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Life Science Nanotechnological Approach in Drug Development for Wound Healing and Critical Care Medicine

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The use of drugs on the basis of nanotechnological heal wounds and Critical Care Medicine.

Key words: Life Science, nanotechnological Approach, drug Development, Wound Healing. Critical Care Medicine

We have to remind ourselves that the first and foremost function of Human Physiology is not well-being or reproduction, but a survival from extreme conditions. Human organism as a part of a mammalian system, during the evolution developed the most sophisticated adaptation mechanisms to post-aggressive reactions of the organism - critical (physical aggression) and extreme (environmental) conditions.

The pathogenesis of post-aggressive reactions of the Organisms (critical/extreme/shock condition after severe trauma) is one of the most vital subject not only for critical medicine, but is the fundamental factor in understanding of mechanisms of how the Living System adapts not only to the critical conditions but also to chronicle malfunction of various organs and daily stress.

The basic mechanisms of adaptation to post-aggressive reactions and thereafter probable tanathogenesis are similar regardless of there initial cause. Understanding the pathogenesis of such processes helps develop strategy how to support self-healing processes of the Organism, but not interfere with them.

Only simultaneous study of Biological Active Substances & Circulation parameters allowed us to understand when the biological information communication starts to be broken between First & Second Degree Messengers, Intracellular Transmitters, Cellular receptors, and other biochemical parameters.

There is no deficit or reduction of the First Degree Messengers during the entire process of post-aggressive reaction of the Organism. Even at latest stages of tanathogenesis majority of biological active substances responsible for maintenance of integral hemodynamic parameters were significantly increased, but by some reason there efforts to maintain blood perfusion and blood pressure was ineffective.

Major cause of interruption/blockage of Biological Information Transfer is insufficient activity and/or quantity of membrane & cytosol receptors. If extensive adaptation to severe trauma continues more than 3.5 – 4 hours than the substantial decline of cellular activity observed.

It was observed paradox situation in adaptation of the Living Organism to severe trauma - if from the initial stages adaptation to extreme condition happens on a lower pace, meaning less aggressive attempt of the Organism to help 'survive' the body, than the Organisms has higher chance to survive and avoid tanathogenesis processes. This happens due to the lower speed of cellular receptors utilization on the initial stages of post-aggressive reaction.

Based on fundamental scientific research it was developed several proprietary life science nanotechnologies, which currently are in use as a technological platform for creating multiple products oriented toward enhancement of self-healing processes with specific curative effects to repair malfunctioning biological information transfer. This technological platform is based on the development of Bioactive Complexes Modeling (NANO-COMPLEXES™), which has the ability to manipulate not only with Nano (10^{-9}), but also with Pico (10^{-12}) quantities of bioactive substances, targeting the problem-specific biochemical pathway.

PATHOGENESES OF POST-AGRESSIVE REACTIONS OF THE LIVING ORGANISM

After severe post-aggressive reactions (traumatic shock) the body mobilizes all possible resources and triggers all available defense mechanisms intend to only one objective – how to survive from extreme condition and/or how to resist the death. The mechanisms of post-aggressive reaction of the Organism and its adaptation to critical condition involve activation of very identical complex of biologically active substances. Only on the latter stages, if the Living Organism is 'survived' the cause of aggression requires specific attention and is subject of special treatment. A similar process happens in less aggressive post-traumatic wound healing processes, but initial steps are similar to 'regular' post-aggressive reaction of the Organism.

