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The relevance of research of regeneration peculiarities in tension of tissues distension

The article shows the importance of studying the regeneration of features at a tensile stress of tissues. The article shows the perspective of this study, the results of which will reveal the underlying mechanisms of the regeneration process at a tensile stress, in particular, and reduction reactions of the organism as a whole, and give sufficient theoretical material to justify the creation of drugs for the treatment of various pathological conditions. Deeper and more detailed study, and further, the management of these processes will create a theoretical background for the preparation of medicaments for the treatment of many diseases, including the aging, we also believe pathology of the whole organism.
Keywords: tissues, distension, regeneration, healing of wounds

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Ұлпалардың созылуының күштенуі кезіндегі регенерация ерекшеліктерін зерттеу өзектілігі

Мақалада ұлпалардың созылуының күштенуі кезіндегі регенерация ерекшеліктерін зерттеу өзектілігі көрсетілген. Алға қойылған мақсаттарды шешуді жүзеге асыру созылудың күштенуі кезінде регенеративтік үдерістің терең механизмдерін ашуға мүмкіндік береді жекелей алғанда ағзаның қайта қалпына келуін, ал мүмкіндігінше толықтай түрлі патологиялық жағдайларды емдеуге дәрілік құралдарды жасап шығаруды негіздеуде ауқымды теориялық материал болып табылады. Терең әрі жекелей зерттеу келешекте көптеген ауруларды біз бүкіл ағзаның патологиясы деп білетін қартаюды да емдеу үшін алынатын дәрілік препараттардың теория жүзіндегі алғышарттарын құрастырып, осы үрдістерді басқаруға мүмкіндік береді.
Кілтті сөздер: созылуының күштенуі, регенерация, ұлпаның созылуы, жараның жазылуы

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Актуальность изучения особенностей регенерации при напряжении растяжения тканей

В статье показана актуальность изучения регенерационных особенностей при напряжении растяжения тканей, а также перспективы исследования, результаты которого позволят раскрыть глубинные механизмы регенерационного процесса при напряжении растяжения, в частности, и восстановительных реакций организма, в целом, и дадут достаточный теоретический материал для обоснования создания лекарственных средств для лечения различных патологических состояний. Более глубокое и детальное изучение, а в дальнейшем управление этими процессами позволят создать теоретические предпосылки для получения лекарственных препаратов для лечения многих болезней, вплоть до старения, которое мы также считаем патологией всего организма.
Ключевые слова: напряжение растяжения, регенерация, растяжение тканей, заживление ран.

Effectiveness in the use of apparatuses of the author's and others design is proved clinically and experimentally in treating wounds and wound complications [1,2,3,4,5]. As a result of this research, it was found that while using these technical device, tissue tension was detected, which had been discovered by G.A. Izmaylov and is observed during the wound regeneration process.

Tissue regeneration problem is one of the key issues in Biology and Medicine. This process provides tissue homeostasis in physiological conditions and structure recovery, impaired functions at injuries [6]. This issue is extremely important for medicine as majority of diseases more or less is connected with organ injury and the efficacy of the administered therapy is defined by the course of restorative processes. At the same time most mechanisms of the regenerative process and especially its regulation are still not studied enough [7].

It is planned to study the cell mitosis stimulation mechanisms in tissue tension. We think that distension tension includes certain genes in cells responsible for their division. Low-response genes are the main ones involving in cell division, the key process of regeneration, the functions of which are still being investigated by many researches [8,9]. Search and identification of these genes will allow understanding deeper the restorative tissue processes.

If the opportunity to strain various body tissues especially the connective tissue is an established fact nowadays and is widely used in clinical practice then the question about how this process is structurally achieved is unclear at present time. There are no doubts that morphological changes occur in the process of tissue distension owing to which the integrity is preserved and tissue mass is deposited. Mechanical tissue distension leads to progressive tissue changes i.e. in its hyperplasia. Although, depending on what cell elements take part in tissue distension and what changes they are exposed to, there are different viewpoints. Some authors think that origin of cell division in tissue distension is cambial and mesenchyme cells that differentiate into that cell type, which is exposed to distension. Cambial cells are supposed to have strict determinacy and specificity for each of new in tissue distension, i.e. hyperplasia of cambial cells, precursor of osteoblasts in bone distension and fibroblasts in skin distension and etc. Another group of authors hold the opinion on the metaplastic development of the single mesenchyme and cambial cell into connective cell i.e. osteoblast, fibroblast, smooth muscle cell and etc.

There is a hypothesis assuming the synthesis of specific and metaplastic cambial theories. It is considered to be important that practically all authors assign a significant part to the vascular system in regeneration and hyperplastic processes and namely to such cells of walls of minute vessels such as pericytes lining the vessel inside [10].

Mechanisms of tissue processes in regeneration process of intracellular changes during mitosis of various aspects were studied in different experimental conditions [11,12]. Though, morphological changes in regenerative processes in bone tissue namely in Distraction osteosynthesis are studied better than in skin distension, but in both cases they demand further research especially regarding more accurate identification of cell elements, which plays a determining role in these processes.

