method, based on a systematic study of the parameter space A uniformly distributed in a multidimensional cube [17,18].

2. MATERIALS AND METHODS

2.1. Mathematical Model for Regenerative Rehabilitation of an Articular Cartilage Defect

Fig.1 shows the layout of a local articular cartilage defect on the articular surface of the knee joint, as well as its element on an enlarged scale and the associated coordinate system.

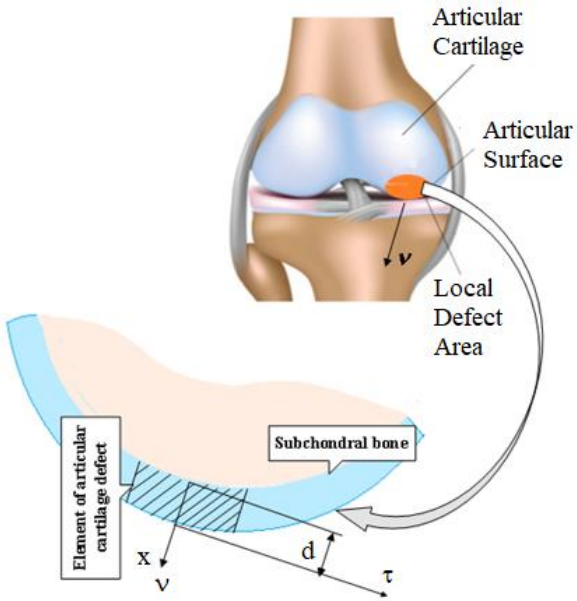

Fig. 1 Representation scheme of the elementary area for the articular cartilage defect and the associated coordinate system

If to assume that the development of the process for articular cartilage regenerative rehabilitation begins with the subchondral bone, and the properties of the tissue or tissue-engineered structure located in the defect area change slightly in planes parallel to the subchondral bone, then, for the purpose of simplification, this problem can be approximately considered as one-dimensional. We will place the origin of the coordinate system on the surface of the subchondral bone in the conditional center of the defect, and direct the x coordinate axis along the outer normal vector ν to the articular surface.

We will assume that the articular cartilage regenerative rehabilitation is performed under conditions of implantation in the area of the MSC defect, i.e., based on the ASI - Articular Stem cell Implantation cellular technology that meets the criteria established by the International Society for Cellular Therapy's Mesenchymal Stromal Cell Committee (ISCT MSC) [19].

To solve this work problems, a mathematical model of the "diffusion-reaction" type is used, the advantages of which were confirmed when modeling various types of biological processes. The history of this type model development is briefly presented in [20]. Initially, starting in the mid-1980s, it was used to simulate the dynamics of tumor-induced angiogenesis. In 1990, it was modified to predict epidermal wound healing by taking into account the interaction between

endothelial cells and a chemoattractant. This took into account the chaotic movement of cells, their proliferation and death, while the chemical substance produced by the cells diffused and underwent natural breakdown. The results of this model were in very good agreement with the results of experimental studies.

In 1993, Chaplain and Stuart developed a model for the chemotactic response of endothelial cells to tumor angiogenesis factor [21], which was modified in 1995 by Byrne and Chaplain [22] to account for the development of blood vessels in response to tumor angiogenesis factor. In addition, in 1996, the same authors established an important link between mathematical models of tumor-induced angiogenesis and wound healing angiogenesis [23], which led to the emergence of numerous mathematical models of wound angiogenesis, which were based on the concept of the "diffusion-reaction" model. The most important among them, according to many researchers, are the models developed in 1996 by Pettet, Byrne, McElwain and Chaplain, which inspired many researchers to study wound angiogenesis [24].

In 2001, Bailón-Plaza and Meulen [25] used a "diffusion-reaction" model to study fracture healing processes taking into account the influence of growth factors, the equations of which had the following structure:

1) MSC density = migration + mitosis + differentiation;
2) chondrocytes density = mitosis + differentiation - endochondral replacement;
3) osteoblasts density = mitosis + differentiation – removal;
4) connective/cartilage ECM density = synthesis – degradation;
5) bone ECM density = synthesis – degradation;
6) osteogenic growth factor concentration = diffusion + production – decay;
7) chondrogenic growth factor concentration = diffusion + production – decay.

The migration of MSC in the model was simulated based on the experimentally observed behavior of randomly populated cells, considering the effects of ECM density and environmental heterogeneity; to account for cell proliferation, the logistic growth law was used, according to which the rate of cell division decreases linearly with increasing cell density due to space and nutrient limitations. The size of the cell population, in accordance with experimental data, stabilized around the maximum density, and cell death due to apoptosis was balanced by the mitosis of new cells. In addition, the enhancing and inhibitory effects observed at low and high ECM densities, respectively, were taken into account. The differentiation of mesenchymal cells into osteoblasts or chondrocytes was regulated by the concentrations of osteogenic and chondrogenic growth factors, respectively. In this case, the dependence of the differentiation rate on the concentration of growth factors was determined considering experimental dose-dependent curves. Other processes occurring in the fracture area during its healing were similarly modeled.

In 2011, adopting the approach proposed by Bailón-Plaza and Meulen, Lutianov, Naire, Roberts and Kuiper presented a "diffusion-reaction" model to predict articular cartilage regeneration using cell therapy [4], which was studied in a modified form by Campbell, Naire and Kuiper in 2019 [5,6]. This model, which previously studied by Popov, Poliakov and Pakhaliuk under other conditions [7], is also used in this work. Its equations are:

$$\frac{\partial C_S}{\partial t} = \underbrace{\nabla[D_S \nabla C_S]}_{diffusion} + \underbrace{p_1 C_S \frac{n}{n+n_0} H(n-n_1)}_{proliferation} - \underbrace{p_2 C_S H(C_S - C_{S_0})}_{differentiation} - \underbrace{p_3 C_S H(n_1 - n)}_{death}, \quad (1)$$

$$\frac{\partial C_C}{\partial t} = \underbrace{\nabla[D_C \nabla C_C]}_{diffusion} + \underbrace{p_4 C_C \frac{n}{n+n_0} H(n-n_1)}_{proliferation} + \underbrace{p_2 C_S H(C_S - C_{S_0})}_{differentiation} - \underbrace{p_5 C_C H(n_1 - n)}_{death}, \quad (2)$$

$$\frac{\partial n}{\partial t} = \underbrace{\nabla\{D_n \nabla n\}}_{diffusion} + \underbrace{\frac{n}{n+n_0}(p_6 C_S + p_7 C_C)}_{reaction}, \quad (3)$$

$$\frac{\partial m}{\partial t} = \underbrace{\nabla[D_m \nabla m]}_{diffusion} + p_8 \underbrace{\frac{n}{n+n_0} C_C}_{reaction}, \quad (4)$$

$$\frac{\partial g}{\partial t} = \underbrace{\nabla[D_g \nabla g]}_{diffusion} + \underbrace{p_9 C_S - p_{11} g}_{reaction}, \quad (5)$$

$$\frac{\partial b}{\partial t} = \underbrace{\nabla[D_b \nabla b]}_{diffusion} + \underbrace{p_{12} C_C - p_{13} g}_{reaction}, \quad (6)$$

where $\nabla = \frac{\partial}{\partial x}\vec{i} + \frac{\partial}{\partial y}\vec{j} + \frac{\partial}{\partial z}\vec{k}$; $\nabla\varphi(x,y,z) = \frac{\partial \varphi}{\partial x}\vec{i} + \frac{\partial \varphi}{\partial y}\vec{j} + \frac{\partial \varphi}{\partial z}\vec{k} = \overrightarrow{grad}\varphi$;

$\nabla\overrightarrow{grad}\varphi = \frac{\partial^2 \varphi}{\partial x^2}\vec{i} + \frac{\partial^2 \varphi}{\partial y^2}\vec{j} + \frac{\partial^2 \varphi}{\partial z^2}\vec{k} = div\overrightarrow{grad}\varphi$; where $\varphi(x,y,z)$ is a scalar function.