Reasonable question is that why today, in modern technology-oriented medicine the problem post-aggressive reactions continues to be actual and why we need deeper knowledge in understanding of details of genetically determined adaptation mechanism to the critical conditions? There are several reasons:

- Historically, people always tried to understand what is going on in Human Body and empirically reproduce some of the processes physiologically existing in normal functional system;
- Pathogenetic mechanisms of post-aggressive reactions (example, traumatic shock) is one the rare processes, when scientists has the ability to monitor the process from its initial stages up to the end - either survival of the Organism, or transformation of adaptation mechanisms into tanathogenesis processes. Such an approach allows for the deep understanding of the systemic and intracellular regulation throughout the adaptation mechanism to the extreme conditions.
- This kind of scientific research provides us with the knowledge of how to imitate/stimulate physiological processes, but not interfere with them. Modern pharmaceutical industry approach is to fix the symptoms, but not to treat existing problem. It often works for short term, thus creating multiple health problems in long term.
- Last, but never the less, very important part of the necessity of this kind of research lays down in a military aspect. Usually, huge amount of traumatic and hemorrhagic shock conditions exists on the battlefield. During all wars the lethality after traumatic and hemorrhagic shock was on a first place. We are speaking about millions and millions of people to whom medicine was powerless to help. This is one of the reasons why in last 200 years the scientific literature referred to different aspects of pathogenesis of shock conditions is one of the most represented in bio-medical field.
- There are no any other more serious extreme conditions in the Human Body than shock process, when all adaptation mechanisms are activated simultaneously for only one purpose – stimulate all internal resources to help the Living System to survive after a severe trauma. During the shock process, body mobilizes its all defense systems, fighting the Death.

As a part of post-traumatic adaptation mechanisms, the subject of wound healing processes related to the same family of problems.

In a Living Organism there is a complex of specific Bioactive Substances involved in normal Biological Information Transfer sustaining normal physiology and homeostasis, thus allowing the Living Organism adaptation to critical conditions. When by a number of reason the adaptation mechanisms becoming ineffective or insufficient than pathogenesis moves toward tanathogenesis (mechanism of death). When it is happens and how it is happens is a critical question for all time medicine. Understanding the mechanism of pathogenesis transformation to tanathogenesis helps identify the level of malfunctioning biological information transfer and thereafter increase the effectiveness of its restoration. Reinstatement of normal biological information transfer increases effectiveness of treatment processes not only in critical conditions, but helps to find new solutions in treatment of chronicle dysfunctions.