Thus, the stated above hypotheses point at cell elements, which might take part in the very effect, but they do not show what impact the studied tissue distension tension has on them.

Distension stimulates the growth of tissue, the number of vessels, cells and intracellular substance increases. The reference list also indicates that in skin distension the number of active fibroblasts goes up and blood supply in tissues increases. Though, molecular and genetic aspects of this problem are not actually investigated.

Thus, the stated above represents that the study of functional activity of the cell's restorative system in view of its possibility to be involved in regeneration is a relevant scientific problem requiring task-oriented and special investigations.

As a result of research, molecular and biological aspects of tissue regeneration in distension tension will be studied. In-depth and thorough study will allow developing theoretical grounding to tissue regeneration process. The effect of genetic apparatus on mitotic process in tissues exposed to mechanical attack will be investigated during the research. For the first time the certain genes of mitotic cells of tissues undergone mechanical attack will be demonstrated

Solving of the set tasks will allow discovering the deepest mechanisms of regeneration process in distension tension namely restorative reactions of the body in whole and will provide sufficient theoretical

background for proving developing medicaments for treating various pathologic conditions.

In-depth and thorough study and at a later stage controlling these processes will let to develop theoretical background for getting medicaments for treating many diseases up to ageing, which we consider to be pathology of organism.

Scientific methods' description used in the project as a means to achieve the set goals, grounding of the selected method;

Research target is histological material taken from laboratory animals, the tissues of which undergone distension tension with the help of apparatus methods (patent of the Republic of Kazakhstan № 3864 dated from 15.08.2007 and etc.) Final research target is biopsy material of the same laboratory animals from the symmetrical skin areas.

Selection representation in the experiment is very high due to the fact that laboratory animals are of the same breed, sex, and age (rabbits of the breed " Belyi Velikan" "White Giant", buck rabbits, age of 1,5-2, weight 4500-5000 gr.). Test and experimental wound will be located in the same animal. The number of animals will reach 280, from which the histologic material will be collected at different time periods (every 6 hours during 7 days).

On the one hand, the animals' wounds will undergo mechanical attack by apparatus method, on the other hand, check sample will be taken from the same laboratory animal.

It is planned to sample biopsy materials by 0, 5-0,7cm thickness being fixed in 10 % normal formalin solution. Preparation staining will be done by the ordinary hematoxylin, eosin and iron hemotoxylin method as well as by Gieson's staining.

The essence of our molecular and genetic investigations is in study of DNA with the use of PCR obtained by reverse transcription polymerase processing of the isolated RNA. RNA driven out from the investigated biopsy material and be the DNA product will indicate the activity of one or another gene being responsible for mitotic process. It is planned to study RNA (DNA) of the following genes – slow-response genes (cyclin D, cyclin-dependent kinase 4, Mys, Cds25a) and p27 gene –inhibitor of the whole range of cyclin-dependent complexes.

Plan of Molecular-Genetic Research

Olegonucleotide Design

For designing oligonucleotide sequences will be used various computer programs: FastPCR, UGENE, BioEdit, Primer Expres, Oligo 7.37, PrimerPremier, VectorNTI, Methyl primer express v1.0 and genome browsers (for example UCSC Genome Browser, Ensembl) and other corresponding data-bases such as RefSeq, GenBank, Nucleotide BLAST, OligoCalc and others.

RNA Isolation

Genome RNA isolation from the biological material will be performed with the use of TRIzol® methods and phenol-chloroform method. These methods are usually used on demand of simultaneous isolation from total cell RNA, genome DNA or total proteins. As far as these methods allow isolating the whole genome RNA and DNA out of the biological material, they can be used to estimate the RNA quality in the analytical sample. Quality of genome RNA isolated by these methods is high enough and can be directly used for different purposes including RT-PCR amplification, restriction analysis and etc.

Amount and purity of RNA preparation will be estimated spectrophotometrically.

RT-PSR

Reverse transcription or information transfer from RNA to DNA will be performed with the use of reverse transcription enzyme.

Complementary DNA (cDNA) being formed on the basis of cell template DNA (mRNA) is formed as a result of RT-PCR activity on the basis of revertase.

Synthesized cDNA will be directly used for PCR amplification method. Purification of the isolated DNA by the method of phenol-chloroform extraction with ethanol precipitation will be carried out, which will allow escaping impurities affecting PCR quality.

PCR

We will carry out PCR having specific primers on cDNA template using heat-stable TaqDNA polymerase and Mastercycler proS (Eppendorf, Germany) amplifier. PCR-products will be separated by us electrophoretically in 15% agaroze gel laced with ethydium bromide. Gene expression level will be estimated by UV-gel analyzer using the program Quantum St4 (Vilber lourmat).

Crucially new facts will be discovered in the field of regeneration process research. Molecular and biological aspects of tissue regeneration in distension tension will be studied owing to investigation. In-depth and thorough investigation will allow

developing theoretical foundation to regeneration process in tissues. The effect of genetic apparatus on mitotic process in tissues in mechanical attack will be studied during the research. Stimulation mechanism of mitotic division in distension tension will be grounded.

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