A detailed description of the model, represented as system of Eqs. (1-6) and the results of its study are presented in [7]. The numerical values of the parameters used in this case corresponded to the values given in the literature [4–6]. Their brief description is presented in Appendix A. Previously, these values were established experimentally or justified theoretically and used in a number of studies on bone fracture healing, implant osseointegration, and articular cartilage regeneration [26,27].

Boundary conditions of the problem [7]:

- at the point $x=0$:

$$-D_S \frac{\partial C_S}{\partial x} = f(t), D_C \frac{\partial C_C}{\partial x} = 0, D_g \frac{\partial g}{\partial x} = 0, D_b \frac{\partial b}{\partial x} = 0, D_n \frac{\partial n}{\partial x} = 0, D_m \frac{\partial m}{\partial x} = 0; \quad (7)$$

- at the point $x=d$:

$$D_S \frac{\partial C_S}{\partial x} = 0, D_C \frac{\partial C_C}{\partial x} = 0, D_g \frac{\partial g}{\partial x} = -\gamma g, D_b \frac{\partial b}{\partial x} = -\chi b, n = N_0, D_m \frac{\partial m}{\partial x} = 0. \quad (8)$$

Initial conditions at t=0 [7]:

$$C_S = C_S^{(0)} h(x), g = 0, b = 0, C_C = 0, n = N_0, m = m_3 + m_s, \quad (9)$$

where $C_S^{(0)}$ is the initial MSC density; $h(x)$ is the seeding profile of MSC by defect depth; m_3 is the initial ECM density; m_s is the scaffold density.

2.2. Methodology for the Systematic Study of a Mathematical Model by the Sobol-Statnikov Method

2.2.1. *Research Objective*

It should be noted that the problem solved in the framework of this work is essentially a multicriteria one. This is due to the fact that it is rather difficult to formulate its global criterion, despite the fact that its qualitative content is interpreted quite unambiguously: complete regeneration of the articular cartilage defect. But, as practice shows, even if it is possible to achieve this result, the ways to achieve it are most likely ambiguous. In addition, there is a possibility that during the regeneration of a tissue defect, undesirable complications may occur or other quality criteria will deteriorate.

Another problem is that the global criterion, the qualitative content of which is formulated above, is quite difficult to give a quantitative form. Therefore, its evaluation should be carried out indirectly along with the evaluation of other criteria. For example, the mathematical model represented as system of Eqs. (1-6) makes it possible to evaluate the rate of ECM synthesis and interstitial cartilage growth by increasing the density of chondrocytes. Therefore, ECM density (m) and chondrocytes density (C_C) on the articular surface can be taken as quality criteria for the process of an articular cartilage defect regenerative rehabilitation, assuming that their increase promotes the regeneration of cartilage tissue. In addition, MSC, which can differentiate into chondrocytes, play an important role in the processes of articular cartilage regeneration. Therefore, the density of these cells in the defect area or on its surface can also be attributed to the quality criterion of the cartilage tissue regeneration process. Thus, the task is to determine the set of model parameters at which the highest density of ECM (m), chondrocytes (C_C) and MSC (C_S) on the articular surface in the area of the defect is achieved over a certain period of time.

2.2.2. *Parametric Constraints*

The solution of the problem is sought taking into account the set of variable parameters of the α_i model, which, depending on the state of the cellular microenvironment, can take different values belonging to some segments $[\alpha_i^*, \alpha_i^{**}]$. Their description is given in Table 1.

The minimum α_i^* and maximum α_i^{**} values of the parameters are accepted to be smaller or larger, respectively, than in [4–6]. This was done in order to evaluate as many options as possible for potentially possible processes of regenerative rehabilitation, in which the parameters of the cellular environment α_i can take extreme values.

Table 1 Variable parameters of the mathematical model

Parameter	Functional dependencies and parameter values	Parameter dimension	Dimensionless parameter values, $\alpha_i^* - \alpha_i^{**}$
D_{S_0} – MSC diffusion constant	$D_{S_0} \approx 2m_1 D_S^*$	$(mm^3/hour)\cdot(g/mm^3)$	0.001 – 0.01
D_{C_0} – chondrocyte diffusion constant	$D_{C_0} \approx 2m_1 D_C^*$	$(mm^3/hour)\cdot(g/mm^3)$	0.0005 – 0.0015
D_n – nutrient diffusion coefficient	$D_n \approx 4.6$	$mm^2/hour$	100 – 300
D_m – ECM diffusion coefficient	$D_m \approx 2.5\cdot10^{-5}$	$mm^2/hour$	0.001 – 0.01
D_g – FGF-1 diffusion coefficient	$D_g \approx 2.0\cdot10^{-3}$	$mm^2/hour$	0.5 – 1.5
D_b – BMP-2 diffusion coefficient	$D_b \approx 2.0\cdot10^{-3}$	$mm^2/hour$	0.5 – 1.5
p_2 – MSC differentiation rate	$p_2 \approx 3.75\cdot10^{-3}$	$1/hour$	0.5 – 1.5
p_3 – MSC death rate	$p_3 \approx 3.75\cdot10^{-3}$	$1/hour$	0.5 – 1.5
p_{4_0} – chondrocyte proliferation constant	$p_{4_0} \approx 4\cdot10^{-9}$	$g/(hour\cdot mm^3)$	0.006 – 0.018
$p_{4_{00}}$ – chondrocyte proliferation rate	$p_{4_{00}} \approx 2\cdot10^{-4}$	$1/hour$	0.006 – 0.018
p_5 – chondrocyte death rate	$p_5 \approx 3.75\cdot10^{-3}$	$1/hour$	0.5 – 1.5
p_{8_0} – ECM production constant	$p_{8_0} \approx 3.75\cdot10^{-13}$	$(g/mm^3)/((cells/mm^3)\cdot(1/hour))$	0.2 – 1.2
$p_{8_{00}}$ – FGF-1 ECM deposition rate	$p_{8_{00}} = 0 \div 1$	-	0.2 – 1.2
p_{8_1} – ECM degradation constant	$p_{8_1} \approx 3.75\cdot10^{-9}$	$(1/hour)\cdot(1/(cells/mm^3))$	0.5 – 1.5
p_9 – FGF-1 production constant	$p_9 \approx 10^{-17}$	$(g/mm^3)/((cells/mm^3)\cdot(1/hour))$	22.0 – 28.0
p_{11} – FGF-1 degradation rate	$p_{11} \approx 5.8\cdot10^{-2}$	$1/hour$	13.0 – 16.0
p_{12} – BMP-2 production constant	$p_{12} \approx 10^{-17}$	$(g/mm^3)/((cells/mm^3)\cdot(1/hour))$	22.0 – 28.0
p_{13} – BMP-2 degradation rate	$p_{13} \approx 5.8\cdot10^{-2}$	$1/hour$	13.0 – 16.0

2.2.3. *Functional Limitations*

The functional limitations of the regenerative rehabilitation process are set directly in the equations of the mathematical model. The following effects are taken into account. Proliferation of MSC and chondrocytes can occur as long as the concentration of nutrients n in the defect area is greater than the critical value n_1, which is ensured by introducing the

Heaviside function into the model equations of the form $H(n-n_1)=\begin{cases}0, n \le n_1\\ 1, n > n_1\end{cases}$. On the contrary, as soon as n becomes less than n_1 due to a lack of nutrients, the cells gradually begin to die, which is ensured by the Heaviside function of the form $H(n_1-n)=\begin{cases}0, n_1 \le n\\ 1, n_1 > n\end{cases}$. The process of MSC differentiation into chondrocytes is explained similarly. It occurs as long as the MSC density (C_S) is greater than the threshold value C_{S_0}. As soon as C_S becomes smaller C_{S_0}, differentiation stops. These effects in the model equations are provided by the Heaviside function of the form

$$H(C_S - C_{S_0}) = \begin{cases}0, C_S \le C_{S_0}\\ 1, C_S > C_{S_0}\end{cases},$$

where $C_{S_0} = (C_{S_{0\max}} - C_{S_{0\min}})e^{-ab}$; $C_{S_{0\max}}$, $C_{S_{0\min}}$ are the maximum/minimum threshold MSC density, respectively, a is the threshold MSC density reduction factor.