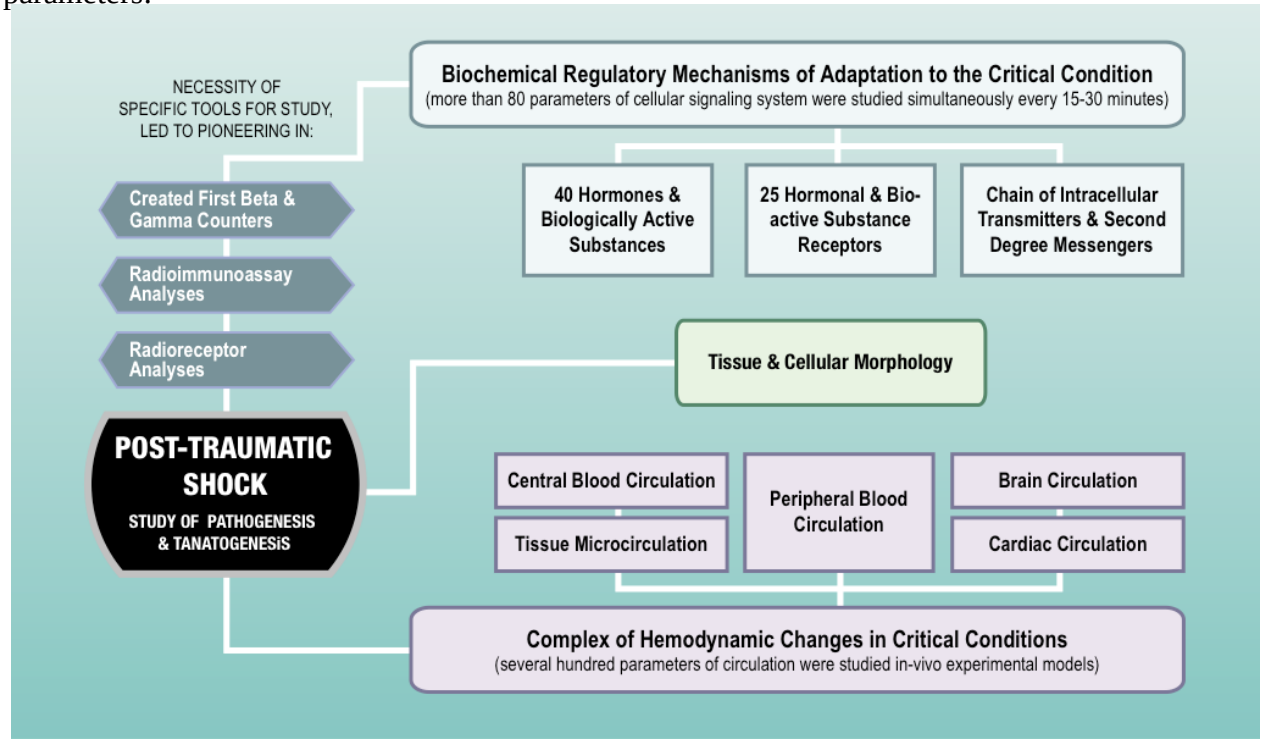
METHODOLOGY OF POST-AGRESSIVE REACTIONS STUDY

A: BASICS CRITERIA

To understand the pathogenesis of shock process as well as the mechanisms of tanathogenesis it was conducted very complex, extensive and expensive experiments through which was studied hundreds of inter-related parameters regulating homeostasis during the post-aggressive reaction of the organism (severe traumatic shock). This study has been conducted in the process of post-aggressive reaction of the Organisms after severe trauma. In the process of post-aggressive reaction it was evaluated:

- More than 80 hormones, biologically active substances, intracellular transmitters and cell receptors were evaluated. Each mentioned above biochemical parameter has been studied in the process of pos-aggressive reaction development of the Organism every 30 minutes – 1hour. Test materials has been collected from multiple arterial and venous systems (local and central blood circulatory system) as well as from different organs/tissues.
- In parallel to biochemical parameters very complex study (more than 100 parameters) of hemodynamics was conducted: cerebral hemodynamics, peripheral circulation, and local microcirculation; contractile function of the myocardium and coronary circulation. Majority of the circulation parameters was continuously monitored and the rest measured every 30 minutes.

- The study included evaluation of the following methodological, biochemical and circulation parameters:



Developed models permits to understand how the Living Organisms regulates itself, down to the minutest level of intracellular processes.

Majority of study of post-aggressive reactions of the Organisms was conducted on various models of experimental traumatic shock. These complex studies was conducted by team of scientists, including medical doctors, biologists, biochemists in a unique laboratory in Georgia, Tbilisi (former USSR) at the Institute of Experimental Morphology Academy of Science and than continued in the USA in MD SCIENCE, Inc. & BIONOVA, Inc.

To analyze enormous amount of the experimental data it was necessary to develop completely new methodology of study, as well as to build-up a new not-existed test systems. With the help of the brightest mathematical and engineering minds we were able to create new equipment, new data processing, and most importunately new methodology of analyses of such a complex pathogenetic

processes as post-aggressive reactions of the organisms. This research conducted over 20 years and developed models would permit us to understand how the Living System regulates itself, down to the minutest level of intracellular processes.

B: METHODS OF BIOLOGICAL ACTIVE SUBSTANCES STUDY

The concentration of hormones and biologically active substance has been evaluated by radioimmunoassay (RIA), which is characterized by high specificity, high sensitivity and highest possible precision. Bellow is presented only some major biological active substances evaluated during years of the experiments. We are listing only substances, which based on our research, are responsible for adaptation mechanisms to extreme conditions.

The names of hormones and biologically active substances are given in accordance with the nomenclature recommended by the International Biological Union.

FIRST DEGREE MESSENGERS

- Adeno-Pituitary (Adenohypophysis) Function: Adrenocorticotropic Hormone (ACTH); Lutropin (Luteinizing Hormone (LH); Follitropin (Follicle-stimulating Hormone (FSH); Somatotropin (Growth Hormone (STH).
- Neuro-Pituitary (Neurohypophysis) Function: Vasopressin (ADH).
- Cortico-Suprarenal Gland Activity: Hydrocortisone (11b,17a,21-trihydro-4-pregnene, 3,20-dion); Corticosteron (17-deoxycortisol, 11β,21-dihydroprogesterone.
- Mineralocorticosteroid: Aldosteron(11b,21-dihydroxy-3,20-dioxo-4pregnene-18al-18-11-hemiacetal).
- Androgens: Testosterone; Dehydroepiandrosrerone; 5a-Dehydrotestosterone.
- Estrogens: 17a-Estradiol; Estron; Estriol.
- Gestagens: Progesterone; 17a-Hydroxyprogesterone.
- Renin-Angiotensin System: Plasma Renin Activity (PRA); Serum Angiotensin Converting Enzyme (SACE).
- Medulla-Suprarenal Gland Activity: Epinephrine; Arterenol.

SECOND DEGREE MESSENGERS: Cyclic Adenosine Monophosphate (c-AMP; 3'5'-AMP); Cyclic Guanosine Monophosphate (c-GMP; 3'5'-GMP); Calmoduline (Calcium Binding Protein); Cyclic Nucleotide Phosphodiesterase Activator.

INTRACELLULAR TRANSMITTERS: Prostaglandin A (PGA); Prostaglandin E (PGE); Prostaglandin E2 (PGE2); Prostaglandin E1 (PGE1); Prostaglandin F2□ (PGF2□); PGF1□-6 keto-13,14-dihydro-15-keto; PGF2□□ 11-Deoxy-13,14-dihydro-15-keto-11□,16e-cyclo-PGE2.

CELL MEMBRANE AND CYTOSOL RECEPTORS ACTIVITY: All membrane and cytosol receptors were evaluated using various types of proprietary radioisotope ligand techniques. Using corresponding isotope (ligand) the following receptors activity and/or amount have been determined:

- **Membrane Type of Receptors:** □1 and □2 – adrenoreceptors; □1 and □2 – adrenoreceptors, Angiotensin II receptors, Prostaglandin E2 receptors, Prostaglandin F2□□receptors.
- **Cytosol Type of Receptors:** Glucocorticosteroid receptors; Mineralocorticosteroid receptors; Estrogen receptors; Androgen receptors.

C: METHODS OF BLOOD CIRCULATION STUDY (HEMODYNAMIC)

Special algorithm and application package for automatic processing over 100 parameters of blood circulation both on-line and off-line systems have been developed. Majority of the hemodynamic parameter was continuously monitored and the rest measured every 30 minutes.

Following parameters of circulation has been monitored:

- Central (systemic) hemodynamics
- Cerebral circulation
- Peripheral circulation
- Microcirculation
- Contractile function of the myocardium

- Coronary circulation

All studies was conducted by parallel monitoring of multiple biochemical changes in different parts of body (hormones, biologically active substances, intracellular transmitters activity, cellular receptors, etc.) with the changes in systemic and local hemodynamics in association with microcirculation.