In addition, the functional limitations that must be taken into consideration in the simulation process can include the initial values of the key parameters of the model given in Table 2 [4].

Table 2 Initial values of the mathematical model key parameters

Parameter	Functional dependencies and parameter value	Parameter dimension	Dimensionless parameter value
$C_S^{(0)}$ – initial MSC density	$C_S^{(0)} \approx 2.5 \cdot 10^5$	*cells/mm*^{3}	0.25
$C_C^{(0)}$ – initial chondrocytes density	$C_C^{(0)} \approx 10^{-2}\%$ of total cell density	*cells/mm*^{3}	0.0001
m_3 – initial ECM density	assumed $m_{max}/10^4$	*g/mm*^{3}	0.0001
g_{init} – initial FGF-1 concentration	$g_{init} \approx 10^{-12}$	*g/mm*^{3}	0.01
b_{init} – initial BMP-2 concentration	$b_{init} \approx 10^{-12}$	*g/mm*^{3}	0.01

It should be noted that in practice these values significantly depend on the state of a particular tissue, as a result of which the processes of regenerative rehabilitation are highly personalized.

2.2.4. *Criteria Constraints*

It is known that after reaching a certain critical value, the reproduction of ECM stops. Therefore, in order to exclude obtaining impossible results of the model study, a criterion constraint of the form $m \le m_{max}=10^{-4}$ *g/mm*^{3} is introduced.

The same applies to the differentiation of MSC into chondrocytes. Those, after reaching the critical density of chondrocytes, their reproduction in the defect area stops, which is taken into consideration in the model by introducing a criterion constraint of the form $C_C \le C_{Cmax0}=10^6$ *moles/mm*^{3}. Finally, it is assumed that the density of MSC in the defect area should also be limited. As a first approximation, one can take $C_S \le C_{Smax0}=10^6$ *moles/mm*^{3}.

Taking into consideration the results of studies presented in [19-21], these criteria restrictions in dimensionless form are presented as follows:

$$m \le m_{max}=1;\ C_C \le C_{Cmax0}=0.4;\ C_S \le C_{Smax0}=0.6. \quad (10)$$

Constraints in Eq. (10) assume that the maximum density of chondrogenic cells in the $C_{total,max0}$ articular cartilage defect should not exceed 10^6 *cells/mm*^{3} at zero ECM (m=0) density

$$C_{total,\max 0}=\left(1-\frac{m}{m_{\max}}\right)^{-1}(C_{S\max 0}+C_{C\max 0}) \le 10^6\, cells/mm^3 .$$

In the dimensionless form $C_{total,max0} \le 1$, therefore, $C_S + C_C \le 1$.

2.2.5. *Selection of Trial Points*

According to the Sobol-Statnikov method, to select test points $A_k = (\alpha_{k1}, \alpha_{k2},\ldots, \alpha_{kr})$, it is advisable to use sequences $Q_1, Q_2, \ldots, Q_k, \ldots$ uniformly distributed in the parameter space with fairly good uniformity characteristics and, if possible, simple algorithms for calculating coordinates [18]. Then, by the Cartesian coordinates of the next point $Q_k = (q_{k1}, q_{k2},\ldots, q_{kr})$, you can find the generalized coordinates of the point A_k using the following relationship

$$\alpha_{ki}=\alpha_i^{*}+q_{ki}(\alpha_i^{**}-\alpha_i^{*}), i=\overline{1,r}, \quad (11)$$

where r is the number of mathematical model variable parameters.

To determine Q_k, LΠ$_\tau$-sequences of points are used, which are based on the concepts of a binary segment, a binary parallelepiped, a multidimensional cube, and a Π$_\tau$-grid.

Definition 1. Any segment that can be obtained by dividing segment [0,1] into 2^m equal parts (m=0,1,2,…) is called binary.

All binary segments can be numbered and denoted as l_s, s=1,2,… Let $k=(k_1,k_2,\ldots,k_r)$, where some or even all of k_j, j=1,2,…,r may coincide.

Definition 2. A set of points with coordinates $(x_1,x_2,\ldots,x_r)$ such that $x_i \in l_{ki}, i=1,2,\ldots,r$ is called a binary parallelepiped Π_k. Any binary box belongs to the unit dimensional cube K^r.

Definition 3 [17]. A grid consisting of $N=2^\nu$ points of an r-dimensional cube K^r is called a Π$_\tau$-grid, if each binary parallelepiped Π_k with volume $V_{\Pi_k}=\frac{2^\nu}{N}$ has 2^τ points of the grid at $\nu > \tau$.

Definition 4 [17]. A sequence of points $P_0,P_1,\ldots,P_i,\ldots$ of an r-dimensional cube K^r is called an LΠ$_\tau$-sequence if any of its binary segments containing at least $2^{\tau+1}$ points is a Π$_\tau$-grid.

It is proved that all LΠ$_\tau$-sequences are uniformly distributed in K^r [17]. Moreover, all LΠ$_\tau$-sequences are perfectly distributed [28]. The main idea of the Sobol-Statnikov method is that for a systematic study of the parameter space of a model, perfectly distributed LΠ$_\tau$-sequences in K^r can be successfully used.

Let $i = e_m\ldots e_2e_1$ be the number of the trial point in the binary system. Then its Cartesian coordinates $Q_k = (q_{k1},q_{k2},\ldots,q_{kr})$ can be calculated by the formula

$$q_{ki}=e_1V_i^{(1)} * e_2V_i^{(2)} * \ldots * e_mV_i^{(m)}, \quad (12)$$

where the symbol (*) denotes the "exclusive OR" operation.

Eq. (12) can also be calculated in the usual arithmetic way. To do this, considering the given k, first calculate the number

$$m = 1 + [\ln k / \ln 2], \tag{13}$$

and then for $i=1,2,\ldots,r$

$$q_{ki} \sum_{l=1}^{m} 2^{-l+1} \left\{ \frac{1}{2} \sum_{s=l}^{m} \left[2\{k 2^{-s}\} \right] \cdot \left[2\{r_i^{(s)} 2^{i-1-s}\} \right] \right\}, \tag{14}$$

where $r_i^{(s)}$ are the numerators of guiding numbers [17].

In formulas (13) and (14), [*] is the integer part of the number *, and {*} is the fractional part of the number indicated as *.

Thus, the set of Cartesian coordinates q_{ki}, calculated by Eq. (14) makes it possible to determine the set of generalized coordinates of test points given in Eq. (11), uniformly distributed in the parameter space of the model for the process of an articular cartilage defect regenerative rehabilitation, which makes it possible to systematically study this process *in silico*.

3. Results of the Study

For a systematic study of the influence the parameters of the mathematical model on the course of the processes for an articular cartilage defect regenerative rehabilitation, according to Eq. (13) and Eq. (14), the Cartesian coordinates $L\Pi_\tau$-sequences of points uniformly distributed in an 18-dimensional cube were calculated, which were used to calculate by the Eq. (11) generalized coordinates of sample points uniformly distributed in the 18-dimensional parameter space, taking into consideration the parametric constraints given in Table 1. As an illustration, Fig.2 shows the distribution of sample points $A_k = (\alpha_{k1}, \alpha_{k2}, \ldots, \alpha_{kr})$ in the 3-dimensional parameter space.

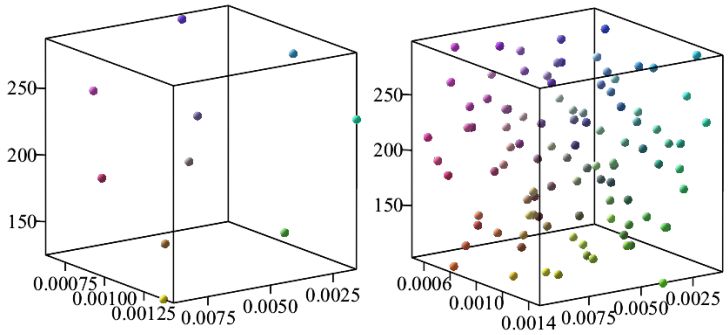

Fig. 2 Illustration of the test points $A_k=\{\alpha_{k1}, \alpha_{k2}, \alpha_{k3}\}$ distribution in a 3-dimensional parameter space: a) 10 test points (k=1..10); b) 100 trial points (k=1..100)

In this work, the study of the mathematical model represented by the system of Eqs. (1-6) was carried out taking into consideration the generalized coordinates of 56 test points uniformly distributed in the 18-dimensional parameter space. The numerical values of the

generalized coordinates of the test points are presented in Appendix B; the results of the mathematical model study are in Appendix C.