CONCEPT OF BIOLOGICAL INFORMATION TRANSFER

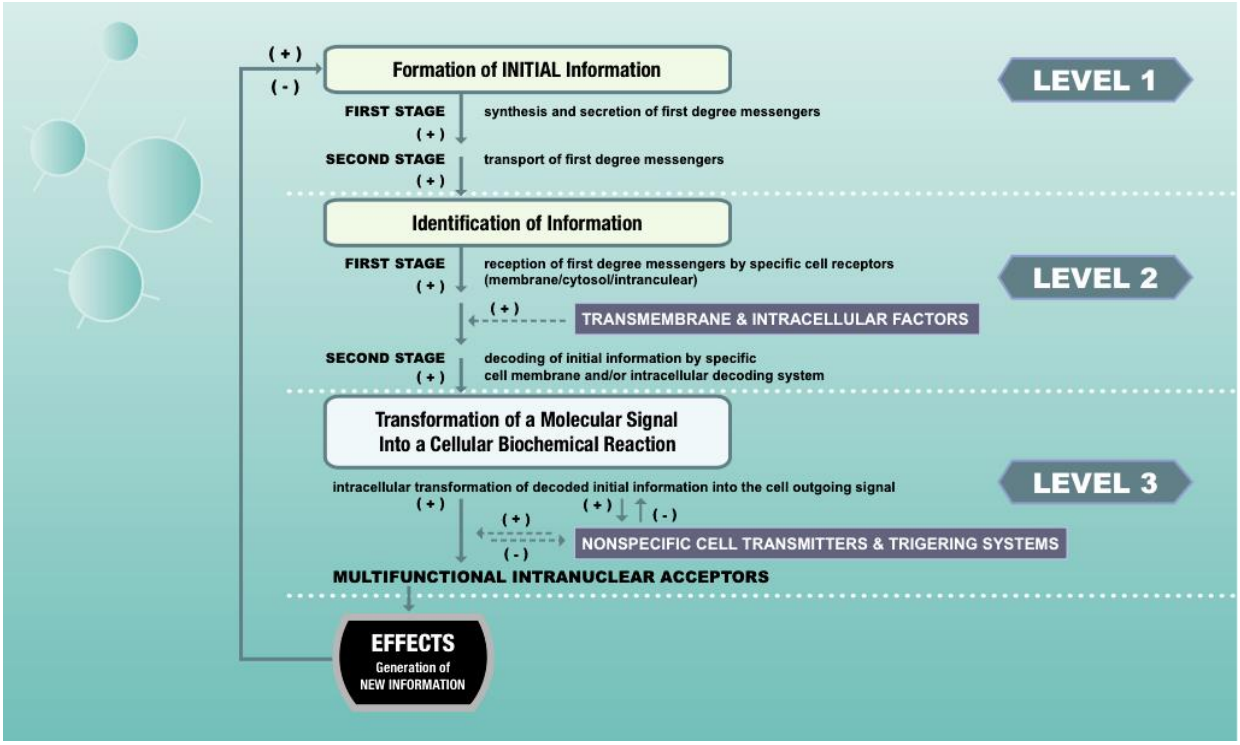
Regardless of the nature of the post-aggressive reaction of the Organisms and/or chronicle disease, Living Organism is facing very complex malfunction in a biological information transfer. It is impossible to restore such complex dysfunction by intervening with high dosage of the singular active substances, which is the strategy of pharmaceutical industry.

Where is the solution? Restoration of broken biological informational transmission requires completely different and complex approach; not to devastate already unstable system of biological information transfer. The only way to restore biological information transfer would be through physiological restoration of self-healing processes.

Even though our original goal was to create new substance (drug) for treatment of patients after severe trauma, after two decades of experiments it become evident that it is impossible to create any new substance that itself could be helpful to reduce lethality after severe aggression on the Living System. With this data, it becomes obvious that no new substances needed to be developed. Everything that our body requires has already been created and placed within and our cells, which know the best how to fix themselves.

The entire system of biological information communication and intricate biochemical pathways become understandable and tangible. This was expensive and logway groundwork to create an absolutely different approach in restoration of malfunctioned biological information transfer.

As a result of this extensive research it was developed a new scientific "Concept of Biological Information Transfer", which reshaped conventional approaches in medicine. The basics of our scientific concept are that everything that human body needs has already been placed within and that our cells are programmed to fix themselves. This scientific concept was the first to reveal that the best approach to the 21st century medicine would be using substances that naturally exist within the body, instead of having to create and administer new molecules that are foreign to the body.



The basic outcome of the discovered scientific concept is based on fundamental rule that the restoration of the information stability in the living organism is possible only by:

- Reconstruction of biological information transfer by restoring the genetically determined chain of information transfer.
- Restore of the genetically determined chain of information transfer by acting simultaneously on all the levels of the information transfer mechanism.
- Ensure the stability of information flow in the Living Organism by supporting all the basic elements responsible for biological information transfer.

Restoration of normal biological information transfer, i.e. reconstruction of the genetically determined chain of biological information transfer is only possible by means of creating biologically active complexes containing all informational substances necessary for normal biological information transfer.