The first stage of the mathematical model study was carried out taking into consideration the time of the regenerative rehabilitation process of about 110 days (in a dimensionless form t=10). At the same time, at 6 test points (Nos. 20, 22, 25, 27, 36, 56), unstable solutions of the system of Eqs. (1-6) were obtained, and therefore they were excluded from consideration.

The analysis of the results for the mathematical model study was carried out under the assumption that all test points are independent and uniformly distributed in the 18-dimensional parameter space. In principle, another 56 sample points evenly distributed in this space could be chosen for the study. Formally, this would have no effect on the results of the numerical experiment. To confirm this fact, in Fig. 3 shows different sets of 10 sample points in 3D parameter space. Their generalized coordinates were determined using $L\Pi_\tau$-sequences, which means that from a mathematical point of view they are uniformly distributed in the parameter space, which, in general, is also confirmed visually. Therefore, we can assume that the test points used to study the mathematical model were randomly selected, which means that the results of the study are also random.

Fig. 4 shows the distributions of the quality criteria values $\{m, C_C, C_S\}$ in 3-dimensional and 2-dimensional criteria spaces, and Table 3 presents the correlation coefficients of these criteria.

The results of the mathematical model study indicate that there is a strong negative relationship between the ECM density and the MSC density in the tissue defect area, which is explained by the essence of the tissue regeneration process. It is known that MSC under certain conditions can differentiate into chondroblasts, which, in turn, provide the secretion of ECM, producing type II collagen and sulfated glycosaminoglycans associated with non-collagen proteins - proteoglycans. Ultimately, chondroblasts clog in the matrix cavities (lacunae) and gradually turn into mature cells with lower synthetic activity - chondrocytes. Thus, MSC produced during proliferation differentiate into chondroblasts, which contribute to the secretion of ECM and increase its density. At the same time, the MSC density decreases.

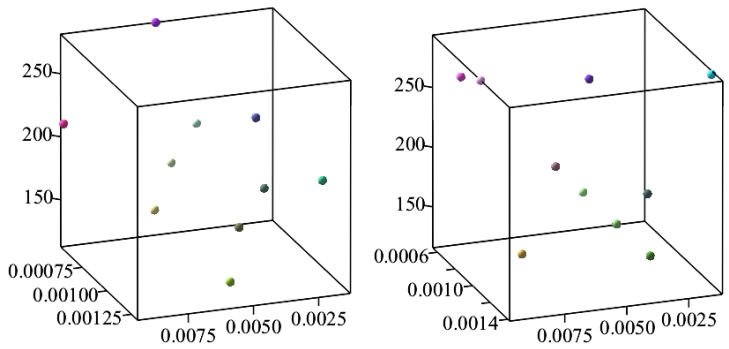

Fig. 3 Illustration of the test points $A_k=\{\alpha_{k1}, \alpha_{k2}, \alpha_{k3}\}$ distribution in a 3-dimensional parameter space: a) 10 test points (k = 11…20); b) 10 test points (k = 31…40)

Table 3 Quality criteria correlation coefficients {m, C_C, C_S}

	m	C_C	C_S
m	1	-0.4186	-0.9977
C_C	-0.4186	1	0.4341
C_S	-0.9977	0.4341	1

At the same time, a moderate negative relationship is observed between the ECM density and the density of chondrocytes, indicating that the dynamics of changes in these parameters are interrelated, but expressed not brightly. That is, there is a tendency for a decrease in the density of ECM with an increase in the density of chondrocytes and vice versa.

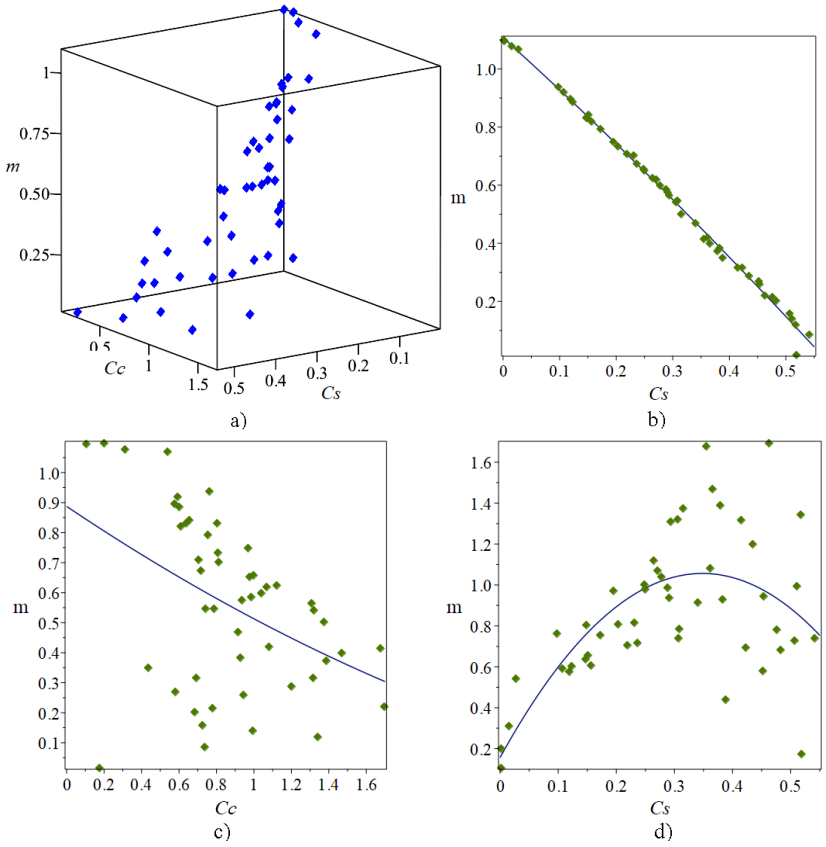

Fig. 4 Distribution of quality criteria values in criterion spaces calculated at 50 trial points: a) 3-dimensional space of criteria {m, C_C, C_S}; b) 2-dimensional space of criteria {m, C_S}; c) 2-dimensional space of criteria {m, C_C }; d) 2-dimensional space of criteria {C_C, C_S}. Regression lines are shown in blue.

And, finally, from the data of Table 3 and the nature of the location of points in the 2-dimensional criterion space {C_S, C_C}, shown in Fig. 4d, it follows that there is a moderate positive relationship between the density of MSC and the density of chondrocytes, which

is also explained by the essence of the regenerative process: an increase in the density of MSC in the defect area contributes to an increase in the density of chondrocytes and, consequently, the regeneration of cartilage tissue.

Table 4 presents the values of the correlation coefficients between the quality criteria of the problem under study and the mathematical model variable parameters.