Based on developed scientific concept it was discovered methods of 'copying' nature of how to 'put together' multiple biological informational substances in physiological concentration (nano-and pico quantities) to imitate biochemical processes between and within the cellular communication. Nano-and pico quantities reflects normal range of biologically active substances needed to repair malfunctioning lines of information communication in the Living Organism.

Restoration of biological information transfer required development of new technologies, based on bioengineering of biologically active NANO-COMPLEXES™.

CONCLUSION OF RESEARCH DATA

Even very complex study of biologically active substances do not allows to objectively understanding the functional condition of the Organism. Only simultaneous study of Biological Active Substances & Circulation parameters allowed us to understand when the biological information communication starts to be broken between First & Second Degree Messengers, Intracellular Transmitters, Cellular receptors, and other biochemical parameters.

Detail analyses of each biochemical parameters and circulation data was a subject of our multiple articles, which has been presented to scientific community in different time. Now we will try to overall present our understanding of mechanisms of post-aggressive reactions of the organisms and there adaptation to critical condition:

1. There is no deficit or reduction of the First Degree Messengers during the entire process of post-aggressive reaction of the Organism. Even at latest stages of tanathogenesis majority of biological (majority of adeno-pituitary hormones, corticosteroids, catecholamines, rennin-angiotensin system, including one of the strongest vasopressors, angiotensin II) responsible for maintenance of integral hemodynamic parameters were significantly increased, but by some reason there efforts to maintain blood perfusion and blood pressure was ineffective.
2. Major cause of interruption/blockage of Biological Information Transfer was insufficient activity ans/or quantity of membrane & cytosol receptors. If extensive adaptation to severe trauma continues more than 3.5 – 4 hours than the substantial decline of cellular activity observed. This is an indication that the cellular receptors have been utilized and there is not enough time and internal resources to re-synthesize new 'batch' of cellular receptors. Then outcome of such decline in receptors activity/quantity is significant reduction of biological information transfer from First Degree Messengers to activate intracellular cascade of Second Degree Messengers, supporting adequate function of cell metabolism and its functional activity.
3. We observed paradox situation in adaptation of the Living Organism to severe trauma - if from the initial stages adaptation mechanisms to extreme condition happens on a lower pace, meaning less aggressive attempt of the Organism to help 'survive' the body, than the Organisms has higher chance to survive and avoid tanathogenesis processes. This happens due to the lower speed of cellular receptors utilization on the initial stages of post-aggressive reaction. It allows sustain

(even on a lower level) the biological information transfer from First Degree Messengers to support biochemical cascade of intracellular Second Degree Messengers functionality.

What is the mechanism of such paradox? Such survival (lover rate of activation) of First Degree Messengers from first the stage of adaptation allows to maintain Biological Information Transfer on a moderate level and maintains its recourses for longer time, thus allowing the cells to re-synthesize vital for normal functioning membrane & intracellular receptors.

This is a part of adaptation mechanism of the Living System, which is genetically determined for moderate trauma, when immediate and intensive increase of catabolic hormones and intracellular transmitters helps Organism to survive on short distance. This mechanisms allow the Living System to easy and quickly adapt to the moderate aggression; at the same time, when the Living Organism reacts the same way to extensive (time-vise) aggression the cell receptors resources comes to the end – they have no time to be re-synthesized to support intracellular transmission of biological signaling from First Degree Messengers.

4. How to fix the problem? The original goal was if not to find 'magic bullet', but at least to develop new drug, which can help to enhance self-healing processes and normalize adaptation mechanism. Soon we realized that there is no such a solution which allows developing new effective molecule to treat the patients under severe post-aggressive reaction of the Organism. Living System is very complex functional system, where hundreds and thousand of substances involved in normal physiological processes and even more bioactive substances is activating during post-aggressive reaction to help the Organism to survive. When we, Humans, looking to find some miracle substance or substances to increase the adaptation mechanisms its sounds very unrealistic. Looking to all bio-physiological parameters involved in adaptation mechanism to support the stability of Biological Information Transfer we realized that it is impossible to develop singular substance/molecule, which can normalize such a complex dysfunction as happening in post-aggressive reactions of the organisms. Even more, if we look carefully to pathogenesis of any chronicle disease and to the complexity of biologically active substances involved in such a processes, than its becoming understandable such low efficacy of drugs.

LIFE SCIENCE NANOTECHNOLOGY IN NEW DRUG DEVELOPMENT

As it mentioned above, the next step in our research was to find the method how to fix malfunctioning biological information transfer. Based on fundamental scientific research it was developed several proprietary nanotechnologies, which currently are in use as a technological platform for creating multiple products oriented toward enhancement of self-healing processes with specific curative effects to repair malfunctioning biological information transfer. This technological platform is based on the development of Bioactive Complexes Modeling (NANO-COMPLEXES™), which has the ability to manipulate not only with Nano (10^{-9}), but also with Pico (10^{-12}) quantities of active substances, targeting the problem-specific biochemical pathway. The usage amounts of bioactive substances in NANO-COMPLEXES are precisely within the physiological range of a Living Organism.

Developed technological platform is based on inventions, which can be grouped into two categories:

- **Opti-Path™:** nanotechnology of Biologically Active Complexes Modeling; this technology allows to bring together (assemble) all necessary bioactive substances supporting restoration of biological information transfer of a specific metabolic pathway. The type, concentration and quantity of the substances in the complex depend on the pathway being corrected.
- **NuCell-Direct™:** nanotechnologically based delivery system; the composition and structure of developed delivery system approximates the structure of a human cell membrane. NuCell-Direct provides entrapped active ingredients with the extraordinary stability to the extreme physical and chemical conditions.

Developed Nanotechnological platform is based on NANO-COMPLEXES containing all necessary Bioactive Substances necessary to restore normal Biological Information Transfer between and within the cellular communication, enabling to create physiological approach for treatment of multiple human dysfunctions and diseases.

NANO-COMPLEXES in comparison with classical drugs (singular molecules with specific effect) triggers adaptation system of the Living System through enhancement of self-healing properties.

WHAT EXATLY ARE NANO-COMPLEXES™?

NANO-COMPLEXES is a complex of naturally existing in Living System biologically active substances composition stabilized and incorporated into specially developed NuCell-Direct Delivery System by means of Nanotechnological processes.

Usually, NANO-COMPLEXES imitate composition and concentration of bioactive substances existence in healthy human bodies. They contain First and Second Degree Messengers together with a cellular signaling substances necessary for re-establishment of inter- and intra- cellular communication. All informational Bioactive Substances are incorporated and stabilized in NuCell-Direct Delivery System, which allows all ingredients to act synergistically as one unit.

- Biologically Active NANO-COMPLEXES™ support activation of adaptation system of Human Organism providing protection / prevention / treatment of multiple diseases.
- NANO-COMPLEXES composed of the Bioactive Substances naturally existing in a Living Organisms with strong and predictable results, without any side effects.
- The usage amount of individual biological active substances in NANO-COMPLEXES is from hundreds to thousand times less than in currently available Drugs. Concentration of Bioactive Substances in NANO-COMPLEXES is not exceeds the buffering range of those bioactive substances in Living Organism and is in range of pico and nano quantities.
- Delivery System: biologically active substances used in billions & trillions of the gram requires new generation of delivery system being able to:
  - stabilize really unstable biologically active substances
  - delivery of bioactive systems to the targeted places

მ.დანიელოვი
სიცოცხლის მეცნიერების ნანოტექნოლოგიური მიდგომა ჭრილობების შეხორცების პრეპარატების შექმნაში და კრიტიკული მედიცინაში

კვლევა მოიცავს ნანოტექნოლოგიის საფუძველძე ჭრილობების შესახორცებელი მედიკამენტების მომზადების პრობლემას. ასევე გაანალიზებულია კრიტიკულ მედიცინაში გამოყენებული ჭამლების ნანოტექნოლოგიის ბაზაზე მომზადების საკითხები.