It is easy to see that p_{8_0} – ECM production constant parameter, which determines the ECM synthesis rate, has the most significant impact on the quality criteria values

$$p_8 = p_{8_0} - p_{8_1} m . \tag{15}$$

Table 4 Correlation coefficients between quality criteria $\{m, C_C, C_S\}$ and the mathematical model variable parameters

	m	C_C	C_S
D_{S_0}	-0.1146	0.1763	0.1311
D_{C_0}	-0.0127	-0.0283	0.0113
D_n	0.1612	0.0400	-0.1738
D_m	0.0132	0.1602	-0.0145
D_g	0.1795	-0.1186	-0.1786
D_b	-0.1130	-0.3195	0.1078
p_2	0.1132	0.2691	-0.1351
p_3	0.1091	-0.1554	-0.0998
p_{4_0}	0.0661	-0.0452	-0.0773
$p_{4_{00}}$	0.1151	0.0201	-0.1216
p_5	-0.0966	-0.0354	0.0881
p_{8_0}	0.8771	-0.5113	-0.8724
$p_{8_{00}}$	-0.0936	0.0031	-0.0916
p_{8_1}	-0.1924	0.3921	0.1985
p_9	0.0274	-0.1558	-0.0415
p_{11}	-0.1151	0.0277	-0.1076
p_{12}	-0.1548	0.2757	0.1574
p_{13}	0.0187	0.1269	-0.0072

This parameter does not depend on the density of the ECM, but has a direct impact on its dynamics. In addition, as follows from Table 3, it has a moderate effect on the change in the density of chondrocytes and a strong effect on the change in the density of MSC. Another parameter: p_{8_1} – ECM degradation constant, has a moderate effect on the change in the density of chondrocytes. It follows from Eq. (15) that its increase contributes to a decrease in the ECM synthesis rate with an increase in the ECM density.

The results of the mathematical model study indicate that other parameters that are important in terms of the implementation of a sustainable process of articular cartilage regeneration have an insignificant effect on its dynamics. At the same time, some of their combination can lead to a violation of the process stability or its atypical development.

By typical is meant a process of regenerative rehabilitation in which an increase in matrix density is constantly observed in the area of the articular cartilage defect, which ultimately ends in the formation of native tissue characterized by a physiologically limited density. Atypical processes are multidirectional during regenerative rehabilitation and, as a rule, do not lead to the formation of tissue with physiological density. In addition, processes that cannot be

implemented within the framework of the mathematical model under study should be considered atypical. That is, in such cases, solving the system of Eqs. (1- 6) is impossible.

An illustration of a typical increase in ECM density and the corresponding pattern of changes in nutrient density in space and time are shown in Fig. 5, and an example of an atypical process is shown in Fig. 6.

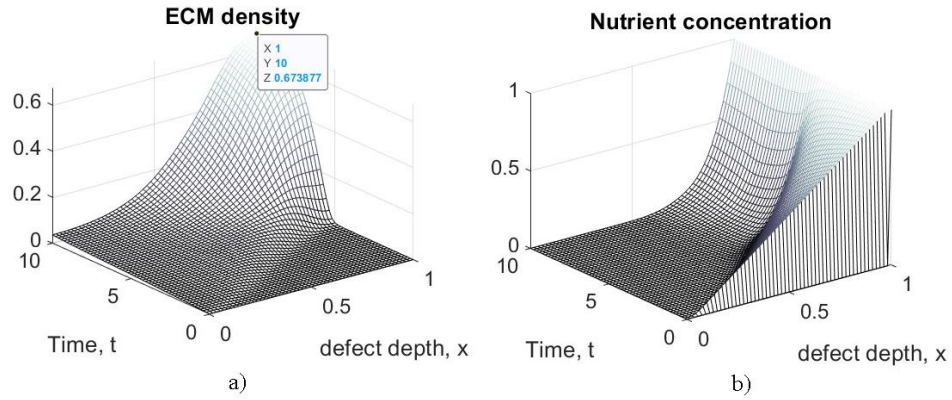

Fig. 5 Illustration of changes in state variables of a mathematical model corresponding to a typical regenerative rehabilitation process (test point No. 51): a) change in ECM density (*m*); b) change in nutrient concentration (*n*)

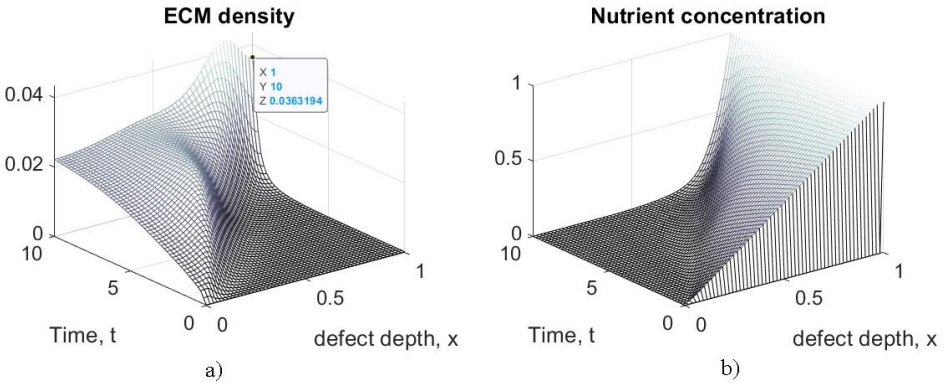

Fig. 6 Illustration of changes in state variables of a mathematical model corresponding to a typical regenerative rehabilitation process (test point No. 56): a) change in ECM density (*m*); b) change in nutrient concentration (*n*)

In typical cases, the growth of ECM density from the very beginning of the process is purposeful. At the same time, the process of nutrient consumption quickly becomes established. In atypical cases, at the initial stage, the process of ECM density growth is multidirectional, and the process of nutrient consumption only after a certain period of time tends to take a stable form. In some cases, the process of an articular cartilage defect regenerative rehabilitation becomes unstable and in most of them cannot be assessed.

4. CONCLUSION

In this paper, a systematic analysis of the regenerative rehabilitation processes of a local articular cartilage defect was performed using a mathematical model represented by a system of non-linear differential equations in partial derivatives (1-6) of the "diffusion-reaction" type. This model is quite plausible and makes it possible to judge the dynamics of the process for given parameters values, which are determined mainly on the basis of the experimental studies results *in vitro*. At the same time, it is known that many parameters of the cellular environment depend on the mechanical stimulation of cells, which largely determines their future fate. Taking into consideration this fact, the concept of regenerative rehabilitation of articular cartilage defects was developed, based on the simultaneous use of cellular and rehabilitation technologies. However, for its successful practical implementation, it is necessary to find answers to a number of questions, including:

- what should be the parameters values of the cellular environment in vivo, at which a stable, irreversible and high-quality regeneration process of damaged tissue is achieved?
- how should mechanical stimulation of cells implanted in the defect area be organized in order to ensure the condition of the damaged tissue with parameters that ensure a stable, irreversible and high-quality regeneration process?

The results of the mathematical model study performed in this work allow us to note that there are parameters of the cellular microenvironment of the tissue, in which the regenerative rehabilitation process of an articular cartilage defect is stable, irreversible and can result in complete regeneration of the damaged tissue. The problem of achieving the values of these parameters under *in vivo* conditions remains topical. One way to find its solution is to compare the parameters of the cellular microenvironment observed after physiotherapeutic procedures with the optimal parameters determined as a result of a mathematical model systematic study. If with the help of the available physiotherapeutic methods and means it will not be possible to ensure the compliance of the parameters, then there will be a need to develop new approaches to the mechanical stimulation of tissues and cells.

It is necessary to note a number of limitations adopted in this study.

1. First of all, this concerns the rationale for choosing a one-dimensional model for studying, generally speaking, the spatial process of regenerative rehabilitation. This was done primarily to simplify solutions to the system of nonlinear partial differential equations representing the model. It should be noted that even in the one-dimensional case, obtaining a set of such solutions for each set of state parameters - a trial point - is a non-trivial task. Due to the impossibility obtaining analytical solutions, in this work, to study the model at 56 test points, uniformly distributed (in the sense of I.M. Sobol) in an 18-dimensional parameter space, has been used the finite element method, implemented in Matlab using the built-in **pdepe** function on supercomputer at the Afalina Shared Use Center of Sevastopol State University.

2. In the mathematical model, the size of the defect was neglected and the change in the parameters for the tissue stress-strain state was not taken into account, which did not allow assessing the value of the chondrogenic index and the probability of achieving the desired result - the formation of native hyaline cartilage at the end of the regenerative rehabilitation process for the defect.

3. The mathematical model did not consider the possibility of MSC differentiation into bone and fibrous tissue cells, because of which the information obtained from the results of its study was incomplete and could potentially contribute to the formulation of incorrect conclusions.

4. The mathematical model did not take into consideration the possibility of using technologies for implanting autologous chondrocytes or their combinations with MSC into the defect area, which limited the obtaining more complete results achieved by methods of regenerative tissue rehabilitation.

5. When determining the density of the formed ECM, the biodegradation for the tissue-engineered structure scaffold populated with cells and implanted into the defect area at the initial stage was not taken into account.

6. The boundaries for the mathematical model variable parameters have been chosen, in general, arbitrarily, considering only a limited number of experimental studies in which they were assessed.

However, the results obtained in this study indicate a high potential for articular cartilage regeneration under conditions for parallel use of cellular and rehabilitation technologies, at least in the initial stages of osteoarthritis. In the context of this work, the use of neural networks and machine learning methods to predict and control the processes of regenerative tissue rehabilitation is of interest. In further studies devoted to this topic, the mathematical model for regenerative rehabilitation will be refined, which will make it possible to obtain more accurate information about the course of this process in vivo and plan more reliable protocols for the treatment of osteoarthritis.

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APPENDIX A
Parameters of the mathematical model for regenerative rehabilitation articular cartilage defect

Parameter	Functional dependencies and parameter value	Parameter dimension	Dimensionless parameter value
C_S – MSC density	$C_S=C_S(x,t)$	*cells/mm*^{3}	-
C_C – chondrocytes density	$C_C=C_C(x,t)$	*cells/mm*^{3}	-
m – ECM density	$m=m(x,t)$	*g/mm*^{3}	-
n – nutrient concentration	$n=n(x,t)$	*moles/mm*^{3}	-
g – FGF-1 concentration	$g=g(x,t)$	*g/mm*^{3}	-
b – BMP-2 concentration	$b=b(x,t)$	*g/mm*^{3}	-
D_S – MSC diffusion coefficient	$D_S = D_{S_0}\dfrac{m}{m^2+m_1^2}$	*mm*^{2}*/hour*	-
D_S^* – maximum MSC diffusion coefficient	$D_S^* \approx 3.6\cdot(10^{-4}\div 10^{-3})$	*mm*^{2}*/hour*	-
D_C – chondrocytes diffusion coefficient	$D_C = D_{C_0}\dfrac{m}{m^2+m_1^2}$	*mm*^{2}*/hour*	-
D_C^* – maximum chondrocytes diffusion coefficient	$D_C^* \approx 3.6\cdot(10^{-4}\div 10^{-3})$	*mm*^{2}*/hour*	-
p_1 – MSC proliferation rate	$p_1 = A_m\left(1-\dfrac{C_S}{C_{S_{\max}}}\right)$	*cells/hour*	-
	$A_m = p_{1_0}\dfrac{m}{m^2+m_2^2}$ $C_{S_{\max}} = C_{S_{\max 0}}\left(1-\dfrac{m}{m_{\max}}\right)$	-	-
p_{1_0} – MSC proliferation constant	$p_{1_0} = 2m_2p_1^* = 4\cdot 10^{-6}$	*g/*(*mm*$^3\cdot$*hour*)	12
p_1^* – maximum MSC proliferation rate	$p_1^* = 0.2$	*cells/hour*	-
m_1 – reference ECM density	m_1=10^{-5}	*g/mm*^{3}	0.1
m_2 – reference ECM density	m_2=10^{-5}	*g/mm*^{3}	0.1
p_4 – chondrocyte proliferation rate	$p_4 = B_m\left(1-\dfrac{C_C}{C_{C_{\max}}}\right)$	1/*hour*	-
	$B_m = p_{4_0}\dfrac{m}{m^2+m_2^2} + p_{4_{00}}\dfrac{g}{g+g_0}$ $C_{C_{\max}} = C_{C_{\max 0}}\left(1-\dfrac{m}{m_{\max}}\right)$	-	-

g_0 – FGF-1 reference concentration	$g_0=10^{-10}$	*g/mm*^{3}	-
p_4^* – maximum chondrocyte proliferation rate	$p_4^* = 2 \cdot 10^{-4}$	1/*hour*	-
n_1 – critical nutrient concentration	$n_1=9.5 \cdot 10^{-12}$	*moles/mm*^{3}	0.1
n_0 – threshold nutrient concentration	$n_0=2.3 \cdot 10^{-11}$	*moles/mm*^{3}	0.24 – 0.81
C_{S_0} – MSC threshold density	$C_{S_0} = (C_{S_0 \max} - C_{S_0 \min})e^{-ab} + C_{S_0 \min}$	*cells/mm*^{3}	-
$C_{S_{0\max}}$ – maximum threshold MSC density	$C_{S_0 \max} = \frac{C_{total,\max 0}}{2}$	*cells/mm*^{3}	0.35
$C_{S_{0\min}}$ – minimum threshold MSC density	$C_{S_0 \min} = 0.9 C_{S_0 \max}$	*cells/mm*^{3}	0.315
p_6 – nutrient uptake constant by MSC	$p_6=1.5 \cdot 10^{-14}$	*moles/*(N_C·*hour*)	10000
p_7 – nutrient uptake constant by chondrocytes	$p_7=1.5 \cdot 10^{-14}$	*moles/*(*cells*·*hour*)	10000
p_8 – ECM synthesis rate	$p_8 = p_{8_0} - p_{8_1} m$	(*g/mm*^{3})/((*cells/mm*^{3})·(1/*hour*))	-
a – threshold MSC density reduction factor	$a = 10^{10}$	1/(*g/mm*^{3})	100
γ – FGF-1 flux coefficient	$\gamma=10^{-2}$	*mm/hour*	0.01
χ – BMP-2 flux coefficient	$\chi=10^{-2}$	*mm/hour*	1
b_0 – BMP-2 reference concentration	$b_0=10^{-10}$	*g/mm*^{3}	-
N_0 – initial nutrient concentration	$N_0 = (2.85 – 9.5) \cdot 10^{-11}$	*moles/mm*^{3}	-

APPENDIX B
General Coordinates of Test points

	1	2	3	4	5	6	7	8
D_{S_0}	0.0055	0.0034	0.0078	0.0021	0.0066	0.0044	0.0089	0.0016
D_{C_0}	0.001	0.0013	0.0008	0.0011	0.0006	0.0009	0.0014	0.0014
D_n	200.0	150.0	250.0	275.0	175.0	225.0	125.0	237.5
D_m	0.0055	0.0078	0.0033	0.0089	0.0044	0.0021	0.0066	0.0038
D_g	1.0	0.75	1.25	1.125	0.625	1.375	0.875	0.6875
D_b	1.0	1.25	0.75	0.625	1.125	1.375	0.875	0.5625
p_2	1.0	0.75	1.25	0.875	1.375	0.625	1.125	0.9375
p_3	1.0	1.25	0.75	0.875	1.375	1.125	0.625	1.0625
p_{4_0}	0.012	0.0150	0.009	0.0165	0.0105	0.0075	0.0135	0.0158
$p_{4_{00}}$	0.012	0.009	0.015	0.0135	0.0075	0.0165	0.0105	0.0143
p_5	1.0	1.25	0.75	1.125	0.625	0.875	1.375	0.5625
p_{8_0}	0.7	0.45	0.95	1.075	0.575	0.825	0.325	0.3875
$p_{8_{00}}$	0.7	0.95	0.4500	1.075	0.575	0.325	0.825	0.6375
p_{8_1}	1.0	0.75	1.2500	0.625	1.125	0.875	1.375	1.0625
p_9	25.0	26.5	23.5	24.25	27.25	25.75	22.75	23.875
p_{11}	14.5	13.75	15.25	14.125	15.625	13.375	14.875	15.4375
p_{12}	25.0	23.5	26.5	27.25	24.25	25.75	22.75	26.875
p_{13}	14.5	15.25	13.75	14.875	13.375	14.125	15.625	15.0625
	9	10	11	12	13	14	15	16
D_{S_0}	0.0061	0.0038	0.0083	0.0027	0.0072	0.0049	0.0094	0.0013
D_{C_0}	0.0009	0.0007	0.0012	0.0008	0.0013	0.0011	0.0006	0.0010
D_n	137.5	287.5	187.5	162.5	262.5	112.5	212.5	181.25
D_m	0.0083	0.0061	0.0016	0.0072	0.0027	0.0049	0.0094	0.0030
D_g	1.1875	0.9375	1.4375	1.0625	0.5625	1.3125	0.8125	0.9688
D_b	1.0625	1.3125	0.8125	0.6875	1.1875	1.4375	0.9375	0.7813
p_2	1.4375	0.6875	1.1875	0.5625	1.0625	0.8125	1.3125	1.4688
p_3	0.5625	0.8125	1.3125	1.4375	0.9375	0.6875	1.1875	0.7813
p_{4_0}	0.0098	0.0068	0.0128	0.0083	0.0143	0.0173	0.0113	0.0071
$p_{4_{00}}$	0.0083	0.0173	0.0113	0.0068	0.0128	0.0098	0.0158	0.0161
p_5	1.0625	1.3125	0.8125	1.1875	0.6875	0.9375	1.4375	0.9688
p_{8_0}	0.8875	0.6375	1.1375	1.0125	0.5125	0.7625	0.2625	1.1063
$p_{8_{00}}$	1.1375	0.8875	0.3875	0.7625	0.2625	0.5125	1.0125	0.8563
p_{8_1}	0.5625	1.3125	0.8125	1.1875	0.6875	1.4375	0.9375	1.2188
p_9	26.875	25.375	22.375	23.125	26.125	27.625	24.625	25.5625
p_{11}	13.9375	14.6875	13.1875	15.0625	13.5625	15.8125	14.3125	14.0313
p_{12}	23.875	25.375	22.375	23.125	26.125	24.625	27.625	26.6875
p_{13}	13.5625	14.3125	15.8125	13.1875	14.6875	15.4375	13.9375	13.6563
	17	18	19	20	21	22	23	24
D_{S_0}	0.0058	0.0035	0.0080	0.0024	0.0069	0.0047	0.0092	0.0018
D_{C_0}	0.0005	0.0008	0.0013	0.0007	0.0012	0.0015	0.0009	0.0010
D_n	281.25	131.25	231.25	206.25	106.25	256.25	156.25	268.75
D_m	0.0075	0.0097	0.0052	0.0086	0.0041	0.0018	0.0063	0.0047
D_g	1.4688	0.7188	1.2188	1.3438	0.8438	1.0938	0.5938	0.7813
D_b	1.2813	1.0313	0.5313	0.9063	1.4063	1.1563	0.6563	0.8438

p_2	0.9688	1.2188	0.7188	1.0938	0.5938	1.3438	0.8438	1.0313
p_3	1.2813	1.0313	0.5313	0.6563	1.1563	1.4063	0.9063	1.3438
p_{4_0}	0.0131	0.0161	0.0101	0.0176	0.0116	0.0086	0.0146	0.0154
$p_{4_{00}}$	0.0101	0.0131	0.0071	0.0116	0.0176	0.0086	0.0146	0.0109
p_5	1.4688	1.2188	0.7188	1.3438	0.8438	0.5938	1.0938	0.9063
p_{8_0}	0.6063	0.8563	0.3563	0.2313	0.7313	0.4813	0.9813	1.0438
$p_{8_{00}}$	0.3563	0.6063	1.1063	0.4813	0.9813	0.7313	0.2313	1.0438
p_{8_1}	0.7188	1.4688	0.9688	1.0938	0.5938	1.3438	0.8438	0.6563
p_9	22.5625	24.0625	27.0625	27.8125	24.8125	23.3125	26.3125	26.6875
p_{11}	15.5313	13.2813	14.7813	13.6563	15.1563	14.4063	15.9063	14.5938
p_{12}	23.6875	25.1875	22.1875	22.9375	25.9375	24.4375	27.4375	22.5625
p_{13}	15.1563	15.9063	14.4063	14.7813	13.2813	14.0313	15.5313	14.5938
	25	26	27	28	29	30	31	32
D_{S_0}	0.0063	0.0041	0.0086	0.0030	0.0075	0.0052	0.0097	0.0011
D_{C_0}	0.0015	0.0012	0.00072	0.0013	0.0008	0.00059	0.0011	0.0013
D_n	168.75	218.75	118.75	143.75	243.75	193.75	293.75	290.625
D_m	0.0092	0.0069	0.0024	0.0058	0.0013	0.0035	0.00803	0.0070
D_g	1.2813	0.5313	1.0313	1.4063	0.9063	1.1563	0.6563	1.2969
D_b	1.3438	1.0938	0.5938	0.9688	1.4688	1.2188	0.7188	1.4219
p_2	0.5313	1.2813	0.7813	1.4063	0.9063	1.1563	0.6563	1.2344
p_3	0.8438	0.5938	1.0938	1.2188	0.7188	0.9688	1.4688	1.3906
p_{4_0}	0.0094	0.0064	0.0124	0.0079	0.0139	0.0169	0.0109	0.0126
$p_{4_{00}}$	0.0169	0.0079	0.0139	0.0154	0.0094	0.0124	0.0064	0.0159
p_5	1.4063	1.1563	0.6563	1.2813	0.7813	0.5313	1.0313	0.7969
p_{8_0}	0.5438	0.7938	0.2938	0.4188	0.9188	0.6688	1.1688	0.9969
$p_{8_{00}}$	0.5438	0.2938	0.7938	0.4188	0.9188	1.1688	0.6688	1.1531
p_{8_1}	1.1563	0.9063	1.4063	0.5313	1.0313	0.7813	1.2813	1.0781
p_9	23.6875	22.1875	25.1875	25.9375	22.9375	24.4375	27.4375	25.0938
p_{11}	13.0938	15.3438	13.8437	15.7188	14.2188	14.9688	13.4688	13.3281
p_{12}	25.5625	24.0625	27.0625	27.8125	24.8125	26.3125	23.3125	22.4688
p_{13}	13.0938	13.8438	15.3438	13.4688	14.9688	15.7189	14.2188	13.5156
	33	34	35	36	37	38	39	40
D_{S_0}	0.0056	0.0034	0.0079	0.0023	0.0068	0.0045	0.0090	0.0017
D_{C_0}	0.0008	0.00054	0.0010	0.0009	0.0014	0.0012	0.00067	0.00073
D_n	190.625	240.625	140.625	115.625	215.625	165.625	265.625	153.125
D_m	0.0025	0.0048	0.0093	0.0037	0.0082	0.0059	0.0014	0.0099
D_g	0.7969	1.0469	0.5469	0.9219	1.4219	0.6719	1.1719	1.4844
D_b	0.9219	0.6719	1.1719	1.2969	0.7969	0.5469	1.0469	1.4844
p_2	0.7344	1.4844	0.9844	1.3594	0.8594	1.1094	0.6094	1.2969
p_3	0.8906	0.6406	1.1406	1.0156	0.5156	0.7656	1.2656	0.9531
p_{4_0}	0.0066	0.0096	0.0156	0.0111	0.0171	0.0141	0.0081	0.0103
$p_{4_{00}}$	0.0099	0.0129	0.0069	0.0114	0.0174	0.0084	0.0144	0.0107
p_5	1.2969	1.0469	0.5469	1.4219	0.9219	0.6719	1.1719	0.8594
p_{8_0}	0.4969	0.7469	0.2469	0.3719	0.8719	0.6219	1.1219	1.1844
$p_{8_{00}}$	0.6531	0.4031	0.9031	0.2781	0.7781	1.0281	0.5281	0.7156
p_{8_1}	0.5781	1.3281	0.8281	1.2031	0.7031	1.4531	0.9531	0.5156
p_9	22.0938	23.5938	26.5937	27.3438	24.3438	22.8438	25.8438	26.9688

p_{11}	14.8281	14.0781	15.5781	14.4531	15.9531	13.7031	15.2031	15.3906
p_{12}	25.4688	23.9688	26.9688	27.7188	24.7188	26.2188	23.2188	26.5938
p_{13}	15.0156	15.7656	14.2656	14.6406	13.1406	13.8906	15.3906	14.8281
	41	42	43	44	45	46	47	48
D_{S_0}	0.0062	0.00395	0.0085	0.0028	0.0073	0.00508	0.0096	0.0014
D_{C_0}	0.0012	0.00148	0.00098	0.0011	0.0006	0.00086	0.00136	0.00077
D_n	253.125	103.125	203.125	228.125	128.125	278.125	178.125	221.875
D_m	0.0054	0.0031	0.0076	0.00198	0.0065	0.0087	0.0042	0.0062
D_g	0.9844	1.2344	0.7344	0.8594	1.3594	0.6094	1.1094	1.2031
D_b	0.9844	0.7344	1.2344	1.3594	0.8594	0.6094	1.1094	1.1406
p_2	0.7969	1.0469	0.5469	1.1719	0.6719	1.4219	0.9219	0.7656
p_3	1.4531	1.2031	0.7031	0.5781	1.0781	1.3281	0.8281	1.1719
p_{4_0}	0.0163	0.01331	0.0073	0.0148	0.0088	0.0118	0.0178	0.0129
$p_{4_{00}}$	0.0167	0.0077	0.0137	0.0152	0.0092	0.0122	0.0062	0.0066
p_5	1.3594	1.1094	0.6094	1.4844	0.9844	0.7344	1.2344	0.7031
p_{8_0}	0.6844	0.9344	0.4344	0.3094	0.8094	0.5594	1.0594	0.3406
$p_{8_{00}}$	0.2156	0.4656	0.9656	0.5906	1.0906	0.8406	0.3406	0.5594
p_{8_1}	1.0156	0.7656	1.2656	0.6406	1.1406	0.8906	1.3906	0.6719
p_9	23.9688	22.4688	25.4688	26.2188	23.2188	24.7188	27.7188	22.6563
p_{11}	13.8906	14.6406	13.1406	15.0156	13.5156	15.7656	14.2656	13.7969
p_{12}	23.5938	25.0938	22.0938	22.8438	25.8438	24.3438	27.3438	27.1563
p_{13}	13.3281	14.0781	15.5781	13.7031	15.2031	15.9531	14.4531	13.2344
	49	50	51	52	53	54	55	56
D_{S_0}	0.0059	0.00367	0.00817	0.00255	0.00705	0.0048	0.0093	0.00198
D_{C_0}	0.00127	0.00102	0.00052	0.00139	0.00089	0.00064	0.00114	0.0012
D_n	121.875	271.875	171.875	196.875	296.875	146.875	246.875	134.375
D_m	0.0017	0.00395	0.00845	0.00508	0.0096	0.0073	0.0028	0.0079
D_g	0.7031	1.4531	0.9531	0.5781	1.0781	0.8281	1.3281	1.0156
D_b	0.6406	0.8906	1.3906	1.0156	0.5156	0.7656	1.2656	1.2031
p_2	1.2656	0.5156	1.0156	0.6406	1.1406	0.8906	1.3906	0.7031
p_3	0.67188	0.9219	1.4219	1.2969	0.7969	0.5469	1.0469	0.7344
p_{4_0}	0.0069	0.0099	0.0159	0.0114	0.0174	0.0144	0.0084	0.0092
$p_{4_{00}}$	0.0126	0.00956	0.0156	0.01406	0.00806	0.0170	0.01106	0.0148
p_5	1.2031	1.4531	0.9531	1.0781	0.5781	0.8281	1.3281	0.6406
p_{8_0}	0.8406	0.5906	1.0906	0.9656	0.4656	0.7156	0.2156	0.2781
$p_{8_{00}}$	1.0594	0.8094	0.3094	0.9344	0.4344	0.6844	1.1844	0.3719
p_{8_1}	1.1719	0.9219	1.4219	0.5469	1.04688	0.7969	1.2969	1.2344
p_9	25.6563	27.1563	24.1563	24.9063	27.9063	26.4063	23.4063	23.7813
p_{11}	15.2969	13.0469	14.5469	13.4219	14.9219	14.1719	15.6719	14.7344
p_{12}	24.1563	25.6563	22.6563	23.4063	26.4063	24.9063	27.9063	22.2813
p_{13}	14.7344	15.4844	13.9844	15.1094	13.6094	14.3594	15.8594	14.9219

Appendix C Results of the mathematical model investigation

	1	2	3	4	5	6	7	8
m	0.5982	0.215	0.7321	1.0776	0.3499	0.5466	0.1400	0.3152
C_C	1.0376	0.7795	0.8062	0.3101	0.4375	0.7856	0.9931	1.316
C_S	0.2779	0.476	0.2032	0.0148	0.3888	0.3077	0.5108	0.4146
*FGF*1	3.6621	3.0659	4.0465	4.4377	1.5657	6.9173	3.1281	1.8207
*BMP*2	3.8201	2.3315	5.3087	1.7329	6.9741	4.6249	2.2303	3.577
	9	10	11	12	13	14	15	16
m	0.9373	0.4199	1.0681	0.5450	0.6240	0.3824	0.2195	0.8316
C_C	0.7614	1.0803	0.5413	0.7406	1.1207	0.9292	1.6943	0.8042
C_S	0.0979	0.3612	0.0273	0.3076	0.2650	0.3829	0.4629	0.1486
*FGF*1	5.0074	4.5112	4.8063	3.1708	2.2640	5.9213	2.4906	3.0667
*BMP*2	5.3259	3.8617	2.6619	1.4368	7.4866	5.185	3.4894	3.9660
	17	18	19	20	21	22	23	24
m	0.6515	0.5012	0.2882	-	0.2686	-	0.8423	1.0990
C_C	0.9789	1.3741	1.1998	-	0.5790	-	0.6557	0.2010
C_S	0.2493	0.3152	0.4348	-	0.4516	-	0.1506	0.0019
*FGF*1	3.8033	2.4157	7.9171	-	3.1099	-	1.9360	1.9905
*BMP*2	2.7256	2.8415	2.8215	-	4.8565	-	2.0348	2.3157
	25	26	27	28	29	30	31	32
m	-	0.7490	-	0.3722	0.7919	0.6564	0.8198	0.8320
C_C	-	0.9692	-	1.3878	0.7556	0.9994	0.6076	0.6377
C_S	-	0.1949	-	0.3794	0.1730	0.2488	0.1567	0.1472
*FGF*1	-	1.3102	-	3.3675	3.8432	3.6281	3.0314	3.9435
*BMP*2	-	4.9400	-	3.8044	7.3184	8.3667	1.9080	6.3305
	33	34	35	36	37	38	39	40
m	0.2013	0.5419	0.0856	-	0.8856	0.3978	0.8963	1.0964
C_C	0.6829	1.3217	0.7386	-	0.6010	1.4700	0.5758	0.1069
C_S	0.4827	0.3055	0.5416	-	0.1229	0.3653	0.1193	0.0023
*FGF*1	2.9481	3.5707	1.6303	-	6.5989	2.4895	4.3165	4.1863
*BMP*2	2.2565	2.6339	7.6867	-	5.0906	3.8176	1.9505	6.6589
	41	42	43	44	45	46	47	48
m	0.5745	0.7012	0.2586	0.0167	0.4678	0.5642	0.7082	0.1572
C_C	0.9367	0.8136	0.9458	0.1744	0.9135	1.3071	0.7062	0.7271
C_S	0.2904	0.2307	0.4538	0.5183	0.3407	0.2943	0.2187	0.5069
*FGF*1	3.4220	3.4029	5.5431	3.4370	6.0843	1.2618	5.6291	4.4711
*BMP*2	2.4714	2.1959	5.4392	4.1516	2.3561	2.6471	4.0417	5.9224
	49	50	51	52	53	54	55	56
m	0.6195	0.3160	0.6739	0.9191	0.4149	0.5855	0.1199	-
C_C	1.070	0.6936	0.7177	0.5920	1.6777	0.9843	1.3427	-
C_S	0.2705	0.4232	0.2367	0.1075	0.3547	0.2877	0.5180	-
*FGF*1	2.1485	9.9384	2.8559	1.9884	4.6092	4.4418	3.3682	-
*BMP*2	2.1416	1.6862	4.2940	2.0489	5.0816	3.9575	4.0476	-